PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION OF SOME SPECIES OF FAMILY LEGUMINOSAE



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INSTITUTE OF CHEMICAL SCIENCES

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DECLARATION

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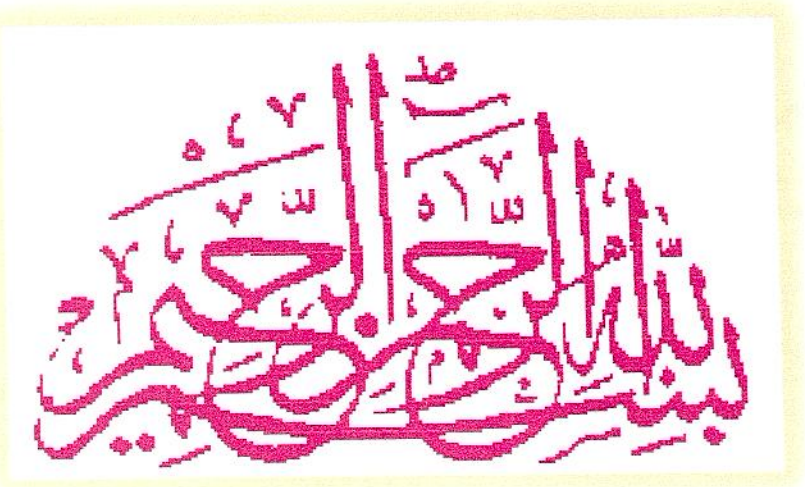
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In the name of ALLAH

The Most Beneficent,

The Most Merciful

This Thesis Is Dedicated TO

MY

FATHER AND MOTHER

(The eternal sources of guidance)

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LIST OF ABBREVIATIONS

^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance Spectroscopy
^{1}H NMR	Hydrogen-1 Nuclear Magnetic Resonance Spectroscopy
2D NMR	Two Dimensional Nuclear Magnetic Resonance Spectroscopy
Ar	Aryl
AcO	Acetate
BB	Broad Band
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
B. subtulis	*Bacillus subtilus*
COSY	Correlation Spectroscopy
CVD	Cardio Vascular Diseases
D	Deuterium
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
DMF	Dimethyl Formamide
DMSO	Dimethy Sulfoxide
DNA	Deoxyribonucleic Acid
DPPH	1,1-Diphenyl Picryl Hydrazyl
EI-MS	Electron Impact-Mass Spectrometer
E. coli	*Escherichia coli*
ES^{+}	Electrospray
Et	Ethyl
FAB-MS	Fast Atom Bombardment Mass Spectrometer
FTC	Ferric Thiocynate
GAE	Gallic Acid Equivalent
GPX	Glutathione Peroxidas
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HTS	High Throughput Screening

HR-EI-MS	High Resolution Electron Impact-Mass spectrometer
HIV	Human Immunodeficiency Virus
Hz	Hertz
IC_{50}	Inhibitory Concentration
IR	Infra Red
J	Coupling Constant
mp	Melting Point
mM	Milli-Mole
m/z	Mass to Charge ratio
Me	Methyl
MeO	Methoxy
MIC	Minimum Inhibitory Concentration
MOA	Monoamic Oxidase
NOE	Nuclear Overhauser effect
NOESY	Nuclear Overhauser Effect Spectroscopy
MS	Mass Spectroscopy
OD	Optical Density
P	Probability
ppm	Parts Per Million
PBS	Phosphate Buffer Solution
R	Alkyl
ROS	Reactive Oxygen species
RSA	Radical Scavenging Activity
TBA	Thiobarbituric Acid
S. aureus	*Staphylococcus aureus*
S. typhi	*Salmonella typhi*
TLC	Thin Layer Chromatography
UV	Ultraviolet
V	Wave Number
µL	Micro Litre
ABTS	2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid

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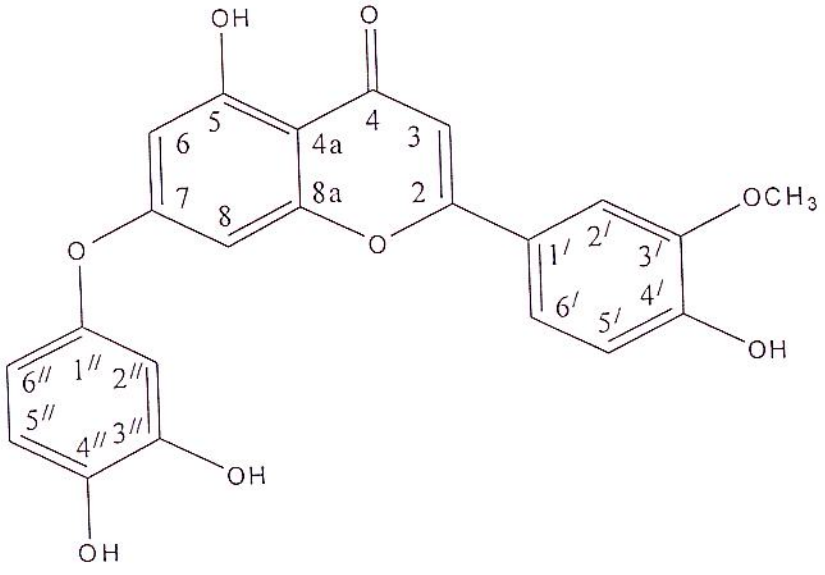
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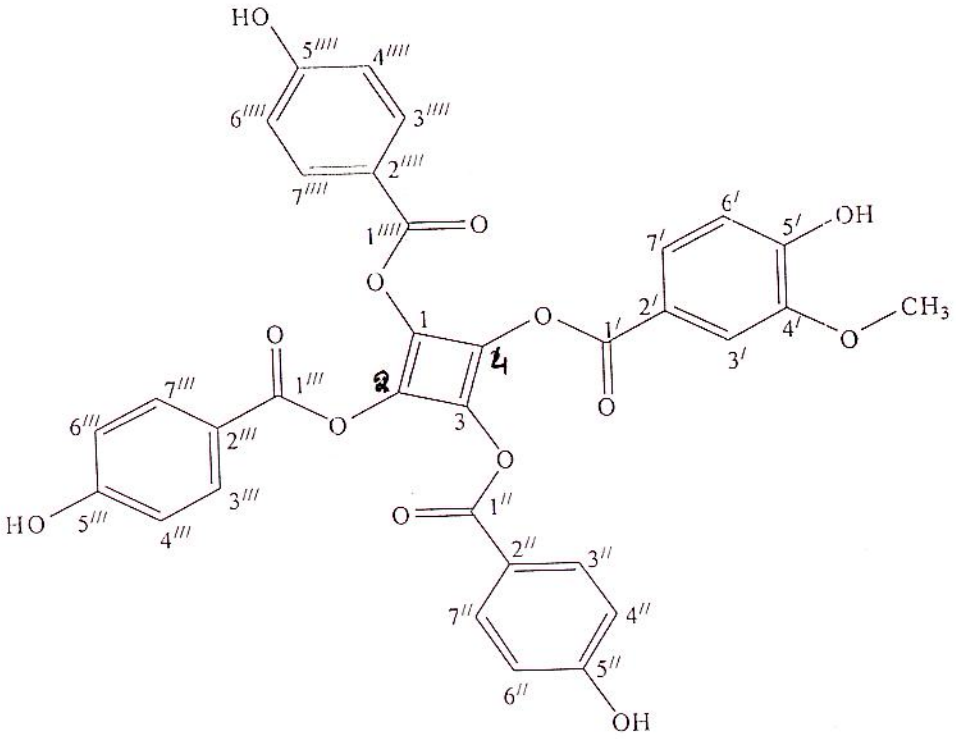
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ABSTRACT

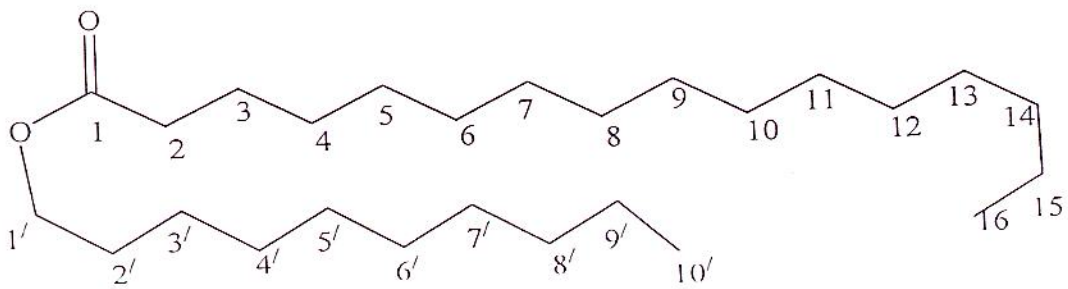
This dissertation is in two parts, **Part A** and **Part B**. Part A describes the Phytochemical investigation of *Cassia artemisioides* and *Cassia nemophila* (Leguminoseae). Nine new (**48-56**) and thirty eight known (**57-94**) compounds have been isolated from the two *Cassia* species of Pakistani origin. From the *Cassia artemisioides* flowers two new (**48-49**) and eight known compounds (**57-64**), while six new (**50-55**) and six known compounds (**65-70**) have been isolated from the stem bark. One new (**56**) and five known compounds (**71-75**) were isolated from root bark. Similarly three known compounds (**76-78**) were isolated from the pods and five known compounds (**79-83**) were isolated from the seeds. The phytochemical investigation of various parts of *Cassia nemophila* has led to the isolation of eleven known compounds (**84-94**). There has been no reports on the two species. Various experimental techniques and extensive spectroscopic studies were used for the structural elucidation of the isolated compounds. The isolated flavonoids, caffeates and anthraquinones were evaluated for their antioxidant and antibacterial activities.



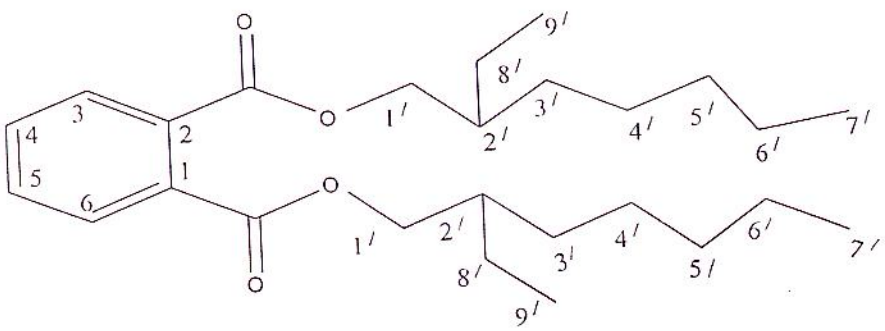
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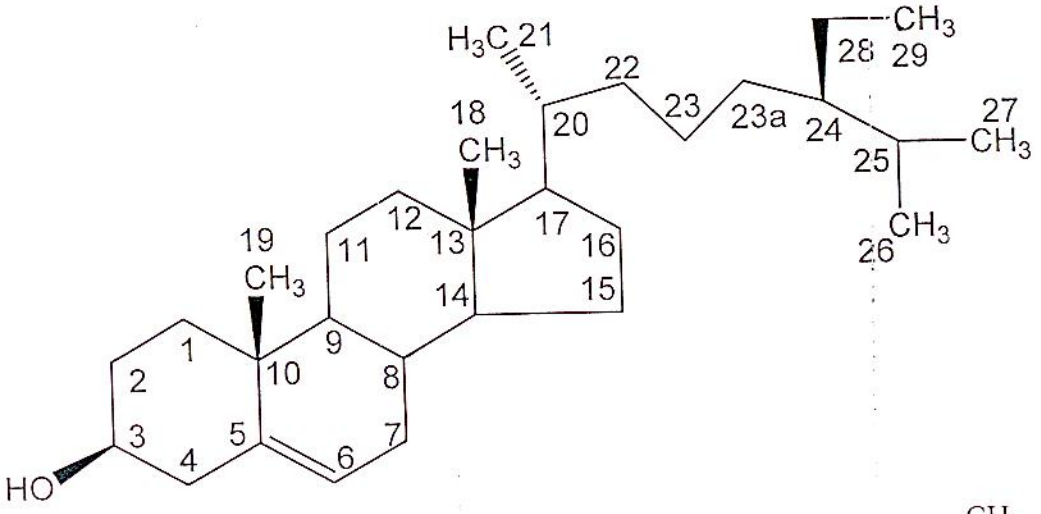
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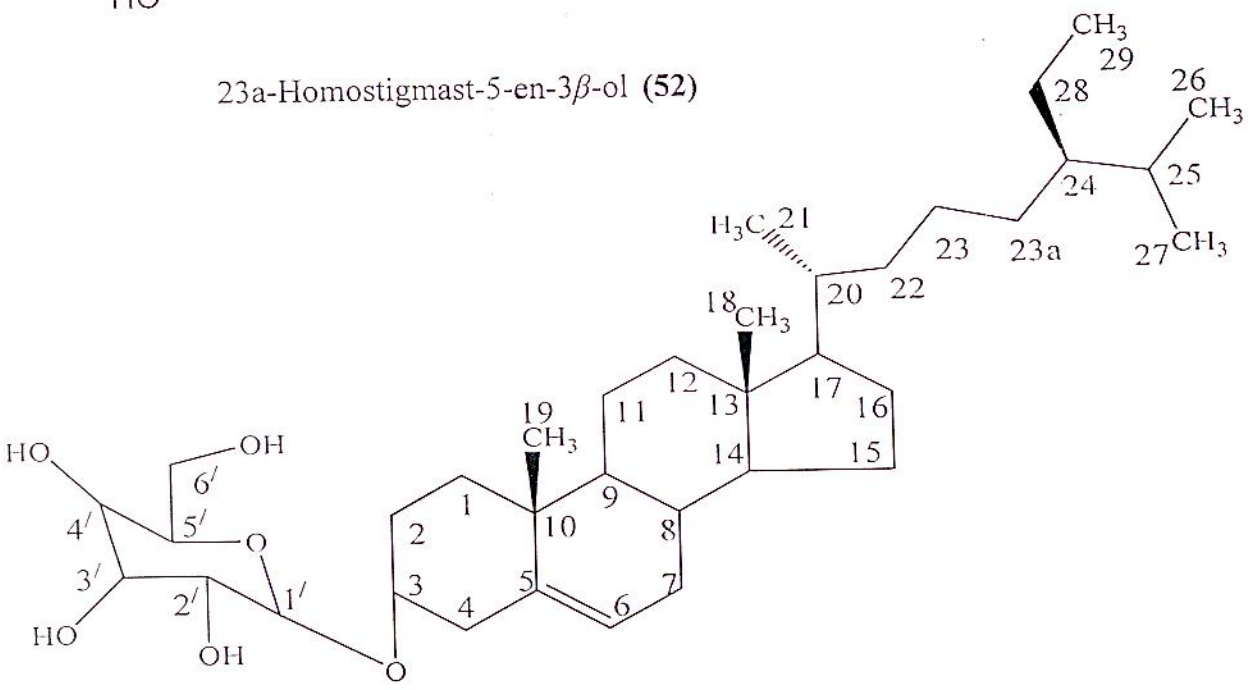
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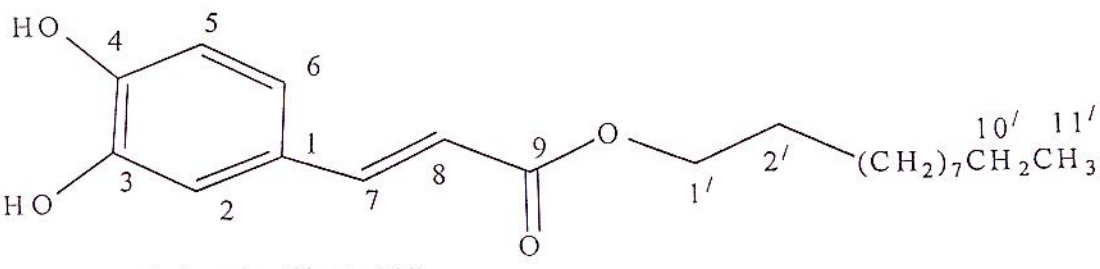
Di-2-ethylheptyl phthalate **(51)**



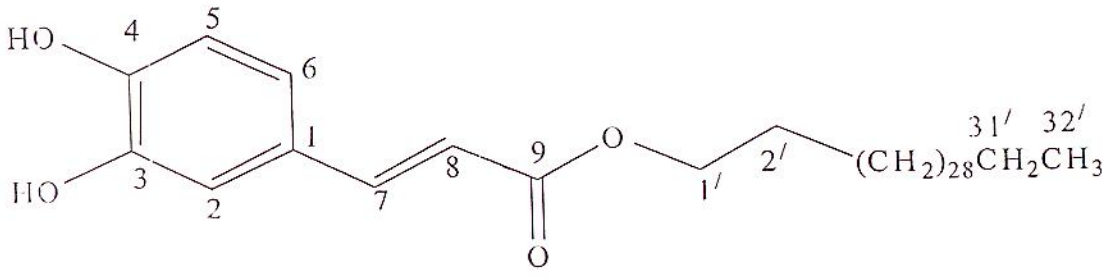
23a-Homostigmast-5-en-3β-ol **(52)**



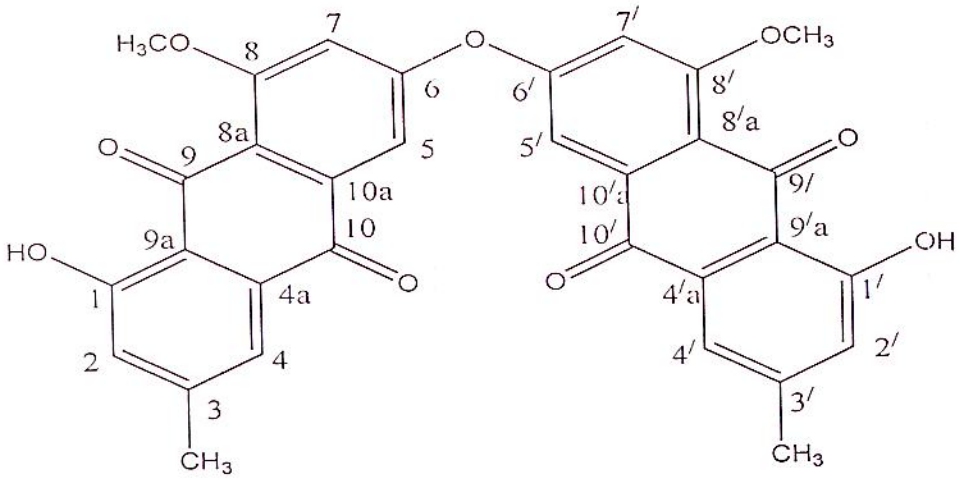
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Undecyl caffeate **(54)**



Dotricontanyl caffeate (55)



1,1′-Dihydroxy-8,8′-dimethoxy-3,3′-dimethyl-6,6′-bianthraquinone (56)

PART B

Part B is concern with evaluation of biological activities of crude extracts, fractions and pure constituents. Different solvents soluble fractions of the selected plants belonging to family Leguminosae (*Cassia artemisioides* and *Cassia nemophila*) were screened for their possible antioxidant activity. Antioxidant activity by DPPH method was used for analysis. We report here the antioxidant and antimicrobial potential of the various extracts and fractions of the listed plants for the first time. The objectives of this study were to explore the biological and medicinal value of the extract/fractions of the above mentioned plants.

Chapter 1

INTRODUCTION (Part A)

1.1. General Introduction

Allah Almighty has blessed the nature with enormous number of precious gifts, the kingdom plantae being one of them. Medicinal plants have provided a basis for the development of modern drug systems. Plants are like factories producing valuable phytochemicals in the most efficient way and with precise selectivity. The search for better, more effective, cheaper and easily available medicinal agents is on as the drug discovery and design research projects in the research laboratories around the world. A number of bioactive principles have been obtained and used as lead molecules in modern medicines.

Almost 80% of the third world population particularly in the rural areas depend on traditional medicine for their health care. These folk treatments are largely based on the use of medicinal plants.

The interest in natural product research has resurged throughout the world due to the failure of alternative drug discovery methods to deliver lead compounds in key therapeutic areas, such as immunosuppression, anti-infectives, arthritis, metabolic diseases etc. In past decades, higher plants were recognized for their ability to produce a wide spectrum of natural products with interesting bioactivities.[1-3] There are more than 400,000 species of higher plants on earth and less than 2% have been studied as potential sources of new drugs. Natural products, particularly the plant-based medicines, will continue to be extremely important as a source of therapeutic agents in the future.

1.2. Drug Discovery from Natural Products

The control of diseases through antibiotics and some other new drugs is a great achievement. In the last two decades, several compounds such as cyclosporin A, clavulanic acid, mevinolin and ivermectin were discovered by natural product screening approaches. A review on natural products as a source of new drug was presented by Clark in 1996[1] while a review related to anticancer and anti-infective natural drugs, has been contributed by Crag, *et al.*[2] The central role of natural products in the discovery and development of new pharmaceuticals has been summarized in these reviews. Some of the recent publications, citing the current literature in bioorganic chemistry,[3] seeking drugs in natural products,[4] new technologies and approaches in natural product drug discovery[5] and natural products for the improvement of the quality of life,[6] are important in understanding the progress in the field of natural products.

There has been a rapid escalation in the discovery of molecular targets that may be applied to discovery of novel tools for the diagnosis, prevention, and treatment of human diseases.[7] The main advantage of natural products as a source of lead compounds is the tremendous molecular diversity found in nature. Advances in the methodologies used to evaluate extracts, fractions and pure compounds for biological activities, have enabled miniaturization and automation of extremely specific biochemical tests. With new chemical methods, it is now possible to isolate and identify interesting biologically active compounds on a milligram scale.[8] Of the new approved drugs reported during 1981-2002, drugs of natural origin are predominant (78 %) in the area of antibacterial while 61 % of the 31 anticancer drugs are naturally derived or are modeled on a natural product parent. Their rich structural and stereo chemical characteristics make them valuable templates for exploring novel molecular diversity.[9]

Natural products are often structurally complex and difficult to synthesize for further structure activity relationship studies. Resultantly, natural product research involves semi-synthetic or biotechnological approaches including microbial transformation.[10] The next breakthrough in drug discovery was the use of mechanism-based screening for

bioassay-guided isolation. Through continual improvement of screening methods, reagent production and data management, mechanism-based screening then becomes the mainstay of high throughput screening (HTS).[11]

The approach adopted to obtain new lead compounds from medicinal plants used by traditional healers, involves interdisciplinary work in botany, chemistry, pharmacology and toxicology. Selection of plant is either by ethno pharmacological knowledge or resource based information. The following steps are involved to obtain new lead compounds from medicinal plants.

- Collection of plant, botanical identification and submission of specimen in herbarium.
- Drying and grinding of plant material.
- Extraction with suitable solvents and preliminary analysis such as toxicology of crude extract.
- Biological and pharmacological screening of crude extracts with low or no toxicity. Special in *vitro* or in *vivo* tests to detect the activity anticipated from the use of the plant in traditional medicine.
- Bioactivity guided fractionation of the biologically active crude extract. Each fraction is tested for the main effect that was found in step 3 and purification of the most biologically active fraction is then carried out.
- Isolation and identification of active compound(s).
- Toxicity and Pharmacological screening of pure compound(s) in *vitro* and then in *vivo*.
- If promising pharmacological activities, then partial or total synthesis, combinatorial chemistry or biotechnological approach.
- Pre-clinical trials. If passed then submitted for clinical trials.
- If approved then authorized for marketing.

Quinine from *Cinchona* bark and resperine from *Rauwolfia serpentina* are the two outstanding examples, which have been acclaimed as effective drugs against malaria and mental ailment/high blood pressure respectively.

Another example is the significant discovery of vinca alkaloids viz. vincristine and vinblastine from rosy periwinkle (*Catharanthus roseus* L.). This plant is used by indigenous physicians for the treatment of haemorrhage, toothache, chronic wounds and diabetic ulcers, because of its hypoglycemic activity.[12]

Even in the latter half of the 20th century, there were significant discoveries made from natural products. These include the taxanes, paclitaxel of the Pacific yew tree (*Taxus brevifolia* Nutt.) (Taxol™) and docetaxel (Taxotere™).[13, 14] Discovery of penicillin by Alexander Fleming in 1928,[15] won the Nobel Prize for Fleming, Chain and Florey. In subsequent years, better antibiotics were developed, based on core penicillin structure making it resistant to enzymatic breakdown and widening its spectrum of activity and includes streptomycin, chloramphenicol, chlortetracycline, cephalosporin, erythromycin and vancomycin.[16, 17]

The secondary metabolites in higher plants mainly comprise of chemical constituents such as coumarins, flavonoids, isoflavonoids, lignins, withanolides, saponins, glycosides, terpenoids, alkaloids, essential oils, fatty acids, resins and gums etc. Natural product chemists have a compelling curiosity to discover those bioactive compounds in a plant extract used as a remedy, which are responsible for the therapeutic effects. To do this it is necessary to isolate pure compounds by different chromatographic techniques such as TLC, HPLC, column chromatography, Chromatotron and GLC etc and elucidate their structures. Similarly chemists can also synthesize those compounds which are present in relatively small quantities in plants to meet the requirements. The combination of phytochemicals investigations and chemical synthesis has resulted in the discovery of drugs and the development of pharmaceutical industries in the world.

Several plant derived medicines are rich in phenolic compounds, which can provide protection against coronary heart diseases and carcinogenesis. Phenols can be classified into flavonoids, isoflavonoids, phenolic acids, lignans, stilbenes and complex phenolic polymers. Family Leguminosae is rich in such compounds, and is the focus of the current investigation.

1.3. Family Leguminosae

Family Leguminosae commonly known as Fabaceae, or pea family, is one of the largest and the most important family of flowering plants with 650 genera and 18,000 species [18] of herbs, shrubs, trees or climbers and showing a great diversity of habitat, being aquatic, xerophytic and mesophytic. Some are fresh-water aquatics but there are no marine species. The species within the family range from dwarf herbs of arctic and alpine vegetation to massive trees of tropical forest. The important unifying characteristics of the family is the fruit, a pod, known as a legume. The family is usually divided into three sub-families. Papilionaceae, Caesalpiniaceae and Mimosaceae, that is recognized as three separate families, identified by their flowers.[18]

1.3.1. Sub-Family Papilionaceae (Pea family)

This sub-family includes the largest number of species in India and Pakistan. Most of our vegetables like pea, bean, pulses and groundnuts which are extensively used in the production of vegetable oil, belong to this family. Plants are herbs, shrubs, trees or climbers of aquatic, xerophytic and mesophytic nature.

Leaves are alternate, simple or compound, the leaflets shows sleep movements and autonomous movements are also observed. Flowers being bisexual, zygomorphic, sepals more or less connate, petals five, stamens ten or nine, carpel one, ovule one, fruit a legume.[18]

1.3.1.1. Important Genera [18]

Abrus. Seeds are marked into red and black areas and are poisonous.

Arachis. The seeds are edible and also yield oil, which is hydrogenated.

Butea. *Butea monospera* and *Butea superba* are use in medicine.

Cajanus. A cultivated shrub, 5-10 ft high, flower yellow, seed used as pulse and the

dried stem as fuel.

Clitoria. The powder seeds are a useful purgative in combination with cream of tartar. The roots are used in croup, it sickens and causes vomiting. It is given as a laxative to children and is diuretic.

Crotrolaria. Flower generally yellow, style long, pod straight, generally flattened. The plants are used for green-manure.

Cyamopsis. Pod linear, straight, divided internally by partition. Pods used as a vegetable, also a good fodder crop.

Dolichos. Pods are eaten, useful for patient suffering from urinary troubles due to its diuretic properties.

Glycyrrhiza. *Glycyrrhiza glabra* (sweet wood) is a medicinal plant used for hoarse throat and cough.

Glycine. Seeds are rich in protein, fats, vitamins and are used an important source of diet.

Sesbania. *Sesbania aegyptica,* a tall quick growing shrub with yellow flowers.

Trifolium. Cultivated as fodder crop, very useful for cattle to increase the milk yield.

Trigonella. Leaves are fragrant, used as pot-herb and fodder, seeds as condiments in pickles, also in medicine.[18]

1.3.2. Sub-Family Mimosaceae (*Acacia* family)

This sub-family includes the plants like *Aacacia arabica* (kikar) *Albizzia lebbek*, *Memosa pudica* etc. Plants are trees or shrubs, rarely climbers of perennial herbs, many are xerophytic. Leaves are bipinnate, flowers small, bisexual rarely polygamous, regular, actinomorphic, calyx tubular valvate, petals also valvate and connate below, stamens numerous, free or connate below, carpel, fruit and seed as in Papilionaceae.[18]

1.3.2.1. Important Genera

Acacia. Trees or shrubs, prickly with white or yellow flowers in round heads or cylindrical spikes, stamens free, pod nearly cylindrical or flat, two-valve or indehiscent. *Acacia arabica* yields fuel and gum, grows in dry localities, branches used as tooth brushes. The gum procured by incisions, made in the branches is used in native medicine, by dyer and cloth printers. The bark is used for tanning and dyeing.

Albizzia. Large unarmed tree, flowers in round heads, stamens united at the base, several times as long as the corolla, pod long, thin strap-shape, large deciduous trees.

Mimosa. Shrub or herbs with sensitive leaflets, flowers in dense round heads, pod flat and jointed.

Entada. Wood climber with tendrils, calyx minute, pod very large, jointed outwardly. Seeds are both edible and medicinal.[18]

1.3.3. Sub–Family Caesalpiniaceae (*Cassia* family)

Mostly trees or shrubs, rarely herbs or climbers, climbing by hooks or stem tendrils. Leaves are pinnate or bipinnate, compound, rarely simple, stipulate, stipules minute or cauducous, flowers zygomorphic, irregular, bisexual, calyx five, corolla five stamens ten, style long, stigma simple, fruit legume.[19]

1.3.3.1. Important Genera

Bauhinia. Erect trees or tendril-bearing climbers, leaves simple, notched or 2-lobed, flowers showy, petals generally clawed, stamens 3-5, some time imperfect or absent, ovary stalked, pod flat, oblong. The bark is astringent and used as a tonic in fever. The dried flower buds are used in diarrhea and for worms. The bark is used for tanning and dyeing purposes. The dried flower buds and leaves of *Bauhinia tomentosa* are used in

dysentery. The bruised bark is applied to tumors and wounds.

Caesalpinia. Prickly shrubs with showy yellow or reddish-yellow flowers, calyx deeply cleft, the lowest lobe largest and hooded, petals spreading, stamens ten, of the same size and in two whorls, leaves bipinnate, cultivated shrubs in gardens.

Haematoxylon. The thick walls of the wood fibers are deeply colored in the heart wood and yield a dye called haematoxylin.

Tamarindus. Seeds yields starch and are used as famine food.[19]

The Genus *Cassia*

Cassia is the main focus of the current investigation. These are trees or herbs, leaf even-pinnate, flowers rather large, yellow, stamens ten, some often imperfect, pod flat, cylindrical petiole or midrib or often with one or two conspicuous glands. *Cassia* which is also known as *Senna* is a major genus of the family, comprising about 600 species, some widely distributed throughout the world.[20] Several species of the subfamily are well-known ornamentals such as flamboyant (*Delonix regia*) and barbados pride (*Caesalpinia pulcherrima*).

1.4. Pharmacological Importance of the Genus *Cassia*

Although the pods and leaves of *Cassia* provide a well known laxative,[21, 22] but as a whole, it became renowned as fabulous ornamental and medicinal plants.[23] Several *Cassia* species have been reported to accumulate phenolic compounds with diverse biological and pharmacological properties[2] and are widely used in traditional medicine for their antimicrobial, laxative, antiulcerogenic, analgesic and antiinflammatory properties.[24, 25, 26, 27]

Cassia species are known to be rich in flavonoids, anthraquinones, anthrones and

triterpenes. The anthraquinone derivatives dianthronic, anthronic and anthraquinone glycosides, 1,8-dihydroxyanthraquinone derivatives particularly their glycosides are shown to have purgative, laxative and cathartic activities are now considered to be potent hepatoprotective drugs.[28] A lot of herbal preparations for the treatment of liver diseases contain hydroxyanthraquinones, whose medicinal properties have been attributed to their laxative activities only. Chrysophenol glycoside isolated from *Cassia tora* also showed potent hepatoprotective activity.[29] *C. tora* is not only important as a source of natural fungicides but is also considered important for its medicinal properties, such as an antiseptic, antidiarrheal, antioxidant and antimutagenic properties.[30, 31,32] All parts of *Cassia absus* are used in folk medicine.[33] The leaves are used for the treatment of tumors and asthma. The roots are used for the treatment of constipation. The alkaloids chaksine and isochaksine are reported from the seeds.[34, 35]

Cassia fistula seeds are used in folk medicine to treat diarrhea and gastritis, it is also an insecticide.[36] Sugars, unsaturated fatty acids, cyclopropenoid fatty acids and triacylglycerols have been reported from the seeds.[37, 38,39] *Cassia fistula* is highly reputed for its strong laxative and purgative properties.[40] In Ayurvedic medicine, it is used against various disorders such as haematemesis, pruritus, leucoderma, and diabetes.[41] The antipyretic, analgesic effects[42] and antitussive potentials of the plant have also been reported, together with their antifungal and antibacterial activities.[43] The plant extract is recommended as a pest-control agent.[44] These properties have been mainly attributed to the presence of alkaloids,[45] triterpene derivatives,[46] anthraquinone derivatives[47] and polyphenolics comprising flavonoids,[48] catechins and proanthocyanidins.[49, 50]

Cassia alata leaf extract has been reported to have a number of pharmacological activities. The methanol extracts of flowers, leaves, root barks and stem of *Cassia alata* showed a broad spectrum antibacterial activity.[51,52] In a study on cancer chemoprevention, the stilbene related compound resveratrol was isolated as a potential cancer chemopreventive agent from *Cassia quinquangulata*.[53]

Cassia auriculata is used in traditional medicine for the treatment of opthalima and conjunctivitis. Seeds are powdered and applied to eyes after mixing with coconut oil.

Flowers are used for the treatment of diabetes mellitus and the stem bark for enema.[54] The leaves, roots and flowers of *Cassia podocarpa* are used in the treatment of female venereal diseases.[55] The fresh leaves are used in headache, opthlmia, swellings and wounds and are used both internally and externally for skin diseases and yaws.[56]

There is a clear evidence indicating that excessive free radical production and lipid peroxidation are actively involved in the pathogenesis of a wide range of diseases including atherosclerosis,[57] cardiac and cerebral ischemia,[58] neurodegenerative disorders,[59] carcinogenesis,[60] diabetes,[61] rheumatic disorders[62] and that play a major role in the aging process.[63] Plant-derived antioxidants such as vitamin E, vitamin C, polyphenols including phenolic acids, phenolic diterpenes, catechins, flavonoids, anthocyanins and procyanidins are becoming increasingly important dietary factors.[64]

Lipid peroxidation is an important deteriorative reaction of foods during processing and storage. Toxic substances formed by lipid peroxidation may lead to other adverse effects such as carcinogensis, mutagenesis and aging.[65] To avoid or delay peroxidation process, addition of antioxidants to foods is the most common method. Synthetic antioxidants (e.g. butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroquinone etc.) have been used as antioxidants in food for years. However, consumers are concerned about the safety of synthetic antioxidants.[66] Although tocopherol and ascorbic acid are extensively used as natural antioxidants but are less effective as compared to synthetic antioxidants.[67] This concern has resulted in increased interest in the investigation of the effectiveness of naturally occurring compounds with antioxidant properties.[68]

Various antioxidants occur naturally in the fruit, vegetables, nuts, seeds, leaves, flowers, roots and barks of a number of plants.[69] Lipid peroxidation causes destabilization and disintegration of the cell membrane, leading to liver injury, atherosclerosis, kidney disorder, aging and susceptibility to cancer.[70] Antioxidants may play a role in preventing the development of vascular disease and some forms of cancer.[71]

The antioxidant activities of *Cassia fistula* L. are due to the presence of phenolic, proanthocyanidin and flavonoid constituents.[72] The seeds of *Cassia tora* L. and *Cassia occidentalis* L. called jue ming zi in Chinese, is conventionally used in Chinese medicine for centuries. It has been reported to contain a number of active substances, including kaempferol **(46)**,[73] emodin **(6)**, rhein, etc.[74] The roasted jue ming zi has a special flavor and colour and is popularly used as a health drink tea. Choi *et al.*[30] reported that jue ming zi exhibited antimutagenic activity. The methanolic extracts of *C. tora* showed good antioxidant activity and their antioxidant activities were higher than that of *C. occidentalis* and also identified an antioxidative compound, emodin from *C. tora*.[31] Moreover, the close relationship between antioxidant activity and antimutagenicity has been demonstrated by Yen and Chen.[75]

The ethanol and methanol extracts of *C. auriculata* flowers showed antioxidant activity, the assay was based on scavenging of ABTS radical cation and DPPH radical. These antioxidant activities are due to the presence of flavonoids and tannins.[76] The alcoholic extract of *Cassia siamea* L. flowers showed a potent antioxidant activity. The chemical examination of alcoholic extract from flowers has shown the presence of a large number of polyphenols, which may be responsible for the antioxidant and antihepatoprotective activities.[77]

1.5. Literature Review of Genus *Cassia*

Previous studies on the genus *Cassia* have resulted in the isolation of large number of various compounds including anthraquinones, xanthones, alkaloids, sesquiterpenes, flavanoids, steroids, lactones and phenyl esters, etc. A comprehensive review (1958-June 2009) of these compounds is provided in **Table 1**.

Table 1. Compounds Isolated from the Plants of the Genus *Cassia*

S.No	Compound *	Formula	M.Mass	Source Ref.
1	Biochanin A (1)	$C_{16}H_{12}O_5$	284.00	*C. fistula*[78]
2	Kaempferol-3-O-[(6‴-O-trans-sinnapoyl)-β-D-glucopyranosyl(1→6)]-β-D-glucopyranoside	$C_{38}H_{40}O_{20}$	815.20	*C.angustifolia*[79]
3	Chrobisiamone A	$C_{26}H_{24}O_8$	465.15	*C. siamea*[80]
4	1,8-Dihydroxy-3-methoxy-6-methylxantone (2)	$C_{15}H_{12}O_5$	272.06	*C. obtusifolia*[81]
5	(4R*,5S*,6E,8Z)-Ethyl-4-((E)-but-1-enyl)-5-hydroxy pentdeca-6,8-dienoate	$C_{21}H_{36}O_3$	336.26	*C. obtusifolia*[81]
6	1-Demethylaurantio-obtusin-2-*O*-*β*-*D*-glucopyranoside	$C_{22}H_{22}O_{12}$	478.00	*C. obtusifolia*[82]
7	Cyclosophoside A	$C_{36}H_{60}O_{11}$	668.00	*C. sophera*[83]
8	(+)-3-*O*-Feruloylcassine	$C_{28}H_{44}NO_5$	474.31	*C. spectabilis*[84]
9	1-Hydroxyl-2-acetyl-3,8-dimethoxy-naphthalene-6-*O*-*β*-*D*-apiofuranosyl-(1→2)-*β*-*D*-glucopyranoside	$C_{25}H_{32}O_{14}$	556.17	*C. obtusifolia*[85]
10	2-Acetyl-3-methyl-8-methoxyl-1,4-naphthoquinone-6-*O*-*β*-*D*-glucopyranoside	$C_{20}H_{22}O_{10}$	422.12	*C. obtusifolia*[85]
11	8-O-[β-D-Glucopyranosyl(1→3)-O-β-D-glucopyranosyl(1→6)-O-β-D-glucopyranoside]	$C_{32}H_{44}O_{19}$	733.00	*C. tora*[86]
12	Toralactone 9-O-[*β*-*D*-glucopyranosyl-(1→3)-*O*-*β*-*D*-glucopyranosyl-(1→6)-*O*-*β*-*D*-glucopyranoside]	$C_{33}H_{42}O_{20}$	759.00	*C. tora*[86]

* Structure are given for the compounds number in parenthesis

13	3,5,4′-Trihydroxy-7-methoxyflavone-5-*O*-*β*-*D*-xylopyranosyl-(1→4)-*O*-*β*-*D*-glucopyranosyl-(1→4)-O-α-L-rhamnopyranoside	$C_{33}H_{40}O_{19}$	740.00	*C. tora*[87]
14	Petersinone 1	$C_{13}H_{12}O_4$	232.23	*C. petersiana*[88]
15	Petersinone 2	$C_{13}H_{14}O_4$	234.25	*C. petersiana*[88]
16	Petersinone 3	$C_{11}H_{12}O_3$	192.21	*C. petersiana*[88]
17	3-(Methoxymethyl)-5-(2-oxopropyl) benzoic acid	$C_{12}H_{14}O_4$	222.24	*C. petersiana*[88]
18	Cassiarins A (**3**)	$C_{13}H_{12}NO_2$	214.08	*C. siamea*[89]
19	Cassiarins B (**4**)	$C_{18}H_{19}NO_4$	314.13	*C. siamea*[89]
20	Clerosterol	$C_{29}H_{48}O$	394.00	*C. fistula*[90]
21	1,5-Dihydroxy-2-hydroxymethyl-anthraquinone	$C_{15}H_{10}O_5$	270.00	*C. italica*[91]
22	3,12-Dihydroxycadalene	$C_{15}H_{19}O_2$	231.13	*C. spectabilis*[92]
23	1,3,5,7,9-Cadinapentaene-3,11,12-triol	$C_{15}H_{18}O_3$	246.30	*C. spectabilis*[92]
24	5,7,3′,4′-Tetrahydroxy-3-methoxy flavone-5-*O*-*α*-*L*-rhamnopyranosyl-7-*O*-*β*-*D*-glucopyranosyl (1→3)-O-β-D-xylopyranoside	$C_{33}H_{40}O_{20}$	756.00	*C. sophera Linn*[93]
25	4′,5-Dihydroxy-7-methylhomoisoflavone (**5**)	$C_{17}H_{14}O_4$	282.29	*C. nodosa*[94]
26	5,8-Octacosanediol	$C_{28}H_{58}O_2$	426.76	*C. fistula*[95]
27	Cis-Heptacosanyl-5-hydroxypentadec-2-enoate	$C_{42}H_{82}O_3$	635.10	*C. fistula*[95]
28	1,5,6-Trihydroxy-3-methylanthraquinone -8-O-α-L-Glucoside	$C_{21}H_{20}O_{11}$	448.00	*C. reingera*[96]
29	Cassiamin	$C_{30}H_{18}O_9$	522.00	*C. siamea*[97]
30	(-)-3-O-Acetylspectaline	$C_{22}H_{42}NO_3$	368.31	*C. spectabilis*[98]
31	(-)-7-Hydroxyspectaline	$C_{20}H_{40}NO_3$	342.30	*C. spectabilis*[98]

32	Iso-6-spectaline	$C_{20}H_{40}NO_2$	326.34	*C. spectabilis*[98]
33	Emodin-1-*O*-*β*-gentiobioside	$C_{27}H_{30}O_{15}$	594.00	*C. obtusifolia*[99]
34	3-Hydroxy-6-methyl-2-(11-oxododecyl)pyridine	$C_{18}H_{29}NO_2$	291.43	*C. spectabilis*[100]
35	Spectamine B	$C_{20}H_{37}NO_3$	339.51	*C. spectabilis*[101]
36	Spectamine A	$C_{25}H_{39}NO_3$	401.58	*C. spectabilis*[101]
37	6-Isocarnavaline	$C_{18}H_{37}NO_2$	299.49	*C. spectabilis*[101]
38	Apigenin-7-alloside **(8)**	$C_{21}H_{20}O_{10}$	432.38	*C. occidentalis*[102]
39	3,2′-Dihydroxy-7,8,4′-trimethoxy--flavvone-5-*O*-{-*β*-*D*-glucopyranosyl (1→2)}-*β*-*D*-galacto-pyranoside	$C_{30}H_{36}O_{18}$	684.60	*C. occidentalis*[102]
40	5,3′,4′-Trihydroxy-6-methoxy-7-*O*-*α*-*L*-rhamnopyranosyl-(1→2)-*O*-*β*-*D*-galactopyranoside	$C_{28}H_{32}O_{16}$	624.00	*C. fistula*[103]
41	Adenine **(7)**	$C_5H_5N_5$	134.00	*C. alata*[104]
42	Kleinioxanthrone 3	$C_{31}H_{42}O_5$	494.67	*C. kleinii*[105]
43	Kleinioxanthrone 4	$C_{27}H_{34}O_8$	487.00	*C. kleinii*[105]
44	Cassiaphenone B 2′-glucoside	$C_{21}H_{20}O_{13}$	480.38	*C. angustifolia*[106]
45	Cassiaphenone A 2′-glucoside	$C_{21}H_{22}O_{12}$	466.39	*C. angustifolia*[106]
46	1-Hydroxy-6,8-dimethoxy-3-methylanthraquinone	$C_{17}H_{14}O_5$	298.00	*C. angustifolia*[107]
47	Quercetin	$C_{15}H_{10}O_7$	302.00	*C. wislizeni*[108]
48	5,7-Dirnethoxyquercetin	$C_{17}H_{14}0_7$	330.00	*C. wislizeni*[108]
49	5,7-Dimethoxyrutin**(12)**	$C_{29}H_{34}0_{16}$	638.00	*C. skinneri*[108]
50	(2′ S)-7-Hydroxy-5-hydroxymethyl-2-(2′-hydroxypropyl)chromone **(13)**	$C_{13}H_{14}O_5$	250.08	*C. fistula*[109]
51	Benzyl-2*β*-*O*-*D*-glucopyranosyl-3,6-dimethoxybenzoate **(14)**	$C_{22}H_{26}O_{10}$	450.00	*C. fistula*[109]
52	5-(2-Hydroxyphenoxymethyl) furfural **(15)**	$C_{12}H_{10}O_4$	218.05	*C. fistula*[109]

53	Benzyl-2-hydroxy-3,6-dimethoxy benzoate **(16)**	$C_{16}H_{16}O_5$	288.29	*C. fistula*[109]
54	3*β*,22*β*,24,29-Tetrahydroxy olean-12-ene-3,24-*O*-*β*-*D*-dixylopyranoside	$C_{40}H_{66}O_{12}$	738.40	*C. sophera*[110]
55	3-(2′-Hydroxy-3′-methyl-3′-butenyl) acetophenone-4-*O*-[*β*-*D*-arabinopyranosyl (1→3)]-*O*-*β*-*D*-galactopyranoside	$C_{24}H_{34}O_{12}$	514.50	*C. sophera*[110]
56	Racemochrysone **(11)**	$C_{18}H_{18}O_4$	298.33	*C. racemosa*[111]
57	Kaempferol-3-*O*-α-L-rhamnopyranosyl (1→2)-*β*-*D*-glucopyranosyl (1→6)-*β*-*D*-galactopyranosides	$C_{33}H_{40}O_{21}$	772.00	*C. marginata*[112]
58	Quercetin-3-*O*-α-L-rhamnopyranosyl (1→2)-*β*-*D*-glucopyranosyl(1→6)-*β*-*D*-galactopyranosides	$C_{33}H_{40}O_{20}$	756.00	*C. marginata*[112]
59	10-Hydroxylanthrone glycoside-1,8,10-trihydroxyl-1-*O*-*β*-*D*-glucopyranosyl-3-methyl-10-C(S)-*β*-*D*-glucopyranosyl-anthrone-9	$C_{27}H_{32}O_{14}$	580.00	*C. siamea*[113]
60	1,3-Dihydroxy-6,7,8-trimethoxy-2-methylanthraquinone-3-*O*-α-rhamno pyranosyl-(1→6)-β-glucopyranosyl (1→6)-*β*-galactopyranoside	$C_{36}H_{46}O_{21}$	814.25	*C. occidentalis*[114]
61	1-Hydroxy-3,6,7,8-tetramethoxy-2-methylanthraquinone-1-*O*-*β*-rhamnopyranosyl (1→6)-*β*-glucop-yranosyl(1→6)-*β*-galactopyranoside	$C_{37}H_{48}O_{21}$	828.26	*C. occidentalis*[114]
62	Quinquangulin	$C_{16}H_{14}O_5$	286.28	*C. quinquangulata*[115]
63	Quinquangulone	$C_{16}H_{14}O_6$	302.28	*C. quinquangulata*[115]

64	Kleinioxanthrone 1 **(9)**	$C_{26}H_{32}O_6$	440.53	*C. kleinii*[28]
65	Kleinioxanthrone 2 **(10)**	$C_{29}H_{38}O_5$	466.61	*C. kleinii*[28]
66	2-Methyl-5-propyl-7,12-dihydroxy chromone-12-O-β-D-glucopyranoside	$C_{19}H_{24}O_9$	396.39	*C. siamea*[116]
67	Kaempferol 3-gentiobioside	$C_{27}H_{30}O_{16}$	609.00	*C. alata*[117]
68	(7-Chloro*C.*min A)	$C_{30}H_{17}ClO_9$	556.91	*C. siamea*[118]
69	1,1′,3,8,8′-Pentahydroxy-3′,6-dimethyl [2,2′-bianthracene]-9,9′,10,10′-tetrone	$C_{30}H_{18}O_9$	522.09	*C. siamea*[118]
70	Aloeemodin	$C_{15}H_{10}O_5$	270.24	*C. tora*[119]
71	7-Methoxy-2,5-dimethylchromone	$C_{12}H_{12}O_3$	204.22	*C. fistula*[120]
72	5-Acetonyl-7-hydroxy-2-hydroxymethylchromone **(22)**	$C_{13}H_{12}O_5$	248.23	*C. siamea*[121]
73	4-(trans)-acetyl-3,6,8-trihydroxy-3-methyldihydronaphthalenone**(23)**	$C_{13}H_{14}O_5$	251.00	*C. siamea*[121]
74	Calendin **(18)**	$C_{11}H_{16}O_3$	196.00	*C. laevigata*[122]
75	3-Hydroxy-1-(4′-hydroxy-3′-methoxyphenyl)propan-1-one **(19)**	$C_{10}H_{12}O_4$	196.00	*C. laevigata*[122]
76	2,3-Dihydroxy-1-(4′-hydroxy-3′-methoxyphenyl)propan-1-one **(20)**	$C_{10}H_{12}O_5$	212.00	*C. laevigata*[122]
77	3-Hydroxy-1-(4′-hydroxy-3′,5′-dimethoxyphenyl)propan-1-one **(21)**	$C_{11}H_{14}O_5$	226.00	*C. laevigata*[122]
78	Cinnamic acid **(17)**	$C_9H_8O_2$	148.00	*C. laevigata*[122]
79	Di-2-ethylhexyl phthalate	$C_{23}H_{36}O_4$	376.00	*C. auriculata*[123]
80	3-*O*-*β*-*D*-Xylopyranosid	$C_{35}H_{56}O_8$	604.30	*C. auriculata*[124]
81	Kaempferol-3-*O*-*α*-*L*-rhamnopyranosyl (1→2)-*α*-*L*-rhamnopyranoside	$C_{27}H_{30}O_{14}$	577.00	*C. hirsuta*[125]
82	Torachrysone Tetraglucoside	$C_{38}H_{54}O_{24}$	895.00	*C. tora*[126]
83	Demethylflavasperone Gentiobioside	$C_{27}H_{32}O_{15}$	597.18	*C. tora*[126]
84	Rubrofusarin triglucoside **(26)**	$C_{33}H_{42}O_{20}$	759.23	*C. tora*[126]
85	Norrubrofusarin-6-*β*-gentiobioside **(27)**	$C_{26}H_{30}O_{15}$	582.51	*C. tora*[126]

86	Torachrysone gentiobioside (**28**)	$C_{26}H_{34}O_{14}$	571.00	*C. tora*[126]
87	Torachrysone apioglucoside	$C_{25}H_{32}O_{13}$	541.00	*C. tora*[126]
88	Cassiaoccidentalin A (**24**)	$C_{27}H_{28}O_{13}$	560.51	*C. occidentalis*[127]
89	Cassiaoccidentalin B (**25**)	$C_{27}H_{28}O_{14}$	576.51	*C. occidentalis*[127]
90	Cassiaoccidentalin C	$C_{28}H_{30}O_{14}$	590.53	*C. occidentalis*[127]
91	1,3-Dihydroxy-6-methoxy-7-methylanthraquinone	$C_{16}H_{12}O_5$	284.26	*C. obtusa*[128]
92	1-Hydroxy-3,7-diformylanthraquinone	$C_{16}H_8O_5$	280.23	*C. obtusa*[128]
93	Torososide B	$C_{40}H_{52}O_{25}$	932.83	*C. torosa*[129]
94	Torosachrysone-8-*O*-6^{II}-malonylgentiobioside	$C_{31}H_{38}O_{18}$	698.63	*C. torosa*[129]
95	1-Hydroxy-3,6,7,8-tetramethoxy-2-methylanthraquinone-1-*O*-(2^{II}-*O*-*β*-*D*-mannopyranosyl)-*β*-*D*-allopyranoside	$C_{31}H_{38}O_{17}$	682.00	*C. occidentalis*[130]
96	1-Hydroxy-7-methoxy-3-methylanthraquinone	$C_{16}H_{12}O_4$	268.26	*C. obtusifolia*[131]
97	1,7,8-Trihydroxy-2,3-dimethoxyanthraquinone	$C_{16}H_{12}O_7$	316.26	*C. obtusifolia*[131]
98	8-Hydroxy-1-methoxy-3-methylanthraquinone	$C_{16}H_{12}O_4$	268.26	*C. obtusifolia*[131]
99	1-Hydroxy-8-methoxy-3-methylanthraquinon	$C_{16}H_{12}O_4$	268.26	*C. speciosa*/ *C. multijuga*[131]
100	Anhydrobarakol (**29**)	$C_{13}H_{10}O_3$	214.22	*C. siamea*[132]
101	Cassiaside B2	$C_{39}H_{52}O_{25}$	920.82	*C. obtusifolia*[133]
102	Cassiaside C2	$C_{39}H_{52}O_{25}$	920.82	*C. obtusifolia*[133]
103	Alaternin-2-*O*-*β*-*D*-glucopyranoside	$C_{21}H_{20}O_{11}$	448.38	*C. tora*[134]
104	1-Hydroxy-2-isopropyl-3,6,7,8-tetramethoxyanthraquinone	$C_{21}H_{22}O_7$	386.40	*C. angustifolia*[135]
105	2,4,8-Trihydroxy-1-methoxy-6-methylanthraquinone	$C_{16}H_{12}O_6$	300.26	*C. angustifolia*[135]

106	2,4-*trans*-7,4′-Dihydroxy-4-methoxyflavane	$C_{16}H_{16}O_4$	272.30	*C. abbreviata*[136]
107	3-Formyl-1-hydroxy-8-methoxyanthraquinone	$C_{16}H_{10}O_5$	282.00	*C. fistula*[137]
108	1,8-Dihydroxy-2,3-dimethoxy-7-methylanthraquinone	$C_{17}H_{14}O_6$	314.29	*C. nodosa*[138]
109	Octamethoxynaphthalene	$C_{18}H_{24}O_8$	368.38	*C. nodosa*[139]
110	Presengulone	$C_{32}H_{26}O_{10}$	570.15	*C. sophera*[140]
111	Floribundone 3	$C_{32}H_{22}O_{11}$	582.51	*C. septemtrionalis*[141]
112	3β-Hydroxy-17-norpimar-8(9)-en-15-one	$C_{19}H_{30}O_2$	290.44	*C. fistula*[142]
113	1-Hydroxy-6,8-dimethoxy-2,3-dimethylanthraquinone	$C_{18}H_{16}O_5$	312.32	*C. grandis*[143]
114	3-Hydroxy-6,8-dimethoxy-2-methyle anthraquinone-1-*O*-β-*D*-galactoside	$C_{23}H_{24}O_{11}$	476.43	*C. auriculata*[144]
115	2-Hydroxy-1,6,8-trimethoxyanthraquinone	$C_{17}H_{14}O_6$	314.29	*C. renigera*[145]
116	Grandisone	$C_{16}H_{12}O$	332.26	*C. grandis*[146]
117	Isorubrofusarin 10-gentiobioside	$C_{27}H_{32}O_{15}$	596.54	*C. tora*[147]
118	(2S)-3′,4′,7-Trihydroxyflavan-(4β→8)-catechin	$C_{30}H_{26}O_{10}$	546.52	*C. nomame*[148]
119	(2S)-3′,4′,7-Trihydroxyflavan-(4α→8)-catechin	$C_{30}H_{26}O_{10}$	546.52	*C. nomame*[148]
120	2-Hentriacontanone	$C_{31}H_{62}O$	450.83	*C. fistula*[46]
121	5-Nonatetracontanone	$C_{49}H_{98}O$	703.31	*C. fistula*[46]
122	Rhamnetin-3-*O*-gentiobioside	$C_{28}H_{32}O_{17}$	640.55	*C. fistula*[149]
123	1,5-Dihydroxy-8-methoxy-2,3-dimethylanthraquinone	$C_{17}H_{14}O_5$	298.29	*C. grandis*[150]
124	1,7,8-Trihydroxy-4-methoxy-2-methyl anthraquinone-3-*O*-β-*D*-galactopyranosid	$C_{22}H_{22}O$	478.40	*C. grandis*[151]

125	1,8-Dimethoxy-3-methylanthraquinone	$C_{17}H_{14}O_4$	282.29	*C. obtusifolia*/ *C. pudibunda*[152]
126	1,3,4-Trihydroxy-6,7,8-trimethoxy-2-methylanthraquinone-3-*O*-*β*-*D*-glucopyranoside	$C_{24}H_{26}O_{13}$	522.46	*C. grandis*[153]
127	Isosengulone	$C_{32}H_{22}O_{10}$	566.12	*C. multiglandulosa*[154]
128	10-Hydroxy-10-(physcion-7′-yl)-chrysophanol-9-anthrone	$C_{31}H_{22}O_9$	538.12	*C. didymobotrya*[155]
129	5,10-Dihydroxy-2-methyl-9-(physcion-7′-yl)-1,4-anthraquinone	$C_{31}H_{20}O_9$	536.11	*C. didymobotrya*[155]
130	Guibourtinidol(4α→6)afzelechin	$C_{30}H_{26}O_9$	530.53	*C. abbreviata*[156]
131	Trans-3-methoxy-4,5-methylenedioxy cinnamaldehyde	$C_{11}H_{10}O_4$	206.19	*C. grandis*[157]
132	1,3,4-Trihydroxy-6,7,8-trimethoxy-2-methylanthraquinone-3-*O*-*β*-*D*-glucopyranoside	$C_{24}H_{26}O_{13}$	522.00	*C. grandis*[153]
133	Leptophyllin A **(30)**	$C_{18}H_{37}NO_3$	315.49	*C. leptophylla*[158]
134	3-Acetylleptophyllin A **(31)**	$C_{20}H_{39}NO_4$	357.53	*C. leptophylla*[158]
135	Leptophyllin B**(32)**	$C_{17}H_{33}NO_3$	299.45	*C. leptophylla*[158]
136	Canavaline **(33)**	$C_{18}H_{37}NO_2$	299.49	*C. leptophylla*[158]
137	(-)-Spectalinine	$C_{20}H_{40}NO_2$	326.30	*C. leptophylla*[158]
138	Lup-20(29)-ene-3*β*-octanoate	$C_{38}H_{64}O_2$	552.92	*C. roxburghii*[159]
139	Torososide A	$C_{38}H_{32}O_{15}$	728.66	*C. torosa*[160]
140	Cassitoroside	$C_{25}H_{32}O_{14}$	556.51	*C. tora*[161]
141	8-Glucopyranosyl-2′,4′,5,7-tetrahydroxyisoflavone	$C_{21}H_{20}O_{11}$	448.38	*C. siamea*[162]
142	1,1′-Bipiperidine	$C_{10}H_{20}N_2$	168.20	*C. grandis*[163]
143	1,3,5,8-Tetrahydroxy-6,7-dimethoxy-2-methylanthraquinone	$C_{17}H_{14}O_8$	346.00	*C. grandis*[164]

144	Cassigarol E	$C_{28}H_{22}O_8$	486.47	*C. garrettiana*[165]
145	Cassigarol F	$C_{28}H_{22}O_8$	486.47	*C. garrettiana*[165]
146	Cassigarol G	$C_{28}H_{20}O_8$	484.46	*C. garrettiana*[165]
147	1,2,4,8-Tetrahydroxy-5,6-dimethoxy-3-methylanthraquinone	$C_{17}H_{14}O_8$	346.29	*C. grandis*[166]
148	Alquinone	$C_{15}H_8O_6$	284.22	*C. alata*[167]
149	Isoziganein-1-rutinoside	$C_{27}H_{30}O_{13}$	562.52	*C. alata*[168]
150	1,5-Dihydroxy-2-methylanthraquino	$C_{15}H_{10}O_4$	254.00	*C. alata*[168]
151	Torosanin-9,10-quinone	$C_{32}H_{26}O_{10}$	570.55	*C. occidentalis*[169]
152	Sengulone	$C_{32}H_{22}O_{10}$	566.12	*C. occidentalis*[169]
153	Alatonal	$C_{15}H_8O_6$	284.22	*C. alata*[170]
154	1,2,4-Trihydroxy-5,6,7-trimethoxy-3-methylanthraquinone	$C_{18}H_{16}O_8$	360.32	*C. grandis*[171]
155	19a,24-Dihydroxyurs-12-ene-28-oic acid-3-*O*-*β*-*D*-xylopyranoside	$C_{35}H_{56}O_9$	502.00	*C. siamea*[172]
156	Torosaol III	$C_{32}H_{30}O_{10}$	574.58	*C. torosa*[173]
157	1,3,4,6-Tetrahydroxy-5,8-dimethoxy-2-methylanthraquinone	$C_{17}H_{14}O_8$	346.29	*C. javanica*[174]
158	5,8-Dihydroxy-1,2,3-trimethoxy-7-methylanthraquinone	$C_{18}H_{16}O_7$	344.32	*C. javanica*[175]
159	1,5,6,7-Tetrahydroxyanthraquinone 3-carboxylic acid	$C_{15}H_{10}O_7$	302.24	*C. didymobotrya*[176]
160	3-Formyl-1,6,8,10-tetrahydroxyanthrone.	$C_{15}H_{10}O_6$	286.24	*C. alata*[177]
161	Emodin-8-*O*-sophoroside	$C_{27}H_{30}O_{14}$	578.00	*C. angustifolia*[178]
162	1,5-Dihydroxy-3-methylanthraquinone	$C_{16}H_{12}O_5$	284.26	*C. italica*[179]
163	Nodosin	$C_{27}H_{30}O_{15}$	594.52	*C. nodosa*[180]
164	Pelargonidin-5-galactoside	$C_{21}H_{21}O_{10}^+$	433.39	*C. auriculata*[181]
165	6,8-Dimethoxy-2-methylanthraquinone-3-*O*-*β*-*D*-galactopyranoside	$C_{23}H_{24}O_{10}$	460.43	*C. siamea*[182]
166	1,5,8-Trihydroxy-2-methylanthraquinone	$C_{21}H_{20}O_{11}$	448.38	*C. siamea*[182]

	-3-O-β-D-galactopyranoside			
167	1,5,7-Trihydroxy-3-methylanthraquinone (34)	$C_{15}H_{10}O_5$	270.00	*C. alata*[183]
166	5,7-Dihydroxy-6-methylflavone-4′-neohesperidoside	$C_{28}H_{32}O_{15}$	608.55	*C. marginata*[184]
168	Rubrofusarin	$C_{15}H_{13}O_5$	273.07	*C. longiracemosa*[185]
169	Nataloeemodin	$C_{15}H_{10}O_5$	270.24	*C. longiracemosa*[185]
170	3-Hydroxy-6,8-dimethoxy-2-methyl anthraquinone-3-*O*-*β*-*D*–glucopyranoside	$C_{23}H_{24}O_{10}$	460.13	*C. grandis*[186]
171	Cassigarol C	$C_{28}H_{22}O_7$	470.47	*C. garrettiana*[187]
172	Cassigarol D	$C_{28}H_{20}O_8$	484.46	*C. garrettiana*[187]
173	2,2′,4,4′-Tetrahydroxy-5,5′,7,7′-tetramethoxy-3,3′-dimethyl-bianthraquinone	$C_{34}H_{26}O_{12}$	626.57	*C. siamea*[188]
174	Tamarixetin 7-rhamnoside 3-rutinoside	$C_{34}H_{42}O_{20}$	770.69	*C. italica*[189]
175	Trachypone	$C_{34}H_{26}O_{10}$	594.57	*C. trachypus*[190]
176	4′-*O*-Methyl-ent-gallocatechin	$C_{16}H_{16}O_7$	320.00	*C. trachypus*[190]
177	Torosaflavone B 3′-glucoside	$C_{28}H_{32}O_{14}$	592.55	*C. torosa*[191]
178	1-Hydroxy-2,6,7,8-tetramethoxy-3-methylanthraquinone	$C_{19}H_{18}O_7$	358.34	*C. lindheimeriana*[192]
179	3,8-Dihydroxy-6-hydroxymethyl-1,2,5-trimethoxyanthraquinone	$C_{18}H_{16}O_8$	360.32	*C. greggii*[193]
180	2,8-Dihydroxy-6-hydroxymethyl-1,3,5-trimethoxyanthraquinone	$C_{18}H_{16}O_8$	360.32	*C. greggii*[193]
181	8-Hydroxy-6-hydroxymethyl-1,2,3,5-tetramethoxyanthraquinone	$C_{19}H_{18}O_8$	374.34	*C. greggii*[193]
182	2,5,8-Trihydroxy-1,3-dimethoxy-6-methylanthraquinone	$C_{17}H_{14}O_7$	330.29	*C. greggii*[193]
183	2,8-Dihydroxy-1,3,5-trimethoxy-6-methylanthraquinone	$C_{20}H_{20}O_7$	372.37	*C. greggii*[193]

184	1,2,3,5,8-Pentamethoxy-6-methylanthraquinone	$C_{20}H_{20}O_7$	372.37	*C. greggii*[193]
185	8-Hydroxy-3,5-dimethoxy-1,2-methylenedioxy-6-methylanthraquinone	$C_{18}H_{14}O_7$	342.30	*C. greggii*[193]
186	De-*O*-Methyltorosaflavone C	$C_{21}H_{14}O_8$	394.33	*C. nomame*[194]
187	De-*O*-Methyltorosaflavone D	$C_{18}H_{12}O_8$	356.28	*C. nomame*[194]
188	3-*O*-*β*-*D*-Glucopyranosyl-1,3-dihydroxy-6,7,8-trimethoxy-2-methylanthraquinone	$C_{24}H_{26}O_{12}$	506.00	*C. grandis*[195]
189	2-(p-Hydroxyphenoxy)-5,7-dihydroxychromone	$C_{15}H_{10}O_6$	286.00	*C. obtusifolia*[196]
190	7-*O*-[*β*-*D*-Mannopyranosyl-(1→2)-*β*-*D*-allopyranoside]	$C_{28}H_{32}O_{16}$	624.50	*C. alata*[197]
191	3-*O*-[*β*-*D*-Mannopyranosyl-(1→2)-*β*-*D*-allopyranoside]	$C_{28}H_{32}O_{17}$	640.50	*C. alata*[197]
192	Kaempferol-7-*O*-(*β*-*D*-galactosyl-rhamnosyl-(1→6)-α-*L*-rhamnopyranoside)	$C_{27}H_{30}O_{15}$	594.52	*C. biflora*[198]
193	Torosaflavone C	$C_{22}H_{16}O_8$	408.36	*C. torosa*[199]
194	Torosaflavone D	$C_{19}H_{14}O_8$	370.31	*C. torosa*[199]
195	Quinquangulin-6-*O*-*β*-*D*-glucopyranoside	$C_{22}H_{24}O_{10}$	448.42	*C. pudibunda*[200]
196	4′,7-Dihydroxy-3′,5-dimethoxyflavone	$C_{17}H_{14}O_6$	314.29	*C. didymobotrya*[201]
197	7-Acetylchrysophanol **(35)**	$C_{17}H_{12}O_5$	296.27	*C. didymobotrya*[201]
198	3′-Hydroxy-3,4,5′-trimethoxystilbene	$C_{17}H_{18}O_4$	286.32	*C. didymobotrya*[201]
199	2,3-Dihydroeleutherinol	$C_{15}H_{14}O_4$	258.27	*C. torosa*[202]
200	5,4′-Dihydroxyflavones-5-*O*-*β*-*D*-galactopyranoside **(36)**	$C_{21}H_{20}O_{10}$	432.00	*C. auriculata*[203]
201	Torosaol I	$C_{34}H_{34}O_{11}$	618.63	*C. torosa*[204]
202	Torosaol II	$C_{34}H_{34}O_{11}$	618.63	*C. torosa*[204]
203	Epiafzelechin(4*β*→8)-ent-epicatechin	$C_{30}H_{62}O_{11}$	561.52	*C. fistula*[205]

204	Ent-Epicatechin(4α→8)ent-epiafzelechin	$C_{30}H_{26}O_{11}$	561.50	*C. fistula*[205]
205	10-*O*-demethylflavasperone	$C_{15}H_{12}O_5$	272.00	*C. pudibunda*[206]
206	Cassiapyrone-10-sulfate	$C_{16}H_{14}O_8S$	366.00	*C. pudibunda*[206]
207	1,4-Benzenedicarboxylic acid	$C_8H_6O_4$	166.02	*C. roxburghii*[207]
208	7-Acetyl-6,8-dihydroxy-3-methoxy-4,4-dimethyl-1(4H)-naphthalenone **(37)**	$C_{15}H_{16}O_5$	276.00	*C. semicordata*[208]
209	2,2′-Methylenebis[7-acetyl-3,6,8-trihydroxy-4,4-dimethyl]1(4H) naphthalenone	$C_{29}H_{28}O_{10}$	536.00	*C. semicordata*[208]
210	Lanceolin-5-neohesperidoside	$C_{27}H_{32}O_{16}$	612.54	*C. fistula*[209]
211	Limocitrol-3-neohesperidoside	$C_{30}H_{36}O_{18}$	684.60	*C. fistula*[209]
212	Toralactone-9-*β*-*D*-gentiobioside	$C_{27}H_{32}O_{15}$	595.00	*C. tora*[210]
213	6-[(α-Apiofuranosyl-(1→6)-*O*-*β*-*D*-glucopyranosyl)oy]-rubrofusarin	$C_{26}H_{30}O_{14}$	565.00	*C. tora*[210]
214	Occidentalol I	$C_{33}H_{32}O_{10}$	588.61	*C. occidentalis*[211]
215	Occidentalol II	$C_{32}H_{30}O_{10}$	574.58	*C. occidentalis*[211]
216	1-O-[*β*-*D*-Glucopyranosyl-(1→3)-*β*-*D*-glucopyranosyl-(1→6)-*β*-*D*-glucopyranoside]	$C_{33}H_{40}O_{19}$	740.66	*C. tora*[29]
217	Chrysophanol-1-tetraglucoside	$C_{39}H_{50}O_{24}$	902.81	*C. tora*[29]
218	Torosaflavone A	$C_{21}H_{20}O_8$	400.38	*C. torosa*[212]
219	Torosaflavone B	$C_{22}H_{22}O_9$	430.41	*C. torosa*[212]
220	1,3-Dihydroxy-6,8-dimethoxy-2-prenylanthraquinone	$C_{21}H_{20}O_6$	368.38	*C. marginata*[213]
221	5,7-Dihydroxy-3′,4′-methylenedioxyflavone	$C_{16}H_{10}O_6$	298.00	*C. marginata*[213]
222	Floribundone 2	$C_{32}H_{24}O_9$	552.53	*C. floribunda*[214]
223	5,7′-Biphyscion	$C_{32}H_{22}O_{10}$	566.52	*C. floribunda*[214]
224	Floribundone 1	$C_{32}H_{22}O_{10}$	566.52	*C. floribunda*[214]
225	Cassigarol A	$C_{28}H_{24}O_8$	488.49	*C. garrettiana*[215]

226	Cassigarol B	$C_{21}H_{16}O_6$	364.35	*C. garrettiana*[215]
227	Cassiaside B	$C_{26}H_{30}O_{14}$	566.51	*C. obtusifolia*[216]
228	Cassiaside C	$C_{27}H_{32}O_{15}$	596.54	*C. obtusifolia*[216]
229	Cassiaflavan(4α→6)epiafzelechin	$C_{30}H_{26}O_8$	514.50	*C. fistula*[49]
230	Epicatechin(4β→8)epiafzelechin	$C_{30}H_{26}O_{11}$	562.52	*C. fistula*[49]
231	1,3,5,8-Tetrahydroxy-7-methoxy-2-methylanthraquinone	$C_{16}H_{12}O_7$	316.26	*C. javanica*[217]
232	1,2-Dihydro-1,3-dihydroxy-6,8-dimethoxy-2-methylanthraquinone	$C_{17}H_{16}O_6$	316.31	*C. javanica*[217]
233	2-*O*-(3-Hydroxy-3-methyl butyl)-4-*O*(4′-*O*-phenacetyl) glucoside (Marginoside)	$C_{27}H_{32}O_{13}$	564.54	*C. marginata*[218]
234	2-*O*-(4-Hydroxy-3-methyl butyl)-6-*O*-phenacetyl phloroacetophenone-4-*O* neohesperidoside (Cassioside)	$C_{33}H_{42}O_{17}$	710.68	*C. marginata*[218]
235	1,3-Dihydroxy-6,8-dimethoxy-2-methyl anthraquinone-3-*O*-rhamnosyl-(1→6)-glucopyranoside	$C_{21}H_{20}O_{11}$	448.38	*C. marginata*[219]
236	1,3,5,8-Tetrahydroxy-2-methylanthraquinone-3-*O*-glucoside	$C_{29}H_{34}O_{16}$	638.57	*C. marginata*[219]
237	1,7-Dihydroxy-3-methylxanthone	$C_{14}H_{10}O_4$	242.23	*C. occidentalis*[220]
238	1,8-Dihydroxy-6-methoxy-2-methylanthraquinone	$C_{16}H_{12}O_5$	284.26	*C. mimosoides*[221]
239	Butyrospermone	$C_{30}H_{48}O$	424.70	*C. javanica*[222]
240	Roxburghin	$C_{14}H_{12}O_4$	244.24	*C. roxburghii*[223]
241	4,4′-bis(1,3,8-Trihydroxy-6-methoxy-2-methylanthraquinone) **(38)**	$C_{32}H_{22}O_{12}$	598.51	*C. hirsuta*[224]
242	3β,16β,22-Trihydroxyisohopane **(39)**	$C_{30}H_{52}O_3$	442.00	*C. hirsuta*[224]
243	Cassiadinine **(40)**	$C_{16}H_{13}N_3O_4$	311.29	*C. siamea*[225]
244	(+)-6-Hydroxyl mullein	$C_{10}H_{10}O_4$	194.00	*C. siamea*[225]

245	1,4-Dihydroxy-8-methoxy-2-methylanthraquinone-3-*O*-*β*-*D*(+)-glucopyranoside	$C_{22}H_{22}O_{11}$	462.00	*C. nodosa*[226]
246	2,5-Dimethoxy-1,4-benzoquinone	$C_8H_8O_4$	168.14	*C. obtusifolia*[227]
247	3,5-Dihydroxy-7,3′,4′-trimethoxy flavanone-3-*O*-arabinopyranoside	$C_{23}H_{24}O_{11}$	476.43	*C. spectabilis*[228]
248	6-Hydroxy-4′-methoxyflavanone-6-*O*-arabinopyranoside	$C_{21}H_{20}O_8$	400.38	*C. spectabilis*[228]
249	Alaternin-1-*O*-*β*-*D*-glucopyranoside	$C_{21}H_{20}O_{11}$	448.00	*C. obtusifolia*[229]
250	Chryso-obtusin-2-*β*-*D*-glucopyranoside	$C_{25}H_{28}O_{12}$	520.00	*C. obtusifolia*[229]
251	7-Methylphyscion	$C_{17}H_{14}O_5$	298.29	*C. singueana*[230, 231]
252	7-Methyltorosachrysone	$C_{17}H_{18}O_5$	302.32	*C. occidentalis*[231]
253	1,8-Dihydroxy-2-methylanthraaquinone 3-neohesperidoide	$C_{27}H_{30}O_{14}$	778.00	*C. sophera*[232]
254	Jaceidin-7-neohesperidoside	$C_{30}H_{36}O_{17}$	668.60	*C. occidentalis*[233]
255	Roxburghinol (**41**)	$C_{15}H_{12}O_5$	272.25	*C. roxburghii*[234]
256	8-Methyltoralactone-10-methylether	$C_{17}H_{16}O_5$	300.09	*C. torosa*[235]
257	8-Methyltoralactone	$C_{16}H_{14}O_5$	286.28	*C. torosa*[235]
258	1-Desmethylobtusin (**42**)	$C_{17}H_{14}O_7$	330.29	*C. obtusifolia*[236]
259	1,2-Dihydroxy-6,7,8-trimethoxy-3-methylanthraquinone (**43**)	$C_{18}H_{16}O_7$	344.32	*C. obtusifolia*[236]
260	1,2,6,8-Tetrahydroxy-7-methoxy-3-methylanthraquinone (**44**)	$C_{16}H_{12}O_7$	316.26	*C. obtusifolia*[236]
261	1,2,7-Trihydroxy-6,8-dimethoxy-3-methyl-anthraquinone	$C_{17}H_{14}O_7$	330.28	*C. sophora*[237]
262	1,2,6-Trihydroxy-7,8-dimethoxy-3-methylanthraquinone	$C_{17}H_{14}O_7$	330.32	*C. sophera*[237]
263	1,3-Dihydroxy-5,7,8-trimethoxy-2-methylanthraquinone	$C_{18}H_{16}O_7$	344.32	*C. sophora*[237]

264	Javanin	$C_{22}H_{22}O_9$	430.41	*C. javanica*[238]
265	Torosachrysone-8-*β*-*D*-gentiobioside	$C_{28}H_{36}O_{15}$	612.58	*C. torosa*[239]
266	Physcion-8-*β*-*D*-gentiobioside	$C_{28}H_{32}O_{15}$	608.50	*C. torosa*[239]
267	1,6,8-Isoquinolinetriol (Siaminine B)	$C_9H_7NO_3$	177.15	*C. siamea*[240]
268	Siaminine C	$C_{10}H_9NO_3$	190.00	*C. siamea*[240]
269	Siaminine A	$C_{11}H_{11}NO_3$	205.21	*C. siamea*[240]
270	Colensenone	$C_{19}H_{30}O_2$	290.44	*C. petersiana*[241]
271	8,13-Epoxy-3-nor-2-colensanone	$C_{19}H_{32}O_2$	292.46	*C. petersiana*[241]
272	Kaempferol **(46)**	$C_{15}H_{10}O_6$	286.00	*C. fistula*[73]
273	1,8-Dihydroxy-2-methylanthraquinone-3-O-rutinoside	$C_{27}H_{30}O_{14}$	578.00	*C. multijuga*[242]
274	1,3,8-Trihydroxy-6-methoxy-2-methylanthraquinone	$C_{16}H_{12}O_6$	300.00	*C. multijuga*[242]
275	1,8-Dihydroxy-6-methoxy-2-methylanthraquinone-3-*O*-rutinoside	$C_{28}H_{32}O_{15}$	608.00	*C. multijuga*[242]
276	Fistacacidin	$C_{15}H_{14}O_5$	274.27	*C. fistula*[243]
277	3,3′,4′,5,5′,8-Hexahydroxyflavone	$C_{15}H_{10}O_8$	318.23	*C. tora*[244]
278	5-Acetonyl-6-glucosyl-7-hydroxy-2-methyl-4H-1-benzopyran-4-one	$C_{19}H_{22}O_9$	394.37	*C. spectabilis*[245]
279	Anhydrophlegmacin B2	$C_{32}H_{28}O_9$	556.56	*C. torosa*[246]
280	Anhydrophlegmacin-9,10-quinone B2	$C_{32}H_{26}O_{10}$	570.55	*C. torosa*[246]
281	Physcion-8-xyloside	$C_{21}H_{20}O_9$	416.38	*C. marginata*[247]
282	Emodin-8-*O*-L-arbinopyranoside	$C_{20}H_{18}O_9$	402.00	*C. marginata*[247]
283	Germitorosone	$C_{17}H_{18}O$	318.32	*C. torosa*[248]
284	Methylgermitorosone	$C_{18}H_{20}O_6$	332.35	*C. torosa*[248]
285	Sopheranin	$C_{17}H_{12}O_6$	312.27	*C. sophora*[249]
286	Guibourtinidol(4→8)epiafzelechin	$C_{30}H_{26}O_9$	530.53	*C. fistula*[250]
287	Rahmntin-3-*O*-[*β*-*D*-galactopyranosyl-(1→4)-*β*-*D*-galactopyranoside]	$C_{28}H_{32}O_{17}$	640.55	*C. laevigata*[251]

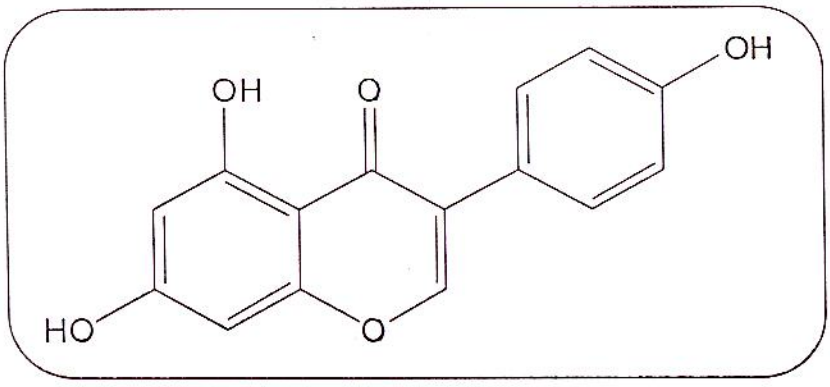
288	Rahmntin-3-O-[*β-D*-galactopyranosyl-(1→6)-*β-D*-galactopyranoside]	$C_{28}H_{32}O_{17}$	640.55	*C. laevigata*[251]
289	1,2,6-Trihydroxy-7,8-dimethoxy-3-methylanthraquinone	$C_{17}H_{14}O_7$	330.29	*C. sophora*[252]
290	1,2,7-Trihydroxy-6,8-dimethoxy-3-methylanthraquinone	$C_{17}H_{14}O_7$	330.00	*C. sophora*[252]
291	Torosanin	$C_{32}H_{28}O_9$	556.56	*C. torosa*[246]
292	Anhyrophlegmacin-9′,10′-quinone	$C_{32}H_{26}O_{10}$	570.52	*C. torosa*[246]
293	Sennoside G	$C_{42}H_{38}O_{20}$	862.00	*C. angustifolia*[253]
294	Cassia lactone	$C_{16}H_{16}O_6$	304.29	*C. obtusifolia*[253]
295	Isotoralactone	$C_{15}H_{12}O_5$	272.06	*C. obtusifolia*[253]
296	Kaempferol-3-*O-β-D*-mannopyranosyl-(1→4)-*O-β-D*-glucopyranoside	$C_{27}H_{30}O_{16}$	610.15	*C. grandis*[254]
297	2,4,5,6,7-Pentahydroxy-1-methoxy-3-methylanthraquinone	$C_{16}H_{12}O_8$	332.26	*C. javanica*[255]
298	1,5-Dihydroxy-4,7-dimethoxy-2-methyl anthraquinone 3-*O-α-L*(-) rhamnopyranoside	$C_{23}H_{24}O_{11}$	476.00	*C. javanica*[255]
299	1,5,8-Trihydroxy-6,7-dimethoxy-2-methylanthraquinone-3-*O-α-L*(-) rhamnopyranoside	$C_{23}H_{24}O_{12}$	492.12	*C. renigera*[256]
300	3-Hydroxy-6,8-dimethoxy-2-methylanthraquinone-1-*O-β-D*(+)glucopyranoside	$C_{23}H_{24}O_{12}$	492.00	*C. multijuga*[256]
301	3-Hydroxy-6,8-dimethoxy-2-methyl anthraquinone-1-*O*-rhamnopyranosyl (1→6)-glucopyranoside	$C_{29}H_{34}O_{16}$	638.00	*C. multijuga*[256]
302	1-Hydroxy-8-methoxy-2-methyl anthraquinone-3-*O-α-L*-(-) rhamnopyranoside	$C_{22}H_{22}O_9$	430.00	*C. multijuga*[256]

303	8-Rhamnosyleuropetin	$C_{22}H_{22}O_{12}$	478.40	*C. sophora*[257]
304	Tinnevellin glucoside	$C_{20}H_{24}O_9$	408.40	*C. angustifolia*[258]
305	6-Nonacosanone	$C_{29}H_{58}O$	422.77	*C. auriculata*[259]
306	Physcion-8-galactoside	$C_{22}H_{22}O_{10}$	446.41	*C. laevigata*[260]
307	Cassiglucin	$C_{28}H_{32}O_{17}$	640.55	*C. multijuga*[261]
308	Singueanol I	$C_{34}H_{34}O_{10}$	602.63	*C. singueana*[262]
309	Singueanol II	$C_{34}H_{34}O_{10}$	602.63	*C. singueana*[262]
310	1,4,5,7-Tetrahydroxy-2,3-dimethoxy-6-methylanthraquinone	$C_{17}H_{14}O_8$	346.29	*C. renigera*[263]
311	Emodin-8-rhamnoside	$C_{21}H_{20}O_9$	416.38	*C. javanica*[264]
312	Chaksine	$C_{22}H_{38}N_6O_4$	450.58	*C. absus*[265]
313	Isochaksine	$C_{11}H_{20}N_3O_2^+$	226.29	*C. absus*[265]
314	Ombuin-3-neohesperidoside	$C_{29}H_{34}O_{16}$	638.50	*C. laevigata*[266]
315	(-)-Epiafzelechin	$C_{15}H_{14}O_5$	274.80	*C. sieberiana*[267]
316	I-Hydroxy-6-methoxy-3-methyl anthraquinone-8-*O*-*β*-*D*-galatctosyl-(1→4)-*O*-*β*-*D*-galactopyranoside	$C_{28}H_{32}O_{15}$	608.00	*C. laeviata*[268]
317	1-(3,4-Dihydroxyphenyl)-2-(3-hydroxyphenyl)ethane	$C_{14}H_{14}O_3$	230.26	*C. garrettiana*[269]
318	2,3,5,7-Tetrahydroxy-9,10-dihydrophenanthrene	$C_{14}H_{12}O_4$	244.24	*C. garrettiana*[269]
319	1,8-Dihydroxy-3-carbo(*β*-*D*-glucopyranosyloxy)-anthraquinone	$C_{21}H_{18}O_{11}$	446.00	*C. marginata*[270]
320	2-Methyl-5-acetonyl-7-hydroxy chromone (Cassiachromone)	$C_{13}H_{12}O_4$	232.23	*C. siamea*[271]
321	2′,5′-Dihydroxy-4-methoxychalcone	$C_{16}H_{14}O_4$	270.28	*C. javanica*[272]
322	Sennoside A	$C_{42}H_{38}O_20$	862.75	*C. angustifolia*[273]
323	Sennoside A1	$C_{42}H_{38}O_{20}$	862.75	*C. angustifolia*[274]
324	Epilactose	$C_{12}H_{22}O_{11}$	342.29	*C. grandis*[275]
325	6,8-Dihydroxy-4-methylbenzanthrone	$C_{18}H_{12}O_3$	276.29	*C. garrettiana*[276]

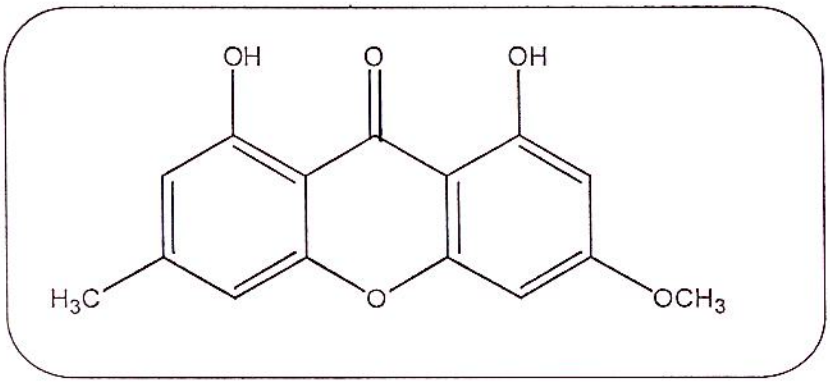
326	6-Isocassine	$C_{18}H_{35}NO_2$	297.48	*C. spectabilis*[277]
327	Emodin (6)	$C_{15}H_{10}O_5$	270.00	*C. tora*[278]
328	Jaceidin-7-rhamnoside	$C_{24}H_{26}O_{12}$	506.46	*C. occidentalis*[279]
329	Matteucinol-7-rhamnoside	$C_{24}H_{28}O$	460.48	*C. occidentalis*[279]
330	Siameanin	$C_{30}H_{18}O_8$	506.46	*C. occidentalis*[280]
331	7-*O*-*β*-*L*-Rhamnopyranoside	$C_{22}H_{24}O_9$	432.42	*C. renigera*[281]
332	Cassialoin	$C_{21}H_{22}O_9$	418.39	*C. garrettiana*[282,276]
333	Torosachrysone	$C_{16}H_{16}O_5$	288.29	*C. torosa*[283]
334	1,8-Dihydroxy-2-methylanthraquinone	$C_{15}H_{10}O_4$	254.00	*C. occidentalis*[284]
335	1,4,5-Trihydroxy-7-methoxy-3-methyl anthraquinone	$C_{16}H_{12}O_6$	300.00	*C. occidentalis*[284]
336	Emodinbianthrone	$C_{30}H_{22}O_8$	510.49	*C. angustifolia*[285]
337	Physcion	$C_{16}H_{12}O_5$	284.28	*C. angustifolia*[285]
338	18-Hydroxy-18-pentadecyl-17-tetratriacontanone	$C_{99}H_{98}O_2$	719.31	*C. auriculata*[286]
339	23-(5-Hydroxypentyl)-22-pentatetracontanone	$C_{50}H_{100}O_2$	733.33	*C. auriculata*[286]
340	26-Hydroxy-21-hexatetracontanon	$C_{46}H_{92}O_2$	677.23	*C. auriculata*[286]
341	Nodolidate	$C_{32}H_{60}O_4$	508.82	*C. nodosa*[287]
342	Auricassidin	$C_{30}H_{26}O_{12}$	578.52	*C. auriculata*[288]
343	Fistulic acid	$C_{18}H_{14}O_8$	358.30	*C. fistula*[289]
344	Azralidoside	$C_{40}H_{74}O_{12}$	747.01	*C. nodosa*[290]
345	1,3,5-Trihydroxy-6,7-dimethoxy-2-methylanthraquinone	$C_{17}H_{14}O_7$	330.29	*C. tora*[291]
346	Nodososide	$C_{22}H_{22}O_{11}$	462.40	*C. nodosa*[292]
347	7-Methoxy-2,5-dimethylchromone	$C_{12}H_{12}O_3$	204.20	*C. siamea*[293]
348	N-Methylmorpholine	$C_5H_{11}NO$	101.08	*C. occidentalis*[294]
349	Quercetin-3′,4′,7-trimethyl ether-3-*O*-*α*-*L*-Rhamnopyranoside	$C_{24}H_{26}O_{11}$	490.46	*C. javanica*[295]
350	Cassiaxanthone	$C_{15}H_8O_7$	300.22	*C. spp*[296]

351	Cassiollin	$C_{16}H_{12}0$	300.00	*C. occidentalis*[297]
352	Cassiamin A	$C_{30}H_{18}O_9$	522.46	*C. siamea*[298]
353	Cassiamin B	$C_{30}H_{18}O_{10}$	538.46	*C. siamea*[298]
354	Cassiamin C	$C_{30}H_{18}O_8$	506.46	*C. siamea*[298]
355	Torachrysone	$C_{14}H_{14}O_4$	246.26	*C. tora*[299]
356	Barakol	$C_{13}H_{12}O_4$	232.23	*C. siamea*[300]
357	Aloeemodin anthrone	$C_{15}H_{12}O_4$	256.25	*C. alata*[301]
358	Fistulin	$C_{30}H_{20}O_{10}$	540.48	*C. fistula*[302]
359	Aloeemodin dianthrone (47)	$C_{30}H_{22}O_8$	510.49	*C. angustifolia*[303]
360	Cassine	$C_{19}H_{37}NO_2$	311.00	*C. excelsa*[304]
361	Glucoaurantioobtusin	$C_{23}H_{24}O_{12}$	492.43	*C. obtusifolia*[305]
362	Chrysoobtusin	$C_{19}H_{18}O_7$	358.34	*C. obtusifolia*[306]
363	Obtusin	$C_{17}H_{13}O_6$	313.00	*C. obtusifolia*[306]
364	Aurantio-obtusin	$C_{17}H_{14}O_7$	330.00	*C. obtusifoloia*[306]
365	Obtusifolin	$C_{16}H_{12}O_5$	284.26	*C. obtusifolia*[307]
366	β-Sitosterol	$C_{29}H_{50}$	414.00	*C. absus*[308]
367	4,5-Dihydroxyanthraquinone-2-carboxylic acid	$C_{15}H_8O_6$	284.22	*C. fistula*[309]

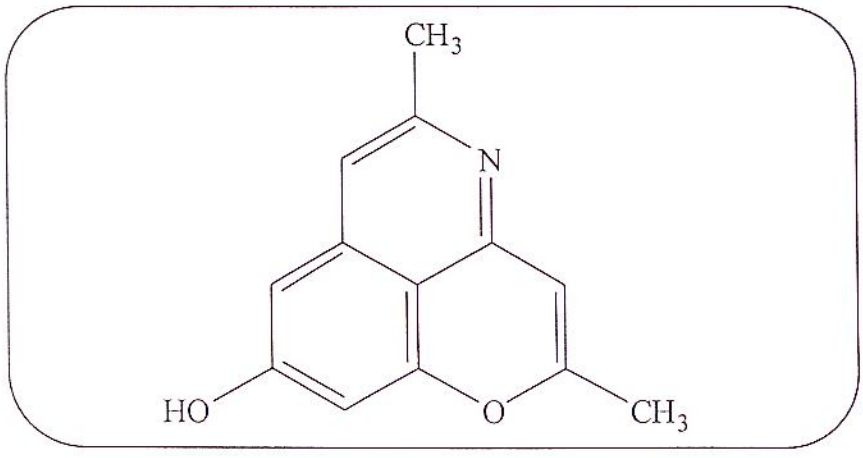
1.6. Structures of Some Isolated Compounds from Genus *Cassia*



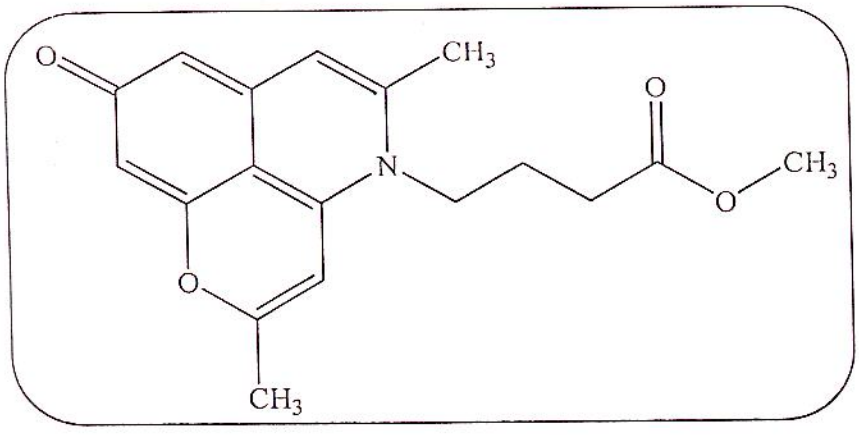
Biochanin A (**1**)[78]



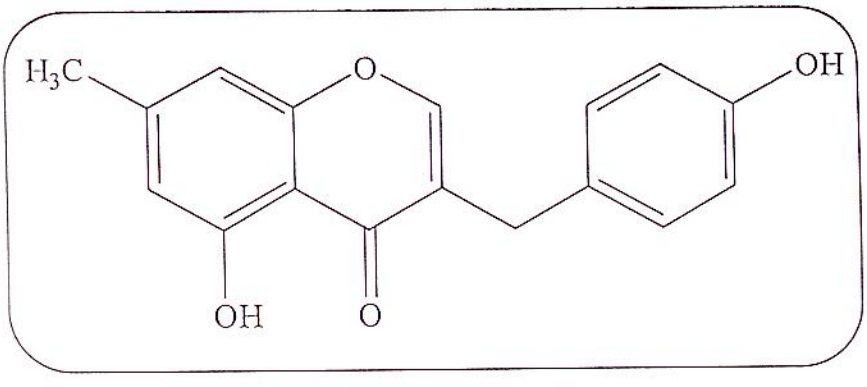
1,8-Dihydroxy-3-methoxy-6-methylxantone (**2**)[81]



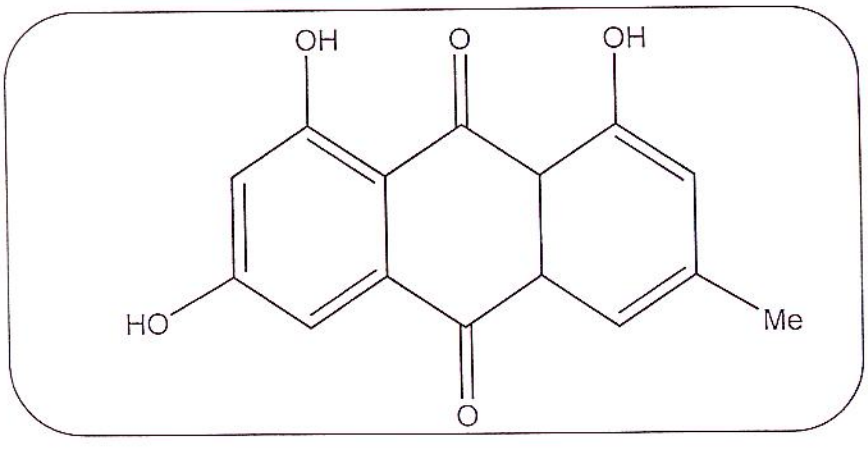
Cassiarins A (**3**)[89]



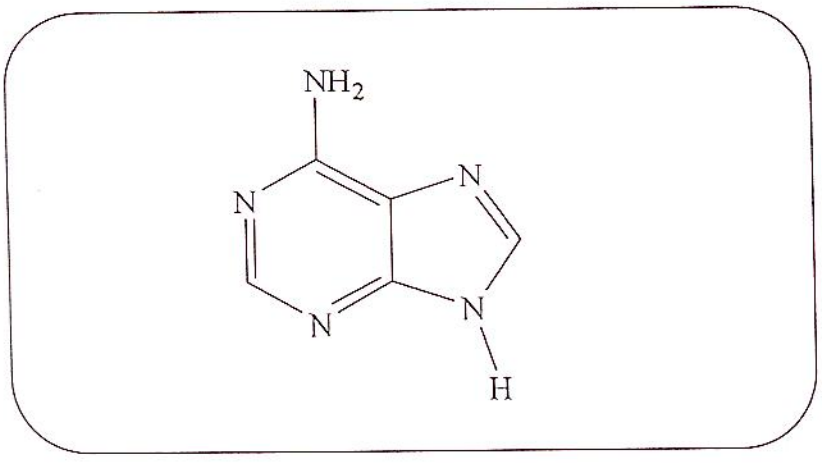
Cassiarins B **(4)**[89]



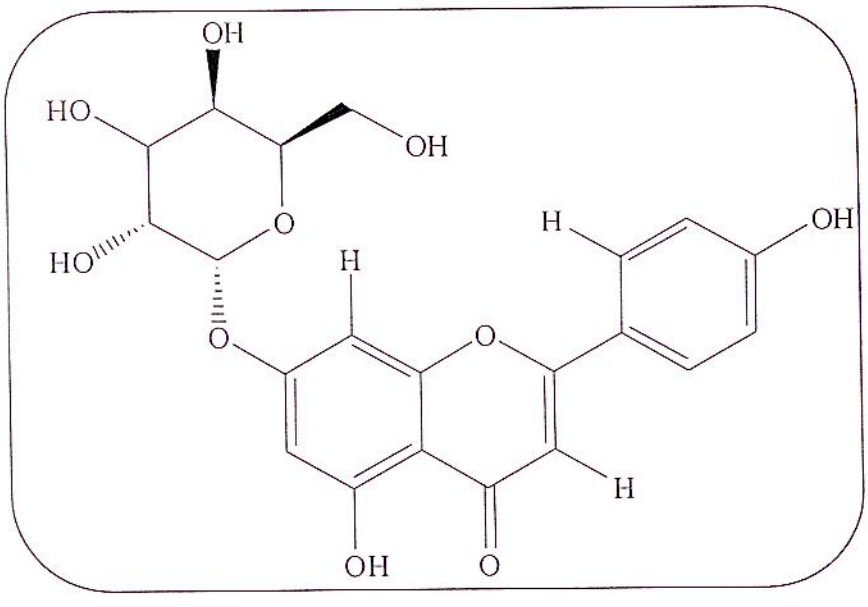
4',5-Dihydroxy-7-methylhomoisoflavone **(5)**[94]



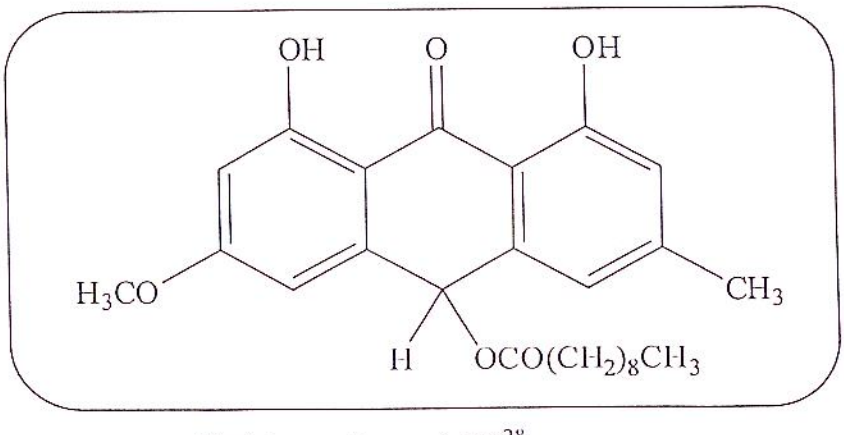
1,8-Dihydroxy-3-methylanthraquinone (Emodin) **(6)**[278]



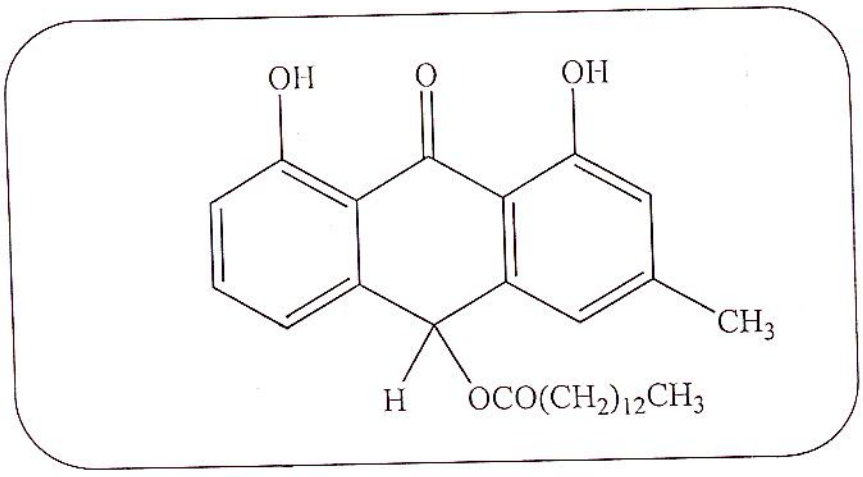
Adenine (7)[104]



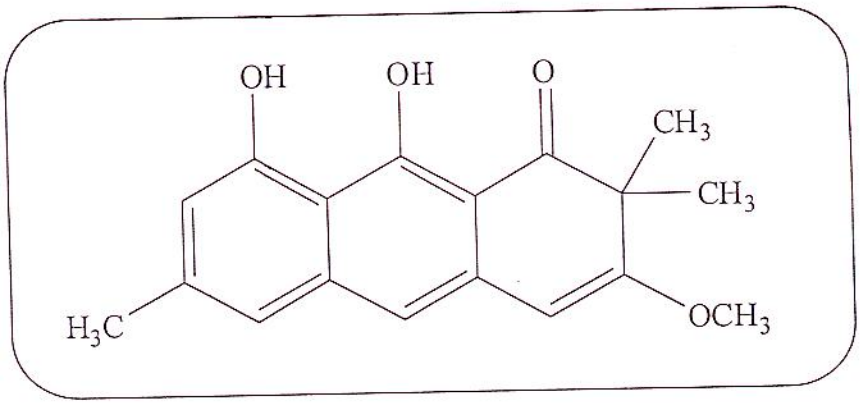
Apigenin 7-alloside (8)[102]



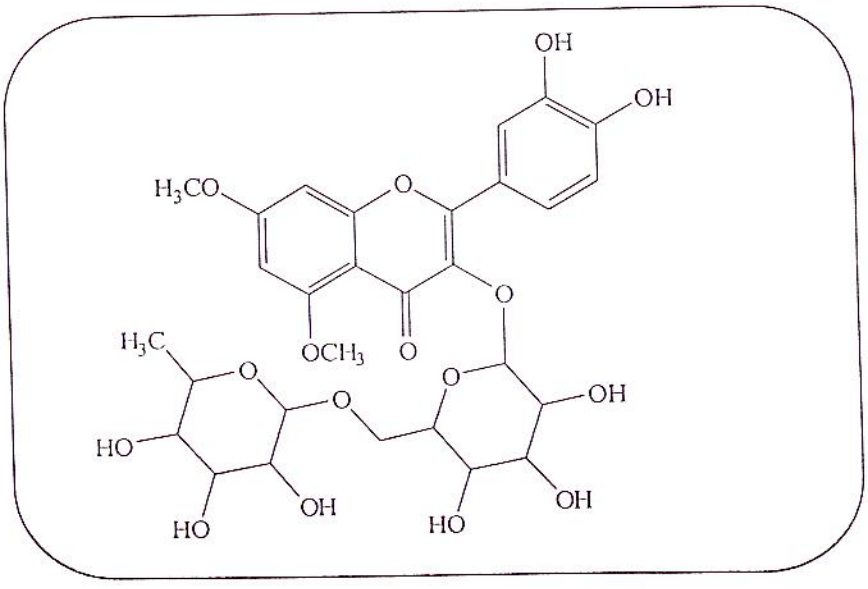
Kleinioxanthrone 1 (9)[28]



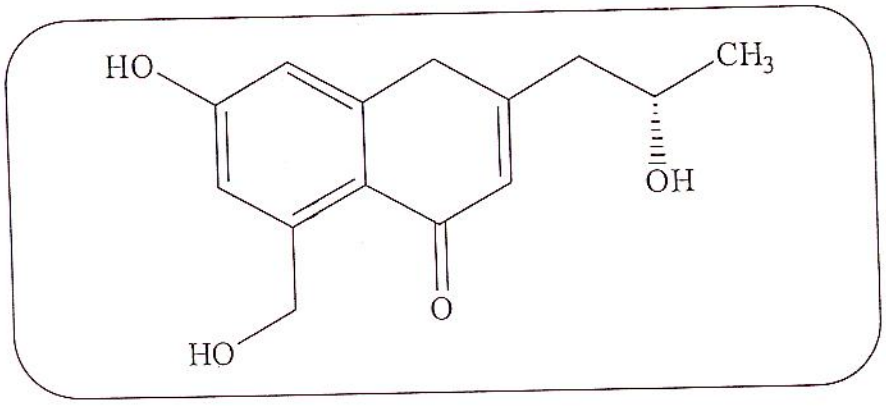
Kleinioxanthrone 2 **(10)**[28]



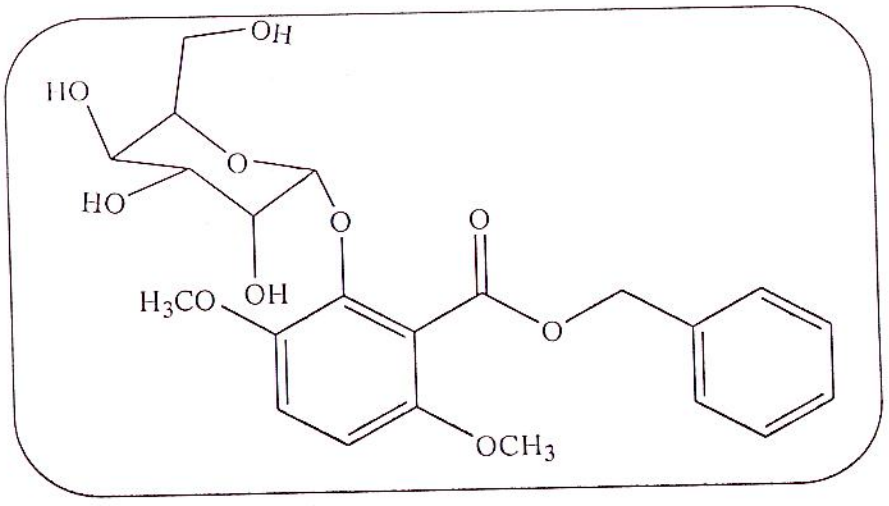
Racemochrysone **(11)**[111]



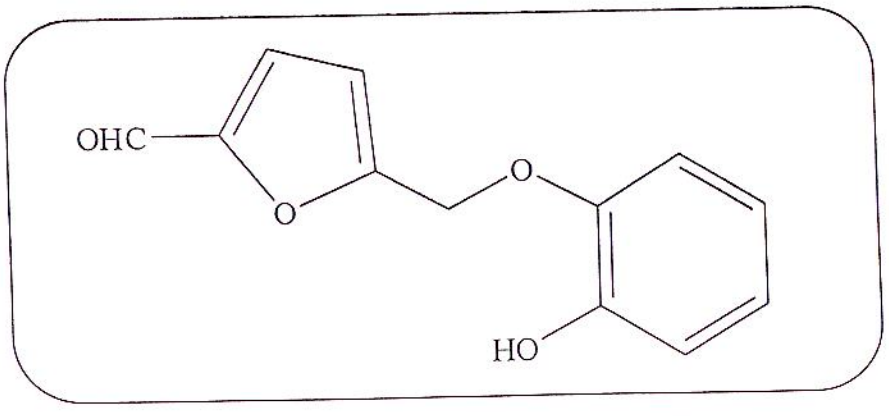
5,7-Dimethoxyrutin **(12)**[108]



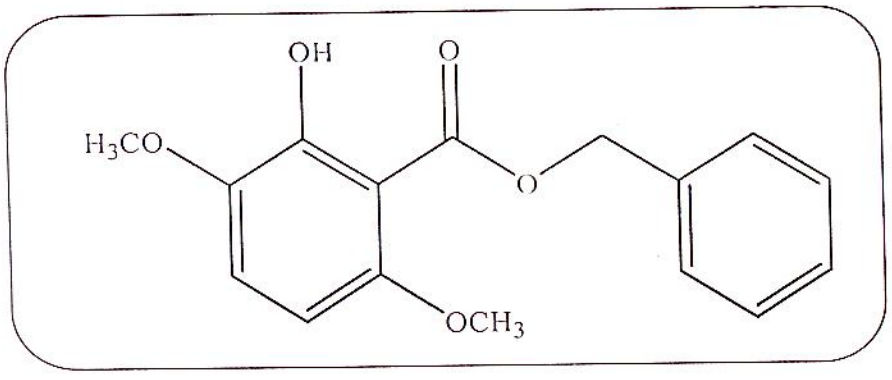
(2′ S)-7-Hydroxy-5-hydroxymethyl-2-(2′-hydroxypropyl)chromone **(13)**[109]



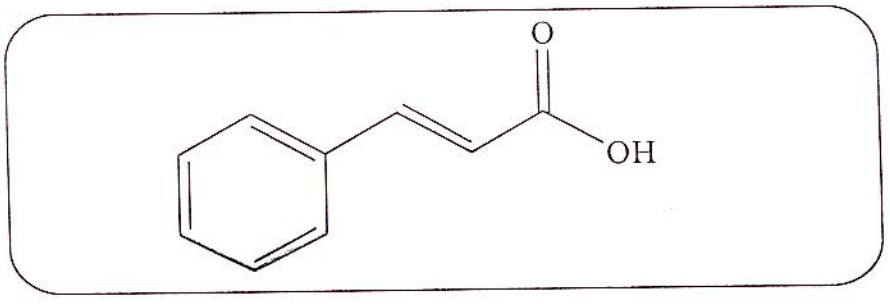
Benzyl 2β-O-D-glucopyranosyl-3,6-dimethoxybenzoate **(14)**[109]



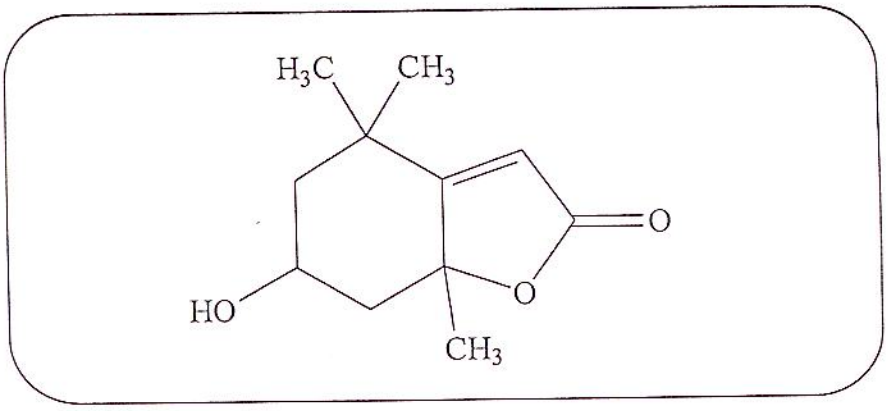
5-(2-Hydroxyphenoxymethyl)furfural **(15)**[109]



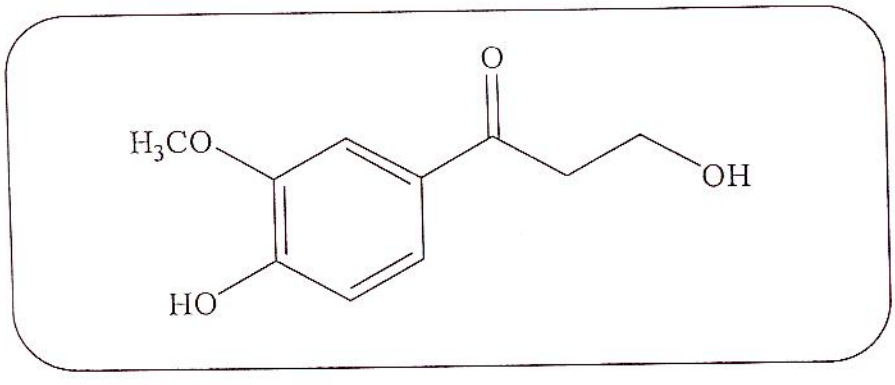
Benzyl 2-hydroxy-3,6-dimethoxybenzoate **(16)**[109]



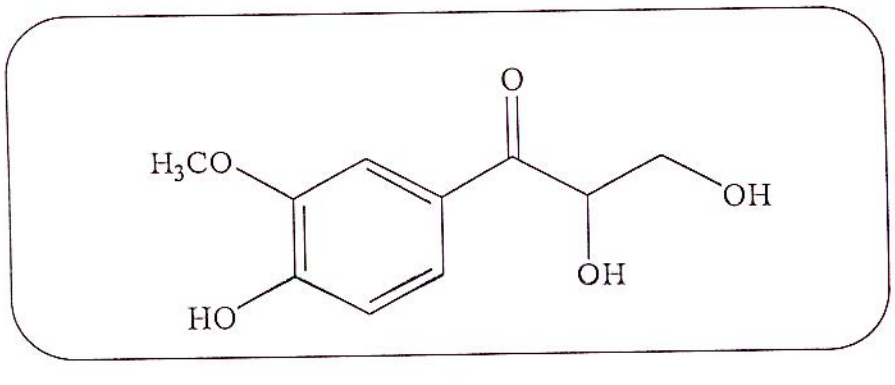
Cinnamic acid **(17)**[122]



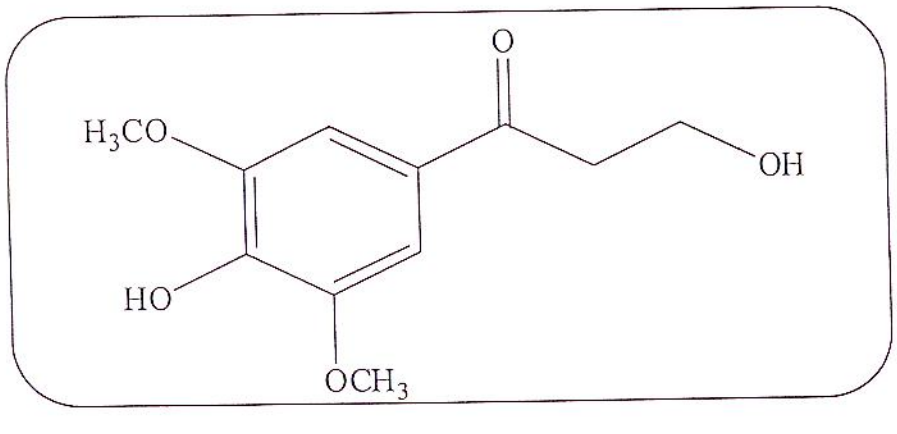
Calendin **(18)**[122]



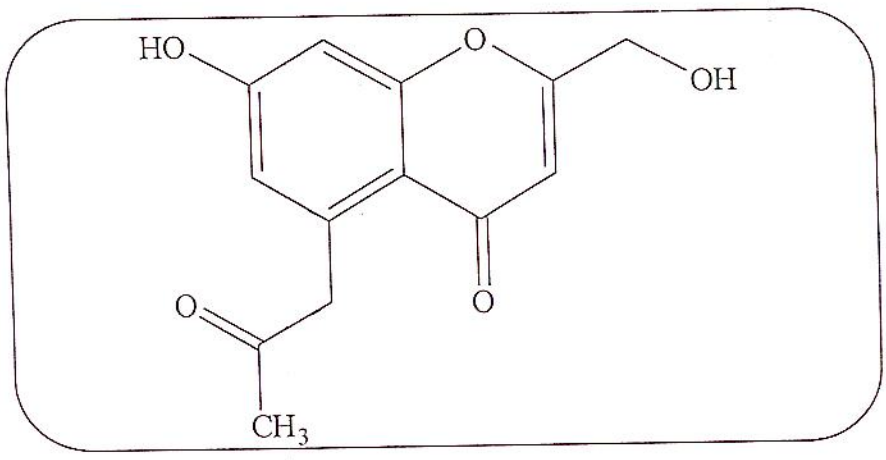
3-Hydroxy-1-(4′-hydroxy-3′-methoxyphenyl)propan-1-one **(19)**[122]



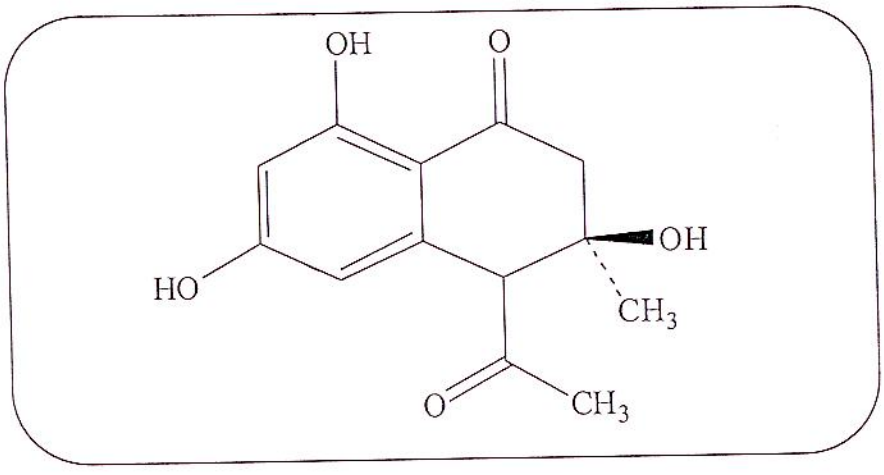
2,3-Dihydroxy-1-(4′-hydroxy-3′-methoxyphenyl)propan-1-one **(20)**[122]



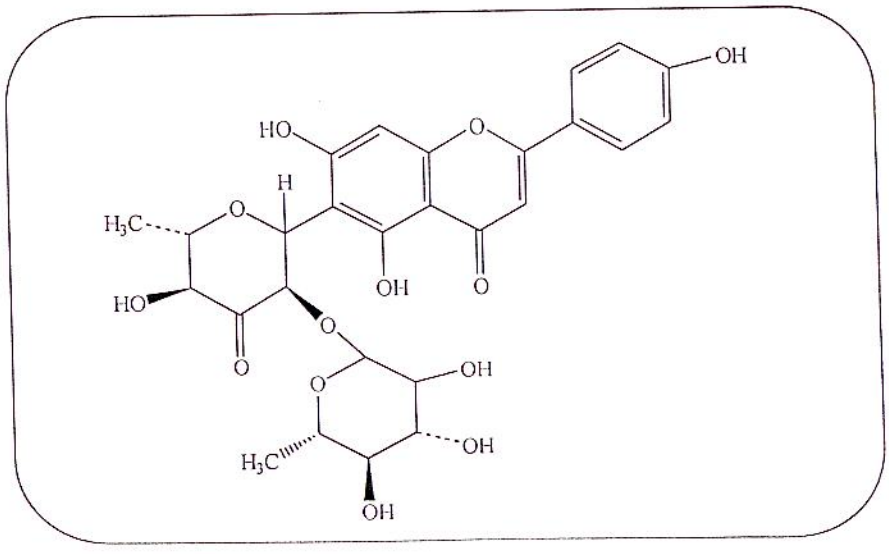
3-Hydroxy-1-(4′-hydroxy-3′,5′-dimethoxyphenyl)propan-1-one **(21)**[122]



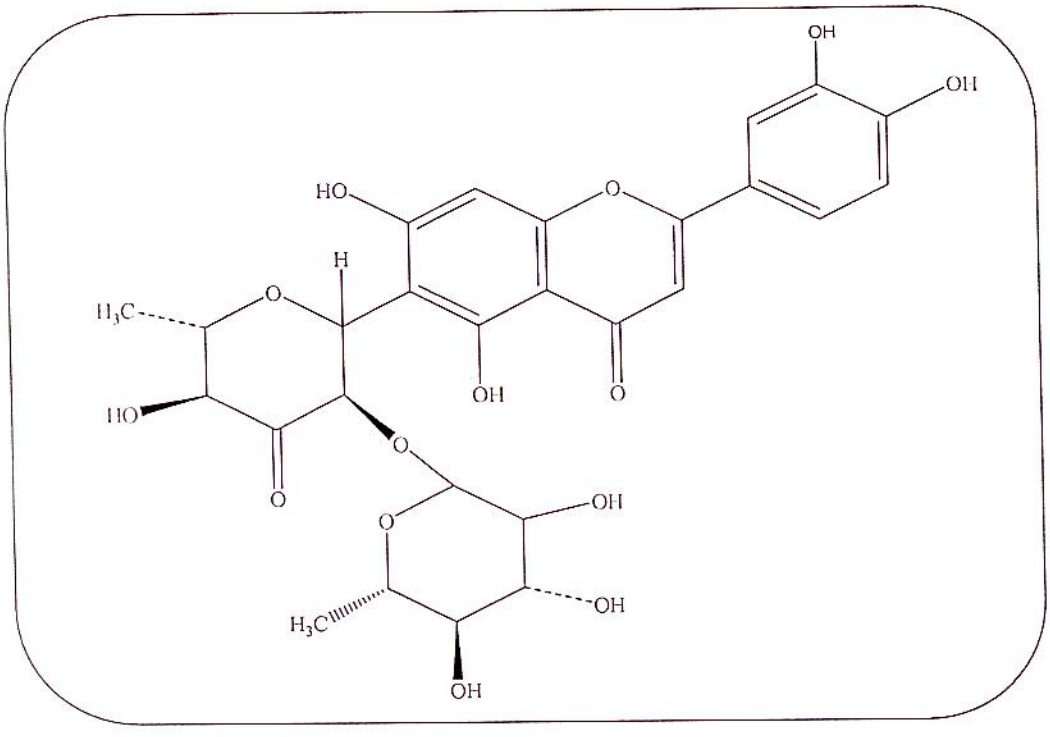
5-Acetonyl-7-hydroxy-2-hydroxymethylchromone **(22)**[121]



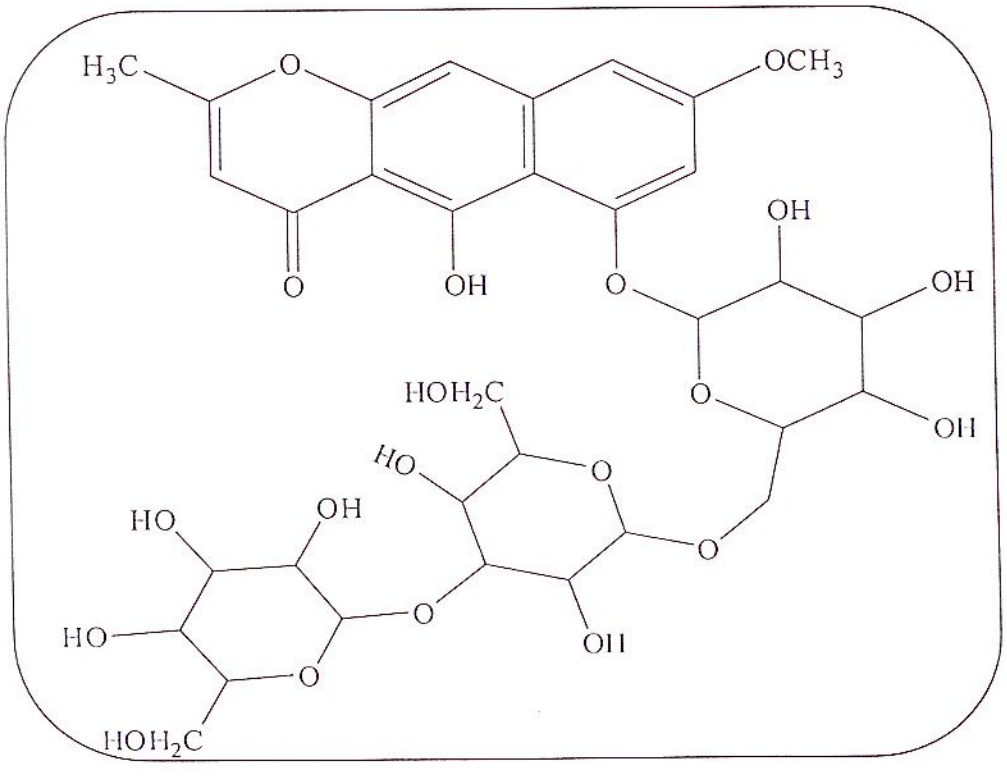
4-(trans)-Acetyl-3,6,8-trihydroxy-3-methyldihydronaphthalenone **(23)**[121]



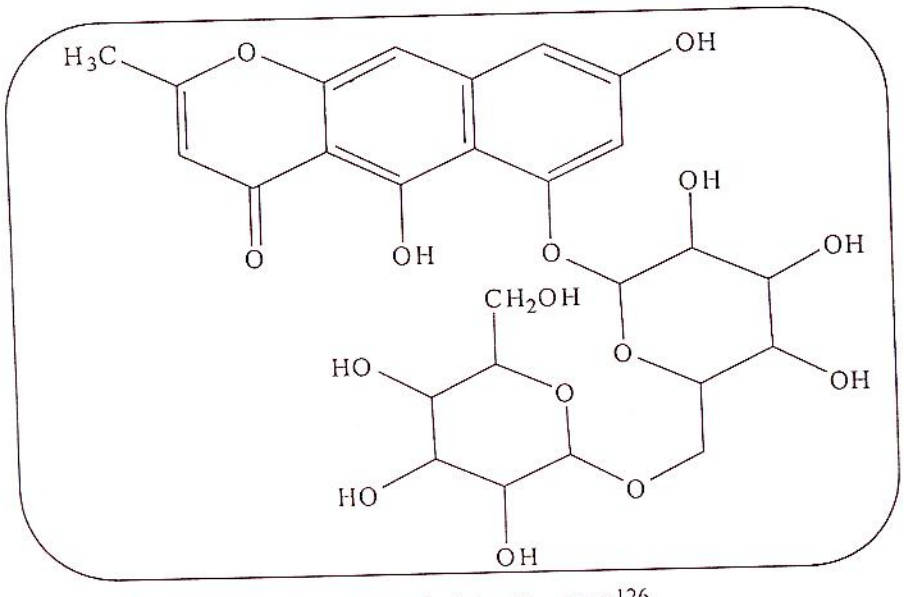
Cassiaoccidentalin A **(24)**[127]



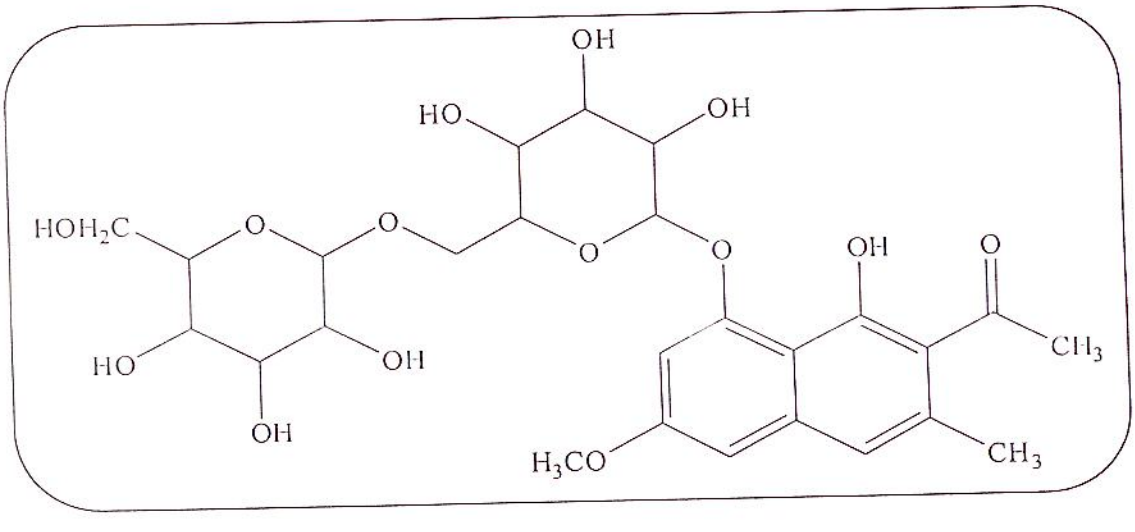
Cassiaoccidentalin B **(25)**[127]



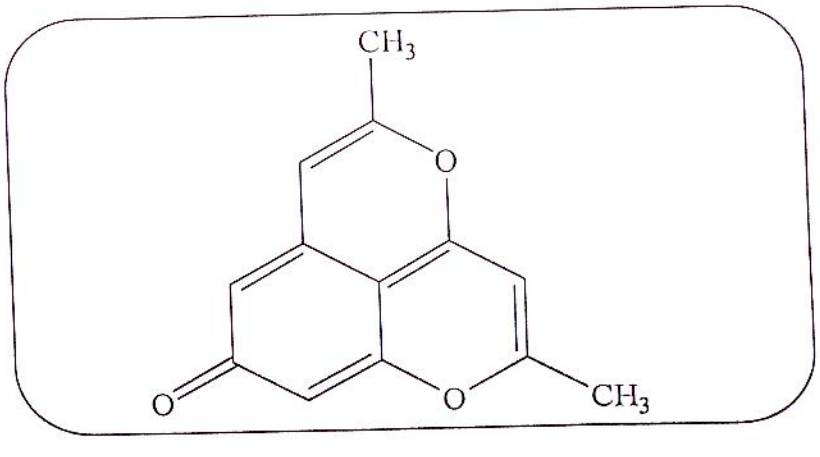
Rubrofusarin triglucoside **(26)**[126]



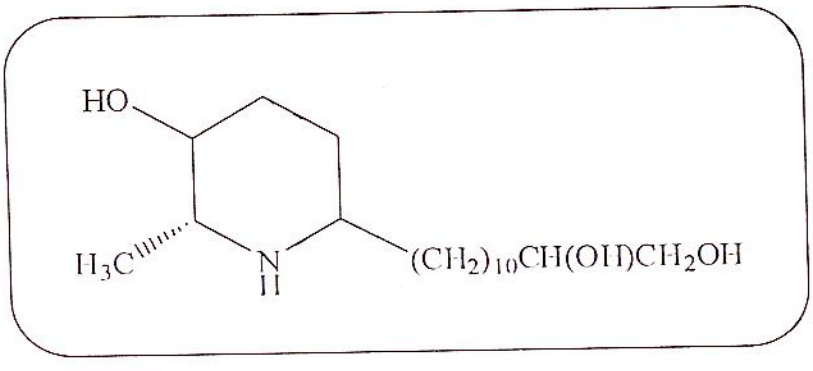
Nor-rubrofusarin gentiobioside **(27)**[126]



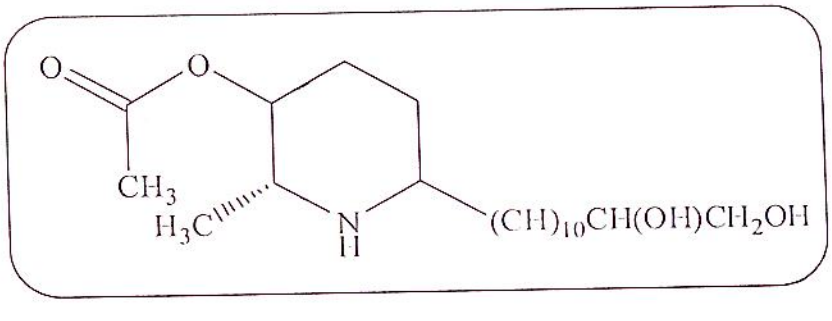
Torachrysone gentiobioside **(28)**[126]



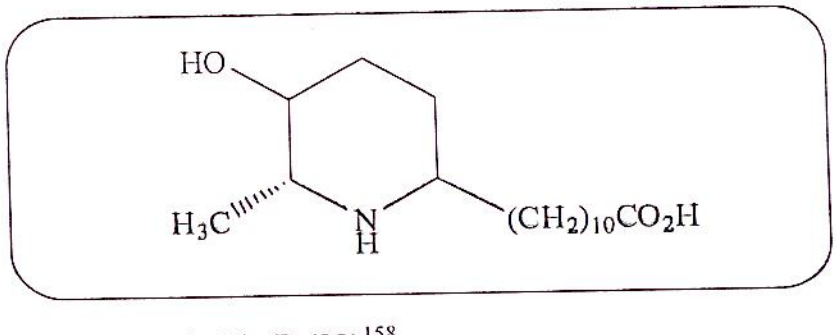
Anhydrobarakol **(29)**[132]



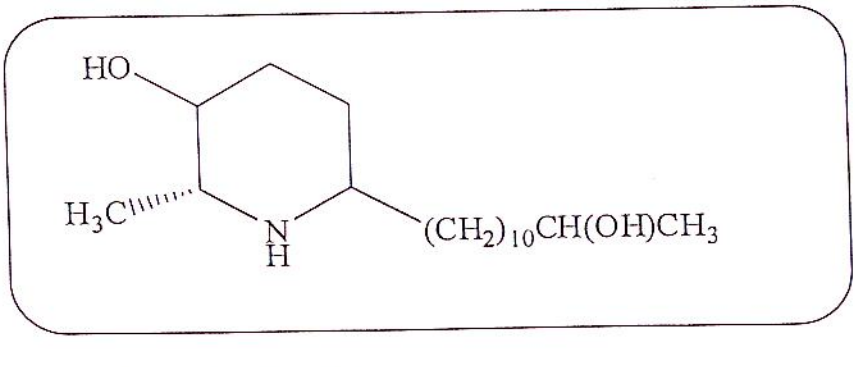
Leptophyllin A **(30)**[158]



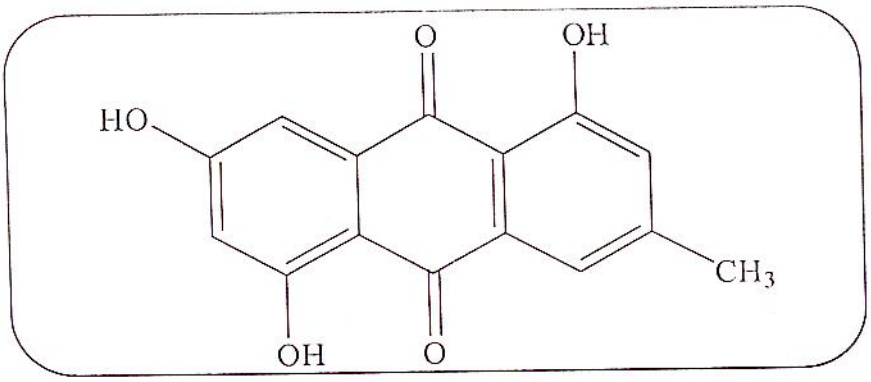
Acetylleptophyllin A **(31)**[158]



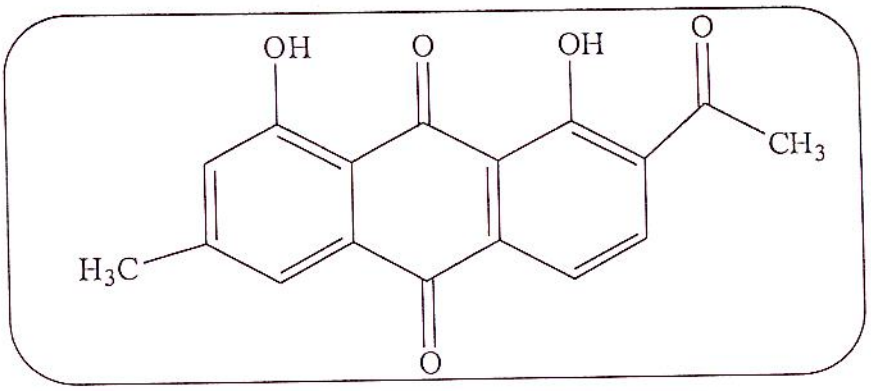
Leptophyllin B **(32)**[158]



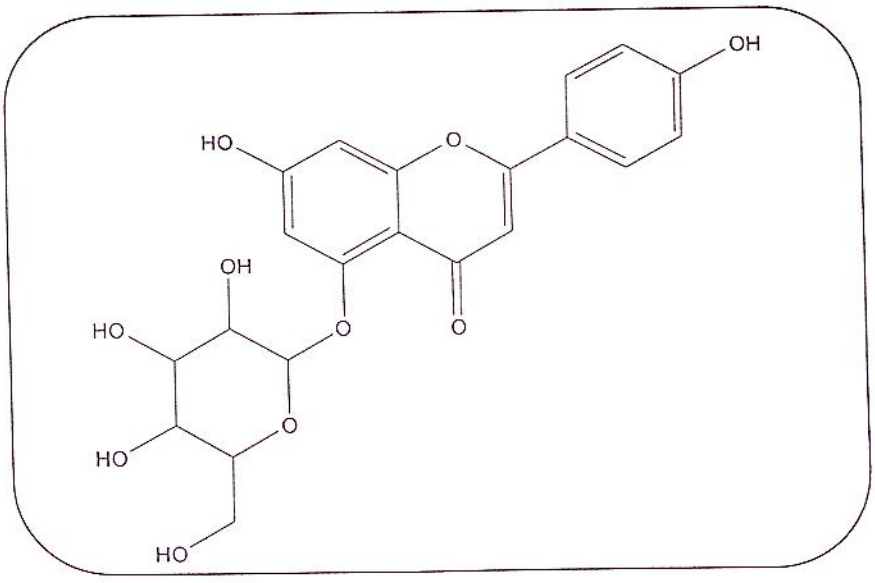
Canavaline **(33)**[158]



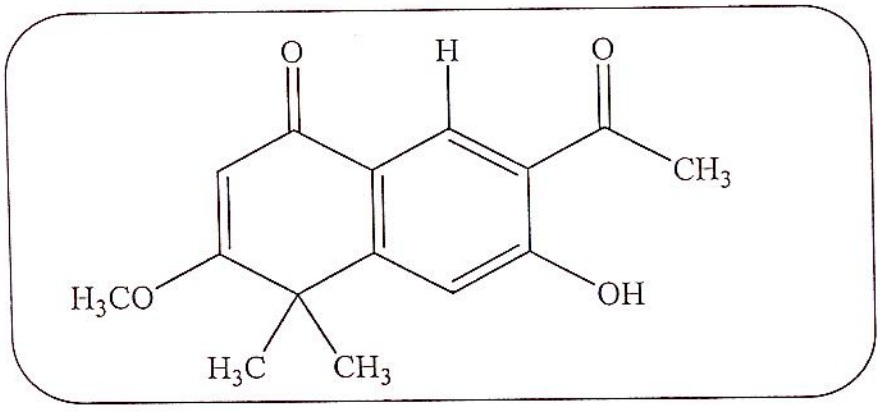
1,5,7-Trihydroxy-3-methylanthraquinone **(34)**[183]



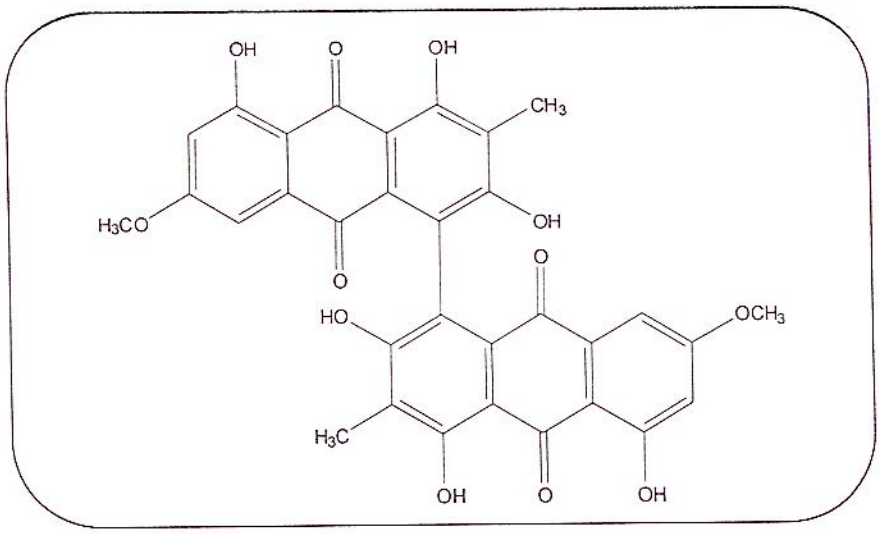
7-Acetylchrysophanol **(35)**[201]



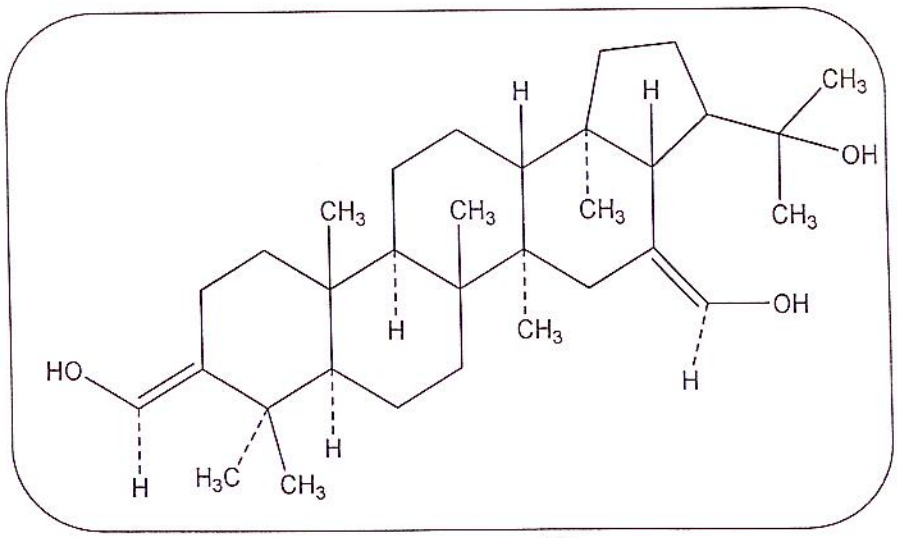
5,4′-Dihydroxyflavone-5-*O*-*β*-*D*-galactopyranoside **(36)**[203]



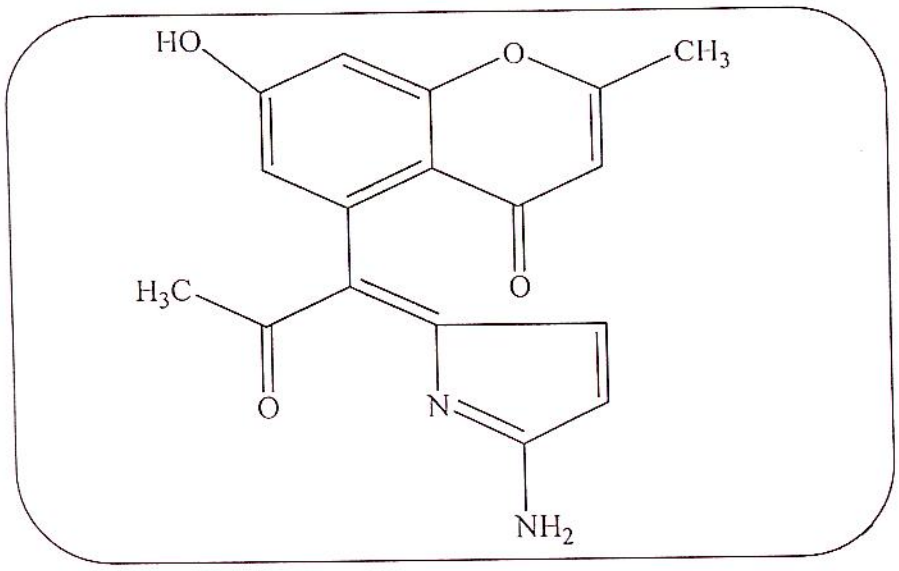
7-Acetyl-6,8-dihydroxy-3-methoxy-4,4-dimethyl-1(4H)-naphthalenone **(37)**[208]



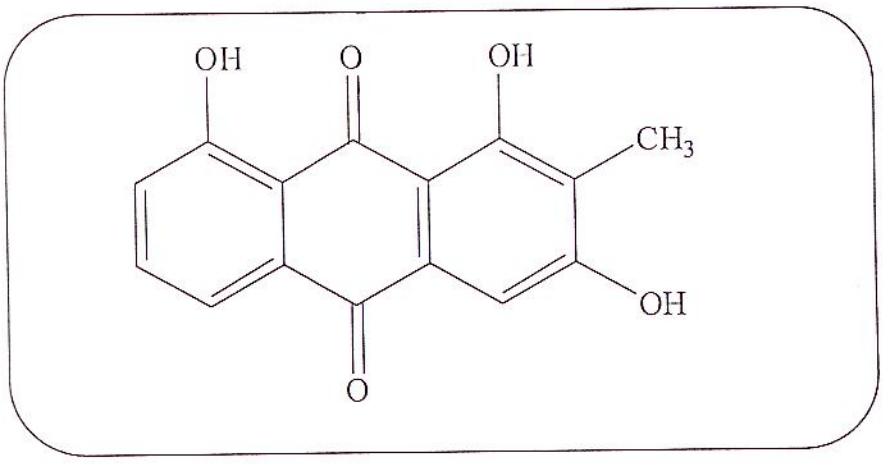
4,4′-bis(1,3,8-Trihydroxy-6-methoxy-2-methylanthraquinone) **(38)**[224]



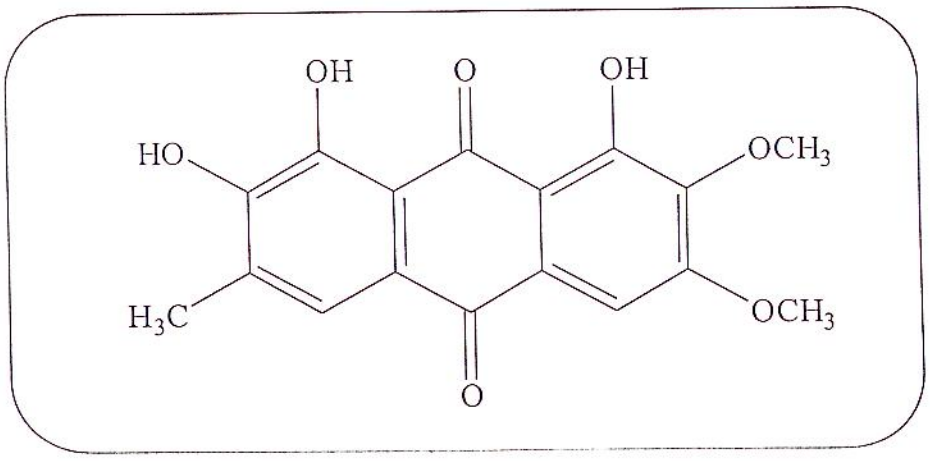
3β,16β,22-Trihydroxyisohopane **(39)**[224]



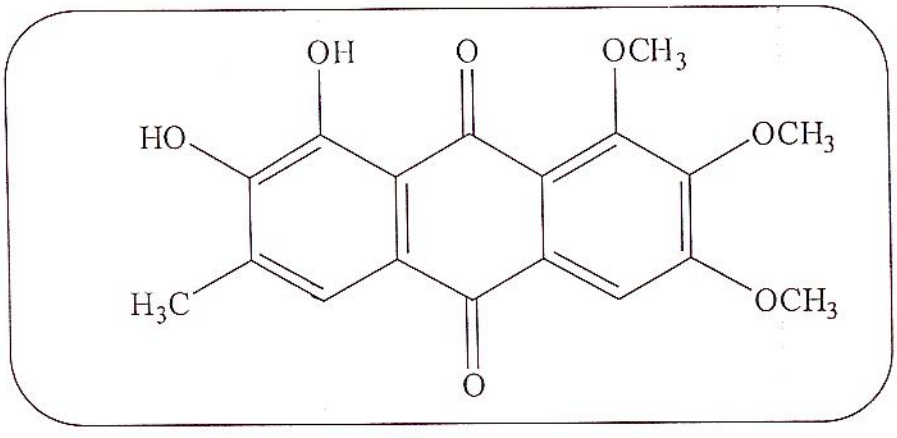
Cassiadinine **(40)**[225]



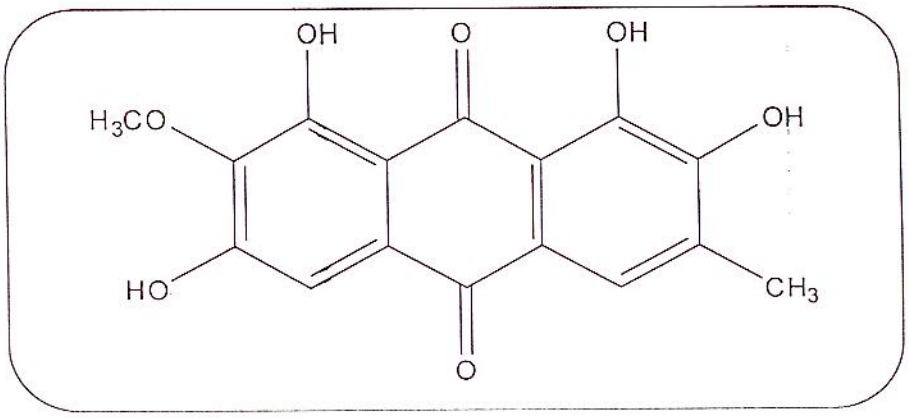
Roxburghinol **(41)**[234]



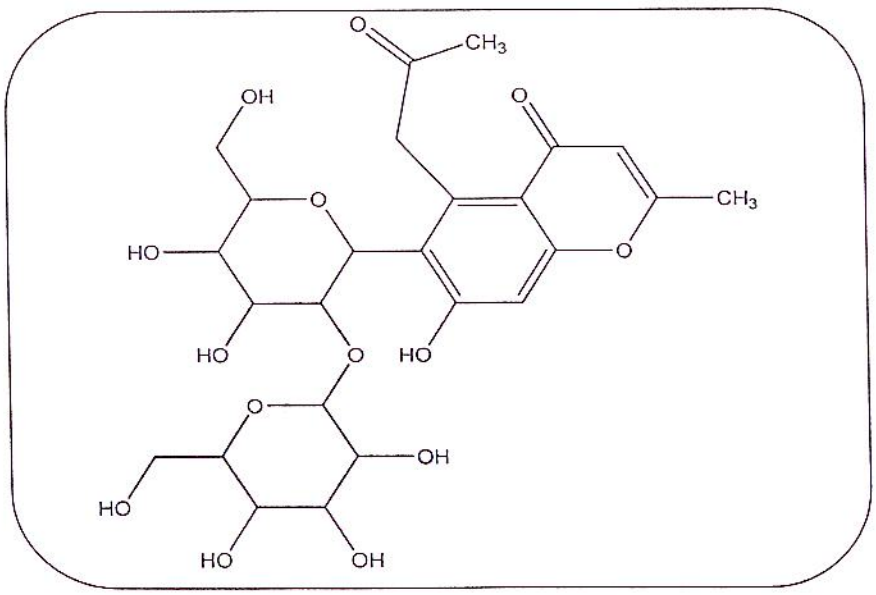
1-Desmethylobtusin **(42)**[236]



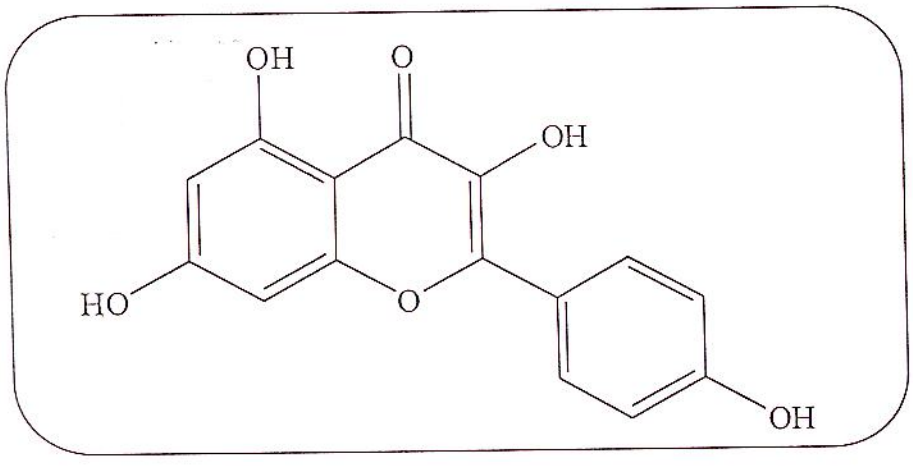
1-Desmethylchryso-obtusin (**43**)[236]



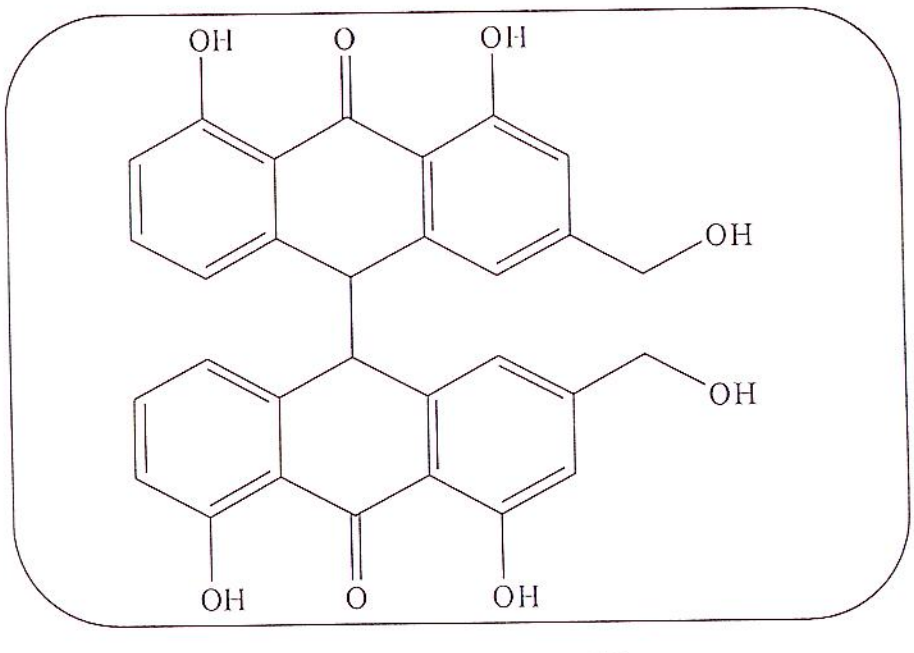
1-Desmethylaurantio-btusin (**44**)[236]



5-Acetonyl-6-glucosyl-7-hydroxy-2-methyl-1-benzopyran-4-one-2′-*O*-*β*-*D*-glucopyranoside (**45**)[310]



Kaempferol **(46)**[73]



Aloeemodin dianthrone **(47)**[303]

1.7. Classification of *Cassia artemisioides* and *Cassia nemophila*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Fabales

Family: Leguminosae / Fabaceae

Subfamily: Caesalpinioideae

Tribe: *Cassieae*

Subtribe: *Cassiinae*

Genus: *Cassia / Senna*

Species: *Cassia artemisioides* (Gaudich. ex DC) Randell and *Cassia nemophila* (Gaudich. ex DC) Randell.

Synonymes: *Senna artemisioides*, Feathery Cassia, Silver Cassia

Origin: Native to Australia, cultivated in NWFP, Pakistan for the last forty years. Evergreen shrub, fast growing 6-9 feet tall, 3-6 feet wide; having pinnate leaves, 0.7 to 1.8 inches long, 4 to 8 pairs of cylindrical gray-green leaflets, 0.4 to 1.6 inches long; little to moderate water, good drainage. In case of *Cassia nemophila* the leaflets is one pair and are needles like. Propagation is through seeds and also by cuttings

Flowering *Cassia artemisioides*(**Figure1.1-1.4**) and *Cassia nemophila* (**Figure1.5-1.6**) are attractive small to medium shrubs with silvery grey, pinnate foliage and bright yellow flowers appear in spring, summer and autumn. Flat, black pods that contain seeds 6-10 in number. Flowers and pods are attractive features. These two *Cassia* species bears an abundance of lightly scented, yellow flowers, 1.2-1.8 cm wide, in small clusters of 4 to 9 flowers, from January to May.

Fruits: Brown pods 1.8 to 3.4 inches long (4-8 cm), 0.25 to 0.4 inch wide (0.6-1 cm). They detached from the shrub in late summer (June-July).



Fig. 1.1. *Cassia artemisioides*

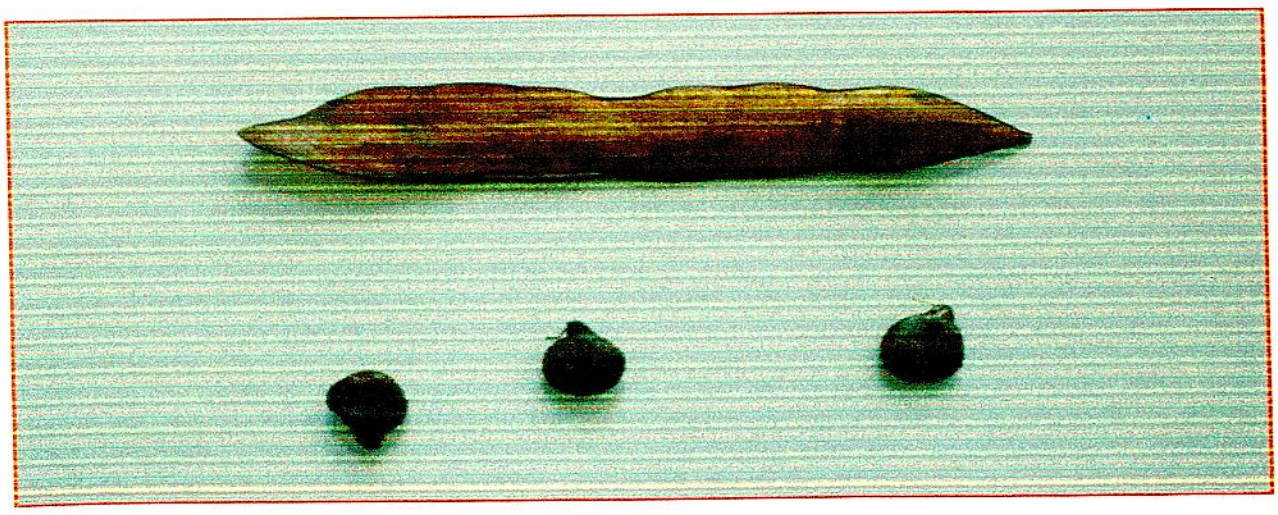


Fig. 1.2. *Cassia artemisioides* Pods and Seeds



Fig.1.3. *Cassia artemisioides* Leaf



Fig. 1.4. *Cassia nemophila*



Fig. 1.5. *Cassia nemophila* Flowers and Leaves



Fig. 1.6. *Cassia nemophila* Pods

Chapter 2

RESULTS AND DISCUSSION (Part A)

2.1. Phytochemicals Isolated from *Cassia* species

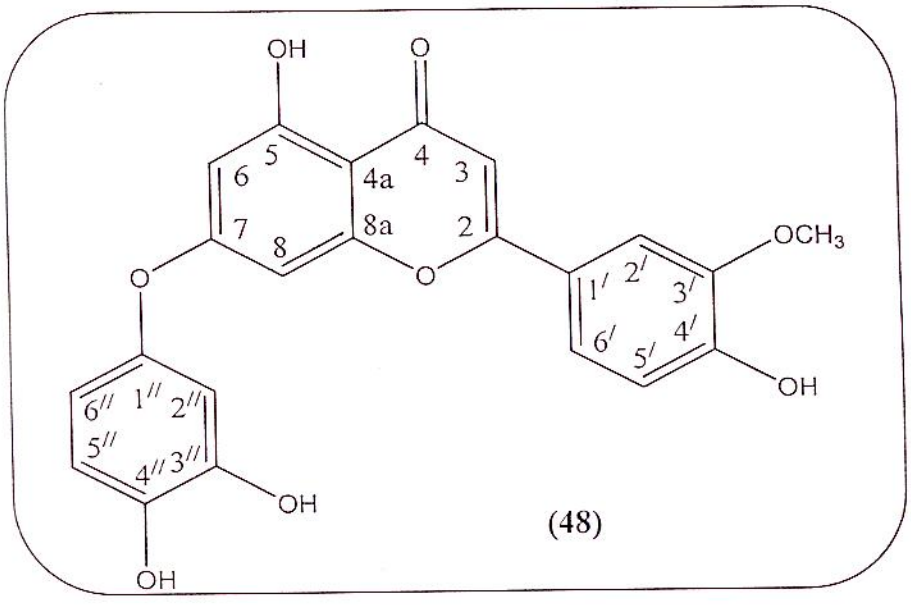
Nine new and thirty eight known compounds have been isolated from *Cassia artemisioides* and *Cassia nemophila* of Pakistani origin. Various experimental techniques and extensive spectroscopic techniques were applied for the structure determination of these compounds. Most of the isolated compounds showed antioxidant activity. The results of these experimental studies are discussed in this chapter. The extraction and isolation procedures have been given in the experimental section (**Chp. 3; Sec.3.4 and 3.5**).

Various parts (flower, stem bark, roots, seeds and pods) of *Cassia artemisioides* were collected from Peshawar. The dried powdered parts were defatted with n-hexane and then extracted with dichloromethane, ethyl acetate and ethanol in increasing order of polarity in a Soxhlet apparatus. After evaporating the solvent, the residue was subjected to column chromatography and preparative TLC. The ethyl acetate fraction of flowers has resulted in two new (**48-49**) and eight known components (**57-64**) while six new (**50-55**) and six known compounds (**65-70**) have been isolated from the stem bark. One new (**56**) and five known compounds (**71-75**) were isolated from the roots. Similarly three known compounds (**76-78**) were isolated from pods and five known compounds (**79-83**) were isolated from the seeds.

The phytochemical investigation of various parts of *Cassia nemophila* has led to the isolation of eleven known compounds (**84-94**), four from the flowers, four from the stem bark and three from the roots. As for as we are aware no phytochemicals or biological investigation has been reported on the two *Cassia* species.

2.1.1. New Compounds Isolated from *Cassia artemisioides* Flowers

2.1.1.1. 7-(3,4-dihydroxyphenoxy)-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-chromen-4-one (48)



Orange red powder (Methanol); mp> 300 °C

The molecular formula was deduced as $C_{22}H_{16}O_8$ from EI mass *m/z* 408 (calcd. 408 for $C_{22}H_{16}O_8$), broad band ^{13}C NMR and DEPT experiments.

The IR absorption at $\tilde{\nu}$ 3005 cm^{-1} for CH st. aromatic and at $\tilde{\nu}$ 2970 cm^{-1} for CH st. saturated. The absorption at $\tilde{\nu}$ 1670 cm^{-1} for C=O stretching of a flavones skeleton, $\tilde{\nu}$ 1601 and 1590 cm^{-1} for C=C st. aromatic.

The ^{13}C NMR specrum showed twenty two carbons in the broad band spectrum including; one methoxy, nine methine and twelve quaternary carbons. The signal at δ 182.17 was assigned to carbonyl carbon of the flavone skeleton. The oxygen deshielded signals at δ 165.29, 164.11 and 161.96 were assigned to C-7, C-2 and C-5 respectively. The signal at δ 161.79 was attributed to C-2″ attached to the flavone skeleton at position 7 through oxygen linkage. The signal at δ 157.92 was assigned to C-8a and the signals at δ 151.38 was assigned to C-4′ and C-4″. The signal at δ 148.60 represents two overlapped signals

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attributed to C-3′ and C-3″. The signals at δ 128.96, 121.68 and 120.88 were assigned to C-6″, C-1′ and C-6′ respectively.

Table 2.1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in DMSO-d6 of 7-(3,4-dihydroxyphenoxy)-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-chromen-4-one **(48)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)*	^{1}H NMR (δ) ($_{HH}$ Hz)**
2	164.11	C	-	
3	103.68	CH	6.60, s	6.71, s
4	182.17	C	-	-
4a	104.01	C	-	-
5	161.96	C	-	12.92, s (OH)
6	99.50	CH	6.17 (d, J = 1.86)	6.13 (d, J = 2.1)
7	165.29	C	-	-
8	94.58	CH	6.42 (d, J = 2.1)	6.44 (d, J = 1.8)
8a	157.92	C	-	-
1′	121.68	C	-	-
2′	110.76	CH	7.45 (d, J = 1.86)	7.51 (d, J = 1.86)
3′	148.60	C	-	-
4′	151.38	C	-	3.27, b s (OH)
5′	116.33	CH	6.90 (d, J = 8.22)	6.90 (d, J = 8.7)
6′	120.88	CH	7.48 (dd, J = 8.22, 1.86)	7.52 (d d, J = 8.7, 1.86)
1″	161.79		-	-
2″	103.31	CH	6.56, s	6.83, s
3″	148.60	C	-	3.27, b s (OH)
4″	151.38	C	-	3.27, b s (OH)
5″	116.52	CH	6.89 (d, J = 8.94)	6.89 (d, J = 8.94)
6″	128.96	CH	7.87 (d, J = 8.94)	7.87 (d, J = 8.94)
3′-OCH_3	56.52	CH_3	3.87 (3H, s, OCH_3)	3.85 (s, 3H)

* ^{1}H NMR in CD_3OD; ** ^{1}H NMR in DMSO-d6

The resonances at δ 116.52, 116.33 and 110.76 were attributed to C-5″, C-5′ and C-2′ respectively. The signals at δ 104.01, 103.68 and 103.31 were attributed to C-4a, C-3 and C-2″ respectively. The signals at δ 99.50 and 94.58 are the characteristic signals for 5,7-dihydroxy flavones and were assigned to C-6 and C-8 respectively. The high field signal at δ 56.52 was assigned to the methoxy group. All the assignments were established on the basis of HMQC and HMBC experiments.

The ^{1}H NMR spectrum was carried out in methanol-d4 with missing hydroxy groups due to the deuterium exchange (with CD_3OD). The spectrum was carried out in DMSO-d6 showed down field sharp signal at δ 12.92 for the chelated hydroxy group at C-5. The broad peak at δ 3.27 with integration for three protons was assigned to hydroxy groups at C-4′, C-3″ and C-4‴ respectively. The solvent effect was observed from 0.02 to 0.9 ppm. The one proton doublet at δ 7.87 (1H, d, J = 8.94 Hz) was assigned to H-6″ and the resonance at δ 7.52 (1H, dd, J = 8.7, 1.86 Hz) was assigned to H-6′. The meta-coupled proton doublet at δ 7.51 (d, J = 1.86 Hz) was assigned to H-2′ and the doublet at δ 6.90 (d, J = 8.7 Hz) was assigned to H-5′. The doublet at δ 6.89 (d, J = 8.94 Hz) was assigned to H-5″ and the singlet at δ 6.71 (1H, s) was attributed to H-3. Two meta-coupled signals at δ 6.44 (d, J = 1.8 Hz) and 6.13 (1H, d, J = 2.1 Hz) were assigned to H-8 and H-6 respectively.

COSY Correlation was observed between H-6 and H-8 and a second correlation between H-3 and H-2′. The third correlation was found among H-2′, H-5′ and H-6′. Similarly H-2″, H-5″ and H-6″ correlated to each other.

From these data (IR, Mass, ^{1}H NMR, ^{13}C NMR, HMQC, COSY and HMBC) the structure was elucidated as 7-(3,4-dihydroxyphenoxy)-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-chromen-4-one or 7-(3″,4″-dihydroxyphenoxy)-5,4′-dihydroxy-3′-methoxyflavone. To the best of our knowledge this is a new compound.

The HMBC correlation is represented in **Fig.2.1**

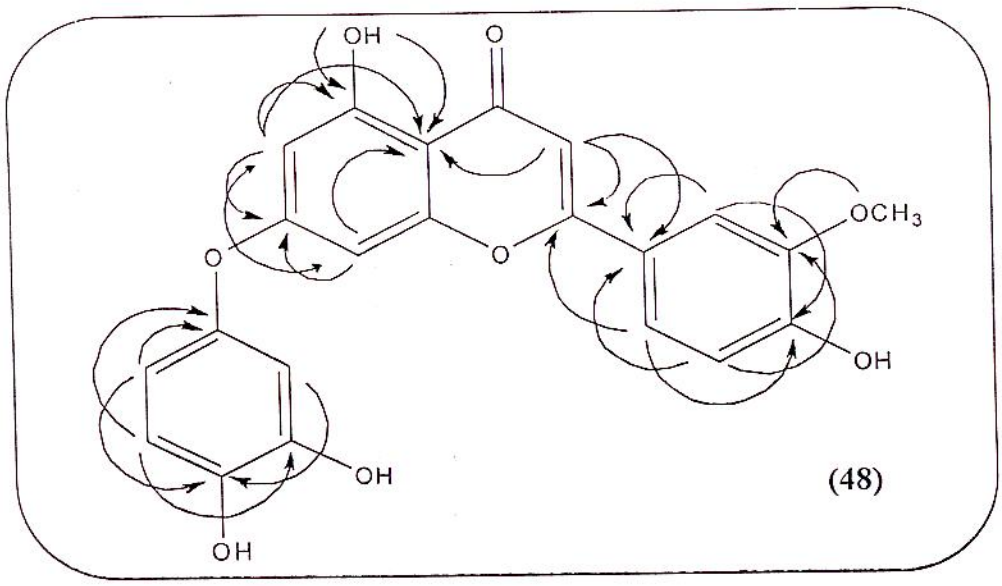
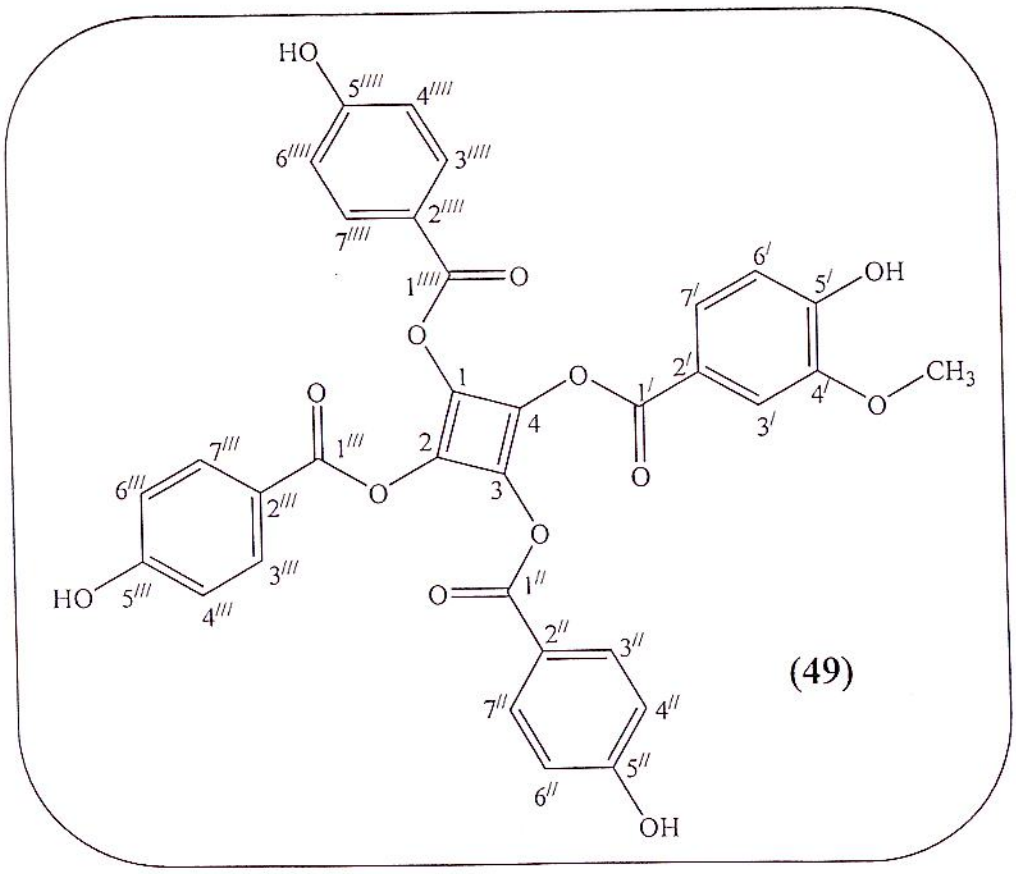


Fig. 2.1. HMBC Correlation of 7-(3,4-dihydroxyphenoxy)-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-chromen-4-one **(48)**

2.1.1.2. 1,2,3-Tri(5-hydroxybenzoyloxy)-4-(5-hydroxy-4-methoxy bezoyloxy)cyclobuta-1,3-diene (49)



Grayish needles (Acetone); mp 282-283 °C

Molecular formula $C_{33}H_{22}O_{13}$, deduced from EI mass spectra *m/z* 626 (M^+) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **49** confirmed the presence of thirty three carbon atoms in the molecule; having twenty three degree of unsaturation. One methoxy, 15 methine, 9 quaternary carbons and 4 carbonyl carbons were found from DEPT experiments. Total assignments were established according to HMQC and HMBC experiments. (**Table 2.2**)

The carbons signal resonating at δ 167.89 were assigned to C-1$^{/}$, C-1$^{//}$ and C-1$^{///}$. The oxygen deshielded aromatic signal at δ 161.97 was assigned to C-5$^{/}$, C-5$^{//}$ and C-5$^{///}$ and the signal at δ 132.08 was assigned to C-4$^{/}$, C-4$^{//}$ and C-4$^{///}$. The signal at δ 132.08 was also assigned to C-6$^{/}$, C-6$^{//}$ and C-6$^{///}$, the four sets of C-4 and C-6 having the same chemical environment and have the same chemical shifts. The signal at δ 121.58 was attributed to C-2$^{/}$, C-2$^{//}$ and C-3$^{///}$. The signal at δ 115.26 was assigned to C-3$^{/}$, C-3$^{//}$ and C-3$^{///}$ and due to the same chemical shift this signal was also assigned to C-7$^{/}$, C-7$^{//}$ and C-7$^{///}$. The aromatic signals resonating at δ 151.41 and 147.25 were assigned to C-5 and C-4 respectively. The resonances at δ 124.26 and 121.82 were assigned to C-7 and C-2 respectively. The signals at δ 114.80 and 112.63 were attributed to C-6 and C-3 respectively. The signal at δ 121.58 was for the four quaternary carbons of cyclobuta-1,3-diene. The high field signal at δ 55.49 was assigned to OCH_3.

The ^1H NMR spectrum of the compound **49** showed signals at δ 3.85 (s, 3H) for a methoxy group, one broad peak at δ 8-9 was assigned to OH-5. Two ortho-coupled aromatic protons at δ 6.90 (1H, d, J = 8.22 Hz, H-6) and 7.58 (1H, d d, J = 8.22, 1.74 Hz, H-7) and one meta-coupled aromatic proton at δ 7.55 (1H, d, J = 1.74 Hz, H-3). Twelve ortho-coupled aromatic proton signals at δ 6.91 (6H, d, J = 8.22 Hz, H-4$^{/}$, H-4$^{//}$, H-4$^{///}$, H-6$^{/}$, H-6$^{//}$ and H-6$^{///}$) and at δ 7.91 (6H, d, J = 8.22 Hz, H-3$^{/}$, H-3$^{//}$, H-3$^{///}$, H-7$^{/}$, H-7$^{//}$ and H-7$^{///}$).

Table 2.2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data and HMBC Correlations of 1,2,3-tri(5-hydroxybenzoyloxy)-4-(5-hydroxy-4-methoxybezoyloxy)cyclobuta-1,3-diene. **(49)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1-4	121.58	C	-	-
$1^{/}$	167.89	C	-	-
$2^{/}$	121.82	C	-	-
$3^{/}$	112.63	CH	7.55 (d, J = 1.74)	C-$1^{/}$, C-$2^{/}$, C-$6^{/}$
$4^{/}$	147.25	C	-	
$5^{/}$	151.47	C	OH	
$6^{/}$	114.80	CH	6.90 (d, J = 8.22)	C-$1^{/}$, C-$2^{/}$, C-$4^{/}$
$7^{/}$	124.26	CH	7.58 (dd, J = 8.22, 1.74)	C-$1^{/}$, C-$3^{/}$, C-$5^{/}$
$4^{/}$-OCH_3	55.49	CH_3	3.85 (3H, s, OCH_3)	C-$4^{/}$
$1^{//}$	167.89	C	-	-
$2^{//}$	121.58	C	-	-
$3^{//}$	115.26	CH	6.91 (d, J = 6.86)	C-$1^{//}$, C-$2^{//}$, C-$5^{//}$
$4^{//}$	132.08	CH	7.91 (d, J = 6.86)	C-$2^{//}$, C-$5^{//}$
$5^{//}$	161.97	C	-	
$6^{//}$	132.08	CH	7.91 (d, J = 6.86)	C-$2^{//}$, C-$5^{//}$
$7^{//}$	115.26	CH	6.91 (d, J = 6.86)	C-$1^{//}$, C-$2^{//}$, C-$5^{//}$
$1^{///}$	167.89	C	-	
$2^{///}$	121.58	C	-	
$3^{///}$	115.26	CH	6.91 (d, J = 6.86)	C-$1^{///}$, C-$2^{///}$, C-$5^{///}$
$4^{///}$	132.08	CH	7.91 (d, J = 6.86)	C-$2^{///}$, C-$5^{///}$
$5^{///}$	161.97	C	-	
$6^{///}$	132.08	C	7.91 (d, J = 6.86)	C-$2^{///}$, C-$5^{///}$
$7^{///}$	115.26	CH	6.91 (d, J = 6.86)	C-$1^{///}$, C-$2^{///}$, C-$5^{///}$
$1^{////}$	167.89	C	-	
$2^{////}$	121.58	C	-	
$3^{////}$	115.26	CH	6.91 (d, J = 6.86)	C-$1^{////}$, C-$2^{////}$, C-$5^{////}$
$4^{////}$	132.08	CH	7.91 (d, J = 6.86)	C-$2^{////}$, C-$5^{////}$
$5^{////}$	161.97	C	-	
$6^{////}$	132.08	CH	7.91 (d, J = 6.86)	C-$2^{////}$, C-$5^{////}$
$7^{////}$	115.26	CH	6.91 (d, J = 6.86)	C-$1^{////}$, C-$2^{////}$, C-$5^{////}$

In the HMBC spectrum, the methoxy protons resonance at δ 3.85 correlated to δ 147.25 (C-4). The signal at δ 6.90 (H-6) correlated to δ 121.82 (C-2). The proton at δ 7.55 (H-3) showed correlation to the signals at δ 167.89 (C-1′) and 151.47 (C-5′), the signal at δ 7.58 (H-7′) correlated with the signals at δ 167.89 (C-1′) and 151.47 (C-5′). The signal at δ 6.91 (H-3″ to H-3‴″ and H-7″ to H-7‴″) showed correlation with carbonyl signal at δ 167.89 (C-1′) and 161.57 (C-5′). The protons at δ 7.91 (H-4″ to H-4‴″ and H-6″ to H-6‴″) correlated with the signals at δ 121.58 (C-2′) and 161.57 (C-5′). These data suggested the presence of one methoxy group at C-3′ and all the hydroxy groups located at C-5′-C5‴″ Therefore the compound **49** was identified as 1,2,3-tri(5-hydroxybenzoyloxy)-4-(5-hydroxy-4-methoxybezoyloxy)cyclobuta-1,3-diene. To the best of our knowledge this is a new compound isolated from the flowers of *Cassia artemisioides*.

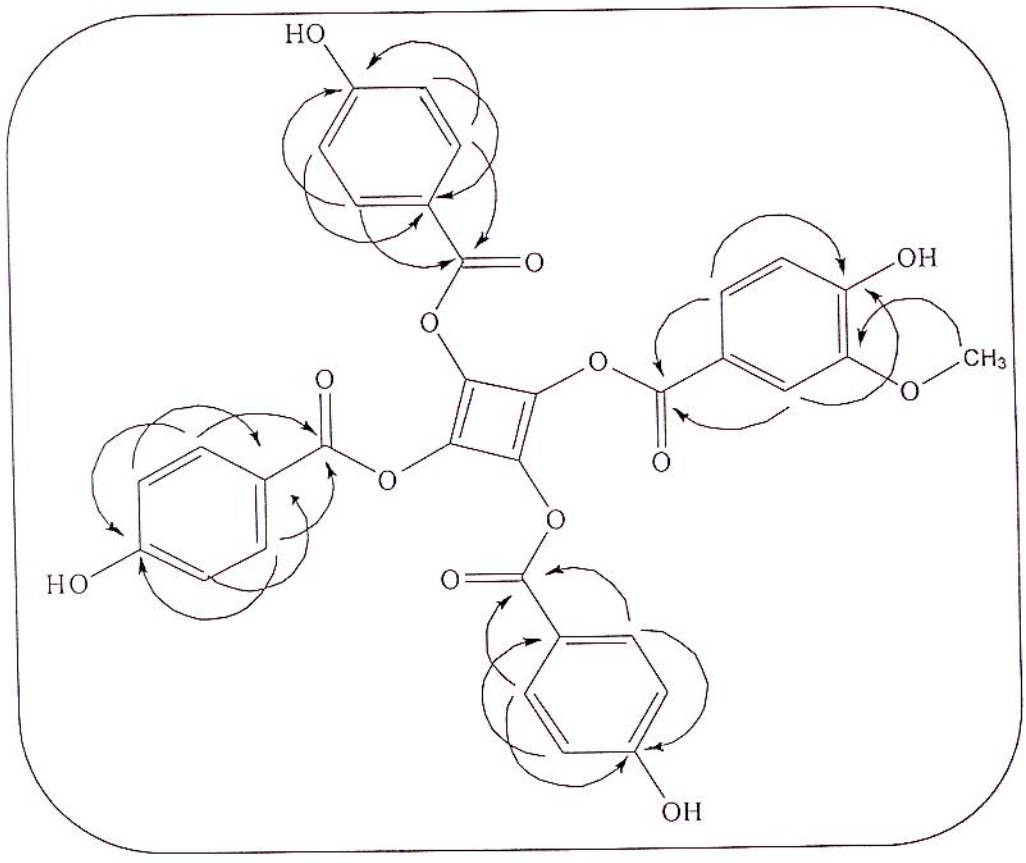
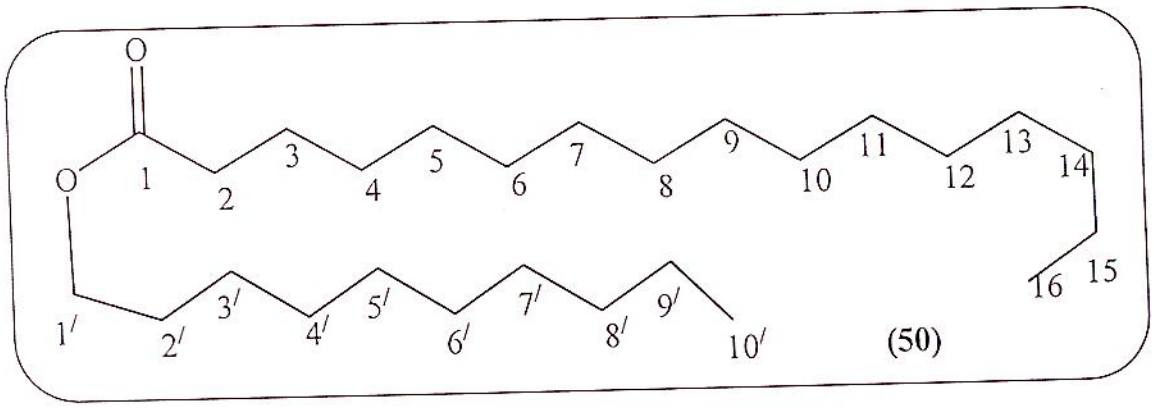


Fig. 2.2. HMBC Correltion of 1,2,3-tri(5-hydroxybenzoyloxy)-4-(5-hydroxy-4-methoxybezoyloxy)cyclobuta-1,3-diene **(49)**

2.1.2. New Compounds from the Stem Bark of *Cassia artemisioides*

2.1.2.1. Decyl palmitate (50)



(50)

White powder; mp 74-76 °C

Molecular formula $C_{26}H_{52}O_2$, deduced through mass spectrum *m/z* 396 (calcd. 396.69 for $C_{26}H_{52}O_2$).

The IR absorption at $\tilde{\nu}$ 1706 cm^{-1} for C=O St. showed the presence an ester.

Table 2.3. ^1H (400 MHz) and ^{13}C (100 MHz) NMR in $CDCl_3$ of decyl palmitate **(50)**

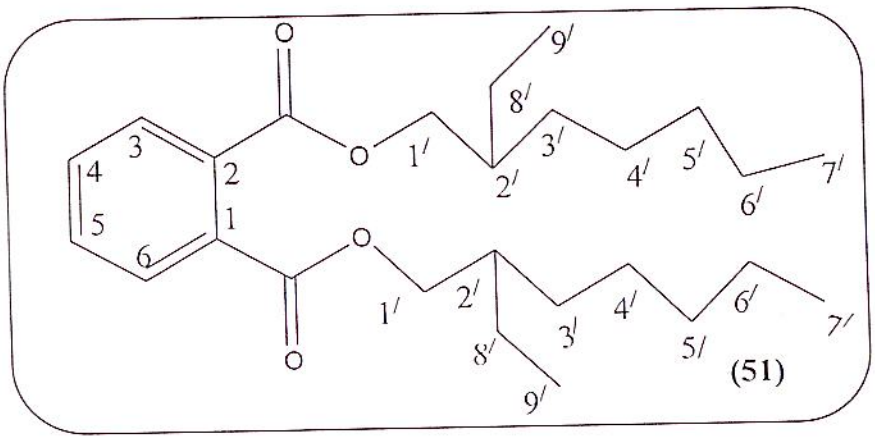
C. No.	^{13}C NMR(δ)	Multiplicity	^1H NMR (δ) (J_{HH} Hz)
1	173.98	C	
1′	64.39	CH_2	4.03 (2 H, t, J = 6.6)
2	34.43	CH_2	2.26 (2 H, t, J = 6.94)
3	31.92	CH_2	1.59, m (2 H)
4-16 and 2′-9′	29.68- 22.68	CH_2	1.23, m (40 H)
16, 10′	14.09	CH_3	0.86 (6 H, t, 6.2)

In the ^1H NMR, a triplet at δ 4.03 was due to CH_2-$\underline{CH_2}$-O (2 H, t, J = 6.6 Hz), a triplet at δ 2.26 (2 H, t, J = 6.94) was a characteristic for a CH_2-$\underline{CH_2}$-C=O group and a multiplet at δ 1.59 was a characteristic for a $CH_2\underline{CH_2}CH_2CO$. The chemical shifts at δ 1.23 (42H) and a six protons triplet at δ 0.86 (6H, t, J = 6.2 Hz) for the terminal CH_3 groups.

The ^{13}C NMR confirmed it as a long chain ester. The signal at δ 173.98 was assigned to carbonyl carbon of the ester (C-1) and the signal at δ 64.39 was assigned to CH_2 (C-1′). The resonances at δ 34.43 and 31.92 were assigned to C-2 and C-2′ respectively. The signals from δ 29.69 to 22.68 ppm were assigned to C-3 and C-3′ to C-15 and C-9′ respectively. The signal at δ 14.08 was assigned to two the terminal CH_3 (C-16 and C-10′).

The position of carbonyl group was established from the mass fragment ion at 257.0 due to the cleavage of ester bond. From the spectroscopic and physical data the structure was proposed as decyl palmitate. To the best of my knowledge this is a new compound.

2.1.2.2. Di-2-ethylheptyl phthalate (51)



(51)

Red colour thick residue (Acetone); melting point 274-276 °C

Molecular formula $C_{26}H_{42}O_4$, deduced from EI mass spectrum *m/z* 418 (calcd. 418.0 for $C_{26}H_{42}O_4$; M^+) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **51** showed the presence of thirteen signals for the carbon atoms in the molecule; due to symmetry in the molecule each peak was consider double in order to arrive at the molecular mass. The broad band decoupled spectrum confirmed the presence of four methyl, six methine, twelve methylene and four quaternary carbons according to DEPT experiments. Total assignments were established according to HMQC, HMBC and DQF COSY experiments (**Table 2.4**).

Table 2.4. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data and HMBC Correlations of di-2-ethylheptyl phthalate **(51)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
	168.00	2C	-	C-1′, C-3, C-6,
1	132.25	C	-	
2	132.25	C	-	
3	128.52	CH	7.69 (d d, J = 5.7, 3.24, H-3, H-6)	COO
4	131.06	CH	7.52 (d d, J = 5.7, 3.24, H-4, H-5)	C-2, C-3
5	131.06	CH	7.52 (d d, J = 5.7, 3.24, H-4, H-5)	C-1, C-3
6	128.52	CH	7.69 (d d, J = 5.7, 3.24, H-3, H-6)	COO
1′	67.75	2 CH_2	4.20, 4.21 (4H, dd, J = 6.18, 6.18)	COO
2′	38.84	2 CH	1.65 (2H, quintet, J = 6.18)	
3′	30.28	2 CH_2	1.41, 1.42 (dt, J = 5.8, 6.18)	
4′	29.79	2CH_2	1.38 (4H, m)	
5′	28.80	2 CH_2	1.36 (4H, m)	
6′	22.69	2 CH_2	1.32 (4H, m)	
7′	13.05	2 CH_3	0.85 (6H, t, J = 6.9)	C-4′
8′	23.61	2 CH_2	1.21, 1.35 (4H, m)	
9′	10.05	2 CH_3	0.90 (6H, t, J = 7.8)	C-2′

The carbon resonating at δ 167.87 was assigned to OC=O where as a signal at δ 132.51 was assigned to C-1 and C-2. The aromatic signals at δ 130.99 and 128.8 were assigned to C-4, C-5 and C-3, C-6 respectively. The resonance at δ 68.24 was assigned to C-1′ next to the ester group. The signals at δ 38.79, 30.42 and 29.79 were assigned to C-2′, C-3′ and C-

4′ respectively. The signals at δ 29.0, 23.81 and 23.08 were attributed to C-5′, C-6′ and C-8′. The high field signals at δ 14.16 and 11.05 were assigned to C-7′ and C-9′ respectively. All the ^{13}C NMR data was in close resemblance with di-2-ethylheptyl phthalate [123] except that it contain two additional CH_2 groups (CH_2-4′).

The ^{1}H NMR spectrum of **51** showed signals at δ 0.85 (t, J = 6.9 Hz, 7′-CH_3) for a methyl group and a second methyl group at δ 0.90 (t, J = 7.8 Hz, 9′-CH_3). The signals at δ 1.21-1.35 (m, H-7′) was assigned to H-7′, which gives multiplet being attached to an asymmetric center on one side and a methyl group on the other. The resonances at δ 1.32 (m, H-5′) and 1.36 (m, H-4′) were attributed to H-5′ and H-4′ respectively. The signals at δ 1.41 (dt, J = 6.18, 5.8 Hz, H-3′) assigned to H-3′ and 1.65 (quintet, J = 6.18 Hz H-2′) was assigned to H-2′ respectively. The down field chemical shift at δ 4.19, 4.20 (dd, J = 6.18, 6.18 Hz, H-1′) for H-1′ attached to an ester group. Two signals for ortho-coupled aromatic protons at δ 7.69 (d d, J = 5.7, 3.24 Hz, H-3, H-6) and 7.52 (d d, J = 5.7, 3.24 Hz, H-4, H-5).

In the HMBC spectrum, the ester carbonyl signal at δ 167.87 correlated with signals at δ 7.69 (H-3, H-6) and 4.19, 4.20 (H-1′). The signal at δ 132.51 (C-1 and C-2) correlated with the signal at δ 7.52 (H-4, H-5). The signal at δ 14.16 (C-9′) showed correlation with signal at δ 1.65 (H-2′) and the resonance at δ 0.90 (H-7′) correlated to δ 29.79 (C-4′). All this data support the structure as di-2-ethylheptyl phthalate. As far as we are aware this is a new compound isolated from the stem bark of *Cassia artemisioides*. The structure was confirmed by using 1D and 2D techniques.

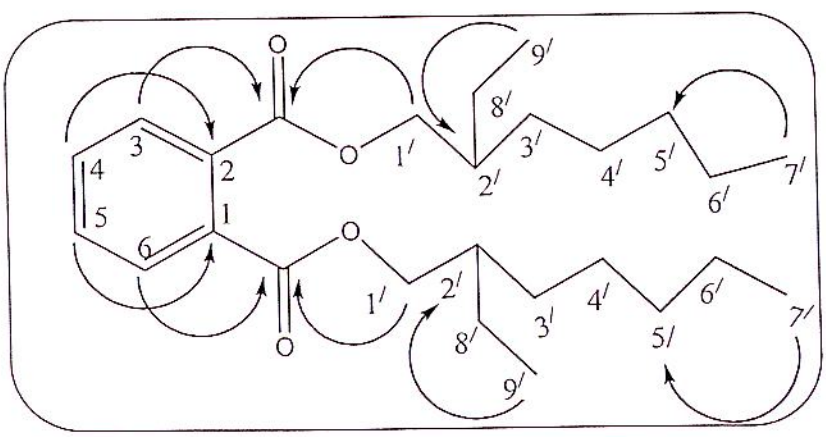
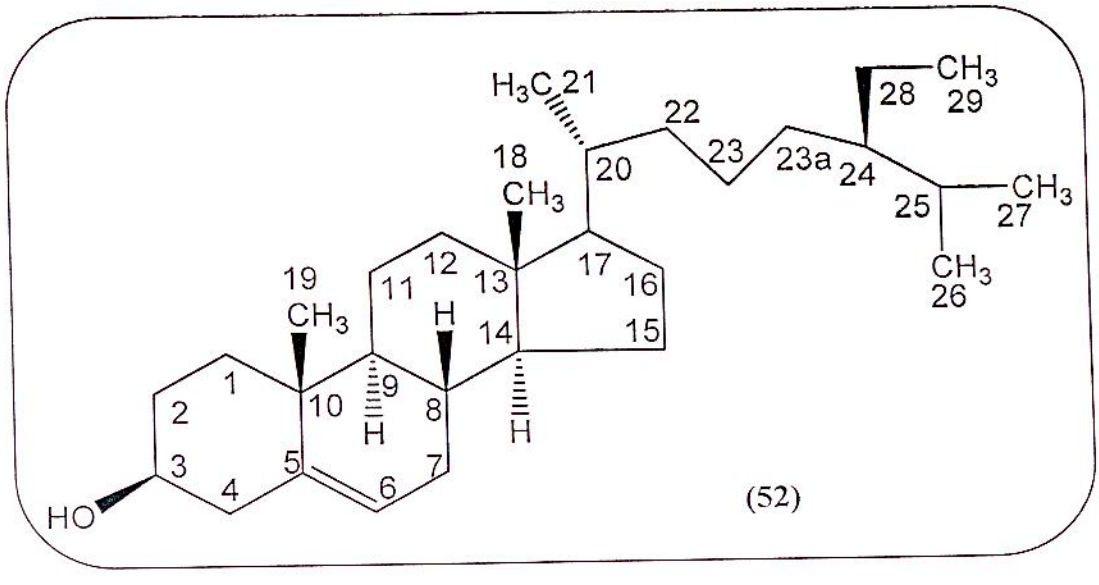


Fig. 2.3. HMBC Correlation of di-2-ethylheptyl phthalate **(51)**

2.1.2.3. 23a-Homostigmast-5-en-3β-ol (17-(6-Ethyl-7-methyloctan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,-11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]-phenanthren-3-ol) (52)



(52)

White amorphous powder (Chloroform), mp 125-127 °C

Molecular formula $C_{30}H_{52}O$, deduced from the EIMS *m/z* 428 (calcd. 428 for $C_{30}H_{52}O$) and ^{13}C NMR.

The IR absorption at $\tilde{\nu}$ 3487.6 cm^{-1} for OH st.; $\tilde{\nu}$ 2918.9 cm^{-1} for CH st satuarated and $\tilde{\nu}$ 1603.1 cm^{-1} for C=C olifinic.

The ^{1}H and ^{13}C NMR spectral data (**Table 2.5**) of the compound **52** was in close agreement to that of the reported for β-sitosterol.[311, 312] The ^{13}C NMR spectra indicated that the main difference between compound **52** and β-sitosterol was the presence of one extra CH_2 group at δ 29.79 (CH_2-23).

The ^{1}H-^{13}C connectivities were determined by the HMQC spectrum, while the long range ^{1}H-^{13}C correlations were assigned through the HMBC technique.

Table 2.5. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of 23a-Homostigmast-5-en-3β-ol **(52)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	37.32	CH_2	1.06 (2H, ddd, J = 3.48, 6.12)	C-3, C-5, C-9
2	31.74	CH_2	1.23, 1.48 (2H, m)	
3	71.90	CH	3.52 (ddt, J = 9.36, 4.88)	
4	42.38	CH_2	2.20 (2H, d, J = 2.1)	C-5, C-6, C-3
			2.33, (2H, m)	
5	140.83	C	-	
6	121.82	CH	5.34, (d, J = 5.22)	C-8, C-10, C-4
7	32.00	CH_2	1.44 (2H, m)	
			1.94, (2H, m)	
8	31.97	CH	1.42, m	
9	50.19	CH	0.84, m	C-5, C-14
10	36.58	C	-	
11	21.16	CH_2	1.49, 1.45 (2H, m)	
12	39.84	CH_2	2.0 (2H, t, J = 9.00)	
			1.98 (2H, d, J = 3.12)	
13	42.39	C	-	
14	56.84	CH	0.97, m	C-9, C-12, C-17
15	24.39	CH_2	1.55 (2H, m)	
16	28.34	CH_2	1.23 (2H, m)	
17	56.11	CH	1.08 (dd, J = 7.8, 3.48)	
18	11.95	CH_3	0.64 (3H, s)	C-12, C-13, C-14, C-17
19	19.49	CH_3	0.99 (3H, s)	C-1, C-5, C-9, C-10
20	36.23	CH	1.34 (m, H-20)	
21	18.86	CH_3	0.91 (3H, d, J = 6.54)	C-20, C-22, C-17
22	34.01	CH_2	1.27 (2H, m)	
			1.55 (2H, m)	

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
23	29.79	CH_2	1.48 (2H, m)	C-20, C-24
23a	26.10	CH_2	1.14 (2H, m)	C-22, C-24, C-25
24	45.89	CH	0.93, m	
25	29.19	CH	1.26, m	
26	19.10	CH_3	0.77 (3H, d, J = 6.8)	C-24, C-25, C-27
27	19.92	CH_3	0.81 (3H, d, J = 5.58)	C-24, C-25, C-26
28	23.13	CH_2	1.25 (2H, m)	
29	12.07	CH_3	0.83 (3H, t, J = 7.5)	C-24, C-28

The ^{13}C NMR showed thirty peaks in broad band spectrum including; 6 methyl, 12 methylene, 9 methine and 3 quaternary carbons. The characteristic peaks at δ 140.83 and 121.82 were assigned to C-5 and C-6 respectively. The signals at δ 71.90, 56.84, 56.11 and 50.19 were attributed to C-3, C-14, C-17 and C-9 respectively. The carbon resonances at δ 45.89, 42.39, 42.38, 39.84 and 37.32 were assigned to C-25, C-13, C-4, C-12 and C-1 respectively. The signal at δ 36.58 was assigned to C-10, 36.23 to C-20 and at δ 34.01 was attributed to C-22. The signals resonances at δ 32.00, 31.97, 31.74, 29.79, 29.19 and 28.34 were assigned to C-7, C-8, C-2, C-23, C-26 and C-16 respectively. While the signals at δ 26.10, 24.39, 23.13 and 21.16 were attributed to C-23a, C-15, C-28 and C-11 respectively. The high field signals at δ 19.92, 19.49, 19.10, 18.86, 12.07 and 11.95 were assigned to C-27, C-19, C-26, C-21, C-29 and C-18 respectively.

The presence of two methyl singlets at δ 0.64 (s, H-18) and 0.99 (s, H-19), three methyl doublets at δ 0.91 (d, J = 6.54 Hz, H-21), 0.81 (d, J = 6.8 Hz, H-28) and 0.77 (d, J = 6.80 Hz, H-27). One methyl triplet at δ 0.83 (t, J = 7.5 Hz, H-30), an olefinic proton broad doublet at δ 5.34 (d, J = 5.22 Hz H-6) and a multiplet at δ 3.51 (H-3) mixed with pyridine peak (^{1}H NMR spectrum in C_5D_5N).

From the IR, mass and NMR spectra the structure of the compound **52** was established as **23a-Homostigmast-5-en-3β-ol (52)** (17-(6-ethyl-7-methyloctan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]-phenanthren-3-ol

(IUPAC). As far as we know it is a new natural product.

The structure was confirmed by using 1D and 2D techniques. In HMBC experiment the resonance at δ 5.29 (H-6) shows correlation to C-4, C-8 and C-10. The resonance at δ 2.20 (H-4) correlated with C-2, C-3, C-5, C-6 and C-10. The signals at δ 1.83 and 1.06 (H-1) correlated with resonates at δ 71.90 (C-3), 140.83 (C-5) and 19.49 (C-19). The signal at δ 0.99 (H-19) correlated to δ 36.58 (C-10), 50.19 (C-9) and 140.83 (C-5). The resonance at δ 0.97 (H-14) showed correlation to C-9, C-12 and C-17 and the signal at δ 0.91 (H-21) correlated to δ 34.01 (C-22), 36.23 (C-20) and 56.11 (C-17). The signal at δ 0.84 (H-9) correlated to C-5 and C-14. The signal at δ 0.81 (H-27) correlated to C-24, C-25, C-26 and the signal at δ 0.77 (H-26) correlated to C-24, C-25 and C-27. The resonance at δ 0.64 (H-18) correlated to resonates at δ 39.84 (C-12), 42.39 (C-13), 56.11 (C-17) and 56.84 (C-14). The DQF COSY correlations are represented in the structure by double arrows.

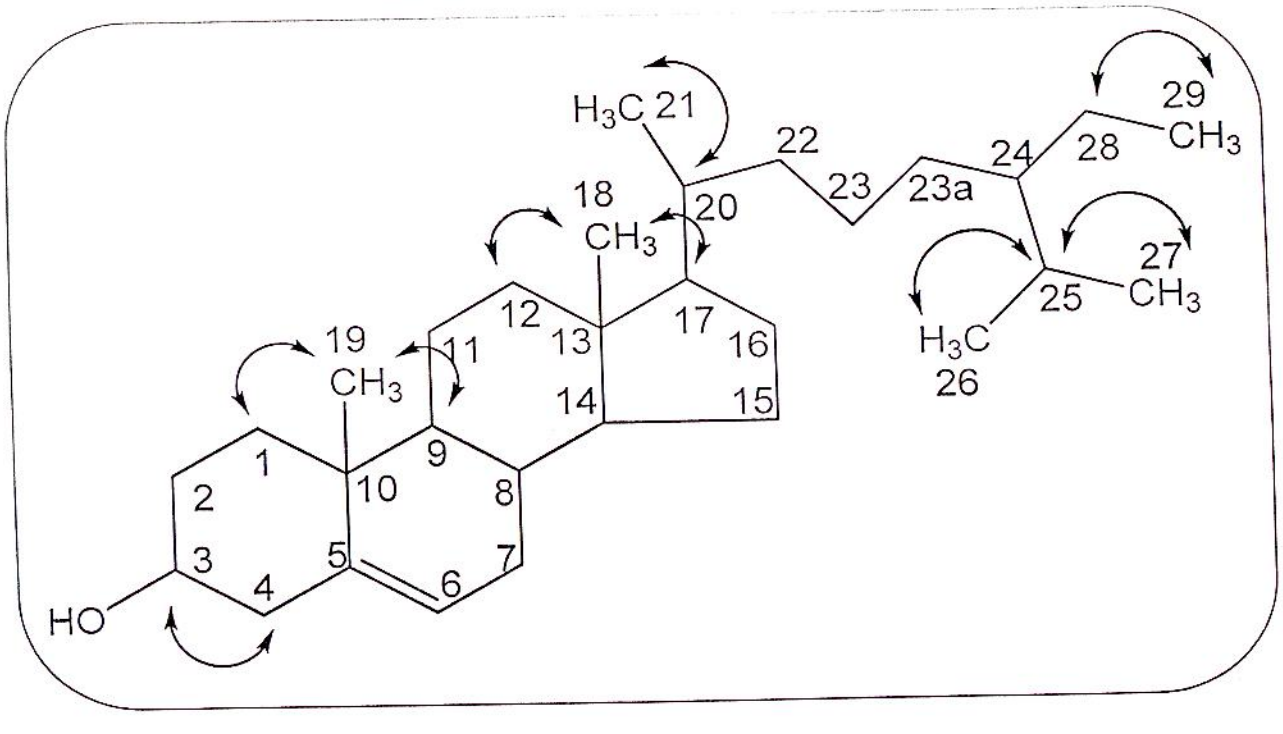
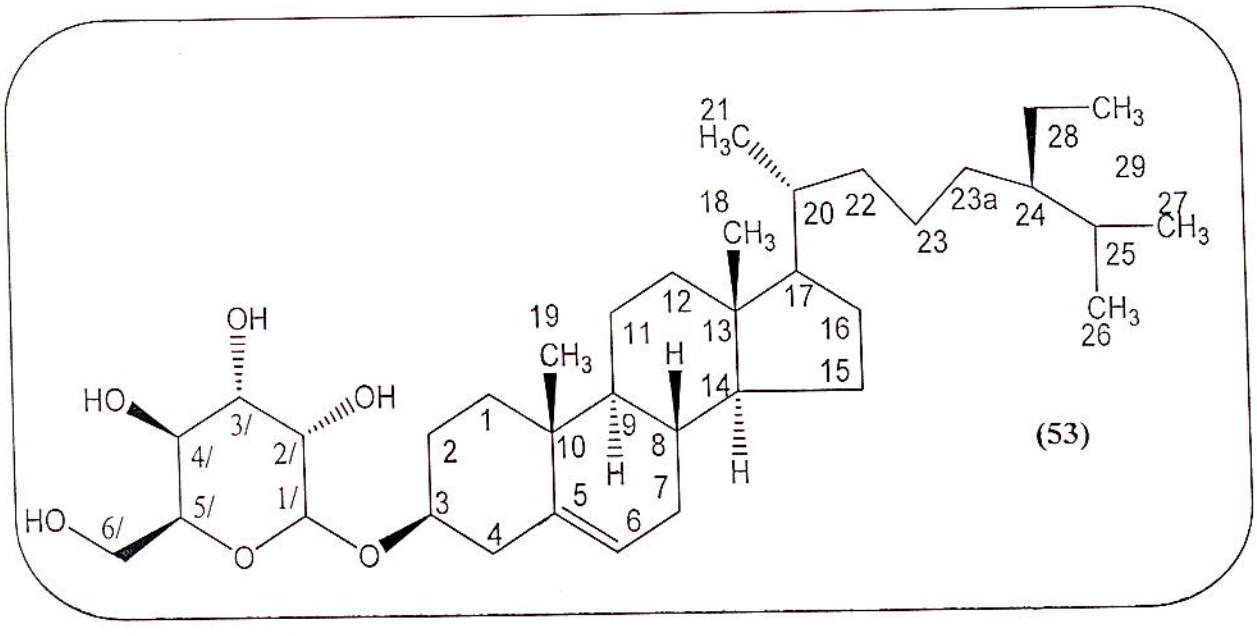


Fig. 2.4. COSY Correlation of 23a-Homostigmast-5-en-3β-ol **(52)**

2.1.2.4. 23a-Homostigmast-5-en-3-*O*-*β*-glucopyranoside (53)



White powder; m. point >290 °C

Molecular formula $C_{36}H_{62}O_6$ was established from ^{13}C NMR and FABMS showed the molecular ion peak at *m/z* 590 (calcd. 590 for $C_{36}H_{62}O_6$)

The IR spectrum of the compound **53** showed absorption at $\tilde{\nu}$ 3450 cm^{-1} for OH st. and at $\tilde{\nu}$ 3025 cm^{-1} for CH st. olefinic.

The ^1H-^{13}C connectivities were determined by the HMQC spectrum, while the long range ^1H-^{13}C correlations was assigned through the HMBC technique. The ^1H and ^{13}C NMR spectral data (**Table 2.6**) of the compound **53** were close to those of 23a-Homostigmast-5-en-3β-ol or 17-(6-ethyl-7-methyloctan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]-phenanthren-3-ol (**52**).

Table 2.6. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in C_5D_5N and HMBC Correlations of 23a-homostigmast-5-en-3-*O*-*β*-glucopyroside **(53)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)
1	37.31	CH_2	1.03, 1.64 (2H, m)
2	30.09	CH_2	1.20, 1.83 (2H, m)
3	78.38	CH	4.26, m
4	39.77	CH_2	2.66 (2H. dd, J = 10.8, 2.4)1.03, m
5	140.73	C	-
6	121.78	CH	5.29 (d, J = 1.74)
7	32.01	CH_2	1.64, 1.89 (2H, m)
8	31.88	CH	1.62,m
9	50.17	CH	0.84, m
10	36.76	C	-
11	21.12	CH_2	1.47, 1.36 (2H, m)
12	39.17	CH_2	2.06, 2.39 (d, J = 11.52)
13	42.31	C	-
14	56.65	CH	1.03, m
15	24.35	CH_2	1.49 (2H, m)
16	28.39	CH_2	1.20 (2H, m)
17	56.01	CH	1.02 (dd, J = 7.8)
18	11.81	CH_3	0.59 (3H, s)
19	19.27	CH_3	0.99 (3H, s)
20	36.23	CH	1.32, m
21	18.85	CH_3	0.91 (3H, d, J = 6.54)
22	34.03	CH_2	1.02, 1.32 (2H, m)
23	29.83	CH_2	1.31, m, 1.47, m
23a	26.19	CH_2	1.17 (2H, m)
24	45.86	CH	0.92, m
25	29.28	CH	1.20, m

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)
26	19.04	CH_3	0.78 (3H, d, J = 6.84)
27	19.83	CH_3	0.81 (3H, d, J = 3.42)
28	23.21	CH_2	1.19, (2H, m)
29	12.00	CH_3	0.82 (3H, t, J = 3.96)
1′	102.40	CH	5.00 *
2′	75.20	CH	4.03 (t, J = 8.04)
3′	78.47	CH	3.90, m
4′	71.51	CH	4.24, m
5′	77.89	CH	3.94, m
6′	62.66	CH_2	4.51 (2H, dd, J = 9.84, 1.86), 4.38 (2H, d, J = 5.16)

* mixed with pyridine peak

The ^{13}C NMR (broad band decoupled and DEPT) showed thirty six peaks in broad band spectrum including; six methyl, thirteen methylene, fourteen methine and three quaternary carbons. For the aglycone part, characteristic peaks at δ 140.73 and 121.78, were assigned to C-5 and C-6 respectively. The signals at δ 78.38, 56.65, 56.01and 50.17 were attributed to C-3, C-14, C-17 and C-9 respectively. The carbon resonates at δ 45.86, 42.31, 39.77, 39.17 and 37.31 were assigned to C-24, C-13, C-4, C-12 and C-1 respectively. The signal at δ 36.76 was assigned to C-10, 36.23 to C-20 and 34.03 to C-22. The signals resonated at δ 32.01, 31.88, 30.09, 29.28 and 28.39 were assigned to C-7, C-8, C-2, C-25 and C-16 respectively. While the signals at δ 26.19, 24.35, 23.21 and 21.12 were attributed to C-23a, C-15, C-28 and C-11 respectively. The high-field signals at δ 19.83, 19.27, 19.04, 18.85, 12.0 and 11.81 were assigned to C-27, C-19, C-26, C-21, C-29 and C-18 respectively.

The ^{1}H NMR indicate the presence of two methyl proton singlets at δ 0.58 (s, H-18) and 0.99 (s, H-19), three methyl doublets at δ 0.91 (d, J = 6.54 Hz, H-21), 0.81, (d, J = 7.0 Hz, H-27) and 0.78 (d, J = 6.8 Hz, H-26). One methyl triplet at δ 0.82 (t, J = 7.32 Hz, H-29), an olefinic proton broad doublet at δ 5.29 (J = 1.74 Hz, H-6) and a multiplet at δ 3.94 (H-3). The large coupling constant of H-3 represents that the *O*-glycoside group at C-3 was *β*-oriented.[313] The anomeric configuration of glucose was found out from J H1-H-2 splitting (J = 8.04 Hz) and the sugar part was also confirmed from the ^{13}C NMR values as glucose.[314] For the aglycone part, all the ^{13}C NMR data was similar to that of the compound **52**.

The proton NMR of the compound **53** in pyridine (C_5D_5N) showed the presence of sugar moiety. The hydroxyl geminal proton of the sugar moiety resonated at δ 5.0 (d, J = 7.8 Hz, H-1′), 4.51 (1H, d, J = 11.46 Hz, H-6′a), 4.38 (1H, dd, J = 11.46, 5.76 Hz, H-6′b), 4.26 (t, J = 5.7 Hz, H-3′), 4.25 (t, J = 5.7 Hz, H-4′), 4.01 (dd, J = 7.8, 7.5 Hz, H-2′) and 3.94 (m, H-3′). All other ^{1}H NMR assignment were established by comparison with the reported data of the related compounds.[312]

The structure was confirmed by using 1 D and 2 D techniques. In HMBC experiment the resonance at δ 5.29 (H-6) showed correlations to C-4, C-8 and C-10. The resonance at 2.66 (H-4) correlated with C-2, C-3, C-5, C-6 and C-10. The signal at δ 1.64, 1.03 (H-1) correlated with resonates at δ 78.38 (C-3), 140.73 (C-5) and 19.27 (C-19). The signal at δ 0.99 (H-19) correlated to δ 36.76 (C-10), 50.17 (C-9), 140.73 (C-5) and the signal at δ 0.91 (H-21) correlated to δ 34.03 (C-22), 36.23 (C-20) and 56.01 (C-17). The signal at δ 0.81 (H-27) correlated to C-24, C-25 and C-26 and the signal at δ 0.78 (H-26) correlated to C-24, C-25 and C-27. The resonance at δ 0.59 (H-18) correlated to resonances at δ 39.17 (C-12), 42.31 (C-13), 56.01 (C-17) and 56.65 (C-14).

From the data given, the structure of the compound was established to be 23a-homostigmast-5-en-3-*O*-*β*-glucopyroside 2-(17-(6-ethyl-7-methylocta-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclo-penta[a]-phenanthren-3-yloxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol.

To the best of our knowledge this was a new natural product.

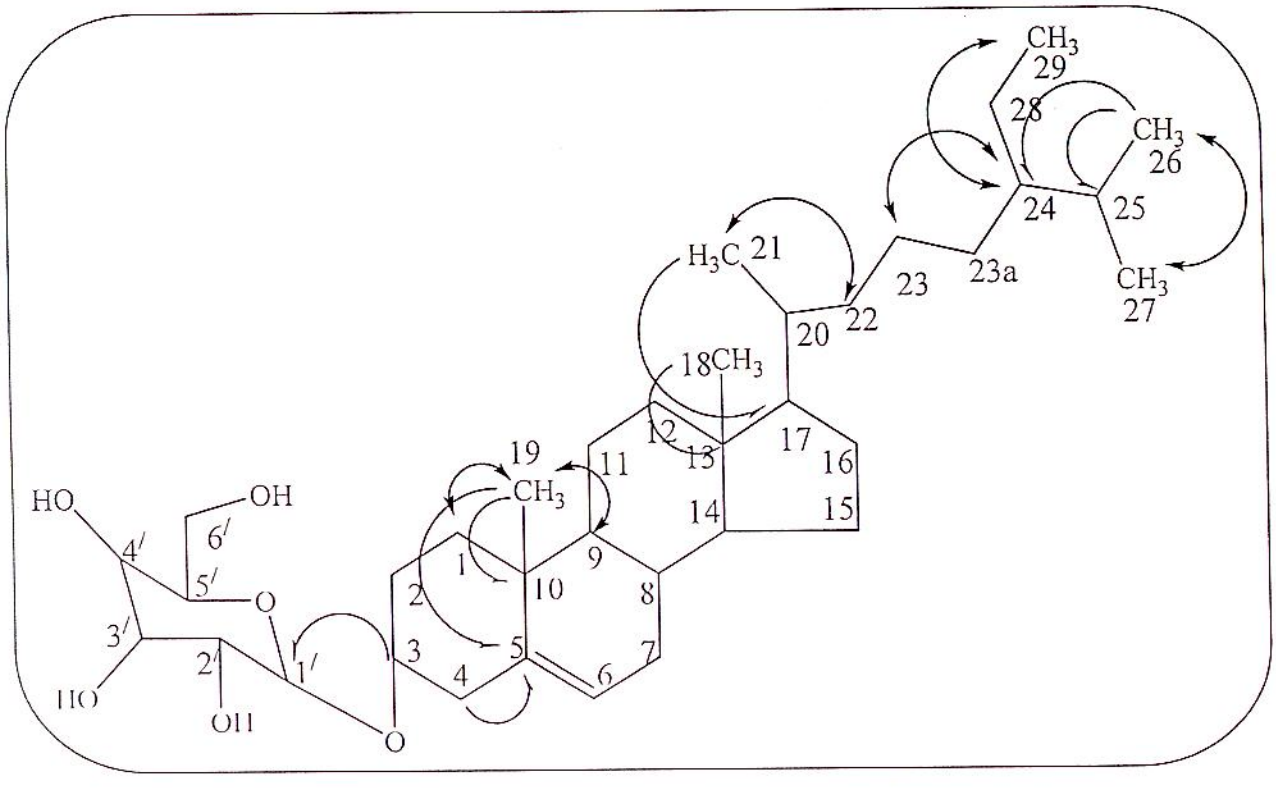
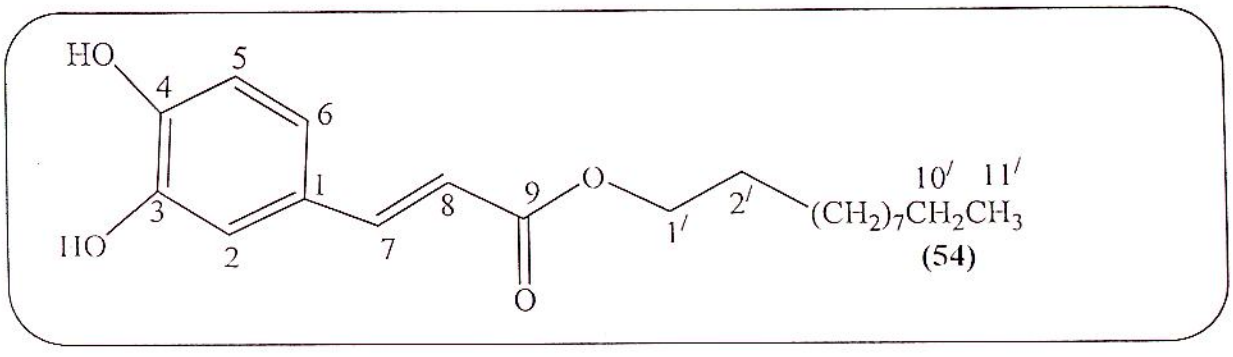


Fig. 2.5. HMBC Correlation of 23a-homostigmast-5-en-3-*O*-*β*-glucopyroside (53)

2.1.2.5. Undecyl caffeate (54)



(54)

White needles (Chloroform); mp 108–109 °C

Molecular formula $C_{20}H_{30}O_4$, deduced from ^{1}H NMR, ^{13}C NMR and mass spectra *m/z* 334 (calcd. for $C_{20}H_{30}O_4$, 334).

The ^{13}C NMR spectrum of compound **54** confirmed the presence of a dihydroxycinnamic acid derivative with a carbonyl signal at δ 167.75. The aromatic region contains two oxygen deshielded quaternary carbons at δ 143.78 and 146.18 were attributed to C-3 and C-4 respectively. The signal at δ 144.61, 127.91 and 122.44 were assigned to C-7, C-1 and C-6 respectively. The resonances at δ 116.11, 115.65 and 114.57 were attributed to C-8, C-5 and C-2 respectively. The olefinic signal at δ 64.88 which was deshielded by oxygen, was assigned to C-1′ and the signal at δ 31.97 was assigned to C-9′. The signals at δ 28.81 and 26.04 were assigned to C-2′ and C-3′. The signals at δ 29.73, 29.63, 29.58, 29.38 and 29.33 were attributed to C-6′, C-7′, C-5′, C-8′ and C-4′. The high field signals at δ 22.71 and 14.10 were assigned to C-10′ and the terminal methyl C-11′.

The ^{1}H NMR ($CDCl_3$) showed two protons signals at δ 7.55 (1H, d, J = 15.78 Hz) and 6.24 (1H, d, J = 15.78 Hz) were assigned to H-7 and H-8. The large coupling between H-7 and H-8 indicated these to be were trans to each other. The signals at δ 7.07 (1H, d, J = 1.6 Hz), 6.99 (1H, dd, J = 8.28, 2.0 Hz) and at 6.85 (1H, d, J = 8.28 Hz) were assigned to H-2, H-6 and H-5 respectively. Two broad singlets at δ 5.62 (s, OH) and 5.55 (s, OH) were assigned to OH at C-3 and C-4 respectively.

Table 2.7. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of undecyl caffeate **(54)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR(δ) (J_{HH} Hz)	HMBC Correlation
1	127.91	C		-
2	114.57	CH	7.07 (d, J = 1.6)	C-4, C-6, C-7
3	143.78	C	5.62, s (OH)	-
4	146.18	C	5.55, s (OH)	-
5	115.65	CH	6.85 (d, J = 8.28)	C-1, C-3
6	122.44	CH	6.99 (dd, J = 8.28, 2.0)	C-2, C-7
7	144.61	CH	7.55 (d, J = 15.78)	C-2, C-6, C-9
8	116.11	CH	6.24 (d, J = 15.78)	-
9	167.75	C	-	-
1′	64.88	CH_2	4.17 (2H, t, J = 6.84)	C-2′, C-3′, C-9
2′	28.81	CH_2	1.66 (2H, pentet, J = 6.84)	C-1′, C-3′ C-9
3′	26.04	CH_2	1.37 (2H, m)	C-2′
4′	29.33	CH_2	1.31-1.21 (2H, m)*	-
5′	29.58	CH_2	1.31-1.21 (2H, m)*	-
6′	29.73	CH_2	1.31-1.21 (2H, m)*	-
7′	29.63	CH_2	1.31-1.21 (2H, m)*	-
8′	29.38	CH_2	1.31-1.21 (2H, m)*	-
9′	31.97	CH_2	1.31-1.21 (2H, m)*	C-26′
10′	22.71	CH_2	1.31-1.21 (2H, m)*	-
11′	14.10	CH_3	0.86 (3H, t, J = 7.08)	C-10′, C-9′

*Overlaped peaks

All the values were assigned through HMQC and HMBC techniques. The proton resonance at δ 7.55 (H-7) showed correlation to δ 114.57 (C-2), 122.44 (C-6) and 167.75 (C-9). The proton signal at δ 7.07 (H-2) correlated to δ 122.44 (C-6), 144.60 (C-7) and 146.18 (C-4). The resonance at δ 6.99 (H-6) correlated to δ 114.57 (C-2), 144.60 (C-7) and 146.18 (C-4). The proton signal at δ 6.85 (H-5) showed correlation to carbon signals at δ 127.91 (C-1), 143.78 (C-3) and 146.18 (C-4), where as the signal at δ 6.24 (H-8) correlated to δ 127.91 (C-1). The proton signal at δ 4.17 (H-1′) correlated to carbonyl carbon at δ 167.75 (C-9), 28.81 (C-2′) and 26.04 (C-3′). The proton resonance at δ 1.66 (H-2′) correlated to carbon signals at δ 64.88 (C-1′), 26.04 (C-3′) and 167.75 (C-9). The methyl signal at δ 0.86 (H-11′) showed correlation to δ 31.97 (C-9′) and 22.71 (C-10′).

On the basis of IR, UV, ^{1}H NMR, ^{13}C NMR and mass spectrum the structure of the compound was elucidated as undecyl caffeate. This was a synthetic derivative [315] and we report it for the first time as a natural product. The DQF COSY and HMBC techniques are not reported before and we report these techniques for the first time.

The DQF COSY correlation is given in **Figure 2.6**, the dotted line represents DQF COSY correlation.

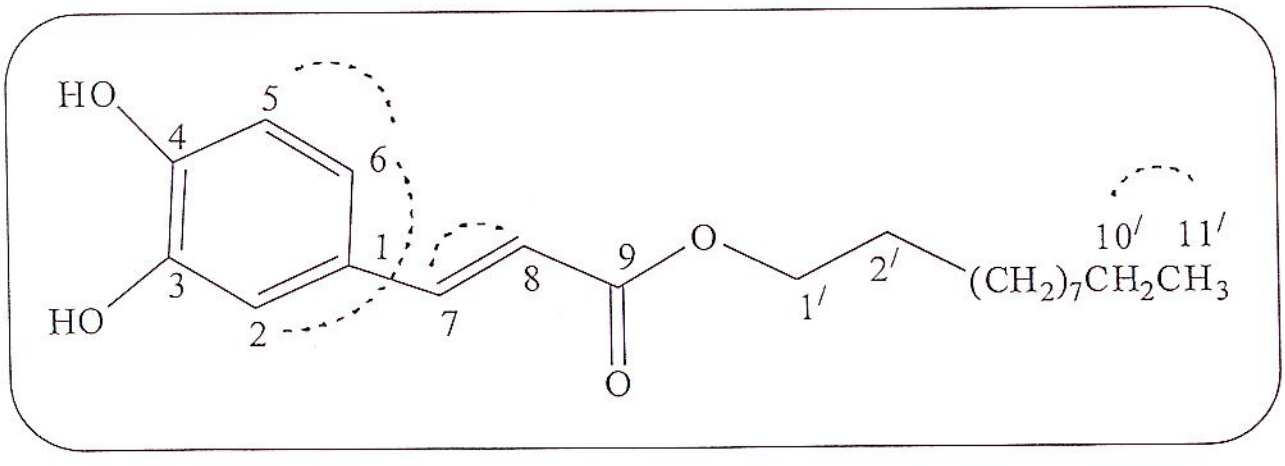
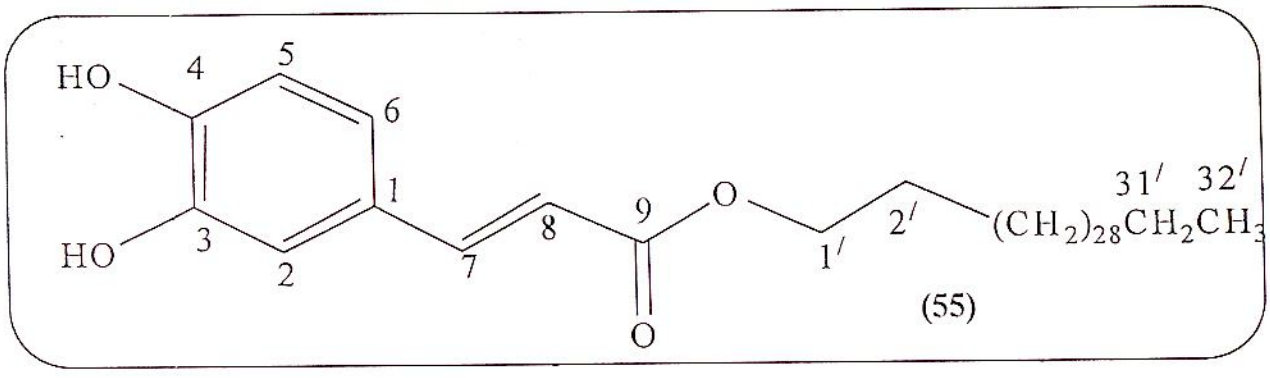


Fig. 2.6 DQF COSY Correlation of undecyl caffeate **(54)**

2.1.2.6. Dotricontanyl caffeate (55)



(55)

White needles (Chloroform); mp 102–103 °C

Molecular formula $C_{41}H_{72}O_4$, deduced from ^{1}H NMR, ^{13}C NMR and mass spectrum *m/z* 628 (M^+), (calcd. 628 for $C_{41}H_{72}O_4$).

Table 2.8. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of dotricontanyl caffeate **(55)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR(δ) (J_{HH} Hz)	HMBC Correlation
1	127.55	C	-	-
2	114.62	CH	7.10, s	C-4, C-6, C-7
3	144.00	C	5.97 (s, OH)	-
4	146.64	C	5.97 (s, OH)	-
5	115.59	CH	6.86 (d, J = 7.8)	C-1, C-3
6	122.43	CH	6.99 (d, J = 7.8)	C-2, C-7
7	145.25	CH	7.56 (d, J = 15.78)	C-2, C-6, C-9
8	115.63	CH	6.24 (d, J = 15.78)	-
9	168.36	C	-	-
1′	55.14	CH_2	4.19 (2H, t, J = 6.8)	C-2′, C-3′, C-9
2′	28.78	CH_2	1.68 (2H, pentet, J = 6.8)	C-1′, C-3′, C-9
3′	26.03	CH_2	1.37 (2H, m)	C-2′
4′-31′	22.72-31.97	CH_2	1.25 (56H, b, s)	-
32′	14.10	CH_3	0.87 (3H, t, J = 7.07)	C-10′, C-11′

The ^{13}C NMR (600MHz, $CDCl_3$) spectrum of the compound **55** confirmed the presence of a dihydroxycinnamic acid derivative with a carbonyl carbon at δ 168.36. The aromatic region contains two oxygen deshielded quaternary carbons at δ 146.64 and 144.00, attributed to C-3 and C-4 respectively. The signal at δ 145.25, 127.55 and 122.43 were assigned to C-7, C-1 and C-6 respectively. The resonances at δ 115.63, 115.59 and 114.62 were attributed to C-8, C-5 and C-2 respectively. The olefinic signal at δ 65.14 which is deshieled by oxygen was assigned to C-1′ and the signal at δ 28.78 was assigned to C-2′. The signals from 22.72 to δ 31.97 were attributed to C-4′ to C-31′. The high field signals at δ 14.10 was assigned to terminal methyl C-32′.

The ^1H NMR spectrum showed two protons signals at δ 7.56 (1H, d, J = 15.88 Hz) and 6.24 (1H, d, J = 15.78 Hz), assigned to H-7 and H-8 having trans configuration due to large coupling. The signals at δ 7.10 (1H, s), 6.99 (1H, d, J = 7.8 Hz) and 6.86 (1H, d, J = 7.8 Hz) were assigned to H-2, H-6 and H-5 respectively. A broad singlet at δ 5.97 (s, OH) was assigned to OH at C-3 and C-4. The two protons triplet at δ 4.19 (2H, t, J = 6.8 Hz) was assigned to (CH_2-1′) attached to an ester group. The signal at δ 1.68 (2H, quintet, J = 6.8 Hz) was assigned to CH-2′, the multiplet at δ 1.37 (2H, m, H-3′) was assigned to CH_2-3′ and the broad peak at 1.25 (56H, b s, CH_2-4′-31) with an integration of fifty six hydrogen was assigned to CH_2-4′-31′. Three proton triplet at δ 0.87 (3 H, t, J = 7.07 Hz) was attributed to the terminal methyl group.

From UV, IR, ^1H NMR and ^{13}C NMR data the structure is established as dotricontanyl caffeate and is reported as new compound.

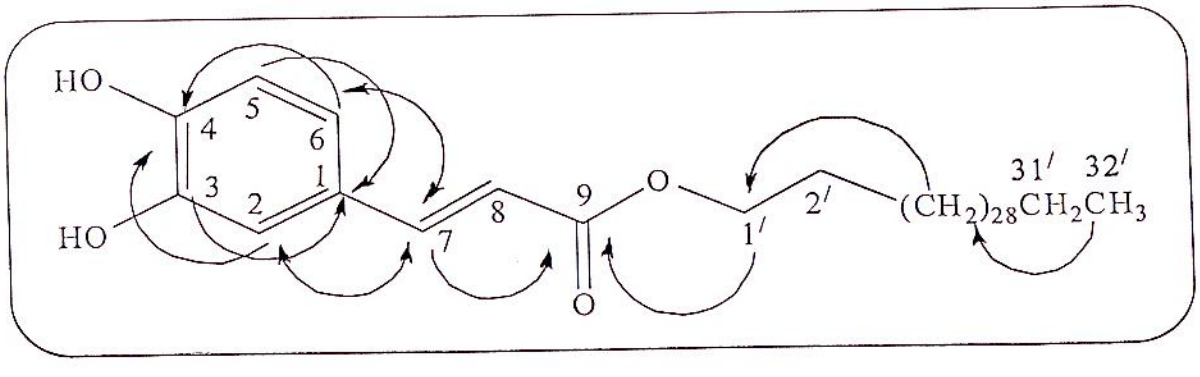
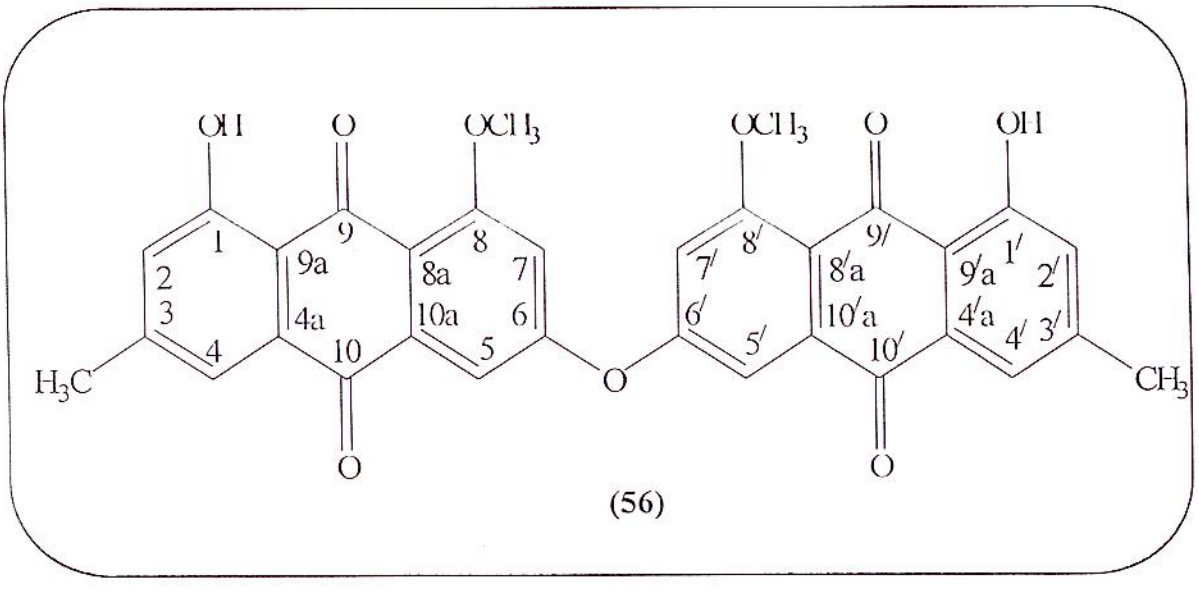


Fig. 2.7. HMBC Correlation of dotricontanyl caffeate (55)

2.1.3. New Isolated Compounds from the Roots of *Cassia artemisioides*

2.1.3.1. 1,1′-Dihydroxy-8,8′-dimethoxy-3,3′-dimethyl-6,6′-bianthraquinone (56)



(56)

Orange red powder (Methanol); mp 288-290 °C

Molecular formula $C_{32}H_{22}O_9$, deduced from broad band decoupled ^{13}C NMR, DEPT experiments and EI mass spectrum *m/z* 564 (calcd. 564 for $C_{32}H_{22}O_9$).

The ^{13}C NMR showed signals for two methyl, two methoxy, eight methine and twenty quaternary carbons. All the assignments were established from the DEPT experiments. The resonances showed similarity to that of 1,6-dihydroxy-8-methoxy-3-methylanthraquinone **(60)** except for C-6 and C-6′ and also most of the carbon peaks splits into two, which indicated that the compound was a symmetrical dimer of 1,6-dihydroxy-8-methoxy-3-methylanthraquinone. The low field signals at δ 186.93, 168.83, 182.45 and 182.45 were attributed to C-9, C-9′, C-10 and C-10′, chelated carbonyl carbons of the anthraquinone moiety. The oxygen deshielded resonances at δ 164.56 was assigned to C-1 and C-1′ whereas the signal at δ 164.03 was assigned to C-8, C-8′. The resonances at δ 162.67 and 162.34 were attributed to C-6 and C-6′ respectively.

Table 2.9. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $(CD_3)_2CO$ and HMBC Correlations of 1,1′-dihydroxy-8,8′-dimethoxy-3,3′-dimethyl-6,6′-bianthraquinone **(56)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	164.56	C	13.33 (s, OH)	C-1
2	124.10	CH	7.07, s	C-4, C-3, C-9a
3	146.72	C	-	
4	119.08	CH	7.47, s	C-2, C-3, C-9a, C-10
4a	132.72	C	-	
5	107.03	CH	7.31 (d, J = 2.28)	C-7, C-6′, C-8a, C-10
6	162.67	C	-	
7	104.92	CH	6.90 (d, J = 2.28)	C-5, C-8a, C-8, C-6′
8	164.03	C	-	
8a	113.73	C	-	
9	186.93	C	-	
9a	114.87	C	-	
10	182.45	C	-	
10a	137.62	C	-	
1′	164.56	C	13.33, s (OH)	OH correlated to C-1′
2′	123.99	CH	7.07, s	C-4′, C-3′, C-9′a
3′	146.68	C	-	
4′	119.05	CH	7.47, s	C-2′, C-3′, C-9′a, C-10′
4′a	132.69	C	-	
5′	106.98	CH	7.31 (d, J = 2.28)	C-6, C-7′, C-8′a, C-10′
6′	162.34	C	-	
7′	104.92	CH	6.90 (d, J = 2.28)	C-6, C-5′, C-8′a, C-8′
8′	164.03	C	-	
8′a	113.73	C	-	
9′	186.83	C	-	
9′a	114.87	C	-	
10	182.45	C	-	
10′a	137.62	C	-	
3-CH_3	20.95	CH_3	2.41 (3H, s, CH_3)	C-2, C-3, C-4
3′-CH_3	20.95	CH_3	2.41 (3H, s, CH_3)	C-2′, C-3′, C-4′
8-OCH_3	56.33	CH_3	3.95 (3H, s, OCH_3)	C-8a
8′-OCH_3	56.33	CH_3	3.95 (3H, s, OCH_3)	C-8′a

The signals at δ 146.72, 148.68 and 137.62 were attributed to C-3, C-3′ and C-10a respectively. The resonances at δ 137.62, 132.72, 132.69 and 124.10 were assigned to C-10′a, C-4a, C-4′a and C-2 respectively. The signals at δ 123.99, 119.08, 119.05 and 114.87 were assigned to C-2′, C-4, C-4′ and C-9a respectively. The signals at δ 114.87, 113.73, 113.73 and 107.03 were attributed to C-9′a, C-8, C-8a and C-5 respectively. The resonances at δ 106.98, 104.92 and 104.92 were attributed to C-5′, C-7 and C-7′ respectively. The carbon signal at δ 56.33 was assigned to the two symmetrical methoxy groups attached at positions C-8 and C-8′. The signal at δ 20.95 was assigned to the two methyl groups attached at C-3 and C-3′.

^{1}H NMR displayed low field signal at δ 13.33 (OH, s, OH-1, OH-1′) was assigned to the chelated hydroxy groups. The position of hydroxyl groups was determined to be at C-1 and C-1′ from the HMBC techniques. The aromatic signal at δ 7.47 (2H, s) was assigned to H-4 and H-4′ and the meta-coupled doublets for aromatic protons at δ 7.31 (2H, d, J = 2.28 Hz) were attributed to H-5 and H-5′. The two protons singlet at δ 7.07 (2H, s) was assigned to H-2 and H-2′. The meta-coupled singlet at δ 6.90 (2H, d, J = 2.28 Hz) integrated for two protons was attributed to H-7 and H-7′. The oxygen deshielded protons at δ 3.95 (6 H, s) was assigned to the two methoxy groups at C-8 and C-8′. The up field singlet at δ 2.41 (6H, s) was assigned to two methy groups at C-3 and C-3′. All the C-H correlations were achieved by using HMQC techniques.

The HMBC correlation of the compound **56** showed some interesting correlations. The OH proton at δ 13.33 showed correlation to δ 164.56 (C-1′). The proton signal at δ 7.47 (H-4) showed correlation to carbon resonance at δ 182.45 (C-10), 146.72 (C-3), 119.08 (C-4) and 114.87 (C-9a). The proton signal at δ 7.31 (H-5) correlated to δ 182.45 (C-10), 162.34 (C-6′), 113.73 (C-8a) and 104.92 (C-7). The proton resonance at δ 7.07 (H-2) showed correlation to carbon signals at δ 119.08 (C-4), 114.87 (C-9a) and 146.72 (C-3). The signal at δ 6.90 (H-7) correlated to resonances at δ 107.03 (C-5), 113.73 (C-8), 162.34 (C-6′) and 164.03 (C-8) The methoxy protons at δ 3.95 showed correlation to C-8 and on the basis of HMBC correlation the position of methoxy groups were assigned at C-8 and C-8′. The up field chemical shift at δ 2.41 (CH_3) showed correlation to δ 146.72 (C-3),

124.10 (C-2) and 119.08 (C-4).

On the bases of these spectral data the structure of the compound **56** was elucidated to be 1,1′-dihydroxy-8,8′-dimethoxy-3,3′-dimethyl-6,6′-bianthraquinone. This is a new natural product and is a dimer of 1,6-dihydroxy-8-methoxy-3-methylanthraquinone. To the best of my knowledge it is a new compound.

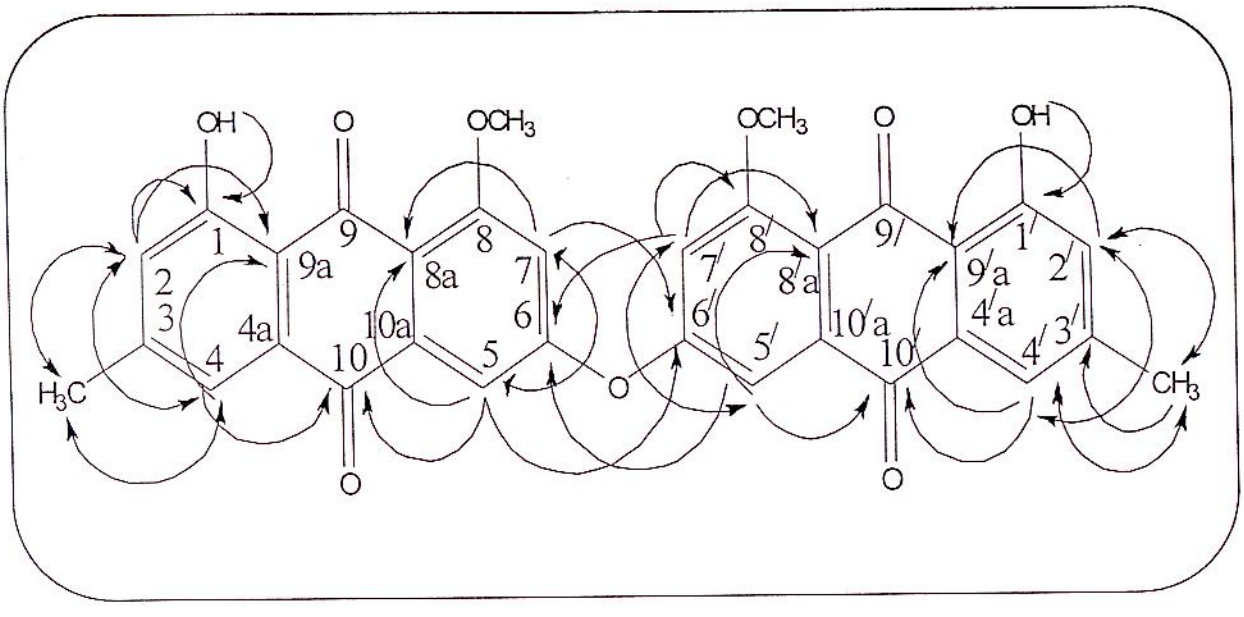
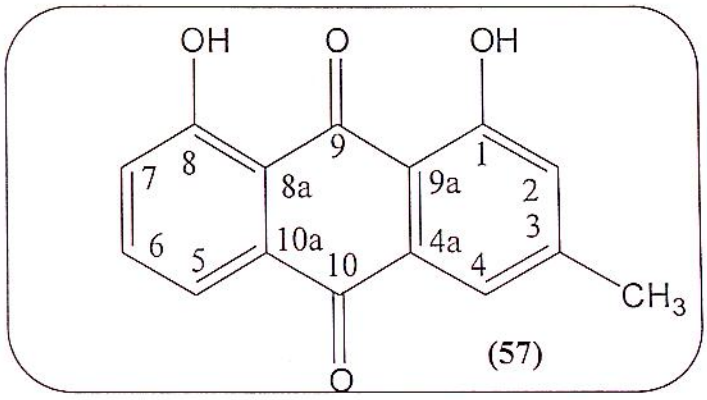


Fig. 2.8. HMBC Correlation of 11,1′-dihydroxy-8,8′-dimethoxy-3,3′-dimethyl-6,6′-bianthraquinone **(56)**

2.1.4. New Source Compounds isolated from *Cassia artemisioides* Flowers

2.1.4.1. 1,8-Dihydroxy-3-methylanthraquinone (57)



(57)

Reddish needles (Chloroform); mp 195-196 °C (Lit. mp 193-194 °C)[316,319]
Molecular formula $C_{15}H_{10}O_4$, deduced from EI mass spectra (calcd. 254 for $C_{15}H_{10}O_4$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **57** confirmed the presence of fifteen carbon atoms in the molecule; one methyl group, five methine and nine quaternary carbons according to DEPT experiments. Total assignments were established according to HMQC and HMBC experiments (**Table 2.I0**). The carbon resonating at δ 192.65 was assigned to C-9 where as a signal at δ 182.14 was assigned to C-10, the two carbonyl carbons of the anthraquinone moiety. The aromatic signals at δ 162.82 and 162.82 were assigned to C-1 and C-8 respectively, confirming the position of the oxygen functionality (OH). The aromatic signals resonating at δ 149.45 and 137.06 were assigned to C-3 and C-6 respectively. The signal at δ 133.74 and 133.38 were assigned to C-10a and C-4a respectively. The signals δ 124.67, 124.47 and 121.48 were assigned to C-7, C-2 and C-4 respectively. The signals at δ 120.04, 115.98 and 113.84 were assigned to C-5, C-8a and C-9a respectively. The high field signal at δ 22.37 was assigned to CH_3 at C-3.

Table 2.10. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of 1,8-dihydroxy-3-methylanthraquinone **(57)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC
1	162.82	C	12.01 (s, OH)	
2	124.47	CH	7.10, s	C-4, C-9a, C-3
3	149.45	C		
4	121.48	CH	7.65, s	C-2, C-9a, C-3
4a	133.38	C		
5	120.04	CH	7.81 (dd, J = 7.5, 0.9)	C-7, C-8a
6	137.06	CH	7.66 (t, J = 7.5)	C-5, C-10a, C-8
7	124.67	CH	7.27 (dd, J = 7.5, 0.9)	C-5, C-8a
8	162.82	C	12.12 (s, OH)	
8a	115.98	C	-	
9	192.65	C	-	
9a	113.84	C	-	
10	182.14	C	-	
10a	133.74	C	-	
3-CH_3	22.37	CH_3	2.46 (3H, s, CH_3)	C-2, C-3, C-4

The ^{1}H NMR spectrum of the compound **57** showed signals at δ 2.46 (s, 3H) for a methyl group, at δ 12.00 and 12.12.11 for the two chelated hydroxy groups at OH-1 and OH-8 respectively. Three ortho-coupled aromatic protons at δ 7.27 (d d, J = 7.5, 0.9 Hz, Hz, H-5), 7.66 (t, J = 7.5 Hz, H-6) and 7.81 (d d, J = 7.5, 0.9 Hz, H-7). The spectrum also showed two isolated aromatic protons signals at δ 7.10 (s, H-2) and at 7.65 (s, H-4).

In the HMBC spectrum, the C-9 which resonate at δ 192.65 correlated with the hydroxyl groups at δ 12.00 (OH-1) and 12.12 (OH-8), confirming the 1,8-dihydroxyanthraquinone moiety. The C-10 signal at δ 182.10 showed correlation with signals at δ 7.65 (H-4) and 7.80 (H-5), the signal at δ 7.66 (H-6) correlated to signals at δ 133.74 (C-10a) and 162.82

(C-8). The signal at δ 7.27 (H-7) correlated with δ 120.04 (C-5) and 133.74 (C-10a) while the signal at δ 7.81 correlated to δ 124.67 (C-7) and 115.98 (C-8a). The signal at δ 7.10 (H-2) correlated with signals at δ 121.48 (C-4) and 113.82 (C-9a). The resonance at δ 7.65 (H-4) correlated with signals δ 124.48 (C-2) and 113.84 (C-9a). The high field signal at δ 2.46 correlated to resonance at δ 149.45 (C-3). The HMBC techniques are reported for the first time.

On the basis of these data, the compound **57** was identified as 1,8-dihydroxy-3-methylanthraquinone (chrysophanol) isolated previously from the *Cassia siamea*[317] and Rhubarb.[318]

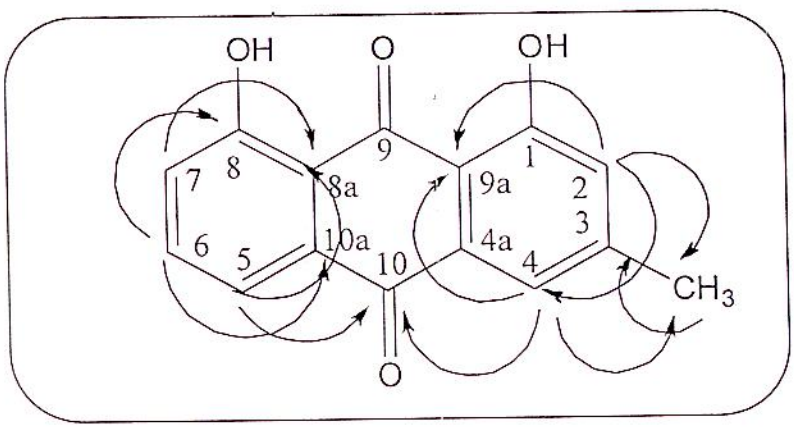
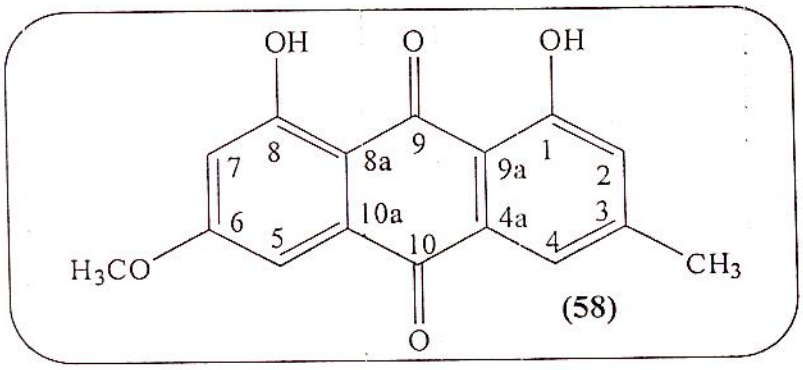


Fig. 2.9. HMBC Correlation of 1,8-dihydroxy-3-methylanthraquinone (57)

2.1.4.2. 1,8-Dihydroxy-6-methoxy-3-methylanthraquinone(58)



(58)

Reddish needles (Acetone); mp 205-206 °C (Lit. mp 206-207 °C)[316]

Molecular formula $C_{16}H_{10}O_5$, deduced from EI mass spectra *m/z* 284 (calcd. 284 for $C_{16}H_{10}O_5$) and ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **58** showed the presence of sixteen carbon atoms in the molecule; one methyl group, one methoxy group, four methine and ten quaternary carbons according to DEPT experiments. Total assignments were established according to HMQC, COSY and HMBC experiments (**Table 2.11**). The carbon resonating at δ 190.86 was assigned to C-9 where as a signal at δ 182.11 was assigned to C-10, both these signals indicated the carbonyl carbons of the anthraquinone moiety. The aromatic signals at δ 166.62, 165.26 and 162.57 were assigned to C-6, C-8 and C-1 respectively which indicated the presence of oxygen functionality, OH at C-8 and C-1. The aromatic signals resonating at δ 148.53 and 135.31 were assigned to C-3 and C-10a respectively. The signals at δ 133.27 and 124.59 were assigned to C-4a and C-2 respectively. The signals at δ 121.38, 113.74 and 110.32 were assigned to C-4, C-9a and C-8a respectively. The signals at δ 108.31 and 106.84 were assigned to C-5 and C-7 respectively. The high field signal at δ 56.17 and 22.25 were assigned to OCH_3 attached at C-6 and C-3 respectively.

Table 2.11. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $(CD_3)_2CO$ and HMBC Correlations of 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (**58**)

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.57	C	12.1 (s, OH)	
2	124.59	CH	7.06, s	C-4, C-9a
3	148.53	C	-	
4	121.38	CH	7.60, s	C-2, C-9a, C-10
4a	133.27	C	-	
5	108.31	CH	7.34 (d, J = 2.52)	C-7, C-8a, C-10
6	166.62	C	-	
7	106.84	CH	6.66 (d, J = 2.52)	C-5, C-8a
8	165.26	C	12.3 (s, OH)	
8a	110.32	C	-	
9	190.86	C	-	
9a	113.74	C	-	
10	182.11	C	-	
10a	135.31	C	-	
3-CH_3	22.25	CH_3	2.43 (3H, s, CH_3)	C-2, C-3, C-4
6-OCH_3	56.17	CH_3	3.91 (3H, s, OCH_3)	C-6

The ^{1}H NMR spectrum of the compound **58** showed signals at δ 2.43 (s, 3H) for a methyl group, at δ 3.91 for a methoxy group (s, 3H). Two sharp signals at δ 12.1 and 12.3 for the two chelated (peri) hydroxy groups; OH-1 and OH-8 respectively (D_2O exchangeable). Two meta-coupled protons at δ 6.66 (d, J = 2.52 Hz, H-7), and 7.34 (d, J = 2.52 Hz, H-5) were assigned to H-7 and H-5 respectively. The spectrum also showed two singlets for the aromatic protons at δ 7.06 (s, H-2) and 7.60 (s, H-4).

In the HMBC spectrum, the C-9 at δ 190.86 correlated with the hydroxy groups at δ 12.1 (OH-1) and 12.3 (OH-8), confirming the 1,8-dihydroxyanthraquinone moiety. The C-10 signal at δ 182.11 showed correlation to signals at δ 7.60 (H-4) and 7.34 (H-5), the signal

at δ 106.84 (H-7) correlated to signals at δ 110.32 (C-8a) and 108.31 (C-5). The signal at δ 7.34 (H-5) correlated with δ 106.84 (C-7) and 110.32 (C-8a). The resonance at δ 7.60 (H-4) correlated with signals at δ 124.59 (C-2) and 113.74 (C-9a). The signal at δ 7.06 (H-2) showed correlation with the signals at δ 121.38 (C-4) and 113.74 (C-9a). The high field absorption at δ 2.45 correlated with resonance at δ 148.53 (C-3) and the one at δ 3.91 correlated to δ 166.62 (C-6). The HMBC Correlation is given **Fig. 2.10**

On the basis of UV-Vis., IR, NMR and EI mass spectra the compound **58** was identified as 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (physcion). All of the spectral data (UV, IR, ^{1}H NMR and ^{13}C NMR) agreed with the physcion isolated from the *Cassia siamea*[319] and Rhubarb.[318]

However this is isolated for the first time from *Cassia artemisioides* flower and the structure was confirmed by using 1D and 2D techniques.

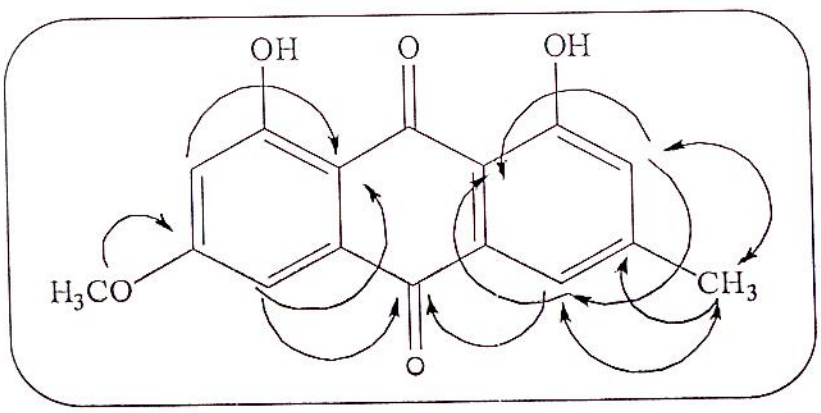
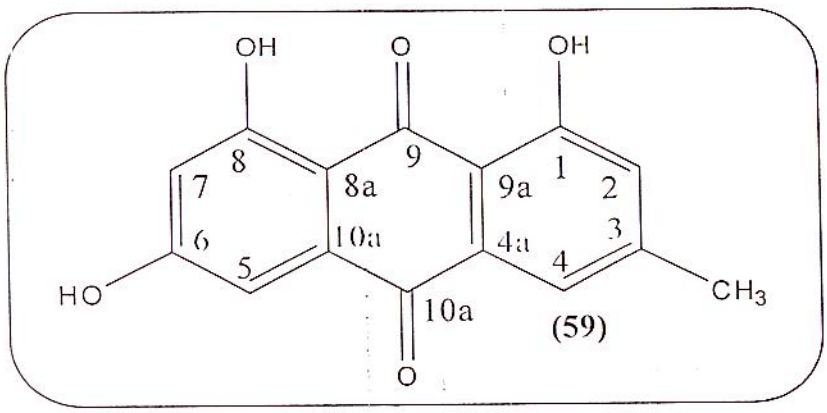


Fig. 2.10. HMBC correlation of 1,8-dihydroxy-6-methoxy-3-methylanthraquinone **(58)**

2.1.4.3. 1,6,8-Trihydroxy-3-methylanthraquinone (emodin) (59)



(59)

Reddish needles (Acetone); mp 268-270 °C (Lit. 267-268 °C)[316]

Molecular formula $C_{15}H_{10}O_5$, deduced from EI mass spectrum *m/z* 270 (M^+) (calcd. 270 for $C_{15}H_{10}O_5$) and ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **59** confirmed the presence of fifteen carbon atoms in the molecule; one methyl, four methine and ten quaternary carbons according to the DEPT experiments. Total assignments were done according to HMQC, COSY and HMBC experiments (**Table 2.12**). The carbon resonating at δ 190.83 was assigned to C-9 where as a signal at δ 181.26 was assigned to C-10, the two carbonyl carbons of the anthraquinone moiety. The aromatic signals at δ 165.55, 165.41 and 162.43 were assigned to C-6, C-8 and C-1 respectively, having oxygen functionality (OH at C-8 and C-1). The aromatic carbons at δ 148.70 and 135.73 were assigned to C-3 and C-10a respectively. The signal at δ 133.33 was attributed to C-4 and the one at δ 124.11 was assigned to C-2. The signals at δ 120.64, 113.59 and 109.58 were assigned to C-4, C-9a and C-8a respectively. The signals at δ 108.83 and 108.02 were assigned to C-5 and C-7 respectively. The high field carbon at δ 21.16 was assigned to the CH_3 at C-3.

Table 2.12. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $(CD_3)_2CO$ and HMBC Correlations of 1,6,8-trihydroxy-3-methylanthraquinone **(59)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.43	C	12.00 (s, OH)	
2	124.11	CH	7.07, s	C-4, C-3, C-9a
3	148.70	C	-	
4	120.64	CH	7.48, s	C-2, C-3, C-9a
4a	133.33	C	-	
5	108.83	CH	7.18 (d, J = 2.1)	C-7, C-8a
6	165.41	C	2.90 (s, OH)	
7	108.02	CH	6.59 (d, J = 2.2)	C-5, C-8, C-8a
8	165.55	C	12.12 (s, OH)	
8a	109.58	C	-	
9	190.83	C	-	
9a	113.59	C	-	
10	181.26	C	-	
10a	135.73	C	-	
3-CH_3	21.16	CH_3	2.42 (3H, s, CH_3)	C-3, C-2, C-4

The ^{1}H NMR spectrum of the compound **59** showed signals at δ 2.42 (s, 3H) for a methyl group, one broad peak at δ 2.90 for the OH, (OH-6) and two sharp signals at δ 12.00 and 12.12 for the two chelated (peri) hydroxy groups; OH-1 and OH-8 respectively (D_2O exchangeable). Two meta-coupled aromatic protons at δ 6.59 (d, J = 2.2 Hz, H-7) and 7.18 (d, J = 2.1 Hz, H-5). The spectrum also showed two singlets for the aromatic protons at δ 7.07 (s, H-2) and 7.48 (s, H-4).

In the HMBC spectrum, the C-9 at 190.83 correlated to the hydroxy group at δ 12.00 (OH-1) and 12.12 (OH-8), confirming the 1,8-dihydroxyanthraquinone moiety. The C-10 signal at δ 181.26 showed correlation to signals at δ 7.48 (H-4) and 7.18 (H-5), the signal at δ 108.02 (H-7) correlated to the signals at δ 109.58 (C-8a) and 108.83 (C-5). The signal at δ

7.18 (H-5) correlated to δ 108.02 (C-7), 109.58 (C-8a) and 181.26. The signal at δ 7.07 (H-2) showed correlation with the signals at δ 120.64 (C-4) and 113.59 (C-9a). The resonance at δ 7.59 (H-4) correlated with the signals at δ 124.59 (C-2) and 113.59 (C-9a). The high field protons at δ 2.42 correlated to carbon at δ 148.70 (C-3). HMBC correlations are given in **Fig. 2.11.**

The COSY spectrum showed correlation between H-2 and H-4 while H-5 correlated to H-7.

On the basis of UV, IR, ^{1}H NMR and ^{13}C NMR, data the compound **59** was identified as 1,6,8-trihydroxy-3-methylanthraquinone (emodin). All of the data agreed with the emodine isolated from the Rhubarb. [316] However first time the structure was confirmed using 1D and 2D techniques.

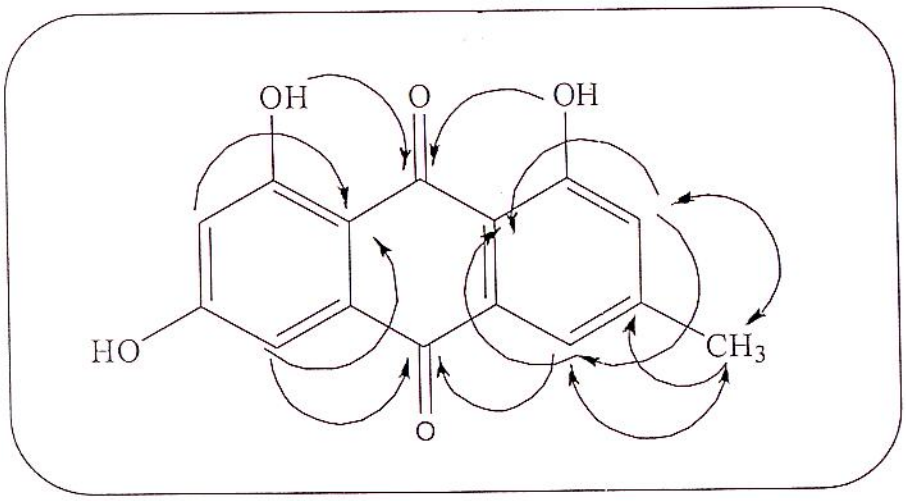
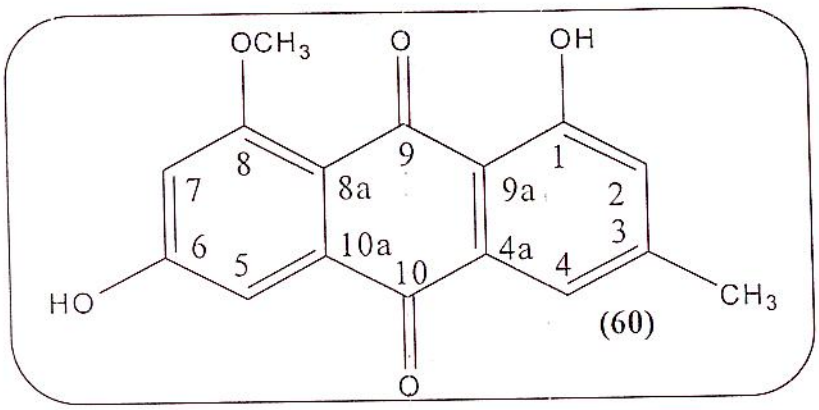


Fig. 2.11. HMBC Correlation of 1,6,8-trihydroxy-3-methylanthraquinone (59)

2.1.4.4. 1,6-Dihydroxy-8-methoxy-3-methylanthraquinone (60)



(60)

Yellow needles (Acetone); mp 300-302 °C (Lit. mp 301-303 °C)[320]

Molecular formula $C_{16}H_{12}O_5$, deduced from EI mass spectrum *m/z* 284 (calcd. 284 for $C_{16}H_{12}O_5$) and ^{13}C NMR

The ^{13}C NMR spectrum of the compound **60** confirmed the presence of sixteen carbons; one methyl, one methoxy group, four methine and ten quaternary carbon atoms according to DEPT experiments. Total assignments were achieved according to HMQC and HMBC experiments (**Table 2.13**). The carbon resonating at δ 186.96 was assigned to C-9 where as a signal at δ 182.40 was assigned to C-10, both these signals indicated the carbonyl carbons of the anthraquinone moiety. The down field aromatic signals at δ 164.38, 163.99 and 162.65 were assigned to C-8, C-6 and C-1 respectively which indicated the presence of oxygen functionality (OH or OCH_3). The aromatic signals at δ 146.76 and 137.60 were assigned to C-3 and C-10a respectively. The signals at δ 132.64, 124.03 and 119.08 were assigned to C-4a, C-2 and C-4 respectively. The reported value for C-2 was δ 120.3 (Acetone d6) which is not correct.[320] The signals at δ 114.82, 113.69, 106.92 and 104.84 were assigned to C-9a, C-8a, C-5 and C-7 respectively. The high field signal at δ 55.90 was assigned to OCH_3 and the one at δ 20.97 to a CH_3.

Table 2.13. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of 1,6-dihydroxy-8-methoxy-3-methylanthraquinone **(60)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.65		13.31, s (OH)	OH, C-1
2	124.03	CH	7.07, s	C-9a, C-4
3	146.76			CH_3 (20.97)
4	119.08	CH	7.47, s	C-2, C-9a
4a	132.64			
5	106.92	CH	7.32 (d, J = 2.3)	C-7, C-8a
6	163.99			
7	104.84	CH	6.90 (d, J = 2.3)	C-5, C-8a
8	164.38			
8a	113.69			
9	186.96			
9a	114.82			
10	182.40			C-4, C-5
10a	137.60			
3-CH_3	20.97	CH_3	2.41 (3H, s, CH_3)	C-2, C-3, C-4
8-OCH_3	55.90	CH_3	3.96 (3H, s, OCH_3)	C-8

The ^{1}H NMR spectrum of the compound **60** showed signals at δ 2.41 (s, 3H) for a methyl group, at δ 3.96 (s, 3H) for a methoxy group and at δ 13.31 for hydrogen bonded hydroxy group (OH-1), two meta-coupled protons at δ 6.90 (d, J = 2.30 Hz, H-7) and 7.32 (d, J = 2.30 Hz, H-5) and two singlets of aromatic protons at δ 7.07 (s, H-2) and at 7.47 (s, H-4).

In the HMBC spectrum, the C-10 signal at δ 181.02 is correlated to protons at δ 7.47 (H-4) and 7.32 (H-5), the signal at δ 164.38 (C-8) correlated with the OCH_3 at δ 3.96 and H-4 at δ 7.47 signals. The signal at δ 162.65 (C-1) correlated with the OH at δ 13.31. The carbon resonance at δ 114.82 (C-9a) showed correlation to the protons at δ 7.07 (H-2) and 7.47

(H-4). The resonance at δ 113.69 (C-8a) correlated with signals at δ 6.90 (H-7) and 7.32 (H-5).

These data suggested the presence of two hydroxyl groups at C-1 and C-6 and a methoxy group at C-8. Therefore compound **60** was identified as 1,6-dihydroxy-8-methoxy-3-methylanthraquinone.

All the spectral data (UV, IR, ^{1}H NMR and ^{13}C NMR) agreed with the reported values for 1,6-dihydroxy-8-methoxy-3-methylanthraquinone, isolated from the fungus *Microascus tardifasiens*[320] and *penicillium frequentans*.[321] However this is reported for the first time from plants. We report for the first time 1D and 2D NMR to determine the correct structure.

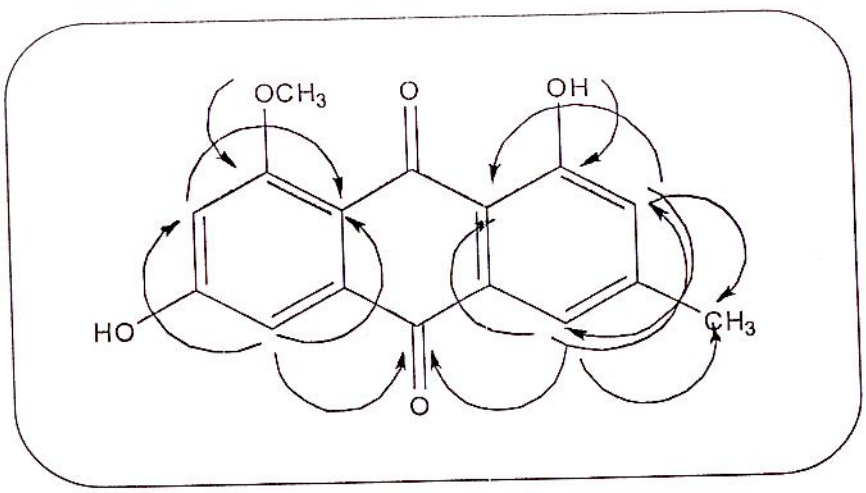
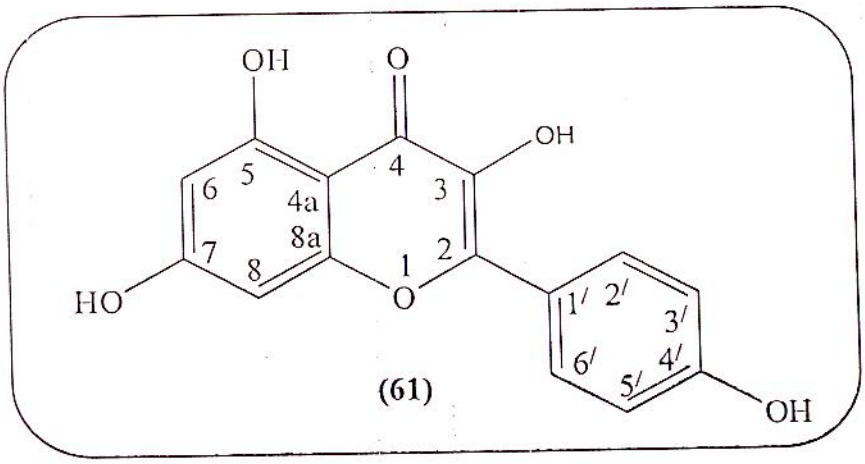


Fig. 2.12. HMBC Correlation of 1,6-dihydroxy-8-methoxy-3-methylanthraquinone **(60)**

2.1.4.5. 3,5,7,4′-Tetrahydroxyflavone (Kaempferol) (61)



(61)

Reddish needles (Acetone); mp 274-276 °C

Molecular formula $C_{15}H_{10}O_6$, calculated from EI mass spectra *m/z* 286 (calcd. 286 for $C_{15}H_{10}O_6$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **61** confirmed the presence of fifteen carbon atoms with six methine and nine quaternary carbons according to DEPT experiments. Total assignments were achieved according to HMQC, DQF COSY and HMBC experiments.

The carbon resonating at δ 175.78 was assigned to C-4 indicating the carbonyl carbon of the flavones moiety. The aromatic signals at δ 164.15, 161.50 and 159.96 were assigned to C-7, C-5 and C-4′ respectively, which indicated the presence of oxygen functionality; OH at C-4′, C-5 and C-7. The aromatic signals resonating at δ 156.96 and 146.19 were assigned to C-8a and C-2 respectively. The signal at δ 135.84 was assigned to C-3 being deshielded by oxygen. The signals at δ 129.65, 129.65 and 122.51 were assigned to C-2′, C-6′ and C-1′ respectively. The signals at δ 115.51, 115.51 and 103.34 were assigned to C-3′, C-5′ and C-4a respectively. The signal at δ 98.28 and 93.68 were assigned to C-6 and C-8 respectively.

Table 2.14. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $(CD_3)_2CO$ and HMBC Correlations of 3,5,7,4′-tetrahydroxyflavone **(61)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR(δ) (J_{HH} Hz)	HMBC Correlation
2	146.19	C	-	C-1′, C-C-6′
3	135.84	C	2.97 (s, OH)	
4	175.78	C	-	
4a	103.34	C	-	
5	161.50	C	12.15 (s, OH)	OH, C-4a, C-6, C-4
6	98.28	CH	6.23 (d, J = 2.04)	C-4a, C-8
7	164.15	C	2.97 (s, OH)	
8	93.68	CH	6.50 (d, J = 1.86)	C-4a, C-6, C-8a
8a	156.96	C	-	
1′	122.51	C	-	
2′	129.65	CH	8.12 (dd, J = 6.6, 2.28)	C-2, C-4′
3′	115.51	CH	6.98 (dd, J = 6.8, 2.1)	C-1′, C-4′
4′	159.96	C	2.97 (s, OH)	
5′	115.51	CH	6.98 (dd, J = 6.8, 2.1)	C-1′, C-4′
6′	129.65	CH	8.12 (dd, J = 6.6, 2.28)	C-2, C-4′

The ^{1}H NMR spectrum of the compound **61** showed broad signal at δ 2.96 (s, OH-3 and OH-7) for the two hydroxyl groups, one sharp signal at δ 12.15 for OH (OH-5). Two meta-coupled protons at δ 6.23 (d, J = 2.04 Hz, H-6), and 6.50 (d, J = 2.04 Hz, H-8). Two para-coupled aromatic protons at δ 6.98 (d d, J = 6.8, 2.1 Hz, H-2′, H-6′) and 8.12 (d d, J = 6.6, 2.28 Hz, H-3′, H-5′).

In the HMBC spectrum, the C-4 resonane at δ 175.78 correlated with hydroxy group at δ 12.15 (OH-5). The signal at δ 8.12 (H-2′) showed correlation to the signals at δ 146.19 (C-2) and 159.96 (C-4′). The signal at δ 6.98 (H-3′) correlated to signals at δ 159.96 (C-4′) and 122.51 (C-1′). The signal at δ 6.23 (H-6) correlated to δ 93.68 (C-8) and 103.34 (C-4a). The signal at δ 6.50 (H-8) showed correlation to the signals at δ 98.28 (C-8) and 103.34 (C-4a).

The COSY spectrum showed correlation between H-6 and H-8 while H-2′ correlated to H-6′ and H-3′correlated to H-6′. The mass spectrum fragmentation results are in agreement with the reported data.[322]

On the basis of spectroscopic data, compound **61** was identified as 3,5,7,4′-tetrahydroxy flavone. All the data is in close agreement to that reported for 3,5,7,4′-tetrahydroxyflavone (kaempferol), from the *Sideritis euboea*.[323]

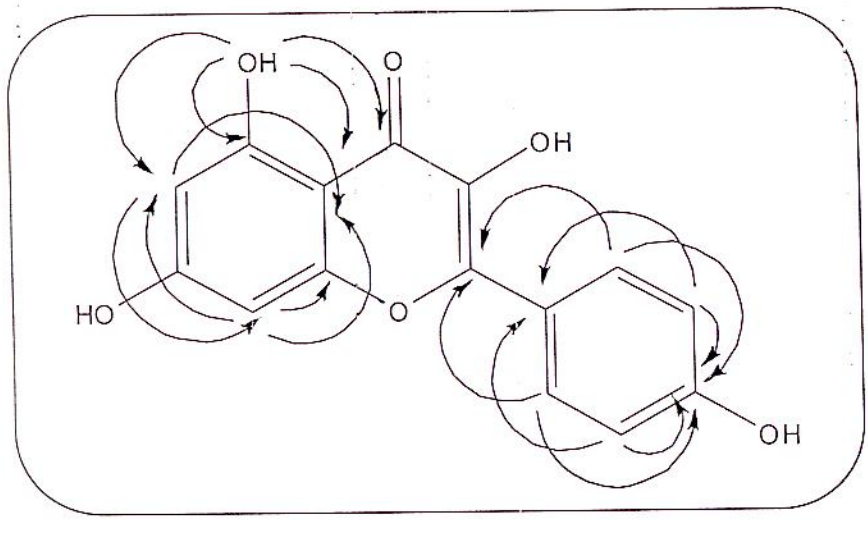
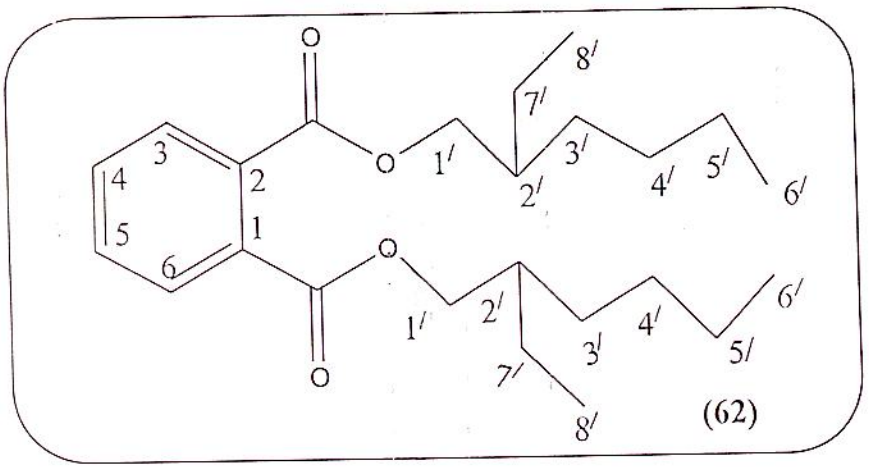


Fig. 2.13. HMBC Correlation of 3,5,7,4′-tetrahydroxyflavone **(61)**

2.1.4.6. Di-2-ethylhexyl phthalate (62)



(62)

Colourless thick residue (Acetone) (272-276)

Molecular formula $C_{24}H_{38}O_4$, deduced from EI mass spectra *m/z* 390, (M^+) (calcd. 390 for $C_{24}H_{38}O_4$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **62** showed the presence of twelve signals in the broad band spectrum due to symmetry in the molecule, each peak was consider double in order to arrive at the molecular mass. The broad band spectrum confirmed the presence of four methyl, four aromatic methine, two methine, ten methylene and four quaternary carbons from the DEPT experiments. Total assignments were established by HMQC and HMBC experiments (**Table 2.15**).

The carbon resonating at δ 168.00 was assigned to the ester CO where as a signal at δ 132.25 was assigned to C-1 and C-2. The aromatic signals at δ 131.06 and 128.52 were assigned to C-4, C-5 and C-3, C-6 respectively. The signal at δ 67.75 was assigned to CH_2-O (C-1′). The signals at δ 38.84, 30.28 and 28.80 were assigned to C-2′, C-3′ and C-4′ respectively. The signals at δ 23.61, 22.69, 13.05 and 10.05 were assigned to C-7′, C-5′ C-6′ and C-8′ respectively.

Table 2.15. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in CD_3OD and HMBC Correlations of di-2-ethylhexyl phthalate **(62)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
	168.00	2C		C-1′, C-3, C-6
1	132.25	C		
2	132.25	C		
3	128.52	CH	7.69 (dd, J = 5.7, 3.24)	COO
4	131.06	CH	7.60 (dd, J = 5.7, 3.24)	C-2, C-3
5	131.06	CH	7.60 (dd, J = 5.7, 3.24)	C-2, C-3
6	128.52	CH	7.69 (dd, J = 5.7, 3.24)	COO
1′	67.75	2 CH_2	4.18, 4.19 (4H, ddd, J = 5.76, 4.23, H-1′)	COO
2′	38.84	2 CH	1.65, m (2H, H-2′)	
3′	30.28	2 CH_2	1.41, 1.68 (4H, d t, J = 5.8, 6.0)	
4′	28.80	2 CH_2	1.36 (4H, m, H-4′)	
5′	22.69	2 CH_2	1.32 (4H, m, H-5′)	
6′	13.05	2 CH_3	0.85 (6H, t, J = 6.9, H-6′)	C-4′
7′	23.61	2 CH_2	1.21, 1.35 (4H, m, H-7′)	
8′	10.05	2 CH_3	0.90 (6H, t, J = 7.8, H-8′)	C-2′

The ^{1}H NMR spectrum of the compound **62** showed signals at δ 0.85 (t, J = 6.9 Hz, 6′-CH_3) for the methyl group and a second methyl at δ 0.90 (t, J = 7.8 Hz, 8′-CH_3). The signals at δ 1.21 and 1.35 (m, H-7′) were assigned to H-7′, which gives multiplet as these are diastereotropic protons and further split by a methyl group. The resonances at δ 1.32 (m, H-5′) and at 1.36 (m, H-4′) were attributed to H-5′ and H-4′ respectively. The signals at δ 1.41 (d, 6.0 Hz, H-3′a), 1.68 (t, J = 5.8, H-3′b) and 1.65 (m, H-2′) were assigned to H-3′a, H-3′b and H-2′ respectively. The chemical shifts at δ 4.18 and 4.19 (dd, J = 5.76, 4.23 Hz, H-1′) for H-1′ as it is diastereotropic proton. Two signal for ortho-coupled protons at δ 7.69 (dd, J = 5.7, 3.24 Hz, H-3, H-6) and 7.60 (dd, J = 5.7, 3.24 Hz, H-4, H-5).

The reported value for H-2′ as a doublet is not correct, it gives ddd or multiplet. Similarly the H-6′ and H-8′ gives clear triplet which are not mention in the literature. The ^{13}C NMR reported chemical shifts for C-3 was δ 30.28 was incorrect, its correct value was δ 38.83 and for C-4 the correct value is δ 30.28 not δ 38.83.[123]

In the HMBC spectrum, the ester C=O signal at δ 168.00 showed correlation to signals at δ 7.69 (H-3, H-4) and 4.18, 4.19 (H-1′). The carbon at δ 132.25 (C-2 and C-3) correlated to the proton at δ 7.60 (H-4, H-5). The signal at δ 13.05 (C-8′) showed correlation to the signal at δ 1.65 (H-2′) and the resonance at δ 0.90 (H-6′) correlated to δ 28.80 (C-4′).

All this data support the correct structure and confirmation of the structure using 1D and 2D techniques. All of the UV, IR, ^{1}H NMR and ^{13}C NMR data agreed with the reported values of di-2-ethylhexyl phthalate, isolated from *Cassia auriculata* L.[123] However corrections in ^{1}H NMR and ^{13}C NMR also the HMBC techiqniques has been reported for the first time. All the spectral data suggest the structure given but the melting point reported is -56 °C but our compound having mp 274-276 °C. Further work is nessary to confirm the structure.

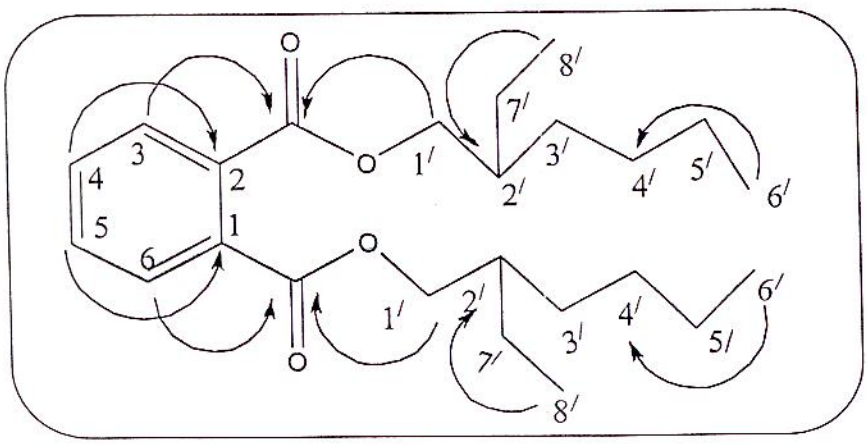
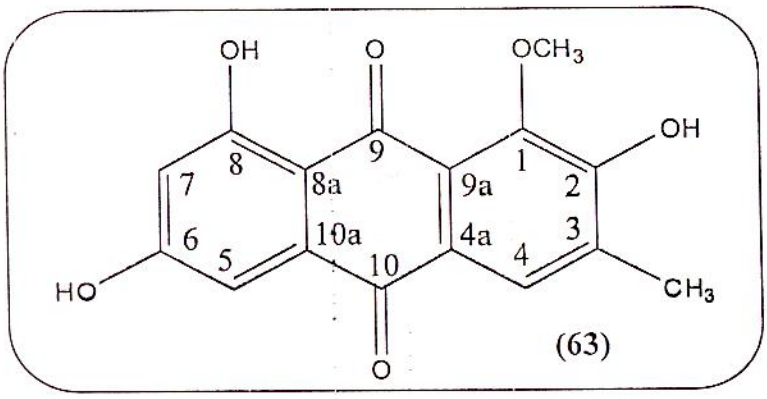


Fig. 2.14. HMBC Correlation of di-2-ethylhexyl phthalate **(62)**

2.1.4.7. 2,6,8-Trihydroxy-1-methoxy-3-methylanthraquinone (63)



(63)

Yellow needles (Acetone); mp 291-293 °C (Lit. mp 292-294 °C)[324]

Molecular formula $C_{16}H_{12}O_6$, deduced from EI mass spectrum *m/z* 300 (calcd. 300 for $C_{16}H_{12}O_6$) and the ^{13}C NMR.

Table 2.16. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $(CD_3)_2CO$ and HMBC Correlations of 2,6,8-trihydroxy-1-methoxy-3-methylanthraquinone (63)

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	147.20	C	-	
2	155.47	C	2.86 (b s, OH)	
3	131.72	C	-	
4	126.19	CH	7.84, s	C-2, C-9a, C-10
4a	125.86	C	-	
5	107.19	CH	7.16 (d, J = 2.34)	C-7, C-8a, C-10
6	164.53	C	2.86 (b s, OH)	
7	107.67	CH	6.56 (d, J = 2.28)	C-5, C-8a
8	165.49	C	13.16 (s, OH)	
8a	110.99	C	-	
9	187.14	C	-	
9a	123.86	C	-	
10	181.02	C	-	
10a	135.25	C	-	
8-OCH_3	61.2	OCH_3	3.90 (3H, s, OCH_3)	C-8
3-CH_3	15.74	CH_3	2.34 (3H, s, CH_3)	C-2, C-3, C-4

The ^{13}C NMR spectrum of the compound **63** confirmed the presence of sixteen carbon atoms, having one methyl group, one methoxy group, three methine and ten quaternary carbons according to DEPT experiments. Total assignments were achieved according to HMQC and HMBC experiments (**Table 2.16**)

In ^{13}C NMR the carbon at δ 187.14 was assigned to C-9 and at δ 181.02 was assigned to C-10, two carbonyl carbons of the anthraquinone moiety. The aromatic signals at δ 165.49 and 164.53 were assigned to C-8 and C-6 respectively, confirming the oxygen functionality (OH or OCH_3). The aromatic carbons at δ 155.47 and 147.20 were assigned to C-2 and C-1 respectively. The signals at δ 135.25 and 131.72 were assigned to C-10a and C-3. The aromatic carbons at δ 126.19 and 125.86 were assigned to C-4 and C-4a respectively. The signal at δ 123.86, 110.99, 107.67 and 107.19 were assigned to C-9a, C-8a, C-7 and C-5 respectively. The high field signal at δ 61.20 was assigned to OCH_3 and at δ 15.74 to a CH_3.

The ^1H NMR spectrum of the compound **63** showed signal at δ 2.34 (s, 3H) for a methyl group, for nonchelated hydroxyl group at δ 2.86 (b s, OH-2 and OH-6), at δ 3.90 (s, 3H) for a methoxy group and at δ 13.16 for a chelated (peri) hydroxy group (OH-8). Two meta-coupled aromatic protons at δ 6.56 (d, J = 2.28 Hz, H-7) and 7.16 (d, J = 2.34 Hz, H-5) and a singlet for an aromatic proton at δ 7.84 (s, H-4) was assigned to H-4.

In the HMBC spectrum, the C-10 signal at δ 181.02 showed correlation with signals at δ 7.84 (H-4) and 7.16 (H-5), the signal at δ 155.47 (C-2) correlated to CH_3-3 (δ 2.34) and H-4 (δ 7.84) signals and the signal at δ 147.20 (C-1) correlated to OCH_3-1 (δ 3.90). These data suggested the presence of three hydroxyl groups at C-2, C-6 and C-8 and the methoxy group correlated to C-1 in HMBC experiment.

Therefore, the compound **63** was identified as 2,6,8-trihydroxy-1-methoxy-3-methylanthraquinone. All the spectral data (UV, IR, ^1H NMR and ^{13}C NMR) agreed with the reported values for 2,6,8-trihydroxy-1-methoxy-3-methylanthraquinone, isolated from *Ventilago leiocarpa* Benth (Rhamnaceae).[324] However this is the first report of the

compound from the family Leguminosae. The compound **63** is also reported to significantly suppresse the growth of Vero, Wish, Calu-1, Raji and HeLa tumor cells.[324]

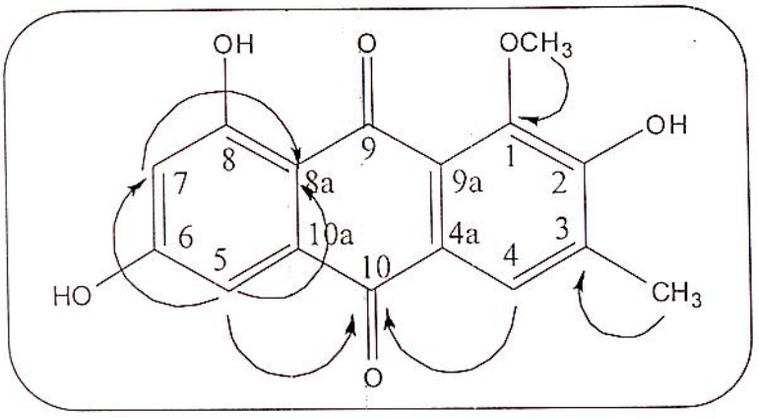
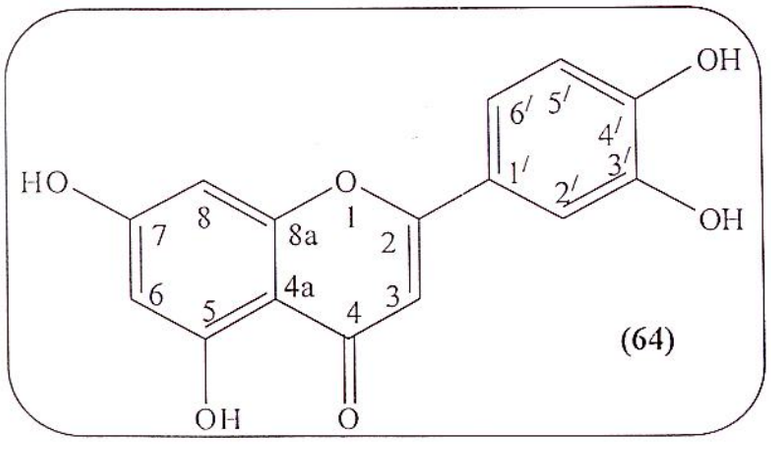


Fig. 2.15. HMBC Correlation of 2,6,8-trihydroxy-1-methoxy-3-methylanthraquinone (**63**)

2.1.4.8. 3′,4′,5,7-Tetrahydroxyflavone (luteolin) (64)



Yellow powder (Methanol); mp > 300 °C (Lit. mp 327-328 °C)[325]

Molecular formula $C_{15}H_{10}O_6$, deduced from EI mass spectra *m/z* 286 (calcd. for $C_{15}H_{10}O_6$ 286) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **64** confirmed the presence of fifteen carbon atoms in the molecule; six methine and nine quaternary carbons according to the DEPT experiments. Total assignments were achieved using HMQC, COSY and HMBC experiments (**Table 2.17**). The carbon resonating at δ 182.41 was assigned to C-4, the

carbonyl carbon of the flavone moiety. The aromatic signals at δ 165.82, 164.91 and 161.80 were assigned to C-7, C-2 and C-5 respectively, having oxygen functionality (OCH_3 or OH). The aromatic carbons resonating at δ 158.14 and 145.77 were assigned to C-8a and C-3′ respectively. The signals at δ 122.25 was assigned to C-1′. The signals at δ 118.92, 115.44 and 112.72 were assigned to C-6′, C-5′ and C-2′ respectively. The carbons at δ 103.62, 102.36 and 99.12 were assigned to C-4a, C-3 and C-6 respectively. The signal at δ 93.22 was assigned to C-8.

Table 2.17. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR* and HMBC Correlations of 3′,5′,7,8-tetrahydroxyflavone **(64)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR(δ) (J_{HH} Hz)	HMBC Correlation
2	164.91	C	-	
3	102.36	CH	6.54, s	C-4a, C-1/, C-2, C-4
4	182.41	C	-	
4a	103.62	C	-	
5	161.80	C	13.02 (s, OH)	
6	99.12	CH	6.21 (d, J = 1.6)	C-8, C-4a, C-5, C-7
7	165.82	C	-	
8	93.92	CH	6.50 (d, J = 1.6)	C-6, C-4a, C-8a, C-7
8a	158.14	C	-	
1′	122.25	C	-	
2′	112.72	CH	7.46 (d, J = 2.04)	C-2, C-4/, C-6/
3′	145.77	C	-	
4′	149.82	C	-	
5′	115.44	CH	6.94 (d, J = 8.2)	C-1′, C-3′
6′	118.92	CH	7.41 (dd, 8.4, 2.04)	C-2′, C-4′, C-2

* Solvent ^{1}H NMR in Acetone-d6 and ^{13}C NMR in Methanol-d4

The ^{1}H NMR spectrum of the compound **64** showed a sharp singlet at δ 13.02 for OH-5. Two meta-coupled aromatic protons at δ 6.21 (d, J = 1.6 Hz, H-6) and 6.50 (d, J = 1.6 Hz, H-8). One proton singlet at δ 6.54 was for H-3. The spectrum showed two aromatic ortho-

coupled protons at δ 6.94 (d, J = 8.2 Hz, H-5′) and 7.41 (dd, J = 8.2, 2.04 Hz, H-6′) and a meta-coupled proton at δ 7.46 (d, J = 2.04 Hz, H-2′).

In the HMBC spectrum, the C-4 at δ 182.41 correlated to the hydroxy group at δ 13.02 (OH-5). The signal at δ 7.46 (H-2′) showed correlation to signals at δ 161.91 (C-2), 149.82 (C-4′) and 118.92 (C-6′). The signal at δ 7.41 (H-6′) showed correlation with signals at δ 161.91 (C-2), 149.82 (C-4′) and 112.72 (C-2′). The signal at δ 6.54 (H-3′) correlated to signals at δ 182.41 C-4), 164.91 (C-2), 122.25 (C-1′) and 103.62 (C-4a). The resonance at δ 6.94 (H-5′) correlated to δ 145.77 (C-4′) and 122.25 (C-1′). The signal at δ 6.21 (H-6) correlated with δ 165.82 (C-7), 161.80 (C-5), 93.92 (C-8) and 103.62 (C-4a). The proton absorption at δ 6.50 (H-8) showed correlation to the carbons at δ 99.12 (C-8) and 103.62 (C-4a).

On the basis of these data, the compound **64** was identified as 3′,4′,5,7-tetrahydroxyflavone (luteolin). All the spectral data (UV, IR and NMR) agreed with the reported values for 3′,4′,5,7-tetrahydroxyflavone isolated from the *Seriphidium santolium* Poljak.[326] However 1D and 2D techniques were applied for the first time.

COSY Correlation of the compound **64** is represented by dotted lines.

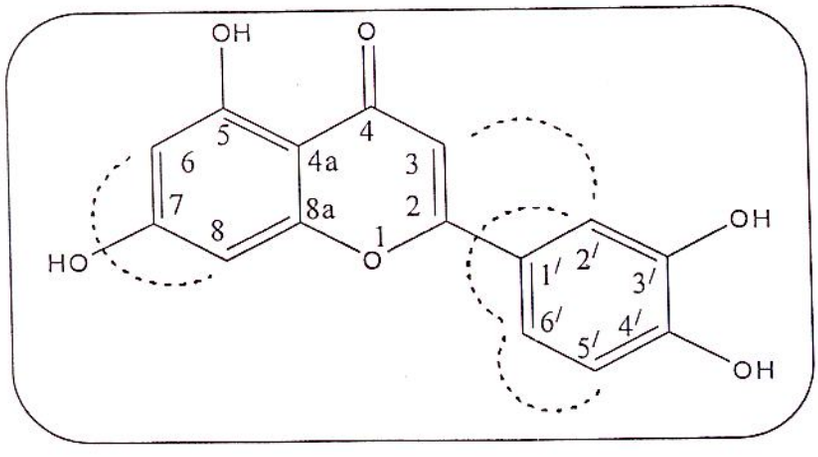


Fig. 2.16. DQF COSY Correlation of the 3′,4′,5,7-tetrahydroxyflavone (64)

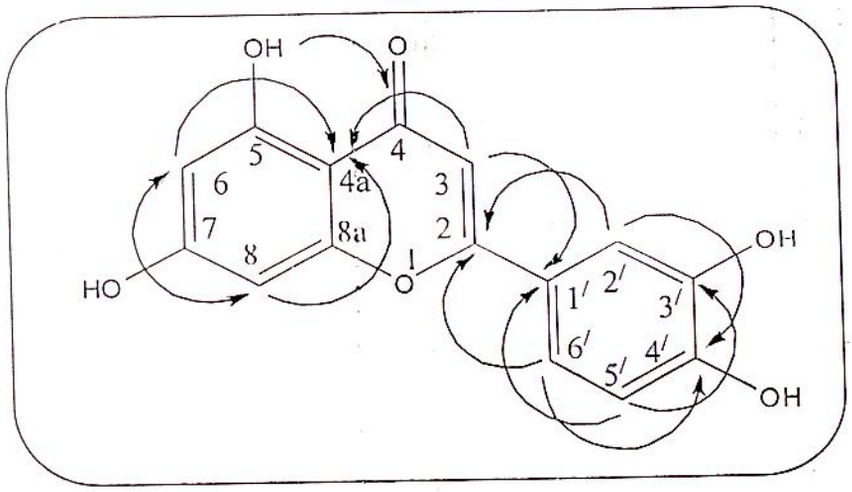
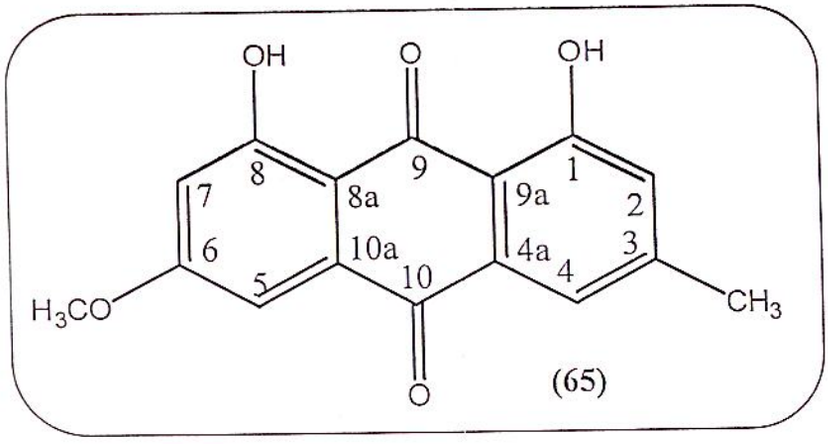


Fig. 2.17. HMBC Correlation of the 3′,4′,5,7-tetrahydroxyflavone (64)

2.1.5. New Source Isolated Compounds from the Stem Bark of *Cassia artemisioides*

2.1.5.1. 1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (65)



Reddish needles (Chloroform); m. point 205-206 °C (Lit. mp 206-207 °C)[316]

Molecular formula $C_{16}H_{10}O_5$, deduced from EI mass spectra *m/z* 284 (calcd. for $C_{16}H_{10}O_5$; 284) and the ^{13}C NMR

Table 2.18. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in CD_3OD and HMBC Correlations of 1,8-dihydroxy-6-methoxy-3-methylanthraquinone **(65)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)
1	162.30	C	-
2	124.19	CH	7.14, s
3	148.97	C	-
4	120.78	CH	7.58, s
4a	132.65	C	-
5	108.03	CH	7.26 (d, J = 2.52)
6	166.97	C	-
7	106.25	CH	6.78 (d, J = 2.28)
8	165.46	C	-
8a	110.32	C	-
9	191.12	C	-
9a	113.72	C	-
10	181.29	C	-
10a	135.45	C	-
3-CH_3	21.15	CH_3	2.45 (3H, s, CH_3)
6-OCH_3	55.98	CH_3	3.99 (3H, s, OCH_3)

The ^{13}C NMR spectrum of the compound **65** confirmed the presence of sixteen carbon atoms in the molecule; one methyl, one methoxy, four methine and ten quaternary carbons from the DEPT experiments. Total assignments were established according to HMQC, HMBC and COSY experiments (**Table 2.18** and **figure 2.18**). The carbon at δ 191.12 was assigned to C-9 and the one at δ 181.29 was assigned to C-10, these two indicating the carbonyl carbons of the anthraquinone moiety. The aromatic carbons at δ 166.97, 165.46 and 162.30 were assigned to C-6, C-8 and C-1 respectively. The aromatic signals at δ 148.97 and 135.45 were assigned to C-3 and C-10a respectively. The signals at δ 132.65 and 124.19 were attributed to C-4a and C-2 respectively. The signals δ 120.78, 113.72 and

110.32 were assigned to C-4, C-9a and C-8a respectively. The signals at δ 108.03 and 106.25 were assigned to C-5 and C-7 respectively. The high field signal at δ 55.97 and 21.15 were assigned to the OCH_3 group at C-6 and methyl group at C-3 respectively.

The ^1H NMR spectrum of the compound **65** showed signals at δ 2.45 (s, 3H, CH_3-3) for a methyl group, at δ 3.99 for a methoxy (s, 3H, OCH_3-6). Two meta-coupled protons at δ 6.78 (d, J = 2.52 Hz, H-7) and 7.26 (d, J = 2.52 Hz, H-5). Two aromatic singlets at δ 7.14 (1H, s, H-2) and at 7.58 (1H, s, H-4) were attributed to H-2 and H-4 respectively.

On the basis of these data, the compound **65** was identified as 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (physcion). The UV, IR, ^1H NMR and ^{13}C NMR data agreed with the reported values for 1,8-dihydroxy-6-methoxy-3-methylanthraquinone, isolated from *Cassia siamea* [319] and Rhubarb. [318] However 1D and 2D techniques were applied for the first time.

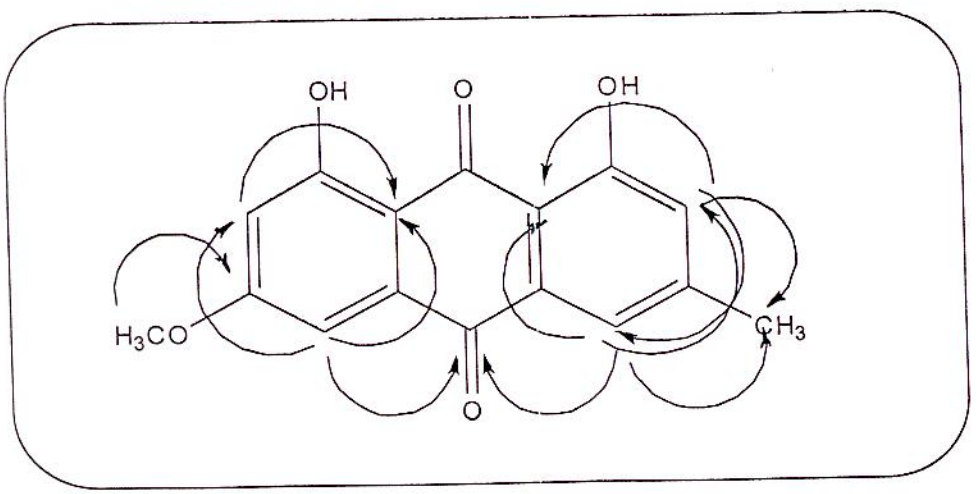
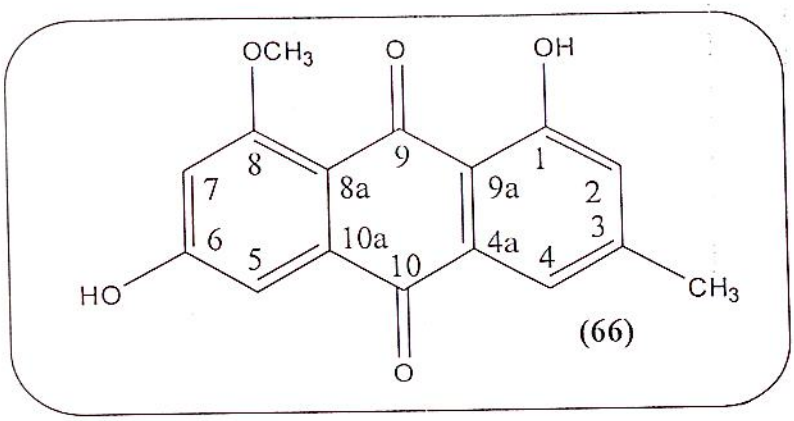


Fig. 2.18. HBMC Correlation of 1,8-dihydroxy-6-methoxy-3-methylanthraquinone **(65)**

2.1.5.2. 1,6-Dihydroxy-8-methoxy-3-methylanthraquinone (66)



(66)

Yellow needles (Acetone); mp 300-302 °C (Lit. mp 301-303 °C)[320]

Molecular formula $C_{16}H_{12}O_5$, deduced from EI mass spectra *m/z* 284 (calcd. 284 for $C_{16}H_{12}O_5$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **66** confirmed the presence of sixteen carbons atoms in the molecule; a methyl, a methoxy, four methine and ten quaternary carbons from the DEPT experiments. Total assignments were established from HMQC and HMBC experiments (**Table 2.19**).

In the ^{13}C NMR the carbon at δ 186.93 was assigned to C-9 and the one at δ 182.41 was assigned to C-10, the two carbonyl of the anthraquinone moiety. The aromatic carbons at δ 164.50, 163.99 and 162.64 were assigned to C-8, C-6 and C-1 respectively, having oxygen functionality (OH or OCH_3). The aromatic carbons at δ 146.73 and 137.58 were assigned to C-3 and C-1 respectively. The signals at δ 132.63, 124.33 and 119.07 were assigned to C-4a, C-2 and C-4 respectively. The signals at δ 114.82, 113.62, 106.97 and 104.84 were assigned to C-9a, C-8a, C-5 and C-7 respectively. The high-field signal at δ 55.89 was assigned to the OCH_3 and the one at δ 20.97 to CH_3 group.

The ^1H NMR spectrum of the compound **66** showed signals at δ 2.40 (s, 3H) for a methyl group, at δ 3.95 (s, 3H) for a methoxy group and at δ 13.31 for chelated hydroxy group (OH-1). One broad peak at δ 2.97 was assigned to OH-3. Two meta-coupled aromatic protons at δ 6.88 (d, J = 2.3 Hz, H-7) and 7.30 (d, J = 2.3 Hz, H-5) and two singlets for

aromatic protons at δ 7.06 (s, H-2) and at δ 7.45 (s, H-4).

Table 2.19. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in CD_3OD and HMBC Correlations of 1,6-dihydroxy-8-methoxy-3-methylanthraquinone **(66)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.64	C	13.31 (s, OH)	OH, C-1
2	124.33	CH	7.06, s	C-9a, C-4
3	146.73	C	-	CH_3 (20.97)
4	119.07	CH	7.45, s	C-2, C-9a
4a	132.63	C	-	
5	106.97	CH	7.30 (d, J = 2.3)	C-7, C-8a
6	163.99	C	2.97 (b s, OH)	
7	104.84	CH	6.88 (d, J = 2.3)	C-5, C-8a
8	164.50	C	-	
8a	113.62	C	-	
9	186.93	C	-	
9a	114.82	C	-	
10	182.41	C	-	C-4, C-5
10a	137.58	C	-	
3-CH_3	20.97	CH_3	2.40 (3H, s, CH_3)	C-2, C-3, C-4
8-OCH_3	55.89	CH_3	3.95 (3H, s, OCH_3)	C-8

In the HMBC spectrum, the C-10 signal at δ 182.41 showed correlation with the protons at δ 7.45 (H-4) and 7.30 (H-5), the signal at δ 164.50 (C-8) correlated with the OCH_3 (δ 3.95) and H-4 (δ 7.45) signals and the signal at δ 162.64 (C-1) correlated with the OH (δ 13.31). The signal at δ 114.82 (C-9a) showed correlation to the signals at δ 7.06 (H-2) and 7.45 (H-4). The resonance at δ 113.63 (C-10a) correlated to signals at δ 6.88 (H-7) and 7.30 (H-5).

These data suggested the presence of two hydroxy groups located at C-1 and C-6 and a methoxy group at C-8. Therefore compound **66** was identified as 1,6-dihydroxy-8-methoxy-3-methylanthraquinone (Questin). All the data (UV, IR, ^{1}H NMR and ^{13}C NMR) agreed with the reported values for 1,6-dihydroxy-8-methoxy-3-methylanthraquinone isolated from the fungi *Microascus tardifasiens*[320] and *penicillium frequentans.*[321] However this is reported for the first time from plants and reporting the 2D NMR techniques.

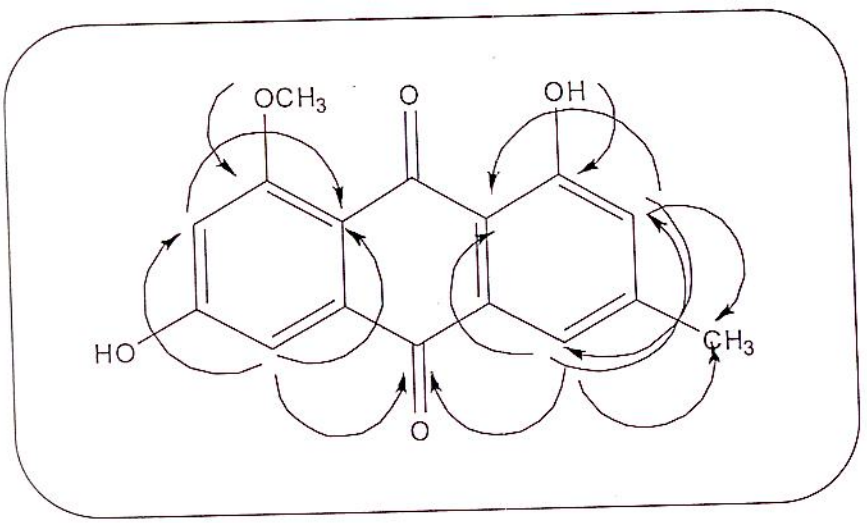
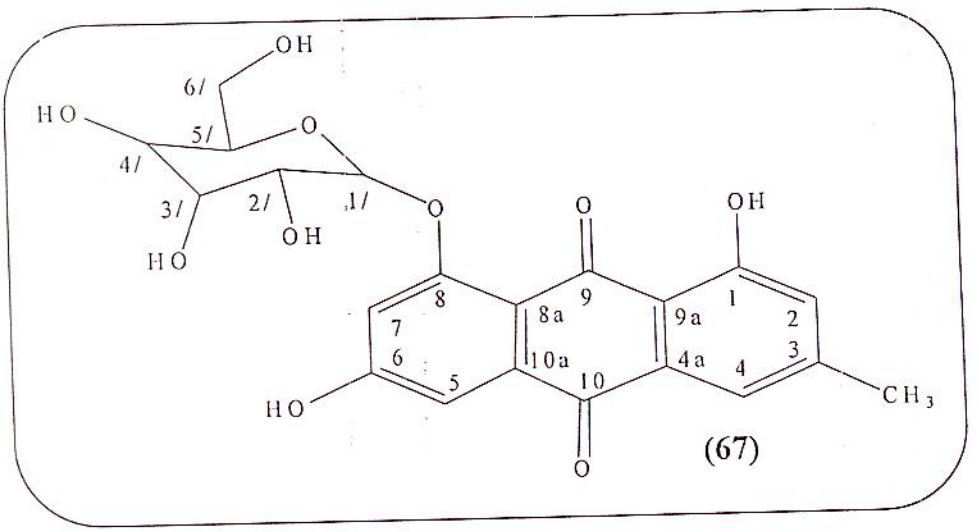


Fig. 2.19. HMBC Correlation of 1,6-dihydroxy-8-methoxy-3-methylanthraquinone **(66)**

2.1.5.3. Emodin-8-*O*-*β*-*D*-glucopyranoside (67)



(67)

Yellow powder (Methanol), mp 221-222 °C (Lit. mp 219-220 °C)[327]

Molecular formula $C_{21}H_{20}O_{10}$, deduced from EI mass spectra *m/z* 432 (calcd. 432 for $C_{21}H_{20}O_{10}$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **67** confirmed the presence of twenty one carbon atoms in the molecule; one methyl, nine methine, one methylene and ten quaternary carbons from the DEPT experiments. Total assignments were made by HMQC, COSY and HMBC experiments (**Table 2.20**).

In ^{13}C NMR the carbon resonating at δ 186.95 was assigned to C-9 while the one at δ 182.68 was assigned to C-10, these are the two carbonyl of the anthraquinone moiety. The aromatic signals at δ 162.26, 161.63 and 158.79 indicating C-O, were assigned to C-6, C-1 and C-8, indicating the presence of oxygen functionality. The aromatic carbons resonating at δ 148.42 and 137.05 were assigned to C-3 and C-10a respectively. The signal at δ 132.66 was assigned to C-4a and at δ 124.71 to C-2. The signals at δ 119.78, 115.04 and 115.05 were assigned to C-4, C-9a and C-8a respectively. The signals at δ 108.92 and 108.92 were assigned to C-5 and C-7 respectively. The high field signal at δ 21.92 was assigned to a CH_3 attached to C-3.

Table 2.20. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in DMSO-d6 and HMBC Correlations of emodin-8-*O*-*β*-*D*-glucopyranoside **(67)**

C. No	^{13}C NMR(δ)	Multiplicity	^{1}H NMR(δ) (J_{HH} Hz)	HMBC Correlation
1	161.63	C	13.14, s (OH)	
2	124.71	CH	7.12, s	C-4, C-3, C-9a
3	148.42	C	-	
4	119.78	CH	7.43, s	C-2, C-3, C-9a
4a	132.66	C	-	
5	108.92	CH	7.25 (d, J = 2.34)	C-7, C-6, C-8a
6	162.26	C	-	
7	108.92	CH	6.96 (d, J = 2.28)	C-7, C-6, C-8a, C-8
8	158.79	C	-	
8a	115.04	C	-	
9	186.95	C	-	
9a	115.04	C	-	
10	182.68	C	-	
10a	137.05	C	-	
3-CH_3	21.92	CH_3	2.42 (3H, s, CH_3)	C-2, C-3, C-4
1′	101.83	CH	5.01 (dd, J = 7.80, 3.2, H-1′)	C-8, C-2″
2′	73.84	CH	3.22, 3.24 (m, H-2′)	
3′	76.90	CH	3.66 (m, H-3′)	
4′	70.04	CH	3.37 (m, H-4′)	
5′	77.86	CH	3.46 (m, H-5′)	
6′	61.15	CH_2	4.55 (1H, dd, J = 4.5, 9.5, H-6′), 3.30 (1H, m, H-6′)	

The ^{1}H NMR spectrum of the compound **67** showed signals at δ 2.42 (s, 3H) for a methyl group, a broad peak at δ 3.38 for an OH (OH-6) and a sharp singlet at δ 13.14 for a chelated hydroxy group (OH-1) (D_2O exchangeable). Two meta-coupled aromatic protons at δ 6.96 (d, J = 2.3 Hz, H-7) and 7.25 (d, J = 2.3 Hz, H-5) were assigned to H-7 and H-5 respectively. Two singlets for aromatic protons at δ 7.12 (s, H-2) and 7.43 (s, H-4).

The ^{1}H NMR of the compound **67** in DMSO-d6 showed the presences of sugar moiety. Theprotons at δ 5.01 (1H, dd, J = 7.8, 3.2 Hz, H-1′), 4.55 (1H, dd, J = 9.5, 4.5 Hz, H-6′a) and 3.30 (1H, m, H-6′b) were assigned to H-1′ and H-6′a and H-6′b respectively. The signals at δ 3.66 (1H, m, H-3′), 3.37 (1H, m, H-4′) and 3.22 (1H, m, H-2′) were assigned to H-3′, H-4′ and H-2′ respectively.

In the HMBC spectrum, the C-9 resonance at δ 186.95 correlated to the hydroxy group at δ 13.14 (OH-1). The C-10 signal at δ 182.68 showed correlation with the signals at δ 7.43 (H-4) and 7.25 (H-5), the signal at δ 108.92 (H-7) correlated to signals at δ 115.04 (C-8a) and 108.92 (C-5). The signal at δ 7.25 (H-5) correlated to δ 108.92 (C-7), 115.05 (C-8a) and 182.68. The signal at δ 7.12 (H-2) correlated with signals at δ 119.78 (C-4) and 115.04 (C-9a). The resonance at δ 7.43 (H-4) correlated with signals at δ 124.71 (C-2) and 115.04 (C-9a). The high field signal at δ 2.46 correlated to signal at δ 148.42 (C-3).

The COSY spectrum showed correlation due to *meta* coupling between H-2 and H-4, while H-5 showed correlation to H-7.

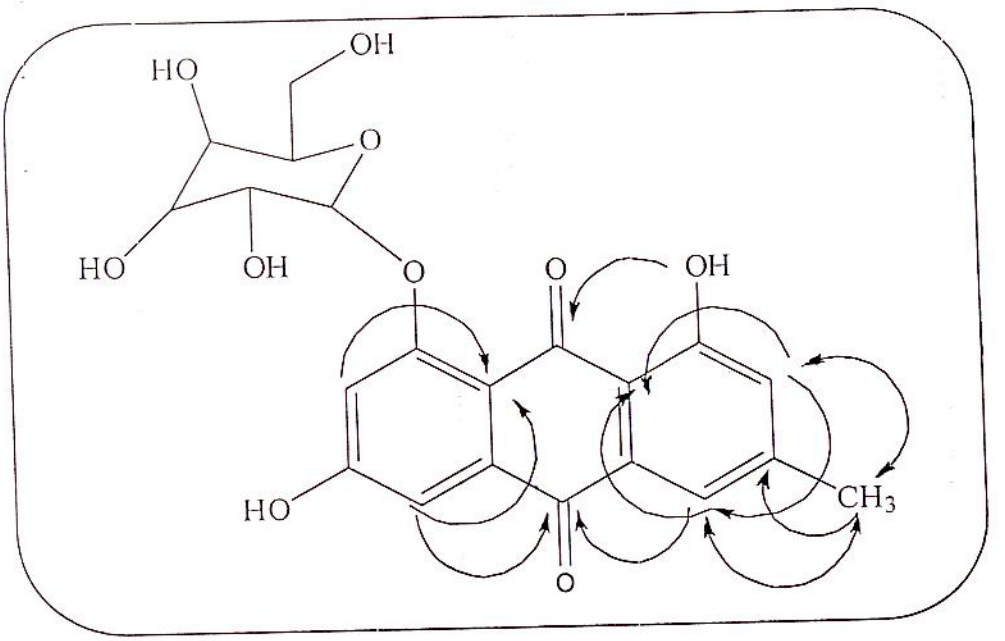
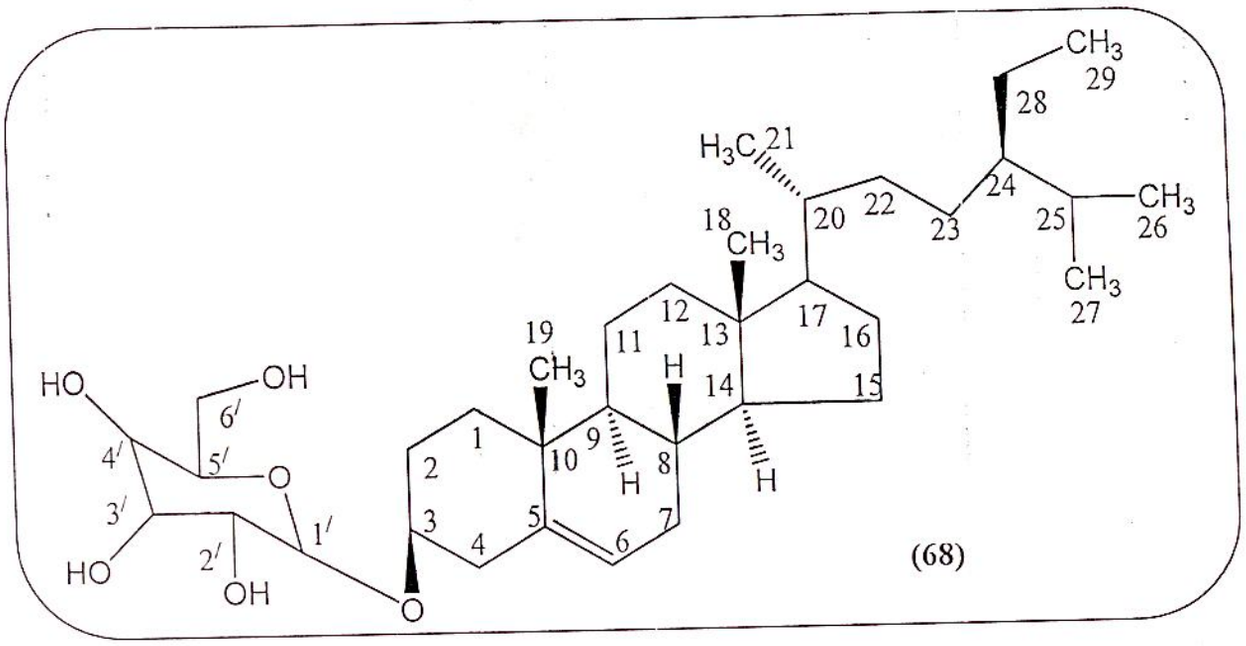


Fig. 2.20. HMBC Correlation of emodin-8-*β*-*D*-glucopyranoside (67)

On the basis of above data, compound **67** was identified as 1,6,dihydroxy-3-methly-anthraquinone-8-*β*-*D*-glucopyranoside or emodin-8-*β*-*D*-glucopyranoside. All the spectral data (UV, IR, ^{1}H NMR and ^{13}C NMR) agreed with the reported values for the compound isolated from Rhubarb[316] and flower of *Reynoutria sachalinensis*[327] (Polygonaceae). However this is isolated for the first time from the genus *Cassia* and the structure was confirmed by using 1D and 2D NMR techniques for the first time.

2.1.5.4. β-Sitosterol-3-*O*-β-D-glucopyranoside (68)



(68)

White powder; mp 236-238 °C (Lit. 232-235 °C)[328]

Molecular formula $C_{35}H_{50}O$, established from the FABMS showed the M^+ at *m/z* 576.43 (calcd. $C_{35}H_{50}O$, 576.43) and the ^{13}C NMR.

The IR spectrum of the compound **68** showed absorption at $\tilde{\nu}$ 3450 cm^{-1} for a hydroxyl group, at $\tilde{\nu}$ 3040 cm^{-1} for CH st. olefinic, $\tilde{\nu}$ 1618, 1596 cm^{-1} for C=C St. olefinic.

The ^{13}C NMR spectrum showed thirty five carbons in the broad band and DEPT experiments including, six methyl, twelve methylene, fourteen methine and three quaternary carbons. The aglycone part gives characteristic peaks at δ 140.73 and 121.78, were assigned to C-5 and C-6 respectively. The signals at δ 78.38, 56.65, 56.01 and 50.17 were attributed to C-3, C-14, C-17 and C-9 respectively. The carbon resonance at δ 45.86, 42.31, 39.77, 39.17 and 37.31 were assigned to C-24, C-13, C-4, C-12 and C-1 respectively. The signal at δ 36.76 was assigned to C-10, at 36.23 to C-20 and 34.03 to C-22. The carbons resonating at δ 32.01, 31.88, 30.09, 29.28 and 28.39 were assigned to C-7, C-8, C-2, C-25 and C-16 respectively.

Table 2.21. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in C_5D_5N and HMBC Correlations of β-sitosterol-3-*O*-β-D-glucopyranoside **(68)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	37.31	CH_2	1.02, 1.64 (2H, m)	C-3, C-5, C-19
2	30.09	CH_2	1.19, m, 1.83, m	
3	78.38	CH	3.94, m	
4	39.77	CH_2	2.66 (2H, dd, J = 13.32, 2.1) 2.39 (2H, t, J = 11.46)	C-2, C-3, C-5, C-6, C-10
5	140.73	C	-	
6	121.78	CH	5.29 (d, J = 1.74)	C-4, C-8, C-10
7	32.01	CH_2	1.64, 1.89 (2H, m)	
8	31.88	CH	1.62, m	
9	50.17	CH	0.84, m	
10	36.76	C	-	
11	21.12	CH_2	1.47, (2H, m) 1.36 (2H, m)	
12	39.17	CH_2	1.89 (2H, dd, J = 12.12, 6.9) 1.17, m	
13	42.31	C	-	
14	56.65	CH	1.03, m	
15	24.35	CH_2	1.47 (2H, m)	
16	28.39	CH_2	1.20 (2H, m) 1.84 (2H, m)	
17	56.01	CH	1.02, m	
18	11.81	CH_3	0.58 (3H, s)	C-12, C-13, C-14, C-17
19	19.27	CH_3	0.99 (3H, s)	C-1, C-9, C-10
20	36.23	CH	1.32, m	
21	18.85	CH_3	0.91 (3H, d, J = 6.54)	C-17, C-20, C-22
22	34.03	CH_2	1.02, 1.28 (2H, m) 1.28 (2H, m)	

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
23	26.19	CH_2	1.17 (2H, m)	
24	45.86	CH	0.92, m	
25	29.28	CH	1.66, m	
26	19.04	CH_3	0.78 (3H, d, J = 6.8)	C-24, C-25, C-27
27	19.83	CH_3	0.81 (3H, d, J = 7.0)	C-24, C-25, C-26
28	23.21	CH_2	1.30, 1.28 (2H, m)	
29	12.00	CH_3	0.82 (3H, t, J = 7.32)	C-24, C-28
1′	102.40	CH	5.0 (d, J = 7.8)	C-3
2′	75.20	CH	4.01 (dd, J = 7.8, 5.7)	
3′	78.47	CH	4.26 (t, J = 5.7)	
4′	71.51	CH	4.25 (t, J = 5.7)	
5′	77.89	CH	3.94, m	
6′	62.66	CH_2	4.51 (1H, d, J = 11.46)	
			4.38 (1H, dd, J = 11.46, 5.76)	

The signals at δ 26.19, 24.35, 23.21 and 21.12 were attributed to C-23, C-15, C-28 and C-11 respectively. The high-field signals at δ 19.83, 19.27, 19.04, 18.85, 12.0 and 11.81 were assigned to C-27, C-19, C-26, C-21, C-29 and C-18 respectively.

In the ^{1}H NMR (C_5D_5N) the presence of two singlets for methyl group at δ 0.58 (s, H-18) and 0.99 (s, H-19), three methyl doublets at δ 0.91 (d, J = 6.54 Hz, H-21), 0.81, (d, J = 7.0 Hz, H-27) and 0.78 (d, J = 6.8 Hz, H-26). One methyl triplet at δ 0.82 (t, J = 7.32 Hz, H-29), an olefinic proton broad doublet at δ 5.29 (J = 1.74 Hz, H-6) and a multiplet at δ 3.94 (H-3). The large coupling constant of H-3 represents that the O-glycoside group at C-3 is β-oriented.[313] The anomeric configuration of glucose was established from J H1-H-2 splitting (7.8 Hz) and the sugar part was also confirmed from the ^{13}C NMR values as glucose.[314] The aglycone part was confirmed to be *β*-sitosterol by comparing ^{13}C NMR data for β-sitosterol.[311, 312] From the above spectral and physical data, the structure of the compound was established as *β*-sitosterol-3-*O*-*β*-D-glucopyranoside.

The ^{1}H NMR (pyridine) of the compound **68** showed the presences of sugar moiety. The anomeric proton resonates at δ 5.0 (d, J = 7.8 Hz, H-1′), 4.51 (1H, ddd, J = 11.46 Hz, H-6′a), 4.38 (1H, dd, J = 11.46, 5.76 Hz, H-6′b), 4.26 (t, J = 5.7 Hz, H-3′), 4.25 (t, J = 5.7, H-4′), 4.01 (dd, J = 7.8, 7.5 Hz, H-2′) and 3.94, m (H-3′). All other ^{1}H NMR assignment was established by comparison with the reported data of the compound.[312]

This compound is reported from a number of species [312, 329] but no 2D NMR techniques were applied. The structure was confirmed by using 2D techniques. In HMBC experiment the resonance at δ 5.29 (H-6) showed correlation to C-4, C-8 and C-10. The resonance at δ 2.66 (H-4) correlated to C-2, C-3, C-5, C-6 and C-10. The signal at δ 1.64, and 1.03 (H-1) correlated to resonates at δ 78.38 (C-3), 140.73 (C-5) and 19.27 (C-19). The signal at δ 0.99 (H-19) correlated to δ 36.76 (C-10), 50.17 (C-9) and 140.73 (C-5) and the signal at δ 0.91 (H-21) correlated to δ 34.03 (C-22), δ 36.23 (C-20) and 56.01 (C-17). The signal at δ 0.81 (H-27) correlated to C-24, C-25, C-26 and the signal at δ 0.78 (H-26) correlated to C-24, C-25 and C-27. The resonance at δ 0.59 (H-18) correlated to resonates at δ 39.17 (C-12), 42.31 (C-13), 56.01 (C-17) and 56.65 (C-14). β-Sitosterol-3-*O*-β-D-glucopyranoside has been reported from a number of species.[312, 330] β-Sitosterol-3-*O*-β-D-glucopyranoside is also reported from *Cassia absus*.[331]

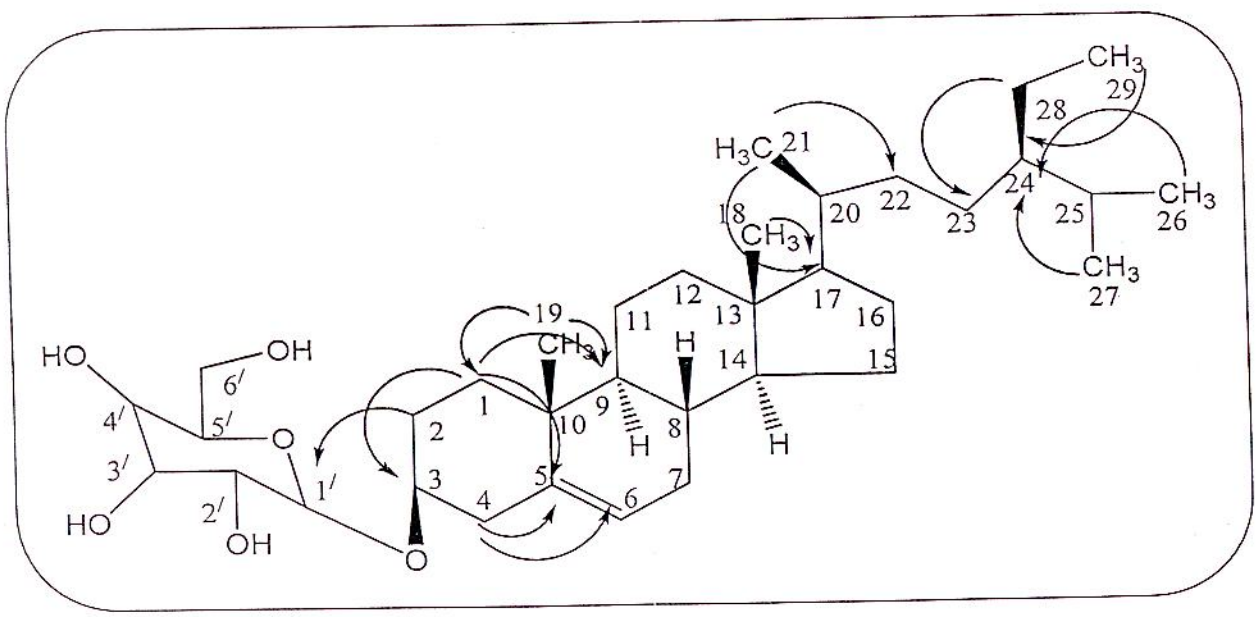
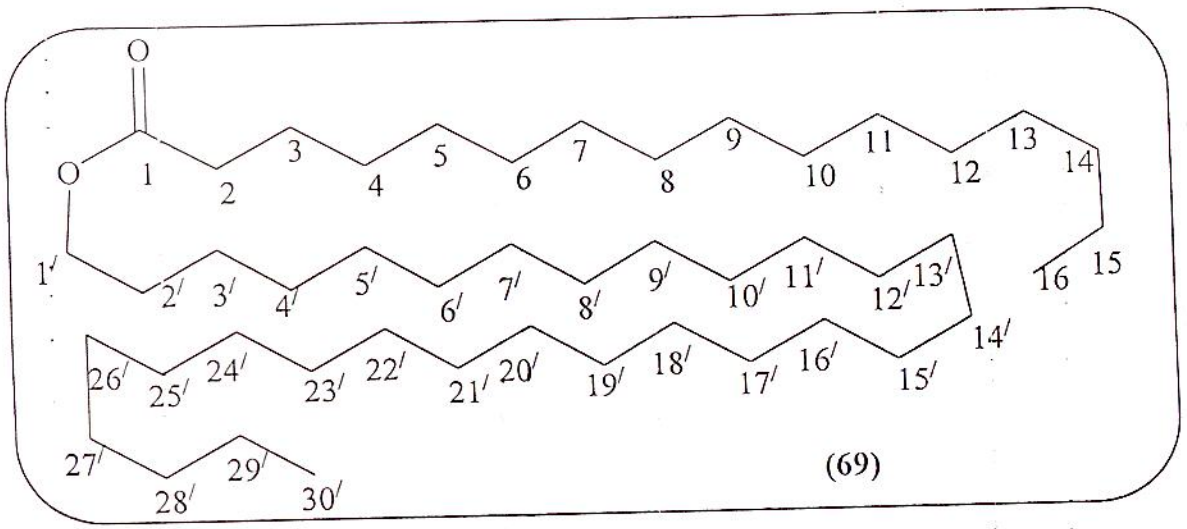


Fig. 2.21. HMBC Correlation of β-Sitosterol-3-*O*-β-D-glucopyranoside (68)

2.1.5.5. Tricontyl palmitate (69)



(69)

White powder (Chloroform) mp 78-79 °C (Not reported in litr.)

Molecular formula $C_{46}H_{92}O_2$, deduced from mass spectrum *m/z* 676 M^+ (calcd. 676 for $C_{46}H_{92}O_2$).

Table 2.22. ^1H (400 MHz) and ^{13}C (100 MHz) NMR data in $CDCl_3$ of the tricontyl palmitate (69)

C. No.	^{13}C NMR(δ)	Multiplicity	^1H NMR (δ) (J_{HH} Hz)
1	174.00	C	-
1′	64.39	CH_2	4.02 (2H, t, J = 6.6)
2	34.43	CH_2	2.26 (2H, t, J = 7.5)
3	31.92	CH_2	1.59, m
3-16 and 2′-29′	29.68- 22.67	CH_2	1.23 (80H, m)
16, 30′	14.09	CH_3	0.86 (6H, t, J = 6.2)

The IR absorption at $\tilde{\nu}$ 1732 cm^{-1} showed the presence of C=O st., for an ester group.

In the ^1H NMR, a triplet at δ 4.02 (2H, t, J = 6.6 Hz) was due to $CH_2\underline{CH_2}OCO$), a triplet at δ 2.26 (2H, t, J = 7.5 Hz) was a characteristic peak for a $CH_2\underline{CH_2}CO$) group and a multiplet at δ 1.59 was a characteristic for a $CH_2\underline{CH_2}CH_2CO$). The chemical shifts at δ 1.23 (42H) for 21 CH_2 groups and a six protons triplet at δ 0.86 (6H, t, J = 6.2 Hz) for the two terminal CH_3 groups.

The ^{13}C NMR spectrum confirmed it as a long chain ester and the mass spectrum also showed a characteristic pattern for a straight chain aliphatic compound. The signal at δ 174.00 was assigned to the ester carbonyl (C-1) and the signal at δ 64.39 was assigned to CH_2 (C-1′). The resonance at δ 34.43 and 31.92 were assigned to C-2 and C-2′ respectively. The signals from δ 29.69 to 22.68 were assigned to C-3 and C-3′ to C-15 and C-29. The signal at δ 14.08 was assigned to two terminal CH_3 groups (C-16 and C-30). The position of carbonyl group was established from the mass fragment ion at 256.9 due to the cleavage of ester bond. From the data the compound was identified as tricontyl palmitate, reported from *Symplocos racemosa* Roxb (Symplocaceae).[332] This is the first report from family Leguminosae.

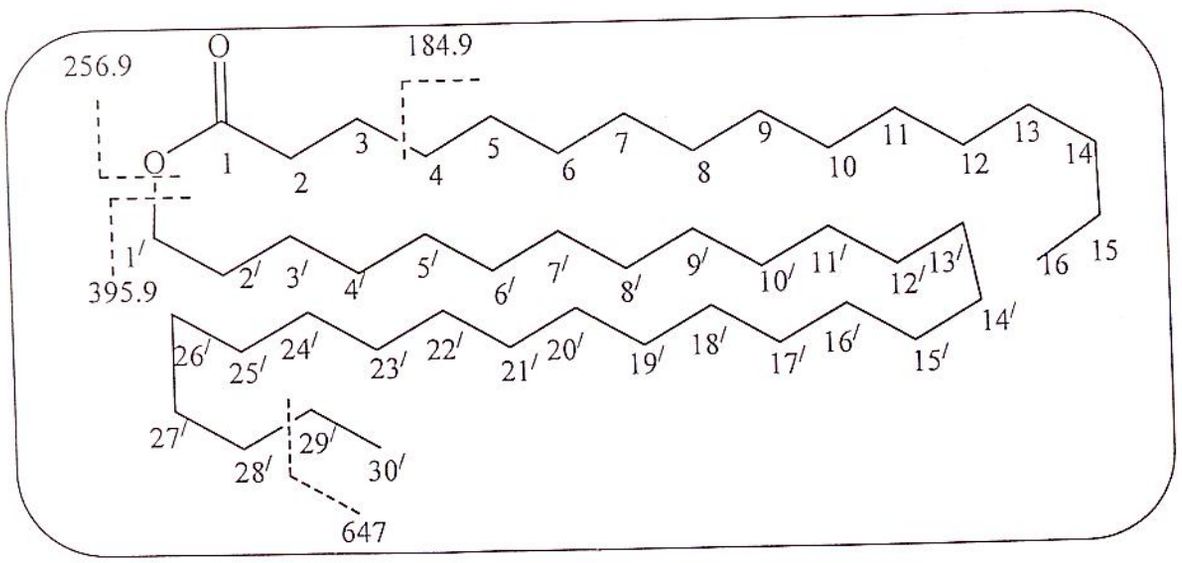
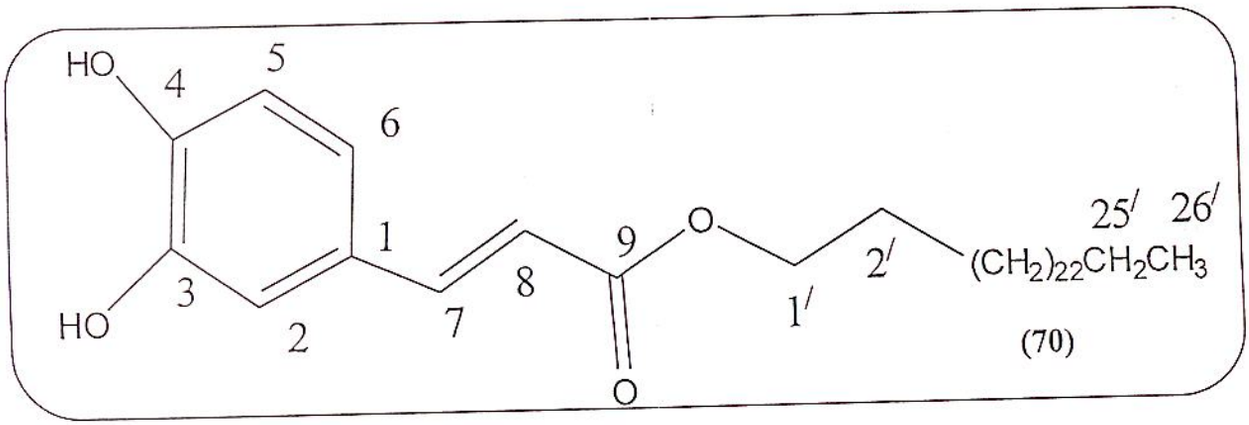


Fig. 2.22. Mass fragmentation tricontyl palmitate **(69)**

2.1.5.6. Hexacosanyl caffeate (70)



(70)

White powder; mp 94-95 °C (mp not reported)

The molecular formula $C_{35}H_{60}O_4$ was deduced from ^1H NMR, ^{13}C NMR and mass spectrum *m/z* 544 (calcd. 544 for $C_{35}H_{60}O_4$).

Table 2.23. ^1H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of hexacosanyl caffeate **(70)**

C No.	^{13}C NMR(δ)	Multiplicity	^1H NMR(δ) (J_{HH} Hz)
1	127.81	C	
2	114.41	CH	7.05 (d, J = 1.6)
3	143.71	C	5.65, s (OH)
4	146.07	C	5.55, s (OH)
5	115.55	CH	6.84 (d, J = 8.28)
6	122.39	CH	6.98 (d, J = 8.28)
7	144.46	CH	7.52 (d, J = 15.88)
8	116.04	CH	6.22 (d, J = 15.88)
9	167.62	C	
1′	64.88	CH_2	4.17 (2H, t, J = 6.84)
2′	28.81	CH_2	1.66 (2H, pentet, J = 6.84)
3′	26.05	CH_2	1.35 (2H, m)
4′-25′	22.68-31.96	CH_2	1.23 (44H, m)
26′	14.09	CH_3	0.86 (3H, t, J = 7.07)

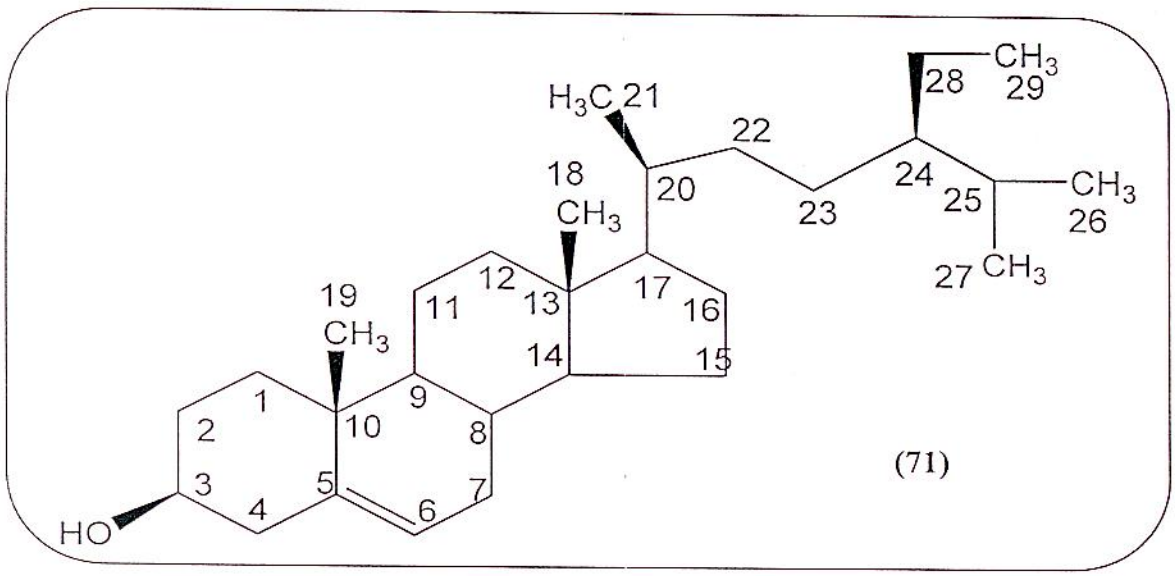
The ^{13}C NMR spectrum of the compound **70** indicated the presence of a dihydroxy cinnamic acid derivative with a carbonyl signal at δ 167.62. The aromatic region contains two oxygen deshielded quaternary carbons at δ 146.07 and 143.71 were attributed to C-3 and C-4 respectively. The signal at δ 144.46, 127.81 and 122.39 were assigned to C-7, C-1 and C-6 respectively. The resonances at δ 116.04, 115.55 and 114.41 were attributed to C-8, C-5 and C-2 respectively. The oxygen deshielded signal at δ 64.88 was assigned to C-1′ and the one at 28.81 was assigned to C-2′. The signals from δ 29.74 to 29.33 were attributed to C-4′ to C-23′ and the one at δ 31.96 was assigned to C-24′. The high field signals at δ 22.68 and 14.10 were assigned to C-25′ and the terminal methyl group C-26′.

The ^{1}H NMR spectrum of the compound **70** showed two *trans* protons signals at δ 7.52 (1H, d, J = 15.88 Hz) and 6.22 (1H, d, J = 15.88 Hz), assigned to H-7 and H-8. The signals at δ 7.05 (1H, d, J = 1.6 Hz), 6.98 (1H, dd, J = 8.28, 2.0 Hz) and 6.84 (1H, d, J = 8.28 Hz) were assigned to H-2, H-6 and H-5 respectively. Two broad singlets at δ 5.65 (s, OH) and 5.55 (s, OH) were assigned to OH at C-3 and C-4 respectively. The two protons triplet at δ 4.17 (2H, t, J = 6.84 Hz) was assigned to the ester methylene group (H-1′). The signal at δ 1.66 (2H, quintet, J = 6.84 Hz) was assigned to H-2′ and the multiplet at δ 1.35 was assigned to H-3′. The multiplet at δ 1.23 (42 H, m) with an integration for forty two protons was assigned to H-4′-H-25′. Three proton triplet at δ 0.87 (3H, t, J = 7.07 Hz) was attributed to the terminal methyl group (H-26′).

From the spectral data (UV, IR, ^{1}H NMR and ^{13}C NMR and mass spectrum) the compound was identified as hexacosanyl caffeate. It was isolated as a mixture form *Acer triflorum* KOM. (Aceraceae).[333] We have reported it from *Cassia artemisioides* bark for the first time in the pure form and reporting the spectral data (UV, IR, ^{1}H NMR and ^{13}C NMR and mass spectrum).

2.1.6. New Source Isolated Compounds from the Roots of *Cassia artemisioides*

2.1.6.1. β-Sitosterol (71)



(71)

White powder; mp 144-145 °C (Lit. 136-137 °C)[328]

Molecular formula $C_{29}H_{50}O$, established from ^{13}C NMR and DEPT and HREIMS. The mass spectrum showed molecular ion peak at 414 (calcd. for $C_{29}H_{50}O$, 414).

The IR spectrum of the compound **71** showed absorption for hydroxyl group at $\tilde{\nu}$ 3440 cm^{-1}, a trisubstituted double bonds at $\tilde{\nu}$ 3045 cm^{-1} CH st. olefinic and 1625 cm^{-1} for C=C St. olefinic st.

The ^{1}H-^{13}C connectivities were determined by the HMQC spectrum, while the long range ^{1}H-^{13}C correlations were assigned through the HMBC technique.

The ^{13}C NMR spectrum of the compound **71** showed twenty nine carbons in the broad band spectrum; including six methyl, eleven methylene, nine methine and three quaternary carbons. The down field signal at δ 140.84 and 121.82 were assigned to C-5 and C-6 respectively. The signals at δ 71.90, 56.84, 56.12 and 50.20 were attributed to C-3, C-14, C-17 and C-9 respectively.

Table 2.24. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of β-sitosterol **(71)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	37.33	CH_2	1.07 (2H, dd, J = 3.48, 6.12)	C-3, C-5, C-19
2	31.75	CH_2	1.23, 1.48, m	
3	71.90	CH	3.52, m	
4	42.38	CH_2	2.00 (2H, t, J = 9.00)	C-2, C-3, C-5, C-6,
			1.98 (d, J = 3.12)	C-10
5	140.84	C	-	-
6	121.82	CH	5.34 (d, J = 2.34)	C-4, C-8, C-10
7	32.00	CH_2	1.44, 1.93, m	
8	31.98	CH	1.42, m	
9	50.20	CH	0.84, m	
10	36.59	C	-	
11	21.16	CH_2	1.49, 1.47, m	
12	42.38	CH_2	2.19 (d, J = 2.1)	-
			2.25 (dd, J = 2.1,3.0)	
13	39.85	C	-	-
14	56.84	CH	0.96, m	
15	24.39	CH_2	1.56, m	
16	28.34	CH_2	1.23, m	
17	56.12	CH	1.07 (dd, J = 7.8, 3.48)	
18	11.95	CH_3	0.66, s	C-12, C-13, C-14,
				C-17
19	19.49	CH_3	0.99, s	C-1, C-9, C-10
20	36.23	CH	1.34, m	
21	18.86	CH_3	0.90 (3H, d, J = 5.26)	C-17, C-20, C-22
22	34.01	CH_2	1.27, 1.55, m	
23	26.12	CH_2	1.14,m	
24	45.90	CH	0.92, m	
25	29.20	CH	1.25, m	

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
26	19.11	CH_3	0.79 (3H, d, J = 6.8)	C-24, C-25, C-27
27	19.91	CH_3	0.81 (3H, d, J = 5.58)	C-24, C-25, C-26
28	23.13	CH_2	1.22, 1.25 (2H, m)	
29	12.07	CH_3	0.82 (3H, t, J = 7.5)	C-24, C-28

The carbon resonances at δ 45.90, 42.38, 42.38, 39.85 and 37.33 were assigned to C-24, C-13, C-4, C-12 and C-1 respectively. The signal at δ 36.59 was assigned to C-10, δ 36.23 to C-20 and δ 34.01 to C-22. The signals at δ 32.00, 31.98, 31.75, 29.20 and 28.34 were assigned to C-7, C-8, C-2, C-25 and C-16 respectively. The signals at δ 26.12, 24.39, 23.13 and 21.16 were attributed to C-23, C-15, C-28 and C-11 respectively. The up-field signals at δ 19.91, 19.49, 19.11, 18.86, 12.07 and 11.95 were assigned to C-27, C-19, C-26, C-21, C-29 and C-18 respectively.

All ^{1}H NMR assignments were established by comparison with the reported data for the *β*-sitosterol.[311, 312] The presence of two methyl groups at δ 0.66 (s, H-18) and 0.99 (s, H-19), three methyl doublets at δ 0.91 (d, J = 5.26 Hz, H-21), 0.80 (d, J = 5.58 Hz, H-27) and 0.79 (d, J = 6.8 Hz, H-26). One methyl triplet at δ 0.82 (t, J = 7.5 Hz, H-29) an olefinic proton broad doublet at δ 5.34 (d, J = 2.34 Hz. H-6) and a multiplet at δ 3.52 (m, H-3).

The IR, NMR and mass spectrum and the physical data of the compound **71** were comparable to the published data for *β*-sitosterol.[311, 312]

The structure was further confirmed by using 2D NMR techniques. In HMBC experiment the resonance at δ 5.34 (H-6) showed correlations to C-4, C-8 and C-10. The resonance at δ 2.0 (H-4) correlated with C-2, C-3, C-5, C-6 and C-10. The signal at δ 1.07 (H-1) correlated with resonates at δ 71.90 (C-3), 140.84 (C-5) and 19.49 (C-19). The signal at δ 0.99 (H-19) correlated to δ 36.59 (C-10), 50.20 (C-9), 140.84 (C-5) and the signal at δ 0.91 (H-21) correlated to δ 34.01 (C-22), 36.23 (C-20) and 56.01 (C-17). The signal at δ 0.81 (H-27) correlated to C-24, C-25, C-26 and the signal at δ 0.78 (H-26) correlated to C-24, C-25 and C-27. The protons at δ 0.59 (H-18) correlated to resonates at δ 39.17 (C-12),

42.31 (C-13), 56.12 (C-17) and 56.84 (C-14).

This is the first report of the 2D NMR of β-sitosterol **(71)**.

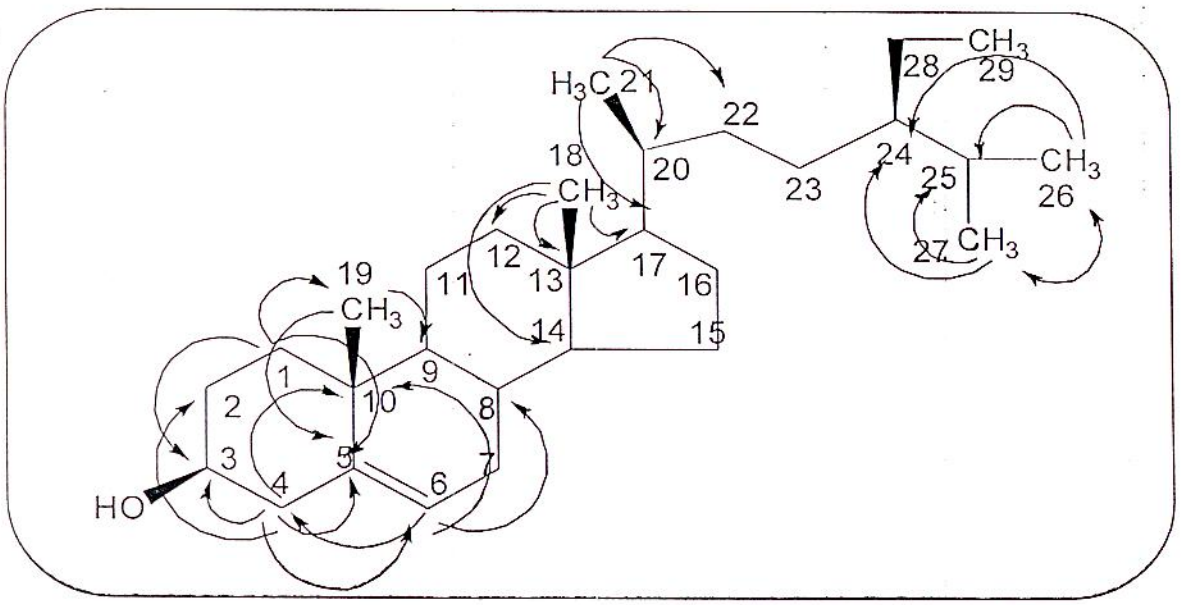
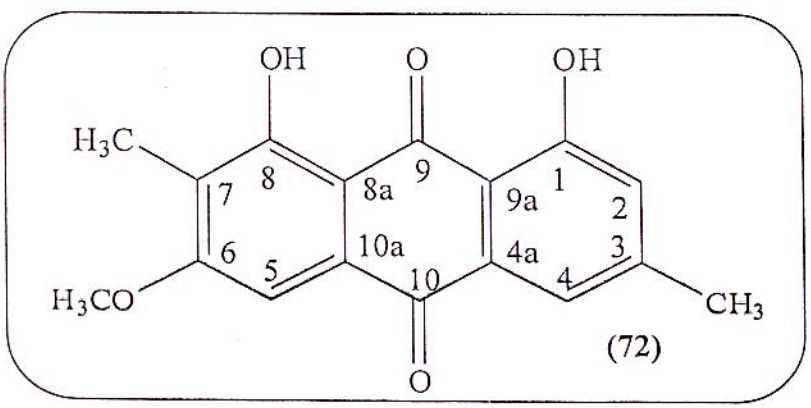


Fig. 2.23. HMBC Correlation of β-sitosterol **(71)**

2.1.6.2. 1,8-Dihydroxy-6-methoxy-3,7-dimethylanthraquinone (72)



(72)

Yellow needles (Acetone); mp 233-234 °C (Lit. mp 233-233.5 °C)[231]

Molecular formula $C_{17}H_{14}O_5$, deduced from EI mass spectra *m/z* 298; M^+ (calcd. 298 for $C_{17}H_{14}O_5$) and the ^{13}C NMR.

Table 2.25. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $(CD_3)_2CO$ and HMBC Correlations of 1,8-dihydroxy-6-methoxy-3,7-dimethylanthraquinone (**72**)

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.53	C	12.14, s (OH)	(OH) C-1, C-2, C-9a
2	124.51	CH	7.06, s	C-4, C-9a, 3-CH_3
3	148.41	C	-	
4	121.23	CH	7.61, s	C-2, C-9a, C-10, 3-CH_3
4a	132.64	C	-	
5	103.03	CH	7.39, s	C-7, C-8a, C-10
6	163.92	C	-	
7	120.88	C	-	
8	161.98	C	12.43, s (OH)	(OH) C-7, C-8a, C-8a
8a	110.53	C	-	
9	191.41	C	-	
9a	113.53	C	-	
10	182.38	C	-	
10a	133.28	C	-	
6-OCH_3	56.33	CH_3	4.00 (3H, s, OCH_3)	C-6
3-CH_3	22.24	CH_3	2.44 (3H, s, CH_3)	C-2, C-3, C-4
7-CH_3	29.79	CH_3	2.18 (3H, s, CH_3)	C-6, C-7, C-8

The ^{13}C NMR spectrum of the compound **72** confirmed the presence of seventeen carbon atoms in the molecule; two methyl, a methoxy, three methine and ten quaternary carbons from the DEPT experiments. Total assignments were established by HMQC and HMBC experiments (**Table 2.25**). The carbon resonating at δ 191.41 was assigned to C-9 while the one at δ 182.41 was assigned to C-10, the two carbonyl of the anthraquinone moiety. The aromatic carbons at δ 163.92, 162.53 and 161.98 were assigned to C-6, C-1 and C-8 respectively, confirming the position of OH or OCH_3. The aromatic carbons at δ 148.41 and 133.28 were assigned to C-3 and C-10a respectively. The signals at δ 132.64, 124.51 and 121.23 were assigned to C-4a, C-2 and C-4 respectively. The signals at δ 120.88,

113.53, 110.53 and 103.03 were assigned to C-7, C-9a, C-8a and C-5 respectively. The high field signal at δ 55.33 was assigned to the OCH_3 group, at 29.79 for a CH_3 at C-7 and at δ 22.24 for a CH_3 at C-3.

The ^1H NMR spectrum of the compound **72** showed singlets at δ 2.18 (3H, s, CH_3-7) for a methyl group, a singlets at δ 2.44 (3H, s, CH_3-3) for a methyl at C-3 and at δ 4.00 (s, 3H) for a methoxy group. The two doublets at δ 12.43 (1H, d, J = 1.32, OH-8) and 12.14 (1H, d, J = 1.32 OH-1) were due to a rare coupling of OH protons. The reason for long rang coupling is the shifting of OH protons with C-9. Three singlets at δ 7.06, 7.39 and 7.61 were assigned to H-2, H-5 and H-4 respectively.

In the HMBC spectrum, the resonance at δ 191.41 correlated to signals at δ 12.43 (OH-8) and 12 14 (OH-1). The C-10 signal at δ 182.38 showed correlation to protons at δ 7.39 (H-5) and 7.61 (H-4). The proton resonates at δ 7.06 (H-2) showed correlation to δ 121.23 (C-4), 113.53 (C-9a) and 22.24 (3-CH_3). The resonance at δ 7.39 (H-5) correlated to δ 182.38 (C-10), 120.88 (C-7) and 110.53 (C-8a) and the signal at δ 7.61 (H-4) correlated to signals at δ 124.51 (C-2), 113.53 (C-9a) and 22.24 (3-CH_3). The proton at δ 4.00 showed correlation to δ 163.92 (C-6) and the one at δ 2.18 showed correlation to δ 120.88 (C-7), 163.92 (C-6) and 161.98 (C-8). The resonance at δ 2.44 correlated to the signals at δ 121.23 (C-4), 124.51 (C-2) and 148.51 (C-3). The protons of OH also showed interesting correlation in the HMBC spectrum; the signal at δ 12.14 showed correlation to δ 162.53 (C-1), 124.51 (C-2) and 113.53 (C-9a) whereas the signal at δ 12.43 showed correction to δ 161.98 (C-8), 120.88 (C-7) and 110.53 (C-8a).

The compound **72** showed DQF COSY correlation between H-2 and H-4.

The above data suggested the presence of two hydroxy groups at C-1 and C-8. Two methyl groups at C-3 and C-7 and a methoxy group at C-6. Therefore, the compound **72** was identified as 1,8-dihydroxy-6-methoxy-3,7-dimethylanthraquinone (7-methyl physcion).

The spectral data (UV, IR, and ^{1}H NMR) agreed with the reported values for 1,8-dihydroxy-6-methoxy-3,7-dimethylanthraquinone, isolated from the tissue culture of *Cassia occidentalis*[231] and *Cassia singueana.*[230]

To the best of our knowledge no ^{13}C NMR data and 2D techniques are published so for, we reporting these for the first time to confirm the structure of 1,8-dihydroxy-6-methoxy-3,7-dimethylanthraquinone.

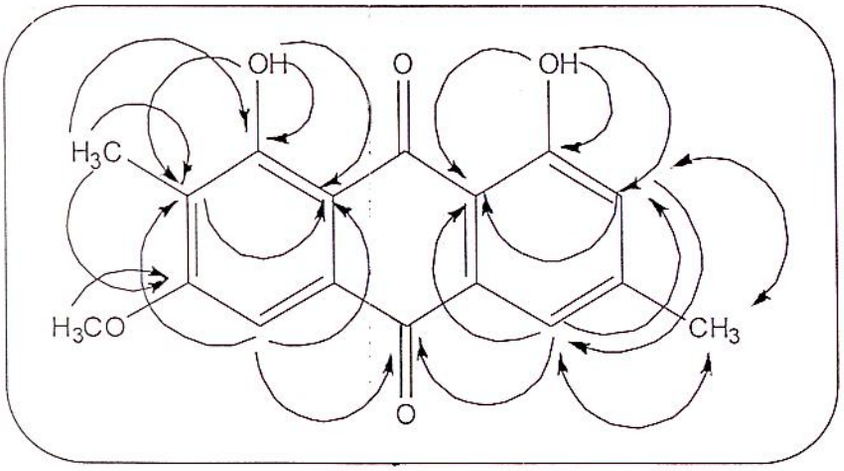
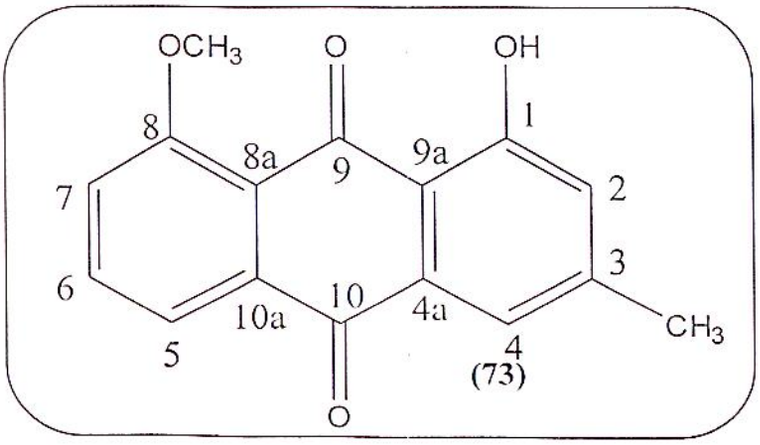


Fig. 2.24. HMBC Correlation of 1,8-dihydroxy-6-methoxy-3,7-dimethylanthraquinone **(72)**

2.1.6.3. 1-Hydroxy-8-methoxy-3-methylanthraquinone (73)



(73)

Orange needles (Acetone); mp 195-196 °C

Molecular formula $C_{16}H_{12}O_4$, deduced from EI mass spectra *m/z* 268 (calcd. 268 for $C_{16}H_{12}O_4$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **73** confirmed the presence of sixteen carbon atoms in the molecule; one methyl, one methoxy, five methine and nine quaternary carbons according to DEPT experiments. Total assignments were established by HMQC and HMBC experiments (**Table 2.26**).

Table 2.26. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR* data and HMBC Correlations of 1-hydroxy-8-methoxy-3-methylanthraquinone **(73)**

C. No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.77	C	12.92 s (OH)	OH-C, C-1, C-2, C-9a
2	124.72	CH	7.08 (d, J = 0.96)	C-4, C-9a, 3-CH_3
3	147.69	C	-	
4	120.26	CH	7.58, s	C-2, C-9a, 3-CH_3
4a	132.47	C	-	
5	120.15	CH	7.36 (d, J = 7.8)	C-7, C-8a, C-10
6	135.89	CH	7.72 (t, J = 7.8)	C-8, C-10a
7	118.21	CH	7.95 (d, J = 7.8)	C-5, C-8a, C-10
8	160.87	C	-	
8a	115.24	C	-	
9	188.64	C	-	
9a	115.06	C	-	
10	183.10	C	-	
10a	135.73	C	-	
3-CH_3	22.16	CH_3	2.42 (3H, s, CH_3)	C-2, C-3, C-4
8-OCH_3	56.75	CH_3	4.01 (3H, s, OCH_3)	C-8

* ^{1}H NMR in acetone d6 and ^{13}C NMR in methanol-d4

In the ^{13}CNMR the carbon resonating at δ 188.64 was assigned to C-9 where the one at δ 183.10 was assigned to C-10, the two carbonyl of the anthraquinone moiety. The aromatic carbons at δ 162.77 and 160.87 were assigned to C-1 and C-8 respectively. Which confirm the position of the hydroxy and methoxy groups. The aromatic carbons at δ 147.69 and

135.89 were assigned to C-3 and C-6 respectively. The signal at δ 135.73 was assigned to C-10a and 132.47 to C-4a. The signals at δ 124.72, 120.26 and 120.15 were assigned to C-2, C-4 and C-5 respectively. The signals at δ 118.21, 115.24 and 115.06 were assigned to C-7, C-8a and C-9a respectively. The high field signal at δ 56.75 was assigned to OCH_3 at C-8 and δ 22.16 to CH_3 at C-3 position.

The ^1H NMR spectrum of the compound **73** showed absorption at δ 2.46 (s, 3H) for a methyl group, 4.01 for a methoxy group (s, OCH_3) and at δ 12.92 for the chelated hydroxy group (OH-1). Three ortho-coupled aromatic protons at δ 7.36 (d, J = 7.82 Hz, H-5), 7.72 (t, J = 7.8 Hz, H-6) and 7.95 (d, 7.8 Hz, H-7). The spectrum also showed two singlets for the aromatic protons at δ 7.08 (s, H-2) and 7.58 (s, H-4).

In the HMBC spectrum, the C-10 signal at δ 183.10 showed correlation to signals at δ 7.58 (H-4) and 7.36 (H-5), the resonance at δ 7.08 (H-2) correlated to signals at δ 120.26 (C-4), 115.06 (C-9a) and 22.16. The signal at δ 7.58 (H-4) correlated to the signals δ 124.72 (C-2), 115.06 (C-9a) and 22.16 (3-CH_3). The resonance at δ 7.36 (H-5) correlated with signals at δ 118.21 (C-7) and 115.24 (C-8a). The signal at δ 7.72 (H-6) showed correlation to signals at δ 135.73 (C-10a) and 160.87 (C-8). The δ 7.95 (H-7) signal showed correlation to carbons at δ 115.24 (C-8a) and 120.15 (C-5). The high field protons at δ 2.42 correlated to resonance at δ 147.69 (C-3) and the signal at δ 4.01 correlated to δ 160.87 (C-8) **(Fig. 2.24)**.

The DQF COSY Correlation of the compound **73** was observed between H-2 and H-4 while H-5, H-6 and H-7 correlated to each other. The methyl protons also showed correlation to H-2 and H-4.

On the basis of UV, IR NMR, DQF COSY and HMBC, the structure of the compound **73** was deduced as 1-hydroxy-8-methoxy-3-methylanthraquinone. All the spectral data (UV, IR, ^1H NMR and ^{13}C NMR) agreed with the reported values for the compound isolated from a marine bacterium, *Nocardia sp. ALAA* 2000.[334]

However this is isolated for the first time from family Leguminosae and the structure was confirmed using 1D and 2D NMR techniques.

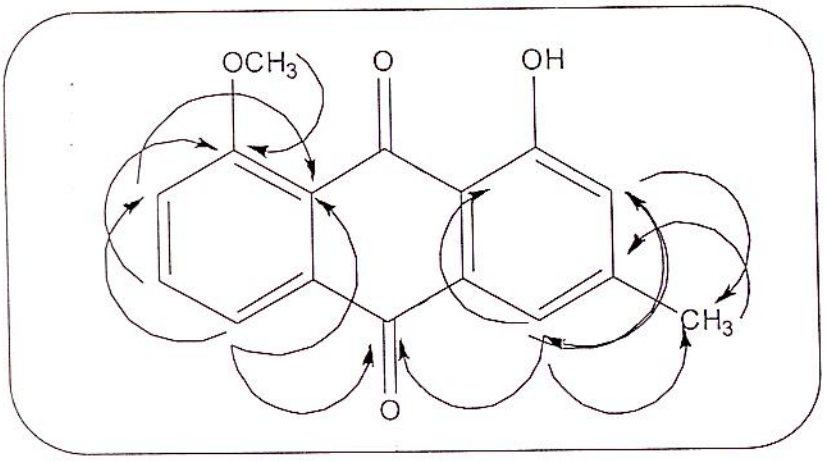
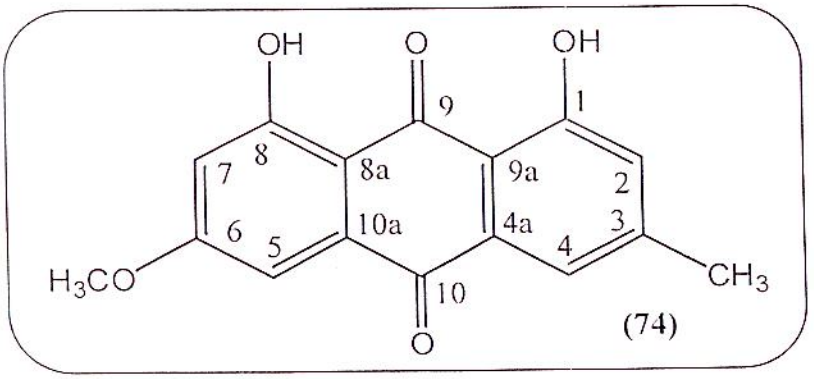


Fig. 2.25. HMBC Correlations of 1-hydroxy-8-methoxy-3-methylanthraquinone **(73)**

2.1.6.4 1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (74)



Reddish needles (Acetone); mp 205-206 °C (Lit. mp 206-207 °C)[316]

Molecular formula $C_{16}H_{10}O_5$, deduced from EI mass spectra *m/z* 284 (calcd. 284 for $C_{16}H_{12}O_5$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **74** confirmed the presence of sixteen carbon atoms in the molecule; having a methyl group, a methoxy group, four methine and ten quaternary carbons from the DEPT experiments. Total assignments were established by HMQC, COSY and HMBC experiments (**Table 2.27**).

Table 2.27. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR* data and HMBC Correlations of 1,8-dihydroxy-6-methoxy-3-methylanthraquinone **(74)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.52	C	12.08 (s, OH)	
2	124.49	CH	7.05, s	C-4, C-9a
3	148.43	C	-	
4	121.27	CH	7.60, s	C-2, C-9a, C-10
4a	132.48	C	-	
5	108.20	CH	7.34 (d, J = 2.34)	C-7, C-8a, C-10
6	166.57	C	-	
7	106.78	CH	6.66 (d, J = 2.34)	C-5, C-8a
8	165.21	C	12.28 (s, OH)	-
8a	113.70	C	-	-
9	190.82	C	-	-
9a	110.28	C	-	-
10	182.01	C	-	-
10a	135.29	C	-	-
3-CH_3	22.13	CH_3	2.42 (3H, s, CH_3)	C-2, C-3, C-4
6-OCH_3	56.06	CH_3	3.91 (3H, s, OCH_3)	C-6

* Solvent $CDCl_3$ and CD_3OD

In the ^{13}CNMR the carbons at δ 190.82 and 182.01 were assigned to C-9 and C-10 respectively, the two carbonyl carbons of the anthraquinone moiety. The aromatic signals at δ 166.57, 165.21 and 162.57 were assigned to C-6, C-8 and C-1 respectively, indicating the presence of OH at C-8, C-1 and OCH_3 at C-6. The aromatic carbons at δ 148.43, 135.29 were assigned to C-3 and C-10a respectively. The signal at δ 132.48 was assigned to C-4a while the one at δ 124.49 was attributed to C-2. The signals at δ 121.27, 113.70 and 110.28 were assigned to C-4, C-9a and C-8a respectively. The signals at δ 108.20 and 106.78 were assigned to C-5 and C-7 respectively. The high field signals at δ 56.06 and 22.13 were assigned to OCH_3 group at C-6 and a methyl group at C-3 respectively.

The ^{1}H NMR spectrum of the compound **74** showed signals at δ 2.42 (s, 3H) for a methyl group and at δ 3.91 for a methoxy group (3H, s, OCH_3). Two sharp singlets at δ 12.08 and 12.28 for two chelated (peri) hydroxy groups (OH-1 and OH-8) respectively (D_2O exchangeable). Two meta-coupled aromatic protons at δ 6.66 (d, J = 2.34 Hz, H-7), 7.34 (d, J = 2.34 Hz, H-5) and two singlets for aromatic protons at δ 7.05 (s, H-2) and 7.60 (s, H-4).

In the HMBC spectrum, the C-9 at δ 190.82 correlated to hydroxy groups at δ 12.08 (OH-1) and 12.28 (OH-8) indicating 1,8-dihydroxyanthraquinone moiety. The C-10 signal at δ 182.01 showed correlation with signals at δ 7.60 (H-4) and 7.34 (H-5), the signal at δ 106.78 (H-7) correlated to δ 110.28 (C-8a) and 108.20 (C-5). The signal at δ 7.34 (H-5) correlated to δ 106.78 (C-7) and 110.28 (C-8a. The proton at δ 7.60 (H-4) correlated to the carbons at δ 124.49 (C-2) and 113.74 (C-9a). The absorption at δ 7.05 (H-2) showed correlation to the signals at δ 121.27 (C-4) and 113.70 (C-9a). The high field resonance at δ 2.43 correlated with resonance at δ 148.43 (C-3) while δ 3.91 showed correlation to δ 166.57 (C-6) **(Fig. 2.25)**.

From the spectra (UV, IR, ^{1}H NMR, ^{13}CNMR, HMQC and HMBC) the structure of the compound **74** was elucidated as 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (Physcion). All the data (UV, IR, ^{1}H NMR and ^{13}C NMR) agreed with the reported values for 1,8-dihydroxy-6-methoxy-3-methylanthraquinone, isolated from *Cassia siamea*[319] and Rhubarb.[318] However the 1D and 2D NMR techniques were applied for the first time.

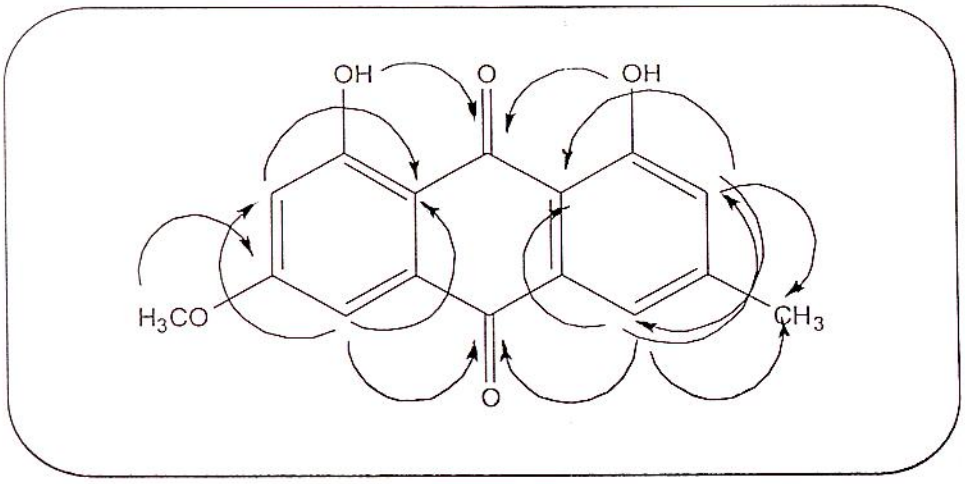
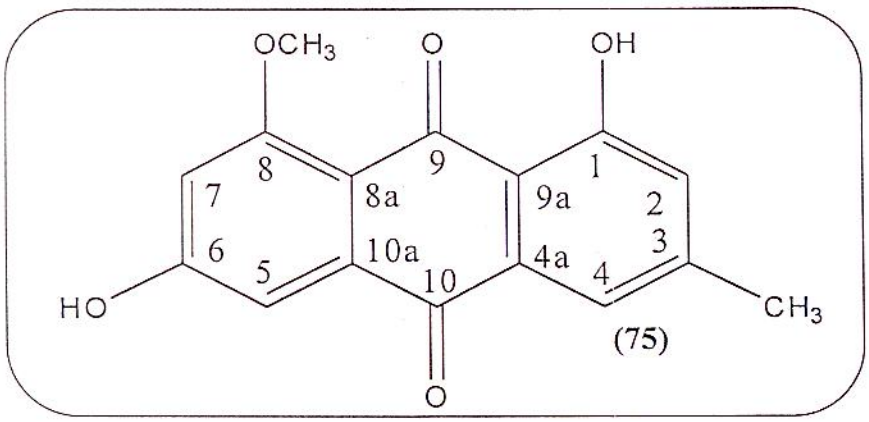


Fig 2.26. HMBC Correlation of 1,8-dihydroxy-6-methoxy-3-methylanthraquinone **(74)**

2.1.6.5. 1,6-Dihydroxy-8-methoxy-3-methylanthraquinone (75)



(75)

Yellow needles (Acetone); mp 300-302 °C (Lit. mp 301-303 °C)[320]

Molecular formula $C_{16}H_{12}O_5$, deduced from EI mass spectra *m/z* 284 (M^+); (calcd. 284 for $C_{16}H_{12}O_5$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **75** confirmed the presence of sixteen carbon atoms in the molecule; one methyl, one methoxy, four methine and ten quaternary carbons from DEPT experiments. Total assignments were established by HMQC and HMBC experiments (**Table 2.28**).

Table 2.28. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $(CD_3)_2CO$ and HMBC Correlations of 1,6-dihydroxy-8-methoxy-3-methylanthraquinone **(75)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.65	C	13.31, s, (OH)	OH, C-1
2	124.03	CH	7.06, s	C-9a, C-4
3	146.76	C	-	CH_3 (20.97)
4	119.08	CH	7.46, s	C-2, C-9a
4a	132.64	C	-	
5	106.92	CH	7.31 (d, J = 2.3)	C-7, C-8a
6	163.99	C	-	
7	104.84	CH	6.89 (d, J = 2.3)	C-5, C-8a
8	164.38	C	-	
8a	113.69	C	-	
9	186.96	C	-	
9a	114.82	C	-	
10	182.40	C	-	C-4, C-5
10a	137.60	C	-	
3-CH_3	20.97	CH_3	2.40 (3H, s, CH_3)	C-2, C-3, C-4
8-OCH_3	55.90	CH_3	3.92 (3H, s, OCH_3)	C-8

In ^{13}C NMR the carbon at δ 186.96 (C-9) and at 182.40 (C-10) were the carbonyl of the anthraquinone moiety. The down field aromatic signals at δ 164.38 and 163.99 were assigned to C-8 and C-6 respectively, confirming the attachment of OH or OCH_3. The aromatic protons at δ 162.65 and 146.76 were assigned to C-1 and C-3 respectively. The signal at δ 137.60 was assigned to C-10a and the ones at δ 132.64, 124.03 and 119.08 were assigned to C-4a, C-2 and C-4 respectively. The absorption at δ 114.82, 113.69, 106.92 and 104.84 were assigned to C-9a, C-8a, C-5 and C-7 respectively. The high field signal at δ 55.90 was assigned to OCH_3 and the one at δ 20.97 to the CH_3 group.

The ^{1}H NMR spectrum of the compound **75** showed signals at δ 2.40 (s, 3H) for a methyl group, at δ 3.92 (s, 3H) for a methoxy group and at δ 13.31 for chelated (peri) hydroxy proton (OH-1), two meta-coupled aromatic protons at δ 6.89 (d, J = 2.30 Hz, H-7) and 7.31 (d, J = 2.30 Hz, H-5) and two non-coupled aromatic protons at δ 7.06 (s, H-2) and 7.46 (s, H-4).

In the HMBC spectrum, the C-10 signal at δ 181.02 showed correlation with signals at δ 7.46 (H-4) and 7.31 (H-5), the one at δ 164.38 (C-8) is correlated to the OCH_3 (δ 3.92) and H-4 (δ 7.46) and the signal at δ 162.65 (C-1) correlated with the OH (δ 13.31) signal. The signal at δ 114.82 (C-9a) showed correlation to the signals at δ 7.06 (H-2) and 7.46 (H-4). The resonance at δ 113.69 (C-10a) correlated to protons at δ 6.89 (H-7) and 7.31 (H-5).

These data suggested the presence of two hydroxy groups at C-1 and C-6 and a methoxy group at C-8. Therefore, compound **75** was identified as 1,6-dihydroxy-8-methoxy-3-methylanthraquinon.

The UV, IR, ^{1}H NMR and ^{13}C NMR data agreed with the reported data for 1,6-dihydroxy-8-methoxy-3-methylanthraquinone, isolated from the fungi *Microascus tardifasiens*[320] and *Penicillium frequentans*.[321] However this is reported for the first time from plants and the use of 2D NMR techniques.

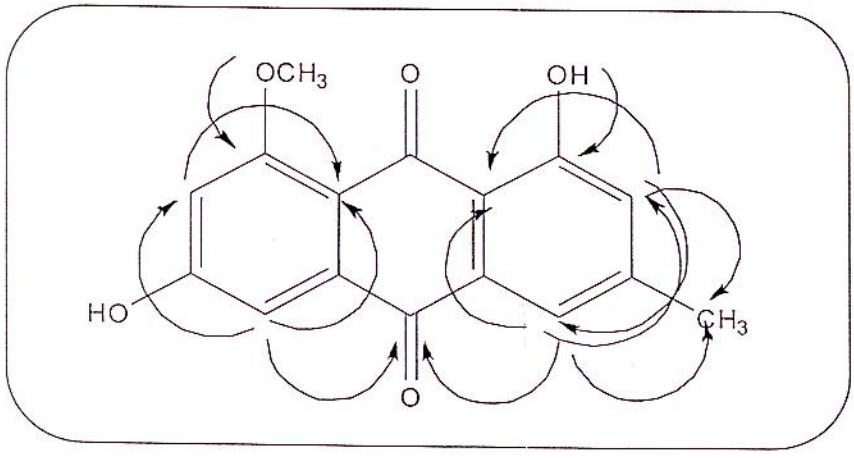
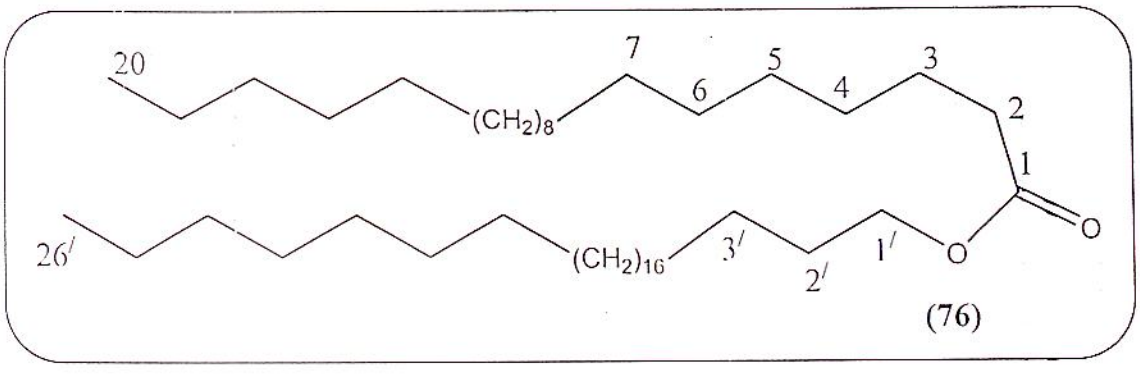


Fig. 2.27. HMBC Correlation of 1,6-dihydroxy-8-methoxy-3-methylanthraquinone **(75)**

2.1.7. New Source Compounds from the Pods of *Cassia artemisioides*

2.1.7.1. Hexacosyl icosaoate (76)



(76)

White powder (Chloroform); mp 76 °C (Not reported in litr.)

Molecular formula was deduced as $C_{46}H_{92}O_2$, through EI-mass spectrum *m/z* 676 (calcd. 676 for $C_{46}H_{92}O_2$), integration of ^{1}H NMR and DEPT experiments.

Table 2.29. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of hexacosyl icosaoate **(76)**

C No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR(δ)[c] Coupling Constants J_{HH} (Hz)
1	174.05	C	-
2	34.49	CH_2	2.27 (2H, t, J = 7.5)
2′	64.45	CH_2	4.04 (2H, t, J = 6.66)
21′	32.00	CH_2	1.22-1.30
22	22.75	CH_2	1.22-1.30, m
3-23	29.77-25.11	CH_2	1.22-1.30 (80 H, complex, 40 × CH_2)
CH_3	14.16	CH_3	0.87 (6H, t, J = 7.0 Hz)

The IR absorption at $\tilde{\nu}$ 1735.9 cm^{-1} showed the presence of C=O st. ester.

In the ^{1}H NMR, a triplet at δ 4.04 (2H, t, J = 6.66 Hz) was a characteristic for a CH_2-O (C-O$\underline{CH_2}$-CH_2) group and a triplet at δ 2.27 (2H, t, J = 7.5 Hz), a characteristic for a CH_2-C=O (CH_2-$\underline{CH_2}$-CO). The chemical shifts at δ 1.59 (2H, quintet, J = 7.5) was attributed to CH_2 ($\underline{CH_2}$-CH_2CO) and at δ 1.59 (2H, quintet, J = 7.5) for CH_2 (CH_2-$\underline{CH_2}CH_2$CO). A complex multiplet at δ 1.22-1.30 for eighty protons, were assigned to CH_2-3 to CH_2-23 and CH_2-3′ to CH_2-23′ (80H, complex, CH_2-3 to CH_2-23 and CH_2-3′ to CH_2-23′). The signal at δ 0.87 (6H, t, J = 7.0 Hz) was for the two terminal CH_3 groups.

The ^{13}C NMR confirmed it as a long chain ester and the position of the ester functional group was at *m/z* 313 for CH_3-$(CH_2)_{19}$-COO and the fragment at *m/z* 44 is also only for COO. The ^{13}C NMR signal at δ 174.05 was assigned to the ester carbonyl (C-1) and the one at δ 64.45 to CH_2-OCO (C-1′). The resonance at δ 34.49 and 32.00 were assigned to CH_2 next to COO(C-2) and C-21′. The absorption from δ 29.77 to 25.11 were assigned to C-3 to C-20 and C-3′ to C-26′. The signal at δ 22.75 was assigned to C-19.

From the UV, IR and ^{1}H NMR and ^{13}CNMR the structure was elucidated to be hexacosyl icosaoate, isolated from the waxes of *Desmodium* sp. and *Scutellaria* sp.[335, 336]

Mass fragmentation is given in **Fig. 2.27**

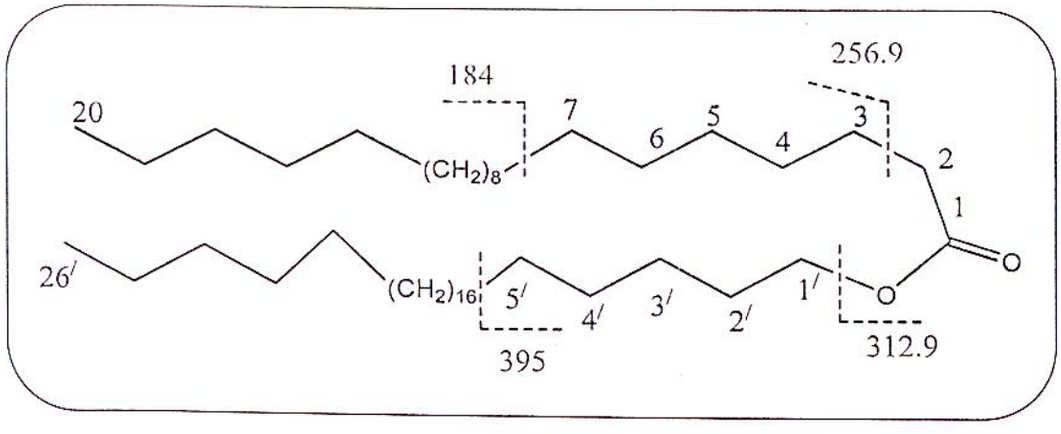
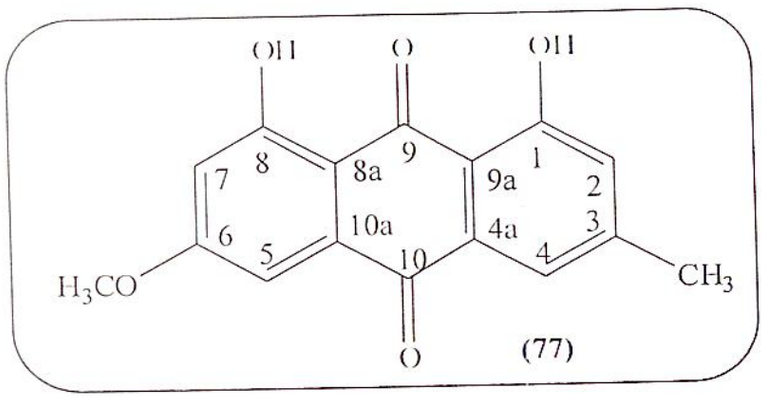


Fig. 2.28. Mass fragmentation of hexacosyl icosaoate **(76)**

2.1.7.2. 1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (77)



(77)

Reddish needles (Acetone); mp 205-206 °C (Lit. mp 206-207 °C)[316]

Molecular formula $C_{16}H_{10}O_5$, deduced from EI mass spectra *m/z* 284 (calcd. 284 for $C_{16}H_{12}O_5$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **77** confirmed the presence of sixteen carbon atoms in the molecule; a methyl group, a methoxy group, four methine and ten quaternary carbons (from DEPT experiments). Total assignments were determined by HMQC, COSY and HMBC experiments (**Table 2.30**).

In ^{13}C NMR the carbons at δ 190.90 (C-9) and 182.15 (C-10) were the carbonyls of the anthraquinone moiety. The aromatic signals at δ 166.63, 165.28 and 162.59 were assigned to C-6, C-8 and C-1 respectively, having oxygen functionality. The aromatic signals at δ 148.54 and 135.34 were assigned to C-3 and C-10a respectively. The signals at δ 133.30 and 124.61 were assigned to C-4a and C-2 respectively. The signals at δ 121.39, 113.76 and 110.35 were assigned to C-4, C-9a and C-8a respectively. The signals at δ 108.33 and 106.86 were assigned to C-5 and C-7 respectively. The high field signal at δ 56.18 and 22.26 were assigned to OCH_3 and CH_3 attached at C-6 and C-3 respectively.

Table 2.30. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of 1,8-dihydroxy-6-methoxy-3-methylanthraquinone **(77)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.59	C	12.11, s, (OH)	
2	124.61	CH	7.07, s	C-4, C-9a
3	148.54	C	-	
4	121.39	CH	7.62, s	C-2, C-9a, C-10
4a	133.30	C	-	
5	108.33	CH	7.36 (d, J = 2.52)	C-7, C-8a, C-10
6	166.63	C	-	
7	106.86	CH	6.67 (d, J = 2.52)	C-5, C-8a
8	165.28	C	12.31, s (OH)	
8a	110.35	C	-	
9	190.90	C	-	
9a	113.76	C	-	
10	182.15	C	-	
10a	135.34	C	-	
3-CH_3	22.26	CH_3	2.44 (3H, s, CH_3)	C-2, C-3, C-4
6-OCH_3	56.18	CH_3	3.92 (3H, s, OCH_3)	C-6

The ^{1}H NMR spectrum of the compound 77 showed signals at δ 2.44 (s, 3H, C-3) for a methyl group and at δ 3.92 for a methoxy group (s, 3H, C-6). Two low field hydroxy protons at δ 12.31 (C-8) and 12.11 (C-1) due to chelated OH (D_2O exchangable). Two meta-coupled aromatic protons at δ 6.67 (d, J = 2.52 Hz, H-7) and 7.36 (d, J = 2.52 Hz, H-5). The spectrum showed two aromatic singlets for two protons at δ 7.07 (s, H-2) and at δ 7.62 (s, H-4).

In the HMBC spectrum, the C-10 signal at δ 182.15 showed correlation with signals at δ 7.62 (H-4) and 7.36 (H-5), the signal at δ 6.67 (H-7) correlated to signals at δ 110.35 (C-8a) and 106.86 (C-5). The signal at δ 7.36 (H-5) correlated with δ 106.86 (C-7) and 113.76

(C-8a). The resonance at δ 7.62 (H-4) correlated to signals at δ 124.61 (C-2) and 113.76 (C-9a). The signal at δ 7.07 (H-2) showed correlation to the signals at δ 121.39 (C-4) and 113.76 (C-9a). The high field absorption at δ 2.44 correlated with resonance at δ 148.54 (C-3) and the signal at δ 3.92 correlated to δ 166.63 (C-6).

On the basis of UV, IR ^{1}H NMR, ^{13}C NMR, HMBC and mass spectra, compound **77** was identified as 1,8-dihydroxy-6-methoxy-3-methylanthraquinone. All the data agreed with the reported values for 1,8-dihydroxy-6-methoxy-3-methylanthraquinone, isolated from *Cassia siamea*[319] and Rhubarb.[318]

The structure was confirmed by HMBC techniques.

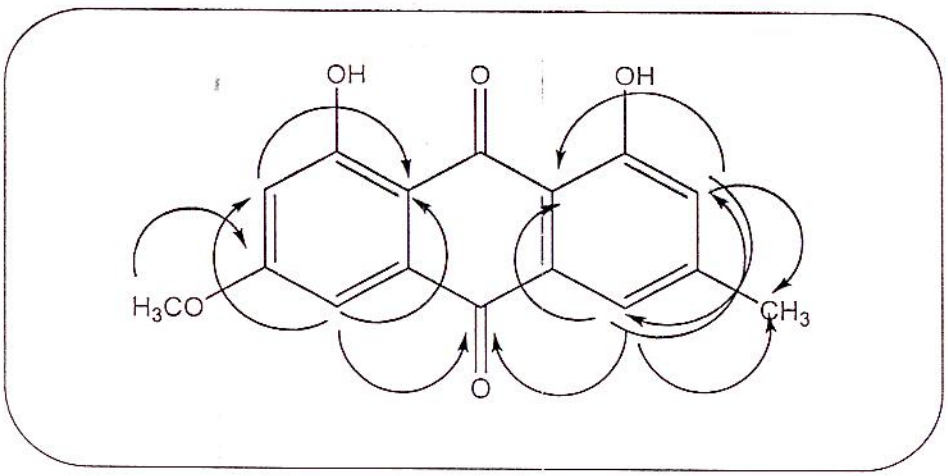
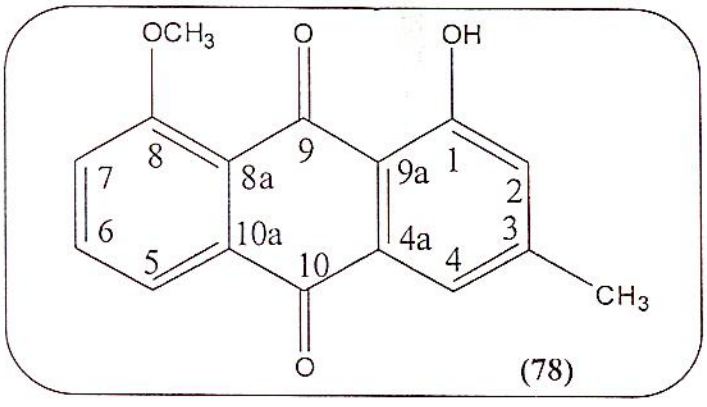


Fig. 2.29. HMBC Correlation of 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (77)

2.1.7.3. 1-Hydroxy-8-methoxy-3-methylanthraquinone (78)



(78)

Orange needles (Acetone); mp 195-196 °C

Molecular formula $C_{16}H_{12}O_4$, deduced from EI mass spectra *m/z* 268 (M^+); (calcd. 268 for $C_{16}H_{12}O_4$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **78** displayed the presence of sixteen carbon atoms in the molecule; a methyl group, a methoxy group, five methine and nine quaternary carbons (from DEPT experiments). Total assignments were achieved by HMQC and HMBC experiments (**Table 2.3I**). The carbonyl carbon resonating at δ 188.49 was assigned to C-9 where as the one at δ 182.64 was assigned to C-10 of the anthraquinone moety. The aromatic signals at δ 162.40 and 161.03 were assigned to C-1 and C-8 respectively, confirming the attachment of OH or OCH_3. The aromatic carbons resonating at δ 147.79 and 135.83 were assigned to C-3 and C-6 respectively. The carbons at δ 135.56 and 132.55 were assigned to C-10a and C-4a respectively. The signals at δ 123.90, 119.42 and 119.37 were assigned to C-2, C-4 and C-5 respectively. The carbon signals at δ 118.64, 114.80 and 112.76 were assigned to C-7, C-8a and C-9a respectively. The high field signal at δ 55.69 was assigned to the OCH_3 at C-8 and 20.63 for the CH_3 group at C-3.

Table 2.31. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR* data and HMBC Correlations of 1-hydroxy-8-methoxy-3-methylanthraquinone **(78)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.40	C	13.00, s (OH)	OH-C, C-1, C-2, C-9a
2	123.90	CH	7.10, s	C-4, C-9a, 3-CH_3
3	147.79	C	-	
4	119.42	CH	7.49, s	C-2, C-9a, 3-CH_3
4a	132.55	C	-	
5	119.37	CH	7.57 (dd, J = 7.5, 2.2)	C-7, C-8a, C-10
6	135.83	CH	7.83 (t, J 7.5)	C-8, C-10a
7	118.64	CH	7.84 (dd, J = 7.5, 2.2)	C-5, C-8a, C-10
8	161.03	C	-	
8a	114.80	C	-	
9	188.49	C	-	
9a	112.76	C	-	
10	182.64	C	-	
10a	135.56	C	-	
3-CH_3	20.63	CH_3	2.42 (3H, s, CH_3)	C-2, C-3, C-4
8-OCH_3	55.69	CH_3	4.01 (3H, s OCH_3)	C-8

* ^{1}H NMR in acetone d_6 and ^{13}C NMR in methanol d_3

The ^{1}H NMR spectrum of the compound **78** showed signals at δ 2.46 (s, 3H, C-3) for a methyl group, 4.01 for a methoxy group (s, CH_3, C-8) and at 13.00 for a chelated hydroxy group (OH-1). Three ortho-coupled aromatic protons at δ 7.57 (dd, J = 7.5, 2.2 Hz, H-5), 7.83 (t, J = 7.5 Hz, H-6) and 7.84 (dd, J = 7.5, 2.2 Hz, H-7). The spectrum also showed two siglets for the two aromatic protons at δ 7.10 (s, H-2) and at 7.49 (s, H-4).

In the HMBC spectrum, the C-10 signal at δ 182.64 showed correlation with signals at δ 7.49 (H-4) and 7.57 (H-5), the proton at δ 7.10 (H-2) correlated to carbons at δ 119.42 (C-4), 112.76 (C-9a) and 20.63. The signal at δ 7.49 (H-4) correlated to δ 123.90 (C-2), 112.76 (C-9a) and 20.63 (3-CH_3). The proton at δ 7.57 (H-5) correlated to the carbons at δ 118.64 (C-7) and 114.80 (C-8a). The signal at δ 7.83 (H-6) showed correlation to carbons at δ 135.56 (C-10a) and 161.03 (C-8). The signal at δ 7.84 (H-7) showed correlation with signals at δ 114.80 (C-8a) and 119.37 (C-5). The high field proton at δ 2.42 correlated to carbon at δ 147.79 (C-3) and signal at δ 4.01 correlated to δ 161.03 (C-8) **(Fig. 2.29)**.

The DQF COSY of the compound **78** showed correlation between H-2 and H-4 and also the two protons are correlated to methyl protons. The third correlation was observed between H-5, H-6 and H-7.

On the basis of above data, compound **78** was identified as 1-hydroxy-8-methoxy-3-methylanthraquinone. All the data (UV, IR, ^{1}H NMR and ^{13}C NMR) agreed with the reported values for 1-hydroxy-8-methoxy-3-methylanthraquinone, isolated from a marine derived bacterium *Nocardia sp. ALAA* 2000.[334] However this is isolated for the first time from plants and the structure was confirmed by using 1D and 2D NMR techniques for the first time.

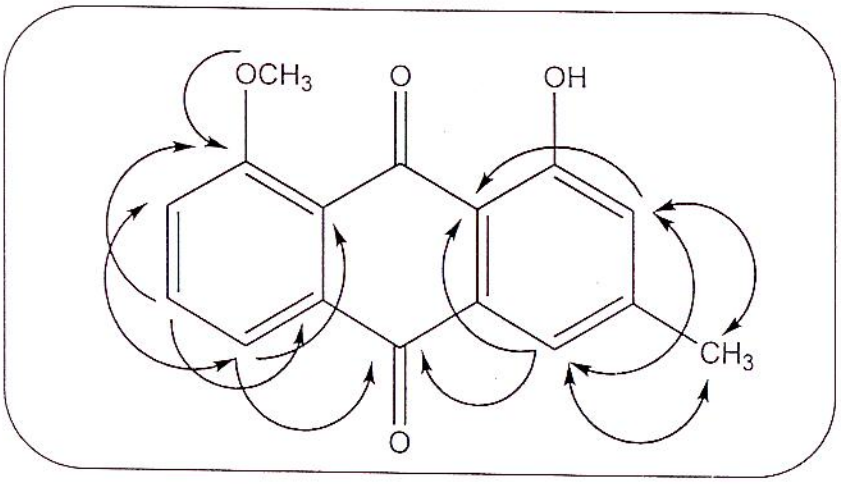
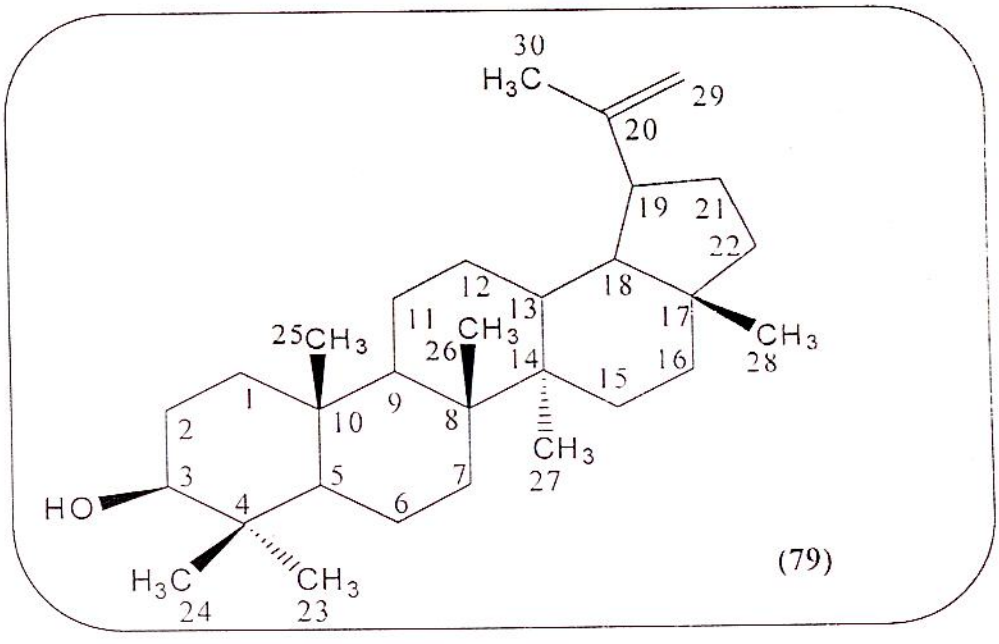


Fig. 2.30 HMBC Correalation of 1-hydroxy-8-methoxy-3-methylanthraquinone **(78)**

2.1.8. New Source Isolated Compounds from the Seeds of Cassia artemisioides

2.1.8.1. 3β-Hydroxylup-20(29)-ene (Lupeol) (79)



(79)

White powder (Chloroform); mp 201-202 °C (Lit. 213-214 °C)[337]

Molecular formula $C_{30}H_{50}O$, determined from the ^{13}C NMR and EI-MS spectra *m/z* 426 (calcd. 426 for $C_{30}H_{50}O$).

The ^{1}H and ^{13}C NMR spectral data (**Table 2.32**) of the compound **79** were assigned using values reported for 3β-Hydroxylup-20(29)-ene (Lupeol).[338]

The ^{13}C NMR indicated thirty carbon signals in the broad band decoupled spectrum; seven methyl, eleven methylene, six methine and six quaternary carbon atoms from DEPT experiments. The chemical shifts at δ 151.06 and 109.42 were the characteristic peaks for lupeol type of skeleton, assigned to C-20 and C-29 respectively. The oxygen deshielding chemical shift at δ 78.08 was assigned to C-3. The resonances at δ 55.37, 50.50, 48.37 and 48.07 were assigned to C-9, C-18 and C-19 respectively.

Table 2.32. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of 3β-hydroxylup-20(29)-ene **(79)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)
1	38.78	CH_2	1.69, m
2	21.01	CH_2	1.37
3	79.08	CH	3.16 (1H, dd, J = 11.46, 4.8)
4	38.94	C	-
5	55.37	CH	0.66 (d, J = 9.6)
6	18.40	CH_2	1.37, 1.49 (2H, m)
7	34.35	CH_2	1.37, 3H
8	40.90	C	-
9	50.50	CH	1.24, 1.27 (1H, m)
10	37.25	C	-
11	27.52	CH_2	1.55, 1.59 (2H, m)
12	25.21	CH_2	1.66, m
13	38.12	CH	0.89, 1.69, (1H, m)
14	42.91	C	-
15	27.50	CH_2	1.55, d, 1.58, d, 1.59, m (2H)
16	35.66	CH_2	1.37, m, 1.45, d, 1.49, d (2H)
17	43.08	C	-
18	48.37	CH	1.32, d, 1.35,d, 1.37 1H
19	48.07	CH	2.37, m (1H)
20	151.06	C	-
21	29.92	CH_2	1.24, d, 1.86, d, 1.90, t, (quentet)
22	40.09	CH_2	1.18, t, 1.37, (2H, m)
23	28.07	CH_3	0.95 (3H, s)
24	16.21	CH_3	0.81 (3H, s)
25	15.46	CH_3	1.01 (3H, s)
26	16.06	CH_3	0.74 (3H, s)
27	14.63	CH_3	0.93 (3H, s)
28	18.09	CH_3	0.77 (3H, s)
29	109.42	CH_2	4.67, 4.55 (2H, m)
30	19.39	CH_3	1.66 (3H, s)

The signals at δ 43.08, 42.91, 40.90 and 40.09 were attributed to C-17, C-14, C-8 and C-22 respectively. The signals at δ 38.94, 38.78, 38.12, 37.25, 35.66, 34.35 and 29.92 were assigned to C-4, C-1, C-13, C-10, C-16, C-7 and C-21 respectively. The chemical shifts at δ 28.07, 27.52, 27.50, 25.21 and 21.01 were assigned to C-23, C-11, C-15, C-12 and C-2 respectively. The high field signals at δ 19.39, 18.40, 18.09 and 16.21 were attributed to C-30, C-6, C-18 and C-24 respectively. Further high field signals at δ 16.06, 15.46 and 14.63 were attributed to C-16, C-25 and C-27 respectively.

^{1}H NMR showed two olefinic protons at δ 4.67 (1H, s, H-29 and at 4.55 (1H, s, H-29). One proton doublet of doublet at δ 3.16 (1H, J = 11.46, 4.8 Hz, H-3) for an oxygen deshielded proton H-3 and one-proton multiplet at δ 2.37 (1H, m, H-19) was assign to H-19.

All the spectral data (IR, UV, ^{1}H NMR ^{13}C NMR and mass spectrum) and other data are in agreement with the reported values for 3β-hydroxylup-20(29)-ene) (Lupeol).[338] The structure was confirmed using 1D and 2D NMR techniques for the first time.

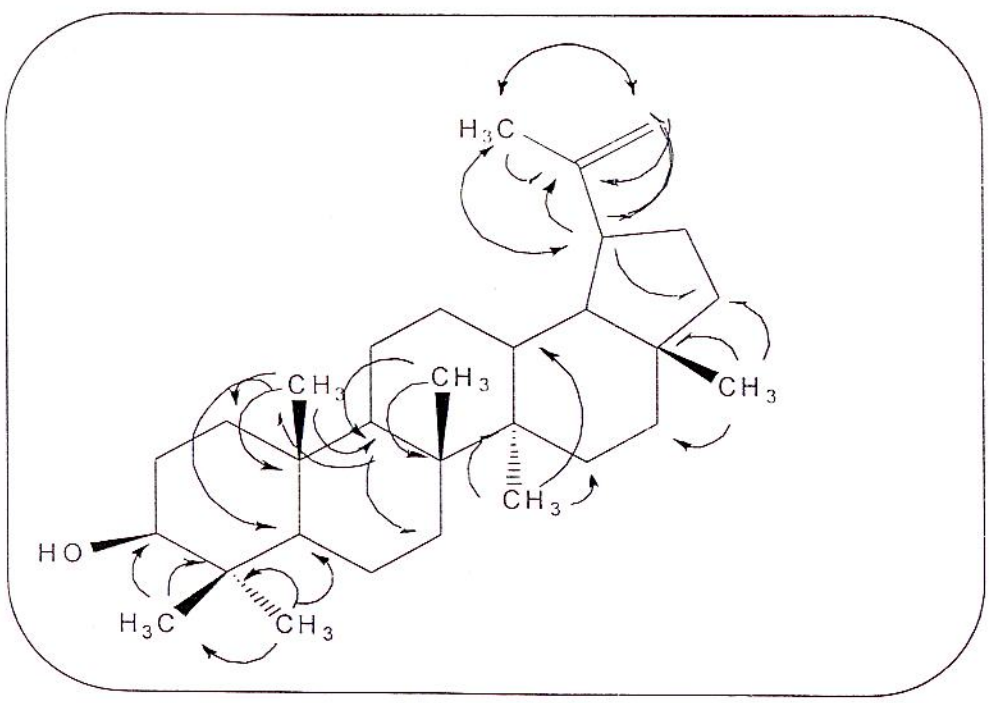
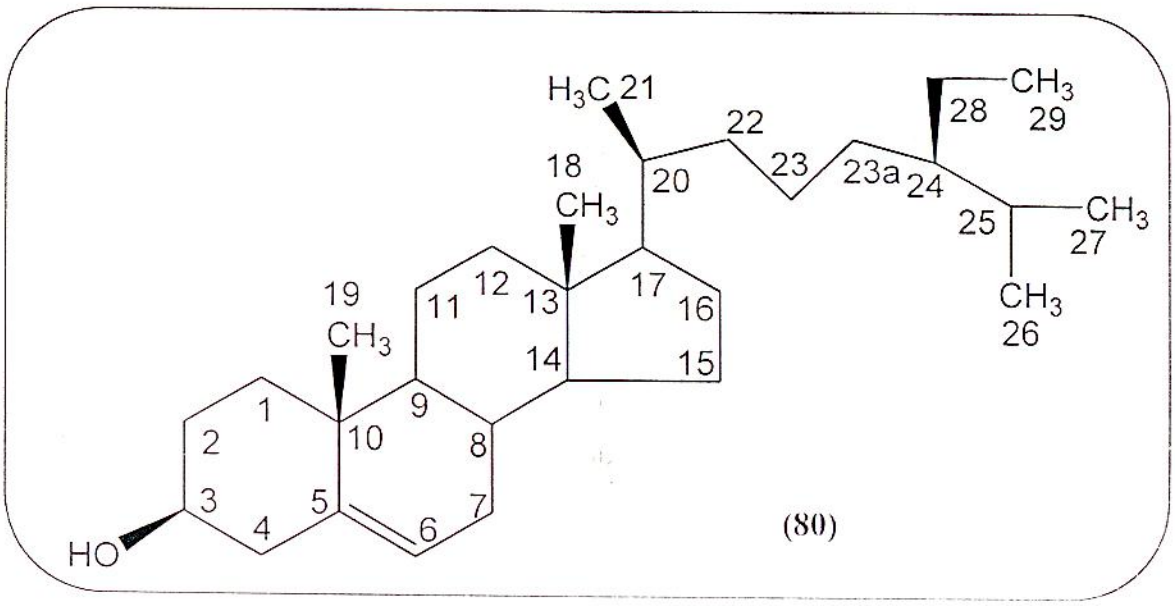


Fig. 2.31. HMBC Correlation of 3β-hydroxylup-20(29)-ene **(79)**

2.1.8.2. 23a-Homostigmast-5-en-3β-ol (80)



White amorphous powder (Chloroform), mp 125-127 °C

Molecular formula $C_{30}H_{52}O$, established from EI mass spectra *m/z* 428 (calcd. 428 for $C_{30}H_{52}O$) and from the ^{13}C NMR.

The IR absorption at $\tilde{\nu}$ 3487.6 cm^{-1} for OH st.: $\tilde{\nu}$ 2918.9 cm^{-1} for CH st satuarated and $\tilde{\nu}$ 1603.1 cm^{-1} for C=C olifinic.

The ^{1}H and ^{13}C NMR spectral data (**Table 2.33**) of the compound **80** was in close agreement to that of the reported for β-sitosterol.[311,312] The ^{13}C NMR spectra indicated that the main difference between the compound **80** and β-sitosterol was the presence of one extra CH_2 group at δ 31.74 (CH_2-23).

Table 2.33. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of 23a-Homostigmast-5-en-3β-ol **(80)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	37.33	CH_2	1.07 (ddd, J = 3.48, 6.12)	C-3, C-5, C-9
2	29.79	CH_2	1.82 (2H, m)	
3	71.86	CH	3.52, m	
4	39.85	CH_2	2.00 (2H, m)	C-5, C-6, C-3
			1.98 (2H, d, J = 2.1)	
5	140.83	C	-	
6	121.80	CH	5.34 (d, J = 5.20)	C-8, C-10, C-4
7	31.99	CH_2	1.44, 1.93 (2H, m)	
8	31.97	CH	1.42, m	
9	50.19	CH	0.84, m	C-5, C-14
10	36.58	C	-	
11	21.16	CH_2	1.49, 1.47 (2H, m)	
12	42.35	CH_2	2.19 (2H, t, J = 9.0)	
			2.26 (2H, d, J = 3.0)	
13	42.39	C	-	
14	56.84	CH	0.96, m	C-9, C-12, C-17
15	24.39	CH_2	1.56 (2H, m)	
16	28.34	CH_2	1.23 (2H, m)	
17	56.12	CH	1.07 (dd, J = 7.8, 3.48)	
18	11.94	CH_3	0.66 (3H, s)	C-12, C-13, C-14, C-17
19	19.49	CH_3	0.99 (3H, s)	C-1, C-5, C-9, C-10
20	36.23	CH	1.34, m	
21	18.86	CH_3	0.90 (3H, d, J = 6.5)	C-20, C-22, C-17
22	34.01	CH_2	1.27 (2H, m)	
			1.55 (2H, m)	
23	31.74	CH_2	1.48 m	C-20, C-24
23a	26.12	CH_2	1.14 (3H, ddd, J = 3.84, 7.44)	
24	45.89	CH	0.92, m	
25	29.20	CH	1.25, m	
26	19.12	CH_3	0.79 (3H, d, J = 6.8)	C-24, C-25, C-27
27	19.92	CH_3	0.81 (3H, d, J = 6.78)	C-24, C-25, C-26
28	23.13	CH_2	1.25 (2H, m)	
29	12.07	CH_3	0.82 (3H, t, J = 7.5)	C-24, C-28

From the IR, mass and NMR spectra of the structure of the compound **80** was established as 23a-homostigmast-5-en-3β-ol (17-(6-ethyl-7-methyloctan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,-11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]-phenanthren-3-ol). This compound is reported as new from the stem bark **(52)** in our investigation.

All the results and discussions are the same as discussed for the compound **52**

The DQF COSY was represented in structure by double arrows.

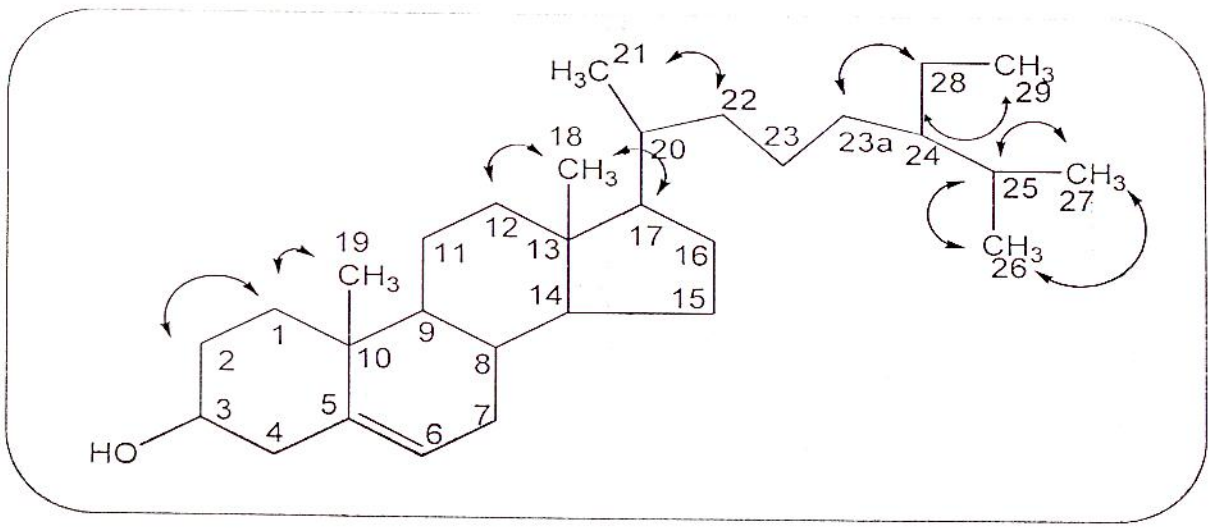
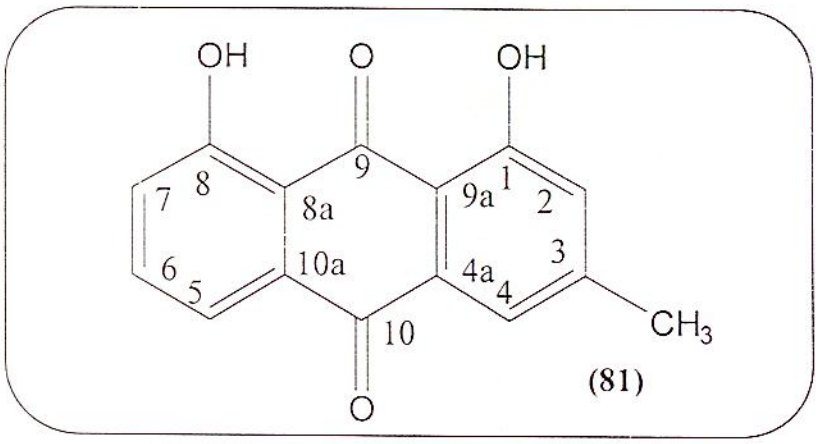


Fig. 2.32. DQF COSY Correlation of 23a-Homostigmast-5-en-3β-ol **(80)**

2.1.8.3. 1,8-Dihydroxy-3-methylanthraquinone (81)



(81)

Reddish needles (Chloroform); mp 195-196 °C (Lit. mp 193-194 °C)[316]

Molecular formula $C_{15}H_{10}O_4$, deduced from EI mass spectra *m/z* 254; (M^+) (calcd. 254 for $C_{15}H_{10}O_4$) and ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **81** confirmed the presence of fifteen carbon atoms in the molecule; including one methyl group, five methine and nine quaternary carbons according to DEPT experiments. Total assignments were established by HMQC and HMBC experiments (**Table 2.34**). The carbonyl carbon at δ 192.62 was assigned to C-9 and the one at δ 182.03 was assigned to C-10 of the anthraquinone moiety. The aromatic carbons at δ 162.80 and 162.49 were assigned to C-1 and C-8 respectively, confirming the attachment of OH. The aromatic signals resonating at δ 149.35 and 136.95 were assigned to C-3 and C-6 respectively. The signals at δ 132.53 and 132.53 were attributed to C-10a and C-4a respectively. The signals δ 124.56, 124.39 and 121.37 were assigned to C-7, C-2 and C-4 respectively. The signals at δ 119.94, 115.95 and 113.82 were assigned to C-5, C-8a and C-9a respectively. The high field signal at δ 22.27 was assigned to the CH_3 at C-3.

Table 2.34. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of 1,8-dihydroxy-3-methylanthraquinone **(81)**

C. No.	^{13}C NMR(δ)	Multiplicity	^{1}H NNMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.80	C	12.00 (s, OH)	
2	124.39	CH	7.09, s	C-4, C-9a, CH_3
3	149.35	C	-	
4	121.37	CH	7.65, s	C-2, C-9a, C-10, CH_3
4a	132.53	C	-	
5	119.94	CH	7.80 (d, J = 7.45)	C-7, C-8a, C-10
6	136.95	CH	7.65 (t, J = 7.45)	C-5, C-7, C-8
7	124.56	CH	7.25 (d, J = 7.45)	C-5, C-8, C-10a
8	162.49	C	12.11 (s, OH)	
8a	115.95	C	-	
9	192.62	C	-	
9a	113.82	C	-	
10	182.03	C	-	
10a	132.53	C	-	
3-CH_3	22.27	CH_3	2.45 (s, CH_3)	C-2, C-3-, C-4

The ^{1}H NMR spectrum of the compound **81** showed absorption at δ 2.45 (s, 3H) for a methyl group, at δ 12.00 and 12.11 for two chelated hydroxy groups, OH-1 and OH-8 respectively. Three ortho-coupled aromatic protons at δ 7.25 (dd, J = 7.5, 0.9 Hz, H-5), 7.65 (t, J = 7.5 Hz, H-6) and 7.80 (dd, J = 7.5, 0.9 Hz, H-5). The spectrum also showed two singlets for aromatic protons at δ 7.09 (s, H-2) and 7.65 (s, H-4).

In the HMBC spectrum, the C-9 resonating at δ 192.62 correlated to the hydroxy groups at δ 12.00 (OH-1) and 12.11 (OH-8), confirming the 1,8-dihydroxyanthraquinone moiety. The C-10 signal at δ 182.03 shows correlation to signals at δ 7.65 (H-4) and 7.80 (H-5), the carbon at δ 124.56 (H-7) correlated to signals δ 132.51 (C-10a) and 115.95 (C-8a). The proton at δ 7.25 (H-7) correlated to δ 119.94 (C-5) and 132.51 (C-10a), while the proton at

δ 7.80 correlated to the carbons at δ 124.56 (C-7) and 115.95 (C-8a). The signal at δ 7.09 (H-2) showed correlation to the carbons at δ 121.37 (C-4) and 113.82 (C-9a). The proton at δ 7.65 (H-4) correlated to the carbons at δ 124.39 (C-2) and 113.82 (C-9a). The high field signal at δ 2.45 correlated to the carbon at δ 149.35 (C-3).

On the basis of the above data, compound **81** was identified as 1,8-dihydroxy-3-methylanthraquinone (chrysophanol). All the UV, IR, ^{1}H NMR and ^{13}C NMR data agreed with the reported values for 1,8-dihydroxy-3-methylanthraquinone, isolated from *Cassia Siamea*[317] and Rhubarb.[318]

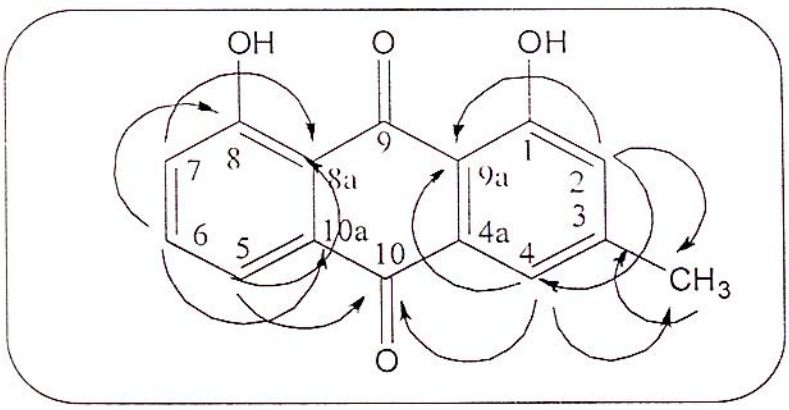
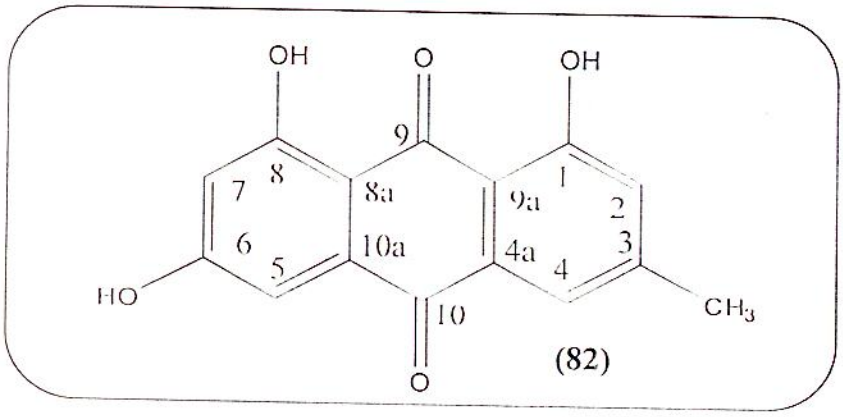


Fig. 2.33. HMBC Correlation of 1,8-dihydroxy-3-methylanthraquinone **(81)**

2.1.8.4. 1,6,8-Trihydroxy-3-methylanthraquinone (emodin) (82)



(82)

Reddish needles (Chloroform); mp 268-270 °C (Lit. mp 267-268 °C)[316]

Molecular formula $C_{15}H_{10}O_5$, deduced from EI mass spectra *m/z* 270 (calcd. 270 for $C_{15}H_{10}O_5$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **82** confirmed the presence of fifteen carbon atoms in the molecule; one methyl, four methine and ten quaternary carbons according to DEPT experiments. Total assignments were achieved by HMQC, COSY and HMBC experiments (**Table 2.35**).

In ^{13}C NMR the carbon resonance at δ 190.91 was assigned to C-9 where as a signal at δ 181.26 was assigned to C-10, typical of the carbonyl carbons of the anthraquinone moiety. The aromatic signals at δ 165.47, 165.70 and 162.47 were assigned to C-6, C-8 and C-1 respectively, indicating the presence of oxygen functionality (OH) at C-6, C-8 and C-1. The aromatic carbons at δ 148.74 and 135.82 were assigned to C-3 and C-10a respectively. The signals at δ 133.44 and 124.14 were attributed to C-4a and C-2 respectively. The signals at δ 120.67, 113.67 and 108.90 were assigned to C-4, C-9a and C-8a respectively. The signals at δ 108.90 and 108.05 were assigned to C-5 and C-7 respectively. The high field signal at δ 21.16 was assigned to the CH_3 at C-3.

Table 2.35. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $(CD_3)_2CO$ and HMBC Correlations of 1,6,8-trihydroxy-3-methylanthraquinone **(82)**

C. No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.47	C	12.06 (s, OH)	
2	124.14	CH	7.12, s	C-4, C-3, C-9a
3	148.74	C	-	
4	120.67	CH	7.55, s	C-2, C-3, C-9a
4a	133.44	C	-	
5	108.90	CH	7.23 (d, J = 2.2)	C-7, C-8a
6	165.47	C		
7	108.05	CH	6.64 (d, J = 2.2)	C-5, C-8, C-8a
8	165.70	C	12.17 (s, OH)	
8a	108.90	C	-	
9	190.91	C	-	
9a	113.67	C	-	
10	181.26	C	-	
10a	135.82	C	-	
3-CH_3	21.16	CH_3	2.44 (3H, s, CH_3)	C-3, C-2, C-4

In the HMBC spectrum, the C-9 resonance at δ 190.91 correlated to the hydroxy groups at δ 12.06 (OH-1) and 12.17 (OH-8) indicating the 1,8-dihydroxy anthraquinone moiety. The C-10 signal at δ 181.26 correlated to δ 7.55 (H-4) and 7.23 (H-5), the signal at δ 108.05 (H-7) correlated to signals at δ 108.90 (C-8a) and 108.90 (C-5). The signal at δ 7.23 (H-5) correlated with δ 108.05 (C-7), 108.90 (C-8a) and 181.26. The proton resonance at δ 7.12 (H-2) showed correlation to the carbons at δ 120.67 (C-4) and 113.67 (C-9a). The resonance at δ 7.55 (H-4) correlated to the signals at δ 124.14 (C-2) and 113.67 (C-9a). The high field absorption at δ 2.44 correlated to the carbons at δ 148.74 (C-3).

The COSY spectrum showed correlation between H-2 and H-4 while H-5 correlated to H-7.

On the basis of these data, compound **82** was identified as 1,6,8-trihydroxy-3-methylanthraquinone (emodin). All the data (UV, IR, ^{1}H NMR and ^{13}C NMR) agreed with the reported values for 1,6,8-trihydroxy-3-methylanthraquinone isolated from Rhubarb.[316]

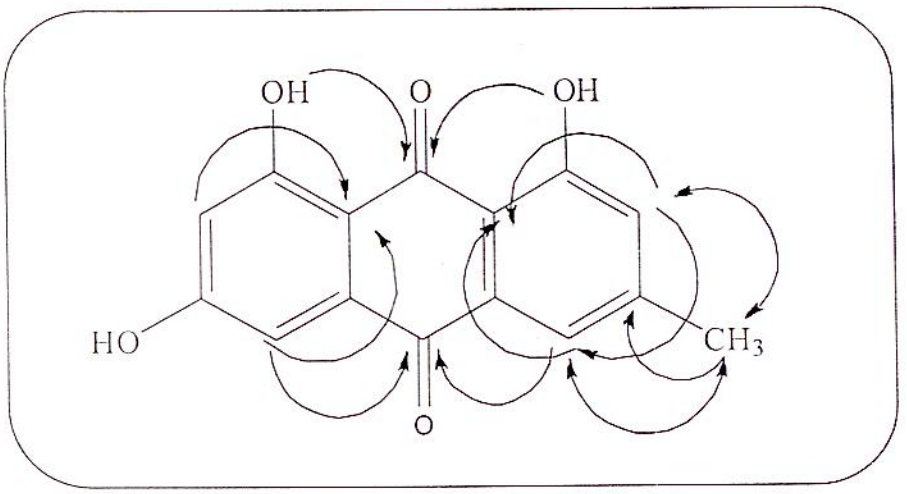
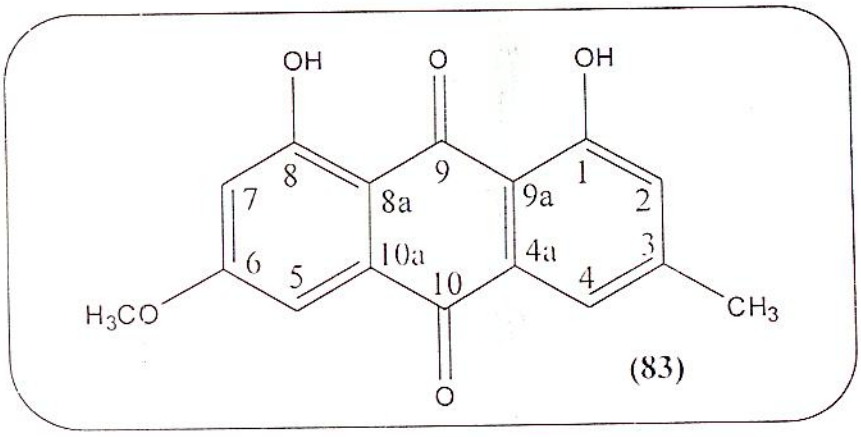


Fig. 2.34. HMBC Correlation of 1,6,8-trihydroxy-3-methylanthraquinone (82)

2.1.8.5. 1,3-Dihydroxy-6-methoxy-3-methylanthraquinone (83)



(83)

Reddish needles (Acetone); mp 205-206 °C (Lit. mp 206-207 °C)[316]

Molecular formula $C_{16}H_{10}O_5$, deduced from EI mass spectra *m/z* 284; (calcd. 284 for $C_{16}H_{10}O_5$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **83** displayed the presence of sixteen carbon atoms; one methyl, one methoxy, four methine and ten quaternary carbons according to DEPT experiments. Total assignments were established by HMQC, COSY and HMBC experiments **(Table 2.36)**.

In ^{13}C NMR the carbon resonance at δ 190.88 was assigned to C-9 and δ 182.14 to C-10, the carbonyl carbons of the anthraquinone moiety. The aromatic signals at δ 166.62, 165.23 and 162.57 were attributed to C-6, C-8 and C-1 respectively, having OH or OCH_3. The aromatic signals at δ 148.54 and 135.32 were assigned to C-3 and C-10a respectively. The signals at δ 133.28 and 124.61 were assigned to C-4a and C-2 respectively. The signals at δ 121.39, 113.75 and 110.33 were attributed to C-4, C-9a and C-8a respectively. The carbons at δ 108.33 and 106.85 were assigned to C-5 and C-7 respectively. The signals at δ 56.17 and 22.25 were assigned to OCH_3 at C-6 and the CH_3 at C-3 respectively.

Table 2.36. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of 1,8-dihydroxy-6-methoxy-3-methylanthraquinone **(83)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.57	C	12.11, s (OH)	
2	124.61	CH	7.06, s	C-4, C-9a
3	148.54	C	-	
4	121.39	CH	7.61, s	C-2, C-9a, C-10
4a	133.28	C	-	
5	108.33	CH	7.30 (d, J = 2.52)	C-7, C-8a, C-10
6	166.62	C	-	
7	106.85	C	6.67 (d, J = 2.52)	C-5, C-8a
8	165.23	C	12.31, s (OH)	
8a	110.33	C	-	
9	190.88	C	-	
9a	113.75	C	-	
10	182.14	C	-	
10a	135.32	C	-	
8-OCH_3	56.18	CH_3	3.92 (3H, s, OCH_3)	C-6
3-CH_3	22.26	CH_3	2.43 (3H, s, CH_3)	C-2, C-3, C-4

The ^{1}H NMR spectrum of the compound **83** showed signals at δ 2.43 (s, 3H) for a methyl group and at δ 3.92 for a methoxy group (s, 3H). In the spectrum two sharp singlets at δ 12.11 and 12.31 for two chelated hydroxy groups, OH-1 and OH-8 respectively, (D_2O exchangeable). Two meta-coupled aromatic protons at δ 6.67 (d, J = 2.52 Hz, H-7), 7.30 (d, J = 2.52 Hz, H-5) were assigned to H-7 and H-5 respectively. The proton spectrum also displayed two singlets for aromatic protons at δ 7.06 (s, H-2) and 7.61 (s, H-4).

In the HMBC spectrum, the C-9 resonance at δ 190.86 correlated with hydroxy groups at δ 12.11 (OH-1) and 12.30 (OH-8), confirming the 1,8-dihydroxyanthraquinone moiety. The C-10 signal at δ 182.14 showed correlation with signals at δ 7.61 (H-4) and 7.30 (H-5), the

signal at δ 106.85 (H-7) correlated to signals at δ 110.33 (C-8a) and 108.33 (C-5). The proton at δ 7.30 (H-5) correlated to δ 106.85 (C-7) and 110.33 (C-8a. The resonance at δ 7.60 (H-4) correlated to signals at δ 124.61 (C-2) and 113.75 (C-9a). The proton at δ 7.06 (H-2) showed correlation to the carbons at δ 121.39 (C-4) and 113.75 (C-9a). The high field proton at δ 2.43 correlated to the resonance at δ 148.54 (C-3) and the signal at δ 3.92 correlated to δ 166.62 (C-6) **(Fig. 2.34)**.

On the basis of these data, compound **83** was identified as 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (physcion). All the data (UV, IR, ^{1}H NMR and ^{13}C NMR) agreed with the reported one for 1,8-dihydroxy-6-methoxy-3-methylanthraquinone, isolated from the *Cassia siamea*[319] and Rhubarb.[318]

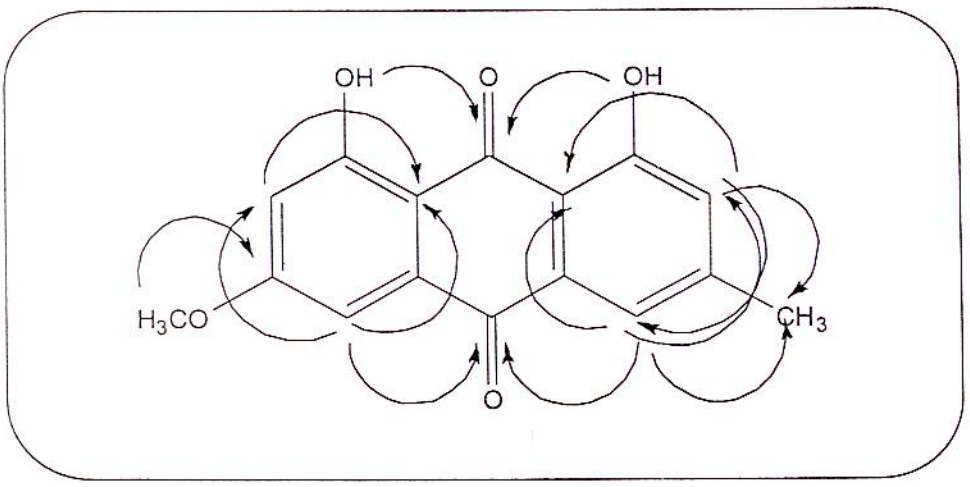
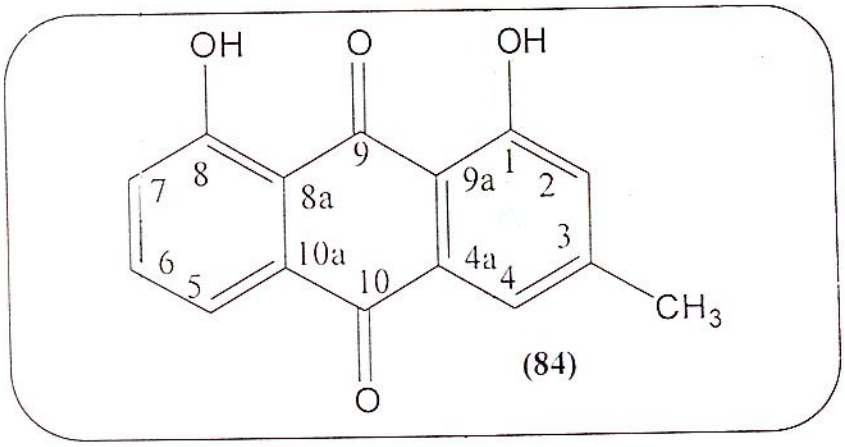


Fig. 2.35. HMBC Correlation of 1,8-dihydroxy-6-methoxy-3-methylanthraquinone **(83)**

2.2. New source Compounds Isolated from *Cassia nemophila* Flower

2.2.1. 1,8-Dihydroxy-3-methylanthraquinone (84)



(84)

Reddish needles (Chloroform); mp 195-196 °C (Lit. mp 193-194 °C)[316]
Molecular formula $C_{15}H_{10}O_4$, deduced from EI mass spectra *m/z* 254 (calcd. 254 for $C_{15}H_{10}O_4$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **84** displayed the presence of fifteen carbon atoms in the molecule; one methyl group, five methine and nine quaternary carbons from the DEPT experiments. Total assignments were made by HMQC and HMBC experiments **(Table 2.37)**.

The anthraquinoid carbonyl carbon resonances at δ 192.63 and δ 182.10 were assigned to C-9 and C-10 respectively. The aromatic carbons at δ 162.80 and 162.50 were assigned to C-1 and C-8 respectively, as expected of the oxygen functionality. The aromatic signals resonating at δ 149.44 and 137.05 were assigned to C-3 and C-6 respectively. The signals at δ 133.73 and 133.38 were assigned to C-10a and C-4a respectively. The signals at δ 124.65, 124.46 and 121.46 were assigned to C-7, C-2 and C-4 respectively. The signals at δ 120.03, 115.95 and 113.82 were assigned to C-5, C-8a and C-9a respectively. The high field carbon at δ 22.37 was assigned to the CH_3 at C-3 position.

Table 2.37. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of 1,8-dihydroxy-3-methylanthraquinone **(84)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.80	C	12.00 (s, OH)	
2	124.46	CH	7.08, s	C-4, C-9a, CH_3
3	149.44	C	-	
4	121.46	CH	7.63, s	C-2, C-9a, C-10, CH_3
4a	133.38	C	-	
5	120.03	CH	7.80 (d, J = 7.5)	C-7, C-8a, C-10
6	137.05	CH	7.65 (t, J = 7.5)	C-5, C-7, C-8
7	124.65	CH	7.27 (d, J = 7.5)	C-5, C-8, C-10a
8	162.50	C	12.11 (s, OH)	
8a	115.95	C	-	
9	192.63	C	-	
9a	113.82	C	-	
10	182.10	C	-	
10a	133.72	C	-	
3-CH_3	22.37	CH_3	2.45 (3H, s, CH_3)	C-2, C-3-, C-4

The ^{1}H NMR spectrum of the compound **84** showed signals at δ 2.45 (s, 3H) for a methyl group, at δ 12.00 and 12.12.11 for the two chelated (peri) hydroxy groups, OH-1 and OH-8 respectively. Three ortho-coupled aromatic protons at δ 7.27 (dd, J = 7.5, 0.9 Hz, H-5), 7.65 (t, J = 7.5 Hz, H-6) and 7.80 (dd, 7.5, 0.9 Hz, H-5). The proton spectrum also displayed two singlets for the aromatic protons at δ 7.08 (s, H-2) and 7.63 (s, H-4).

In the HMBC spectrum, the C-9 resonating at δ 192.63 correlated to the hydroxy groups at δ 12.00 (OH-1) and 12.11 (OH-8), confirming 1,8-dihydroxyanthraquinone moiety. The C-10 signal at δ 182.10 showed correlation to the signals at δ 7.63 (H-4) and 7.80 (H-5) and the signal at δ 137.05 (H-7) correlated to the signals at δ 133.72 (C-10a) and 115.95

(C-8a). The proton at δ 7.27 (H-7) correlated with δ 120.03 (C-5) and 133.72 (C-10a) while the one at δ 7.80 correlated to δ 124.65 (C-7) and 115.95 (C-8a). The signal at δ 7.08 (H-2) showed correlation to the signals at δ 121.46 (C-4) and 113.82 (C-9a). The proton resonance at δ 7.63 (H-4) correlated to the signals at δ 124.46 (C-2) and 113.82 (C-9a). The high field absorbance at δ 2.45 correlated with the resonance at δ 149.44 (C-3).

From the above spectral data, compound **84** was identified as 1,8-dihydroxy-3-methylanthraquinone (chrysophanol). All the data (UV, IR, ^{1}H NMR and ^{13}C NMR) agreed with the reported values for the compound isolated from *Cassia siamea*[317] and Rhubarb.[318]

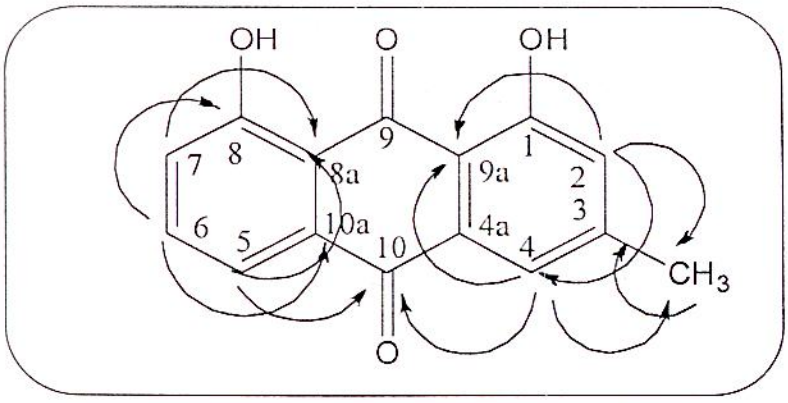
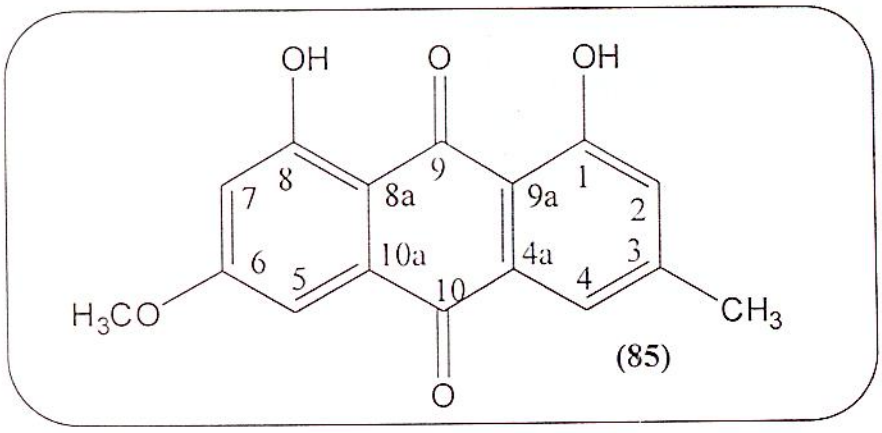


Fig. 2.36. HMBC Correlation of 1,8-dihydroxy-3-methylanthraquinone **(84)**

2.2.2. 1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (85)



(85)

Reddish needles (Chloroform); mp 205-206 °C (Lit. mp 206-207 °C)[316]

Molecular formula $C_{16}H_{10}O_5$, deduced from EI mass spectra *m/z* 284 (calcd. 284 for $C_{16}H_{12}O_5$) and ^{13}C NMR.

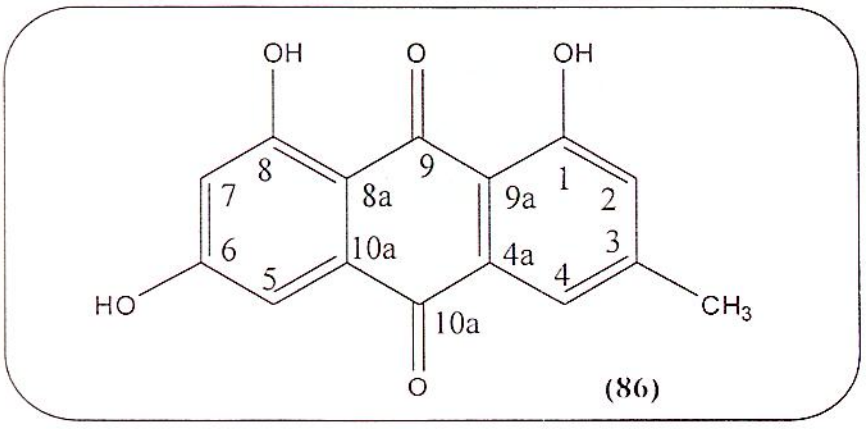
The ^{13}C NMR spectrum of the compound **85** confirmed the presence of sixteen carbon atoms in the molecule; one methyl group, one methoxy group, four methine and ten quaternary carbons (from DEPT experiments). Total assignments were established according to HMQC, COSY and HMBC experiments.

From the data (UV, IR, ^1H NMR and ^{13}C NMR) the compound **85** was identified as 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (physcion). All the spectral data (UV, IR, ^1H NMR and ^{13}C NMR) were the same as reported for the compound **83**, isolated from the seeds. For comparison the data is given in **Table 2.38**.

Table 2.38. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of 1,8-dihydroxy-6-methoxy-3-methylanthraquinone **(85)**

C. No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.53	C	12.08 (s, OH)	
2	124.50	CH	7.06, s	C-4, C-9a
3	148.44	C	-	
4	121.28	CH	7.60, s	C-2, C-9a, C-10
4a	133.25	C	-	
5	108.21	CH	7.34 (d, J = 2.36)	C-7, C-8a, C-10
6	166.57	C	-	
7	106.79	CH	6.66 (d, J = 2.34)	C-5, C-8a
8	165.21	C	12.28 (s, OH)	
8a	110.29	C	-	
9	190.83	C	-	
9a	113.78	C	-	
10	182.01	C	-	
10a	135.29	C	-	
3-CH_3	22.13	CH_3	2.43 (3H, s, CH_3)	C-2, C-3, C-4
6-OCH_3	56.07	CH_3	3.91 (3H, s, OCH_3)	C-6

2.2.3. 1,6,8-Trihydroxy-3-methylanthraquinone (86)



(86)

Reddish needles (Chloroform); mp 268-270 °C (Lit. mp 267-268 °C)[116]

Molecular formula $C_{15}H_{10}O_5$, deduced from EI mass spectra *m/z* 270 (calcd. 270 for $C_{15}H_{10}O_5$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **86** confirmed the presence of fifteen carbon atoms in the molecule; one methyl group, four methine and ten quaternary carbons according to DEPT experiments. Total assignments were established according to HMQC, COSY and HMBC experiments (**Table 2.39**).

The ^1H NMR spectrum of the compound **86** showed signals at δ 2.31 (s, 3H) for a methyl group and two sharp siglets at δ 12.09 and 12.18 for the two chelated (peri) hydroxy groups (OH-1, OH-8) (D_2O exchangeable) respectively. Two meta-coupled aromatic protons at δ 6.48 (d, J = 2.2 Hz, H-7) and δ 7.10 (d, J = 2.2 Hz, H-5). The spectrum also showed two singlets for aromatic protons at δ 6.94 (s, H-2) and 7.45 (s, H-4).

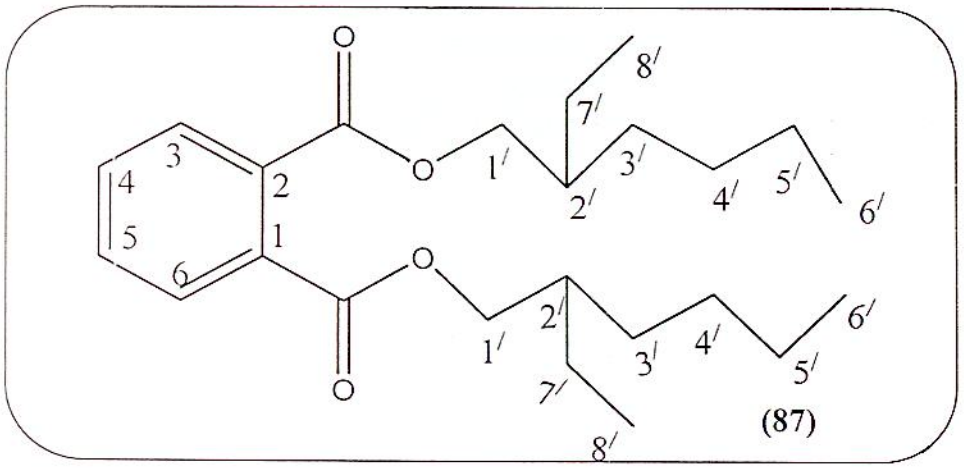
From the data (UV, IR, ^1H NMR and ^{13}C NMR), compound **86** was identified as 1,6,8-trihydroxy-3-methylanthraquinone. All the specrtal data (UV, IR, ^1H NMR and ^{13}C NMR) values were the same as for compound **82**. The slight differences in the chemical shifts were due to the effect of the solvents used for the spectral analasis.

Table 2.39. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR* data and HMBC Correlations of 1,6,8-trihydroxy-3-methylanthraquinone **(86)**

C. No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	161.88	C	12.09 (s, OH)	
2	124.21	CH	6.94, s	C-4, C-3, C-9a
3	147.97	C	-	
4	120.93	CH	7.45, s	C-2, C-3, C-9a
4a	133.01	C	-	
5	109.31	CH	7.10 (d, J = 2.2)	C-7, C-8a
6	165.30	C		
7	108.27	CH	6.48 (d, J = 2.2)	C-5, C-8a
8	165.30	C	12.18 (s, OH)	
8a	113.51	C	-	
9	190.28	C	-	
9a	113.51	C	-	
10	182.51	C	-	
10a	135.16	C	-	
3-CH_3	21.77	CH_3	2.31 (3H, s, CH_3)	C-2, C-3, C-4

* Solvent CD_3OD+$CDCl_3$

2.2.4. Di-2-ethylhexyl phthalate (87)



(87)

White thick residue (Acetone); (274-276)

Molecular formula $C_{24}H_{38}O_4$, deduced from EI mass spectra *m/z* 390 (calcd. 390 for $C_{24}H_{38}O_4$) and the ^{13}C NMR.

The IR spectrum showed absorption at $\tilde{\nu}$ 1730 cm^{-1} for an ester carbonyl stretching.

The ^{13}C NMR spectrum of the compound **87** showed the presence of twelve signals for the 24 carbon atoms in the molecule; due to symmetry in order to account for the molecular mass. The broad band spectrum confirmed the presence of four methyl, six methine, ten methylene and four quaternary carbons from the DEPT experiments. Total assignments were made by HMQC and HMBC experiments (**Table 2.40**). The carbon resonating at δ 168.00 was assigned to the ester carbonyl while the one at δ 132.25 was assigned to C-1 and C-2. The aromatic carbons at δ 131.07 and 128.52 were assigned to C-4, C-5 and C-3, C-6 respectively. The signal resonates at δ 67.76 was assigned to CH_2 (C-1′) next to an ester group. The signals at δ 38.83, 30.28 and 28.80 were assigned to C-2′, C-3′ and C-4′ respectively. The signals at δ 23.61, 22.83, 13.06 and 10.06 were assigned to C-7′, C-5′ and C-6′ and C-8′ respectively.

Table 2.40. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in CD_3OD and HMBC Correlations of di-2-ethylhexyl phthalate **(87)**

C.No	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
	168.00	2C		C-1′, C-3, C-6,
1	132.25	C		
2	132.25	C		
3	128.52	CH	7.69 (dd, J = 5.7, 3.24, H-3, H-6)	COO
4	131.06	CH	7.60 (dd, J = 5.7, 3.24,H-4, H-5)	C-2, C-3
5	131.06	CH	7.60 (dd, J = 5.7, 3.24, H-4, H-5)	C-2, C-3
6	128.52	CH	7.69 (dd, J = 5.7, 3.24,H-3, H-6)	COO
1′	67.75	2 CH_2	4.18, 4.19 (4H, dd, J = 5.76, 4.23, H-1′)	COO
2′	38.84	2 CH	1.65, m (2H, H-2′)	
3′	30.28	2 CH_2	1.41, 1.67 (4H, d t, J = 5.8, 6.0)	
4′	28.80	2 CH_2	1.36, m (4H, H-4′)	
5′	22.69	2 CH_2	1.32, m (4H, H-5′)	
6′	13.05	2 CH_3	0.85 (6H, t, J = 6.9, CH_3-6′)	C-4′
7′	23.61	2 CH_2	1.21, 1.35, m (4H, H-7′)	
8′	10.05	2 CH_3	0.90 (6H, t, J = 7.8, CH_3-6′)	C-2′

The ^{1}H NMR spectrum of the compound **87** showed signals at δ 0.85 (t, J = 6.9 Hz, 6′-CH_3) for a methyl group and a second methyl at δ 0.90 (t, J = 7.8 Hz, 8′-CH_3). The signals at δ 1.21, 1.35 (m, H-7′) was assigned to diastereotropic protons H-7′a and H-7′b, giving a multiplet (one side it is attached to asymmetric center and other side to a methyl group). The resonances at δ 1.32 (m, H-5′) and 1.36 (m, H-4′) were attributed to H-5′ and H-4′ respectively. The protons at δ 1.41 (d, 6.0 Hz, H-3′a), 1.68 (t, J = 5.8, H-3′b) and 1.65 (m, H-2′). The up field absorption at δ 4.18, 4.19 (dd, J = 5.76, 4.23 Hz H-1′) diastereotropic protons for H-1′. Two signal for ortho-coupled aromatic protons at δ 7.69 (7.69 (d d, J =

5.7, 3.24 Hz, H-3, H-6) and 7.60 (d d, J = 5.7, 3.24 Hz, H-4, H-5).

The reported values for H-2′ in the literature is a doublet, which is not correct, it gives multiplet. Similarly the H-6′ and H-8′ gives clear triplet which are not mention in the literature. The ^{13}C NMR value reported for C-3 was δ 30.28, which was incorrect, its correct value is δ 38.83. Similarly for C-4 the correct value was δ 30.28 rather than δ 38.83.[123]

In the HMBC spectrum, the ester carbonyls at δ 168.00 are correlated to signals at δ 7.69 (H-3, H-6) and 4.18, δ 4.19 (H-1′). The signal at δ 132.25 (C-2, C-3) correlated with the signal at δ 7.60 (H-4, H-5). The signal at δ 13.06 (C-8′) shows correlation with the proton at δ 1.65 (H-2′) and the resonance at δ 0.90 (H-6′) correlated to δ 28.46 (C-4′). All these data support the correct structure also confirmed by 1D, 2D NMR techniques. As for as we are aware no HMQC or HMBC techniques are reported so for.

All the IR, ^{1}H NMR and ^{13}C NMR data agreed with the literature value of di-2-ethylhexyl phthalate, isolated from *Cassia auriculata* L.[123] However we are reporting some corrections in ^{1}H NMR and ^{13}C NMR also for the first reporting the HMBC techniques.
All the spectral data suggest the structure given but the melting point reported is -56 °C but our compound having mp 274-276 °C. Further work is nessary to confirm the structure.

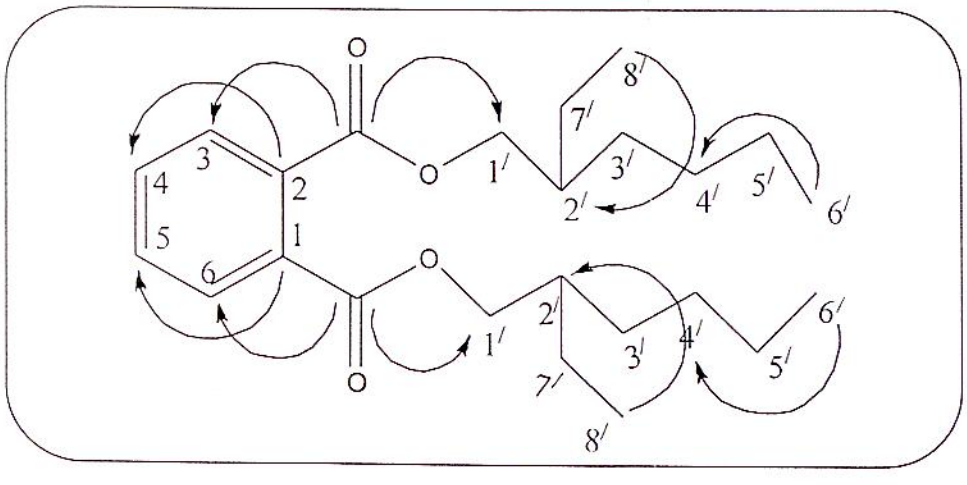
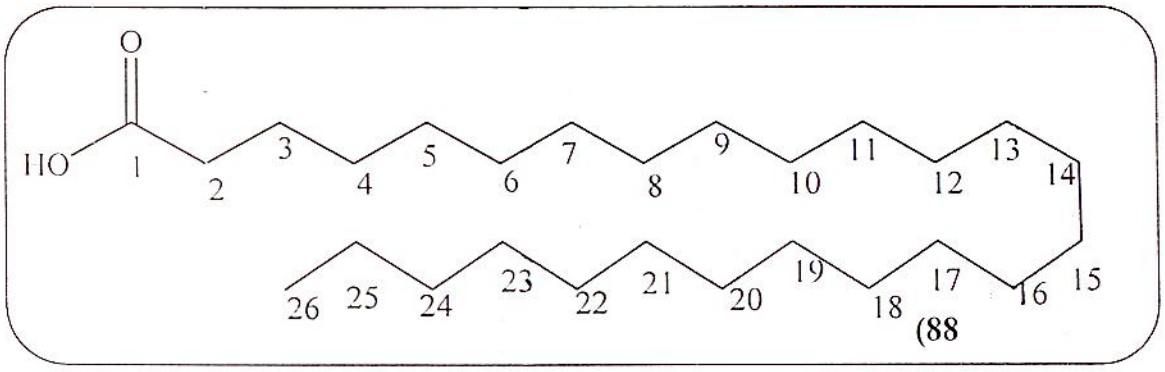


Fig. 2.37. HMBC Correlation of di-2-ethylhexyl phthalate **(87)**

2.3. New source Isolated Compounds from the Bark of *Cassia nemophila*

2.3.1. Hexacosanoic acid (88)



(88

White residue (Chloroform); m. Point 74-75°C

Molecular formula $C_{26}H_{52}O_2$, deduced through mass spectrum *m/z* 396 (calcd. 396 for $C_{26}H_{52}O_2$) and the ^{13}C NMR.

The IR absorption at $\tilde{\nu}$ 3305-2610 cm^{-1} for carboxylic OH stretching, $\tilde{\nu}$ 1707 cm^{-1} for C=0 stretching (COOH) showed the presence of carboxylic acid group.

Table 2.41. ^1H (400 MHz) and ^{13}C (100 MHz) NMR in $CDCl_3$ of hexacosanoic acid **(88)**

C.No.	^{13}C NMR(δ)	Multiplicity	^1H NMR (δ) (J_{HH} Hz)
1	178.00	C	-
2	33.50	CH2	2.30 (2H, t, J = 7.4 Hz)
3	31.90	CH2	1.61, m
4-25	29.68- 22.60	CH2	1.23, m
26	14.09	CH3	0.86 (3H, t, J = 6.8 Hz)

The ^{13}C NMR gives characteristic peak at δ 178.00 for the carboxylic acid carbonyl and the signal at δ 33.50 for the CH_2 next to carbonyl carbon. The signal at δ 31.90 was assigned to C-3. The multiplet from δ 22.60 to 29.60 were assigned to C-4 to C-25. The signal at δ 14.09 was attributed to the terminal CH_3 group.

In the ^1H NMR, a triplet at δ 2.30 (2H, t, J = 6.94 Hz) was a characteristic for a CH_2 (CH_2CO) group and a multiplet at δ 1.61 was a characteristic for a $CH_2\underline{CH_2}CH_2CO$). The forty four protons broad singlet at δ 1.23 (44H, H-4 to H-25) was attributed to protons from H-4 to H-25 and a triplet at δ 0.86 for a terminal CH_3 group.

From the above data (IR, UV, ^1H NMR, ^{13}C NMR and mass spectrum) the structure of the compound was elucidated to be hexacosanoic acid. The compound is reported from Lycoperdaceae mushrooms, *Bovista plumbea* Pers. and *Calvatia utriformis* Pers.[339] from Bryophytes,[340,341] *Eucalyptus* species *Eucalyptus urograndis* and *E. camaldulensis*[342] and from *Musa textilis*[343] family Musaceae through GC/Ms but no ^1H NMR or ^{13}C NMR was reported. As for as we know this is the first report from family Legminoseae and the first report of its ^1H NMR, ^{13}C NMR and mass spectral data.

Mass fragmentation of the compound **88** is given in Figure 2.39

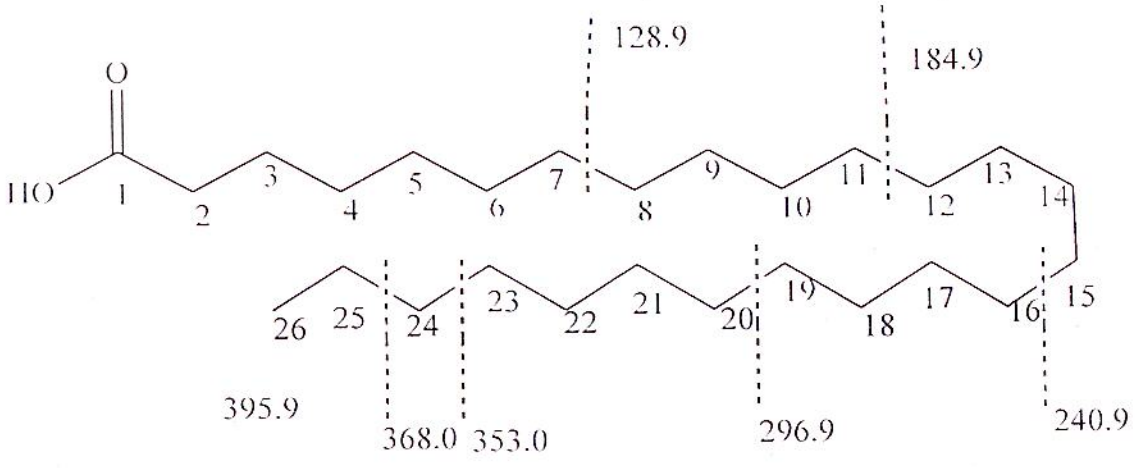
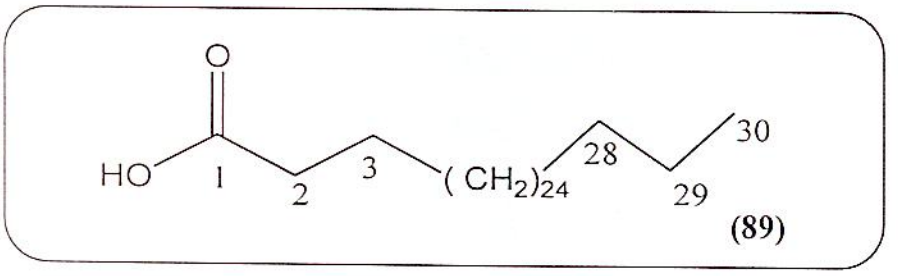


Fig. 2.38. Mass fragmentation of hexacosanoic acid **(88)**

2.3.2. Triacontanoic acid (Melissic acid) (89)



(89)

White powder (Chloroform) m p 92-93 °C (Lit. mp 92-93 °C)[344]

Molecular formula $C_{30}H_{60}O_2$, deduced through mass spectrum *m/z* 452 (calcd. for $C_{30}H_{60}O_2$ (452).

The IR absorption at $\tilde{\nu}$ 1710 cm^{-1}, C=O St. showed the presence of carboxylic group.

Table 2.42. ^1H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ of the compound triacontanoic acid **(89)**

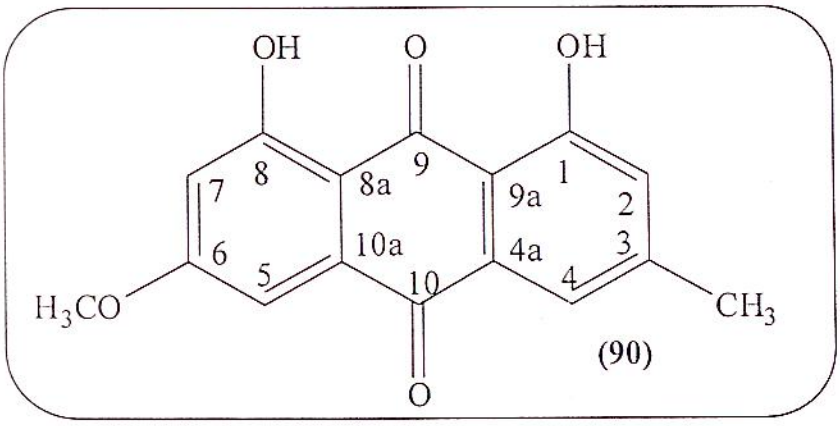
C.No.	^{13}C NMR(δ)	Multiplicity	^1H NMR (δ) Coupling Constants J_{HH} (Hz)
1	178.51	C	5.34, s (OH)
2	33.78	CH_2	2.26 (2H, t, J = 7.4 Hz)
3	31.91	CH_2	1.59, m
4-27	29.68- 29.06	CH_2	1.23, m
28	24.69	CH_2	1.23, m-
29	22.67	CH_2	1.23, m
30	14.09	CH_3	0.83 (3H, t, 6.8 Hz)

In the ^1H NMR a triplet at δ 2.26 (2H, t, J = 7.4 Hz, CH_2-2) was a characteristic for a CH_2 (CH_2CO) group, a multiplet at δ 1.59 for a CH_2 (2H, CH_2-3) and a fifty two protons multiplet at δ 1.23 (52H, b s, 26×CH_2, CH_2-4-29) for 26 CH_2. The triplet at δ 0.83 (3H, t, J = 6.8 Hz) was assigned to the terminal CH_3 group.

The ^{13}C NMR confirmed this as a long chain carboxylic acid and the position of acid functional group is terminal as there is only one CH_2 group attached to CO and one terminal CH_3 group. The signal at δ 178.51 was assigned to carbonyl carbon of the carboxylic acid and a signal at δ 33.78 was assigned to CH_2 next to the carbonyl carbon (C-2). The signal at δ 31.91 was assigned to C-3 and the signals from δ 29.68 to 29.06 were assigned to C-4 to C-27. The up field signals at δ 24.69, 22.67 and 14.09 were assigned to C-28, C-29 and a terminal CH_3 (C-30) respectively.

All the spectral data (UV, IR, ^1H NMR and ^{13}C NMR) agreed with the reported values for triacontanoic acid, isolated from *Fagonia cretica* L. (Zygophyllaceae)[345] However this is reported for the first time from family Leguminosae.

2.3.3. 1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (90)



(90)

Reddish needles (Acetone); mp 205-206 °C (Lit. mp 206-207 °C)[316]

Molecular formula $C_{16}H_{10}O_5$, deduced from EI mass spectra *m/z* 284 (calcd. 284 for $C_{16}H_{10}O_5$) and the ^{13}C NMR.

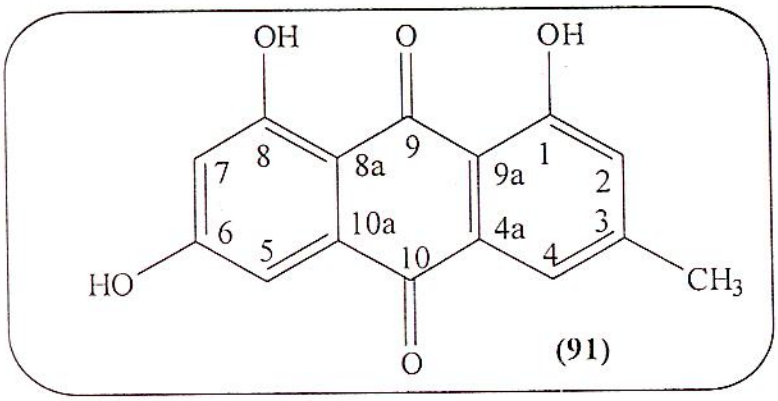
The ^{13}C NMR spectrum of the compound **90** displayed the presence of 16 carbon atoms, including a methyl group, a methoxy group, four methine and ten quaternary carbons from the DEPT experiments. Total assignments were established from the HMQC, COSY and HMBC experiments (**Table 2.43**).

Table 2.43. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in CD_3OD and HMBC Correlations of 1,8-dihydroxy-6-methoxy-3-methylanthraquinone **(90)**

C. No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	163.98	C	-	
2	123.92	CH	7.05, s	C-4, C-9a
3	146.79	C	-	
4	119.17	CH	7.49, s	C-2, C-9a, C-10
4a	132.63	C	-	
5	107.78	CH	7.23 (d, J = 2.28)	C-7, C-8a, C-10
6	162.67	C	-	
7	104.53	CH	6.76 (d, J = 2.28)	C-5, C-8a
8	163.98	C	-	
8a	114.73	C	-	
9	192.12	C	-	
9a	114.73	C	-	
10	182.91	C	-	
10a	137.40	C	-	
3-CH_3	20.51	CH_3	2.39 (3H, s, CH_3)	C-2, C-3, C-4
8-OCH_3	55.41	CH_3	3.93 (3H, s, OCH_3)	C-6

On the basis of IR, UV ^{1}H NMR ^{13}CNMR, HMBC and mass spectrum the compound **90** was identified as 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (physcion). All of the data (UV, IR, ^{1}H NMR and ^{13}C NMR) were the same as discussed for the compound **83**.

2.3.4. 1,6,8-Trihydroxy-3-methylanthraquinone (91)



(91)

Reddish needles (Chloroform); mp 268-270 °C (Litr. mp 267-268 °C)[316]
Molecular formula $C_{15}H_{10}O_5$, deduced from EI mass spectra *m/z* 270 (calcd. 270 for $C_{15}H_{10}O_5$).

The ^{1}H NMR spectrum of the compound **91** showed signals at δ 2.44 (s, 3H) for a methyl group and at 3.92 (s, 3H) for a methoxy group. Two meta-coupled protons at δ 6.67 (d, J = 2.52 Hz, H-7), δ 7.35 (d, J = 2.52 Hz, H-5). The spectrum also displayed two singlets for aromatic protons at δ 7.07 (s, H-2) and at δ 7.61 (s, H-4).

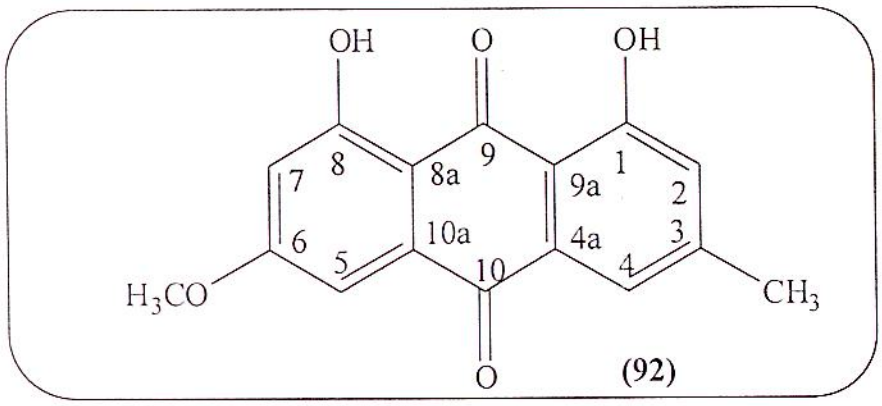
Table 2.44. ^{1}H NMR (600 MHz in $(CD_3)_2CO$) data of the 1,6,8-trihydroxy-3-methylanthraquinone **(91)**

H.No.	^{1}H NMR (δ) Coupling Constants J_{HH} (Hz)
2	7.17, s
4	7.55, s
5	7.23 (d, J = 2.1)
7	6.62 (d, J = 2.2)
3-CH_3	2.44 (3H, s, CH_3)

The ^{1}H NMR values were the same as reported for 1,8-trihydroxy-3-methylanthraquinone **(59)** discussed above reported from *Cassia artemisioides*, reported in literature from Rhubarb.[316]

2.4. New source Isolated Constituents from the Roots of *Cassia nemophila*

2.4.1. 1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (92)



(92)

Reddish needles (Chloroform); mp 205-206 °C (Lit. mp 206-207 °C)[316]

The compound **92** has the molecular formula $C_{16}H_{10}O_5$, deduced from EI mass spectra *m/z* 284 (calcd. 284 for $C_{16}H_{10}O_5$) and the ^{13}C NMR.

The ^{13}C NMR spectrum of the compound **92** confirmed the presence of sixteen carbon atoms in the molecule; one methyl group, one methoxy group, four methine and ten quaternary carbons according to DEPT experiments. Total assignments were made from the HMQC, COSY and HMBC experiments **(Table 2.45)**.

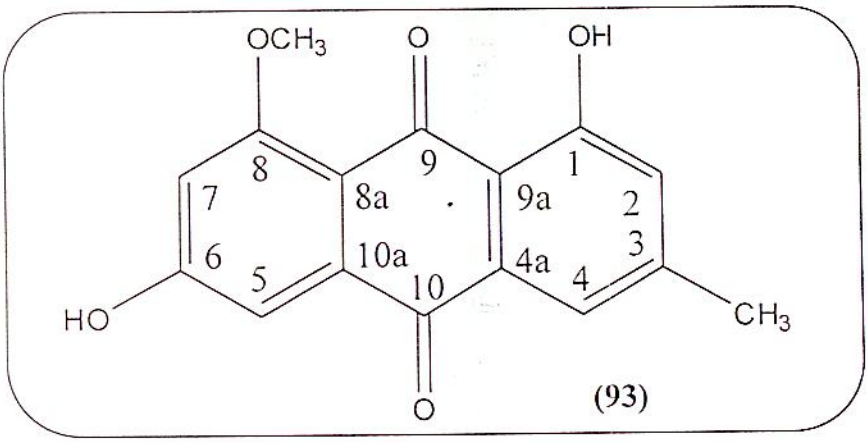
From the data (IR, UV, ^1H NMR, ^{13}C NMR and HMBC) the compound **92** was identified as 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (physcion). All the results were the same as discussed for the compound **90**.

The ^1H NMR and ^{13}C NMR data are given in the table for the sake of comparison.

Table 2.45. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ and HMBC Correlations of 1,8-dihydroxy-6-methoxy-3-methylanthraquinone **(92)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.58	C	12.11 (s, OH)	
2	124.61	CH	7.07, s	C-4, C-9a
3	148.54	C	-	
4	121.39	CH	7.61, s	C-2, C-9a, C-10
4a	133.28	C	-	
5	108.33	CH	7.35 (d, J = 2.52)	C-7, C-8a, C-10
6	166.62	C	-	
7	106.85	CH	6.67 (d, J = 2.52)	C-5, C-8a
8	165.27	C	12.31 (s, OH)	
8a	110.33	C	-	
9	190.88	C	-	
9a	113.75	C	-	
10	182.15	C	-	
10a	135.32	C	-	
3-CH_3	22.26	CH_3	2.44 (3H, s, CH_3)	C-2, C-3, C-4
6-OCH_3	56.18	CH_3	3.92 (3H, s, OCH_3)	C-6

2.4.2. 1,6-Dihydroxy-8-methoxy-3-methylanthraquinone (93)



(93)

Yellow needles (Acetone); mp 300-302 °C (Lit. mp 301-303 °C)[320]

Molecular formula $C_{16}H_{12}O_5$, deduced from EI mass spectrum *m/z* 284 (calcd. 284 for $C_{16}H_{12}O_5$) and ^{13}C NMR

The ^{13}C NMR spectrum of the compound **93** displayed the presence of sixteen carbon atoms in the broad band spectrum; one methyl group, one methoxy group, four methine and ten quaternary carbons were achieved from the DEPT experiments. Total assignments were established according to HMQC and HMBC experiments (**Table 2.46**).

The carbon resonating at δ 187.17 was assigned to C-9 while the one at δ 182.82 to C-10, the two carbonyl carbons of the anthraquinone moiety. The aromatic signals at δ 165.79, 162.32 and 163.92 were assigned to C-8, C-6 and C-1 respectively, confirming the oxygen functionality (OH or OCH_3). The aromatic carbons resonating at δ 146.76 and 137.39 were assigned to C-3 and C-10a respectively. The signals at δ 132.59, 123.94 and 119.20 were assigned to C-4a, C-2 and C-4 respectively. The signals at δ 114.68, 112.75, 107.53 and 104.45 were attributed to C-9a, C-8a, C-5 and C-7 respectively. The high field resonance at δ 55.89 was assigned to OCH_3 and the one at δ 20.52 for a CH_3 group.

Table 2.46. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in CD_3OD and HMBC Correlations of 1,6-dihydroxy-8-methoxy-3-methylanthraquinone **(93)**

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR (δ) (J_{HH} Hz)	HMBC Correlation
1	162.32	C	-	
2	123.94	CH	7.04, s	C-9a, C-4
3	146.83	C	-	CH_3 (20.97)
4	119.20	CH	7.48, s	C-2, C-9a
4a	132.59	C	-	
5	107.53	CH	7.23 (d, J = 2.3)	C-7, C-8a
6	163.92	C	-	
7	104.45	CH	6.76 (d, J = 2.3)	C-5, C-8a
8	165.79	C	-	
8a	112.75	C	-	
9	187.17	C	-	
9a	114.68	C	-	
10	182.82	C	-	C-4, C-5
10a	137.39	C	-	
3-CH_3	20.52	CH_3	2.39 (3H, s, CH_3)	C-2, C-3, C-4
8-OCH_3	55.89	CH_3	3.93 (3H, s, OCH_3)	C-8

The ^{1}H NMR spectrum of the compound **93** showed a singlet integrated for three protons at δ 2.39 (3H, s, CH_3) for a methyl group and another three proton singlet at δ 3.93 (3H, s, OCH_3) for methoxy group. Two meta-coupled aromatic protons at δ 6.76 (d, J = 2.3 Hz, H-7) and 7.23 (d, J = 2.3 Hz, H-5). The spectrum also displayed two singlets for aromatic protons at δ 7.04 (s, H-2) and 7.48 (s, H-4).

In the HMBC spectrum, the C-10 signal at δ 182.82 showed correlation with signals at δ 7.48 (H-4) and 7.23 (H-5), the signal at δ 165.79 (C-8) correlated to the OCH_3 (δ 3.93). The signal at δ 114.68 (C-9a) showed correlation to the signals at δ 7.04 (H-2) and 7.48

(H-4). The resonance at δ 112.75 (C-8a) correlated with the signals at δ 6.76 (H-7) and 7.23 (H-5). The signal at δ 2.39 (CH_3) showed correlation to δ 146.83 (C-3), 123.94 (C-2) and 119.20 (C-4). These data suggested the presence of two hydroxy groups located at C-1 and C-6 and the methoxy group located at C-8. Therefore, the structure of the compound **93** was elucidated as 1,6-dihydroxy-8-methoxy-3-methylanthraquinone.

All the UV, IR, ^{1}H NMR and ^{13}C NMR data agreed with the reported values for 1,6-dihydroxy-8-methoxy-3-methylanthraquinone, isolated from the fungi *Microascus tardifasiens*[320] and *penicillium frequentans*.[321] However this is reported for the first time from plant source.

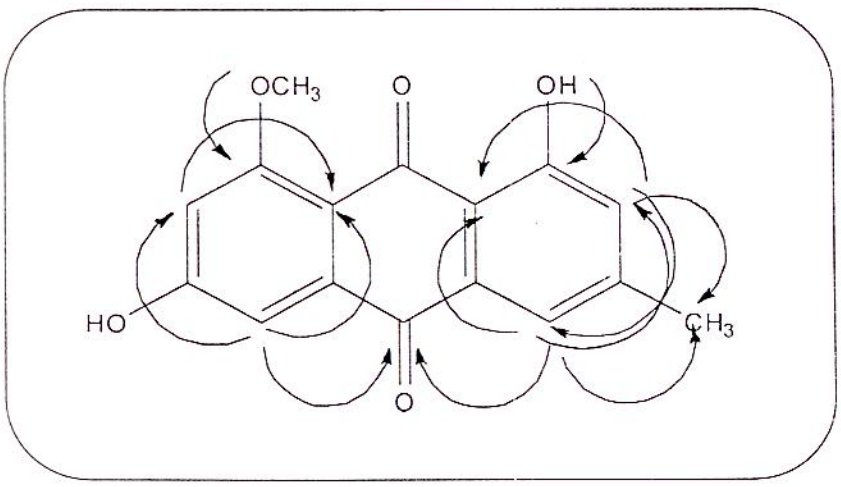
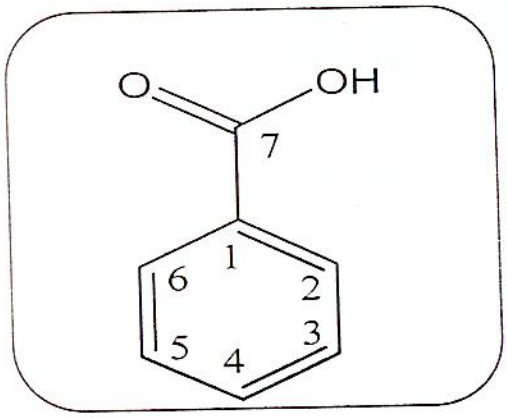


Fig. 2.39. HMBC Correlation of 1,6-dihydroxy-8-methoxy-3-methylanthraquinone (**93**)

2.4.3. Benzoic acid (94)



Colourless crystalline solid; mp 122-123 °C (Lit. mp 121-124 °C)[236]

Molecular formula $C_7H_6O_2$ deduced from EI Mass spectra *m/z* at 122 and ^{13}C NMR.

The IR spectrum, showed absorption at $\tilde{\nu}$ 3260-2700 cm^{-1} for carboxylic OH and at $\tilde{\nu}$ 1704 cm^{-1} for C=O stretching and at $\tilde{\nu}$ 1610 cm^{-1} for aromatic C=C bond.

Table 2.47. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR in $CDCl_3$ of the benzoic acid (94)

C.No.	^{13}C NMR(δ)	Multiplicity	^{1}H NMR(δ) (J_{HH} Hz)
1	129.42	C	-
2	128.59	CH	8.11 (dd, *J* = 8.0, 1.3, H-2)
3	130.32	CH	7.48 (t, *J* =. 8.0, 7.3, H-3)
4	133.93	CH	7.60 (1H, t d, *J* = 7.3, 1.3, H-4)
5	130.32	CH	7.48 (t, *J* =. 8.0, 7.3, H-5)
6	128.59	CH	8.11 (dd, *J* = 8.0, 1.3, H-2)
7	172.58		12.56, b s (OH)

The ^{13}C NMR showed five signals, including three methine and two quaternary carbons.

In ^{13}C NMR the signal at δ 172.58 was assigned to C-7 of the carboxylic carbon. The resonance at δ 133.93 was assigned to C-4 and the signal at δ 130.32 was assign to C-3 and C-4. The signal at δ 129.42 was assigned to C-1 and the resonance at δ 128.59 was

assigned to C-2 and C-6.

The proton NMR showed one up field broad signal at δ 12.56 for a carboxyl OH group and a doublet of doublet at δ 8.11 (2H, dd, J = 8.0, 1.3 Hz, H-2 and H-6). The two triplets with long range douplets at δ 7.60 (1H, t d, J = 7.3, 1.3 Hz, H-4) and 7.48 (2H, t, d J =. 8.0, 7.3 Hz, H-3 and H-5). The signal at δ 7.48 gives douplet of douplet and these douplet overlap which gives a triplet). The structure of the compound was elucidated as benzoic acid, all the IR and ^{1}H and ^{13}C NMR data agreed with the literature.[346]

Chapter 3

EXPERIMENTAL (Part A)

3.1. General Experimental Conditions

3.1.1. Melting Points

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

3.1.2. Optical Rotations

Optical rotations were measured on JASCO DIP-360 digital polarimeter.

3.1.3. UV-Vis Spectrophtometry

Ultraviolet (UV) spectra were recorded in methanol on Shimadzu U-3200 spectrophotometer.

3.1.4. IR Spectra

IR spectra were taken either on a Perkin–Elmer 681 spectrometer using KBr pellets or neat on Nicolet-380 FT-IR Spectrometer were used.

3.1.5. Mass Spectra

Mass spectra (Electrospray) were determined on a Micromass Quatto Ultima, or on an AEI MS 902 spectrometer (EI) using alcohol as matrix. Low-resolution electron impact mass spectra were recorded on a MAT 311A mass spectrophotometer or Finnigan MAT 312 double-focusing mass spectrophotometer, coupled with PDP 11/34 computer system. High resolution mass measurements and fast atom bombardment (FAB) mass measurement were carried out on JEOL JMS HX 110 mass spectrometer.

3.1.6. Nuclear Magnetic Resonance (NMR) studies

^{1}H-NMR and ^{13}C-NMR spectra spectra were recorded in CD_3OD, $CDCl_3$, $(C_3D)_2CO$ and C_5D_5N on a JEOL ECA 600 FT NMR spectrometer (600 MHz) fitted with an autotune 5 mm probe and field gradient coils at the University of Bradford, UK. J values were measured in Hz. Carbon atom types were assigned by employing a combination of ^{13}C NMR spectra, broadband proton decoupled and distortionless enhancement by polarization transfer (DEPT) experiments. Assignments were done by employing a combination of 1-D and 2-D NMR experiments. 2-D NMR spectra were acquired and processed by JEOL DELTA software. ^{1}H-^{1}H correlations by double quantum–filtered COSY. ^{1}H-^{13}C NMR correlations were established by using HMQC and HMBC pulse sequences.

3.1.7. Solvents

All commercial grade solvents were used after distillation. For column chromatography laboratory grade solvents (Fisher) were used.

3.2. Techniques employed for the purification of compounds

3.2.1. Column Chromatography (CC)

All chromatography columns thick layer plates (PLC) were made up using Merck Kieselgel GF_{254} precoated silica gel on glass plate were also used as preparative TLC. Column chromatography (CC) was carried out using silica gel (Si 60, 70-230 mesh, E. Merck) as stationary phase and organic solvents as mobile phase.

3.2.2. Thin Layer Chromatography (TLC/ PTLC)

All preparative thick layer (PTLC) and thin layer (TLC) plates were using Merck Kieselgel 60 F_{254} precoated silica gel GF_{254} preparative plates (20×20, 0.5 mm 20×10, 0.5mm and 10×5, 0.5 mm thick) E. Merck, were also used to checked the purity of the samples.

3.3. Spray Reagents for TLC

3.3.1. Ceric sulphate

The TLC plates were visualized by spraying with ceric sulphate solution made by dissolving .26 g of ceric sulphate in 10ml of sulphuric acid and diluted to 100 mL.

3.3.2. Anisaldehyde

Reagent used for detecting spots on plates were anisaldehyde spray reagent; made by mixing glacial acetic acid, anisaldehyde and concentrated sulphuric acid (98:1:1). 4-methoxy benzaldehyde, 1 ml H_2SO_4 and 98 ml of glacial acetic acid.

3.4. Plant Material

The flowers of *Cassia artemisioides* were collected from Peshawar district (N.W.F.P), Pakistan during the month of January-February 2006 and the plant was identified by Prof. Dr. S.I. Ali Department of botany, University of Karachi. A voucher herbarium specimen was deposited in the herbarium of Department of Botany, Govt. Post Graduate Jehanzeb College Swat Pakistan. The stem bark and roots of *Cassia artemisioidies* were collected from Peshawar district (N.W.F.P), Pakistan during the month of March 2006. The pods and seeds of *Cassia artemisioides* were collected from Peshawar district (N.W.F.P), Pakistan during the month of June-July 2006.

The flowers, stem bark and roots of *Cassia nemophila* were collected from Peshawar and Nowshera districts (N.W.F.P), Pakistan during the month of February-March 2006.

3.4.1. Extraction and fractionation of various parts of *Cassia aremisioides*

Dried and powder parts (flowers, stem bark, roots, pods and seeds) of *Cassia artemisoides* were extracted with hexane, dichloromethane, ethyl acetate and ethanol

using soxhlet apparatus. The general scheme for the extraction and fractionation is given in **Figure 3.1**.

Extraction and fractionation scheme for *Cassia artemisioides*

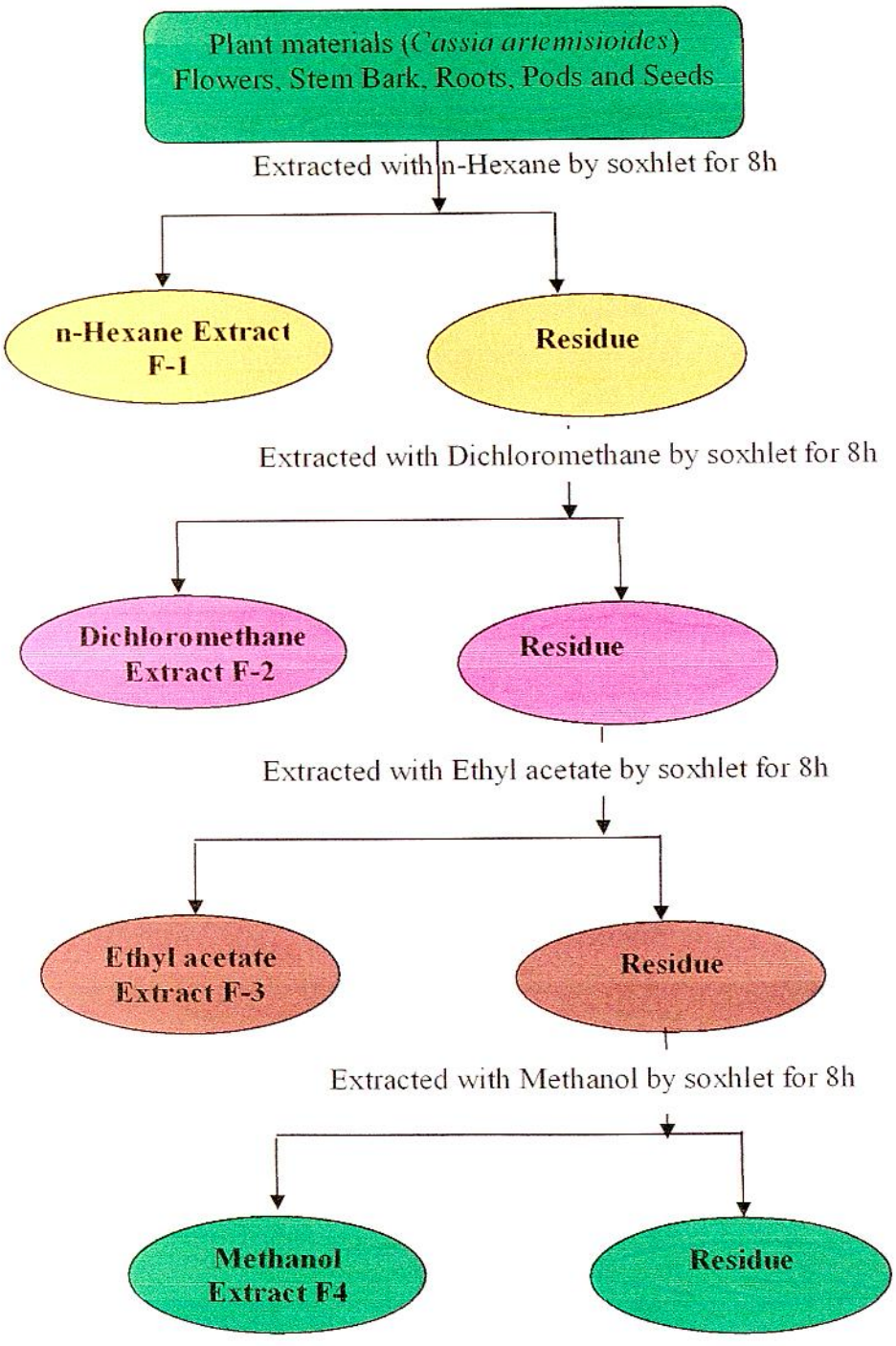


Fig 3.1. General scheme for the extraction of various parts of *Cassia artemisioides* (flowers, stem bark, roots, pods and seeds)

3.4.2. Extraction and isolation of constituents from the Flowers of *Cassia artemisioides*

The air-dried, powdered flowers (2 kg) were extracted with n-hexane, dichloromethane, ethyl acetate and methanol (each for 8 hour) in a soxhlet apparatus. The extracts were concentrated on a rotary evaporator under vacuum to yield the crude residue (**Fig. 3.1**). The ethyl acetate fraction (20 g) was subjected to column chromatography over silica gel eluting with *n*-hexane:ethyl acetate in increasing order of polarity to afford 141 fractions collected in test tubes, using a fraction collector. Similar fractions (TLC) were combined into six major fractions. Fraction **1** was purified through column chromatography (hexane:ethyl acetate, 1:9) and yielded pure compound **57** (21 mg). Fraction **2** was subjected to column chromatography, eluted with hexane:ethyl acetate in increasing order of polarity started at 8:2 and yielded two sub fractions (**2.1**, **2.2**), which were further purified by preparative TLC using hexane:ethyl acetate (7:3) as eluting solvent and purified to give compounds **58** (8 mg) and **59** (11 mg). Fraction **3** was subjected to column chromatography eluted with hexane: ethyl acetate (7:3 to 4:6) yielded 2 fractions (**3.1**, **3.2**), on further purification by preparative TLC using n-hexane:ethyl acetate as eluting solvent system, yielded compound **60** (12 mg) and **61** (7 mg). Fraction **4** was subjected to column chromatography eluted with hexane:ethyl acetate (6:4) yielded 3 fractions (**3.1**, **3.2**, **3.3**), on further purification by preparative TLC using n-hexane:ethyl acetate as eluting solvent system, yielded copmound **61** (2 mg), **62** (7 mg) and **63** (9 mg). Fraction **5** was also subjected to column chromatography eluted with hexane:ethyl acetate in increasing order of polarity started at 1:1 and yielded two sub fractions (**5.1**, **5.2**), these were further purified on preparative TLC using methanol:chloroform (3:97) as eluting system and yielded copmounds **49** (9 mg) and **64** (5 mg). Fraction **6** was subjected to preparative TLC using methanol:chloroform (4:96) as eluting solvent system, yieldeded compound **65** (11 mg). The isolation scheme is given in **Figure 3.2**.

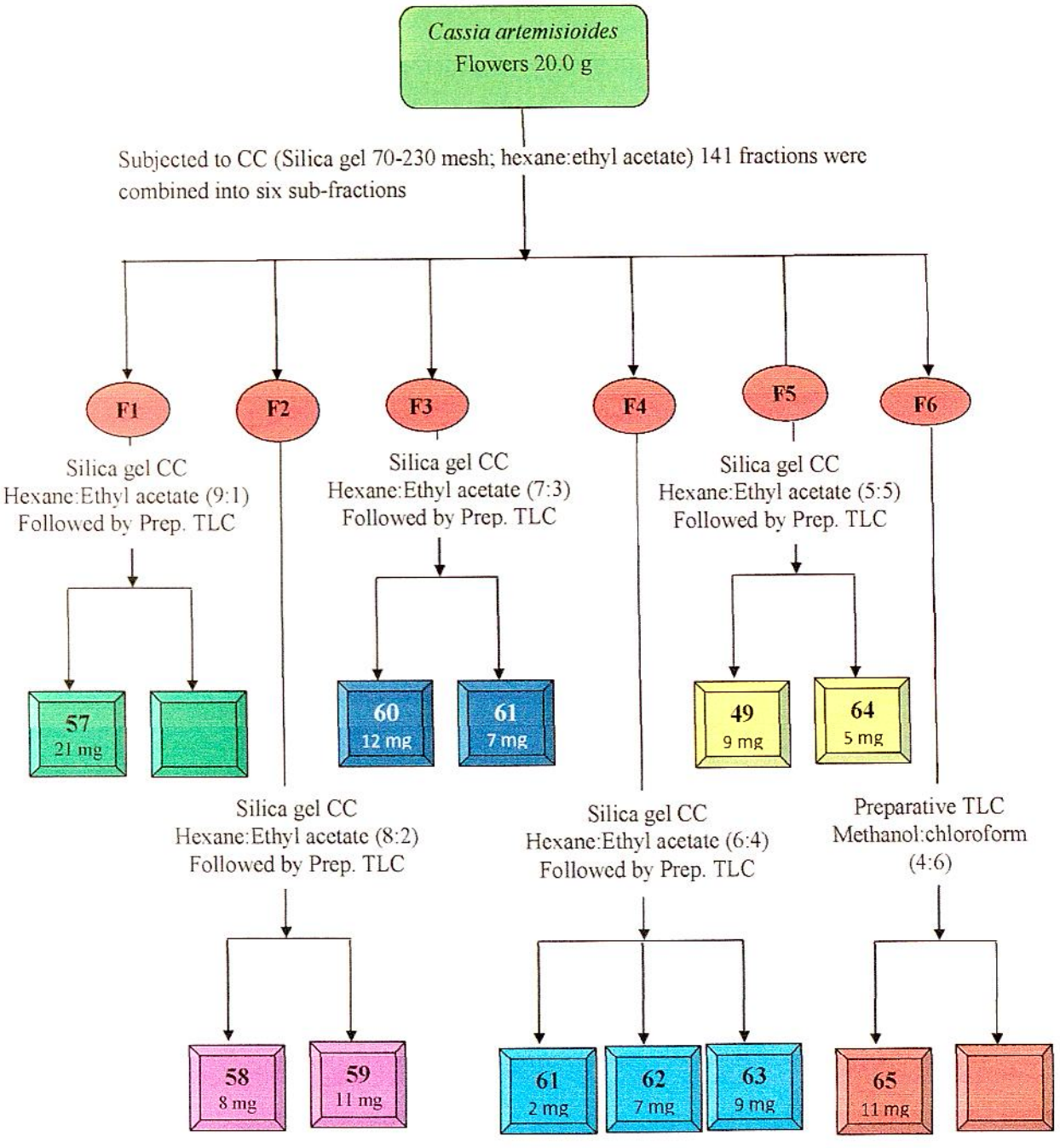


Fig 3.2. Scheme for the isolation of compounds from the Flowers of *Cassia artemisioides*.

3.4.3. Extraction and isolation of the constituents of the Stem Bark of *Cassia artemisioides*

The air-dried, powdered stem bark (1.7 kg) was extracted with n-hexane, dichloromethane, ethyl acetate and methanol (each for 8 hour) in a soxhlet apparatus. The extracts were concentrated using rotary evaporator under reduced pressure yielded the crude extract (**Fig. 3.1**). The ethyl acetate fraction (16 g) was subjected to column chromatography on silica gel eluting with *n*-hexane:ethyl acetate in increasing order of polarity to afford 195 fractions collected in test tubes using fraction collector, Similar fractions (TLC) were combined to get seven major fractions. Fraction **1** was subjected to column chromatography eluted with hexane:ethyl acetate in increasing order of polarity started at 9:1 and yielded two sub fractions (**1.1** and **1.2**), which were further purified by preparative TLC using hexane:ethyl acetate (8:2) as eluting solvent and solvent washing resulted purified compounds **50** (11 mg) and **65** (14 mg). Fraction **2** was purified through column chromatography using hexane:ethyl acetate (8:2) and yielded pure compounds **66** (7 mg) and **67** (19 mg). Fraction **3** was subjected to column chromatography eluted with hexane:ethyl acetate (7:3 to 4:6) yielded **2** fractions **(3.1, 3.2)**, on further purification by preparative TLC using n-hexane:ethyl acetate as eluting solvent system, yielded compoounds **51** (6 mg) and **68** (8 mg). Fraction **4** was also subjected to preparative TLC afforded compound **52** (4 mg). Fraction **5** was also subjected to column chromatography eluted with n-hexane:ethyl acetate in increasing order of polarity started at 5:5 and yielded two sub fractions (**5.1, 5.2**), these were further purified on preparative TLC using methanol:chloroform (3:97) as eluting solvent system and yielded **52** (3 mg), **53** (8 mg) and **69** (7 mg). Fraction **6** was subjected to column chromatography eluted with hexane:ethyl acetate (5:5 to 4:6) yielded pure compound **54** (9 mg). Compounds **55** (5) and **70** (13 mg) were purified in the same way from fraction **7**. The isolation scheme is given in **Fig. 3.3**.

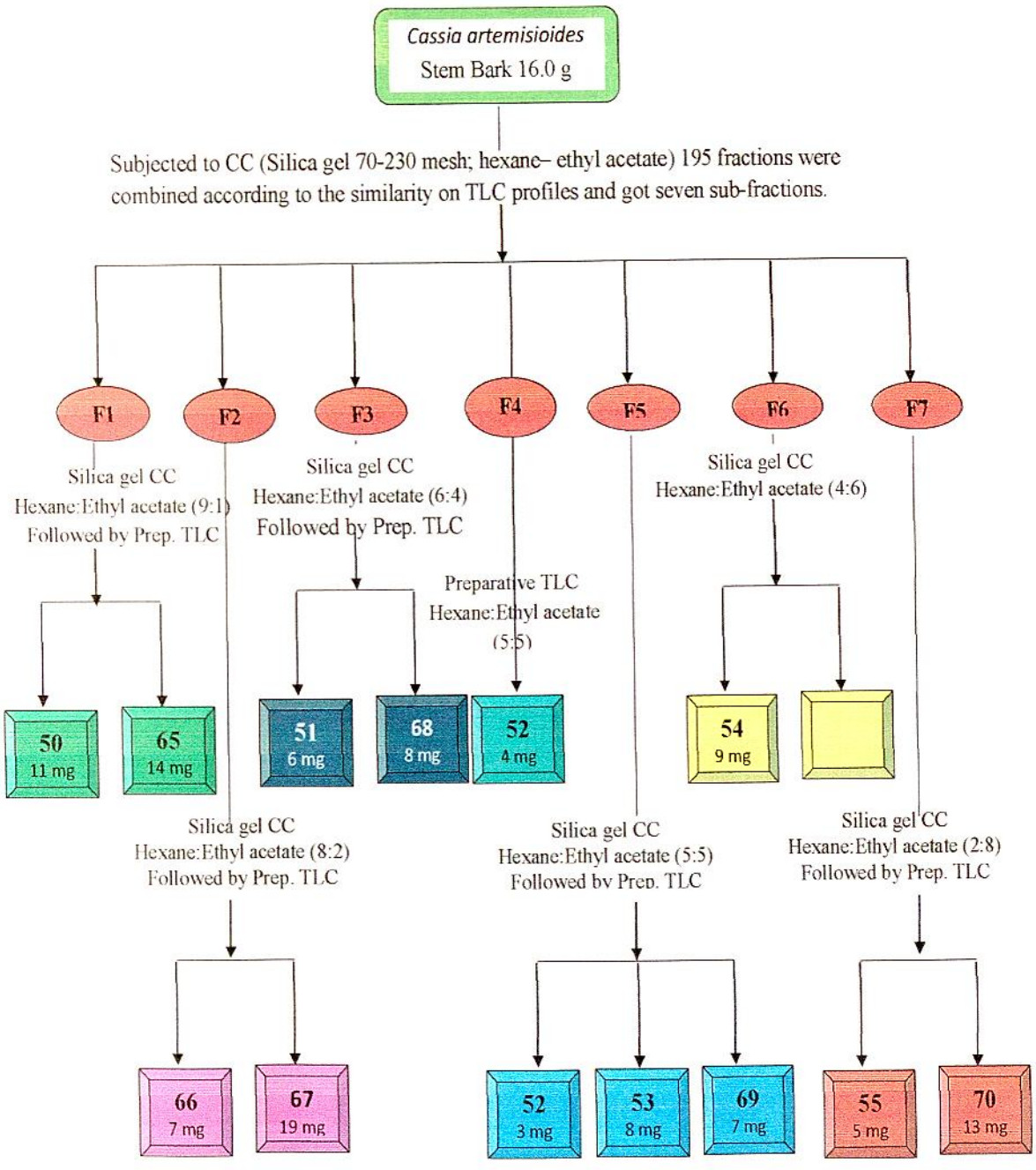


Fig. 3.3 Scheme for the isolation of compounds from *Cassia artemisioides* Stem Bark

3.4.4. Extraction and isolation of the constituents of the Roots of *Cassia artemisioides*

The air-dried ground root (200 g) was extracted with n-hexane, dichloromethane, ethyl acetate and methanol (each for 8 hour) in a soxhlet apparatus. The extracts were concentrated on rotary evaporator under reduced pressure to yield the crude extract (**Fig. 3.1**). The ethyl acetate fraction (7.2 g) was subjected to column chromatography (CC) on silica gel eluting with *n*-hexane and ethyl acetate mixture as a solvent system in increasing order of polarity to afford 165 fractions collected in test tubes using fraction collector. Similar fractions were combined according to the TLC profiles similarity and got three major fractions. Fraction **1** was subjected to column chromatography eluted with hexane:ethyl acetate in increasing order of polarity started at 9:1 and yielded two sub-fractions (**1.1-1.2**), which were further purified by preparative TLC using hexane:ethyl acetate (7:3) as eluting solvents to give purified compounds **71** (12 mg) and **72** (7 mg). Fraction **2** was subjected to column chromatography eluted with hexane:ethyl acetate (6:4 to 5:5) yielded two fractions (**2.1**, **2.2**), on further purification by preparative TLC using n-hexane:ethyl acetate as eluting solvent system, yielded compounds **73** (6 mg) and **74** (5 mg). Fraction **3** was also subjected to column chromatography eluted with hexane:ethyl acetate in increasing order of polarity started at 4:6 and yielded two sub-fractions (**3.1, 3.2**), these were further purified on preparative TLC using methanol:chloroform (5:95) as eluting system and yielded compounds **56** (4 mg) and **75** (16 mg). The isolation scheme is given in **Fig. 3.4**.

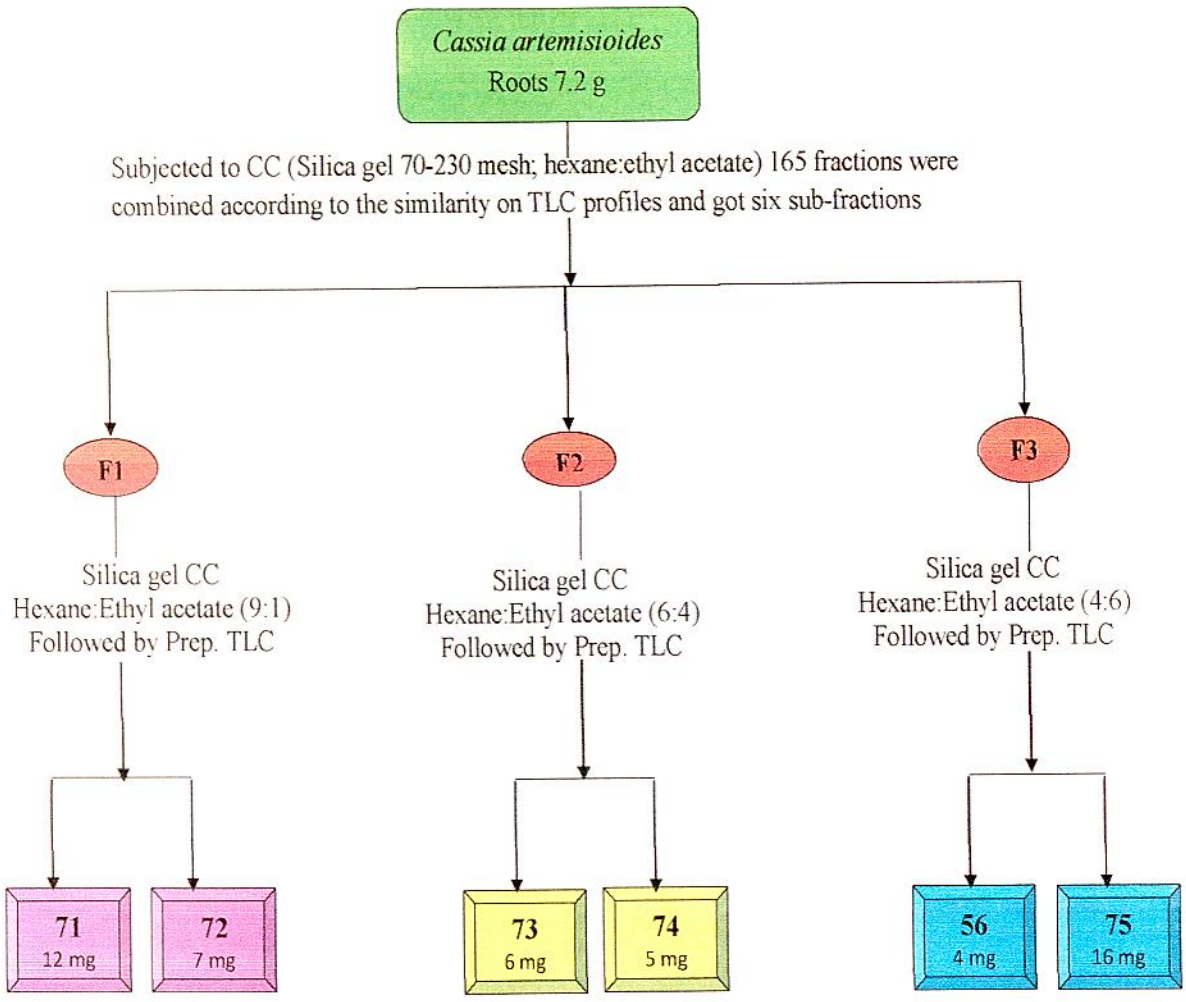


Fig. 3.4 Scheme for the isolation of compounds from the roots *of Cassia artemisioides*

3.4.5. Extraction and isolation from the Pods of *Cassia artemisioides*

The air-dried powdered pods (150 g) were extracted with n-hexane, dichloromethane, ethyl acetate and methanol each for 8 hour in soxhlet apparatus. The extracts were concentrated in a rotary evaporator under vacuum to yield the crude residue (**Fig. 3.1**). The ethyl acetate fraction (6 g) was subjected to column chromatography over silica gel eluting with *n*-hexane:ethyl acetate in increasing order of polarity to afford 73 fractions, which were combined according to the similarity on TLC profiles and obtained two major fractions. Fraction **1** was purified by column chromatography (hexane:ethyl

acetate; 8:2) and yielded pure compound **76** (7 mg).

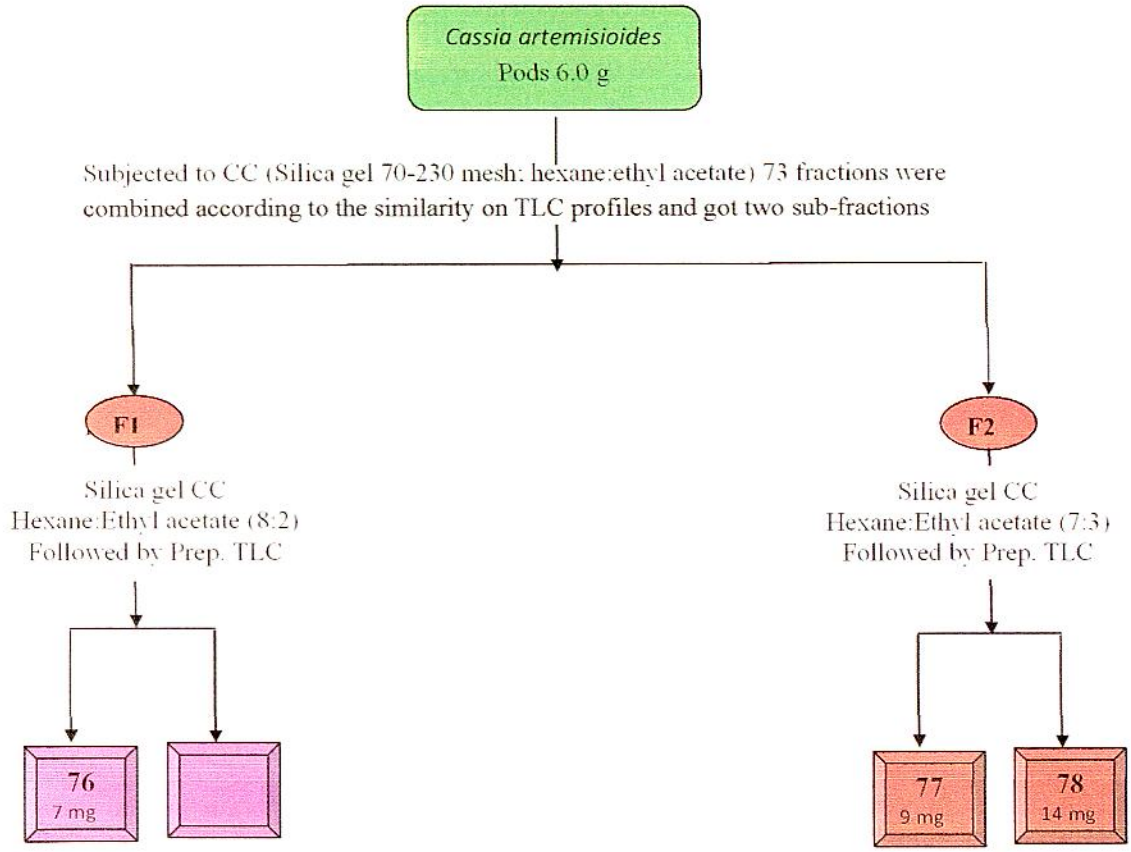


Fig. 3.5 Scheme for the isolation of compounds from the Pods of *Cassia artemisioides*

Fraction **2** was subjected to column chromatography eluted with hexane:ethyl acetate in increasing order of polarity started at 8:2 and yielded two sub fractions (**2.1** and **2.2**), which were further purified by preparative TLC using hexane:ethyl acetate (6:4) as eluting solvent and got purified compounds **77** (9 mg) and **78** (14 mg). The isolation scheme is given in **Fig. 3.5.**

3.4.6. Extraction and isolation from the Seeds of *Cassia artemisioides*

The air-dried and ground seeds (1.3 g) were extracted with n-hexane, dichloromethane, ethyl acetate and methanol each for 8 hour in a soxhlet apparatus. The extracts were concentrated on a rotary evaporator under reduced pressure to yield the crude residue

(**Fig. 3.1**). The ethyl acetate fraction (13 g) was subjected to column chromatography over silica gel eluting with *n*-hexane:ethyl acetate in increasing order of polarity to afford 134 fractions collected in test tubes using fraction collector. Similar (TLC) fractions were combined and got three major fractions. Fraction **1** was subjected to column chromatography eluted with n-hexane:ethyl acetate in increasing order of polarity started at 9:1 which yielded two sub-fractions (**1.1** and **1.2**).

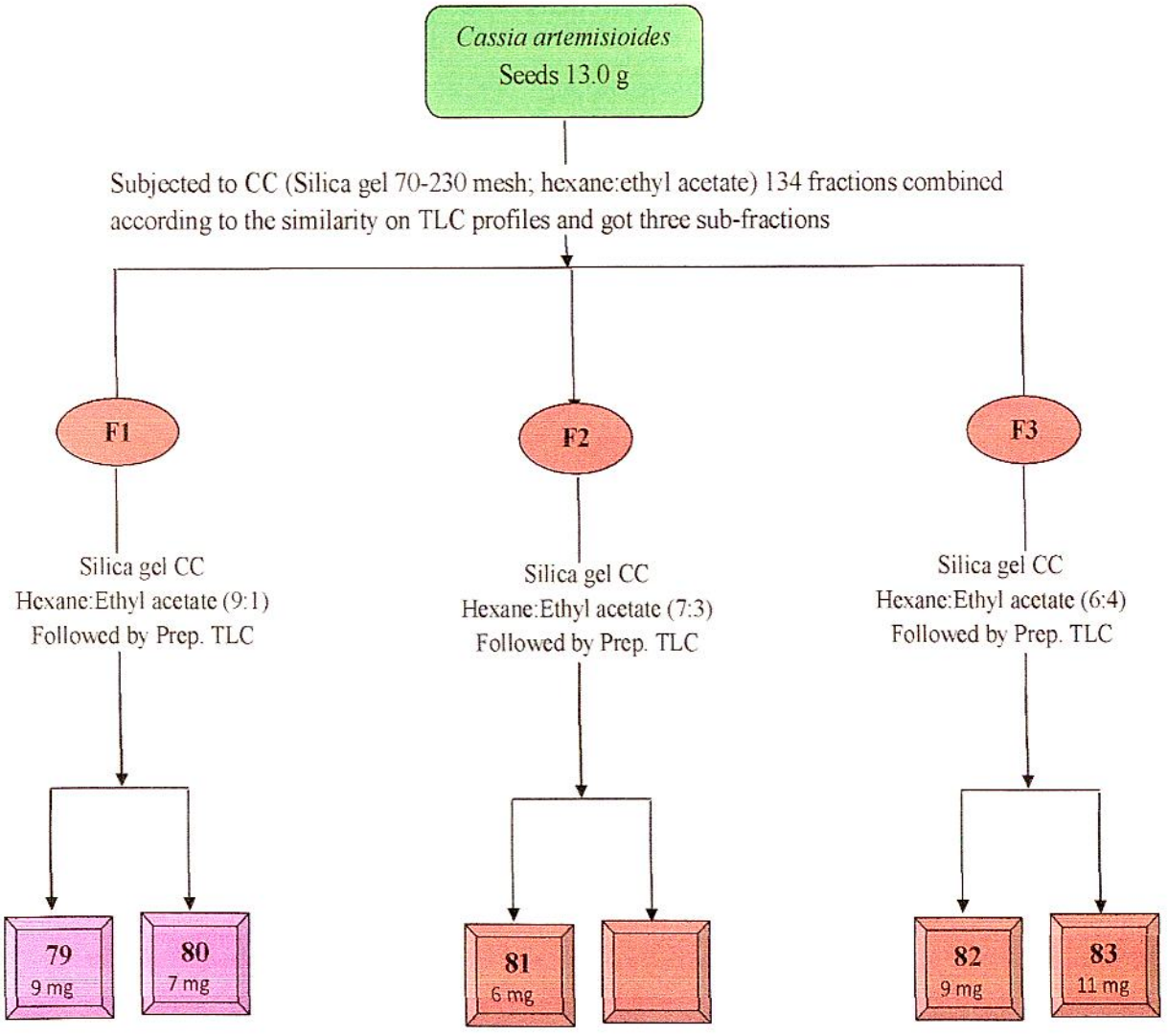


Fig. 3.6 Scheme for the isolation of compounds from the Seeds of *Cassia artemisioides*

These sub-fractions were further purified by preparative TLC using n-hexane:ethyl acetate (7:3) as eluting solvent system, that lead to the isolation of pure compounds **79** (9

These sub-fractions were further purified by preparative TLC using n-hexane:ethyl acetate (7:3) as eluting solvent system, that lead to the isolation of pure compounds **79** (9 mg) and **80** (7 mg). Fraction **2** was subjected to column chromatography eluted with hexane:ethyl acetate (1:1) yielded **81** (6 mg) as a pure compound. Fraction **3** was also subjected to column chromatography eluted with hexane:ethyl acetate in increasing order of polarity started at 6:4 and yielded two sub-fractions (**3.1**, **3.2**), these were further purified on preparative TLC using hexane:ethyl acetate (1:1) as eluting system and yielded compounds **82** (9 mg) and **83** (11 mg). The isolation scheme is given in **Fig. 3.6.**

3.5. Scheme for Extraction and Fractionation of *Cassia nemophila*

Various dried parts (flowers, stem bark and roots) of *Cassia nemophila* were extracted with hexane, dichloromethane, ethyl acetate and ethanol using soxhlet apparatus. The general scheme for extraction and fractionation is given in **Figure 3.7**.

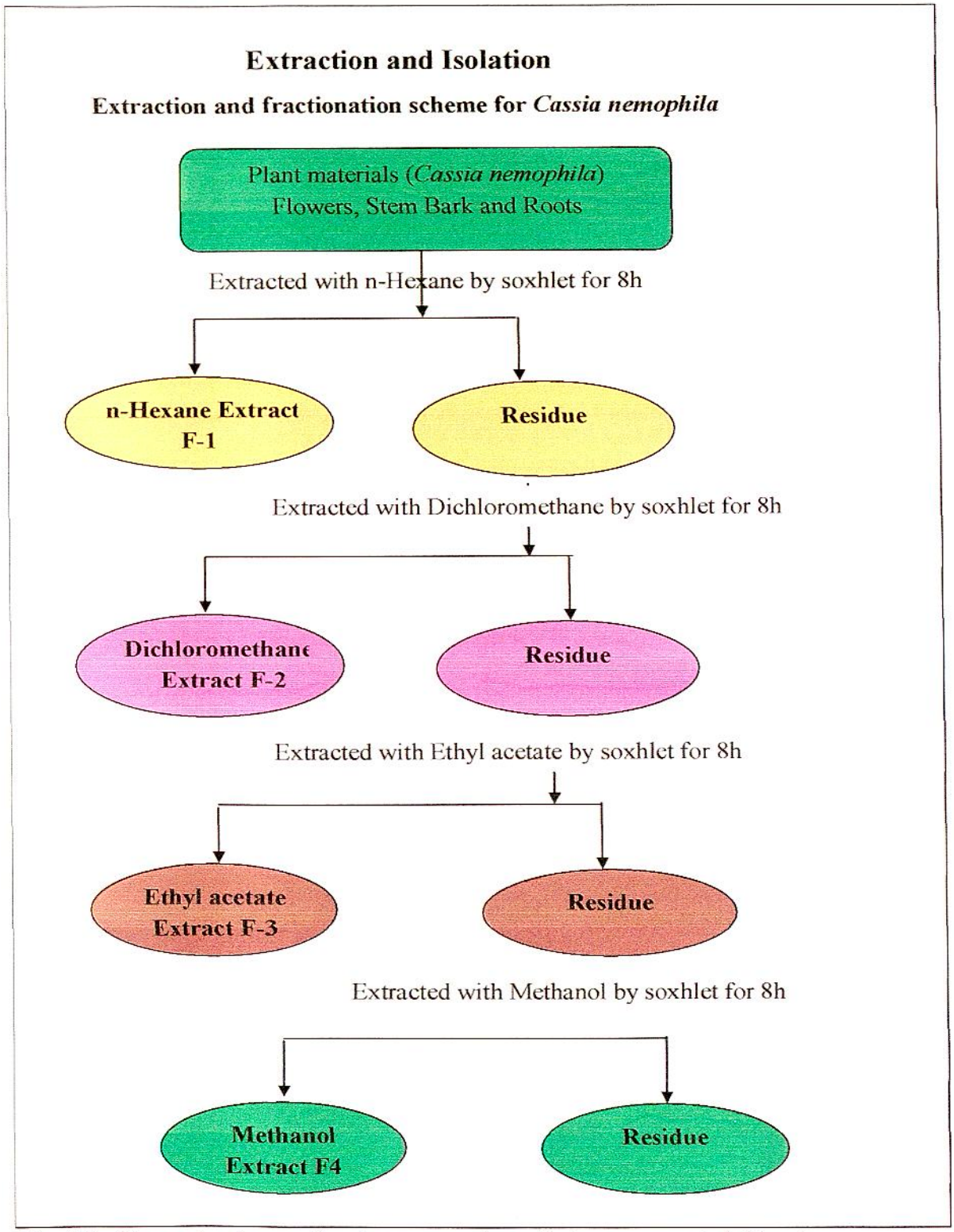


Fig 3.7. General scheme for the extraction of plants material *Cassia nemophila* (flowers, stem bark and roots)

3.5.1. Extraction and isolation from the Flowers of *Cassia nemophila*

The air-dried and powdered seeds (0.9 kg) were extracted with n-hexane, dichloromethane, ethyl acetate and methanol (each for 8 hour) in a soxhlet apparatus. The extracts were concentrated in a rotary evaporator under vacuum to yield the crude extract (**Fig. 3.7**). The ethyl acetate fraction (9 g) was subjected to column chromatography over silica gel eluting with *n*-hexane:ethyl acetate in increasing order of polarity to afford 117 fractions collected in test tubes using fraction collector. The fractions with similar TLC were combined to give three major fractions. Fraction **1** was subjected to column chromatography eluted with hexane:ethyl acetate (9:1) yielded **84** (7 mg) as a pure compound.

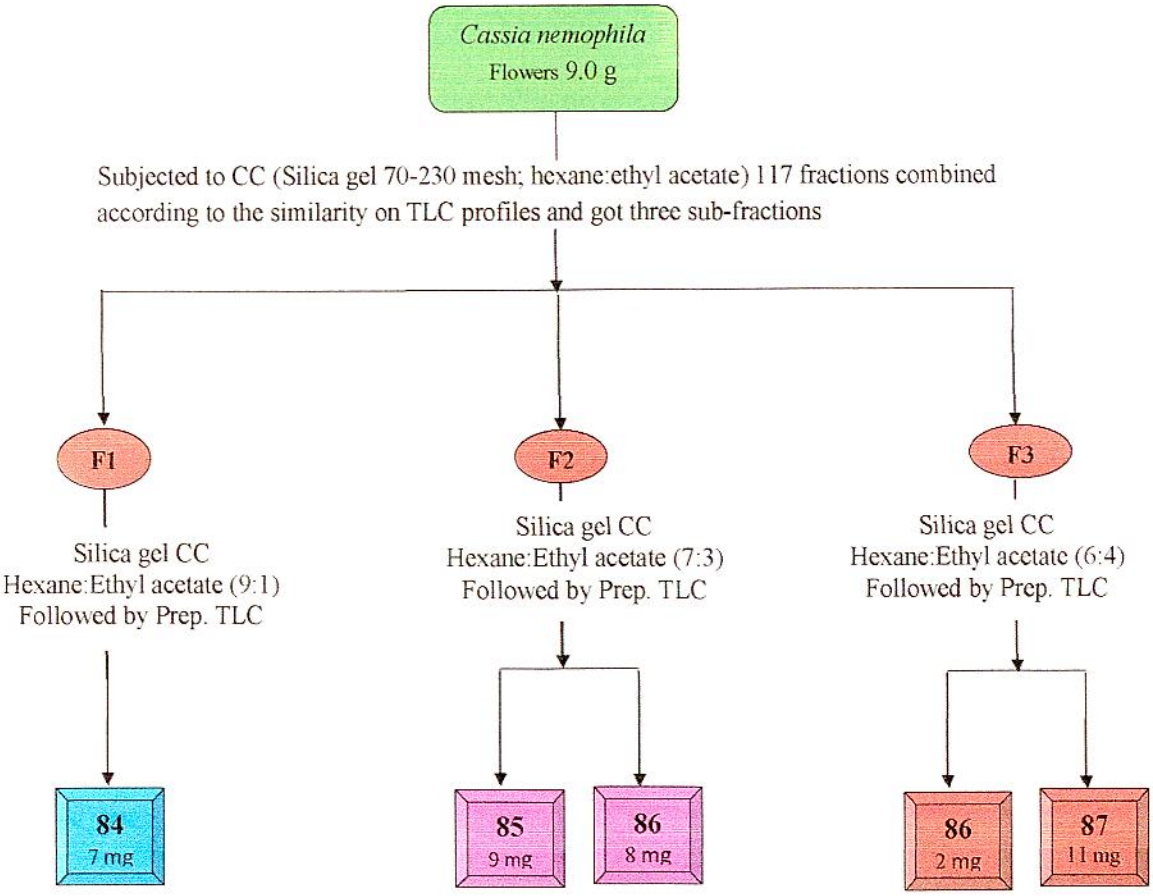


Fig. 3.8. Scheme for the isolation of compounds from the Flowers of *Cassia nemophila*

Fraction **2** was subjected to column chromatography eluted with hexane:ethyl acetate in

Fraction **2** was subjected to column chromatography eluted with hexane:ethyl acetate in increasing order of polarity started at 7:3 and yielded two sub fractions (**1.1, 1.2**), which were further purified by preparative TLC using hexane:ethyl acetate (6:4) as eluting solvent and purified **85** (9 mg) and **86** (8 mg). Fraction **3** was also subjected to column chromatography eluted with hexane:ethyl acetate in increasing order of polarity started at 7:3 and yielded two sub-fractions **(3.1, 3.2)**, these were further purified on preparative TLC using hexane:ethyl acetate (6:4) as eluting system and yielded compounds **86** (2 mg) and **87** (11 mg). The separation scheme is given in **Fig. 3.8.**

3.5.2. Extraction and isolationof the constituents from the Stem Bark of *Cassia nemophila*

The air-dried and ground stem bark (0.2 kg) was extracted with n-hexane, dichloromethane, ethyl acetate and methanol (each for 8 hour) in a soxhlet apparatus. The extracts were concentrated in a rotary evaporator under vacuum to yield the crude extract (**Fig. 3.7**). The ethyl acetate fraction (4 g) was subjected to column chromatography over silica gel eluting with *n*-hexane:ethyl acetate in increasing order of polarity to afford 93 fractions using fraction collector. Similar (TLC) fractions were combined to give three major fractions. Fraction **1** was subjected to column chromatography eluted with mixture of hexane:ethyl acetate in increasing order of polarity started at 8:2 and yielded two sub--fractions (**1.1**, **1.2**), which were further purified by preparative TLC using hexane:ethyl acetate (6:4) as eluting solvent to give pure compounds **88** (4 mg) and **89** (6 mg). Fraction **2** was subjected to column chromatography eluted with hexane:ethyl acetate (7:3) yielded **90** (5 mg) as pure compound. Fraction **3** was also subjected to column chromatography eluted with hexane:ethyl acetate (6:4) yielded **91** (4) in pure form. Isolation scheme is given in **Fig. 3.9**.

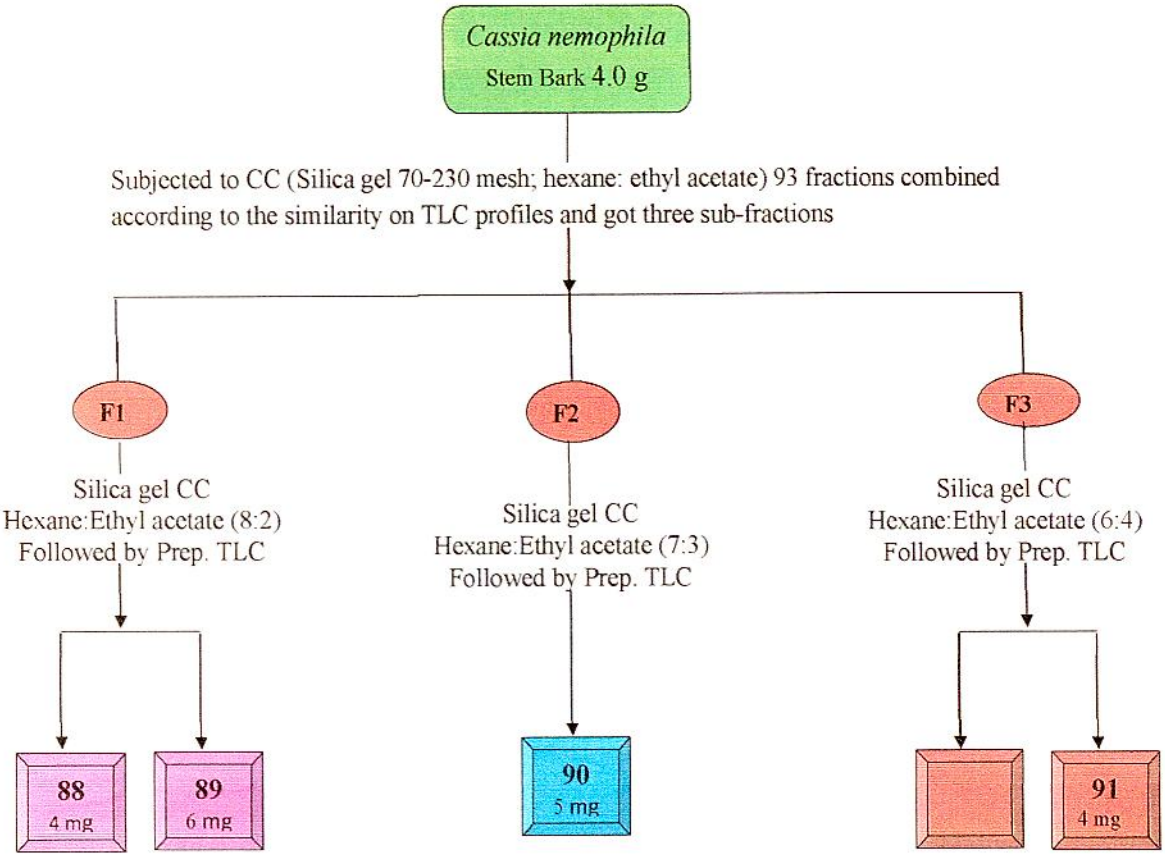


Fig. 3.9. Scheme for the isolation of compounds from the Stem Bark of *Cassia nemophila*

3.5.3. Extraction and isolation of the Constituents from the Roots of *Cassia nemophila*

The air-dried and powdered roots (0.15 kg) were extracted with n-hexane, dichloromethane, ethyl acetate and methanol (each for 8 hour) in a soxhlet apparatus. The extracts were concentrated under vacuum in a rotary evaporator to yield the crude extract (**Fig. 3.7**). The ethyl acetate fraction (3 g) was subjected to column chromatography using silica gel eluting with *n*-hexane:ethyl acetate in increasing order of polarity to afford 76 fractions. The fractions were combined into three major fractions on the basis of TLC similarity.

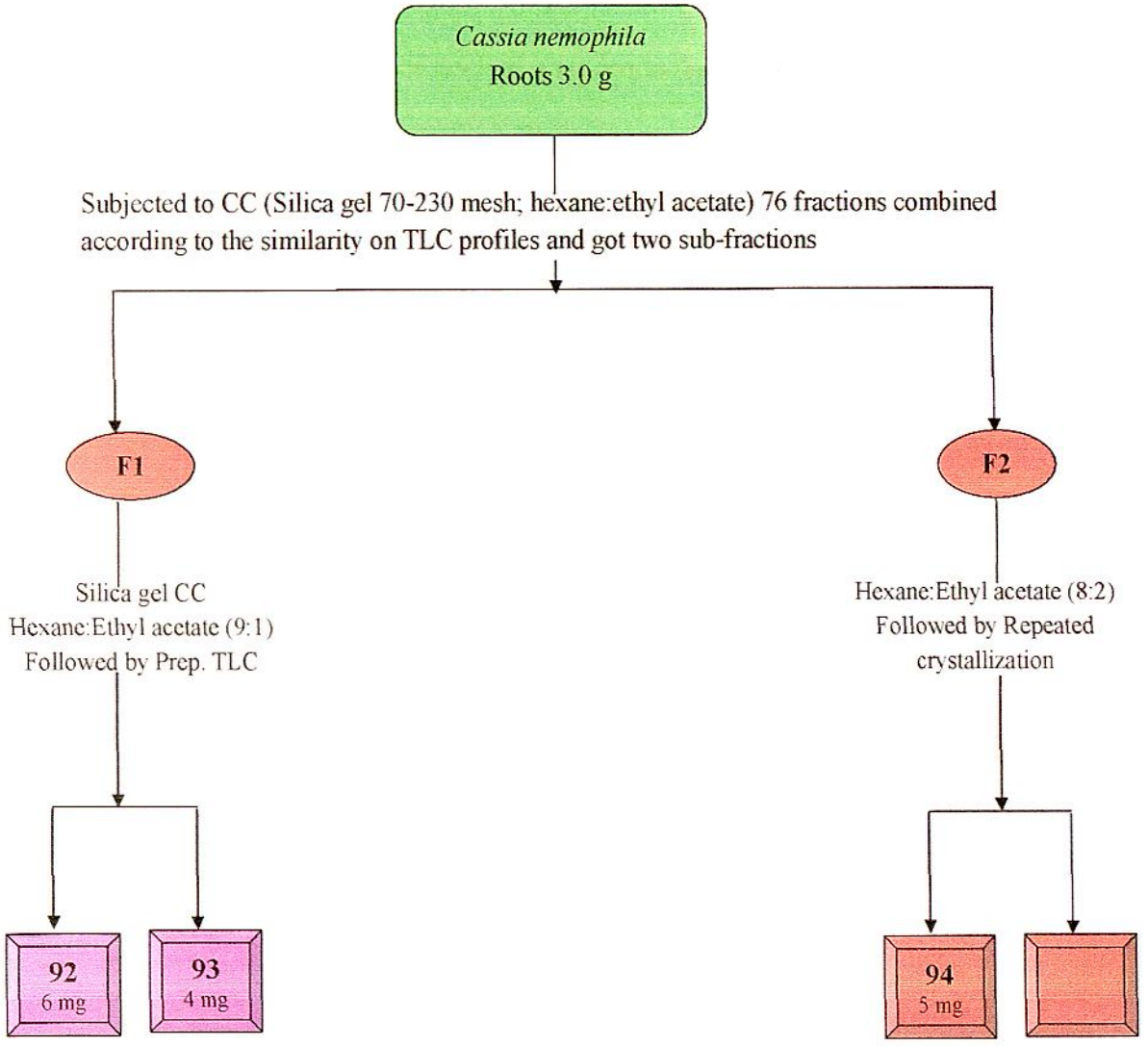
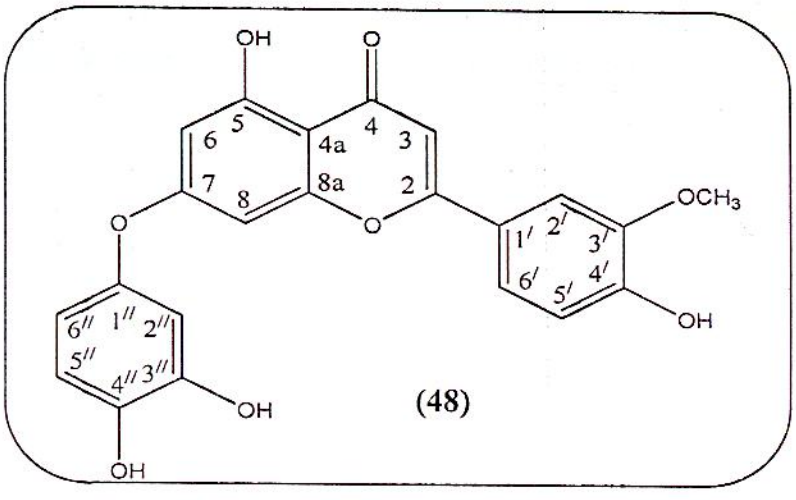


Fig. 3.10. Scheme for the isolation of compounds from the Roots of *Cassia nemophila*.

Fraction 1 was subjected to column chromatography eluted with hexane:ethyl acetate in increasing order of polarity starting at 9:1 and yielded two sub fractions **(1.1, 1.2)**, which were further purified by preparative TLC using hexane:ethyl acetate (6:4) as eluting solvent and to give purified **92** (6 mg) and **89** (4 mg). Fraction **2** was washed with a mixture of n-hexane and dichloromethane resulted the white crystals of **94** (5 mg) in pure form. Isolation scheme is given in **Figure 3.10**.

3.6. Experimental data of new compounds isolated from *Cassia artemisioides* flowers

3.6.1. 7-(3,4-dihydroxyphenoxy)-5-hydroxy-2-(4-hydroxy-3-methoxy phenyl)-4*H*-chromen-4-one (48)



(48)

Orange red powder (Methanol)

Melting point; > 300 °C

Molecular formula $C_{22}H_{16}O_8$

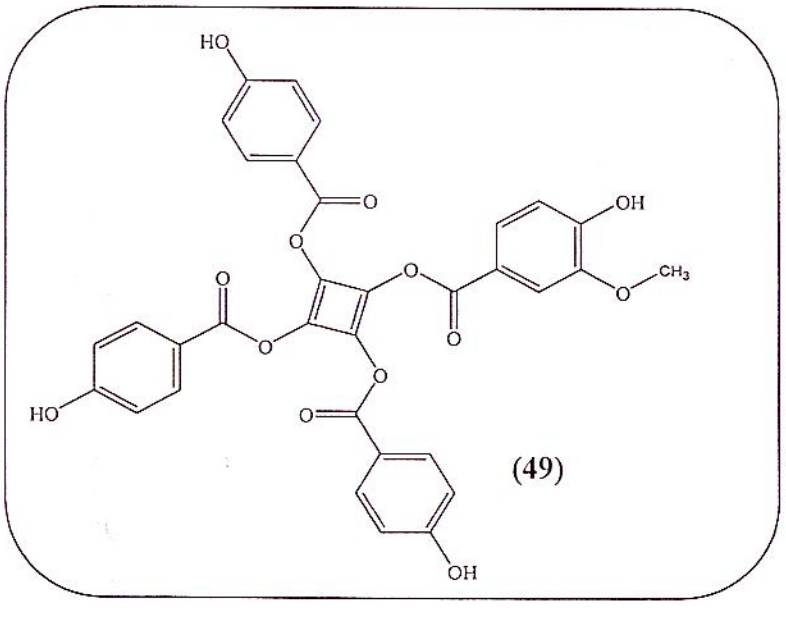
EI mass *m/z*; 407.9

IR (KBr) $\tilde{\nu}_{max}$; 3005. 2970, 1670, 1601, 1590 cm^{-1}

^{13}C NMR; Given in **Table 2.1**

^{1}H NMR; Given in **Table 2.1**

3.6.2. 1,2,3-Tri(5-hydroxybenzoyloxy)-4-(5-hydroxy-4-methoxy bezoyloxy)cyclobuta-1,3-diene (49)



(49)

Grayish needles (Acetone)

Melting point; 272-283 °C

Molecular formula $C_{33}H_{22}O_{13}$

UV (MeOH) λ_{max} (log ε); 232.3 (4.4), 262.6 (4.2) nm

IR (KBr) $\tilde{\nu}_{max}$; 3443, 1744, 1612, 1527 cm^{-1}

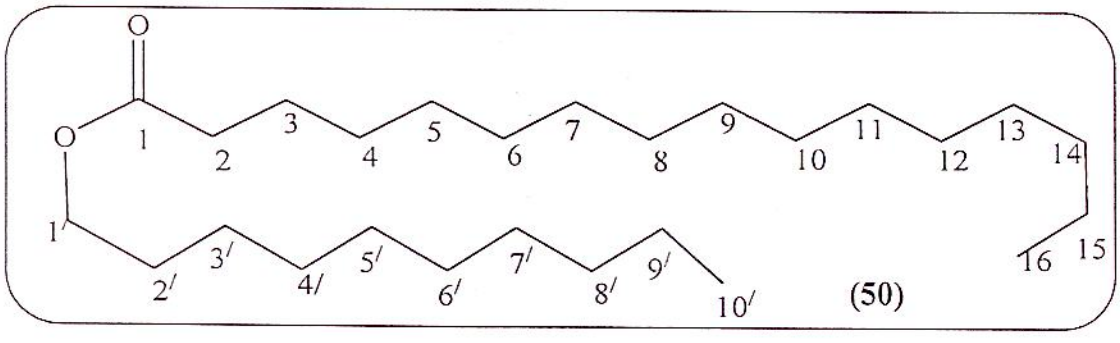
^{13}C NMR; Given in **Table 2.2**

^{1}H NMR; Given in **Table 2.2**

EI-MS (rel. int); 626 (M^+), 182.0, 166.9 137.9, 121.1, 93.14

3.7. Experimental data of new isolated compounds from Stem Bark of *Cassia artemisioides*

3.7.1. Decyl palmitate (50)



(50)

White powder (Chloroform)

Melting point; 74-76 °C

Molecular formula $C_{26}H_{52}O_2$.

IR (neat); $\tilde{\nu}$ 1706 cm^{-1} showed the presence of carboxylic ester functional group.

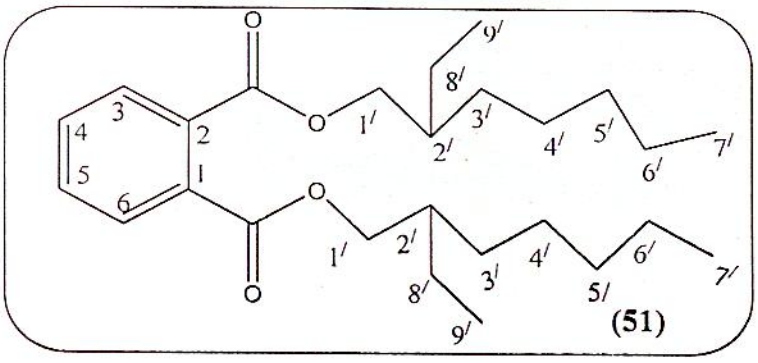
^{13}C NMR; Given in **Table 2.3**

^{1}H NMR; Given in **Table 2.3**

EI-MS; 396 (calcd. 396 for $C_{26}H_{52}O_2$)

EI-MS (rel. int.); 397 ($m+H^+$) 369, 341, 313, 285, 257, 148, 125, 83

3.7.2. Di-2-ethylheptyl phthalate (51)



(51)

Red colour thick residue (Acetone)

Molecular formula $C_{26}H_{42}O_4$

Melting point; 274-276 °C

UV λ_{max} (CH_2Cl_3); 278, 23 nm

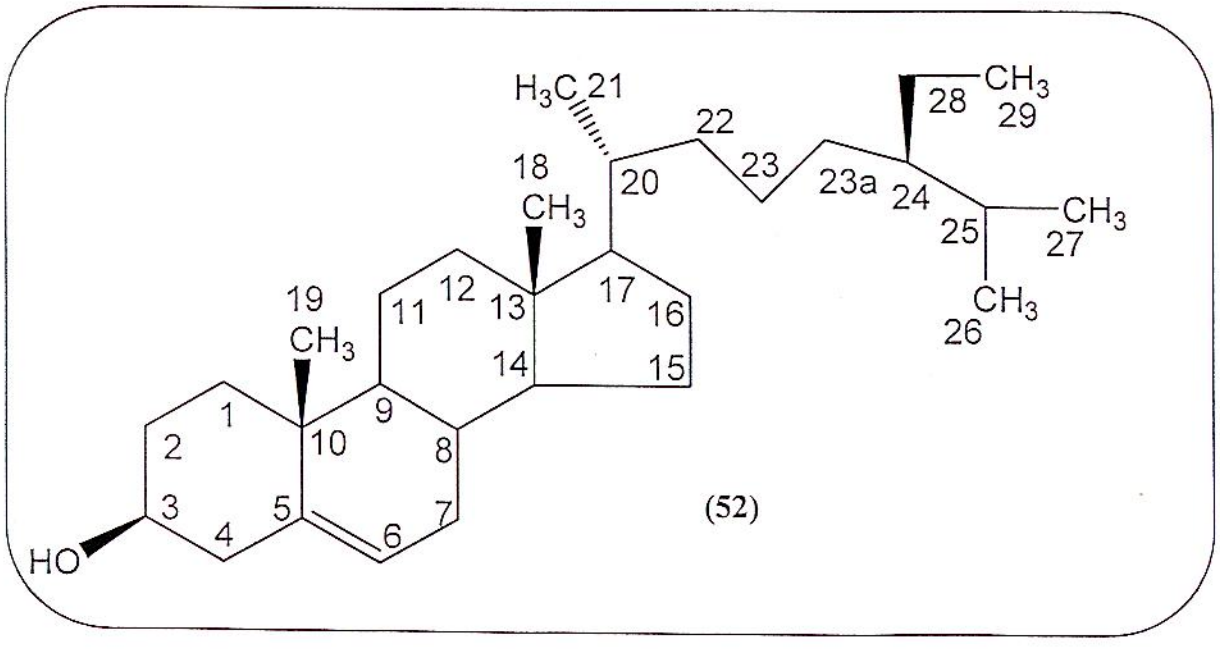
IR (neat) $\tilde{\nu}_{max}$; 3025, 2927, 2885, 28654, 1730, 1605, 1580 cm^{-1}

^{13}C NMR; Given in **Table 2.4**

^{1}H NMR; Given in **Table 2.4**

EI-MSS *m/z* 418 (M^+) (calcd. 418 for $C_{26}H_{42}O_4$)

3.7.3. 23a-Homostigmast-5-en-3*β*-ol (52)



(52)

White amorphous powder (Chloroform)

Melting point; 125-127 °C

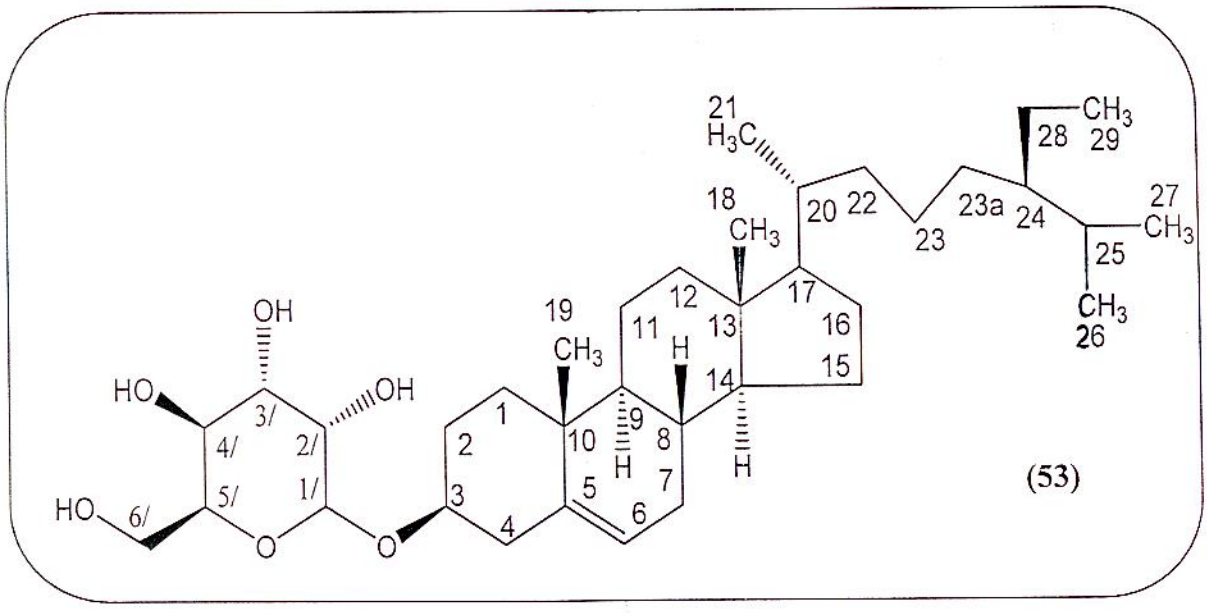
IR (neat) $\tilde{\nu}_{max}$; 3487.6, 3338.6, 2918.9, 2849.4, 1688.2, 1603.1, 1363.8, 1301.9, 1276.9 cm^{-1}

^{13}C NMR; Given in **Table 2.5**

^{1}H NMR; Given in **Table 2.5**

EI-MS; 428 (The intensities of other peaks were less than 5%)

3.7.4. 23a-Homostigmast-5-en-3-*O*-*β*-glucopyranoside (53)



White powder (Chloroform)

Melting point; >290 °C

Molecular formula; $C_{35}H_{50}O$

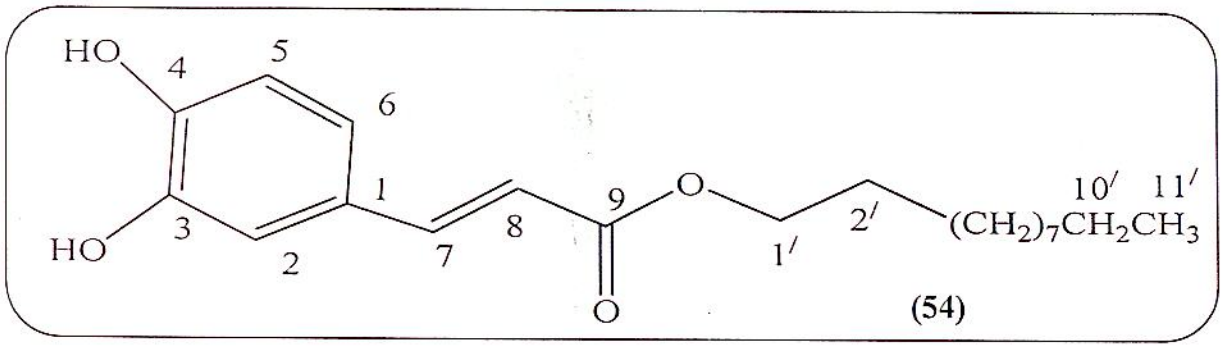
FT-IR $\tilde{\nu}_{max}$; 3487.6, 3338.6, 2918.9, 2849.4, 1688.2, 1603.1, 1363.8, 1301.9 cm^{-1}

^{13}C NMR; Given in **Table 2.6**

^{1}H NMR; Given in **Table 2.6**

EI-MS; 575 [M^+] (calcd. 576 for $C_{35}H_{50}O$)

3.7.5. Undecyl caffeate (54)



(54)

White powder (Chloroform)

Melting point; 108–109 °C

Molecular formula; $C_{20}H_{30}O_4$

FT-IR $\tilde{\nu}_{max}$ (neat); 3485, 3328, 2952, 1690, 1605 cm^{-1}

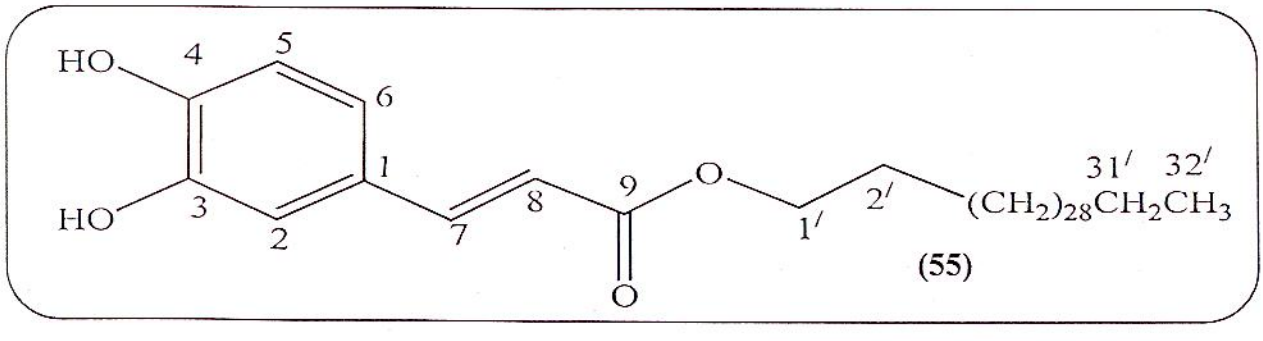
^{13}C NMR; Given in **Table 2.7**

^{1}H NMR; Given in **Table 2.7**

EI-MS; 334 [M^+] (calcd. 334 for $C_{20}H_{30}O_4$)

EI-MS (rel. int.); 334 [M^+], 292, 278, 236, 193, 179.9 (100%), 163, 136, 97.0

3.7.6. Dotricontanyl caffeate (55)



(55)

Colourless needles (Chloroform)

Melting point; m. point 102–103 °C

Molecula formula; $C_{41}H_{72}O_4$

FT-IR (neat) $\tilde{\nu}_{max}$; 3485, 3311, 2950, 1607, 1533, 1445 and 1355 cm^{-1}

^{13}C NMR; Given in **Table 2.8**

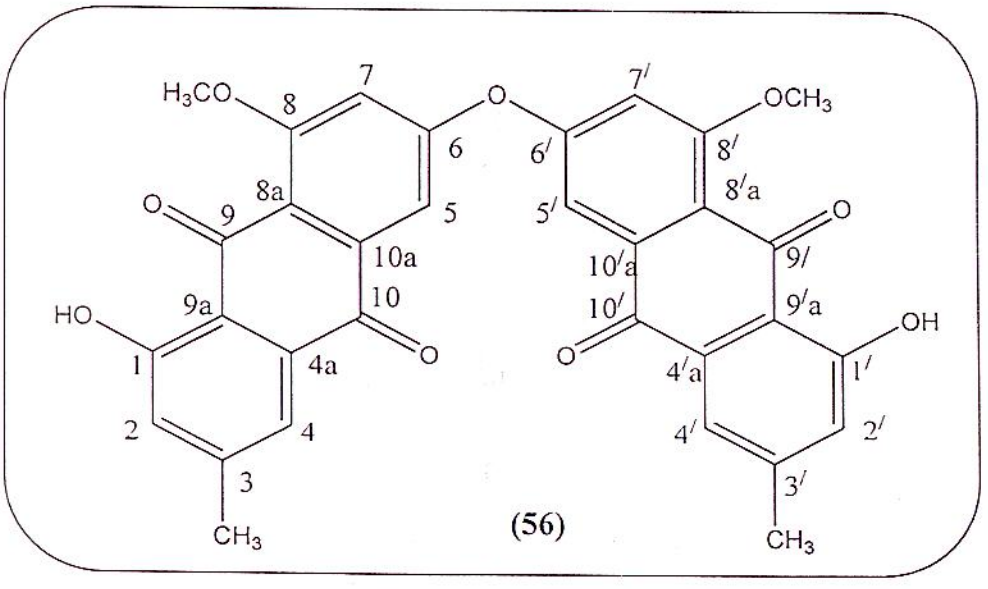
^{1}H NMR; Given in **Table 2.8**

EI-MS; 628 [M^+], (calcd. 628 for $C_{41}H_{72}O_4$)

EI-MS (re lint.); 628 (2.6), 600 (4.8), 572 (39.2), 544 (73.6), 516 (14.6), 488 (10.6), 460 (3.5), 396 (6.1), 219 (2.6), 180 (100), 163 (95.7), 136 (24.8), 123 (22.1), 111 (20.3), 97 (38.4), 83 (44.8).

3.8. Experimental data of new isolated Compounds from the Roots of *Cassia artemisioides*

3.8.1. 1,1′-Dihydroxy-3,3′-dimethyl-8,8′-dimethoxy-6,6′-bianthraquinone (56)



(56)

Orange red powder (Methanol)

Melting point; 288-290 °C

Molecular formula; $C_{32}H_{22}O_9$

IR (neat) $\tilde{\nu}_{max}$; 3340, 2912, 1592, 1485, 1380, 1250 cm^{-1}

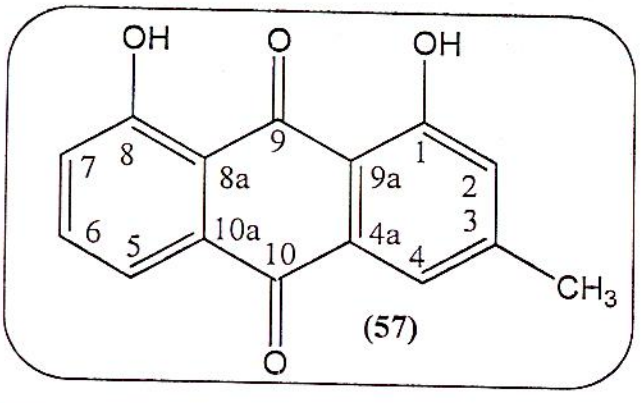
^{13}C NMR; Given in **Table 2.9**

^{1}H NMR; Given in **Table 2.9**

EI-MS; *m/z* 564, [M^+] (calcd. 564 for $C_{32}H_{22}O_9$)

3.9. Experimental data of new Source Compounds isolated from *Cassia artemisioides* Flowers

3.9.1. 1,8-Dihydroxy-3-methylanthraquinone (57)



Reddish needles (Chloroform)

Melting point; 195-196 °C

Molecular formula; $C_{15}H_{10}O_4$

UV (EtOH) λ_{max}; 429, 287, 277, 257 nm

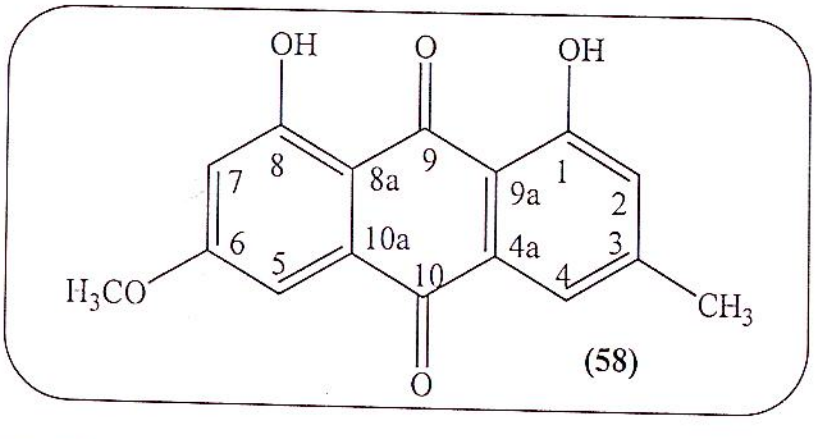
IR (KBr) $\tilde{\nu}_{max}$; 3400, 2970, 1670, 1620 cm^{-1}

^{13}C NMR; Given in **Table 2.10**

^{1}H NMR; Given in **Table 2.10**

EI MS *m/z* (rel. int.); 254 (100) (M^+), 237 (3.8), 226 (7.9), 197 (4.6), 152 (4.0)

3.9.2. 1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (58)



Reddish needles (Acetone)

Melting point; 205-206 °C

Molecular formula; $C_{16}H_{10}O_5$

UV (MeOH); λ_{max} (log ε) 432, 287, 262, 257 nm

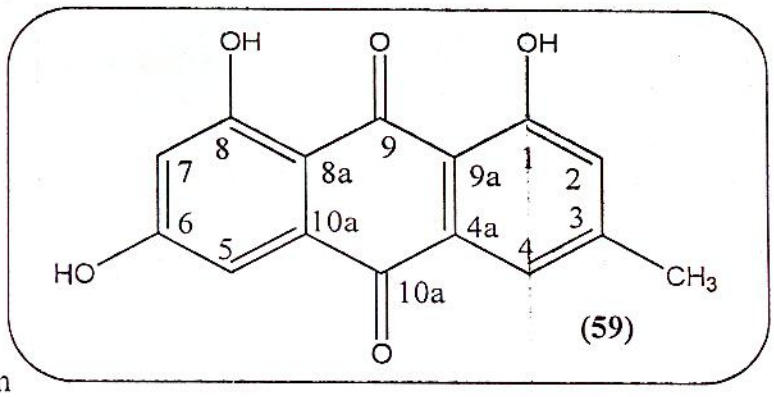
IR (KBr) $\tilde{\nu}_{max}$; 3394, 2917, 1582, 1455, 1377, 1314, 1267 cm^{-1}

^{13}C NMR; Given in **Table 2.11**

^{1}H NMR; Given in **Table 2.11**

EI-MS *m/z* (rel. int.); 284 (100), 266 (49.09), 238 (75.63), 226 (8.89), 197 (17.89), 149.0 (12.64).

3.9.3. 1,3,8-Trihydroxy-3-methylanthraquinone (59)



Reddish needles (Acetone)

Melting point; 268-270 °C

Molecular formula; $C_{15}H_{10}O_5$

^{13}C NMR; Given in Table

UV (MeOH) λ_{max}; 202, 208, 218 nm

IR (KBr) $\tilde{\nu}_{max}$; 3422, 1629, 1560, 1475, 1340, 1260 cm^{-1}

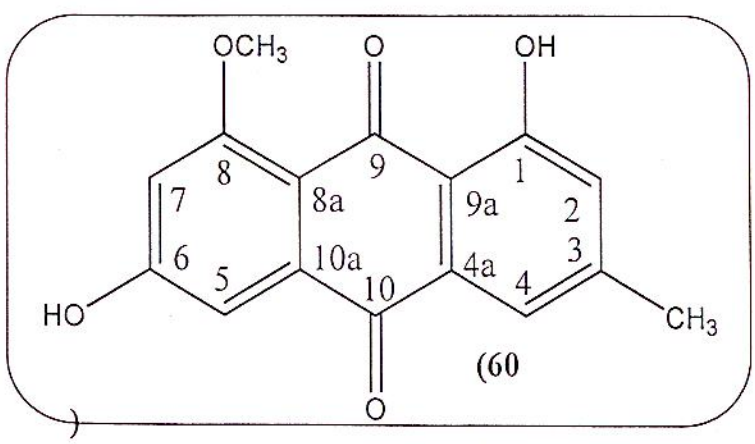
^{13}C NMR; Given in **Table 2.12**

^{1}H NMR; Given in **Table 2.12**

EI-MS; *m/z* 270 [M$^+$] (calcd. 270 for $C_{15}H_{10}O_5$)

EI-MS *m/z* (rel. int.) (%); 270 (100) 242 (7.1), 213 (5.5), 139 (3.1), 121 (3.2)

3.9.4. 1,6-Dihydroxy-8-methoxy-3-methylanthraquinone (60)



Yellow needles (Acetone)

Melting point; 300-302 °C

Molecular formula; $C_{16}H_{12}O_5$

UV (MeOH) λ_{max} (log ε); 225 (4.53) 247 (4.09), 286 (4.43) nm

IR (KBr) $\tilde{\nu}_{max}$; 3427 (OH), 2921, 1680, 1589, 1484, 1441, 1261 cm^{-1}

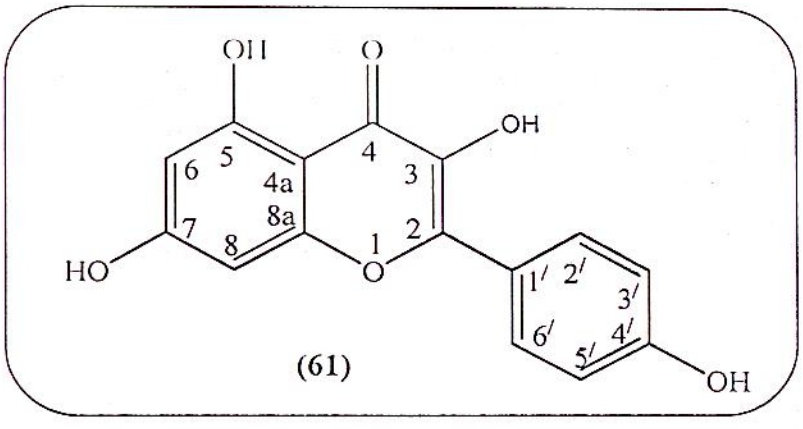
^{13}C NMR; Given in **Table 2.13**

^{1}H NMR; Given in **Table 2.13**

EI-MS; *m/z* 284 [M^+] (calcd. 284 for $C_{16}H_{12}O_5$)

EI-MS *m/z* (rel. int.) (%); 284 (100), 266 (49.09), 238 (75.63), 226 (8.89), 197 (17.89), 149 (12.64).

3.9.5. 3,5,7,4′-Tetrahydroxyflavone (61)



(61)

Reddish needles (Acetone)

Melting point; 274-276 °C

Molecular formula; $C_{15}H_{10}O_6$

UV (MeOH) λ_{max} (log ε); 364 (3.23), 295 (3.90), 261 (3.45) nm

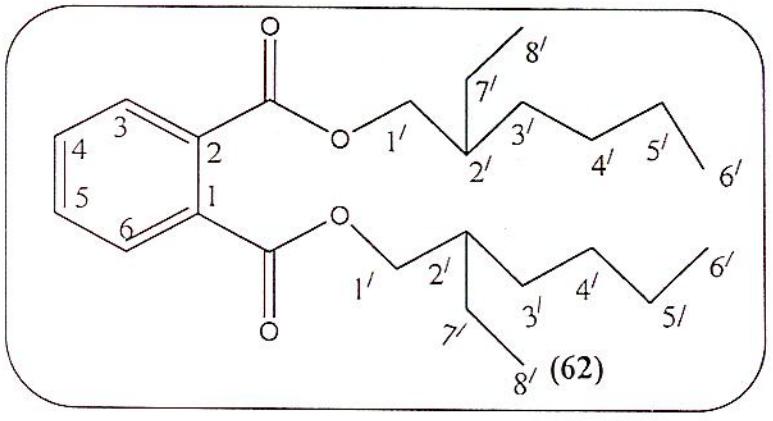
IR (KBr) $\tilde{\nu}_{max}$; 3245, 1646, 1603, 1525, 1183 cm^{-1}

^{13}C NMR; Given in **Table 2.14**

^{1}H NMR; Given in **Table 2.14**

EI-MS; *m/z* 286 [M^+] (calcd. 286 for $C_{15}H_{10}O_6$)

3.9.6. Di-2-ethylhexyl phthalate (62)



Colourless thick residue (Acetone)

M. point 274-276 °C

Molecular formula; $C_{24}H_{38}O_4$

UV (CH_2Cl_3) λ_{max}; 278, 234 nm

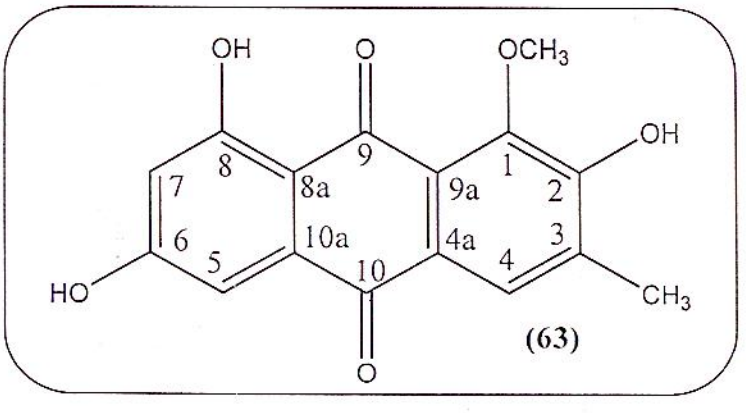
IR (neat) $\tilde{\nu}_{max}$; 3025, 2927, 2885, 1730, 1605, 1580 cm^{-1}

^{13}C NMR; Given in **Table 2.15**

^{1}H NMR; Given in **Table 2.15**

EI-MS; *m/z* 390 [M^+] (calcd. 390 for $C_{24}H_{38}O_4$)

3.9.7. 2,6,8-Trihydroxy-1-methoxy-3-methylanthraquinone (63)



Yellow needles (Acetone)

Melting point; 291-293 °C

Molecular formula; $C_{16}H_{12}O_6$

UV (MeOH) λ_{max} (log ε); 393 (3.92), 311 (4.08), 284 (4.42), 228 (4.36) nm

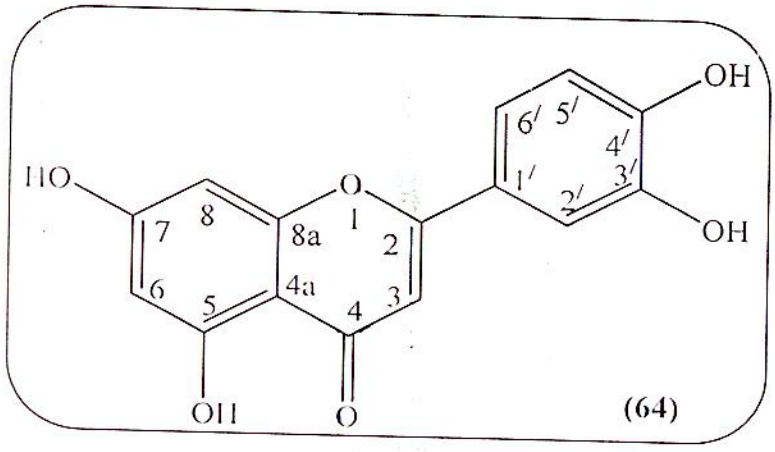
IR (K Br) $\tilde{\nu}_{max}$; 3422 (OH), 1629, 1582, 1455, 1377, 1314, 1267 cm^{-1}

^{13}C NMR; Given in **Table 2.16**

^{1}H NMR; Given in **Table 2.16**

HREIMS; *m/z* 300.0652 [M^+] (calcd. 300.0634 for $C_{16}H_{12}O_6$)

3.9.8. 3′,4′,5,7-Tetrahydroxyflavone (luteolin) (64)



(64)

Reddish needles (Acetone)

Melting point; >300 °C

Molecular formula; $C_{15}H_{10}O_6$

UV (MeOH) λ_{max} (log ε); 349 (3.23), 295 (3.90), 261 (3.45) nm

IR (KBr) $\tilde{\nu}_{max}$; 3422, 1654, 1612, 1520, 1083 cm^{-1}

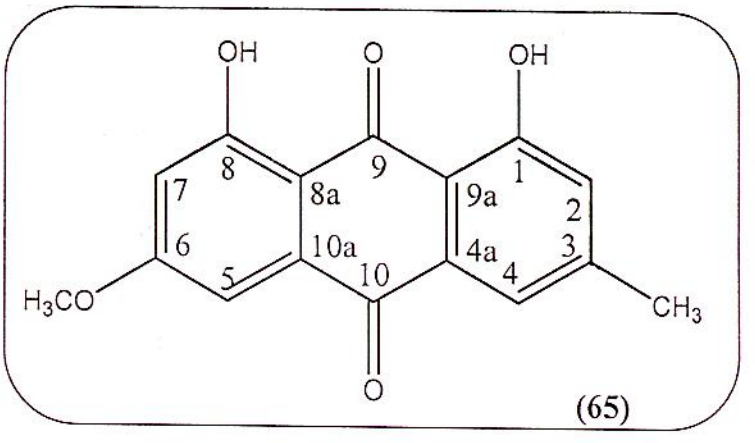
^{13}C NMR; Given in **Table 2.17**

^{1}H NMR; Given in **Table 2.17**

EI-MS; *m/z* (rel. int.) (%); 286 (100) [M^+], 258 (15), 153 (26), 134 (10)

3.10. Experimental data of new source isolated compounds from *Cassia artemisioides* Bark

3.10.1. 1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (65)



(65)

Reddish needles (Chloroform)

Melting point; 205-206 °C

Molecular formula; $C_{16}H_{10}O_5$

UV (MeOH) λ_{max} (log ε); 432, 287, 262, 257 nm

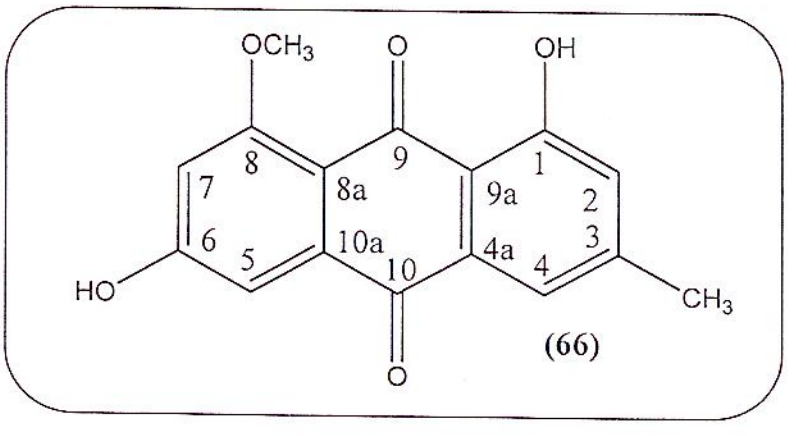
IR (KBr); $\tilde{\nu}_{max}$; 3394, (OH), 2917, 1660, 1455, 1377, 1314, 1267cm^{-1}

^{13}C NMR; Given in **Table 2.18**

^{1}H NMR; Given in **Table 2.18**

EI-MS; *m/z*, (rel. int.) (%); 284 (100), 266 (49.09), 238 (75.63), 226 (8.89), 197 (17.89), 149 (12.64).

3.10.2. 1,6-Dihydroxy-8-methoxy-3-methylanthraquinone (66)



(66)

Yellow needles (Acetone)

Molecular formula; $C_{16}H_{12}O_5$

Melting point; 300-302 °C

UV (MeOH) λ_{max} (log ε); 225 (4.53) 247 (4.09), 286 (4.43) nm

IR (KBr) $\tilde{\nu}_{max}$; 3427 (OH), 2921, 1680, 1589, 1484, 1441, 1261 cm^{-1}

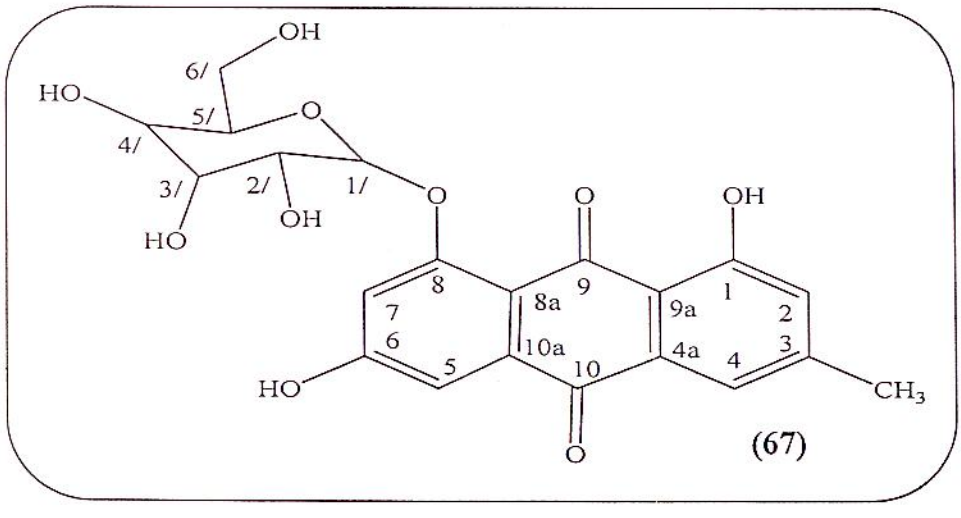
^{13}C NMR; Given in **Table 2.19**

^{1}H NMR; Given in **Table 2.19**

EI-MS; *m/z* 284 [M^+] (calcd. 284 for $C_{16}H_{12}O_5$)

EI-MS; *m/z* (rel. int.) (%); 284 (65), 267 (15), 213 (28), 198 (25), 182 (36), 77 (100)

3.10.3. Emodine-8-*O*-*β*-*D*-glucopyranoside (1,6-dihydroxy-3-methylanthraquinone-8-*O*-*β*-*D*-glucopyranoside) (67)



(67)

Yellow powder (Methanol)

Melting point; 221-222 °C

Molecular formula; $C_{21}H_{20}O_{10}$

UV (MeOH) λ_{max}; 203, 207 and 220 nm

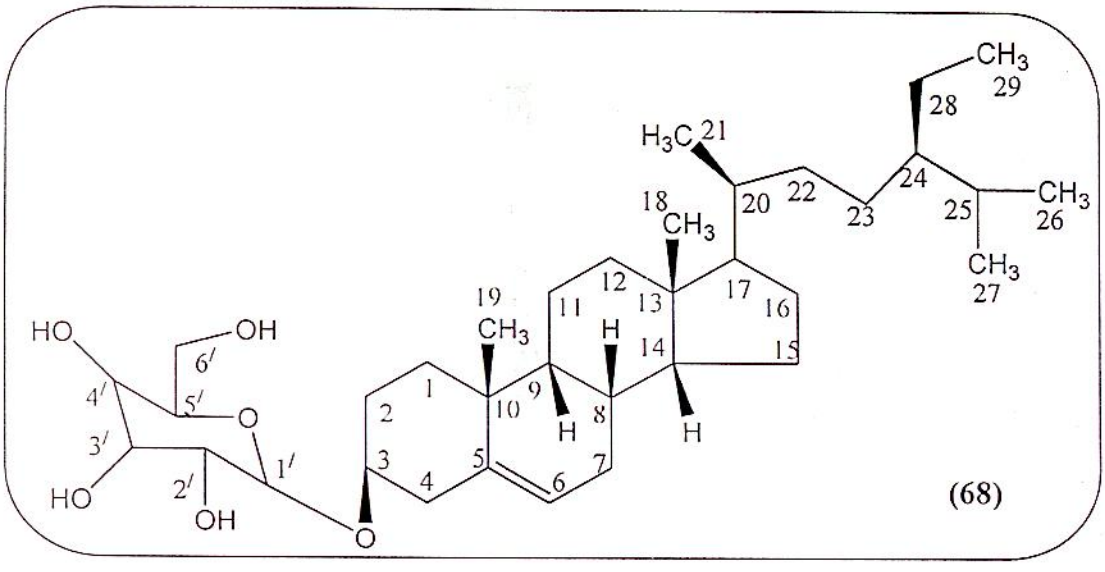
IR (KBr) $\tilde{\nu}_{max}$; 3335, 1665, 1615, 1585, 1448 cm^{-1}

^{13}C NMR; Given in **Table 2.20**

^{1}H NMR; Given in **Table 2.20**

HR-MS; *m/z* 432 [M^+] (calcd. 432 for $C_{21}H_{20}O_{10}$)

3.10.4. *β*-Sitosterol-3-*O*-*β*-D-glucopyranoside (68)



(68)

White powder (Chloroform)

Melting point; 236-238 °C

$[\alpha]^{25}D$; -14.5 (c=0.3, Methanol)

IR (neat) $\tilde{\nu}_{max}$; 3450, 3040, 1618, 1596 cm^{-1}

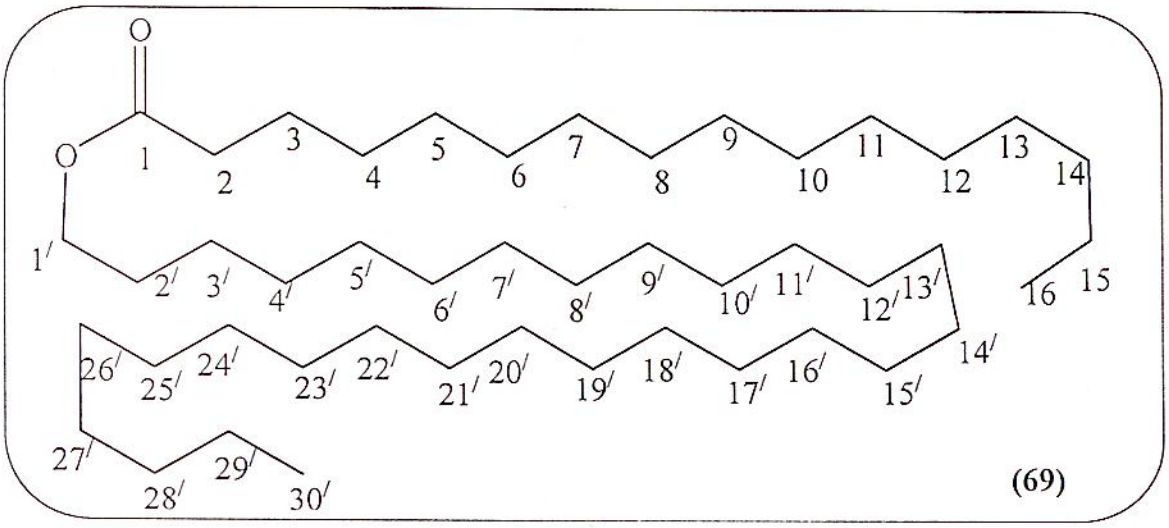
^{13}C NMR; Given in **Table 2.21**

^{1}H NMR; Given in **Table 2.21**

EIMS; *m/z* $[M]^+$ 414 (15), 399 (10), 396 (12), 381 (72), 329 (28), 275 (12), 273 (17), 255 (36).

HR-FABMS; *m/z* 576.4386 $[M^+]$ (calcd. 576.4389 for $C_{35}H_{60}O_6$)

3.10.5. Tricontyl palmitate (69)



(69)

White powder (Chloroform)

Melting point; 78-79 °C

Molecular formula; $C_{46}H_{92}O_2$

$[\alpha]_D^{23}$= 27.2 (c=0.04, chloroform)

UV ($CHCl_3$) λ_{max}; (log ε) 204.2 (3.70) 196.6 (3.20) nm

IR (Neat) $\tilde{\nu}_{max}$; 2914, 2850, 1730, 1165 cm^{-1}

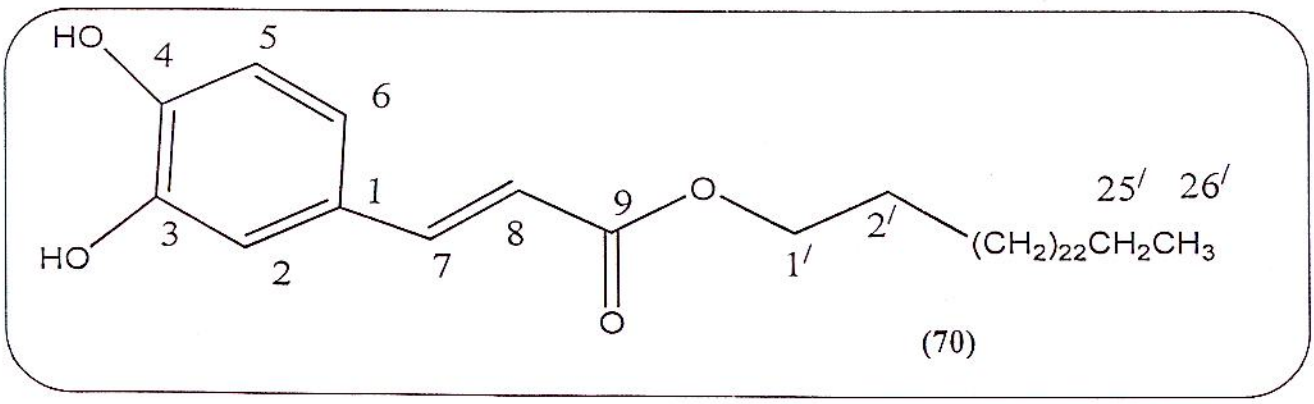
^{13}C NMR; Given in **Table 2.22**

^{1}H NMR; Given in **Table 2.22**

EI-MS; *m/z* 676 [M^+] (Calculated 676 for $C_{46}H_{92}O_2$)

EI-MS; *m/z* (rel. int.) 676 [M^+], 647, 396, 313, 257, 185, 125, 71.0

3.10.6. Hexacosanyl caffeate (70)



(70)

White powder (Chloroform)

Melting point; 94-95 °C

Molecular formula; $C_{35}H_{60}O_4$

IR (KBr) $\tilde{\nu}_{max}$; 3478, 3315, 1695, 1658, 1603, 1518, 1465, 1280 cm^{-1}

^{13}C NMR; Given in **Table 2.23**

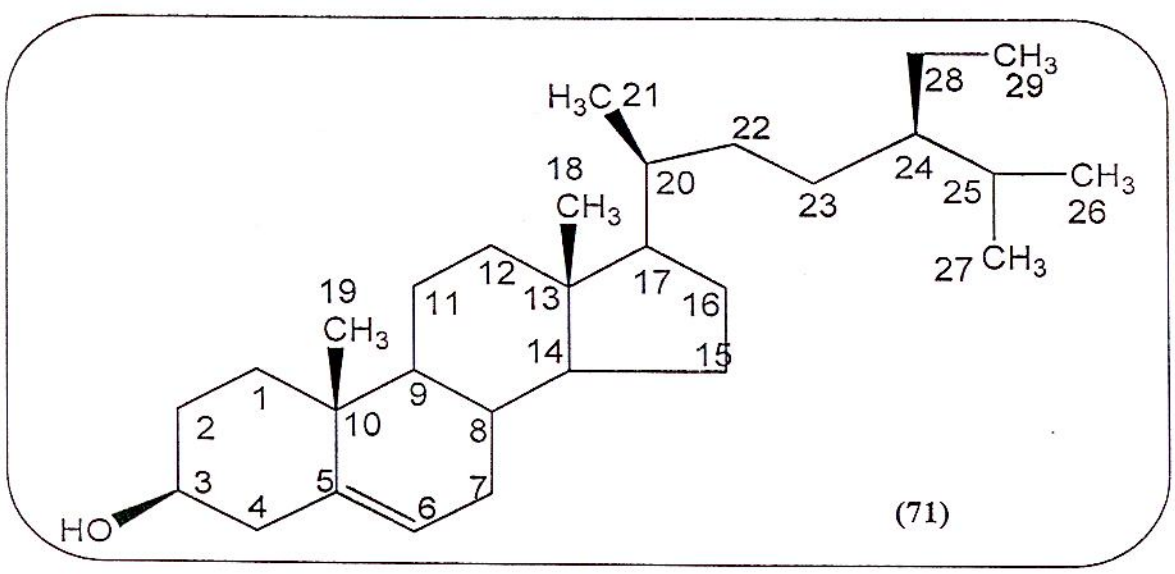
^{1}H NMR; Given in **Table 2.23**

EI-MS *m/z*; 544 [M^+] (calcd. 544 for $C_{35}H_{60}O_4$)

EI-MS; *m/z* (rel. int.); 544, 488, 396, 364, 285, 257, 189, 180, 97

3.11. Experimental data of new source isolated compounds from the Roots of *Cassia artemisioides*

3.11.1. *β*-Sitosterol (71)



(71)

Molecular formula $C_{29}H_{50}O$

Melting point; 144 °C

$[\alpha]_D^{25}$:+35.5 (c 0.22, $CHCl_3$)

IR (KBr) $\tilde{\nu}_{max}$; 3406, 2905 and 1640 cm^{-1}

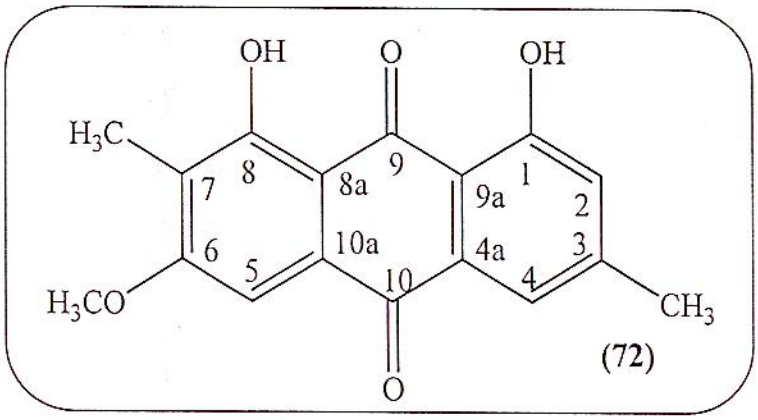
^{13}C NMR; Given in **Table 2.24**

^{1}H NMR; Given in **Table 2.24**

EI-MS; *m/z* 414 [M^+] (calcd. 414 for $C_{29}H_{50}O$)

EI-MS *m/z* (rel. int.); 414, 399, 396, 381, 329, 303

3.11.2. 1,8-Dihydroxy-3,7-dimethyl-8-methoxyanthraquinone (72)



Yellow needles (Acetone)

Melting point; 233-234 °C

Molecular formula; $C_{17}H_{14}O_5$

UV (MeOH) λ_{max} (log ε); 235 (4.12), 248 (4.25), 278 (4.48), 310 (4.00), 434 (4.11) nm

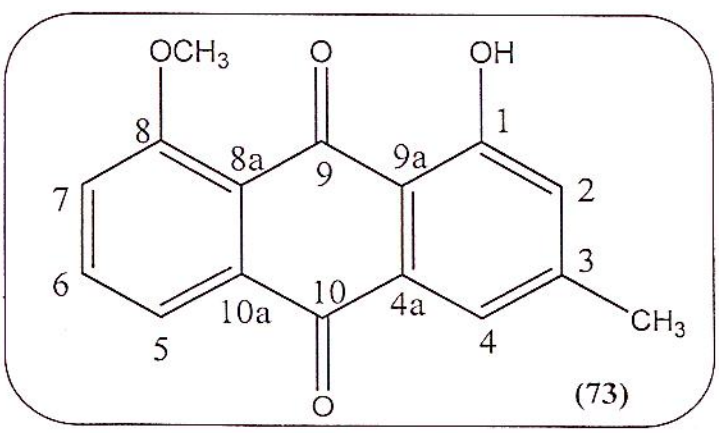
IR (KBr) $\tilde{\nu}_{max}$; 3415 (OH), 16218, 1610, 1575, 1550, 1480, 1267 cm^{-1}

^{13}C NMR; Given in **Table 2.25**

^{1}H NMR; Given in **Table 2.25**

EI-MS; *m/z* (rel. int) (%); 298 (100) [M^+] 283 (19), 280 (58), 269 (27)

3.11.3. 1-Hydroxy-8-methoxy-3-methylanthraquinone (73)



Orange needles (Acetone)

Melting point; 195-196 °C

Molecular formula; $C_{16}H_{12}O_4$

UV (MeOH); λ_{max} (log ε) 221 (4.50), 255 (4.30), 280 (4.10), 413 (3.90) nm

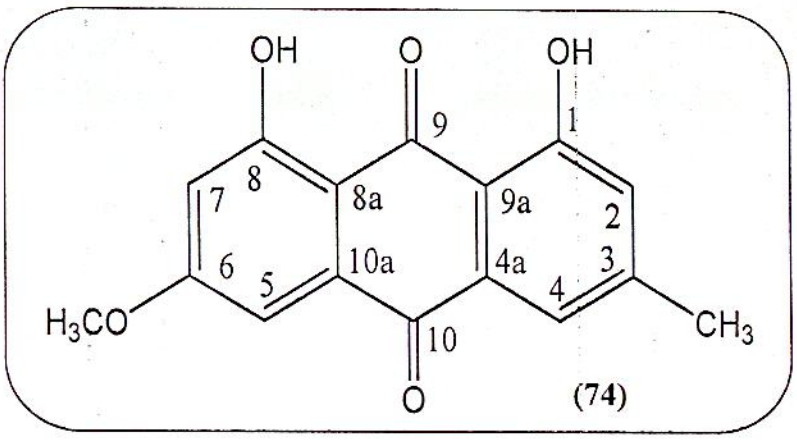
IR (KBr) $\tilde{\nu}_{max}$; 3422 (OH), 2945, 1675, 1630, 1586, 1495, 1440, 1373 cm^{-1}

^{13}C NMR; Given in **Table 2.26**

^{1}H NMR; Given in **Table 2.26**

EI-MS *m/z* (rel. int.) (%); 268 (100) [M$^+$], 250 (40), 239 (18), 222 (48), 152 (86.56)

3.11.4. 1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (74)



Reddish needles (Acetone)

Melting point; 205-206 °C

Molecular formula; $C_{16}H_{10}O_5$

UV (MeOH) λ_{max} (log ε); 432, 287, 262, 257 nm

IR (KBr) $\tilde{\nu}_{max}$; 3394 (OH), 2917, 1582, 1455, 1377, 1314, 1267 cm^{-1}

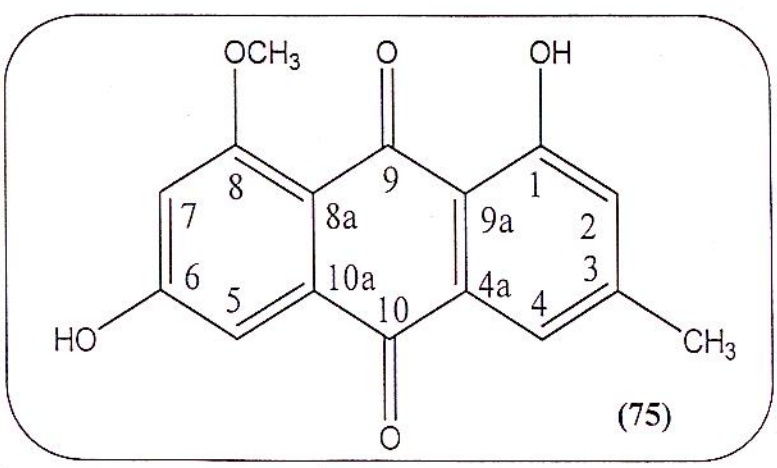
^{13}C NMR; Given in **Table 2.27**

^{1}H NMR; Given in **Table 2.27**

EI-MS; (*m/z*) 284 [M]$^+$ (calcd. 284 for $C_{16}H_{12}O_5$)

EI-MS; *m/z* (rel. int.) (%); 284 (100), 266 (49.09), 238 (75.63), 226 (8.89), 197 (17.89)

3.11.5. 1,6-Dihydroxy-8-methoxy-3-mthylanthraquinone (75)



Yellow needles (Acetone)

Melting point; 300-302 °C

Molecular formula; $C_{16}H_{12}O_5$

UV (MeOH) λ_{max} (log ε); 225 (4.53) 247 (4.09), 286 (4.43) nm

IR (KBr) $\tilde{\nu}_{max}$; 3427 (OH), 2921, 1680, 1589, 1484, 1441, 1261 cm^{-1}

^{13}C NMR; Given in **Table 2.28**

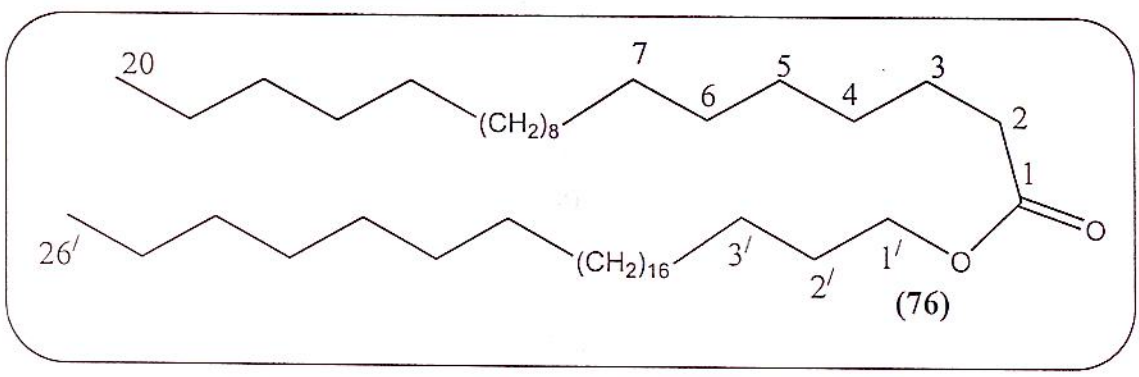
^{1}H NMR; Given in **Table 2.28**

EI-MS; *m/z* 284 [M^+] (calcd. 284 for $C_{16}H_{12}O_5$)

EI-MS *m/z* (rel. int.) (%); 284 (65), 267 (15), 213 (28), 198 (25), 182 (36), 77 (100)

3.12. Experimental data of new source isolated compounds from the Pods of *Cassia artemisioides*

3.12.1. Hexacosyl icosaoate (76)



(76)

White powder (Chloroform)

Melting point; 76 °C

Molecular formula; $C_{46}H_{92}O_2$

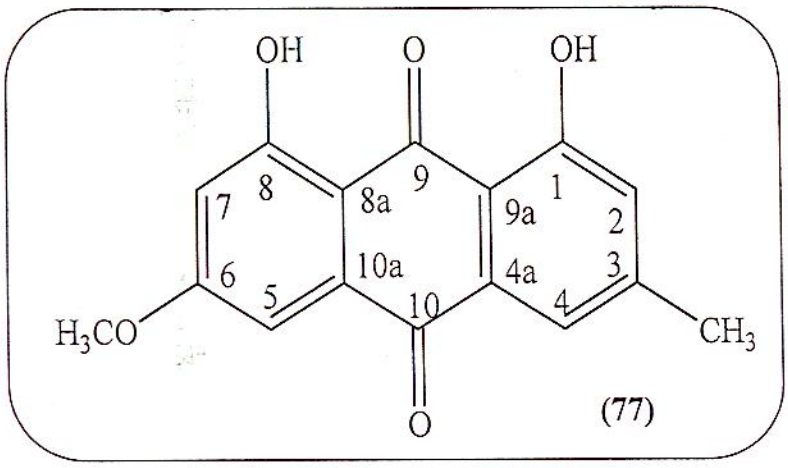
IR (KBr) $\tilde{\nu}_{max}$; 3434, 2919, 2850.3, 1735.9, 1622.1, 1477.5, 1368.7, 1269, 1220.9 cm^{-1}

^{13}C NMR; Given in **Table 2.29**

^{1}H NMR; Given in **Table 2.29**

EI-MS *m/z* (rel. int.) (%); 676 (3.45), 649 (2.25), 313 (18.25), 257 (7.54), 185 (2.87), 125 (14.23)

3.12.2. 1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (77)



Reddish needles (Acetone)

Melting point; 205-206 °C

Molecular formula; $C_{16}H_{10}O_5$

UV (CH_3OH): λ_{max} (log ε) 432, 287, 262, 257 nm

IR (K Br) $\tilde{\nu}_{max}$; 3441.1, 2933.2, 2856.6, 1736, 1694.9, 1637.6, 1458, 1367.6 cm^{-1}

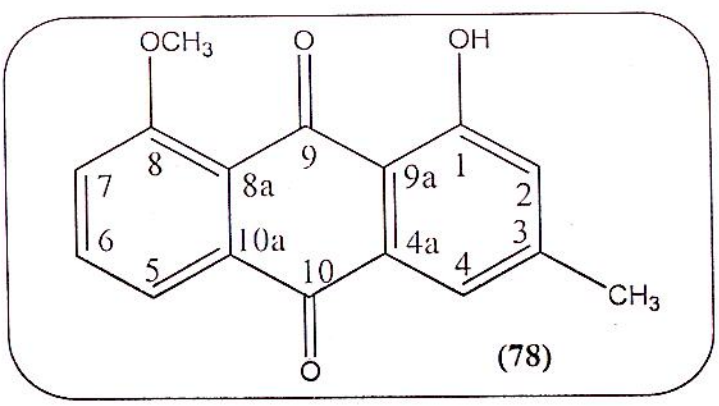
^{13}C NMR: Given in **Table 2.30**

^{1}H NMR: Given in **Table 2.30**

EI-MS *m/z* 284 $[M]^+$ (calcd. 284 for $C_{16}H_{12}O_5$)

EI-MS (rel. int.) (%); 284 (100), 266 (49.09), 238 (75.63), 226 (8.89), 197 (17.89), 149 (12.64)

3.12.3. 1-Hydroxy-8-methoxy-3-methylanthraquinone (78)



Yellow needles (Acetone)

Melting point: 195-196 °C

Molecular formula; $C_{16}H_{12}O_4$

UV (MeOH): λ_{max} (log ε) 221 (4.50), 255 (4.30), 280 (4.10), 413 (3.90) nm

IR (KBr) $\tilde{\nu}_{max}$; 3419 (OH), 2945, 1675, 1630, 1586, 1495, 1440, 1373 cm^{-1}

^{13}C NMR: Given in **Table 2.31**

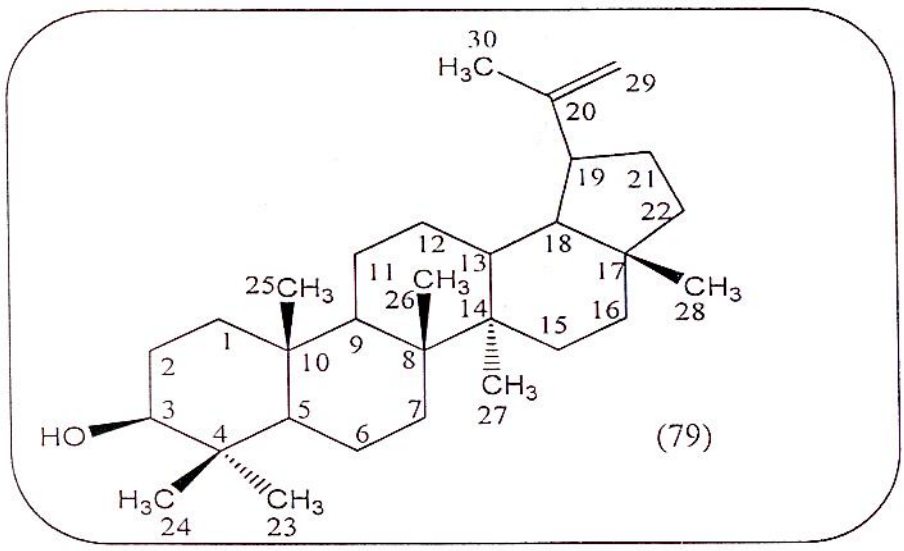
^{1}H NMR: Given in **Table 2.31**

EI-MS *m/z*; 268 [M^+] (calcd. 268 for $C_{16}H_{12}O_4$)

EI-MS *m/z* (rel. int.) (%); 268 (100), 250 (40), 239 (18), 222 (48), 152 (86.56)

3.13. Experimental data of new Source isolated Compounds from the Seeds of *Cassia artemisioides*

3.13.1. 3*β*-Hydroxylup-20(29)-ene (lupeol) (79)



(79)

White powder (Chloroform)

Melting point; 212-213 °C

Molecular formula; $C_{30}H_{50}O$

$[\alpha]_D^{25}$ + 26.4 (*c* = 0.19, $CHCl_3$)

FT-IR (neat) $\tilde{\nu}_{max}$; 3406, 1645, 1495, 1381, 1183, 1104, 1039, 985, 940 cm^{-1}

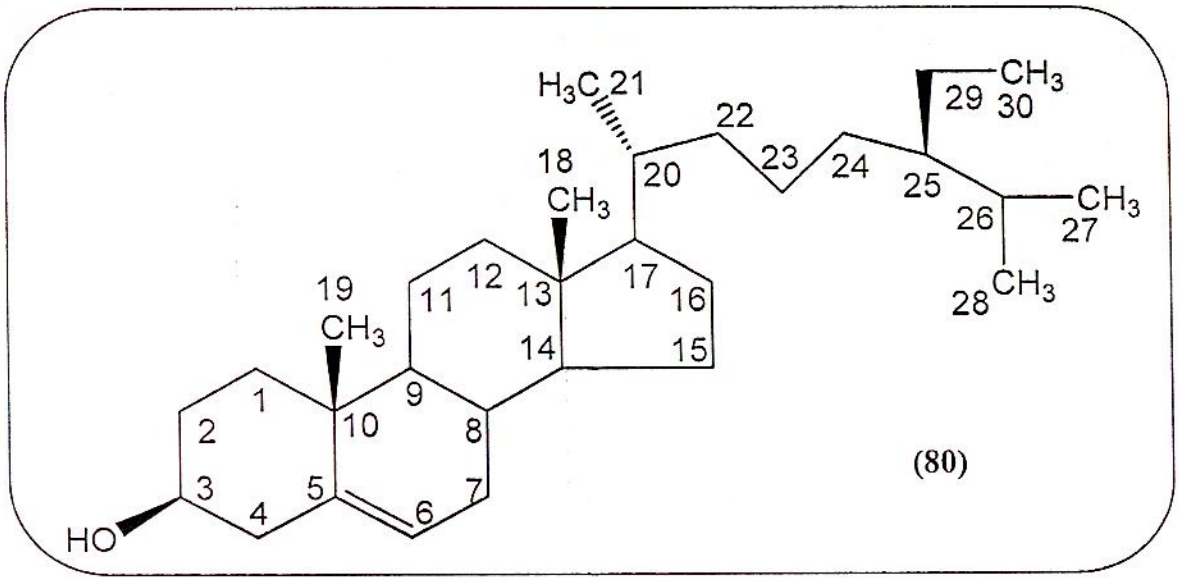
^{13}C NMR: Given in **Table 2.32**

^{1}H NMR: Given in **Table 2.32**

EI-MS *m/z*: 426 [M^+] (calcd. 426 for $C_{30}H_{50}O$)

EI-MS *m/z* (rel. int.) (%): 426 (55.45), 393 (3.71), 315 (13.57), 257 (10.04), 234 (18.16), 189 (68.11), 161 (30.98), 135 (63.03)

3.13.2. 23a-Homostigmast-5-en-3β-ol (80)



White powder (Chloroform)

Melting point; 125-127 °C

Molecular formula; $C_{30}H_{52}O$

FT-IR (neat) $\tilde{\nu}_{max}$; 3487.6, 3338.6, 2918.9, 2849.4, 1688.2, 1603.1, 1363.8, 1301.9 cm^{-1}

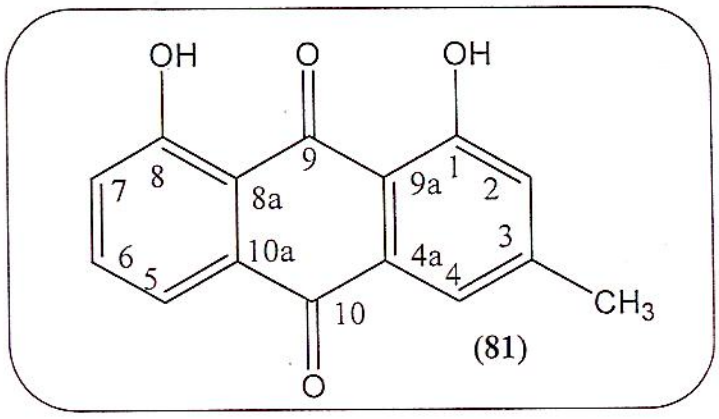
^{13}C NMR: Given in **Table 2.33**

^{1}H NMR: Given in **Table 2.33**

EI-MS *m/z*: 428 [M^+] (calcd. 428 for $C_{30}H_{52}O$)

EI-MS *m/z* (rel. int.) (%): 428.3 (55.40), 393.4 (3.70), 315.5 (13.55), 257.3 (10.5), 234.0 (19.13), 189.0 (67.34), 161.4 (31.05

3.13.3. 1,8-Dihydroxy-3-methylanthraquinone (81)



(81)

Reddish needles (Chloroform

Melting point; 195-196 °C

Molecular formula; $C_{15}H_{10}O_4$

UV (EtOH): λ_{max} (log ε): 429, 287, 277, 257 nm

IR (KBr) $\tilde{\nu}_{max}$; 3400, 1670, 1620 cm^{-1}

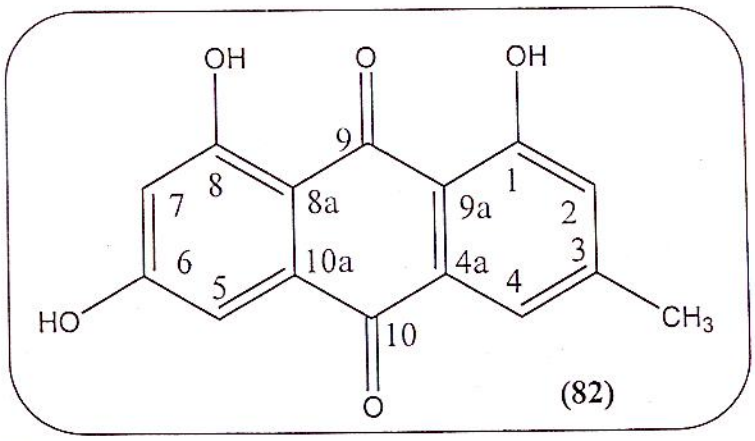
^{13}C NMR: Given in **Table 2.34**

^{1}H NMR: Given in **Table 2.34**

EI-MS *m/z* 254 [M^+] (calcd. 254 for $C_{15}H_{10}O_4$)

EI-MS *m/z* (rel. int.) (%); 254 (100), 237 (3.8), 226 (7.9), 197 (4.6), 152 (4.0)

3.13.4. 1,6,8-Trihydroxy-3-methylanthraquinone (82)



(82)

Reddish needles (Chloroform)

Melting point; 268-270 °C

Molecular formula; $C_{15}H_{10}O_5$

UV (MeOH): λ_{max} 202, 208, 218 nm

IR (KBr) $\tilde{\nu}_{max}$; 3422 (OH), 1629, 1560, 1475, 1340, 1260 cm^{-1}

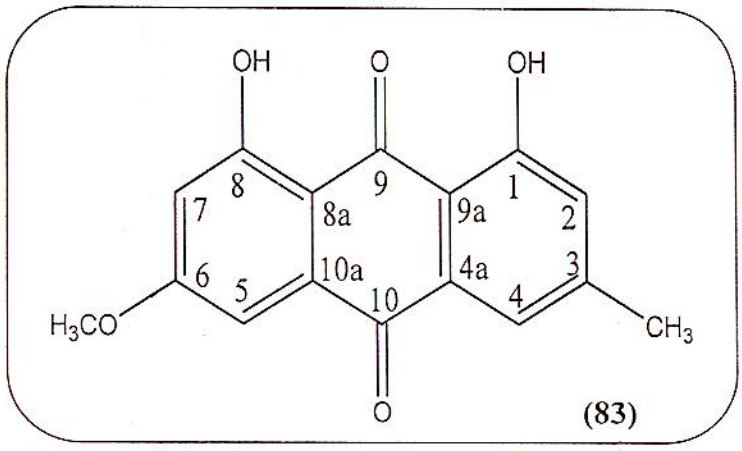
^{13}C NMR Given in **Table 2.35**

^{1}H NMR Given in **Table 2.35**

EI-MS *m/z* 270 [M^+] (calcd. 270 for $C_{15}H_{10}O_5$)

EI-MS *m/z* (rel. int.) (%); 270 (100) 242 (7.1), 213 (5.5), 139 (3.1), 121 (3.2)

3.13.5. 1,8-Dihydroxy-3-methylanthraquinone (83)



(83)

Reddish needles (Acetone)

Melting point; 205-206 °C

Molecular formula $C_{16}H_{10}O_5$

UV (MeOH): λ_{max} (log ε) 432, 287, 262, 257 nm

IR (KBr) $\tilde{\nu}_{max}$; 3441.1 (OH), 2933.2, 2856.6, 1736, 1694.9, 1637.6, 1458, 1367.6 cm^{-1}

^{13}C NMR Given in **Table 2.36**

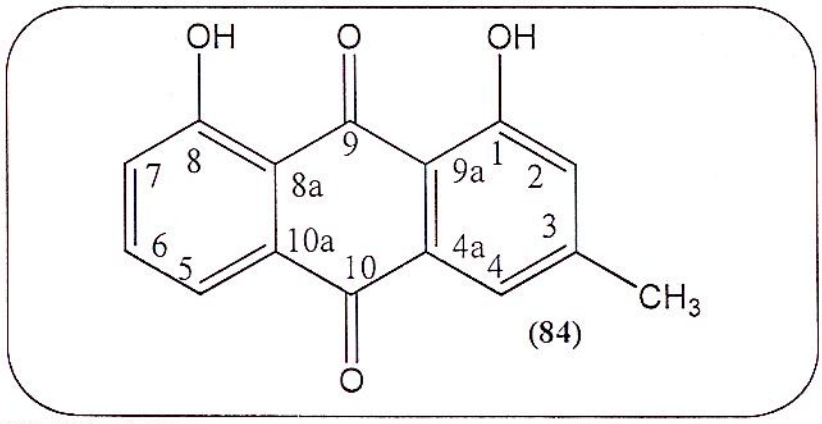
^{1}H NMR Given in **Table 2.36**

EIMS *m/z* 284 $[M]^+$ (calcd. 284 for $C_{16}H_{12}O_5$)

EI-MS (rel. int.) (%); 284 (100), 266 (49.09), 238 (75.63), 226 (8.89), 197 (17.89), 149 (12.64)

3.14. Experimental data of new source compounds isolated from *Cassia nemophila* Flowers

3.14.1. 1,8-Dihydroxy-3-methylanthraquinone (84)



(84)

Reddish needles (Chloroform)

Melting point: 195-196 °C

Molecular formula; $C_{15}H_{10}O_4$

UV (EtOH): λ_{max} (log ε); 429, 287, 277, 257 nm

IR (KBr) $\tilde{\nu}_{max}$; 3400, 1670, 1620 cm^{-1}

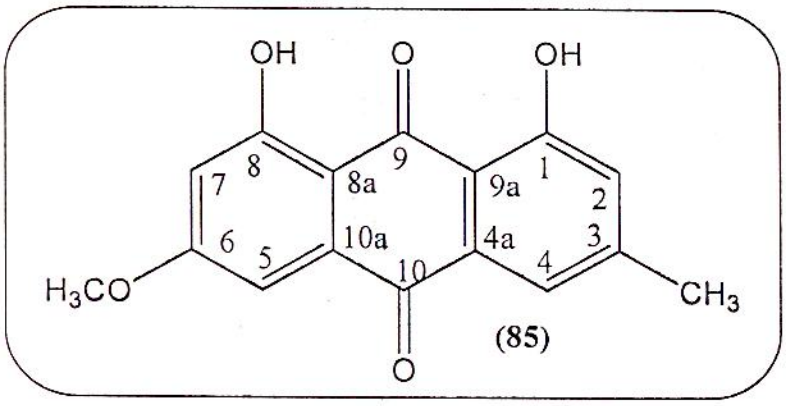
^{13}C NMR: Given in **Table 2.37**

^{1}H NMR: Given in **Table 2.37**

EI-MS *m/z* 254 $[M^+]$ (calcd. 254 for $C_{15}H_{10}O_4$)

EI-MS *m/z* (rel. int.) (%): 254 (100), 237 (3.8), 226 (7.9), 197 (4.6), 152 (4.0)

3.14.2. 1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (85)



(85)

Reddish needles (Chloroform)

Melting point; 205-206 °C

Molecular formula; $C_{16}H_{10}O_5$

UV (MeOH): λ_{max} (log ε); 432, 287, 262, 257 nm

IR (KBr) $\tilde{\nu}_{max}$: 3394, 2917, 1582, 1455, 1377, 1314, 1267 cm^{-1}

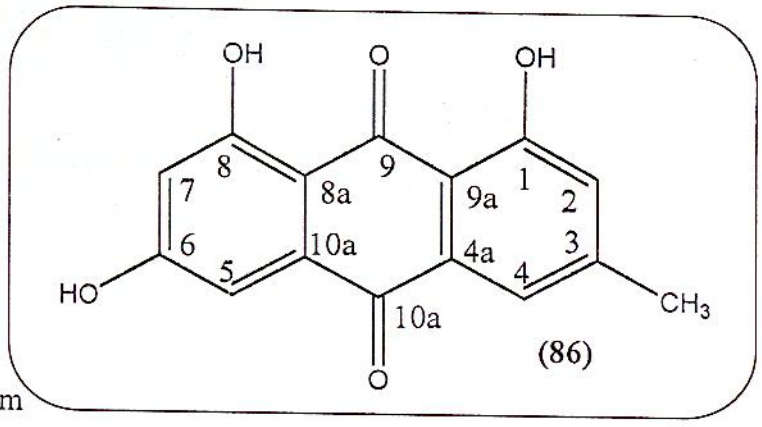
^{13}C NMR: Given in **Table 2.38**

^{1}H NMR: Given in **Table 2.38**

EI-MS *m/z* 284 $[M]^+$ (calcd. 284 for $C_{16}H_{12}O_5$)

EI-MS *m/z* (rel. int.) (%); 284 (100), 266 (49.09), 238 (75.63), 226 (8.89), 197 (17.89)

3.14.3. 1,8-Dihydroxy-3-methylanthraquinone (86)



(86)

Redish needles (Chloroform)

Melting point; 268-270 °C

Molecular formula; $C_{15}H_{10}O_5$

UV (MeOH): λ_{max}; 202, 208, 218 nm

IR (KBr) $\tilde{\nu}_{max}$; 3422, 1629, 1560, 1475, 1340, 1260 cm^{-1}

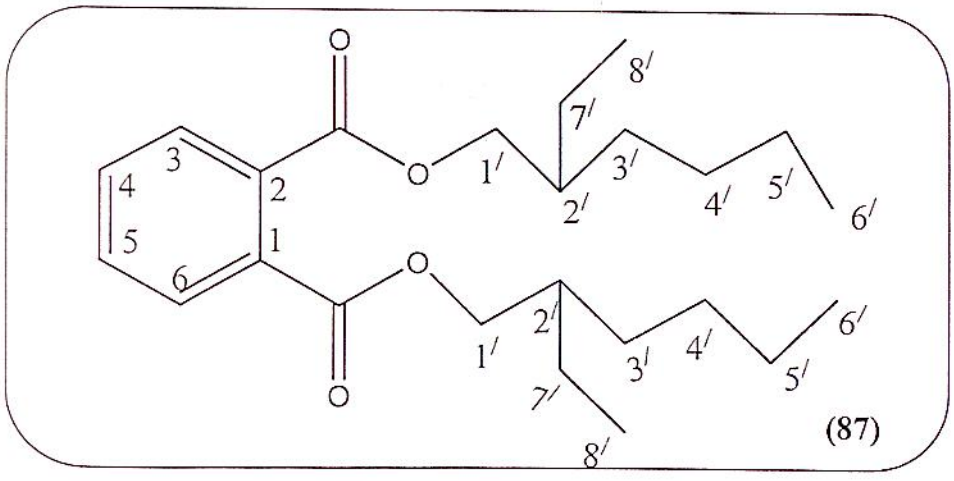
^{13}C NMR: Given in **Table 2.39**

^{1}H NMR: Given in **Table 2.39**

EI-MS *m/z* 270 [M^+] (calcd. 270 for $C_{15}H_{10}O_5$)

EI-MS *m/z*; (rel. int.) (%); 270 (100) 242 (7.1), 213 (5.5), 139 (3.1), 121 (3.2)

3.14.4. Di-2-ethylhexyl phthalate (87)



(87)

Colourless thick residue (Acetone)

Molecular formula; $C_{24}H_{38}O_4$

UV (CH_2Cl_3) λ_{max}; 278 and 234 nm

IR (neat) $\tilde{\nu}_{max}$: 3025, 2927, 2885, 1730, 1605 and 1580 cm^{-1}

^{13}C NMR: Given in **Table 2.40**

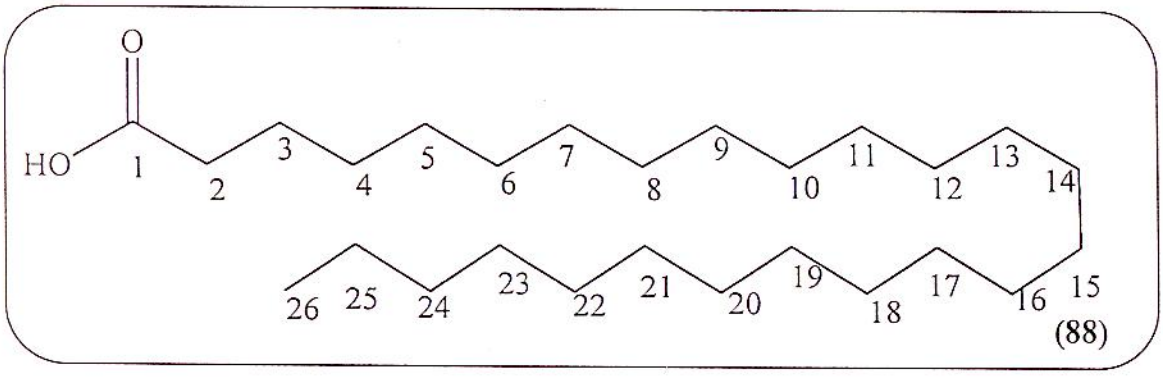
^{1}H NMR: Given in **Table 2.40**

EI-MS *m/z* 390 [M^+] (calcd. 390 for $C_{15}H_{10}O_5$)

EI-MS, *m/z* (rel. int.) (%); 390 (4), 279 (11), 261 (4), 167 (40), 149 (100), 113 (14), 104 (8)

3.15. New source isolated compounds from the Bark of *Cassia nemophila*

3.15.1. Hexacosanoic acid (88)



(88)

White residue (Chloroform)

Molecular formula; $C_{26}H_{52}O_2$

IR (neat) $\tilde{\nu}_{max}$; 3305-2610, 1707 cm^{-1}

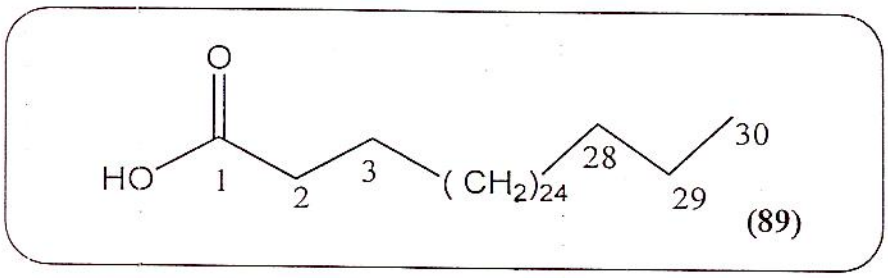
^{13}C NMR Given in **Table 2.41**

^{1}H NMR Given in **Table 2.41**

EI-MS *m/z*: 396 [M^+] (calcd. 396 for $C_{26}H_{52}O_2$)

EI-MS *m/z* (rel. int.) (%): 396, 368, 353, 297, 241, 185, 129, 85

3.15.2. Triacontanoic acid (Melissic acid) (89)



(89)

White powder (Chloroform)

Melting point; 92-93 °C

Molecular formula; $C_{30}H_{60}O_2$

IR (neat) $\tilde{\nu}_{max}$; 3280-2620, 1710, 918 cm^{-1}

^{13}C NMR: Given in **Table 2.42**

^{1}H NMR: Given in **Table 2.42**

EI-MS *m/z*; 451.9 [M^+] (calcd. 452 for $C_{30}H_{60}O_2$)

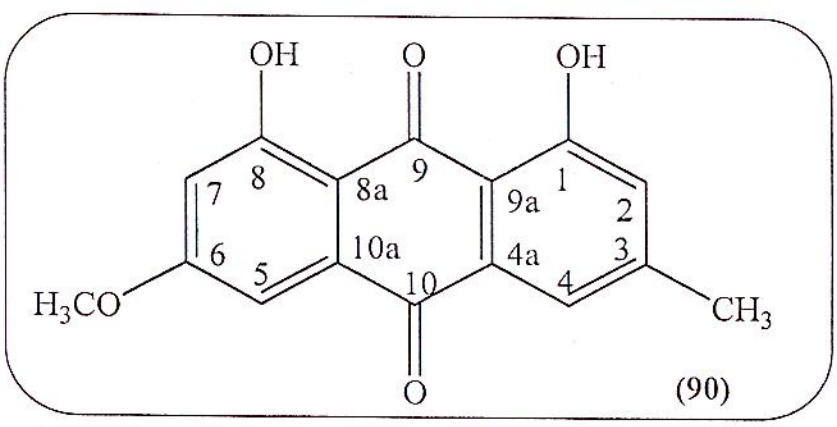
EI-MS *m/z* (rel. int.): 452, 424, 381, 353, 297, 269, 241, 185, 129, 97

3.15.3. 1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (90)

Reddish needles (Acetone)

Melting point 205-206 °C

Molecular formula $C_{16}H_{10}O_5$



(90)

UV (MeOH): λ_{max} 432, 287, 262, 257 nm

IR (KBr) $\tilde{\nu}_{max}$; 3441.1 (OH), 2933.2, 2856.6, 1736, 1694.9, 1637.6, 1458, 1367.6 cm^{-1}

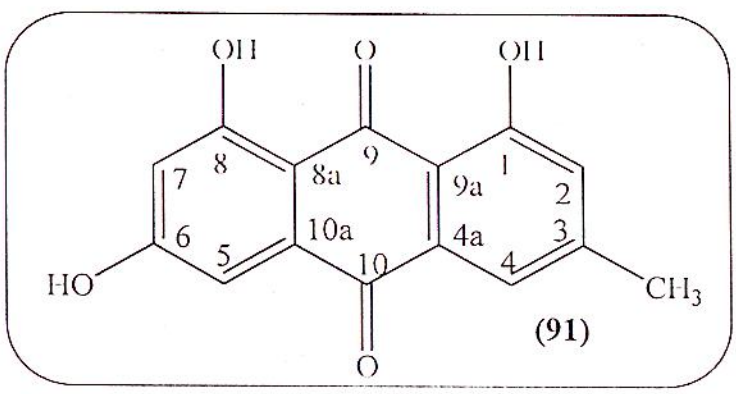
^{13}C NMR Given in **Table 2.43**

^{1}H NMR Given in **Table 2.43**

EIMS *m/z* 284 $[M]^+$ (calcd. 284 for $C_{16}H_{12}O_5$)

EI-MS *m/z*, (rel. int.) (%); 284 (100), 266 (49.09), 238 (75.63), 226 (8.89), 197 (17.89)

3.15.4. 1,8-Trihydroxy-3-methylanthraquinone (91)



(91)

Reddish needles (Chloroform)

Melting point; 268-270 °C

Molecular formula; $C_{15}H_{10}O_5$

UV (MeOH): λ_{max} 202, 208, 218 nm

IR (KBr) $\tilde{\nu}_{max}$; 3422 (OH), 1629, 1560, 1475, 1340, 1260 cm^{-1}

^{13}C NMR: Given in **Table 2.44**

^{1}H NMR: Given in **Table 2.44**

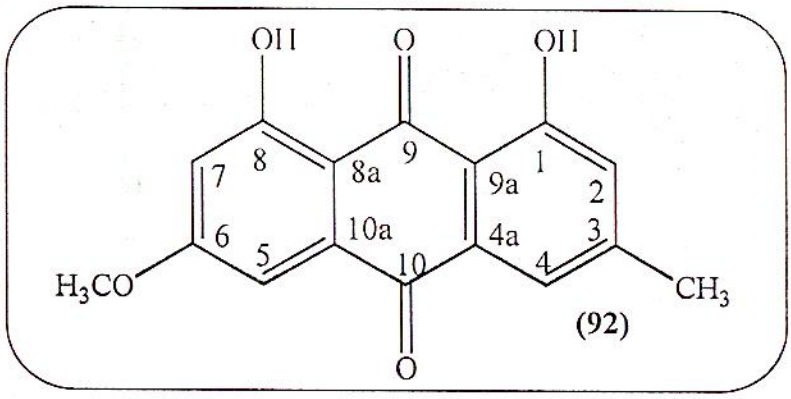
EI-MS *m/z* 270 $[M^+]$ (calcd. 270 for $C_{15}H_{10}O_5$)

EI-MS *m/z*; (rel. int.) (%); 270 (100) 242 (7.1), 213 (5.5), 139 (3.1), 121 (3.2)

347

3.16. Isolated compounds from the Roots of *Cassia nemophila*

3.16.1. 1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (92)



(92)

Reddish needles (Chloroform)

Melting point 205-206 °C

Molecular formula $C_{16}H_{10}O_5$

UV (MeOH): λ_{max} (log ε) 432, 287, 262, 257 nm

IR (KBr) $\tilde{\nu}_{max}$; 3394, 2917, 1582, 1455, 1377, 1314, 1267 cm^{-1}

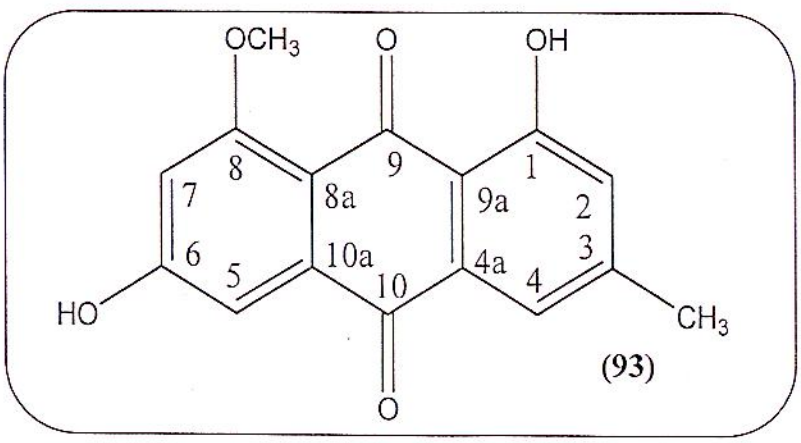
^{13}C NMR: Given in **Table 2.45**

^{1}H NMR: Given in **Table 2.45**

EI-MS *m/z* 284 [M^+] (calcd. 284 for $C_{16}H_{12}O_5$)

EI-MS *m/z*, (rel. int.) (%); 284 (100), 266 (49.09), 238 (75.63), 226 (8.89), 197 (17.89), 149 (12.64)

3.16.2. 1,6-Dihydroxy-8-methoxy-3-methylanthraquinone (93)



(93)

Yellow needles (Acetone)

Melting point; 300-302 °C

Molecular formula; $C_{16}H_{12}O_5$

UV (MeOH): λ_{max} (log ε) 225 (4.53) 247 (4.09), 286 (4.43) nm

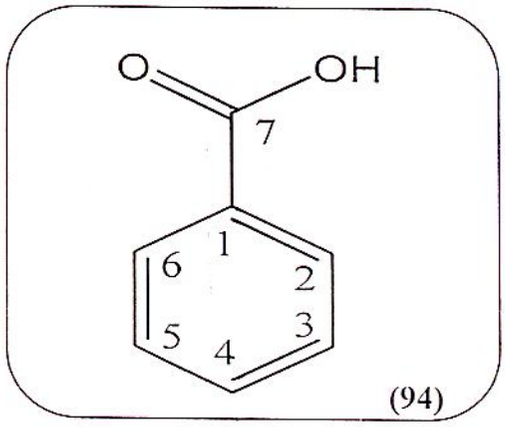
IR (KBr) $\tilde{\nu}_{max}$; 3427 (OH), 2921, 1680, 1589, 1484, 1441, 1261 cm^{-1}

^{13}C NMR Given in **Table 2.46**

^{1}H NMR Given in **Table 2.46**

EI-MS *m/z* 284; [M^+] (calcd. 284 for $C_{16}H_{12}O_5$)

3.16.3. Benzoic acid (94)



(94)

Crystalline solid (water)

Melting point 122-123 °C

UV(Methanol): λ_{max} log ε; 231 (3.70), 275 (3.82), 304 (4.12) nm

IR (Neat) $\tilde{\nu}_{max}$; 3250-2700, 1704, 1610 cm^{-1}

^{1}H NMR Given in **Table 2.47**

^{13}C NMR Given in **Table 2.47**

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Chapter 4

INTRODUCTION (Part B)

4.1. Biological screening of medicinal plants

Plants have been used as medicines for thousands of years. These medicines initially taken in the form of crude drugs such as teas, tinctures, poultices, powder, and other herbal formulations.[1,2] The specific plants for particular purpose and the methods of application for particular use were passed down through oral history. Recently the use of plants as medicines has involved the isolation of active compounds, resulted in the isolation of morphine from opium in the early 19th century for the first time[1] and later on drug discovery from medicinal plants have led to the isolation of early drugs such as codeine, digitoxin, cocaine and quinine.[1,2]

Phytochemists prepare extracts from the plant materials, subject these extracts to biological screening in pharmacologically relevant assays and commence the process of isolation and characterization of the active compounds through bioassay-guided fractionation. Molecular biology has become essential to medicinal plant drug discovery through the determination and implementation of appropriate screening assays directed towards physiologically relevant molecular targets.[2] A large number of assays and protocols are developed for the screening of medicinal plants for their activities. Some of the important biological activities are mentioned here.

4.2. The role of antioxidant in treatment of cancer

In recent years the role of free radicals and reactive oxygen species (ROS) in human degenerative disease of aging, cancer, cardiovascular disease, cataracts, immune system decline and brain dysfunction has become apparent. Oxidant or free radicals are energetically unstable and highly reactive molecules which are constantly generated during the body function such as respiration, oxidative energy metabolism and immune activity. Free radicals are also produced from other sources like UV radiations, smoke pollution heavy metals etc.

The investigations for finding new anticancer compounds are imperative and interesting. After taking into consideration the immense side effects of synthetic anticancer drugs, many researchers are making concerted efforts to find new and natural anticancer compounds.[3] Therefore a number of studies have been carried out on various medicinal plants including fruits and vegetables. It has been shown that dietary patterns were significantly associated with the prevention of chronic diseases such as heart disease, cancer, diabetes and other fatal diseases.[4, 5] The use of fruits and vegetables has been highly associated with the reduced risk of cancer.[6,7] The levels of oxidants and antioxidants in humans are maintained in balance at normal metabolism, which is important for sustaining optimal physiological conditions.[7,8] Overproduction of oxidants like super oxide anion ($O_2^{\bullet-}$), hydroxyl ($HO^{\bullet}$), peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$) and nitric oxide can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA and proteins.[9] These oxidant have been regarded as the fundamental cause of different kinds of diseases, including aging, coronary heart disease, inflammation, stroke, diabetes mellitus, rheumatism, liver disorders, renal failure, cancer and neuro degeneration.[10] Phytochemicals can have complementary and overlapping mechanisms of oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism and antibacterial and antiviral effects.[7,9] Phytochemicals in fruits, vegetables, spices and traditional herbal medicinal plants have been found to play protective role against many human chronic diseases including cancer and cardiovascular diseases (CVD). These diseases are associated with oxidative stresses caused by excess free radicals and other reactive oxygen species. Antioxidant phytochemicals exert their effect by neutralizing these highly reactive radicals.[11]

4.3. Antioxidants from plants in the treatment of cancer

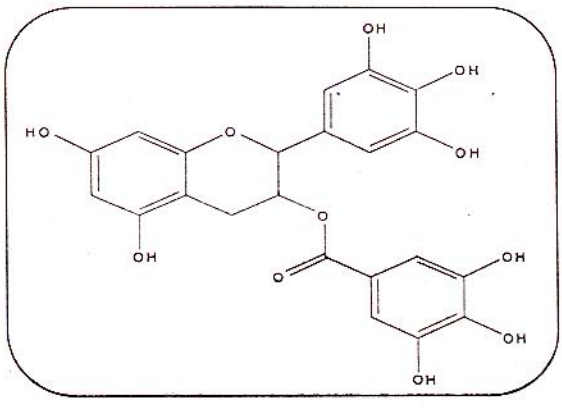
Antioxidants come in various forms. They are classified broadly into two groups; **a. antioxidant enzyme** such as superoxide dismutase, catalase and glutathione peroxidase etc. and **b. molecular enzyme** such as vitamin (Vitamin A, C, several of the B-complex and vitamin E), the minerals (selenium, zinc and manganes), carotenoides and flavonoids which are found in plants. It has been found that a diet rich in carotenoides reduce the risk of many disease, including cancer and

carotenoides can prevent cell division in cancer.[12] Biflavonoids also known as flavonoids, occur in many plants. They can be divided into six groups such as isoflavones, flavanols, flavones, catechins and proanthocyanidines. All of these have potent antioxidant activity.[13] Epigallocatechin gallate (**95**) one of the antioxidant has been shown to accelerate the disappearance of benzo [α] pyrene diol 2-epoxide which is mutagenic and carcinogenic.[14] The polymethoxyflavones, tangeretin (**96**) and nobiletin (**97**) occur in the citrus fruits, were found to enhance carcinogen removal.[15] Quercetin (**98**) also inhibits initiation and promotion of chemically-induced tumors.[16] Flavonoids are abundant in fruits, teas, vegetables and medicinal plants as antioxidants. Since they are highly effective free radical scavengers and are assumed to be less toxic than synthetic antioxidants such as BHA and BHT, which are suspected of being carcinogenic and may cause liver damage.[17]

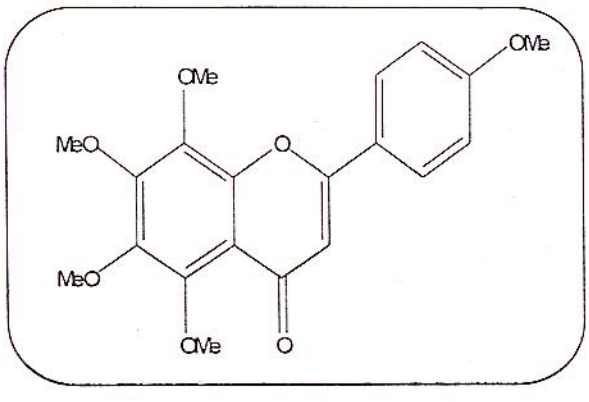
Phytochemicals in fruits, vegetables, spices and traditional herbal medicinal plants have been found to play protective role against many human chronic diseases including cancer and cardiovascular diseases (CVD). Antioxidant phytochemicals exert their effect by neutralizing these highly reactive radicals.[11] An inverse relationship has been shown between dietary intake of antioxidant rich foods and the incidence of a number of human diseases.[18] Thus the search for natural antioxidant sources and their antioxidant potential is becoming more and more important. A number of antioxidants have been derived from plants such as *Cassia tora* L.[19] and *Cassia fistula.*[20] Moreover, the close relationship between antioxidant activity and antimutagenicity has been demonstrated by Yen and Chen.[21]

The ethanol and methanol extracts of *C. auriculata* flowers showed potent antioxidant activity, the assay was based on scavenging of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic (ABTS) radical cation and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. These antioxidant activities are due to the presence of flavonoids and tannins.[22] The alcoholic extract of *Cassia siamea* L. flowers showed good antioxidant activity. The chemical examination of alcoholic extract from flowers has shown the presence of a large number of polyphenols, which may be responsible of the antioxidant and antihepatoprotective activities.[23]

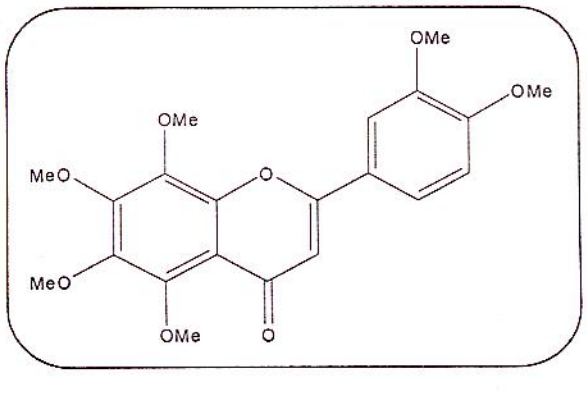
4.3.1. Structures of some antioxidants from plants.



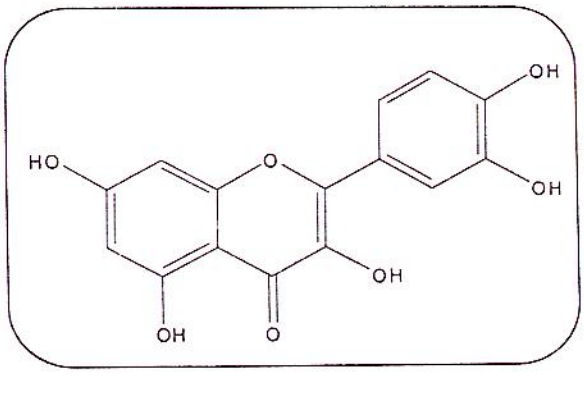
Epigallocatechin gallate **(95)**



Tangatitin **(96)**



Nobiletin **(97)**



Quercetin **(98)**

4.4. Antimicrobial activity

In the last few years, probably an increase drug resistance has been observed in human pathogenic organisms as well as the appearance of undesirable side effects of certain antibiotics and the emergence of previously uncommon infections.[24,25] Antimicrobial properties have been reported more frequently in a wide range of plant extracts and natural products in an attempt to discover new chemical classes of antibiotics that could resolve these problems. A detailed review was published describing the antifungal properties of natural products.[26] This include the cyclic lipopeptide echinocandins from *Aspergillus* sp., pneumocandins produced by directed-biosynthesis using the fungus *Zalerion arhohcola,*[27] the cyclopeptide aureobasidins from the fungus *Aureobasidiiim pultutans,*[26] the pradimicins from

Actiitomadiira hibisca[28] and the vankomycins from *Streptoniyces letnlae.*[26] New antibacterial agents were also discussed in this review[29] which clearly outlined the important classes of known antibiotics in providing templates for chemical modification and listed novel targets against which natural products screening might be productive. Promising recent developments in this field include semi-synthetic glycopeptides antibiotics with greatly improved potency against vancomycin-resistant enterococci and ziracin, a novel oligosaccharide from *Microiuonospora carhonacea* var. *africann* which has good activity against drug-resistant bacteria and which is undergoing phase 1 clinical studies.[30] Screening plants for antiviral activities has resulted in the discovery of agents such as michellamine B and SP-303. Michellamine B inhibits HIV-induced cell killing by at least two distinct mechanisms and is currently in preclinical phases.[31] SP-303 is a plant flavonoid discovered by an ethanobotanical approach which is currently being evaluated for use against influenza virus. Some recent studies have revealed the antimicrobial activity of various Cassia species.[32-33]

4.5. Pharmacological importance of the plant species of the genus *Cassia.* (See Section 1.4 and 4.3)

Chapter 5

RESULTS AND DISCUSSION (Part B)

5.1. Biological screening of the selected species of genus *Cassia* i.e *Cassia artemisioides* and *Cassia nemophila* and the pure isolated compounds.

Different solvents soluble fractions of the various parts of *Cassia artemisioides* and *Cassia nemophila* were subjected to screening for their possible antioxidant and antimicrobial activities. The activities of the pure compounds isolated from *Cassia artemisioides* and *Cassia nemophila* were also studied. The DPPH radicals scavenging power were determined by measuring the decay in absorbance at 517 nm its radical reduction.[34] Herein we report the first study on the antioxidant and antimicrobial potential of the various extracts and fractions of the two plants. The objectives of this study were to explore the biological and medicinal value of the extract/fractions of stem bark, flowers, pods, seeds and roots bark of *Cassia artemisioides* and *Cassia nemophila*.

5.2. DPPH radical-scavenging activity

Phenolic compounds are generally showing good antioxidant activity. The antioxidants can be determined in a number of methods. The DPPH radical scavenging is the straight forward and easy method for determining the antioxidant activity. 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) is a stable free radical having purple color with adsorption band at 516-527 nm in the UV-Vis. It vanishs its colour when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow which can be determind spectrophotometrically.[17] The radical scavenging power of pure isolated compounds from *Cassia* species, and standards were studied. **(Fig. 5.1 & Table 5.1)**. Excellent scavenging activities were shown by flavonoids **(48, 61** and **64)**. Kaempferol **(61)** showed excellent radical scavenging activity which has already been studied for its

antioxidant activity while the activity of 3′,4′,5,7-tetrahydroxyflavone (**64**) and a new flavone are studied for the first time.

All the three flavonoids were found potent even at the lower concentration (20 *u*g/ml) and at higher concentration (100*u*g/ml) nearly touched the lower end of the activity shown by the standards. Generally flavonoids are considered good radical scavenger.[35-39] Second class of isolated compounds evaluated for DPPH radical scavenging activity are cinnmic acid derivatives (**54, 55** and **70**). A very good activity was shown by caffeates (**54, 55** and **70**) isolated from the stem bark of *Cassia artemisioides* (73.11 % at 100 *u*g/mL). The highest % DDPH activity up to 73.11 % at 100 ug/mL (**Table 5.1**) and exhibited IC_{50} up to 35-61 µg/mL (**Table 5.2**) were observed in caffeates (**54, 55** and **70**). The significant DPPH radical scavenging activities of caffeates against DPPH radical were due the number of OH groups, greater the number of hydroxy groups high will be its antioxidant activity.[40] The activities of caffeates were found closed to the literature values reported.[41]

Anthraquinone were also evaluated for DPPH free radical scavenging activity (% RSA). Generally anthraquinone (**57-60, 72** and **73**) showed weak scavenging activity. Among anthraquinones only 2,6,8-trihydroxy-1-methoxy-3-methylanthraquinone (**63**) showed comparatively good activity. The activity of 2,6,8-trihydroxy-1-methoxy-3-methylanthraquinone (**63**) may be attributed to the greater number of hydroxy groups. Several studies concerning the relationship between the phenolic nature of flavonoids, lignans, phenol and anthraquinone have been conducted.[42] Hung et al. studied the relationship of anthraquinone derivatives and antioxidant activity,[42] and concluded that hydroxy group either at meta or para position are responsible for antioxidant activity.[42] It has also been reported that not only hydroxy group but carbonyl group of anthraquinone skeleton is responsible for antioxidant potential.[19]

Table. 5.1. Antioxidant activities (% DPPH Radical Scavenging Activity) of compounds isolated from various parts of *Cassia artemisioides.*

Compound	% DPPH Radical Scavenging Activity				
	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml
(48)	89.20	92.17	94.23	96.50	97.60
(54)	20.50	32.23	51.40	62.52	71.12
(55)	21.50	33.23	55.41	68.21	75.14
(56)	3.7	5.24	7.5	9.53	10.80
(57)	5.1	8.2	9.5	10.5	15.4
(58)	4.50	6.57	7.28	10.53	12.43
(59)	10.40	12.23	15.41	22.2	35.9
(60)	5.5	7.2	9.6	10.5	12.7
(61)	86.3	93.11	95.21	96.10	97.14
(63)	12.42	15.10	18.71	22.6	40.3
(64)	88.2	92.15	94.1	95.3	96.2
(67)	4.7	6.6	7.8	8.9	10.6
(70)	23.14	35.24	53.43	65.41	73.11
(72)	3.40	5.6	7.2	8.3	9.7
(78)	3.42	5.6	7.4	8.7	9.6

The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations.

Table 5.2. EC_{50} values[a] (*u*g/mL) of compounds isolated from various parts of *Cassia artemisioides*

Compound	IC_{50}
7-(3,4-dihydroxyphenoxy)-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-chromen-4-one (**48**)	3.5 µ g/mL
Undecyl caffeate (**54**)	61.1 µ g/mL
Dotricontanyl caffeate (**55**)	55.21 µ g/mL
1,1′-Dihydroxy-3,3′-dimethyl-8,8′-dimethoxy-6,6′-bianthraquinone (**56**)	-
1,8-Dihydroxy-3-methylanthraquinone (**57**)	-
1,8-Dihydroxy-6-methoxy-3-methylanthraquinone (**58**)	-
1,6,8-Trihydroxy-3-methylanthraquinone (**59**)	150.10 µ g/mL
1,6-Dihydroxy-8-methoxy-3-methylanthraquinone (**60**)	-
3,5,7,4′-Tetrahydroxyflavone (**61**)	3.20 µ g/mL
2,6,8-Trihydroxy-1-methoxy-3-methylanthraquinone (**63**)	130.23 µ g/mL
3′,4′,5,7-Tetrahydroxy flavone (**64**)	3.12µ g/mL
1,6-Dihydroxy-3-methylanthraquinone-8-*O*-*β*-*D*-glycoside (**67**)	-
Hexacosanyl caffeate (**70**)	58.10 µ g/mL
1,8-Dihydroxy-3,7-dimethyl-8-methoxyanthraquinone (**72**)	-
1-Hydroxy-8-methoxy-3-methylanthraquinone (**78**)	-

[a] IC_{50} (mg/mL): Effective concentration at which 50% of DPPH radicals are scavenged.

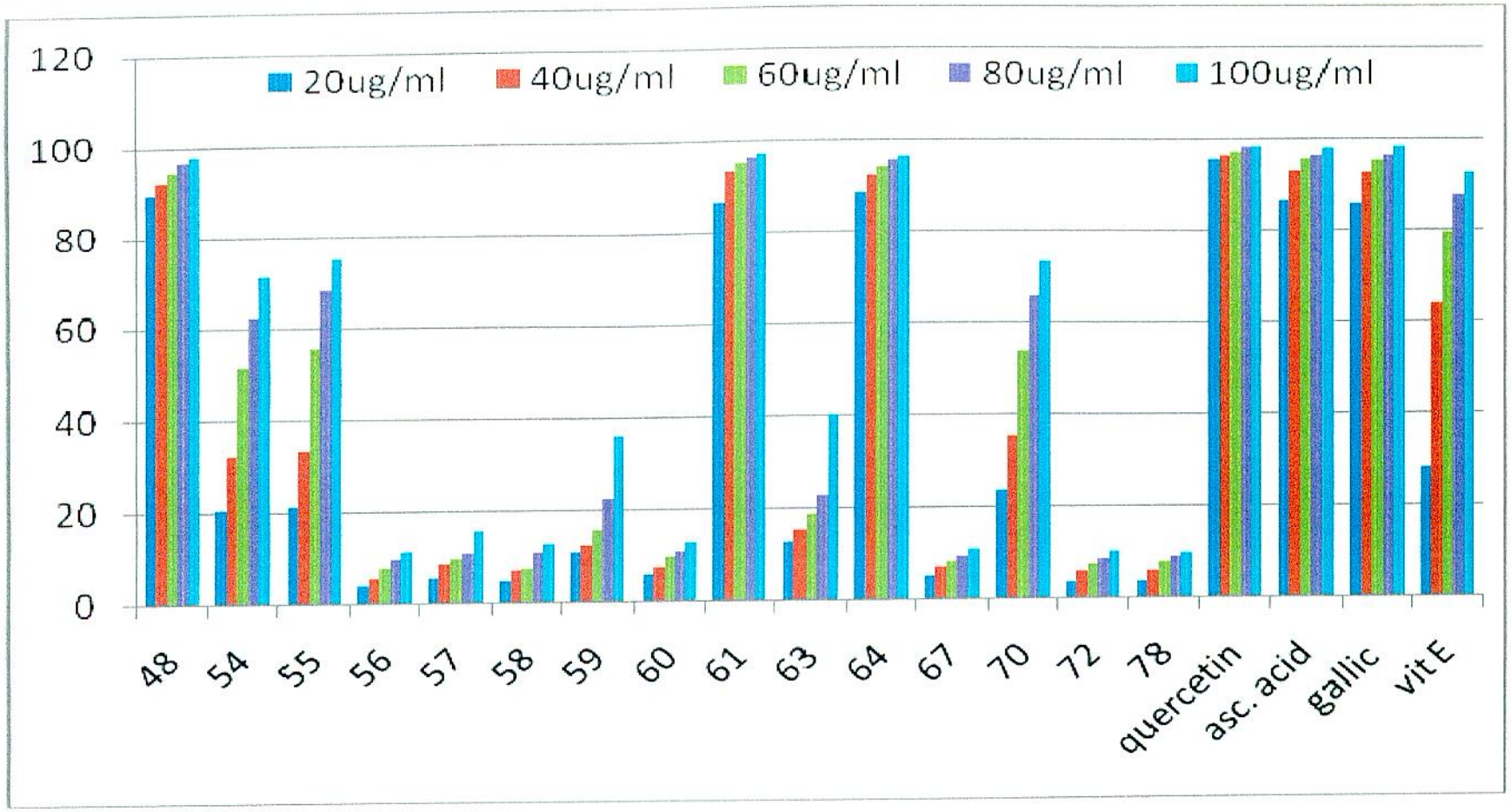


Fig. 5.1. DPPH radical scavenging activity of pure components isolated from various parts of *Cassia artemisioides*

5.3. Antibacterial activity

The antibacterial potential of various extracts from various parts of the two *Cassia* species and pure compounds have been studied (**Table 5.3**). All the analytes showed antibacterial activities against the tested microorganisms to different extent having zone of inhibition in the range between 08 and 28 mm. The significant difference ($P < 0.05$) was found in the activities of various fractions (**F1, F2, F3, F4 and F5**) against all the tested strains. Ethanol fraction (**F1**) was found to be the most active fraction obtained from the flowers of *Cassia nemophila* against *Enterobacter aerogenes* and *Pseudomonas aeruginons*. The methanolic (**F1**) fraction obtained from the pod of *Cassia artemisioides* exhibited considerable activities against *Escherichia coli* and *Enterobacter aerogenes*. Fraction obtained from the stem heart wood, bark, flowers and pods of *Cassia artemisioides* showed good activities against *Enterobacter aerogenes* and *Pseudomonas aeruginons*. Ethyl acetate (**F2**) and dichloromethane (**F3**) fractions of the bark and flowers of both species were found active against *Pseudomonas aeruginons*, *Salmonella typhi* and *Escherichia coli*. n-Hexane (**F4**) and dichloromethane (**F3**) fractions of all the parts showed moderate activity against *Proteus vulgaris* and *Escherichia coli*.

All other fractions obtained from various parts of both the plants showed weak activities against the tested strains. The pure compounds which were isolated in enough quantities were also evaluated for their antibacterial activities. Among the tested compounds 2,6,8-trihydroxy-1-methoxy-3-methyl anthraquinone (**63**) showed good activity against *Enterobacter aerogenes* and *Staphylococcus aureus*. All the bacterial strain in this study were found sensitive to streptomycin specially *Staphylococcus aureus* and *Pseudomonas aeruginosa* was the most sensitive (inhibition zone values of 31.2 and 32.23 mm respectively). *Escherichia coli* and *Salmonella typhi* were resistant to erythromycin.

Table 5.3. Antibacterial activities (diameter of growth inhibition zone) of ethanol **(F1)**, ethyl acetate **(F2)**, dichloromethane **(F3)** and n-hexaane **(4)** extracts (10 mg/ml) of two *Cassia* species.

Plant species	Bacteriaa tested zone of inhibition (mm)						
	Fractions/ Standards	*Ec*	*Sa*	Ea	*St*	*Pv*	*Pa*
Cassia artemisioides Flowers	F1	10.4	18.7	22.3	10.8	12.7	16.3
	F2	0	12.4	17.3	11.5	12.2	15.6
	F3	11.6	17.5	12.2	12.4	11.8	19.2
	F4	11.2	19.1	11.1	10.5	11.3	15.5
Cassia artemisioides Stem Bark	F1	0.0	20.4	15.6	12.8	18.5	12.4
	F2	11.1	16.6	15.5	11.2	14.1	20.4
	F3	11.2	19.2	11.1	10.8	11.2	17.2
	F4	11.5	11.2	17.3	12.7	12.5	11.4
Cassia artemisioides Roots bark	F1	12.3	17.1	11.7	14.3	13.2	21.3
	F2	14.2	13.4	16.2	13.4	14.6	15.7
	F3	15.1	16.2	13.4	15.8	17.2	15.84
	F4	11.7	12.6	11.4	13.6	10.4	11.1
Cassia artemisioides Pods	F1	19.3	17.2	24.1	12.3	11.5	16.5
	F2	10.2	10.3	11.2	11.1	12.6	13.7
	F3	11.2	14.3	18.2	11.3	17.3	16.7
	F4	10.4	10.2	18.0	10.4	17.5	11.4
Cassia artemisioides Stem heart wood	F1	17.6	31.2	17.4	16.7	12.3	15.2
	F2	23.1	12.6	11.5	12.3	14.5	13.4
	F3	0.0	0.0	11.8	0.0	11.2	12.2
	F4	14.3	13.2	18.4	10.2	10.5	14.2
Cassia artemisioides Leaves	F1	13.6	16.3	14.5	11.6	12.5	13.7
	F2	15.4	0.0	13.6	14.8	13.9	14.6
	F3	14.0	11.6	12.3	14.6	11.4	13.8
	F4	0.0	12.6	10.6	11.4	13.6	11.2
Cassia nemophila Flowers	F1	12.4	15.7	28.2	14.6	12.9	21.6
	F2	0.0	11.4	16.7	0.0	12.4	12.6
	F3	20.3	16.2	14.6	16.4	12.6	14.7
	F4	14.1	13.1	12.3	13.2	11.2	12.5

Pure compound	**(48)**	15.1	14.5	13.6	12.4	11.2	14.2
	(57)	9.1	11.3	13.1	12.4	13.6	12.4
	(58)	8.2	15.1	12.9	11.2	10.2	11.2
	(59)	10.12	13.2	15.2	10.8	11.3	10.9
	(60)	14.2	13.1	12.4	14.7	9.4	15.1
	(61)	15.7	13.1	12.4	13.8	12.2	11.2
	(63)	15.5	14.2	13.2	12.1	10.3	14.6
	(64)	12.2	13.2	14.1	12.6	11.4	13.8
Standard	Streptomycin	30.3	31.2	25.3	30.2	28.5	32.1

[a] Bacteria: *Sa, Staphylococcus aureus; Pa, Pseudomonas aeruginosa;St, Salmonella typhi;Ec, Escherichia coli;Pv, Proteus vulgaris;Ea, Enterobacter aerogenes*

Chapter 6

EXPERIMENTAL (Part B)

6.1. Plant material

The collected plant species belonging to famaily Leguminosae genus *Cassia* authenticated by Dr. S.I. Ali, University of Karachi, collected from Peshawar and Nowshera, NWFP Pakistan. Voucher specimens have been retained at the herbarium, Department of Botany, Jehanzeb College Swat.

6.2. Preparation of extracts and fractions

The collected species were extracted with ethanol by soxhlet apparatus (for antioxidant and antimicrobial activities). The respective extracts were filtered and dried under reduce pressure below 50°C. Various fractions were obtained by solvent extraction (**Fig. 6.1**).

6.3. Antioxidant activities

6.3.1. Chemicals

All the chemicals used were of analytical grade, Gallic acid and quecetein were purchased from Acros (USA), 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) from Fluka (Germany) , α-tocopherol from E.Merck (Germany), trichloro acetic acid from Riedal–deHaen (Germany) sodium phosphate from Panreac (Spain), ammonium molybdate from ABSCO (UK Spectra were recorded on a SP-3000 PLUS Spectrophotometer (Optima, Japan) and the commercial solvents used for extraction were re-distilled.

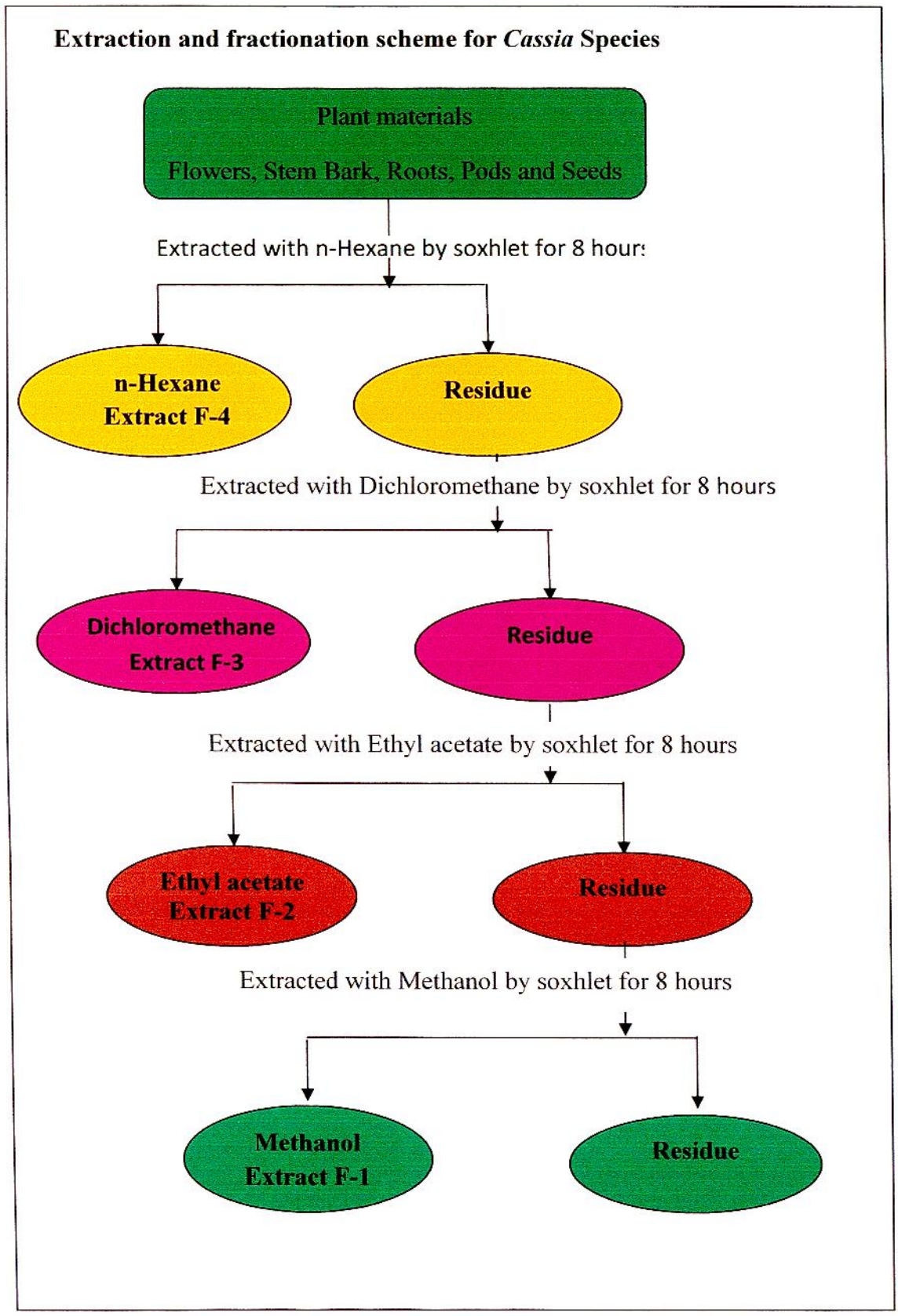


Fig. 6.1. General scheme of the plants material extraction and solvent fractionation for antioxidant and antimicrobial activities.

6.3.2. DPPH radical-scavenging activity

The hydrogen atom or electron donation abilities of the corresponding extracts/fractions and standards were measured from the bleaching of the purple-coloured methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH.) Experiments were carried out according to the method of Blois[43,44] with a slight modification. Briefly, a 1mM solution of DPPH radical solution in methanol was prepared and 1ml of this solution was mixed with 3ml of sample solutions in ethanol (containing 20-100*ug*) and control (without sample). After 30 min, the absorbance at 517 nm was measured. An increase of the DPPH radical-scavenging activity is correlated with decreasing of the DPPH solution absorbance. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated as follow.

$$\% \text{ RSA} = \frac{\text{Control absorbance - Sample absorbance}}{\text{Control absorbance}} \times 100$$

The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations. The EC_{50} (extract concentration showing 50% inhibition) was calculated from the graph of % RSA against extract concentration and quercetin, ascorbic acid, Gallic acid and α-tocopherol used as standards.

6.4. Antimicrobial activities

6.4.1. Test organisms for bioassays

Gram-positive bacteria; *Staphylococcus aureus*, Gram-ive bacteria; *Salmonella typhi*, *Pseudomonas aeruginosa*, *Proteus vulgaris*,, *Escherichia coli*, *Enterobacter aerogenes*. Pure bacterial cultures (clinical isolates) were obtained from Centre of Biotechnology and Microbiology University of Peshawar. Different bacterial strains were kept on nutrient agar (NA, Oxide, UK). Bacterial cultures were prepared by inoculation of two to three colonies into a tube containing 20 ml nutrient broth (Oxide, UK) and grown overnight at 37 °C. Fresh cultures suspensions at McFarland 0.5 density (10^8 CFU/mL) were used for inoculation.

6.4.2. Antibacterial screening

The antibacterial tests were performed using agar-well diffusion assay.[45-47] Petri dishes were prepared from the sterile nutrient agar (Oxide, UK). Standardized bacterial cultures were evenly spread onto the surface of the agar dishes with the help of sterile swab sticks. Five wells (6 mm diameter) were bored in each plate with sterile borer. 100 μL of ethanol, ethyl acetate, dichloromethane and n-hexane extracts (10 mg/mL) of the flowers, stem bark, pods, seeds and roots of the investigated plants were added in each well. 100 μL of absolute alcohol per well was used as a negative and control. For positive control 100 μL of Streptomycin (2 mg/mL) was used. The agar plates were then covered with lids and incubated at 37 °C for 24 hours. The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the wells. The size of the zones of inhibition was measured and the antibacterial activity expressed in terms of the average diameter of the zone inhibition in millimeters. The absence of a zone inhibition was interpreted as the absence of activity.

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