

**PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION ON  
*BERBERIS JAESCHKEANA* SCHNEID VAR. *JAESCHKEANA* AND  
*BERBERIS GLAUCOCARPA* STAPF**



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UNIVERSITY OF PESHAWAR, PAKISTAN  
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**BY  
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**Submitted in partial fulfillment of the requirements for the degree of  
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**INSTITUTE OF CHEMICAL SCIENCES  
UNIVERSITY OF PESHAWAR, PAKISTAN  
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**“Start from the name of ALLAH, the most beneficent, the most merciful.”**

## DECLARATION

This is to certify that this dissertation prepared by MUHAMMAD ALAMZEB entitled as “Phytochemical and biological investigation on *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf.” is accepted in the present form by the Institute of Chemical Sciences, University of Peshawar as fulfilling this part of the requirements for the degree of Doctor of Philosophy in Chemistry.

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### EXTERNAL EXAMINER

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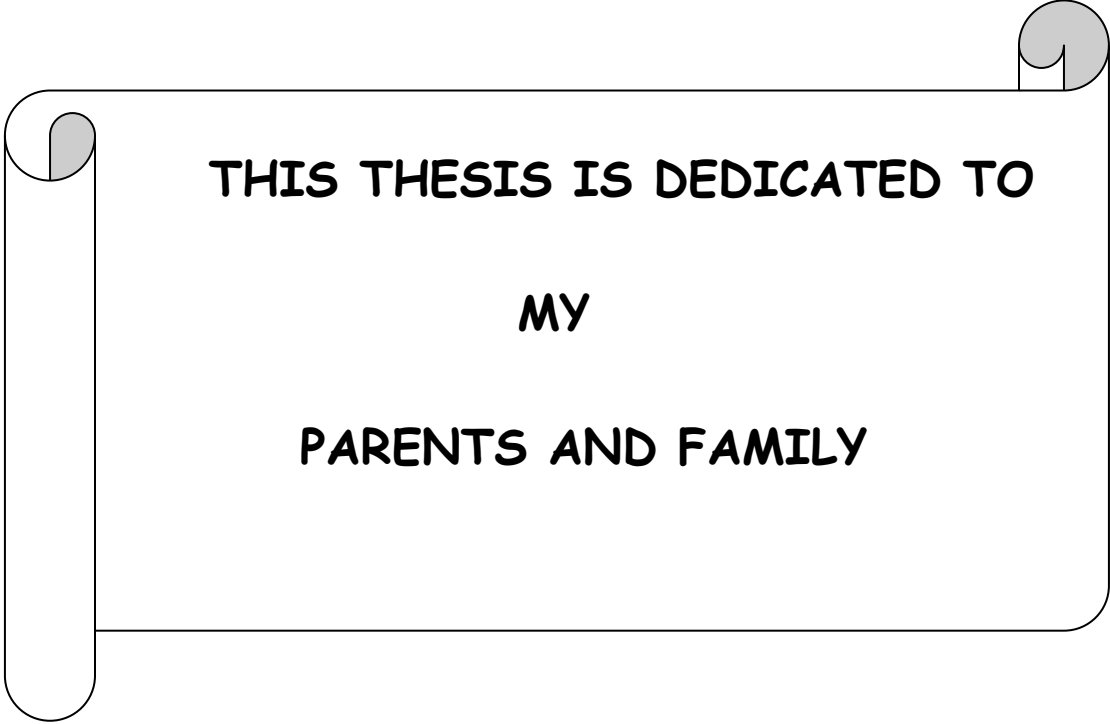
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**THIS THESIS IS DEDICATED TO**

**MY**

**PARENTS AND FAMILY**

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## List of Abbreviations

|                     |                                                           |
|---------------------|-----------------------------------------------------------|
| $^{13}\text{C}$ NMR | Carbon-13 Nuclear Magnetic Resonance Spectroscopy         |
| $^1\text{H}$ NMR    | Proton Nuclear Magnetic Resonance Spectroscopy            |
| 1D NMR              | One Dimensional Nuclear Magnetic Resonance Spectroscopy   |
| 2D NMR              | Two Dimensional Nuclear Magnetic Resonance Spectroscopy   |
| st                  | Stretching                                                |
| COSY                | Correlation Spectroscopy                                  |
| NOESY               | Nuclear Overhauser Effect Spectroscopy                    |
| MS                  | Mass Spectroscopy                                         |
| EIMS                | Electron Mass Impact Spectroscopy                         |
| ESI                 | Electrospray Ionization                                   |
| ESI-MS              | Electrospray Ionization Mass Spectroscopy                 |
| HR-ESI-MS           | High Resolution Electrospray Ionization Mass Spectroscopy |
| UV-Vis              | Ultraviolet-Visible Spectroscopy                          |
| IR                  | Infrared                                                  |
| NMR                 | Nuclear Magnetic Resonance                                |

|             |                                                      |
|-------------|------------------------------------------------------|
| $\bar{\nu}$ | Wave number                                          |
| ppm         | Parts Per Million                                    |
| m/z         | Mass to Charge ratio                                 |
| mp          | Melting Point                                        |
| MIC         | Minimum Inhibitory Concentration                     |
| $J$         | Coupling Constant                                    |
| Hz          | Hertz                                                |
| HMBC        | Heteronuclear Multiple Bond Correlation              |
| HMQC        | Heteronuclear Multiple Quantum Coherence             |
| HSQC        | Heteronuclear Single Quantum Coherence               |
| FAB-MS      | Fast Atomic Bombardment Mass Spectrometry            |
| DEPT        | Distortion less Enhancement by Polarization Transfer |

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## ABSTRACT

This dissertation consists of two parts, **Part A** and **Part B**. Part A describes the phytochemical investigations of *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf (Berberidaceae). A total of twenty two compounds (**111-132**) were isolated from the two plant species. These include four new compounds namely; 10-hydroxy chondrofoline (**111**), 8,12,12a-trihydroxy-11,13a-berberin-dione (**112**) 10-methoxy chondrofoline (**113**) and docosyl-2-hydroxytetracosanoate (**114**) and eighteen new source compounds from these plant species.

The new source compounds are: berberine (**115**), palmatine (**116**), columbamine (**117**), pseudoberberine (**118**), gallic acid (**119**),  $\beta$ -sitosterol (**120**), 8-hydroxy-7,8-dihydroberberine (**121**), docosanoic acid (**122**), methyl gallate (**123**), oxyacanthine (**124**), jatrorrhizine (**125**), 8-oxoberberine (**126**),  $\beta$ -sitosterol glucoside (**127**), 8-trichloromethyldihydroberberine (**128**), pseudopalmatine (**129**), syringic acid (**130**), syringaresinol (**131**) and stigmasterol (**132**). The structure of all the isolated compounds were determined by various spectroscopic techniques like  $^1\text{H}$ NMR,  $^{13}\text{C}$ NMR, HMBC, HSQC, NOESY, COSY, DEPT, EIMS, ESIMS and HRESIMS. The structures of the new source compounds were confirmed by comparing their spectral data with the literature.

Part B deal with the biological activities of crude extracts, fractions and compounds. Most of the compounds and fractions were evaluated for their anti-microbial, anti-trypanosomal, anti-fungal and anti-diabetic properties. Anti-microbial properties of fractions and compounds were determined using Alamar blue <sup>TM</sup> 96-well plate and agar

well diffusion method. Anti-fungal properties were evaluated using the tube dilution method. The anti-diabetic properties of fraction and compounds were evaluated by Protein Tyrosine Phosphatase 1B (**PTP1B**) enzyme method. Berberine (**115**), 8-trichloromethyldihydroberberine (**128**), jatrorrhizine (**125**), columbamine (**117**), and syringaresinol (**131**) and the fractions from which these compounds were isolated showed good anti-microbial properties. Similarly berberine (**115**), berberine chloroform (**128**), 8-oxoberberine (**126**), 10-hydroxy chondrofoline (**111**), syringaresinol (**131**) and their fractions showed good anti-trypanosomal properties.

Most of the isolated compounds and fractions were also tested for their anti-fungal and anti-diabetic properties but none of them displayed significant anti-fungal or anti-diabetic properties. Some of them were moderately active.

## FORMAT OF THE THESIS

This thesis consists of two parts **Part A** and **Part B**. **Part A** deals with the isolation, purification and structure elucidation of compounds isolated from *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf.

**Part B** deals with the bioassay studies of the crude extracts, sub-fractions and compounds from *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf.

### **1.1. General.**

Medicinal plants are used for the treatment of many diseases from the beginning of the human civilization. Recently as the pathogens have developed resistance against various antibiotics. So the use of traditional medicines as alternative medicines have reopened the door for research on medicinal plants and their bioactive components.<sup>1</sup>

Almost all modern medicines have their roots in natural products, particularly through traditional medicine. Such medicines are still practised in most of the developing countries. In Pakistan more than 70 % of the population in the rural areas still depend on traditional medicines obtained from the plants.<sup>2</sup> The demand for plant extracts by cosmetics, food and pharmaceutical industries are increasing day by day, demonstrating that systematic research on medicinal plants is important in order to find effective drugs for the treatment of various diseases.<sup>3</sup> In the developing countries the use of herbal medicines seems to be more popular for the treatment of minor ailments due to its low cost.<sup>4</sup> About 80 % population of the third world countries depend on plant based traditional medicines for their health care. Higher plants have the ability to produce a large variety of natural products with diverse activities.<sup>5,6</sup>

### **1.2. Natural products as a source of medicines.**

Since the beginning of the human civilization, natural products are playing an important role in the health care and prevention of many diseases. The ancient civilizations of China, India and North Africa have written evidences of treating various diseases using natural products.<sup>7</sup> About one hundred anticancer agents have been developed from 1981

to 2006, twenty five among them are natural product derivatives, eighteen are natural products mimics and nine of them are pure compounds.<sup>8</sup> A few examples of drugs obtained from plants are: morphine and codeine from *Papaver somniferum*, quinine from *Cinchona* spp, atropine from *Atropa belladonna* and vincristine and vinblastine from *Catharanthus roseus*. About 60 % of the anti-tumor drugs in the market or under clinical trials are derived from plants.<sup>9</sup> A large number of such drugs cannot be synthesized economically so they are still obtained from the wild and cultivated medicinal plants.<sup>10</sup> Compounds like forskolin, cannabinoids, muscarine, yohimbine, phorbol esters, colchicine and physostigmine have been isolated from plants and have shown pharmacological and biochemical properties.<sup>11</sup>

Recently there has been an increased in the therapeutic uses of natural products of plant origin. This increasing interest has several reasons for example conventional medicines may be inefficient, majority of population around the world does not have access to conventional medicines and abusive use of allopathic drugs has several side effects. Higher plants are still poorly explored to find new drugs. Higher plants have 250,000-500,000 species but only around 5000 of these have been phytochemically investigated. An even smaller percentage has been properly explored for their pharmacological properties. In most of the cases only pharmacological screening have been carried out.<sup>12</sup>

From 1998 to 2004 a total of 21 natural products and natural products derived drugs were launched in USA, Japan and Europe which included three natural products, eight natural products derived drugs and ten semi-synthetic natural products (**Appendix I**),<sup>13</sup> similarly from 2005 to 2010 a total of nineteen natural products based drugs were approved for

marketing which include seven natural products, two natural products derivatives and ten semi synthetic natural products (**Appendix II**).<sup>13</sup>

Alkaloids are pharmacologically active and isoquinoline alkaloids are abundant in the family Berberidaceae, the topic of the current investigation.

### **1.3. Family Berberidaceae (Family of the current study).**

Family Berberidaceae has four genera and 500 species, mostly found in tropical mountains, South America and Temperate regions. In Pakistan it is represented by three genera and 22 species. Plants of the family Berberidaceae are either shrubs or perennial herbs having reddish-brown to pale branches. The branches may be verruculose, armed or unarmed. Leaves may be simple or compound crowded on short stems. Inflorescence may be cymose, fasciculate or racemose. Flowers are regular, bisexual and mostly yellow. Stamens 4-6, often in 2 whorls of 3 each and usually shorter than petals. Ovary is oblong to suborbicular having several ovules on basal or lateral placenta. Fruit is a berry and seeds are large with copious endosperm. The three genera of Berberidaceae in Pakistan are *Berberis* Linn (subject of the current study), *Epimedium* Linn and *Mahonia* Nutt.<sup>14</sup>

#### **1.3.1. The genus *Berberis* Linn (Genus of the current study).**

*Berberis* Linn is a large genus with 400 species, found in Asia, North Africa, Mediterranean region and America. In Pakistan *Berberis* has 20 species and are found in the mountainous parts of the country. *Berberis* species are evergreen and armed shrubs having yellow wood. Stem and branches are reddish-brown to pale or whitish. On long

shoots are usually modified into 1-3-7 spines while on dwarf shoots normal and crowded. Inflorescence is often short. Flowers are yellow to orange and berries usually red or bluish-black.<sup>15</sup>

### **1.3.2. *Berberis jaeschkeana* Schneid var. *jaeschkeana* (Species of the current study).**

*Berberis jaeschkeana* Schneid var. *jaeschkeana* is a shrub and is around 1m tall. Its stem is stout and deciduous having slightly reddish to yellow-brown color. Spines are 3 fid and 10-15 mm long. Leaves are 10-23 mm long, 5-8 mm broad and generally green on both sides. Inflorescence is umbellate or sub-umbellate. Flowers are 9-12 mm across; pedicels are 6-9 mm long. *Berberis jaeschkeana* Schneid var. *jaeschkeana* is distributed in Kashmir, Pakistan and N.W. Himalaya to Nepal.<sup>16</sup>

### **1.3.3. *Berberis Glaucocarpa* Stapf (Species of the current study).**

It is a shrub 3-4 m tall; stem is glabrous and pale yellow. Spines are simple and rarely 3 fid having a length of 5-10 mm. Leaves are 3-7 cm long and 1-3 cm broad. Flowers 10-12 mm across and pale yellow, pedicels 5-10 mm long in fruit, berries oblong and globose, distributed in W. Himalaya.<sup>17</sup>

## **1.4. Literature review of the genus *Berberis* Linn.**

### **1.4.1. Phytoconstituents reported.**

The genus *Berberis* has a large number of medicinal uses in the traditional systems across the world. The phytochemical investigations on the genus revealed a number of classes of compounds including alkaloids, anthocyanins, flavonoids, carotenoids,

phenolic compounds and vitamins. Detailed review (From 1991 to 2010) listing the compounds isolated from the genus *Berberis* so far is given in **Appendix III**.

While biosynthetic pathways of a number of classes of compounds of *Berberis* is as follows.

## **1.5. Biosynthesis.**

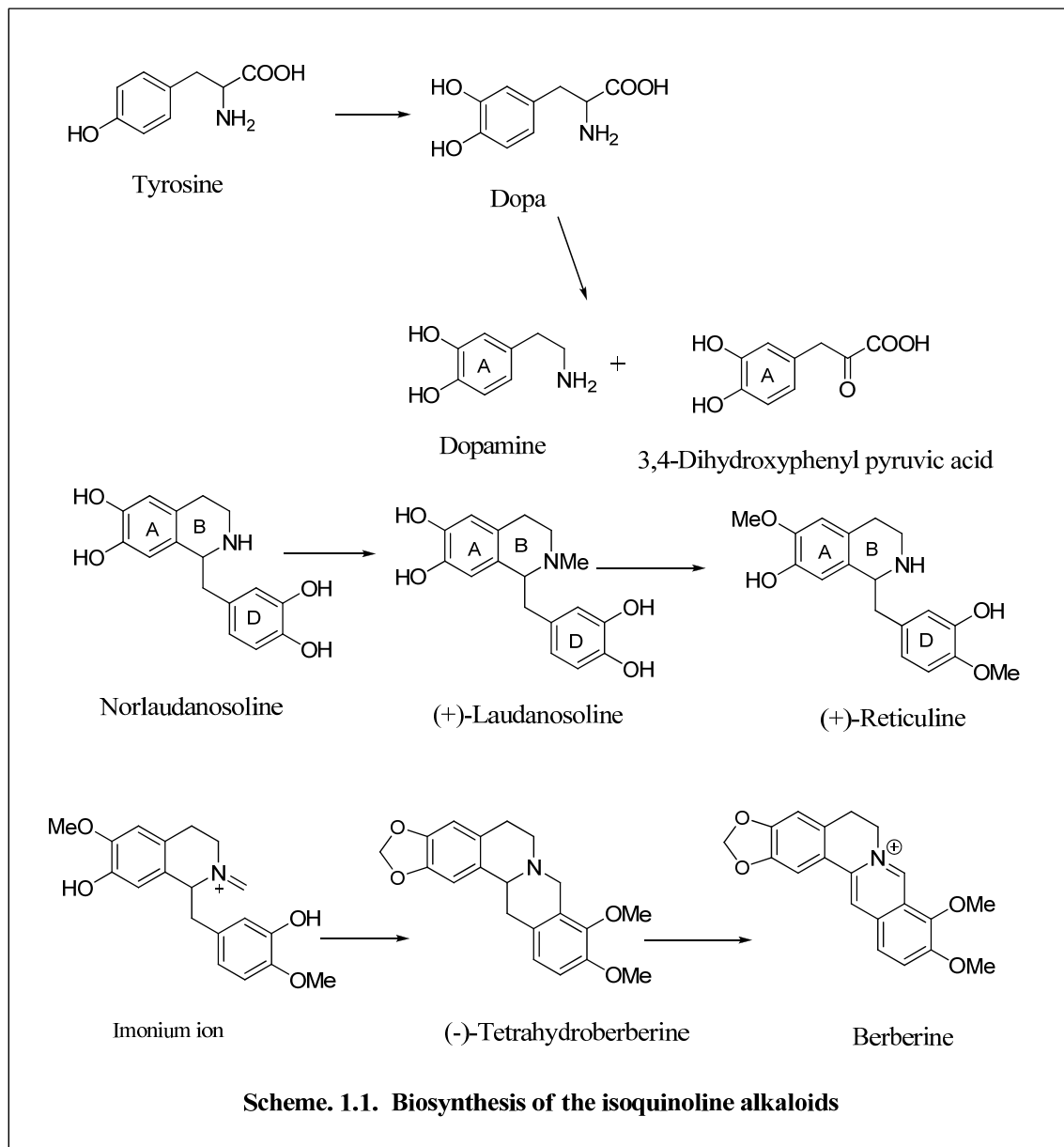
### **1.5.1. Biosynthesis of the isoquinoline alkaloids (berberine).<sup>25</sup>**

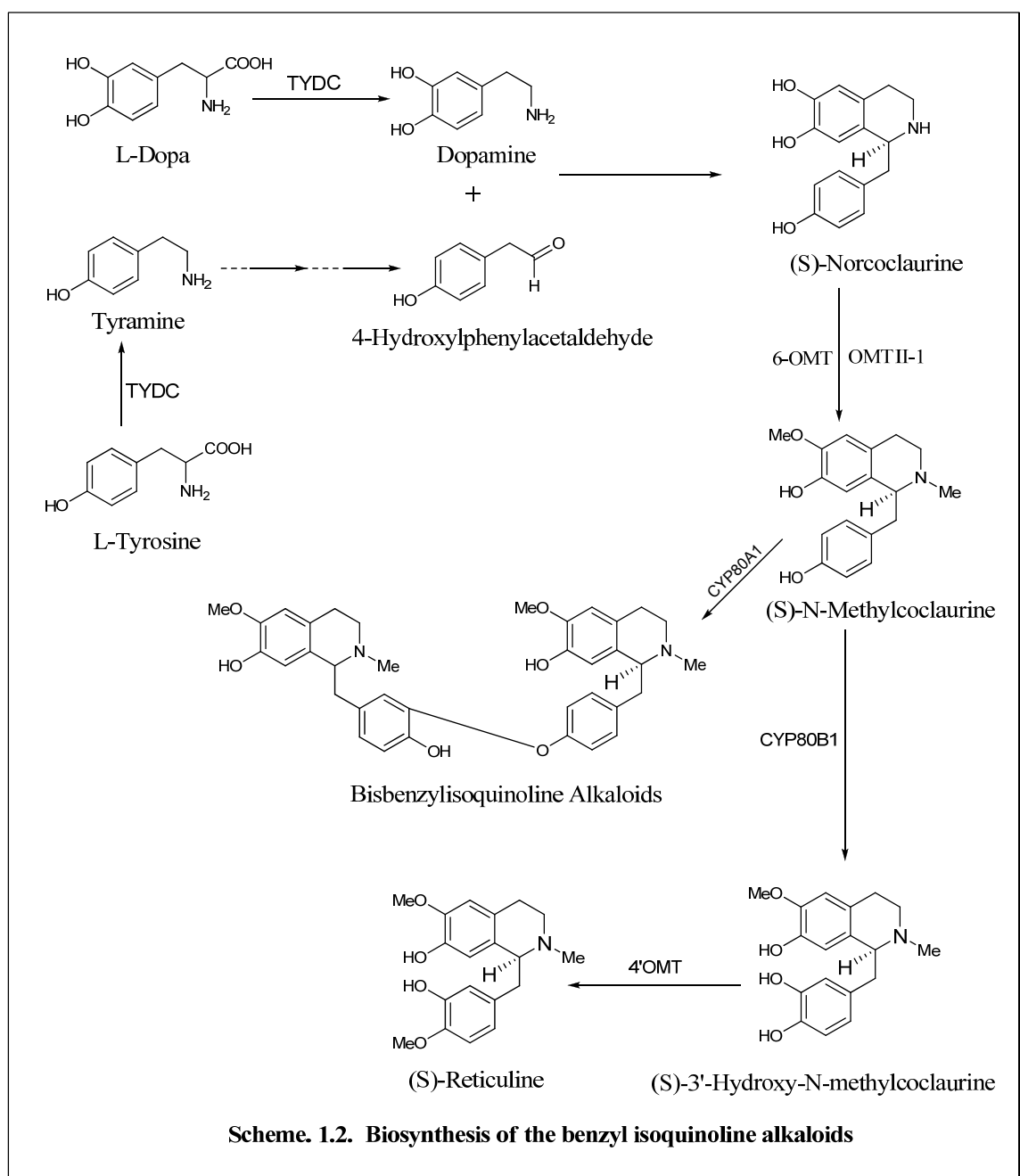
Tyrosine is believed to be an efficient precursor of berberine (an example of isoquinoline alkaloids). Tyrosine is incorporated into the ring A, part of the ring B, also into the ring C and part of the ring D. Tyrosine produces two intermediates during biosynthesis. One of the two intermediates is dopamine. Although all the steps involved in the biosynthesis of berberine are not yet fully established, however the general scheme for the biosynthesis of berberine is given here (**Scheme. 1.1**).

### **1.5.2. Biosynthesis of the benzyloquinoline alkaloids.<sup>26</sup>**

Benzyloquinoline alkaloids are a large group of alkaloids having more the 2500 defined structures. The decarboxylation, orthohydroxylation and deamination reactions convert tyrosine to dopamine and 4-hydroxyphenylacetaldehyde. The only enzyme for this step is L-amino acid decarboxylase (TYDC). This enzyme converts tyrosine and dopa to their corresponding amines. Norcoclaurine synthase (NCS) condense dopamine and 4-hydroxyphenylacetaldehyde to produce trihydroxy benzyloquinoline alkaloid (s)-norcoclaurine which is the central precursor for all benzyloquinoline alkaloids in plants.

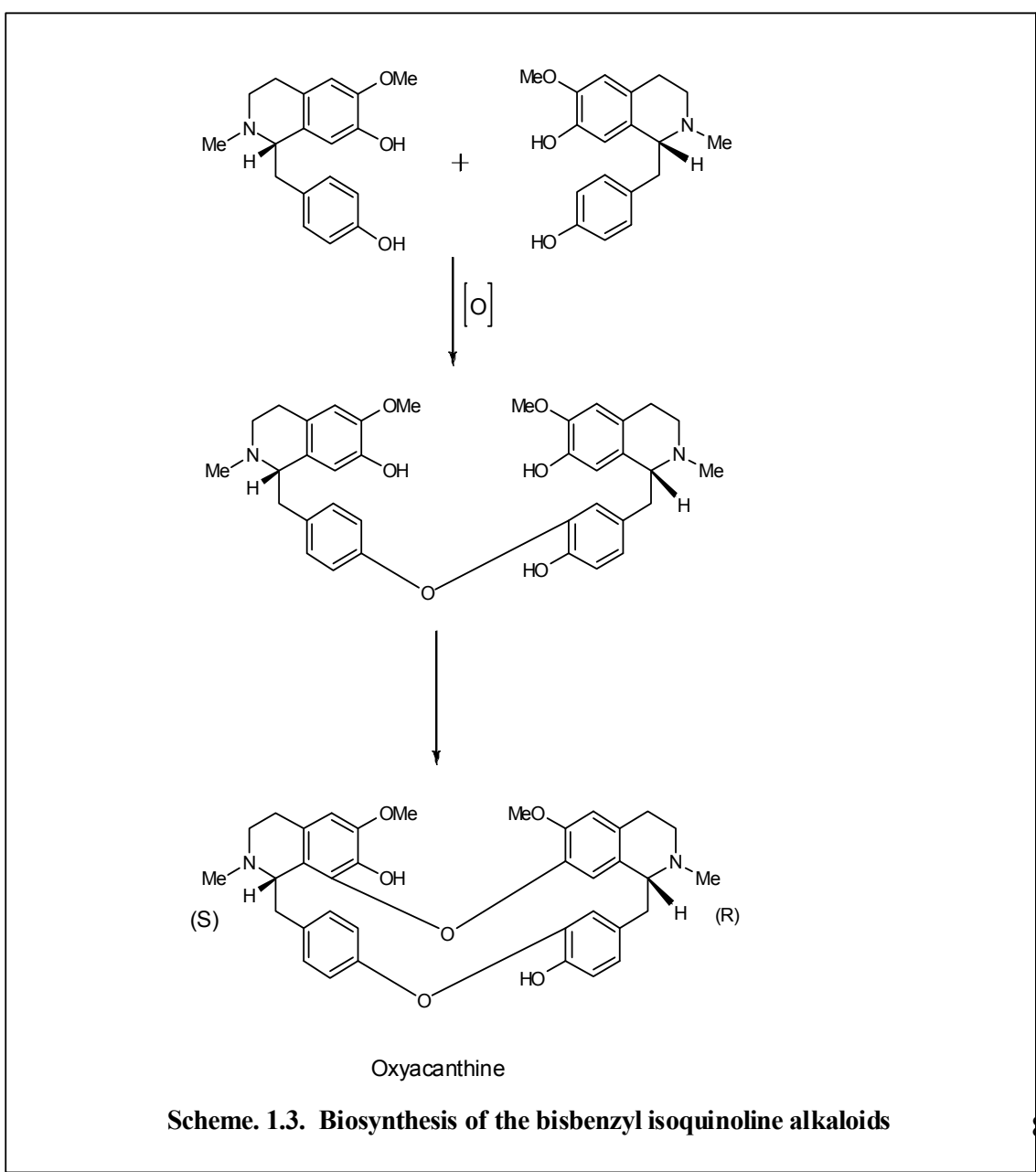
Then (S)-norcoclaurine is converted into (S)-coclaurine. The pathway continues to produce different benzylisoquinoline alkaloids, bisbenzyl isoquinoline alkaloids and aporphine alkaloids (**Scheme. 1.2**).





### 1.5.3. Biosynthesis of the bisbenzyl isoquinoline alkaloids (oxycanthine).<sup>27</sup>

In nature oxycanthine is formed from coclaurine derivatives. Dauricine type of intermediate is formed by the ether linkage between the two benzylic halves of N-methylcoclaurine through intermolecular oxidative coupling. Another oxidative coupling of dauricine type intermediate produces the second ether linkage. Finally the methylation at C-6 in the diether intermediate produces oxycanthine (**Scheme. 1.3**).



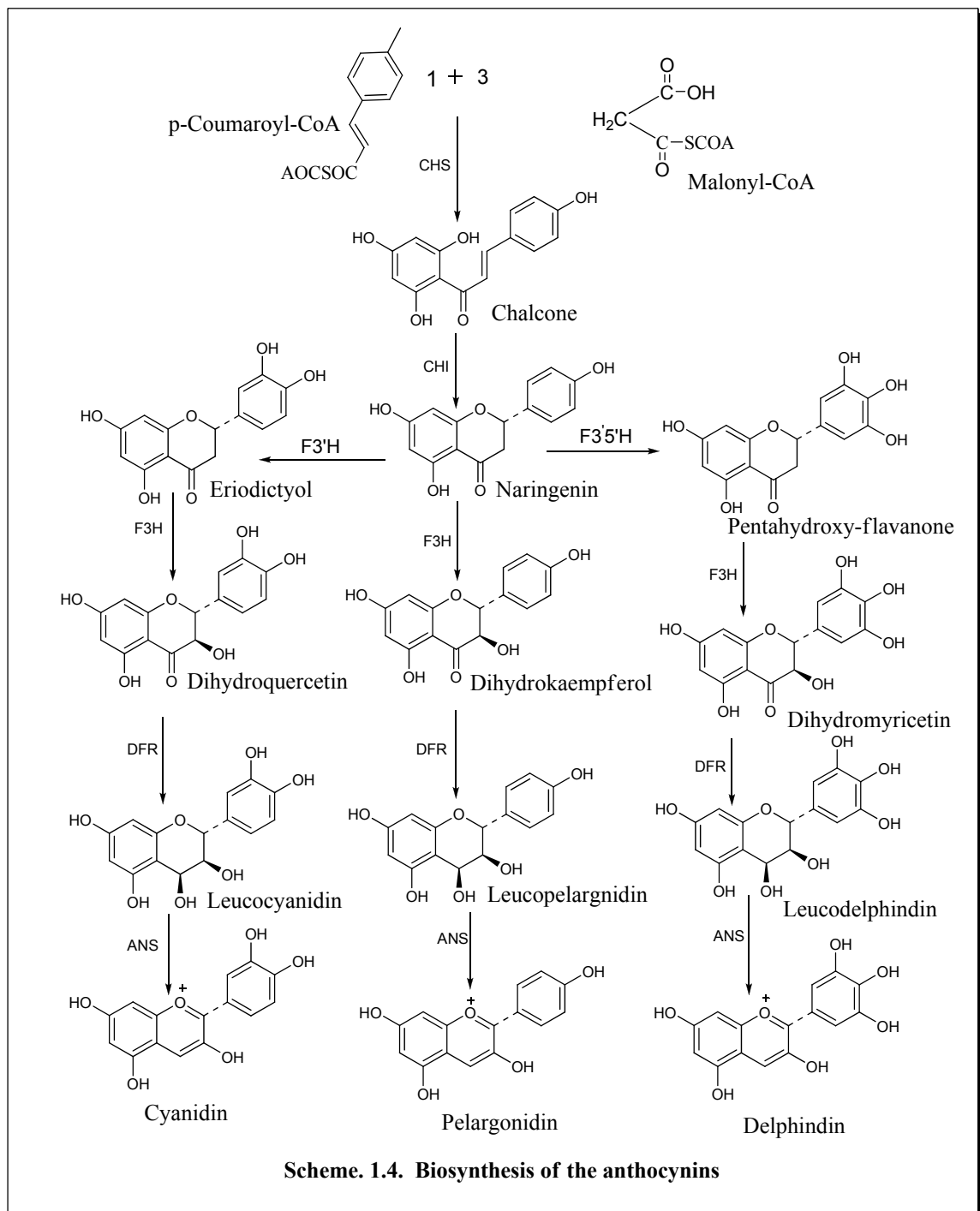
#### 1.5.4. Biosynthesis of the anthocynins.<sup>28</sup>

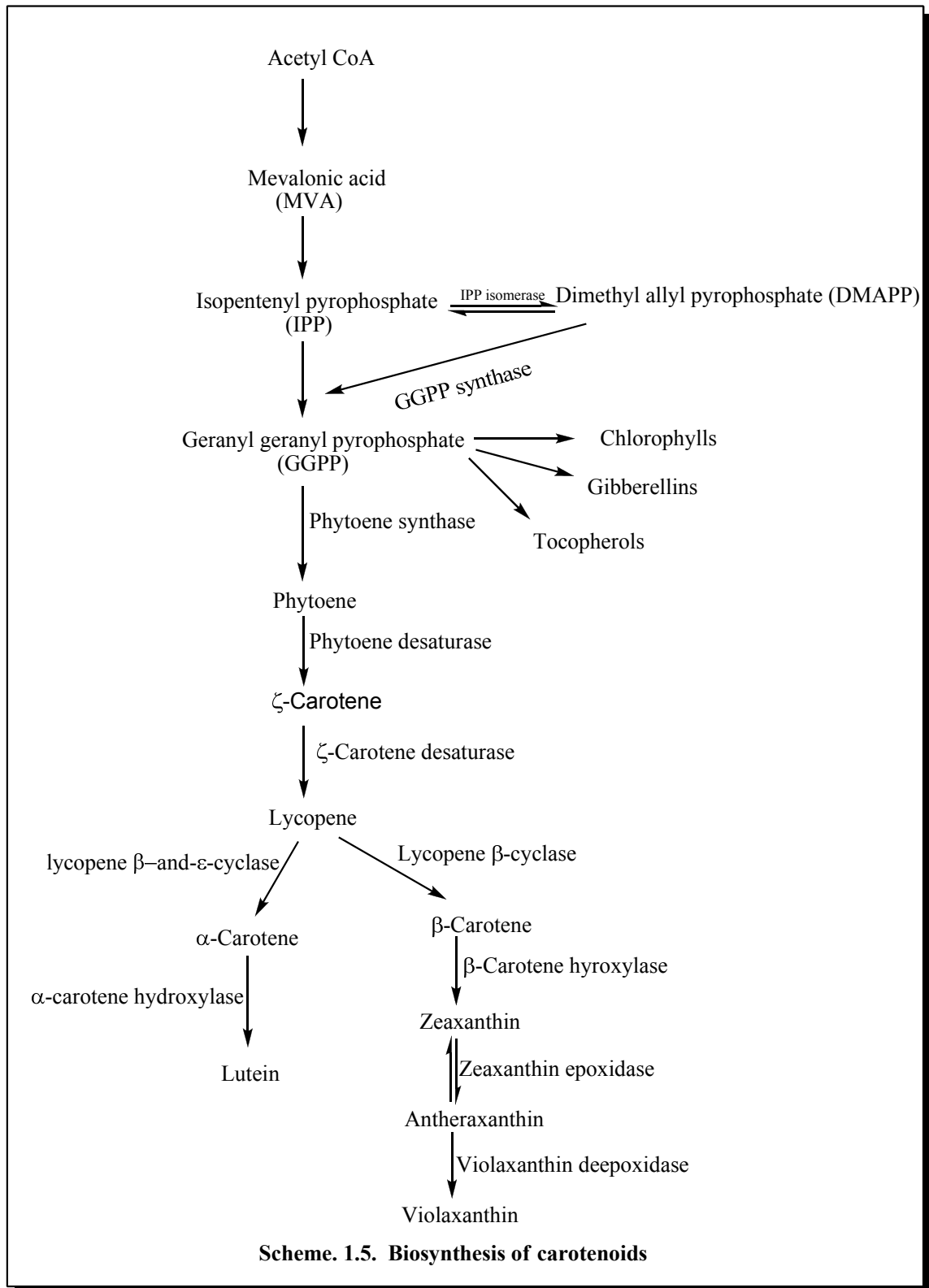
Anthocynins are synthesized in plants through flavonoids pathway. Naringenin is produced from the condensation of three molecules of malonyl-CoA and one molecule of p-coumaroyl-CoA by the action of chalcone synthase (CHS). Naringenin acts as a precursor for pentahydroxy-flavanone, eriodictyol and several other anthocynins. The various enzymes involved in the biosynthetic pathway of anthocynins are: chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3 $\beta$ -hydroxylase (F3H), flavonoid 3'-hydroxylase (F3'H), flavonoid 3',5'-hydroxylase (F3' 5'H), dihydroflavonol 4-reductase (DFR) and anthocyanidin synthase (ANS) (**Scheme. 1.4**).

#### 1.5.5. Biosynthesis of the carotenoids.<sup>29</sup>

Carotenoids are complex C<sub>40</sub> tetraterpenes found in higher plants. These are produced by the central isoprenoid pathway. The common precursor for all isoprenoid compounds is isopentenylpyrophosphate (IPP), which is produced from three molecules of acetyl-CoA through 3-hydroxy-3-methyl glutaryl-CoA (HMG-CoA) and mevalonic acid (MVA). Isopentenylpyrophosphate (IPP) isomerizes to produce its allylic isomer dimethylallyl pyrophosphate (DMAPP). This then condenses with a molecule of IPP to produce geranyl pyrophosphate. Further addition of two IPP units produce geranyl geranyl pyrophosphate (GGPP). Then two molecules of GGPP reacts to produce phytoene. Phytoene then produces phytofluene, zeta-carotene ( $\zeta$ -carotene), neurosporene and lycopene through four desaturation reactions. Lycopene then cyclize to produce  $\beta$  and  $\epsilon$

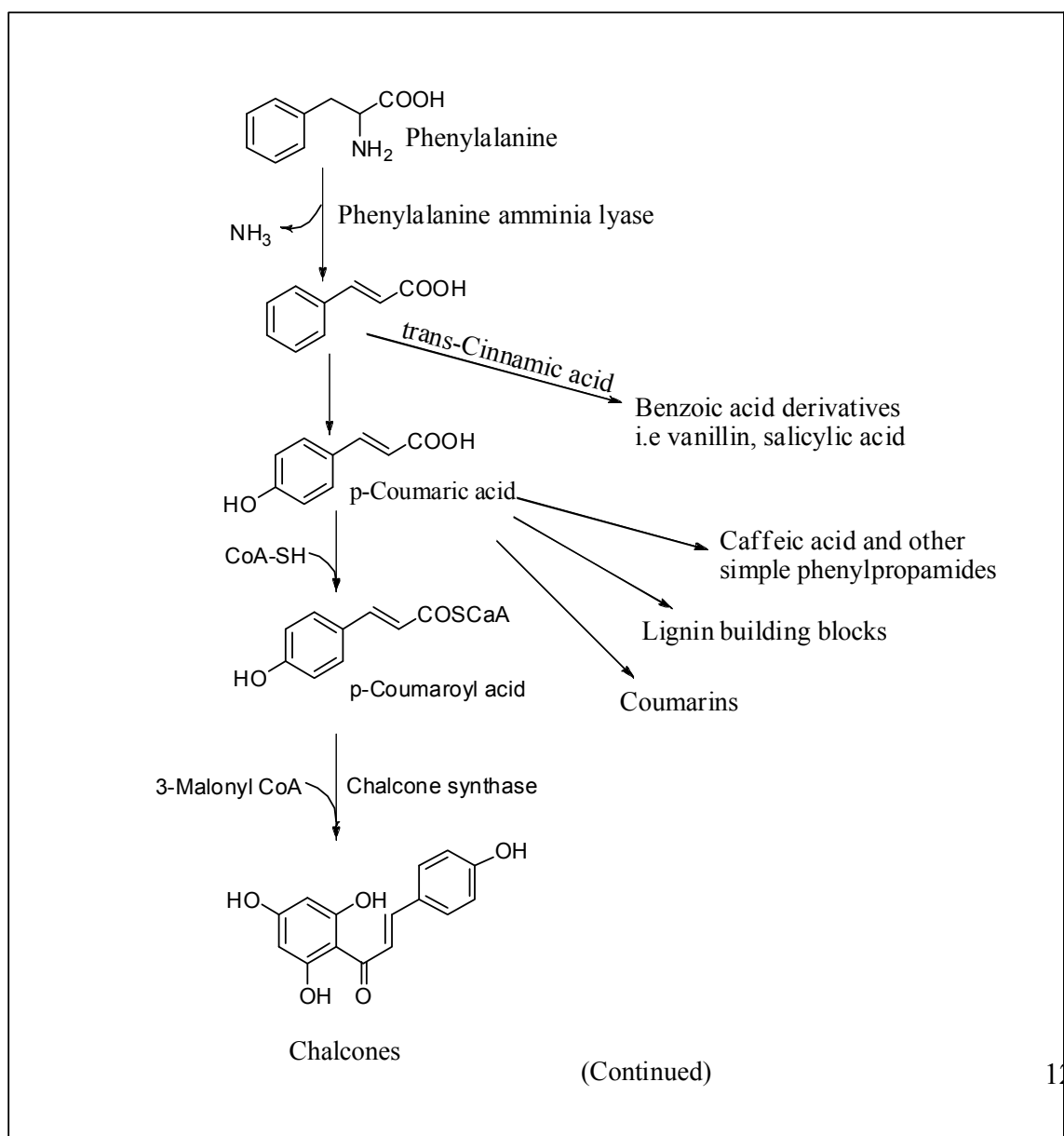
carotenes.  $\beta$ -carotene acts as a precursor for several other plant carotenoids.  $\alpha$ -carotene is the precursor for lutein (**Scheme. 1.5**).

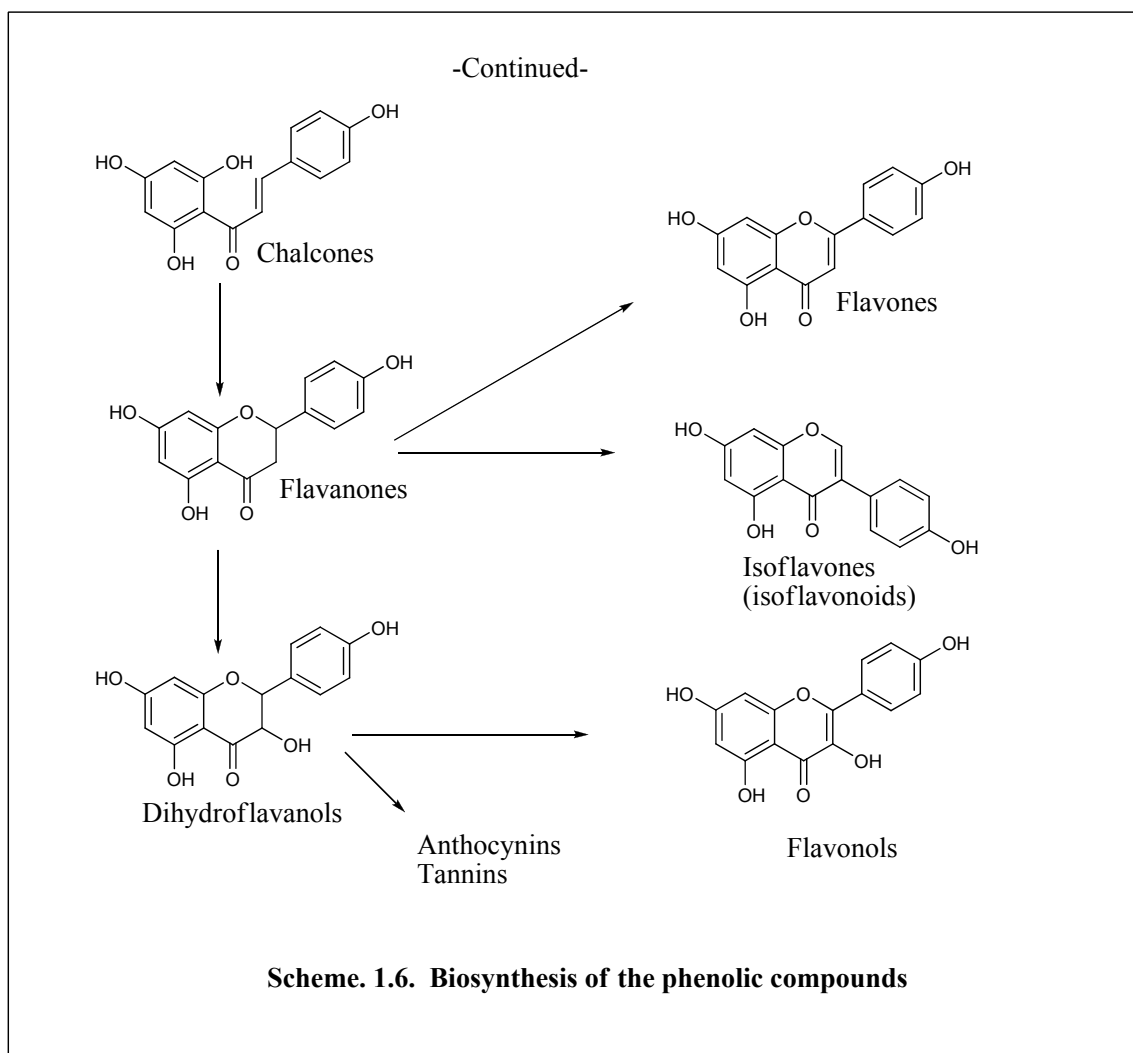




### 1.5.6. Biosynthesis of the phenolic compounds.<sup>30</sup>

Phenylalanine is believed to be the major precursor for almost all phenolic compounds. It is first converted to cinnamic acid under the action of phenylalanine ammonia lyase (PAL). Cinnamic acid is then converted to p-coumaric acid which produce p-coumaroyl acid. Then p-coumaroyl acid combines with 3-malonyl-CoA under the action of chalcone synthase (CHS) to produce chalcone, which then acts as a precursor for other phenolic compounds (**Scheme. 1.6**).





### 1.6. Biological activities reported for the genus *Berberis*.

(These are given in Part B Ch 4 Page 145)

## 2.0. Phytochemicals isolated from *Berberis* species.

Four new and eighteen new source compounds were isolated from *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf. Various experimental and spectroscopic techniques were used to elucidate the structures of the isolated compounds. Various fractions from the two plants and pure compounds were tested for their anti-bacterial, anti-trypanosomal, anti-fungal and anti-diabetic properties (these are given in Part B).

The details of extraction, isolation and purifications of compounds have been given in the experimental section. Different parts of *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf (roots with roots bark and stem with stem bark) were collected from Azad Jammu and Kashmir. Then crude extracts of different parts were obtained with methanol (*Berberis jaeschkeana* Schneid var. *jaeschkeana*) and ethanol (*Berberis glaucocarpa* Stapf). The crude extracts were fractionated using acidification and basification process for alkaloids and non-alkaloids (**Ch 3, Scheme. 3.1**). After evaporating the solvent the crude fractions were subjected to thin layer chromatography, column chromatography, preparative thin layer chromatography and separation over sephadex LH-20. The roots bark and roots wood of *Berberis jaeschkeana* Schneid var. *jaeschkeana* has resulted in two new (**111, 112**) and six reported compounds (**115-120**), stem bark and stem wood of *Berberis jaeschkeana* Schneid var. *jaeschkeana* resulted in one new (**114**) and three reported compounds (**121-123**). While the roots bark and wood of *Berberis glaucocarpa* Stapf has resulted in one new (**113**) and four reported compounds (**124-127**), stem bark and stem wood has resulted in the isolation of five

reported compounds (**128-132**). Similarly there were a number of compounds which were the same and isolated from both the species.

New alkaloids isolated from both the plants are presented first, followed by the new source alkaloids. Similarly the new non-alkaloids and then new source non-alkaloids.

## **2.1. New alkaloids from *Berberis jaeschkeana* Schneid var. *jaeschkeana* roots (with bark).**

### **2.1.1. 10-Hydroxy chondrofoline (111).**

Off white amorphous powder, m.p.: 195-197 °C.

Molecular formula:  $C_{37}H_{40}N_2O_7$  deduced from HR-ESIMS: 623.2772  $[M-H]^+$  (Calculated 623.2757).

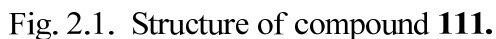
UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ): 282 (4.27) nm.

IR  $\bar{\nu}$ : 3337 (O-H, st.), 2930 (C-H, st. sat.), 2840 (C-H, st. N-methyl), 1601, 1559 (C=C, st. aromatic.), 1030 (finger print region)  $cm^{-1}$

$^1H$ NMR: see **Table. 2.1.**

$^{13}C$ NMR: see **Table. 2.1.**

HMBC: see **Table. 2.1.**



16

**Table. 2.1.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **10-hydroxy chondrofoline (111)** (in  $\text{CDCl}_3$ )

| C.No     | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)                         |
|----------|---------------------|------------------------------------|--------------------------------------------------|
| 1        | 63.68               | 3.86, (br.s)                       | C- $\alpha$ , N-Me, C-4a, C-8,<br>C-8a, C-9, C-3 |
| N-Me     | 41.97               | 2.26, (s)                          | C-1, C-3                                         |
| 3        | 45.01               | 2.87 and 3.46, (m)                 | C-1, N-Me, C-4, C-4a                             |
| 4        | 25.34               | 2.81 and 2.95, (m)                 | C-3, C-5, C-8a, C-4a,                            |
| 4a       | 128.71              | ---                                | ---                                              |
| 5        | 105.43              | 6.28, (s)                          | C-4a, C-8a, C-6, C-7, C-4                        |
| 6        | 151.77              | ---                                | ---                                              |
| 7        | 136.90              | ---                                | ---                                              |
| 6-OMe    | 55.55               | 3.76 (s)                           | C-6                                              |
| 7-OMe    | 60.51               | 3.12 (s)                           | C-7                                              |
| 8        | 148.66              | ---                                | ---                                              |
| 8a       | 120.24              | ---                                | ---                                              |
| $\alpha$ | 38.58               | 2.58 and 3.01, (m)                 | C-1, C-14,C-9, C-8a, C-10                        |
| 9        | 133.63              | ---                                | ---                                              |
| 10       | 147.63              | ---                                | ---                                              |
| 11       | 143.44              | ---                                | ---                                              |

Continue

| C.No      | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)                                |
|-----------|------------|---------------------------|---------------------------------------------------------|
| 12        | 148.20     | ---                       | ---                                                     |
| 13        | 115.34     | 6.79, (d, 8.0)            | C-14, C-9, C-11, C-12                                   |
| 14        | 123.50     | 6.75, (d, 8.0)            | C-13, C-9, C-10, C-12, C- $\alpha$                      |
| 1'        | 62.23      | 3.89, (br.s)              | C- $\alpha'$ , N'-Me, C-3', C-8',<br>C-8'a, C-4'a, C-9' |
| N'-Me     | 42.71      | 2.58, (s)                 | C-1', C-3'                                              |
| 3'        | 45.69      | 2.83 and 3.27, (m)        | C-4', N'-Me, C-1', C-4'a                                |
| 4'        | 24.97      | 2.77 and 2.93, (m)        | C-3', C-1', C-4'a, C-5'                                 |
| 4'a       | 127.33     | ---                       | ---                                                     |
| 5'        | 111.19     | 6.53, (s)                 | C-4'a, C-6', C-7', C-8'a, C-4'                          |
| 6'        | 149.92     | ---                       | ---                                                     |
| 6'-OMe    | 55.76      | 3.59, (s)                 | C-6'                                                    |
| 7'        | 143.89     | ---                       | ---                                                     |
| 8'        | 119.84     | 5.99, (br.s)              | C-1', C-4'a, C-7', C-6'                                 |
| 8'a       | 127.75     | ---                       | ---                                                     |
| $\alpha'$ | 37.66      | 2.93 and 3.28, (m)        | C-1', C-9', C-14', C-10'                                |
| 9'        | 135.42     | ---                       | ---                                                     |
| 10'       | 130.22     | 7.29, (dd, 8.4, 2.4)      | C-11', C-14', C-12', C- $\alpha'$                       |

Continue

| C.No | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)  |
|------|------------|---------------------------|---------------------------|
| 11'  | 121.35     | 7.13, (dd, 8.4, 2.4)      | C-13', C-10', C-9', C-12' |
| 12'  | 153.90     | ---                       | ---                       |
| 13'  | 121.72     | 6.64, (dd, 8.4, 2.4)      | C-11', C-14', C-9', C-12' |
| 14'  | 132.71     | 6.43, (dd, 8.4, 2.4)      | C-13', C-10', C-12'       |

The  $^{13}\text{C}$  NMR spectrum of the compound **111** showed the presence of thirty seven carbon atoms which include three methoxys at  $\delta$  60.51, 55.76 and 55.55, six methylene at  $\delta$  45.69, 38.58, 25.34, 45.01, 37.66 and 24.97, eleven methines at  $\delta$  132.71, 130.22, 123.50, 121.72, 121.35, 119.84, 115.34, 111.19, 105.43, 63.68 and 62.23, fifteen quaternary carbons at  $\delta$  153.90, 151.77, 149.92, 148.66, 148.20, 147.63, 143.89, 143.44, 136.90, 135.42, 133.63, 128.71, 127.75, 127.33 and 120.24 and two N-CH<sub>3</sub> peaks at  $\delta$  42.71 and 41.97.

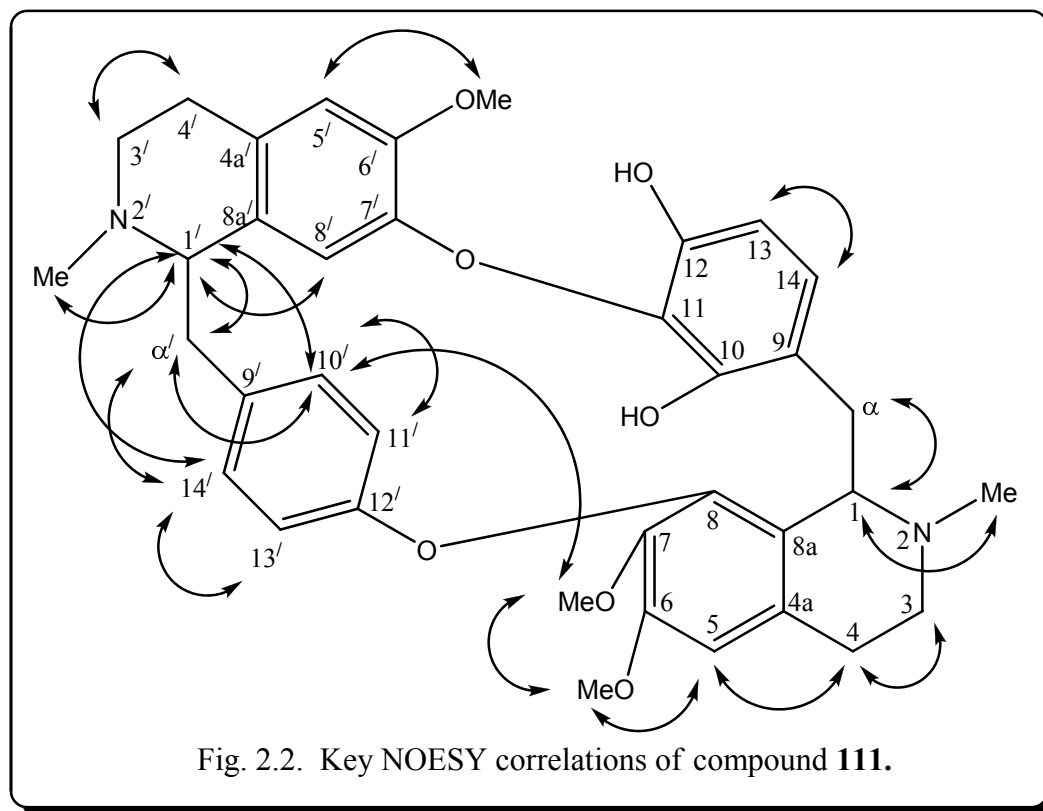
In the HMBC spectrum the signal appearing at  $\delta$  2.26 (N-Me) showed correlations with  $\delta$  63.68 (C-1) and 45.01 (C-3), the methoxy signal at  $\delta$  3.76 (6-OMe) correlated to  $\delta$  151.77 (C-6) while the methoxy singlet at  $\delta$  3.12 (7-OMe) correlated to  $\delta$  136.90 (C-7), the signal at  $\delta$  3.86 (H-1) showed correlations with  $\delta$  38.58 (C- $\alpha$ ), 41.97 (N-Me), 128.71 (C-4a), 148.66 (C-8), 120.24 (C-8a), 133.63 (C-9) and 45.01 (C-3), the signals appearing at  $\delta$  2.87 and 3.46 (H-3) showed correlations with  $\delta$  63.68 (C-1), 25.34 (C-4), 128.71 (C-4a) and 41.97 (N-CH<sub>3</sub>).

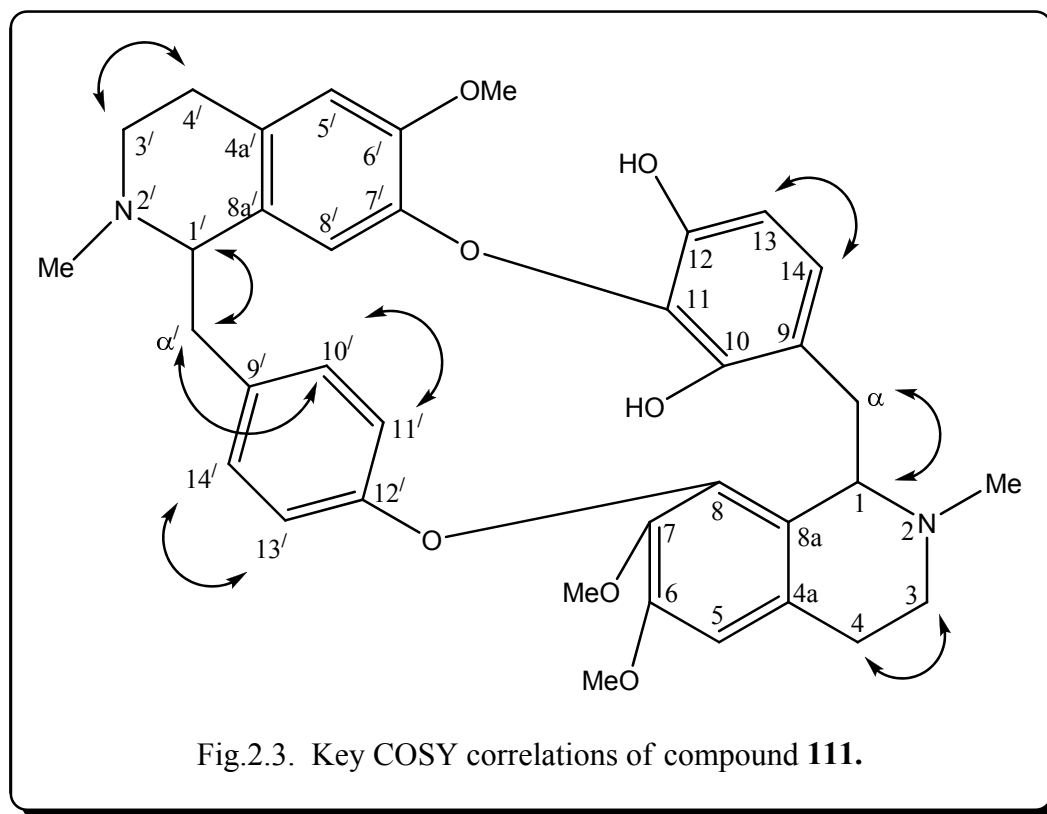
The proton signals appearing at  $\delta$  2.81 and 2.95 (H-4) showed correlations with  $\delta$  45.01 (C-3), 105.43 (C-5), 120.24 (C-8a) and 128.71 (C-4a); the proton appearing at  $\delta$  6.28 (H-5) correlated to  $\delta$  128.71 (C-4a), 120.24 (C-8a), 151.77 (C-6), 136.90 (C-7) and 25.34; the proton signal appearing at  $\delta$  2.58 and 3.01 (H- $\alpha$ ) showed correlations with  $\delta$  63.68 (C-1), 123.50 (C-14), 133.63 (C-9), 120.24 (C-8a) and 147.63 (C-10); the signal appearing at  $\delta$  6.79 (H-13) showed correlations with  $\delta$  123.50 (H-14), 133.63 (H-9), 143.44 (C-11) and 148.20 (H-12); the signal at  $\delta$  6.75 (H-14) showed correlations with  $\delta$  115.34 (C-13), 133.63 (C-9), 147.63 (C-10), 148.20 (C-12) and 38.58 (C- $\alpha$ ).

The signal at  $\delta$  2.58 (N'-Me) showed correlations with  $\delta$  62.23 (C-1') and 45.69 (C-3'); the methoxy signal at  $\delta$  3.59 (6'-OMe) correlated to  $\delta$  149.92 (C-6'); the proton signal at  $\delta$  3.89 (H-1') correlated to  $\delta$  37.66 (C- $\alpha'$ ), 42.71 (N'-Me), 45.69 (C-3'), 119.84 (C-8'), 127.75 (C-8'a), 127.33 (C-4'a) and 135.42 (C-9'); the proton signal at 2.83 and 3.27 (H-3') correlated to  $\delta$  24.97 (C-4'), 42.71 (N'-Me), 62.23 (C-1') and 127.33 (C-4'a); the proton at  $\delta$  2.77 and 2.93 (H-4') showed correlations with  $\delta$  45.69 (C-3'), 62.23 (C-1'), 127.33 (C-4'a) and 111.19 (C-5'); the proton signal at  $\delta$  6.53 (H-5') correlated to  $\delta$  127.33 (C-4'a), 149.92 (C-6'), 143.89 (C-7'), 127.75 (C-8'a) and 24.97 (C-4'); the proton signal at  $\delta$  5.99 (H-8') correlated to  $\delta$  62.23 (C-1'), 127.33 (C-4'a), 149.92 (C-6') and 143.89 (C-7'); the signal at  $\delta$  2.93 and 3.28 (H- $\alpha'$ ) correlated to  $\delta$  62.23 (C-1'), 135.42 (C-9'), 132.71 (C-14'), 127.33 (C-4'a) and 130.22 (C-10').

The signal at  $\delta$  7.29 (H-10') correlated to  $\delta$  121.35 (C-11'), 132.71 (C-14'), 153.90 (C-12') and 37.66 (C- $\alpha'$ ); the proton signal at  $\delta$  7.13 (H-11') correlated to  $\delta$  121.72 (C-13'), 130.22 (C-10'), 135.42 (C-9') and 153.90 (C-12'); the proton signal appearing at  $\delta$  6.64

correlated to  $\delta$  121.35 (C-11'), 132.71 (C-14'), 135.42 (C-9') and 153.90 (C-12'). Similarly the proton signal appearing at  $\delta$  6.43 (H-14') correlated to  $\delta$  121.72 (C-13'), 121.72 (C-13') and 121.72 (C-13').





### 2.1.2. 8,12,12a-Trihydroxy-11,13a-berberin-dione (**112**).

Amorphous orange yellow solid turns to brown on exposure to light.

Molecular formula:  $C_{18}H_{16}NO_8$  deduced from HR-ESIMS: 374.0996  $[M+H]^+$  (Calculated 374.0878).

UV (MeOH)  $\lambda_{max}$  (log  $\epsilon$ ): 300nm (5.84), 260 (5.87) nm.

IR  $\bar{\nu}$ : 3341 (O-H, st.), 1723, 1720 (C=O, st.)  $cm^{-1}$

$^1H$ NMR: see **Table. 2.2**.

$^{13}C$ NMR: see **Table. 2.2**.

HMBC: see **Table. 2.2**.

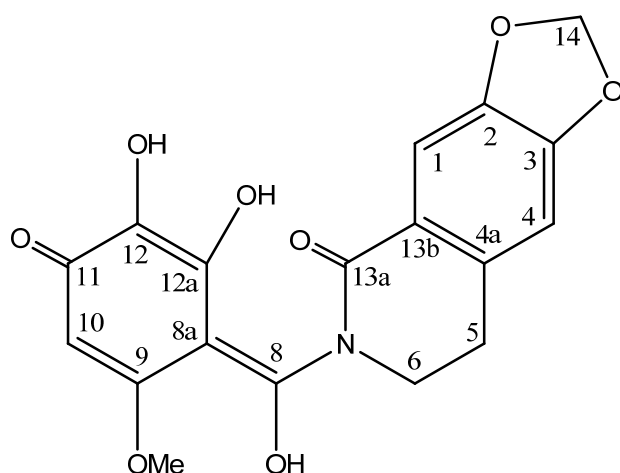


Fig. 2.4. Structure of compound **112**.

The  $^1\text{H}$  NMR spectrum of compound **112** showed three aromatic protons at  $\delta$  7.53 (1H, s, H-1), 7.41 (1H, s, H-10) and 6.68 (1H, s, H-4), three methylene at  $\delta$  6.03 (2H, s, H-14), 3.57 (2H, dt,  $J$  = 6.8, 2.8 Hz, H-6) and 2.94 (2H, t,  $J$  = 6.8 Hz, H-5) and one methoxy singlet at  $\delta$  3.97 (3H, s, 9-OCH<sub>3</sub>).

The  $^{13}\text{C}$  NMR showed 18 carbon atoms including three methine carbons at  $\delta$  107.97, 107.27 and 107.07, three methylene carbons at  $\delta$  101.43, 40.28 and 28.42, one methoxy carbon at  $\delta$  56.50 and eleven quaternary carbon atoms at  $\delta$  170.32, 166.64, 151.01, 146.63, 146.96, 140.56, 139.71, 134.60, 133.75, 122.37 and 106.50.

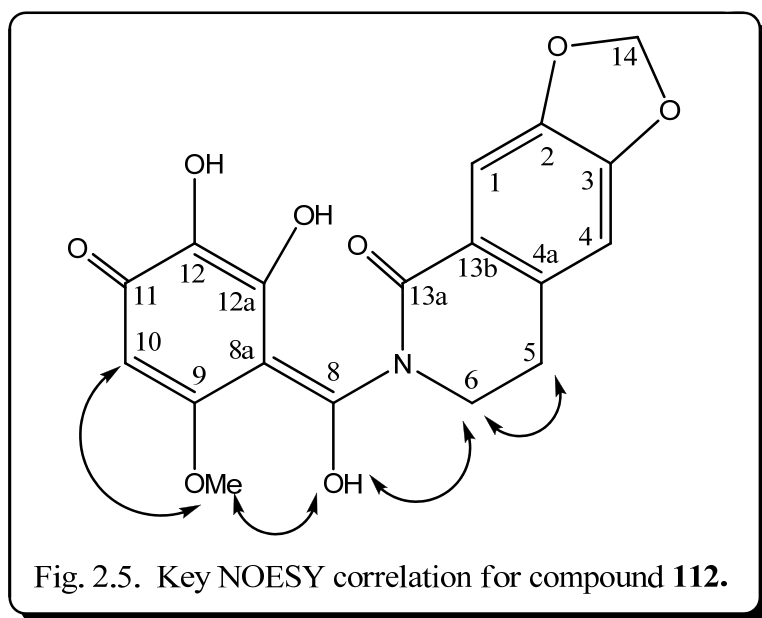
**Table. 2.2.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **8,12,12a-trihydroxy-11,13a-berberin-dione (112)** (in  $\text{CDCl}_3$ )

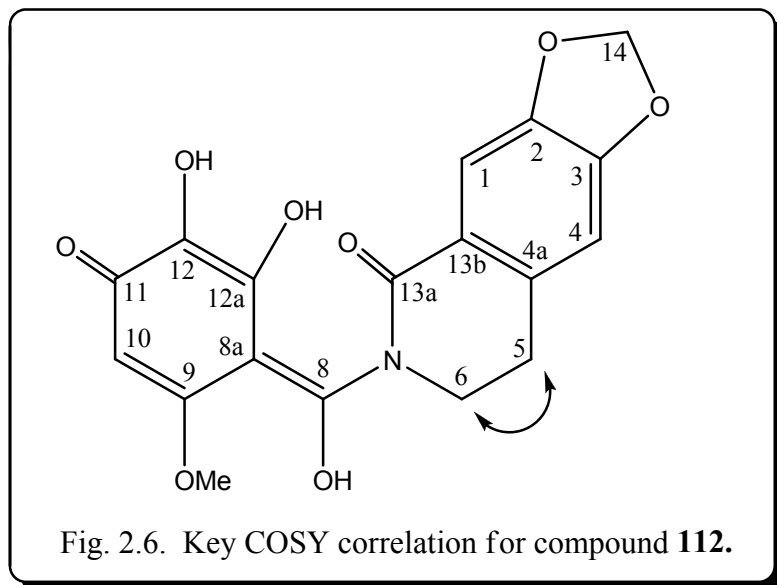
| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|------|---------------------|------------------------------------|--------------------------|
| 1    | 107.07              | 7.53, (s)                          | C-4a, C-2, C-3, C-13a    |
| 2    | 146.96              | ---                                | ---                      |
| 3    | 151.01              | ---                                | ---                      |
| 4    | 107.27              | 6.68, (s)                          | C-5, C-13b, C-2, C-3     |
| 4a   | 133.75              | ---                                | ---                      |
| 5    | 28.42               | 2.94, (t, 6.8)                     | C-6, C-4, C-13b, C-4a    |
| 6    | 40.28               | 3.57, (dt, 6.8, 2.8)               | C-5, C-8, C-13a          |
| 8    | 134.60              | 6.82, (br.s, -OH)                  | ---                      |
| 8a   | 106.50              | ---                                | ---                      |
| 9    | 146.63              | ---                                | ---                      |
| 10   | 107.97              | 7.41, (s)                          | C-8a, C-12, C-9, C-11    |
| 11   | 170.32              | ---                                | ---                      |
| 12   | 139.71              | ---                                | ---                      |
| 12a  | 140.56              | ---                                | ---                      |
| 13   | ---                 | ---                                | ---                      |
| 13a  | 166.64              | ---                                | ---                      |
| 13b  | 122.37              | ---                                | ---                      |

Continue

| C.No                   | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H→C) |
|------------------------|---------------------|------------------------------------|------------|
| -O-CH <sub>2</sub> -O- | 101.43              | 6.03, (s)                          | C-2, C-3   |
| 9-OMe                  | 56.50               | 3.97, (s)                          | C-9        |

In the HMBC spectrum of compound **112** the proton signal at  $\delta$  7.53 (H-1) showed correlations with  $\delta$  133.75 (C-4a), 146.96 (C-2), 151.01 (C-3) and 166.64 (C-4). The proton signal at  $\delta$  6.68 (H-4) correlated to 28.42 (C-5), 122.37 (C-13b), 146.96 (C-2) and 151.01 (C-3), the signal appearing at  $\delta$  2.94 (H-5) showed correlations with  $\delta$  40.28 (C-6), 107.27 (C-4), 122.37 (C-13b) and 133.75 (C-4a), the signal at  $\delta$  3.57 (H-6) showed correlations with  $\delta$  28.42 (C-5), 134.60 (C-8) and 166.64 (C-13a), the signal at  $\delta$  7.41 (H-10) correlated to  $\delta$  106.50 (C-8a), 139.71 (C-12), 146.63 (C-9) and 170.32 (C-11), the signal appearing at  $\delta$  6.03 (H-14) correlated to  $\delta$  146.96 (C-2) and 151.01 (C-3) and the methoxy signal at  $\delta$  3.97 (9-OMe) correlated to 146.63 (C-9).





## 2.2. A new alkaloid from *Berberis glaucocarpa* Stapf roots.

### 2.2.1. 10-Methoxy chondrofoline (**113**).

White amorphous solid, m.p.: 186-188 °C.

Molecular formula:  $C_{38}H_{42}N_2O_7$  deduced from HR-ESIMS 639.2702  $[M+H]^+$  (Calculated 639.2992).

UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 281 (5.28) nm.

IR  $\bar{\nu}$ : 3351 (O-H, st.), 2987 (C-H, st. sat.), 2386 (C-H, st. N-methyl) and 1600, 1501 (C=C st. aromatic.), 1030  $\text{cm}^{-1}$ .

$^1\text{H}$ NMR: see **Table 2.3**.

$^{13}\text{C}$ NMR: see **Table 2.3**.

HMBC: see **Table 2.3**.

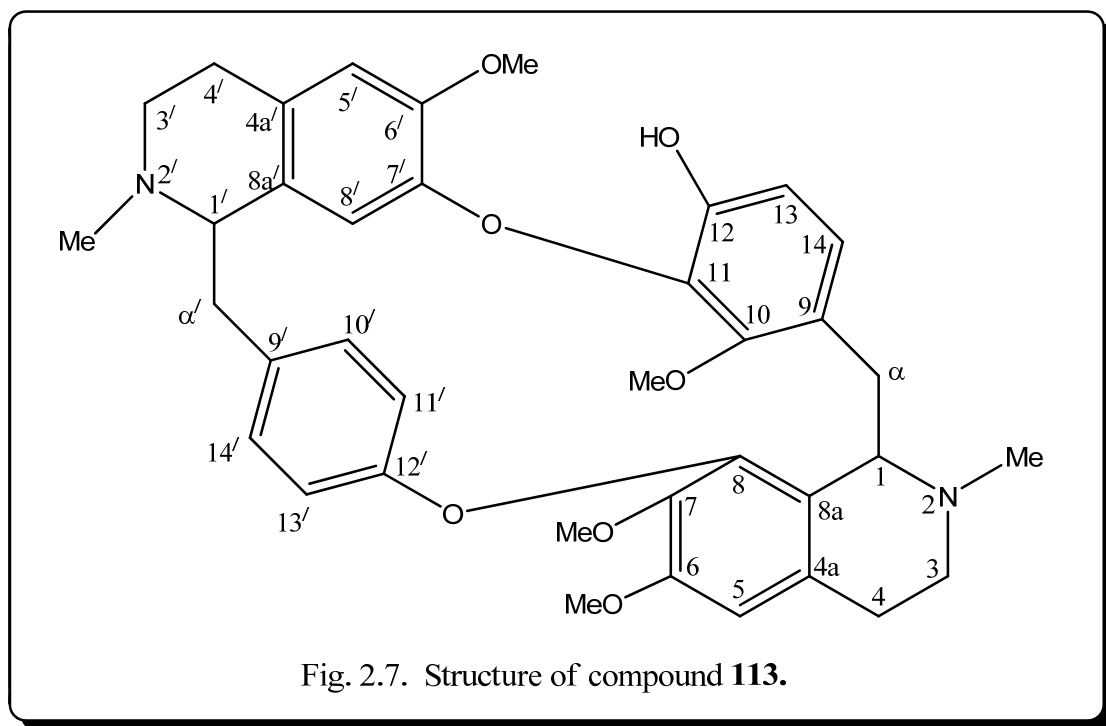


Fig. 2.7. Structure of compound **113**.

The  $^1\text{H}$  NMR spectrum of compound **113** showed nine aromatic protons at  $\delta$  7.29 (1H, dd,  $J = 8.4, 2.6$  Hz, H-10'), 7.10 (1H, dd,  $J = 8.4, 2.6$  Hz, H-11'), 6.78 (1H, d,  $J = 6.4$  Hz, H-13), 6.75 (1H, d,  $J = 6.4$  Hz, H-14), 6.63 (1H, dd,  $J = 8.4, 2.6$  Hz, H-13'), 6.48 (1H, s, H-5'),  $\delta$  6.44 (1H, dd,  $J = 8.4, 2.6$  Hz, H-14'), 6.19 (1H, s, H-5) and 5.97 (1H, br.s, H-8'), two non aromatic methine protons at  $\delta$  3.85 (1H, br.s, H-1) and 3.87 (1H, br.s, H-1'), twelve methylene protons at  $\delta$  2.85 and 3.49 (2H, m, H-3), 2.85 and 3.23 (2H m, H-3'), 2.95 and 3.30 (2H, m, H- $\alpha'$ ), 2.58 and 3.06 (2H, m, H- $\alpha$ ), 2.74 and 2.93 (2H m, H-4), 2.72 and 2.91 (2H, m, H-4'), four methoxy peaks at  $\delta$  3.73 (3H, s, 6-OMe), 3.55 (3H, s, 6'-OMe), 3.12 (3H, s, 7-OMe) and 3.73 (3H, s, 10-OMe), two N-CH<sub>3</sub> peaks at  $\delta$  2.51 (3H, s, N-CH<sub>3</sub>) and 2.26 (3H, s, N'-CH<sub>3</sub>).

**Table. 2.3.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **10-methoxy chondrofoline (113)** (in  $\text{CDCl}_3$ )

| C.No     | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)                        |
|----------|---------------------|------------------------------------|-------------------------------------------------|
| 1        | 63.89               | 3.85, (br.s)                       | C- $\alpha$ , N-Me, C-4a, C-8<br>C-8a, C-9, C-3 |
| N-Me     | 42.59               | 2.17, (s)                          | C-1, C-3                                        |
| 3        | 46.32               | 2.85 and 3.49, (m)                 | C-1, N-Me, C-4, C-4a                            |
| 4        | 25.77               | 2.74 and 2.93, (m)                 | C-3, C-5, C-8a, C-4a                            |
| 4a       | 128.57              | ---                                | ---                                             |
| 5        | 106.47              | 6.19, (s)                          | C-4a, C-8a, C-6, C-7, C-4                       |
| 6        | 151.45              | ---                                | ---                                             |
| 7        | 136.97              | ---                                | ---                                             |
| 6-OMe    | 55.35               | 3.73, (s)                          | C-6                                             |
| 7-OMe    | 59.69               | 3.11, (s)                          | C-7                                             |
| 8        | 148.84              | ---                                | ---                                             |
| 8a       | 120.41              | ---                                | ---                                             |
| $\alpha$ | 38.27               | 2.50 and 3.06, (m)                 | C-1, C-14, C-9, C-8a, C-10                      |
| 9        | 133.43              | ---                                | ---                                             |
| 10       | 145.11              | ---                                | ---                                             |

Continue

| C.No      | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)                                  |
|-----------|------------|---------------------------|-----------------------------------------------------------|
| 10-OMe    | 55.70      | 3.73, (s)                 | C-10                                                      |
| 11        | 144.09     | ---                       | ---                                                       |
| 12        | 148.20     | ---                       | ---                                                       |
| 13        | 115.43     | 6.78, (d, 6.4)            | C-14, C-9, C-11, C-12                                     |
| 14        | 123.09     | 6.72, (d, 6.4)            | C-13, C-9, C-10, C-12, C- $\alpha$                        |
| 1'        | 63.75      | 3.87, (br.s)              | C- $\alpha'$ , N'-Me, C-3', C-8',<br>C-8'/a, C-4'/a, C-9' |
| N'-Me     | 42.34      | 2.51, (s)                 | C-1', C-3'                                                |
| 3'        | 46.30      | 2.85 and 3.23, (m)        | C-4', N'-Me, C-1', C-4'/a                                 |
| 4'        | 24.87      | 2.72 and 2.91, (m)        | C-3', C-1', C-4'/a, C-5'                                  |
| 4'/a      | 127.62     | ---                       | ---                                                       |
| 5'        | 111.64     | 6.48, (s)                 | C-4'/a, C-6', C-7', C-8'/a, C-4'                          |
| 6'        | 148.56     | ---                       | ---                                                       |
| 6'-OMe    | 55.56      | 3.55, (s)                 | C-6'                                                      |
| 7'        | 143.29     | ---                       | ---                                                       |
| 8'        | 119.12     | 5.97, (br.s)              | C-1', C-4'/a, C-7', C-6'                                  |
| 8'/a      | 129.20     | ---                       | ---                                                       |
| $\alpha'$ | 37.61      | 2.95 and 3.30, (m)        | C-1', C-9', C-14', C-10', C-4'/a                          |

Continue

| C.No | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)          |
|------|------------|---------------------------|-----------------------------------|
| 9'   | 135.61     | ---                       | ---                               |
| 10'  | 130.66     | 7.40, (dd, 8.4, 2.6)      | C-11', C-14', C-12', C- $\alpha'$ |
| 11'  | 121.35     | 7.10, (dd, 8.4, 2.6)      | C-13', C-10', C-9', C-12'         |
| 12'  | 153.50     | ---                       | ---                               |
| 13'  | 121.60     | 6.63, (dd, 8.4, 2.6)      | C-11', C-14', C-9', C-12'         |
| 14'  | 132.90     | 6.44, (dd, 8.4, 2.6)      | C-13', C-10', C-12'               |

The  $^{13}\text{C}$  NMR spectrum of compound **113** showed the presence of thirty eight carbon atoms which included four methoxys at  $\delta$  59.69, 55.56, 55.70 and 55.35, six methylenes at  $\delta$  46.32, 38.27, 25.77, 46.30, 37.61 and 24.87, eleven methines at  $\delta$  132.90, 130.66, 123.09, 121.60, 121.35, 119.12, 115.43, 111.64, 106.47, 63.89 and 63.75, fifteen quaternary carbons at  $\delta$  153.50, 151.45, 148.56, 148.84, 148.20, 145.11, 143.29, 144.09, 136.97, 135.61, 133.43, 128.57, 127.62, 129.20 and 120.41 and two N-CH<sub>3</sub> peaks at  $\delta$  42.69 and 42.34.

In the HMBC spectrum of the compound **113**, the signal appearing at  $\delta$  2.17 (N-Me) showed correlations with  $\delta$  63.89 (C-1) and 46.32 (C-3), the signal at  $\delta$  2.51 (N'-Me) showed correlations with  $\delta$  63.75 (C-1') and 46.30 (C-3'), two methoxy signals appeared at  $\delta$  3.73 (6-OMe, 10-OMe) correlated to  $\delta$  151.45 (C-6) and 145.11 (C-10) respectively, the methoxy signal at  $\delta$  3.55 (6'-OMe) correlated to  $\delta$  148.56 (C-6') and the methoxy singlet at  $\delta$  3.11 (7-OMe) correlated to  $\delta$  136.97 (C-7).

The methine signal at  $\delta$  3.85 (H-1) showed correlations with  $\delta$  38.27 (C-  $\alpha$ ), 42.69 (N-Me), 128.57 (C-4a), 148.84 (C-8), 120.41 (C-8a), 133.43 (C-9) and 46.32 (C-3), the methine proton signal at  $\delta$  3.87 (H-1') correlated to  $\delta$  37.61 (C-  $\alpha'$ ), 42.34 (N'-Me), 46.30 (C-3'), 119.12 (C-8'), 129.20 (C-8'a), 127.62 (C-4'a) and 135.61 (C-9').

The methylene proton signals appearing at  $\delta$  2.85 and 3.49 (H-3) showed correlations with  $\delta$  63.89 (C-1), 25.77 (C-4), 128.57 (C-4a) and 42.69 (N-CH<sub>3</sub>), the methylene proton signals appearing at  $\delta$  2.74 and 2.93 (H-4) showed correlations with  $\delta$  46.32 (C-3), 106.47 (C-5), 120.41 (C-8a) and 128.57 (C-4a), the methylene proton signal appearing at  $\delta$  2.50 and 3.06 (H-  $\alpha$ ) showed correlations with  $\delta$  63.89 (C-1), 123.09 (C-14), 133.43 (C-9), 120.41 (C-8a) and 145.11 (C-10), the methylene proton signals at  $\delta$  2.85 and 3.23 (H-3') correlated to  $\delta$  24.87 (C-4'), 42.34 (N'-Me), 63.75 (C-1') and 127.62 (C-4'a), another set of methylene protons at  $\delta$  2.72 and 2.91 (H-4') showed correlations with  $\delta$  46.30 (C-3'), 63.75 (C-1'), 127.62 (C-4'a) and 111.64 (C-5'), the methylene signal at  $\delta$  2.95 and 3.30 (H-  $\alpha'$ ) correlated to  $\delta$  63.75 (C-1'), 135.61 (C-9'), 132.90 (C-14'), 127.62 (C-4'a) and 130.66 (C-10').

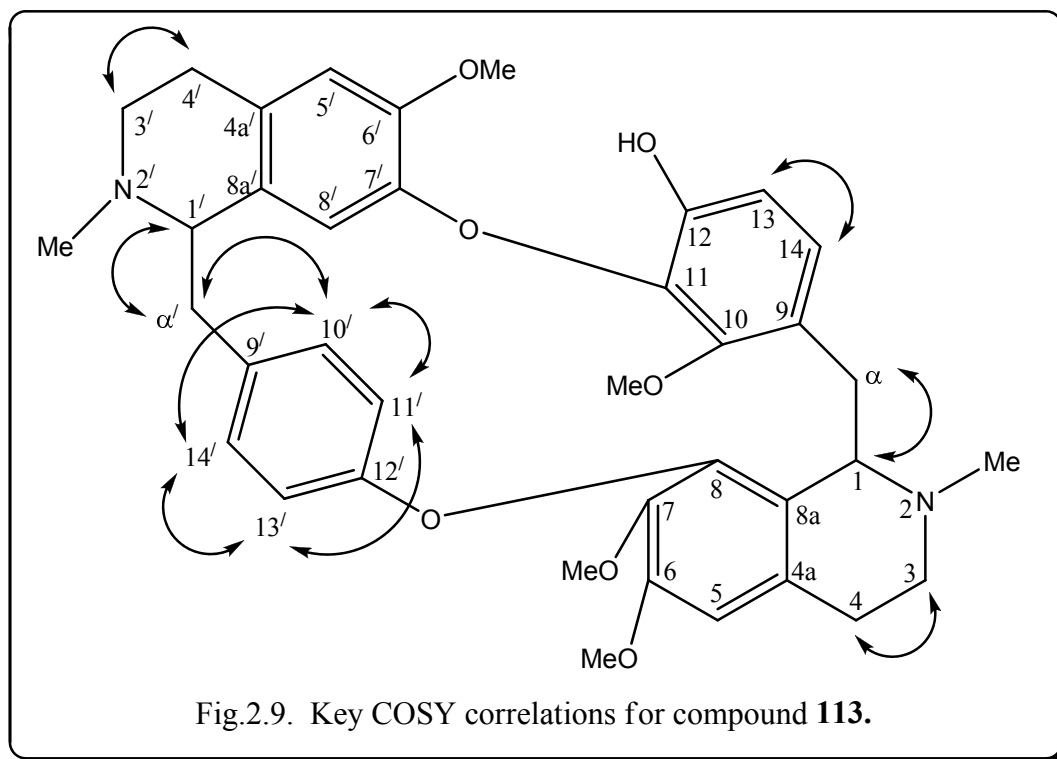
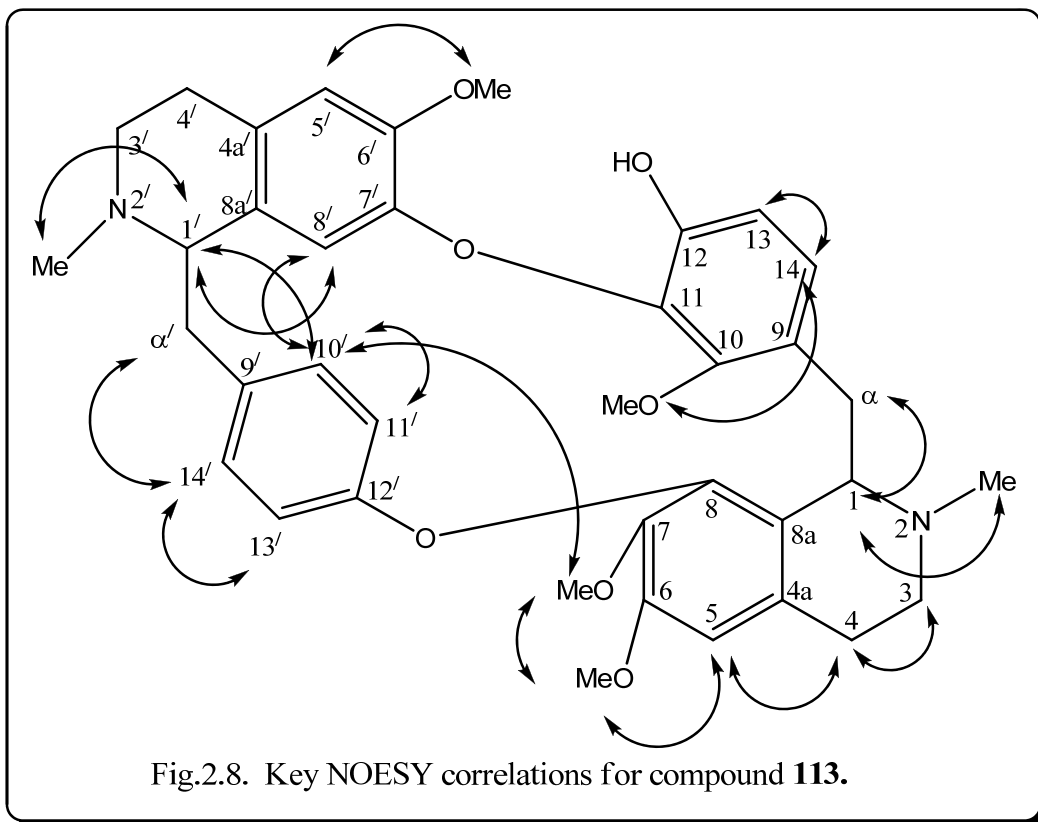
The methine proton appearing at  $\delta$  6.19 (H-5) correlated to  $\delta$  128.57 (C-4a), 120.41 (C-8a), 151.45 (C-6), 136.97 (C-7) and 25.77 (C-1), the signal appearing at  $\delta$  6.78 (H-13) showed correlations with  $\delta$  123.09 (H-14), 133.43 (H-9), 144.09 (C-11) and 148.20 (H-12), the signal at  $\delta$  6.72 (H-14) showed correlations with  $\delta$  115.43 (C-13), 133.43 (C-9), 145.11 (C-10), 148.20 (C-12) and 38.27 (C-  $\alpha$ ), the proton signal at 6.48 (H-5') correlated to  $\delta$  127.62 (C-4'a), 148.56 (C-6'), 143.29 (C-7'), 129.20 (C-8'a) and 24.87 (C-4'), the proton signal at  $\delta$  5.97 (H-8') correlated to  $\delta$  63.75 (C-1'), 127.62 (C-4'a), 148.56

(C-6') and 143.29 (C-7'), the signal at  $\delta$  7.40 (H-10') correlated to  $\delta$  121.35 (C-11'), 132.90 (C-14'), 153.50 (C-12') and 37.61 (C-  $\alpha'$ ).

The proton signal at  $\delta$  7.10 (H-11') correlated to  $\delta$  121.60 (C-13'), 130.66 (C-10'), 135.61 (C-9') and 153.50 (C-12'), the proton signal appearing at  $\delta$  6.63 correlated to  $\delta$  121.35 (C-11'), 132.90 (C-14'), 135.61 (C-9') and 153.50 (C-12'), similarly the proton signal appearing at  $\delta$  6.44 (H-14') showed correlations with  $\delta$  121.60 (C-13'), 153.50 (C-12'), 130.66 (C-10') and 37.61 (C-  $\alpha'$ ).

The spectral data obtained for compound **113** was very similar to that of compound **111** except the presence of an extra methoxy in the  $^1\text{H}$ NMR and extra carbon atom in  $^{13}\text{C}$ NMR spectrum. The HR-ESIMS for compound **113** confirmed it to be having an extra methoxy group instead of one of the hydroxyl groups. The structure and attachment of elements in compound **113** were further confirmed from NOESY and COSY spectra.

The major NOESY and COSY correlations for compound **113** are shown in **Fig. 2.8.** and **Fig. 2.9.** respectively.



### 2.3. A new non-alkaloid from *Berberis jaeschkeana* Schneid var. *jaeschkeana* stem.

#### 2.3.1. Docosyl-2-hydroxytetracosanoate (114).

White powder (CHCl<sub>3</sub>), m.p.: 83-84 °C.

Molecular formula: C<sub>46</sub>H<sub>92</sub>O<sub>3</sub>

UV (CH<sub>2</sub>Cl<sub>2</sub>) λ<sub>max</sub> (log ε): 237 (5.58) nm.

IR  $\bar{\nu}$ : 3400 (O-H, st.), 2915 (C-H, st. sat.), 1729 (C=O, st.), 1462, 1219, 1082,

729 (finger print region) cm.<sup>-1</sup>

EI-MS m/z: 692 [M<sup>+</sup>] (calculated for C<sub>46</sub>H<sub>92</sub>O<sub>3</sub>)

EI-MS m/z (rel. int. %): 692 (38, M<sup>+</sup>), 678 (18), 664 (23), 398 (13), 384 (75), 370 (36), 356 (37), 337 (61), 311(25), 309 (22) 292 (7), 181 (5), 153 (11), 125 (35), 111 (69), 97 (100), 83 (85), 71 (68), 57 (95).

<sup>1</sup>HNMR: see **Table. 2.4.**

<sup>13</sup>CNMR: see **Table. 2.4.**

HMBC: see **Table. 2.4.**

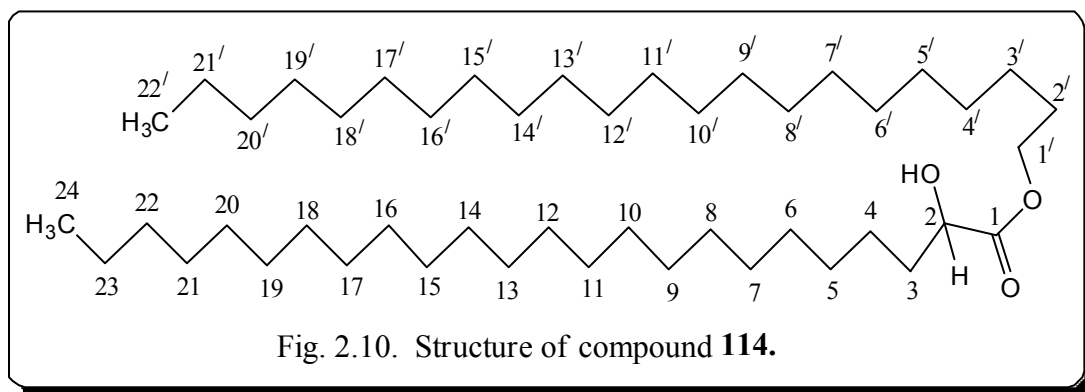


**Table. 2.4.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **docosyl -2-hydroxytetracosanoate (114)**  
(in  $\text{CDCl}_3$ )

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|------|---------------------|------------------------------------|--------------------------|
| 1    | 173.01              | ---                                | ---                      |
| 2    | 70.49               | 4.14, (m)                          | ---                      |
| 3    | 31.94               | 1.63, (m)                          | ---                      |
| 4    | 25.85               | 1.61, (m)                          | ---                      |
| 5    | 29.57               | 1.23, (m)                          | ---                      |
| 6    | 29.57               | 1.23, (m)                          | ---                      |
| 7    | 29.57               | 1.23, (m)                          | ---                      |
| 8    | 29.57               | 1.23, (m)                          | ---                      |
| 9    | 29.57               | 1.23, (m)                          | ---                      |
| 10   | 29.57               | 1.23, (m)                          | ---                      |
| 11   | 29.57               | 1.23, (m)                          | ---                      |
| 12   | 29.57               | 1.23, (m)                          | ---                      |
| 13   | 29.57               | 1.23, (m)                          | ---                      |
| 14   | 29.57               | 1.23, (m)                          | ---                      |
| 15   | 29.57               | 1.23, (m)                          | ---                      |
| 16   | 29.57               | 1.23, (m)                          | ---                      |

Continue

| C.No | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|------|------------|---------------------------|--------------------------|
| 17   | 29.57      | 1.23, (m)                 | ---                      |
| 18   | 29.57      | 1.23, (m)                 | ---                      |
| 19   | 29.57      | 1.23, (m)                 | ---                      |
| 20   | 29.57      | 1.23, (m)                 | ---                      |
| 21   | 29.57      | 1.23, (m)                 | ---                      |
| 22   | 29.57      | 1.23, (m)                 | ---                      |
| 24   | 14.08      | 0.85, (m)                 | ---                      |
| 23   | 22.68      | 1.52, (m)                 | ---                      |
| 1'   | 65.76      | 4.16, (m)                 | ---                      |
| 2'   | 24.72      | 1.60, (m)                 | ---                      |
| 3'   | 29.57      | 1.23, (m)                 | ---                      |
| 4'   | 29.57      | 1.23, (m)                 | ---                      |
| 5'   | 29.57      | 1.23, (m)                 | ---                      |
| 6'   | 29.57      | 1.23, (m)                 | ---                      |
| 7'   | 29.57      | 1.23, (m)                 | ---                      |
| 8'   | 29.57      | 1.23, (m)                 | ---                      |
| 9'   | 29.57      | 1.23, (m)                 | ---                      |
| 10'  | 29.57      | 1.23, (m)                 | ---                      |

Continue

| C.No | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|------|------------|---------------------------|--------------------------|
| 11'  | 29.57      | 1.23, (m)                 | ---                      |
| 12'  | 29.57      | 1.23, (m)                 | ---                      |
| 13'  | 29.57      | 1.23, (m)                 | ---                      |
| 14'  | 29.57      | 1.23, (m)                 | ---                      |
| 15'  | 29.57      | 1.23, (m)                 | ---                      |
| 16'  | 29.57      | 1.23, (m)                 | ---                      |
| 17'  | 29.57      | 1.23, (m)                 | ---                      |
| 18'  | 29.57      | 1.23, (m)                 | ---                      |
| 19'  | 29.57      | 1.23, (m)                 | ---                      |
| 20'  | 29.57      | 1.23, (m)                 | ---                      |
| 21'  | 22.68      | 1.52, (m)                 | ---                      |
| 22'  | 14.08      | 0.85, (m)                 | ---                      |
| - OH | ---        | 2.69 (d, 5.2)             | C-1, C-2                 |

The  $^1\text{H}$ NMR of compound **114** showed resonance at  $\delta$  4.16 (2H, m, H-1'), 4.14 (1H, m, H-2), 2.69 (1H, d,  $J$  = 5.2 Hz, -OH), 1.63 (2H, m, H-3), 1.61 (2H, m, H-4), 1.60 (2H, m, H-2'), 1.52 (4H, m, H-23, H-21'), 1.23 (72H, m, H-5 to H-22 and H-3' to H-20') and at  $\delta$  0.85 (6H, m, H-24 and H-22').

The  $^{13}\text{C}$ NMR of compound **114** gave nine signals for forty six carbon atoms which include two methyls, forty two methylenes, one methine and one quaternary carbon atom

on the basis of DEPT-135 and DEPT-90 spectra. The carbon signal at  $\delta$  14.08 was assigned to C-24 and C-22', the resonance at  $\delta$  22.68 was for C-23 and C-21', the carbon signal at  $\delta$  24.72 was allocated to C-2', the carbon at  $\delta$  25.85 was given to C-4,

the peak at  $\delta$  31.94 was for C-3, the carbon resonating at  $\delta$  65.76 was allocated to C-1', the peak at  $\delta$  70.49 was given to C-2, the carbon peak at  $\delta$  29.57 was allocated for C-5 to C-22 and C-3' to C-20' and the carbon peak at  $\delta$  173.01 was allocated to the quaternary carbon (C-1).

HMBC, HSQC, COSY and NOESY spectra were also recorded for compound **114**. All the spectral data confirmed it to be a long chain molecule. In the  $^1\text{H}$ NMR the peak appearing at  $\delta$  2.69 was not hydrogen according to the HSQC spectrum which indicates it to be a hydroxyl group. In the HMBC this peak ( $\delta$  2.69) showed correlation with  $\delta$  173.01 (C-1) and 70.46 (C-2) which confirmed to be attached to the carbon atom next to carbonyl group. In the NOESY spectrum the peak at  $\delta$  2.69 showed correlation with the proton peak at  $\delta$  1.23.

The problem of carbon chain length on both sides of the carbonyl group was resolved by considering the two mass fragments in the EIMS spectrum at 384 and 309. These two fragments were believed to be formed if the oxygen linkage between C-1 and C-1' was broken. Several other fragments in the EIMS spectrum were also evaluated for the length of the chain length on both sides of the carbonyl group but they did not give satisfactory results. Based on the above the compound is confirmed to be docosyl-2-hydroxytetracosanoate.

## 2.4. New source alkaloids from *Berberis jaeschkeana* Schneid var. *jaeschkeana* roots.

### 2.4.1. Berberine (115).<sup>31</sup>

Yellow needles (MeOH), m.p.: 207-208 °C (Lit 206-208 °C).<sup>33</sup>

Molecular formula: C<sub>20</sub>H<sub>18</sub>NO<sub>4</sub><sup>+</sup>

UV λ<sub>max</sub> (MeOH) (log ε): 266 (5.39), 349 (5.37), 427 (4.82) nm.

IR ν̄: 3020 (C-H, st. aromatic.), 1634, 1601 (C=C, st. aromatic.),

1458 (finger print region) cm.<sup>-1</sup>

EI-MS m/z: 336 [M<sup>+</sup>] (calculated for C<sub>20</sub>H<sub>18</sub>NO<sub>4</sub><sup>+</sup>).

EI-MS m/z (rel. int. %): 336 (6, M<sup>+</sup>), 335 (7), 321 (100), 306 (38), 292 (32), 278 (75), 263 (10), 248 (6), 219 (7), 191(7), 138(7), 95(3), 44(11).

<sup>1</sup>HNMR: see Table. 2.5.

<sup>13</sup>CNMR: see Table. 2.5.

HMBC: see Table. 2.5.

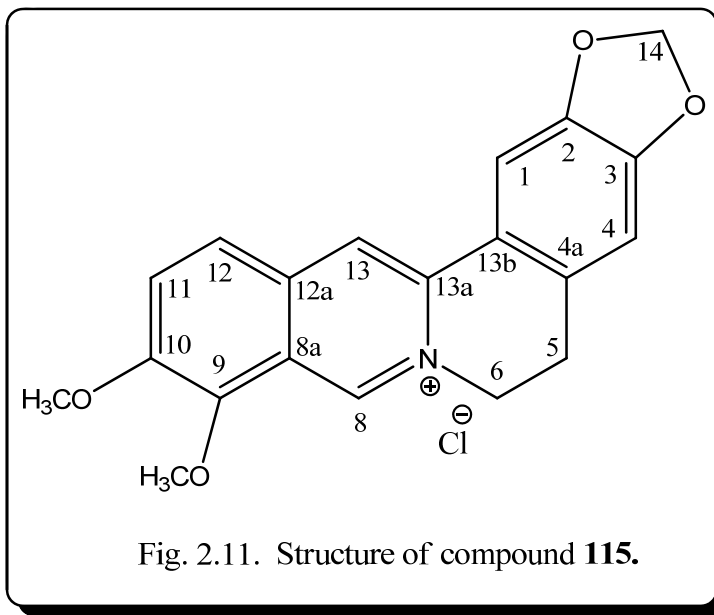


**Table. 2.5.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **berberine (115)** (in MeOD)

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)         |
|------|---------------------|------------------------------------|----------------------------------|
| 1    | 106.53              | 7.62, (s)                          | C-2, C-3, C-4a, C-13b, C-13a     |
| 2    | 149.90              | ---                                | ---                              |
| 3    | 152.16              | ---                                | ---                              |
| 4    | 109.39              | ---                                | ---                              |
| 4a   | 131.88              | ---                                | ---                              |
| 5    | 28.19               | 3.24, (t, 10.0)                    | C-6, C-4a, C-13b, C-4            |
| 6    | 57.19               | 4.92, (t, 10.0)                    | C-13a, C-8, C-4a, C-5            |
| 8    | 146.40              | 9.75, (s)                          | C-13a, C-6, C-8a, C-12a, C-9     |
| 8a   | 123.30              | ---                                | ---                              |
| 9    | 145.73              | ---                                | ---                              |
| 10   | 152.01              | ---                                | ---                              |
| 11   | 128.03              | 8.10, (d, 9.0 Hz)                  | C-10, C-12, C-12a, C-9           |
| 12   | 124.52              | 7.98, (d, 9.0 Hz)                  | C-11, C-10, C-12a,<br>C-8a, C-13 |
| 12a  | 135.12              | ---                                | ---                              |
| 13   | 121.47              | 8.67, (s)                          | C-12a, C-8a, C-13a, C-12         |
| 13a  | 139.63              | ---                                | ---                              |
| 13b  | 121.85              | ---                                | ---                              |

Continue

| C.No   | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|--------|------------|---------------------------|--------------------------|
| 14     | 103.68     | 6.09 (s)                  | C-2, C-3                 |
| 9-OMe  | 62.56      | 4.19, (s)                 | C-9                      |
| 10-OMe | 57.63      | 4.09, (s)                 | C-10                     |

The  $^1\text{H}$ NMR of compound **115** showed six aromatic protons at  $\delta$  7.62, (1H, s, H-1), 6.94(1H, s, H-4), 9.75 (1H, s, H-8), 8.10 (1H, d,  $J$  = 9.0 Hz, H-11), 7.98 (1H, d,  $J$  = 9.0 Hz, H-12) and 8.67(1H, s, H-13); three methylene at  $\delta$  6.09 (2H, s, H-14), 3.24 (2H, t,  $J$  = 10.0 Hz, H-5) and 4.92 (2H, s,  $J$  = 10.0 Hz, H-6) and two methoxy peaks at  $\delta$  4.19 (3H, s, 9-OMe) and 4.09 (3H, s, 10-OMe).

The  $^{13}\text{C}$ NMR of compound **115** showed twenty carbon atoms which included six methine, three methylenes, two methoxy and nine quaternary carbon atoms on the basis of DEPT-90 and DEPT-135 spectra.

In the HMBC spectra of compound **115** the methine proton at  $\delta$  7.62 (H-1) correlated to  $\delta$  149.90 (C-2), 152.16 (C-3), 131.88 (C-4a), 121.85 (C-13b) and 139.63 (C-13a), the methine proton appearing at  $\delta$  6.94 (H-4) showed correlations with  $\delta$  149.90 (C-2), 152.16 (C-3), 131.88 (C-4a) 121.85 (C-13b) and 28.19 (C-5), the methine proton at  $\delta$  9.75 (H-8) showed correlations with  $\delta$  139.63 (C-13a), 57.19 (C-6), 123.30 (C-8a), 135.12 (C-12a) and 145.73 (C-9), the methine proton chemical shift at  $\delta$  8.10 (H-11) correlated to  $\delta$  152.01 (C-10), 124.52 (C-12), 135.12 (C-12a) and 145.73 (C-9), the methine proton appearing at  $\delta$  7.98 (H-12) showed correlations with  $\delta$  128.03 (C-11),

152.01 (C-10), 135.12 (C-12a), 123.30 (C-8a) and 121.47 (C-13), the methine proton at  $\delta$  8.67 (H-13) correlated to  $\delta$  135.12 (C-12a), 123.30 (C-8a), 139.63 (C-13a) and 124.52 (C-12).

The methylene protons appearing at  $\delta$  6.09 (H-14) correlated to  $\delta$  149.90 (C-2) and 152.16 (C-3), the methylene protons appearing at  $\delta$  3.24 (H-5) correlated to  $\delta$  57.19 (C-6), 131.88 (C-4a), 121.85 (C-13b) and 109.39 (C-4), similarly the methylene protons at  $\delta$  4.92 (H-6) showed correlations with  $\delta$  139.63 (C-13a), 146.40 (C-8), 131.88 (C-4a) and 28.19 (C-5) and the two methoxys at  $\delta$  4.19 (9-OMe) and 4.09 (10-OMe) correlated to  $\delta$  145.73 (C-9) and 152.01 (C-10) respectively.

The above spectral data for compound **115** was in complete agreement with the reported values for berberine.<sup>31</sup>

#### 2.4.2. Palmatine (116).<sup>32</sup>

Brownish yellow needles (MeOH), m.p.: 202-203°C (Lit: 202-204°C).<sup>33</sup>

Molecular formula: C<sub>21</sub>H<sub>22</sub>NO<sub>4</sub><sup>+</sup>

UV  $\lambda_{\max}$  (MeOH) (log  $\epsilon$ ): 347 (5.42), 430 (4.88) nm.

IR  $\bar{\nu}$ : 2992 (C-H, st. sat.) 1624, 1522 (C=C, st. aromatic.), 1390,

1240 (finger print region) cm.<sup>-1</sup>

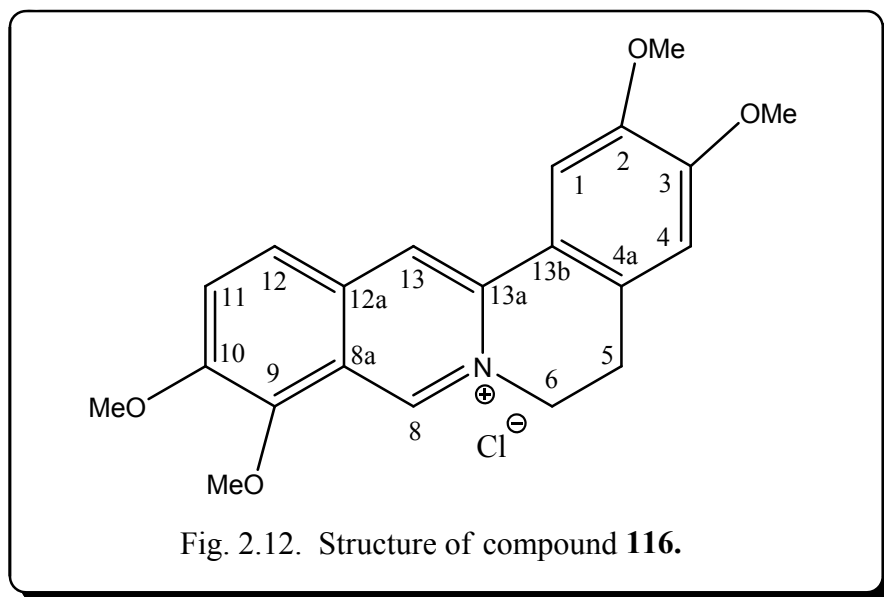
EI-MS m/z: 352 [M<sup>+</sup>] (calculated for C<sub>21</sub>H<sub>22</sub>NO<sub>4</sub><sup>+</sup>).

EI-MS m/z (rel. int. %): 352 (11, M<sup>+</sup>), 351 (35), 337 (100), 294 (46), 278 (34), 219 (7), 190(8), 138(5), 94(5), 43(10).

$^1\text{H}$ NMR: see Table. 2.6.

$^{13}\text{C}$ NMR: see Table. 2.6.

HMBC: see Table. 2.6.



$^1\text{H}$ NMR of compound **116** showed six aromatic protons at  $\delta$  7.72, (1H, s, H-1), 7.08 (1H, s, H-4), 9.89 (1H, s, H-8), 8.20 (1H, d,  $J$  = 9.0 Hz, H-11), 8.04 (1H, d,  $J$  = 9.0 Hz, H-12) and 9.09 (1H, s, H-13), three methylenes at  $\delta$  6.09 (2H, s, H-14), 3.24 (2H, t,  $J$  = 10.0 Hz, H-5) and 4.92 (2H, s,  $J$  = 10.0 Hz, H-6) and two methoxy peaks at  $\delta$  4.19 (3H, s, 9-OMe) and 4.09 (3H, s, 10-OMe).

The  $^{13}\text{C}$ NMR spectrum of compound **116** showed twenty one carbon atoms which included six methine, two methylene, four methoxy and nine quaternary carbon atoms on the basis of DEPT-q spectral data.

**Table. 2.6.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **palmatine (116)** (in DMSO- $d_6$ )

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)         |
|------|---------------------|------------------------------------|----------------------------------|
| 1    | 108.78              | 7.72, (s)                          | C-2, C-3, C-4a, C-13b, C-13a     |
| 2    | 150.20              | ---                                | ---                              |
| 3    | 151.46              | ---                                | ---                              |
| 4    | 111.27              | 7.08, (s)                          | C-2, C-3, C-13b, C-4a, C-5       |
| 4a   | 128.56              | ---                                | ---                              |
| 5    | 25.94               | 3.20, (t, 7.0 Hz)                  | C-6, C-4a, C-13b, C-4            |
| 6    | 55.32               | 4.95, (t, 7.0 Hz)                  | C-13a, C-8, C-4a, C-5            |
| 8    | 145.39              | 9.89, (s)                          | C-13a, C-6, C-8a, C-12a, C-9     |
| 8a   | 121.31              | ---                                | ---                              |
| 9    | 143.60              | ---                                | ---                              |
| 10   | 149.78              | ---                                | ---                              |
| 11   | 126.73              | 8.20, (d, 9.0 Hz)                  | C-10, C-12, C-12a, C-9           |
| 12   | 123.40              | 8.04, (d, 9.0 Hz)                  | C-11, C-10, C-12a,<br>C-8a, C-13 |
| 12a  | 133.10              | ---                                | ---                              |
| 13   | 119.91              | 9.09, (s)                          | C-12a, C-8a, C-13a, C-12         |
| 13a  | 137.65              | ---                                | ---                              |

Continue

| C.No   | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|--------|------------|---------------------------|--------------------------|
| 13b    | 118.90     | ---                       | ---                      |
| 2-OMe  | 56.17      | 3.93, (s)                 | C-2                      |
| 3-OMe  | 55.83      | 3.86, (s)                 | C-3                      |
| 9-OMe  | 61.88      | 4.09, (s)                 | C-9                      |
| 10-OMe | 57.02      | 4.06, (s)                 | C-10                     |

The methine proton at  $\delta$  9.89 (H-8) showed correlations with  $\delta$  137.65 (C-13a), 55.32 (C-6), 121.31 (C-8a), 133.10 (C-12a) and 143.60 (C-9), the methine proton at  $\delta$  8.20 (H-11) correlated to  $\delta$  149.78 (C-10), 123.40 (C-12), 133.10 (C-12a) and 143.60 (C-9), the methine proton appearing at  $\delta$  8.04 (H-12) showed correlations with  $\delta$  126.73 (C-11), 1149.78 (C-10), 133.10 (C-12a), 121.31 (C-8a) and 119.91 (C-13), the methine proton at  $\delta$  9.09 (H-13) correlated to  $\delta$  133.10 (C-12a), 121.31 (C-8a), 137.65 (C-13a) and 123.40 (C-12).

The methylene protons appearing at  $\delta$  3.20 (H-5) correlated to  $\delta$  55.32 (C-6), 128.56 (C-4a), 118.90 (C-13b) and 111.27 (C-4), the methylene protons at  $\delta$  4.95 (H-6) showed correlations with  $\delta$  137.65 (C-13a), 145.39 (C-8), 128.56 (C-4a) and 25.94 (C-5) and four methoxy peaks at  $\delta$  4.09 (9-OMe), 4.06 (10-OMe), 3.93 (2-OMe) and 3.86 (3-OMe) correlated to  $\delta$  143.60 (C-9), 149.78 (C-10), 150.20 (C-2) and 151.46 (C-3) respectively.

Based on the above Compound **116** was established as palmatine and confirmed by comparison with the literature.<sup>32</sup>

**2.4.3. Columbamine (117).**<sup>33</sup>

Yellow needle like crystals (MeOH), m.p.: 281-283 °C (Lit: 280-282 °C).<sup>33</sup>

Molecular formula: C<sub>20</sub>H<sub>20</sub>NO<sub>4</sub><sup>+</sup>

UV λ<sub>max</sub> (MeOH) (log ε): 276 (5.30), 349 (5.43) nm.

IR ν̄: 3310 (O-H, st.), 1605, 1510 (C=C, st. aromatic.), 1456,

1316 (finger print region) cm.<sup>-1</sup>

EI-MS: m/z 338 [M<sup>+</sup>] (calculated for C<sub>21</sub>H<sub>22</sub>NO<sub>4</sub><sup>+</sup>).

EI-MS: m/z (rel. int. %): 338 (7, M<sup>+</sup>), 323 (100), 305 (35), 291 (30), 278 (73), 264 (10), 217 (6), 191(8), 139 (5), 94(5), 44(8).

<sup>1</sup>HNMR: see **Table. 2.7.**

<sup>13</sup>CNMR: see **Table. 2.7.**

HMBC: see **Table. 2.7.**

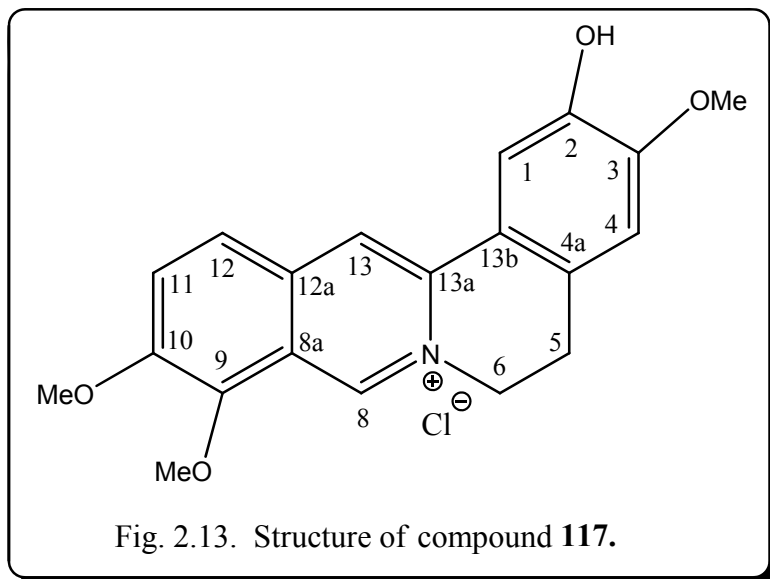


**Table. 2.7.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **columbamine (117)** (in  $\text{CDCl}_3$ )

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)         |
|------|---------------------|------------------------------------|----------------------------------|
| 1    | 115.10              | 7.35, (s)                          | C-2, C-3, C-4a, C-13b, C-13a     |
| 2    | 144.21              | ---                                | ---                              |
| 3    | 150.78              | ---                                | ---                              |
| 4    | 110.20              | 6.54, (s)                          | C-2, C-3, C-13b, C-4a, C-5       |
| 4a   | 134.01              | ---                                | ---                              |
| 6    | 56.11               | 4.87, (m)                          | C-13a, C-8, C-4a, C-5            |
| 8    | 145.11              | 9.52, (s)                          | C-13a, C-6, C-8a, C-12a, C-9     |
| 8a   | 118.27              | ---                                | ---                              |
| 9    | 148.88              | ---                                | ---                              |
| 10   | 150.86              | ---                                | ---                              |
| 11   | 127.91              | 7.84, (d, 8.0 Hz)                  | C-10, C-12, C-12a, C-9           |
| 12   | 124.83              | 7.94, (d, 8.0 Hz)                  | C-11, C-10, C-12a,<br>C-8a, C-13 |
| 12a  | 129.55              | ---                                | ---                              |
| 13   | 121.01              | 8.45, (s)                          | C-12a, C-8a, C-13a, C-12         |
| 13a  | 139.60              | ---                                | ---                              |
| 13b  | 122.21              | ---                                | ---                              |

Continue

| C.No   | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|--------|------------|---------------------------|--------------------------|
| 3-OMe  | 57.01      | 3.90, (s)                 | C-2, C-3                 |
| 9-OMe  | 57.94      | 4.05, (s)                 | C-9                      |
| 10-OMe | 62.88      | 4.15, (s)                 | C-10                     |

The  $^1\text{H}$ NMR of compound **117** showed six aromatic protons at  $\delta$  7.35 (1H, s, H-1), 6.54 (1H, s, H-4), 9.52 (1H, s, H-8), 7.84 (1H, d,  $J$  = 8.0 Hz, H-11), 7.94 (1H, d,  $J$  = 8.0 Hz, H-12) and 8.45 (1H, s, H-13), two methylenes at  $\delta$  3.20 (2H, t,  $J$  = 7.0 Hz, H-5) and 4.87 (2H, t,  $J$  = 7.0 Hz, H-6) and three methoxy peaks at  $\delta$  4.05 (3H, s, 9-OMe), 4.15 (3H, s, 10-OMe) and 3.90 (3H, s, 3-OMe).

The  $^{13}\text{C}$ NMR of compound **117** showed twenty carbon atoms which include six methine, two methylene, three methoxy and nine quaternary carbon atoms on the basis of DEPT-q spectra.

In the HMBC spectra of compound **117** the methine proton at  $\delta$  7.35 (H-1) correlated to  $\delta$  144.21 (C-2), 150.78 (C-3), 134.01 (C-4a), 122.21 (C-13b) and 139.33 (C-13a), the methine proton appearing at  $\delta$  6.54 (H-4) showed correlations with  $\delta$  144.21 (C-2), 150.78 (C-3), 134.01 (C-4a), 122.21 (C-13b) and 27.12 (C-5), the methine proton at  $\delta$  9.52 (H-8) showed correlations with  $\delta$  139.33 (C-13a), 56.11 (C-6), 118.27 (C-8a), 129.55 (C-12a) and 148.88 (C-9), the methine proton at  $\delta$  7.84 (H-11) correlated to  $\delta$  150.86 (C-10), 124.83 (C-12), 129.55 (C-12a) and 148.88 (C-9), the methine proton appearing at  $\delta$  7.94 (H-12) showed correlations with  $\delta$  127.91 (C-11), 150.86 (C-10),

129.55 (C-12a), 118.27 (C-8a) and 121.01 (C-13), the methine proton at  $\delta$  8.45 (H-13) correlated to  $\delta$  129.55 (C-12a), 118.27 (C-8a), 139.33 (C-13a) and 124.83 (C-12), the methylene protons appearing at  $\delta$  3.20 (H-5) correlated to  $\delta$  56.11 (C-6), 134.01 (C-4a), 122.21 (C-13b) and 110.20 (C-4), similarly the methylene protons at  $\delta$  4.87 (H-6) showed correlations with  $\delta$  139.33 (C-13a), 145.11 (C-8), 134.01 (C-4a) and 27.12 (C-5) and three methoxy peaks at  $\delta$  4.05 (9-OMe), 4.15 (10-OMe) and 3.90 (3-OMe) correlated to  $\delta$  148.88 (C-9), 150.86 (C-10) and 150.78 (C-3) respectively.

The HMBC and other spectral data for compound **117** on comparison with the literature confirmed it to be columbamine.<sup>33</sup>

#### 2.4.4. Pseudoberberine (118).<sup>31</sup>

Yellow needles (MeOH), m.p.: 206-208 °C.

Molecular formula: C<sub>20</sub>H<sub>18</sub>NO<sub>4</sub><sup>+</sup>

UV  $\lambda_{\max}$  (MeOH) (log  $\epsilon$ ): 347 (5.48), 429 (5.07) nm.

IR  $\bar{\nu}$ : 3037 (C-H, st. aromatic.), 1630, 1601 (C=C, st. aromatic.),

1458 (finger print region) cm.<sup>-1</sup>

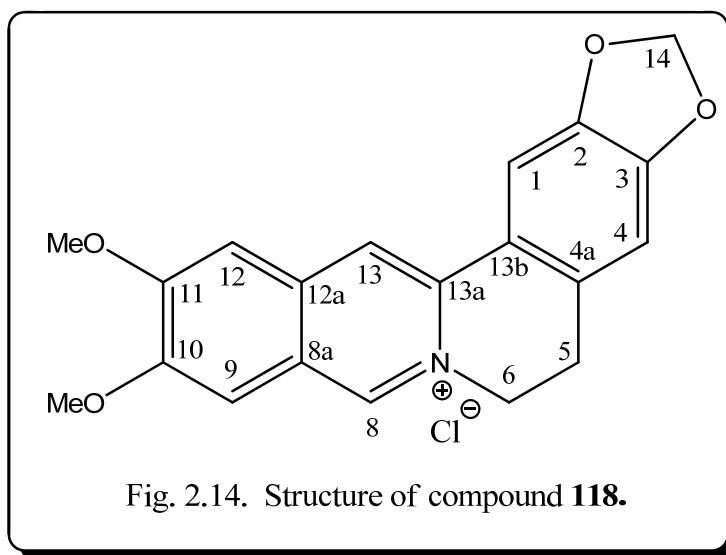
EI-MS m/z: 336 [M<sup>+</sup>] (calculated for C<sub>20</sub>H<sub>18</sub>NO<sub>4</sub><sup>+</sup>).

EI-MS m/z (rel. int. %): 336 (10, M<sup>+</sup>), 335 (6), 321 (100), 306 (36), 292 (37), 278 (71), 263 (10), 248 (8), 218 (6), 192(9), 138(5), 96(3), 44(10).

<sup>1</sup>HNMR: see **Table. 2.8.**

$^{13}\text{C}$ NMR: see **Table 2.8**.

HMBC: see **Table 2.8**.



The  $^1\text{H}$ NMR of compound **118** showed six aromatic protons at  $\delta$  7.33 (1H, s, H-1), 6.81 (1H, s, H-4), 10.40 (1H, s, H-8), 7.72 (1H, s, H-9), 7.81 (1H, s, H-12) and 8.51 (1H, s, H-13), three methylenes at  $\delta$  6.09 (2H, s, H-14), 3.31 (2H, t,  $J$  = 9.4 Hz, H-5) and 4.95 (2H, s,  $J$  = 9.4 Hz, H-6) and the two methoxy peaks at  $\delta$  3.96 (3H, s, 10-OMe) and 3.83 (3H, s, 11-OMe).

The  $^{13}\text{C}$ NMR of compound **118** showed twenty carbon atoms which included six methine, three methylene, two methoxy and nine quaternary carbon atoms according to DEPT-q spectra.

**Table. 2.8.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **pseudoerberine (118)** (in MeOD)

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)         |
|------|---------------------|------------------------------------|----------------------------------|
| 1    | 107.22              | 7.33, (s)                          | C-2, C-3, C-4a, C-13b, C-13a     |
| 2    | 150.15              | ---                                | ---                              |
| 3    | 153.10              | ---                                | ---                              |
| 4    | 108.98              | 6.81, (s)                          | C-2, C-3, C-13b, C-4a, C-5       |
| 4a   | 129.88              | ---                                | ---                              |
| 5    | 27.65               | 3.31, (t, 9.4 Hz)                  | C-6, C-4a, C-13b, C-4            |
| 6    | 56.33               | 4.95, (t, 9.4 Hz)                  | C-13a, C-8, C-4a, C-5            |
| 8    | 145.51              | 10.40, (s)                         | C-13a, C-6, C-8a, C-12a, C-9     |
| 8a   | 124.30              | ---                                | ---                              |
| 9    | 127.71              | 7.72, (s)                          | C-10, C-8a, C-12a, C-11, C-8     |
| 10   | 151.06              | ---                                | ---                              |
| 11   | 147.02              | ---                                | ---                              |
| 12   | 123.22              | 7.81, (s)                          | C-11, C-10, C-12a,<br>C-8a, C-13 |
| 12a  | 134.10              | ---                                | ---                              |
| 13   | 119.88              | 8.51, (s)                          | C-12a, C-8a, C-13a, C-12         |
| 13a  | 138.60              | ---                                | ---                              |
| 13b  | 120.86              | ---                                | ---                              |

| C.No   | $\delta_C$ | $\delta_H$ (mult, J Hz) | HMBC (H $\rightarrow$ C) |
|--------|------------|-------------------------|--------------------------|
| 14     | 103.54     | 6.09, (s)               | C-2, C-3                 |
| 10-OMe | 56.76      | 3.96, (s)               | C-10                     |
| 11-OMe | 55.61      | 3.83, (s)               | C-11                     |

In the HMBC spectra of compound **118** the methine proton at  $\delta$  7.33 (H-1) correlated to  $\delta$  150.15 (C-2), 153.10 (C-3), 129.88 (C-4a), 120.86 (C-13b) and 138.60 (C-13a), the methine proton appearing at  $\delta$  6.81 (H-4) showed correlations with  $\delta$  150.15 (C-2), 153.10 (C-3), 129.88 (C-4a), 120.86 (C-13b) and 27.65 (C-5), the methine proton at  $\delta$  10.40 (H-8) showed correlations with  $\delta$  138.60 (C-13a), 56.33 (C-6), 124.30 (C-8a), 134.10 (C-12a) and 127.71 (C-9), the methine proton at  $\delta$  7.72 (H-9) correlated to  $\delta$  151.06 (C-10), 124.30 (C-8a), 134.10 (C-12a), 147.02 (C-11) and 145.51 (C-8), the methine proton appearing at  $\delta$  7.81 (H-12) showed correlations with  $\delta$  147.02 (C-11), 151.06 (C-10), 134.10 (C-12a), 124.30 (C-8a), and 119.88 (C-13), the methine proton at  $\delta$  8.51 (H-13) correlated to  $\delta$  134.10 (C-12a), 124.30 (C-8a), 138.60 (C-13a) and 123.22 (C-12).

The methylene protons appearing at  $\delta$  6.09 (H-14) correlated to  $\delta$  150.15 (C-2) and 153.10 (C-3), the methylene protons appearing at  $\delta$  3.31 (H-5) correlated to  $\delta$  56.33 (C-6), 129.88 (C-4a), 120.86 (C-13b) and 108.98 (C-4), similarly the methylene protons at  $\delta$  4.95 (H-6) showed correlations with  $\delta$  138.60 (C-13a), 145.51 (C-8), 129.88 (C-4a) and 27.65 (C-5) and two methoxy peaks at  $\delta$  3.96 (10-OMe) showed correlation with  $\delta$  151.06 (C-10) and the methoxy peak at  $\delta$  3.83 (10-OMe) correlated to  $\delta$  147.02 (C-11).

The above spectral data for compound **118** was in complete agreement with the literature values for pseudoberberine.<sup>31</sup>

## 2.5. New source non-alkaloids from *Berberis jaeschkeana* Schneid var. *jaeschkeana* roots.

### 2.5.1. Gallic acid (**119**).<sup>34</sup>

White crystalline powder (MeOH), m.p.: 249-251 °C (Lit: 250-252 °C).<sup>34</sup>

Molecular formula: C<sub>7</sub>H<sub>6</sub>O<sub>5</sub>

UV  $\lambda_{\text{max}}$  (MeOH) (log  $\epsilon$ ): 273 (4.83) nm.

IR  $\bar{\nu}$ : 3370 (O-H, st.), 2996 (C-H, st. sat.), 1715 (C=O, st.) cm.<sup>-1</sup>

EI-MS m/z: 170 [M<sup>+</sup>] (calculated for C<sub>7</sub>H<sub>6</sub>O<sub>5</sub>).

EI-MS m/z (rel. int. %): 170 (98, M<sup>+</sup>), 154 (94), 127 (15), 106 (7), 79 (18).

<sup>1</sup>HNMR: see **Table. 2.9**.

<sup>13</sup>CNMR: see **Table. 2.9**.

HMBC: see **Table. 2.9**.

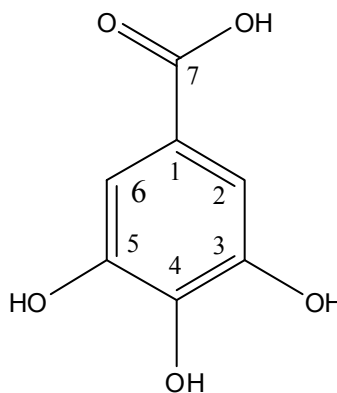


Fig. 2.15. Structure of compound **119**.

**Table. 2.9.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **gallic acid (119)** (in  $\text{DMSO-d}_6$ )

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|------|---------------------|------------------------------------|--------------------------|
| 1    | 121.56              | ---                                | ---                      |
| 2    | 109.77              | 6.94, (s)                          | C-1, C-3, C-4, C-7, C-6  |
| 3    | 146.13              | ---                                | ---                      |
| 4    | 138.89              | ---                                | ---                      |
| 5    | 146.13              | ---                                | ---                      |
| 6    | 109.77              | 6.94, (s)                          | C-1, C-3, C-4, C-7, C-6  |
| 7    | 169.11              | ---                                | ---                      |

The  $^1\text{H}$ NMR spectrum of compound **119** showed one aromatic proton at  $\delta$  6.94 (2H, s, H-2, 6).

The  $^{13}\text{C}$ NMR for compound **119** showed the presence of seven carbon atoms which include two methine and five quaternary carbon atoms according to DEPT-q spectra.

The obtained spectral data for compound **119** was compared with literature and compound **119** was found to be gallic acid.<sup>34</sup>

### 2.5.2. $\beta$ -Sitosterol (**120**).<sup>35</sup>

White powder ( $\text{CHCl}_3$ ), m.p.: 134-136 °C (Lit: 134-135 °C).<sup>35</sup>

Molecular formula:  $\text{C}_{29}\text{H}_{50}\text{O}$

UV  $\lambda_{\text{max}}$  ( $\text{CH}_2\text{Cl}_2$ ) (log  $\epsilon$ ): 240.5 (5.22) nm.

IR  $\bar{\nu}$ : 3376 (O-H, st.), 2931 (C-H, st. sat.), 1636 (C=C, st.), 1463, 1442,

1375 (finger print region)  $\text{cm}^{-1}$

EI-MS:  $m/z$  414 [ $M^+$ ] (calculated for  $C_{29}H_{50}O$ ).

EI-MS:  $m/z$  (rel. int. %): 414 (100,  $M^+$ ), 396 (89), 382 (45), 381(71), 329 (72), 303 (81), 255 (72), 213 (76), 161 (57), 145 (65), 107(65), 81(55), 55 (56).

$^1\text{H}$ NMR: see **Table. 2.10**.

$^{13}\text{C}$ NMR: see **Table. 2.10**.

HMBC: see **Table. 2.10**.

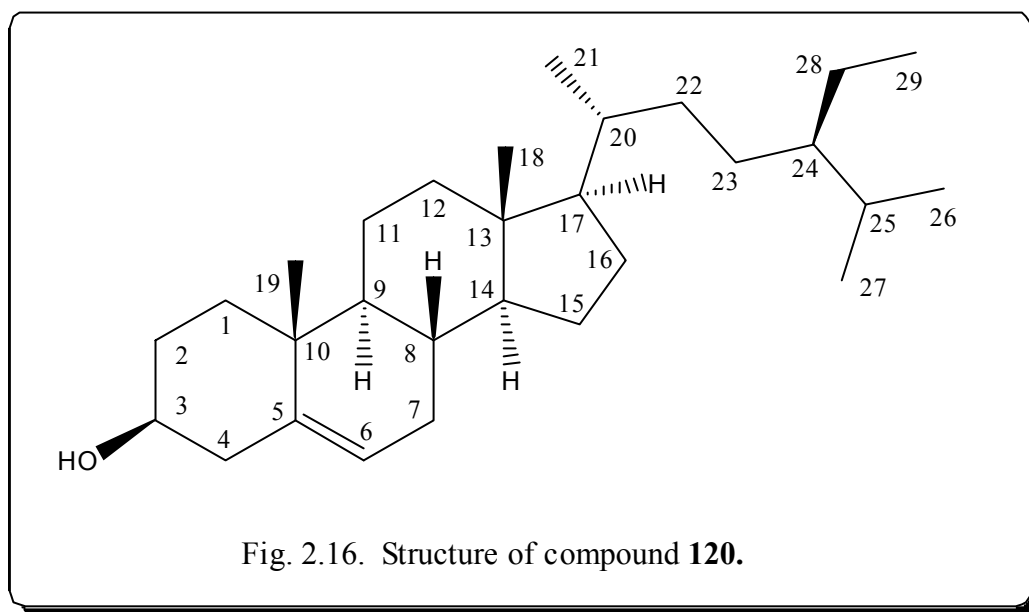


**Table. 2.10.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of  **$\beta$ -sitosterol (118)** (in  $\text{CDCl}_3$ )

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|------|---------------------|------------------------------------|--------------------------|
| 1    | 37.27               | 1.06, (2H, m)                      | ---                      |
| 2    | 31.68               | 1.23 and 1.47, (m)                 | ---                      |
| 3    | 71.82               | 3.50, (m)                          | ---                      |
| 4    | 39.79               | 1.98, (br.t, 12.4)                 | C-2, C-3, C-5, C-6, C-10 |
| 5    | 140.77              | ---                                | ---                      |
| 6    | 121.72              | 5.33, (1H, d, 2.5)                 | C-4, C-8, C-10           |
| 7    | 31.92               | 1.42 and 1.93, (m)                 | ---                      |
| 8    | 33.72               | 1.42, (m)                          | ---                      |
| 9    | 50.15               | 0.84, (m)                          | ---                      |
| 10   | 36.52               | ---                                | ---                      |
| 11   | 21.10               | 1.47, (m)                          | ---                      |
| 12   | 42.32               | 2.21, (m)                          | ---                      |
| 13   | 38.85               | ---                                | ---                      |
| 14   | 56.78               | 0.94, (br.s)                       | ---                      |
| 15   | 24.31               | 1.54, (m)                          | ---                      |
| 16   | 28.25               | 1.19, (m)                          | ---                      |
| 17   | 56.07               | 1.06, (m)                          | ---                      |
| 18   | 11.87               | 0.65, (s)                          | C-12, C-13, C-14, C-17   |

Continue

| C.No | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|------|------------|---------------------------|--------------------------|
| 19   | 19.40      | 0.98, (s)                 | C-1, C-9, C-10           |
| 20   | 36.15      | 1.32, (m)                 | ---                      |
| 21   | 18.79      | 0.89, (d, 6.4)            | C-17, C-20, C-22         |
| 22   | 33.96      | 1.26 and 1.54, (m)        | ---                      |
| 23   | 26.10      | 1.11, (m)                 | ---                      |
| 24   | 45.85      | 0.90, (m)                 | ---                      |
| 25   | 29.17      | 1.23, (m)                 | ---                      |
| 26   | 19.05      | 0.78, (d, 8.4)            | C-24, C-25, C-27         |
| 27   | 19.82      | 0.82, (d, 8.4)            | C-24, C-25, C-26         |
| 28   | 23.08      | 1.19 and 1.23, (m)        | ---                      |
| 29   | 11.99      | 0.80, (t, 8.4)            | C-24, C-28               |

The  $^1\text{H}$ NMR of compound **120** showed two methyl groups at  $\delta$  0.65 (3H, s, H-18) and  $\delta$  0.98 (3H, s, H-19), three methyl doublets at  $\delta$  0.89 (3H, d,  $J$  = 6.4 Hz, H-21), 0.78 (3H, d,  $J$  = 8.4 Hz, H-26) and 0.82 (3H, d,  $J$  = 8.4 Hz, H-27), one methyl triplet at  $\delta$  0.80 (3H, t,  $J$  = 8.4 Hz, H-29), an olefinic proton broad doublet at  $\delta$  5.33 (1H, d,  $J$  = 2.5 Hz, H-6) and a multiplet at  $\delta$  3.50 (H-3).

The  $^{13}\text{C}$ NMR of compound **120** showed the presence of twenty nine carbon atoms which included six methyls at  $\delta$  11.87 (C-18), 19.40 (C-19), 18.79 (C-21), 19.05 (C-26), 19.82 (C-27) and 11.99 (C-29), eleven methylene carbons at  $\delta$  37.27 (C-1), 31.68 (C-2), 39.79

(C-4), 31.92 (C-7), 21.10 (C-11), 42.32 (C-12), 24.31 (C-15), 28.25 (C-16), 33.96 (C-22), 26.10 (C-23), 23.08 (C-28).

Nine methine carbons at  $\delta$  71.82 (C-3), 121.72 (C-6), 33.72 (C-8), 50.15 (C-9), 56.78 (C-14), 56.07 (C-17), 36.15 (C-20), 45.85 (C-24) and 11.99 (C-29) and three quaternary carbons at  $\delta$  140.77 (C-5), 36.52 (C-10) and 38.85 (C-13) according to DEPT-135 and DEPT-90 spectra.

The  $^1\text{H}$ NMR,  $^{13}\text{C}$ NMR and EIMS data for compound **120** was compared with the literature which confirmed compound **120** to be  $\beta$ -sitosterol.<sup>35</sup>

## 2.6. A new source alkaloid from *Berberis jaeschkeana* Schneid var. *jaeschkeana* stem.

### 2.6.1. 8-Hydroxy-7, 8-dihydroberberine (121).<sup>31</sup>

Physical state: yellow amorphous powder (MeOH).

Melting point: 145-147 °C (Lit: 144-149 °C).<sup>40</sup>

Molecular formula:  $\text{C}_{20}\text{H}_{19}\text{NO}_5$

UV  $\lambda_{\text{max}}$  (MeOH) (log  $\epsilon$ ): 254, 340 (5.32) nm.

IR  $\bar{\nu}$ : 3356 (O-H, st.), 2998 (C-H, st. sat.), 1574 (C=C, st. aromatic.),

1431 (finger print region)  $\text{cm}^{-1}$

EI-MS  $m/z$ : 353 [ $\text{M}^+$ ] (calculated for  $\text{C}_{20}\text{H}_{19}\text{NO}_5$ ).

EI-MS  $m/z$  (rel. int. %): 353 (19,  $M^+$ ), 352 (7), 336 (98), 321 (76), 306 (33), 291 (26), 276 (40), 254 (11), 190(7), 138(6), 95(2).

$^1\text{H}$ NMR: see **Table. 2.11.**

$^{13}\text{C}$ NMR: see **Table. 2.11.**

HMBC: see **Table. 2.11.**

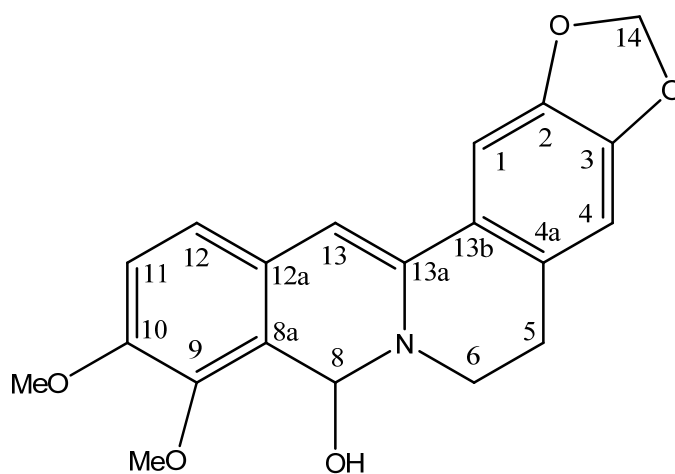


Fig. 2.17. Structure of compound **121**.

**Table. 2.11.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **8-hydroxy-7, 8-dihydroberberine (121)**  
(in MeOD)

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)         |
|------|---------------------|------------------------------------|----------------------------------|
| 1    | 105.10              | 7.28, (s)                          | C-2, C-3, C-4a, C-13b, C-13a     |
| 2    | 147.66              | ---                                | ---                              |
| 3    | 148.03              | ---                                | ---                              |
| 4    | 108.41              | 6.71, (s)                          | C-2, C-3, C-13b, C-4a, C-5       |
| 4a   | 129.78              | ---                                | ---                              |
| 5    | 29.39               | 2.79, (t, 6.0)                     | C-6, C-4a, C-13b, C-4            |
| 6    | 46.22               | 3.60, (t, 6.0)                     | C-13a, C-8, C-4a, C-5            |
| 8    | 77.80               | 6.27, (s)                          | C-13a, C-6, C-8a, C-12a, C-9     |
| 8a   | 124.02              | ---                                | ---                              |
| 9    | 147.09              | ---                                | ---                              |
| 10   | 151.01              | ---                                | ---                              |
| 11   | 117.08              | 7.02, (d, 7.2)                     | C-10, C-12, C-12a, C-9           |
| 12   | 120.12              | 6.97, (d, 7.2)                     | C-11, C-10, C-12a,<br>C-8a, C-13 |
| 12a  | 127.29              | ---                                | ---                              |
| 13   | 97.05               | 6.13, (s)                          | C-12a, C-8a, C-13a, C-12         |
| 13a  | 137.61              | ---                                | ---                              |

Continue

| C.No   | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|--------|------------|---------------------------|--------------------------|
| 13b    | 125.80     | ---                       | ---                      |
| 14     | 101.64     | 5.95, (s)                 | C-2, C-3                 |
| 9-OMe  | 62.07      | 3.94, (s)                 | C-9                      |
| 10-OMe | 56.22      | 3.87, (s)                 | C-10                     |

The  $^1\text{H}$ NMR of compound **121** showed six methine protons at  $\delta$  7.28 (1H, s, H-1), 6.71 (1H, s, H-4), 6.27 (1H, s, H-8), 7.02 (1H, d,  $J$  = 7.2 Hz, H-11), 6.97 (1H, d,  $J$  = 7.2 Hz, H-12) and 6.13 (1H, s, H-13), three methylene protons at  $\delta$  5.95 (2H, s, H-14), 2.79 (2H, t,  $J$  = 6.0 Hz, H-5) and 3.60 (2H, s,  $J$  = 6.0 Hz, H-6) and two methoxy resonances at  $\delta$  3.94 (3H, s, 9-OMe) and 3.87 (3H, s, 10-OMe).

The  $^{13}\text{C}$ NMR of compound **121** showed twenty carbon atoms which included six methine, three methylenes, two methoxys and nine quaternary carbon atoms on the basis of DEPT-135 and DEPT-90 spectra.

In the HMBC spectra of compound **121** the methine proton at  $\delta$  7.28 (H-1) showed correlations with  $\delta$  147.66 (C-2), 148.03 (C-3), 129.78 (C-4a), 125.80 (C-13b) and 137.61 (C-13a), the methine proton appearing at  $\delta$  6.71 (H-4) correlated to  $\delta$  147.66 (C-2), 148.03 (C-3), 129.78 (C-4a), 125.80 (C-13b) and 29.39 (C-5), the methine proton appearing at  $\delta$  6.27 (H-8) showed correlations with  $\delta$  137.61 (C-13a), 46.22 (C-6), 124.02 (C-8a), 127.29 (C-12a) and 147.09 (C-9), the methine proton chemical shift at  $\delta$  7.02 (H-11) correlated to  $\delta$  151.01 (C-10), 120.12 (C-12), 127.29 (C-12a) and 147.09 (C-9), the methine proton at  $\delta$  6.97 (H-12) showed correlations with  $\delta$  117.08 (C-11), 151.01

(C-10), 127.29 (C-12a), 124.02 (C-8a) and 97.05 (C-13), the methine proton at  $\delta$  6.13 (H-13) showed correlations with  $\delta$  127.29 (C-12a), 124.02 (C-8a), 137.61 (C-13a) and 120.12 (C-12).

The methylene protons appearing at  $\delta$  5.95 (H-14) correlated to  $\delta$  147.66 (C-2) and 148.03 (C-3), the methylene protons appearing at  $\delta$  2.79 (H-5) correlated to  $\delta$  46.22 (C-6), 129.78 (C-4a), 125.80 (C-13b) and 108.41 (C-4), the methylene protons at  $\delta$  3.60 (H-6) showed correlations with  $\delta$  137.61 (C-13a), 77.80 (C-8), 129.78 (C-4a) and 29.39 (C-5), the methoxy peak at  $\delta$  3.94 (9-OMe) correlated to  $\delta$  147.09 (C-9) and the methoxy peak at  $\delta$  3.87 (10-OMe) correlated to  $\delta$  151.01 (C-10) respectively.

Compound **121** on comparison with the literature confirmed it to be 8-Hydroxy-7, 8-dihydroberberine.<sup>31</sup>

## 2.7. New source non-alkaloids from *Berberis jaeschkeana* Schneid var. *jaeschkeana* stem

### 2.7.1. Docosanoic acid (122).<sup>36</sup>

White amorphous powder (CHCl<sub>3</sub>), m.p.: 70-71 °C (Lit: 70-72 °C).<sup>36</sup>

Molecular formula: C<sub>22</sub>H<sub>44</sub>O<sub>2</sub>

UV (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{\max}$  (log  $\epsilon$ ): 235 (5.13) nm.

IR  $\bar{\nu}$ : 3452 (O-H, st.), 2928 (C-H, st. sat.), 1691 (C=O, st.) cm.<sup>-1</sup>

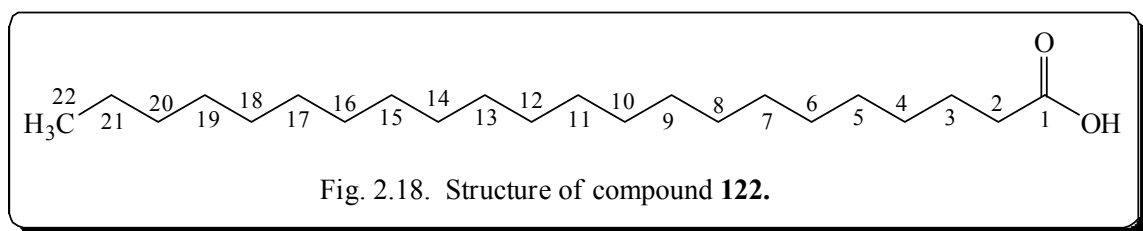
EI-MS m/z: 340 [M<sup>+</sup>] (calculated for C<sub>29</sub>H<sub>50</sub>O).

EI-MS  $m/z$  (rel. int. %): 340 (84,  $M^+$ ), 313 (12), 297 (30), 280 (14), 257 (100), 241 (21), 227 (12), 213 (19), 185 (27), 171 (15), 147 (22), 129 (51), 111 (38), 97 (63), 83 (65), 57 (88), 43 (77).

$^1\text{H}$ NMR: see **Table. 2.12.**

$^{13}\text{C}$ NMR: see **Table. 2.12.**

HMBC: see **Table. 2.12.**



The  $^1\text{H}$ NMR of compound **122** showed five signals resonating at  $\delta$  2.34 (2H, t,  $J$  = 7.2 Hz, H-2), 1.61 (2H, br.s, H-3), 1.30 (34H, br.s, H-4 to H-20), 0.86 (5H, m, H-21, H-22) and a small broad singlet at  $\delta$  5.34 (1H, s, -OH).

The  $^{13}\text{C}$ NMR of compound **122** displayed six signals for twenty two carbon atoms which include one methyl, twenty methylene and one quaternary carbon atoms. The carbon signal at  $\delta$  14.11 (C-22) was assigned to a methyl, the carbon appearing at  $\delta$  29.70 (C- 4 to C-20) was for the seventeen methylenes, the carbon signal at  $\delta$  34.01 (C-2) was allocated to the methylene next to carbonyl group, the carbon signal at 24.68 (C-3) was allocated to one of the methylene carbon and the carbon signal appearing at  $\delta$  22.69 (C-21) was allocate to the methylene next to methyl group. Methyl, methylene, methine and quaternary carbons were determined from DEPT-135 and DEPT-90 spectra.

**Table. 2.12.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **docosanoic acid (122)** (in  $\text{CDCl}_3$ )

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|------|---------------------|------------------------------------|--------------------------|
| 1    | 179.89              | ---                                | ---                      |
| 2    | 34.01               | 2.34, (2H, t, 7.2)                 | C-3, C-1, C-4            |
| 3    | 24.68               | 1.61, (2H, m)                      | C-4, C-2, C-1            |
| 4    | 29.70               | 1.30, (2H, m)                      | ---                      |
| 5    | 29.70               | 1.30, (2H, m)                      | ---                      |
| 6    | 29.70               | 1.30, (2H, m)                      | ---                      |
| 7    | 29.70               | 1.30, (2H, m)                      | ---                      |
| 8    | 29.70               | 1.30, (2H, m)                      | ---                      |
| 9    | 29.70               | 1.30, (2H, m)                      | ---                      |
| 10   | 29.70               | 1.30, (2H, m)                      | ---                      |
| 11   | 29.70               | 1.30, (2H, m)                      | ---                      |
| 12   | 29.70               | 1.30, (2H, m)                      | ---                      |
| 13   | 29.70               | 1.30, (2H, m)                      | ---                      |
| 14   | 29.70               | 1.30, (2H, m)                      | ---                      |
| 15   | 29.70               | 1.30, (2H, m)                      | ---                      |
| 16   | 29.70               | 1.30, (2H, m)                      | ---                      |
| 17   | 29.70               | 1.30, (2H, m)                      | ---                      |

Continue

| C.No | $\delta_C$ | $\delta_H$ (mult, J Hz) | HMBC (H $\rightarrow$ C) |
|------|------------|-------------------------|--------------------------|
| 18   | 29.70      | 1.30, (2H, m)           | ---                      |
| 19   | 29.70      | 1.30, (2H, m)           | ---                      |
| 20   | 29.70      | 1.30, (2H, m)           | ---                      |
| 21   | 22.69      | 0.86, (2H, m)           | C-22                     |
| 22   | 14.11      | 0.86, (3H, m)           | C-21                     |
| 1-OH | ---        | 5.34, (br.s)            | ---                      |

The HMBC and HSQC spectra for compound **122** were very simple. In the HMBC spectrum the proton triplet appearing at  $\delta$  2.34 (H-2) correlated to  $\delta$  24.68 (C-3), 29.70 (C-4) and 179.89 (C-1), the proton signal appearing at  $\delta$  1.61 (H-3) correlated to  $\delta$  29.70 (C-4), 34.01 (C-2) and 179.89 (C-1) similarly the proton signal appearing at 0.86 (H-21, H-22) correlated to carbon signal appearing at  $\delta$  22.69 (C-21) and 14.11 (C-22).

The spectral data obtained for compound **122** on comparison with the literature confirmed it to be Docosanoic acid.<sup>36</sup>

### 2.7.2. Methyl gallate (123).<sup>37</sup>

Physical state: off white crystalline powder (MeOH).

m.p.: 202-204 °C (Lit: 203).<sup>37</sup>

Molecular formula: C<sub>8</sub>H<sub>8</sub>O<sub>5</sub>

UV  $\lambda_{\max}$  (MeOH) (log  $\epsilon$ ): 273 (4.96) nm.

IR  $\bar{\nu}$ : 3412 (O-H, st.), 2988 (C-H, st. sat.), 1736 (C=O, st.)  $\text{cm}^{-1}$

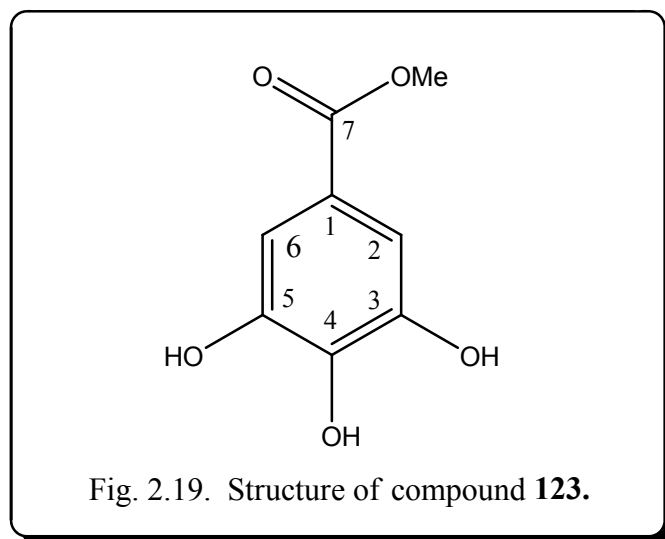
EI-MS  $m/z$ : 184  $[\text{M}^+]$  (calculated for  $\text{C}_8\text{H}_8\text{O}_5$ ).

EI-MS  $m/z$  (rel. int. %): 184 (41,  $\text{M}^+$ ), 154 (100), 127 (33), 107 (12), 79 (24).

$^1\text{H}$ NMR: see **Table 2.13**.

$^{13}\text{C}$ NMR: see **Table 2.13**.

HMBC: see **Table 2.13**.



The  $^1\text{H}$ NMR spectrum of compound **123** showed a singlet for aromatic proton at  $\delta$  7.10 (2H, s, H-2, 6).

The  $^{13}\text{C}$ NMR for compound **123** showed five carbon atoms at  $\delta$  121.01 (C-1), 110.33 (C-2, 6), 146.53 (C-3, 5), 138.89 (C-4) and 171.21 (C-7).

**Table. 2.13.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **methyl gallate (123)** (in MeOD)

| C.No              | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|-------------------|---------------------|------------------------------------|--------------------------|
| 1                 | 121.01              | ---                                | ---                      |
| 2                 | 110.33              | 7.10, (s)                          | C-1, C-3, C-4, C-7, C-6  |
| 3                 | 146.53              | ---                                | ---                      |
| 4                 | 138.89              | ---                                | ---                      |
| 5                 | 146.53              | ---                                | ---                      |
| 6                 | 110.33              | 7.10, (s)                          | C-1, C-3, C-4, C-7, C-6  |
| 7                 | 171.21              | ---                                | ---                      |
| O-CH <sub>3</sub> | 52.02               | ---                                | ---                      |

The spectra for compound **123** was similar to that of compound **119** except and extra peak for methoxy in  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR spectra.

From the above, compound **123** is established as methyl gallate which is further confirmed by comparison with the literature.<sup>37</sup>

## 2.8. New source alkaloids from *Berberis glaucocarpa* Stapf roots.

### 2.8.1. Oxyacanthine (124).<sup>38</sup>

Off white powder ( $\text{CHCl}_3$ ), m.p.: 214-216 °C (Lit: 216-217 °C).<sup>77</sup>

Molecular formula:  $\text{C}_{37}\text{H}_{40}\text{N}_2\text{O}_6$

UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ): 281 (5.38) nm.

IR  $\bar{\nu}$ : 3313 (O-H, st.), 2921 (C-H, st. sat.), 2831 (C-H, st. N-methyl), 1607,

1516 (C=C st. aromatic.)  $\text{cm}^{-1}$

HR-ESIMS = 609.2958  $[\text{M}+\text{H}]^+$  (calculated for  $\text{C}_{37}\text{H}_{40}\text{N}_2\text{O}_6$  = 608.2886).

EI-MS:  $m/z$  608  $[\text{M}^+]$  (calculated for  $\text{C}_{37}\text{H}_{40}\text{N}_2\text{O}_6$ ).

EI-MS:  $m/z$  (rel. int. %): 608 ( $\text{M}^+$ , 100), 593 (29), 577 (6), 416 (12), 395 (81), 381(69), 379(28), 365(10), 335(6), 198(51), 192(17), 174(31), 146(8).

$^1\text{H}$ NMR: see **Table. 2.14.**

$^{13}\text{C}$ NMR: see **Table. 2.14.**

HMBC: see **Table. 2.14.**

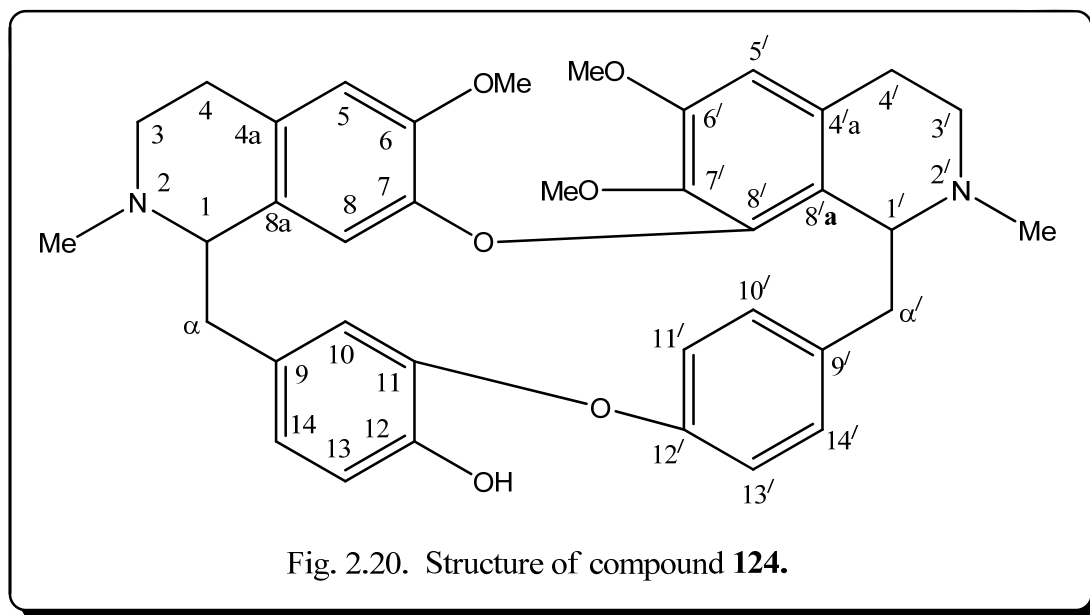


**Table. 2.14.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of oxyacanthine (**124**) (in  $\text{CDCl}_3$ )

| C.No     | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)                         |
|----------|---------------------|------------------------------------|--------------------------------------------------|
| 1        | 63.71               | 3.76, (m)                          | C- $\alpha$ , N-Me, C-4a, C-8,<br>C-8a, C-9, C-3 |
| N-Me     | 42.70               | 2.22, (s)                          | C-1, C-3                                         |
| 3        | 45.87               | 2.85 and 3.39, (m)                 | C-1, N-Me, C-4, C-4a                             |
| 4        | 25.46               | 2.75 and 2.90, (m)                 | C-3, C-5, C-8a, C-4a                             |
| 4a       | 128.10              | ---                                | ---                                              |
| 5        | 105.46              | 6.24, (br.s)                       | C-4a, C-8a, C-6, C-7, C-4                        |
| 6        | 151.81              | ---                                | ---                                              |
| 7        | 136.95              | ---                                | ---                                              |
| 6-OMe    | 55.53               | 3.72, (s)                          | C-6                                              |
| 7-OMe    | 60.50               | 3.08, (s)                          | C-7                                              |
| 8        | 147.53              | ---                                | ---                                              |
| 8a       | 120.74              | ---                                | ---                                              |
| $\alpha$ | 38.56               | 2.78 and 3.24, (m)                 | C-1, C-14, C-9, C-8a, C-10                       |
| 9        | 133.51              | ---                                | ---                                              |
| 10       | 114.67              | 6.38, (br.s)                       | ---                                              |
| 11       | 143.53              | ---                                | ---                                              |

Continue

| C.No      | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)                                 |
|-----------|------------|---------------------------|----------------------------------------------------------|
| 12        | 148.18     | ---                       | ---                                                      |
| 13        | 115.30     | 6.78, (d, 7.4)            | C-14, C-9, C-11, C-12                                    |
| 14        | 123.52     | 6.71, (dd, 7.4, 2.2)      | C-13, C-9, C-10, C-12, C-                                |
| 1'        | 62.20      | 3.78, (m)                 | C- $\alpha'$ , N'-Me, C-3', C-8',<br>C-8'a, C- 4'a, C-9' |
| N'-Me     | 42.75      | 2.51, (s)                 | C-1', C-3'                                               |
| 3'        | 44.89      | 2.54 and 2.97, (m)        | C-4', N'-Me, C-1', C-4'a                                 |
| 4'        | 22.69      | 2.79 and 2.89, (m)        | C-3', C-1', C-4'a, C-5'                                  |
| 4'a       | 127.41     | ---                       | ---                                                      |
| 5'        | 111.23     | 6.49, (s)                 | C-4'a, C-6', C-7', C-8'a, C-4'                           |
| 6'        | 149.90     | ---                       | ---                                                      |
| 6'-OMe    | 55.77      | 3.55, (s)                 | C-6'                                                     |
| 7'        | 143.76     | ---                       | ---                                                      |
| 8'        | 119.84     | 5.95, (br.s)              | C-1', C-4'a, C-7', C-6'                                  |
| 8'a       | 128.74     | ---                       | ---                                                      |
| $\alpha'$ | 37.77      | 2.79 and 3.26, (m)        | C-1', C-9', C-14', C-10', C-4'a                          |
| 9'        | 135.57     | ---                       | ---                                                      |
| 10'       | 130.25     | 7.25, (dd, 8.6, 2.4)      | C-11', C-14', C-12', C- $\alpha'$                        |

Continue

| C.No | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)  |
|------|------------|---------------------------|---------------------------|
| 11'  | 121.38     | 7.08, (dd, 8.6, 2.4)      | C-13', C-10', C-9', C-12' |
| 12'  | 153.82     | ---                       | ---                       |
| 13'  | 121.73     | 6.58, (dd, 8.6, 2.4)      | C-11', C-14', C-9', C-12' |
| 14'  | 132.25     | 6.40, (dd, 8.6, 2.4)      | C-13', C-10', C-12'       |

The  $^1\text{H}$  NMR spectrum of compound **124** showed ten aromatic methines at  $\delta$  7.25 (1H, dd,  $J$  = 8.6, 2.4 Hz, H-10'), 7.08 (1H, dd,  $J$  = 8.6, 2.4 Hz, H-11'), 6.78 (1H, d,  $J$  = 7.4 Hz, H-13), 6.71 (1H, dd,  $J$  = 7.4, 2.2 Hz, H-14), 6.58 (1H, dd,  $J$  = 8.6, 2.4 Hz, H-13'), 6.49 (1H, s, H-5'), 6.40 (1H, dd,  $J$  = 8.6, 2.4 Hz, H-14'), 6.24 (1H, s, H-5), 6.38 (1H, br.s, H-10) and 5.95 (1H, br.s, H-8'), two non aromatic methines at  $\delta$  3.76 (1H, m, H-1) and 3.78 (1H, m, H-1'). Six methylenes at  $\delta$  2.85 and 3.39 (2H, m, H-3), 2.54 and 2.97 (2H, m, H-3'), 2.78 and 3.24 (2H, m, H- $\alpha'$ ), 2.78 and 3.24 (2H, m, H- $\alpha$ ), 2.75 and 2.90 (2H, m, H-4) 2.79 and 2.89 (2H, m, H-4'), three methoxy peaks at  $\delta$  3.72 (3H, s, 6-OMe) 3.55 (3H, s, 6'-OMe) and 3.08 (3H, s, 7-OMe), two N-CH<sub>3</sub> peaks at  $\delta$  2.22 (3H, s, N-CH<sub>3</sub>) and 2.51 (3H, s, N'-CH<sub>3</sub>).

The  $^{13}\text{C}$  NMR spectrum of compound **124** showed the presence of thirty seven carbon atoms including three methoxys at  $\delta$  60.50, 55.77 and 55.53, six methylene at  $\delta$  45.87, 38.56, 25.46, 44.89, 37.77 and 22.69, twelve methines at  $\delta$  132.25, 130.25, 123.52, 121.73, 121.38, 119.84, 115.30, 114.67, 111.23, 105.46, 63.71 and 62.20, fourteen quaternary carbons at  $\delta$  153.82, 151.81, 149.90, 148.18, 147.53, 143.76, 143.53,

136.95, 135.57, 133.51, 128.74, 128.10, 127.41 and 120.74 and two N-CH<sub>3</sub> at  $\delta$  42.70 and 41.75.

In the HMBC spectrum the signal appearing at  $\delta$  2.22 (N-Me) showed correlations with 63.71 (C-1) and 45.87 (C-3), the methoxy signal at  $\delta$  3.72 (6-OMe) correlated to  $\delta$  151.81 (C-6) while the methoxy group at  $\delta$  3.08 (7-OMe) correlated to  $\delta$  136.95 (C-7), the signal at  $\delta$  3.76 (H-1) showed correlations with  $\delta$  38.56 (C- $\alpha$ ), 42.70 (N-Me), 128.10 (C-4a), 148.18 (C-8), 120.74 (C-8a), 133.51 (C-9) and 45.87 (C-3), the signals appearing at  $\delta$  2.85 and 3.39 (H-3) showed correlations with  $\delta$  63.71 (C-1), 25.46 (C-4), 128.10 (C-4a) and 42.70 (N-CH<sub>3</sub>).

The proton resonance appearing at  $\delta$  2.75 and 2.90 (H-4) showed correlations with  $\delta$  45.87 (C-3), 105.46 (C-5), 120.74 (C-8a) and 128.10 (C-4a), the proton appearing at  $\delta$  6.24 (H-5) correlated to  $\delta$  128.10 (C-4a), 120.74 (C-8a), 151.81 (C-6), 136.95 (C-7) and 25.46 (C-4), the proton signal appearing at  $\delta$  2.78 and 3.24 (H- $\alpha$ ) showed correlations with  $\delta$  63.71 (C-1), 123.52 (C-14), 133.53 (C-9), 120.74 (C-8a) and 114.67 (C-10), the signal appearing at  $\delta$  6.78 (H-13) showed correlations with  $\delta$  123.52 (H-14), 133.51 (H-9), 143.53 (C-11) and 148.18 (H-12), the proton signal at  $\delta$  6.71 (H-14) showed correlations with  $\delta$  115.30 (C-13), 133.51 (C-9), 114.67 (C-10), 148.18 (C-12) and 38.56 (C- $\alpha$ ), the signal appearing at  $\delta$  6.38 showed correlations with the carbon signals at  $\delta$  143.53 (C-11), 148.18 (C-12), 123.52 (C-14), 133.51 (C-9) and 38.56 (C- $\alpha$ ), the proton signal at  $\delta$  2.51 (N'-Me) showed correlations with  $\delta$  62.20 (C-1') and 44.89 (C-3'), the methoxy signal at  $\delta$  3.55 (6'-OMe) correlated to 149.90 (C-6').

The proton signal at  $\delta$  3.78 (H-1') correlated to  $\delta$  37.77 (C-  $\alpha'$ ), 42.75 (N'-Me), 44.89 (C-3'), 119.84 (C-8'), 128.74 (C-8'a), 127.41 (C-4'a) and 135.57 (C-9'), the proton resonating at  $\delta$  2.54 and 2.97 (H-3') correlated to  $\delta$  22.69 (C-4'), 42.75 (N'-Me), 62.20 (C-1') and 127.41 (C-4'a), the proton at  $\delta$  2.79 and 2.89 (H-4') showed correlations with  $\delta$  44.89 (C-3'), 62.20 (C-1'), 127.41 (C-4'a) and 111.23 (C-5'), the proton resonance at  $\delta$  6.49 (H-5') correlated to  $\delta$  127.41 (C-4'a), 149.90 (C-6'), 143.76 (C-7'), 128.74 (C-8'a) and 22.69 (C-4'), the proton appearing at  $\delta$  5.95 (H-8') correlated to  $\delta$  62.20 (C-1'), 127.41 (C-4'a), 149.90 (C-6') and 143.76 (C-7').

The signal at  $\delta$  2.79 and 3.26 (H-  $\alpha'$ ) correlated to  $\delta$  62.20 (C-1'), 135.57 (C-9'), 132.25 (C-14'), 127.41 (C-4'a) and 130.25 (C-10'), the signal at  $\delta$  7.25 (H-10') correlated to  $\delta$  121.38 (C-11'), 132.25 (C-14'), 153.82 (C-12') and 37.77 (C-  $\alpha'$ ), the proton signal at  $\delta$  7.08 (H-11') correlated to  $\delta$  121.73 (C-13'), 130.25 (C-10'), 135.57 (C-9') and 153.82 (C-12'), the proton signal appearing at  $\delta$  6.58 (C- 13') correlated to  $\delta$  121.38 (C-11'), 132.25 (C-14'), 135.57 (C-9') and 153.82 (C-12'). Similarly the proton signal appearing at  $\delta$  6.40 (H-14') correlated to  $\delta$  121.73 (C-13'), 130.25 (C-10'), 153.82 (C-12'), 135.77 (C-9') and 37.77 (C-  $\alpha'$ ).

From the above, compound **124** is elucidated as oxyacanthine and further confirmed by comparison with the reported data.<sup>38</sup>

### 2.8.2. Jatrorrhizine (125).<sup>33</sup>

Yellow needles (MeOH), m.p.: 280-281 °C (Lit: 280-282 °C).<sup>33</sup>

Molecular formula: C<sub>20</sub>H<sub>20</sub>NO<sub>4</sub><sup>+</sup>

UV  $\lambda_{\max}$  (MeOH) ( $\log \epsilon$ ): 281(5.60), 349 (5.13) nm.

IR  $\bar{\nu}$ : 3310 (O-H, st.), 3031 (C-H, st. aromatic.), 1610 (C=C, st. aromatic.),

1465 (finger print region)  $\text{cm}^{-1}$

EI-MS  $m/z$ : 338 [ $M^+$ ] (calculated for  $C_{20}H_{20}NO_4^+$ ).

EI-MS  $m/z$  (rel. int. %): 338 (24,  $M^+$ ), 323 (100), 307 (37), 294 (39), 278 (70), 265 (11).

$^1\text{H}$ NMR: see **Table. 2.15.**

$^{13}\text{C}$ NMR: see **Table. 2.15.**

HMBC: see **Table. 2.15.**

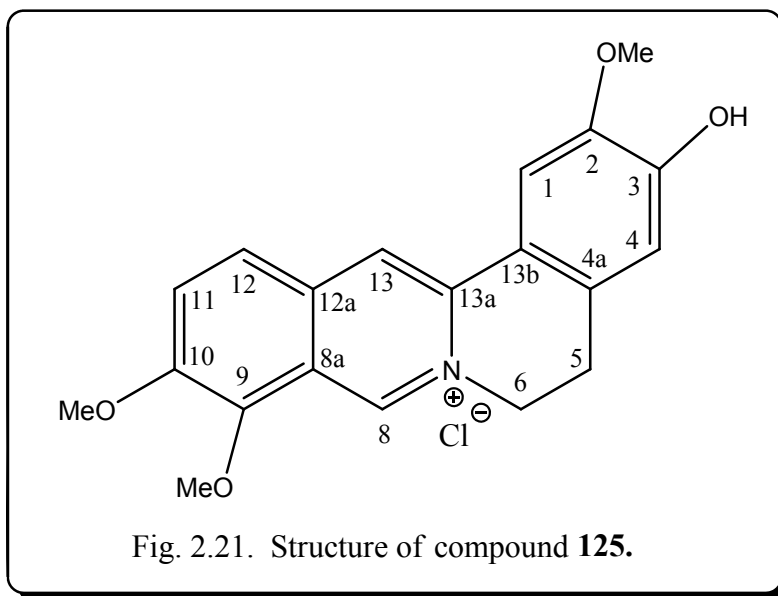


**Table. 2.15.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **jatrorrhizine (125)** (in DMSO- $d_6$ )

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)         |
|------|---------------------|------------------------------------|----------------------------------|
| 1    | 112.76              | 7.58, (s)                          | C-2, C-3, C-4a, C-13b, C-13a     |
| 2    | 145.33              | ---                                | ---                              |
| 3    | 151.71              | ---                                | ---                              |
| 4    | 110.88              | 6.72, (s)                          | C-2, C-3, C-13b, C-4a, C-5       |
| 4a   | 134.72              | ---                                | ---                              |
| 5    | 26.88               | 3.28, (t, 7.0)                     | C-6, C-4a, C-13b, C-4            |
| 6    | 57.31               | 4.89, (t, 7.0)                     | C-13a, C-8, C-4a, C-5            |
| 8    | 145.66              | 9.74, (s)                          | C-13a, C-6, C-8a, C-12a, C-9     |
| 8a   | 118.99              | ---                                | ---                              |
| 9    | 149.98              | ---                                | ---                              |
| 10   | 150.26              | ---                                | ---                              |
| 11   | 126.23              | 8.14, (d, 8.0)                     | C-10, C-12, C-12a, C-9           |
| 12   | 125.83              | 7.95, (d, 8.0)                     | C-11, C-10, C-12a,<br>C-8a, C-13 |
| 12a  | 130.65              | ---                                | ---                              |
| 13   | 122.09              | 8.82, (s)                          | C-12a, C-8a, C-13a, C-12         |
| 13a  | 139.89              | ---                                | ---                              |
| 13b  | 123.41              | ---                                | ---                              |

Continue

| C.No   | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|--------|------------|---------------------------|--------------------------|
| 2-OMe  | 58.01      | 3.91, (s)                 | C-2, C-3                 |
| 9-OMe  | 62.55      | 4.09, (s)                 | C-9                      |
| 10-OMe | 57.68      | 4.05, (s)                 | C-10                     |

The  $^1\text{H}$ NMR of compound **125** showed six aromatic protons at  $\delta$  7.58 (1H, s, H-1), 6.72 (1H, s, H-4), 9.74 (1H, s, H-8), 8.14 (1H, d,  $J$  = 8.0 Hz, H-11), 7.95 (1H, d,  $J$  = 8.0 Hz, H-12) and 8.82 (1H, s, H-13), two methylenes at  $\delta$  3.28 (2H, t,  $J$  = 7.0 Hz, H-5) and 4.89 (2H,  $J$  = 7.0 Hz, H-6) and three methoxys at  $\delta$  4.09 (3H, s, 9-OMe), 4.05 (3H, s, 10-OMe) and 3.91 (3H, s, 2-OMe).

The  $^{13}\text{C}$ NMR of compound **125** showed twenty carbon atoms which include six methine, two methylenes, three methoxys and nine quaternary carbon atoms on the basis of DEPT-135 and DEPT-90 spectra.

In the HMBC spectra of compound **125** the methine proton at  $\delta$  7.58 (H-1) correlated to  $\delta$  145.33 (C-2), 151.71 (C-3), 134.72 (C-4a), 123.41 (C-13b) and 139.89 (C-13a), the methine proton appearing at  $\delta$  6.72 (H-4) showed correlations with  $\delta$  145.33 (C-2), 151.71 (C-3), 134.72 (C-4a), 123.41 (C-13b) and 26.88 (C-5), the methine proton at  $\delta$  9.74 (H-8) showed correlations with  $\delta$  139.89 (C-13a), 57.31 (C-6), 118.99 (C-8a), 130.65 (C-12a) and 149.98 (C-9), the methine proton chemical shift at  $\delta$  8.14 (H-11) correlated to  $\delta$  150.26 (C-10), 125.83 (C-12), 130.65 (C-12a) and 149.98 (C-9), the methine proton at  $\delta$  7.95 (H-12) showed correlations with  $\delta$  126.33 (C-11), 150.26 (C-

10), 130.65 (C-12a), 118.99 (C-8a) and 122.09 (C-13), the methine proton at  $\delta$  8.82 (H-13) correlated to  $\delta$  130.65 (C-12a), 118.99 (C-8a), 139.89 (C-13a) and 125.83 (C-12).

The methylene protons appearing at  $\delta$  3.28 (H-5) correlated to  $\delta$  57.11 (C-6), 134.72 (C-4a), 123.41 (C-13b) and 110.88 (C-4), similarly the methylene protons at  $\delta$  4.89 (H-6) showed correlations with  $\delta$  139.89 (C-13a), 145.66 (C-8), 134.72 (C-4a) and 26.88 (C-5), while the three methoxys at  $\delta$  4.09 (9-OMe), 4.05 (10-OMe) and 3.91 (2-OMe) correlated to  $\delta$  149.98 (C-9), 150.26 (C-10) and 145.33 (C-2) respectively.

From the above data compound **125** was established as jatrorrhizine which was confirmed by comparison with the literature.<sup>33</sup>

### 2.8.3. 8-Oxoberberine (126).<sup>31</sup>

Yellow needles (MeOH), m.p.: 191-192 °C (Lit: 190-192°C).<sup>33</sup>

Molecular formula: C<sub>20</sub>H<sub>17</sub>NO<sub>5</sub>

UV  $\lambda_{\max}$  (MeOH) (log  $\epsilon$ ): 315 (5.5), 370 (5.32) nm.

IR  $\bar{\nu}$ : 3020 (C-H, st. aromatic.), 1643 (C=O, st.), 1501 (C=C, st. aromatic.),

1293, 1240 (finger print region) cm.<sup>-1</sup>

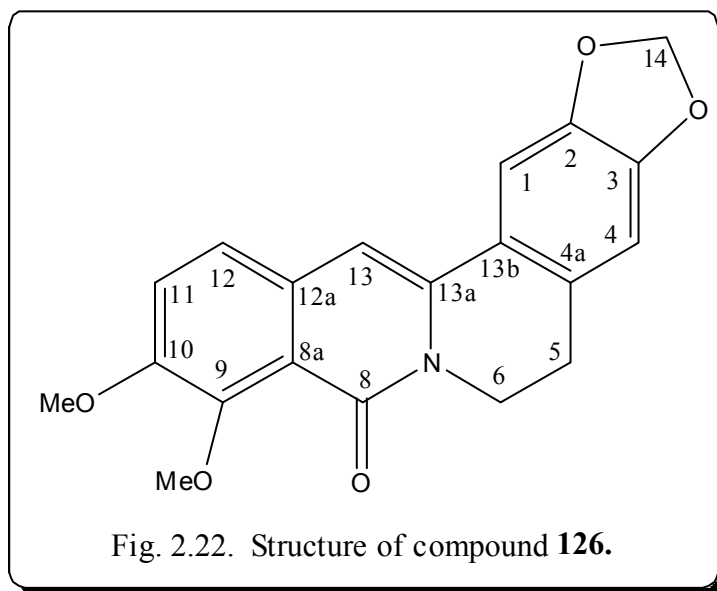
EI-MS m/z: 351 [M<sup>+</sup>] (calculated for C<sub>20</sub>H<sub>17</sub>NO<sub>5</sub>).

EI-MS m/z (rel. int. %): 351 (100, M<sup>+</sup>), 336 (71), 322 (45), 292 (37), 160 (24).

<sup>1</sup>HNMR: see **Table. 2.16.**

<sup>13</sup>CNMR: see **Table. 2.16.**

HMBC: see **Table. 2.16**.



The  $^1\text{H}$ NMR of compound **126** showed five aromatic protons at  $\delta$  7.40 (1H, s, H-1), 6.63 (1H, s, H-4), 7.54 (1H, d,  $J = 8.4$  Hz, H-11), 7.23 (1H, d,  $J = 8.4$  Hz, H-12) and 7.01 (1H, s, H-13), three methylenes at  $\delta$  6.03 (2H, s, H-14), 2.91 (2H, t,  $J = 8.2$  Hz, H-5) and 4.13 (2H, s,  $J = 8.4$  Hz, H-6) and two methoxys at  $\delta$  3.74 (3H, s, 9-OMe) and 3.89 (3H, s, 10-OMe).

DEPT-q spectra of compound **126** showed twenty carbon atoms which include five methine, three methylene, two methoxy and ten quaternary carbon atoms.

**Table. 2.16.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **8-oxoberberine (126)** (in DMSO- $d_6$ )

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)         |
|------|---------------------|------------------------------------|----------------------------------|
| 1    | 104.69              | 7.40, (s)                          | C-2, C-3, C-4a, C-13b, C-13a     |
| 2    | 146.98              | ---                                | ---                              |
| 3    | 148.11              | ---                                | ---                              |
| 4    | 108.01              | 6.63, (s)                          | C-2, C-3, C-13b, C-4a, C-5       |
| 4a   | 129.92              | ---                                | ---                              |
| 5    | 27.87               | 2.91, (t, 10.0)                    | C-6, C-4a, C-13b, C-4            |
| 6    | 38.90               | 4.13, (t, 10.0)                    | C-13a, C-8, C-4a, C-5            |
| 8    | 159.11              | ---                                | ---                              |
| 8a   | 118.61              | ---                                | ---                              |
| 9    | 148.50              | ---                                | ---                              |
| 10   | 150.95              | ---                                | ---                              |
| 11   | 119.44              | 7.54, (d, 8.4)                     | C-10, C-12, C-12a, C-9           |
| 12   | 122.67              | 7.23, (d, 8.4)                     | C-11, C-10, C-12a,<br>C-8a, C-13 |
| 12a  | 131.92              | ---                                | ---                              |
| 13   | 101.33              | 7.01, (s)                          | C-12a, C-8a, C-13a, C-12         |
| 13a  | 135.22              | ---                                | ---                              |

Continue

| C.No   | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|--------|------------|---------------------------|--------------------------|
| 13b    | 123.34     | ---                       | ---                      |
| 14     | 101.89     | 6.03, (s)                 | C-2, C-3                 |
| 9-OMe  | 60.80      | 3.74, (s)                 | C-9                      |
| 10-OMe | 56.71      | 3.89, (s)                 | C-10                     |

In the HMBC spectra of compound **126** the methine proton at  $\delta$  7.40 (H-1) correlated to  $\delta$  146.98 (C-2), 148.11 (C-3), 129.92 (C-4a), 123.34 (C-13b) and 135.22 (C-13a), the methine proton resonance at  $\delta$  6.63 (H-4) showed correlations with  $\delta$  146.98 (C-2), 148.11 (C-3), 129.92 (C-4a), 123.34 (C-13b) and 27.87 (C-5), the methine proton chemical shift at  $\delta$  7.54 (H-11) correlated to  $\delta$  150.95 (C-10), 122.67 (C-12), 131.92 (C-12a) and 148.50 (C-9), the methine proton at  $\delta$  7.23 (H-12) showed correlations with  $\delta$  119.44 (C-11), 150.95 (C-10), 131.92 (C-12a), 118.61 (C-8a) and 101.33 (C-13), the methine proton at  $\delta$  7.01 (H-13) correlated to  $\delta$  131.92 (C-12a), 118.61 (C-8a), 135.22 (C-13a) and 122.67 (C-12).

The methylene protons appearing at  $\delta$  6.03 (H-14) correlated to  $\delta$  146.98 (C-2) and 148.11 (C-3), the methylene protons appearing at  $\delta$  2.91 (H-5) correlated to  $\delta$  38.90 (C-6), 129.92 (C-4a), 123.34 (C-13b) and 108.01 (C-4), similarly the methylene protons at  $\delta$  4.13 (H-6) showed correlations with  $\delta$  135.22 (C-13a), 159.11 (C-8), 129.92 (C-4a) and 27.87 (C-5) and two methoxys resonating at  $\delta$  3.74 (9-OMe) correlated to  $\delta$  148.50 (C-9) and  $\delta$  3.89 (10-OMe) correlated to  $\delta$  150.95 (C-10) respectively.

On comparison of all the spectral data for compound **126** with the literature confirmed it to be 8-oxoberberine.<sup>33</sup>

## 2.9. A new source non-alkaloid from *Berberis glaucocarpa* Stapf roots.

### 2.9.1. $\beta$ -Sitosterol glucoside (**127**).<sup>37</sup>

Colorless amorphous powder (C<sub>5</sub>H<sub>5</sub>N), m.p.: 270-272 °C (Lit: 272-274 °C).<sup>37</sup>

FABMS [M<sup>+</sup>] 576 (calculated for C<sub>35</sub>H<sub>50</sub>O 576.4390).

Molecular formula: C<sub>35</sub>H<sub>60</sub>NO<sub>6</sub>

IR  $\bar{\nu}$ : 3483(O-H, st.), 2928 (C-H, st. sat.) 1014 cm.<sup>-1</sup>

EI-MS: m/z (rel. int. %): 414 (28), 396 (100), 382 (88), 367 (11), 354 (8), 329 (14), 303(17), 288(24), 275 (30), 255(48), 229 (12), 213 (37), 201(12), 175 (18), 161 (42), 147 (59), 121 (34), 105 (31), 81 (39), 69 (22), 43(29).

<sup>1</sup>HNMR: see Table. 2.17.

<sup>13</sup>CNMR: see Table. 2.17.

HMBC: see Table. 2.17.

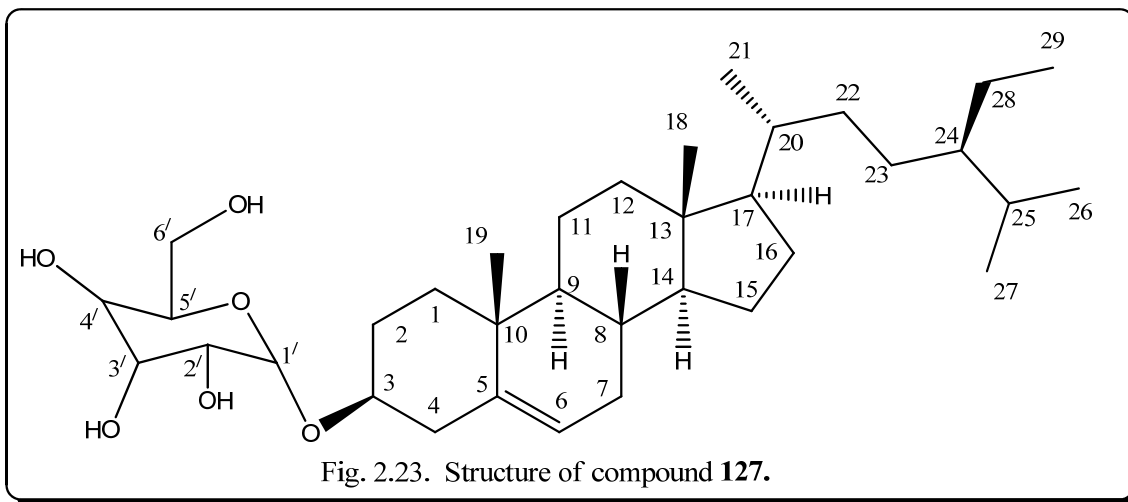


**Table. 2.17.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of  **$\beta$ -sitosterol glucoside (127)** (in  $\text{C}_5\text{D}_5\text{N}$ )

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz)       | HMBC (H $\rightarrow$ C) |
|------|---------------------|------------------------------------------|--------------------------|
| 1    | 37.51               | 1.07, 1.41, m                            | C-3, C-5, C-19           |
| 2    | 30.28               | 1.30, (m)                                | ---                      |
| 3    | 78.46               | 3.97, (m)                                | C-2, C-1'                |
| 4    | 39.99               | 2.72, (dd, 13.0, 2.5)<br>2.46, (t, 11.5) | C-2, C-3, C-5, C-6, C-10 |
| 5    | 140.96              | ---                                      | ---                      |
| 6    | 121.92              | 5.34, (d, 2.5)                           | C-4, C-8, C-10           |
| 7    | 32.20               | 1.34, 1.53, (m)                          | ---                      |
| 8    | 32.09               | 1.55, (m)                                | ---                      |
| 9    | 50.39               | 0.87, (m)                                | ---                      |
| 10   | 36.95               | ---                                      | ---                      |
| 11   | 21.31               | 1.49, (m)                                | ---                      |
| 12   | 39.37               | 1.97, (dt, 12.5, 3.0)                    | ---                      |
| 13   | 42.51               | ---                                      | ---                      |
| 14   | 56.87               | 1.09, (m)                                | ---                      |
| 15   | 24.53               | 1.51, (m)                                | ---                      |
| 16   | 28.55               | 1.34, 1.84, (m)                          | ---                      |

Continue

| C.No | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|------|------------|---------------------------|--------------------------|
| 17   | 56.29      | 1.06, (m)                 | ---                      |
| 18   | 11.99      | 0.64, (s)                 | C-12, C-13, C-14, C-17   |
| 19   | 19.43      | 0.92, (s)                 | C-1, C-9, C-10           |
| 20   | 36.40      | 1.37, (m)                 | ---                      |
| 21   | 19.03      | 0.98, (d, 6.0)            | C-17, C-20, C-22         |
| 22   | 34.25      | 1.27, (m)                 | ---                      |
| 23   | 26.47      | 1.24, (m)                 | ---                      |
| 24   | 46.09      | 0.97, (m)                 | ---                      |
| 25   | 29.53      | 1.24, (m)                 | ---                      |
| 26   | 19.24      | 0.87, (d, 7.0)            | C-24, C-25, C-27         |
| 27   | 19.98      | 0.85, (d, 6.4)            | C-24, C-25, C-26         |
| 28   | 23.44      | 1.36, (m)                 | ---                      |
| 29   | 12.18      | 0.80, (t, 7.0)            | C-24, C-28               |
| 1'   | 102.61     | 5.04, (d, 7.5)            | C-3                      |
| 2'   | 75.35      | 4.06, (t, 8.0)            | C-3', C-1'               |
| 3'   | 78.62      | 4.28, (t, 7.0)            | ---                      |
| 4'   | 71.76      | 4.27, (t, 7.0)            | ---                      |
| 5'   | 78.18      | 4.41, (dd, 11.0, 5.0)     | ---                      |
| 6'   | 62.89      | 4.57, (dd, 9.5, 5.0)      | ---                      |

The  $^1\text{H}$ NMR of compound **127** showed the presence of glucose moiety attached to  $\beta$ -sitosterol. The peaks for glucose appeared at  $\delta$  5.04 (1H, d,  $J = 7.5$  Hz, H-1'), 4.06 (t,  $J = 8.0$  Hz, H-2'), 4.28 (t,  $J = 7.0$  Hz, H-3'), 4.27 (t,  $J = 7.0$  Hz, H-4'), 4.41 (dd,  $J = 11.0, 5.0$  Hz, H-5') and 4.57 (2H, dd,  $J = 9.5, 5.0$  Hz, H-6'), while the remaining peaks were similar to those of  $\beta$ -sitosterol which include the presence of two methyl singlets at  $\delta$  0.64 (3H, s, H-18) and  $\delta$  0.92 (3H, s, H-19), three methyl group doublets at  $\delta$  0.98 (d,  $J = 6.0$  Hz, H-21), 0.87 (d,  $J = 7.0$  Hz, H-26) and 0.85 (d,  $J = 6.4$  Hz, H-27), one methyl triplet at  $\delta$  0.80 (t,  $J = 7.0$  Hz, H-29), an olefinic proton broad doublet at  $\delta$  5.34 (1H, d,  $J = 2.5$  Hz, H-6) and a multiplet at  $\delta$  3.97 (1H, m, H-3).

The  $^{13}\text{C}$ NMR of compound **127** showed the presence of thirty five carbon atoms, which included six methyl carbons resonating at  $\delta$  11.99 (C-18), 19.43 (C-19), 19.03 (C-21), 19.24 (C-26), 19.98 (C-27) and 12.18 (C-29), twelve methylene carbon atoms at  $\delta$  37.51 (C-1), 30.28 (C-2), 39.99 (C-4), 32.20 (C-7), 21.31 (C-11), 39.37 (C-12), 24.53 (C-15), 28.55 (C-16), 34.25 (C-22), 26.47 (C-23), 23.44 (C-28) and 62.89 (C-6'), fourteen methine carbon atoms at  $\delta$  78.46 (C-3), 121.92 (C-6), 32.09 (C-8), 50.39 (C-9), 56.87 (C-14), 56.29 (C-17), 36.40 (C-20), 46.09 (C-24), 12.18 (C-29), 102.61 (C-1'), 75.35 (C-2'), 78.62 (C-3'), 71.76 (C-4') and 78.18 (C-5') and three quaternary carbons at  $\delta$  140.96 (C-5), 36.95 (C-10) and 42.51 (C-13) according to DEPT-135 and DEPT-90 spectra.

The 1D and 2D data of compound **127** was compared with the literature which confirmed it to be  $\beta$ -sitosterol glucoside.<sup>37</sup>

## 2.10. New source alkaloids from *Berberis glaucocarpa* Stapf stem.

### 2.10.1. 8-Trichloromethyldihydroberberine (128).<sup>32</sup>

Light yellow crystals (CHCl<sub>3</sub>), m.p.: 186-187 °C (Lit: 186-188 °C).<sup>32</sup>

Molecular formula: C<sub>21</sub>H<sub>18</sub>Cl<sub>3</sub>NO<sub>4</sub>

UV λ<sub>max</sub> (MeOH) (log ε): 231(5.95), 285 (5.69), 381 (5.83) nm.

IR ν̄: 2980 (C-H, st. sat.), 1610 (C=C, st. aromatic.), 1501(C=C, st. aromatic.),

1290, 1240 (finger print region) cm.<sup>-1</sup>

ESI-MS: 454.0313 [M+H]<sup>+</sup> (calculated for C<sub>21</sub>H<sub>19</sub>Cl<sub>3</sub>NO<sub>4</sub> = 454.0381).

EI-MS m/z (rel. int. %): 420 (78), 383 (82), 368 (75), 336 (100), 321 (26), 292 (24), 278 (27).

<sup>1</sup>HNMR: see Table. 2.18.

<sup>13</sup>CNMR: see Table. 2.18.

HMBC: see Table. 2.18.

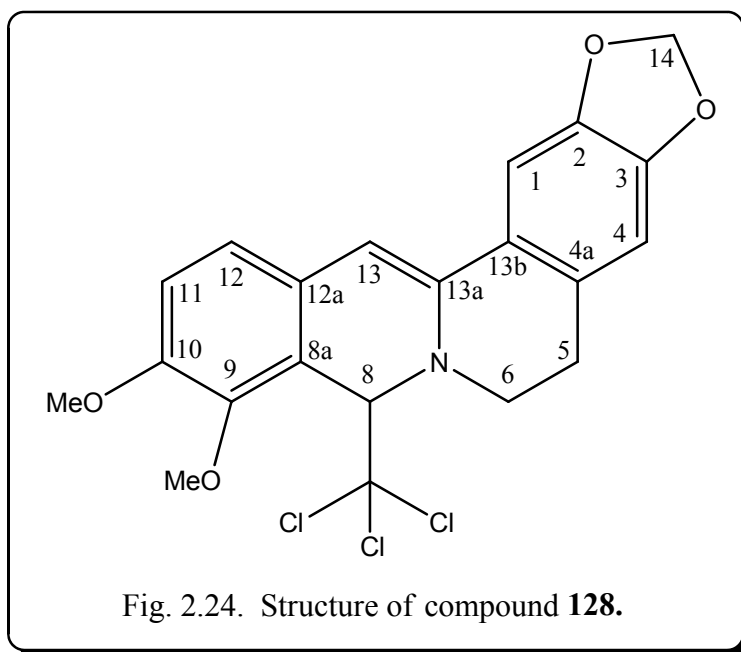


**Table. 2.18.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **8-trichloromethyldihydroberberine**(in  $\text{CDCl}_3$ )

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)         |
|------|---------------------|------------------------------------|----------------------------------|
| 1    | 104.16              | 7.14, (s)                          | C-2, C-3, C-4a, C-13b, C-13a     |
| 2    | 146.76              | ---                                | ---                              |
| 3    | 147.48              | ---                                | ---                              |
| 4    | 108.15              | 6.59, (s)                          | C-2, C-3, C-13b, C-4a, C-5       |
| 4a   | 128.73              | ---                                | ---                              |
| 5    | 30.52               | 2.73 and 3.33, (m)                 | C-6, C-4a, C-13b, C-4            |
| 6    | 51.61               | 3.71 and 3.86, (m)                 | C-13a, C-8, C-4a, C-5            |
| 8    | 73.53               | 5.62, (s)                          | C-13a, C-6, C-8a, C-12a, C-9     |
| 8a   | 114.89              | ---                                | ---                              |
| 9    | 146.41              | ---                                | ---                              |
| 10   | 149.69              | ---                                | ---                              |
| 11   | 114.62              | 6.97, (d, 8.4)                     | C-10, C-12, C-12a, C-9           |
| 12   | 118.62              | 6.86, (d, 8.4)                     | C-11, C-10, C-12a,<br>C-8a, C-13 |
| 12a  | 129.36              | ---                                | ---                              |
| 13   | 97.52               | 6.08, (s)                          | C-12a, C-8a, C-13a, C-12         |
| 13a  | 137.60              | ---                                | ---                              |

Continue

| C.No   | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|--------|------------|---------------------------|--------------------------|
| 13b    | 125.07     | ---                       | ---                      |
| 14     | 101.07     | 5.93, (d, 3.2)            | C-2, C-3                 |
| 9-OMe  | 61.88      | 3.92, (s)                 | C-9                      |
| 10-OMe | 57.02      | 3.86, (s)                 | C-10                     |

$^1\text{H}$ NMR spectrum of compound **128** showed five aromatic protons at  $\delta$  7.14, (1H, s, H-1), 6.59 (1H, s, H-4), 6.97 (1H, d,  $J$  = 8.4 Hz, H-11), 6.86 (1H, d,  $J$  = 8.4 Hz, H-12) and 6.08 (1H, s, H-13), one non aromatic proton at  $\delta$  5.62 (1H, s, H-8), three methylenes at  $\delta$  5.93 (2H, d,  $J$  = 3.2 Hz, H-14), 2.73 and 3.33 (2H, m, H-5) and 3.71 and 3.86 (2H, m, H-6) and two methoxy peaks at  $\delta$  3.92 (3H, s, 9-OMe) and 3.86 (3H, s, 10-OMe).

The  $^{13}\text{C}$ NMR spectrum of compound **128** showed twenty one carbon atoms which include six methine, three methylenes, two methoxy and nine quaternary carbon atoms according to DEPT-135 and DEPT-90 spectra.

In the HMBC spectra of compound **128** the methine proton at  $\delta$  7.14 (H-1) correlated to  $\delta$  146.76 (C-2), 147.48 (C-3), 128.73 (C-4a), 125.07 (C-13b) and 137.60 (C-13a), the methine proton appearing at  $\delta$  6.59 (H-4) showed correlations with  $\delta$  146.76 (C-2), 147.48 (C-3), 128.73 (C-4a), 125.07 (C-13b) and 30.52 (C-5), the methine proton at  $\delta$  5.62 (H-8) showed correlations with  $\delta$  137.60 (C-13a), 51.61 (C-6), 114.89 (C-8a), 129.36 (C-12a) and 146.41 (C-9), the methine proton chemical shift at  $\delta$  6.97 (H-11) correlated to  $\delta$  149.69 (C-10), 118.62 (C-12), 129.36 (C-12a) and 146.41 (C-9), the

methine proton appearing at  $\delta$  6.86 (H-12) showed correlations with  $\delta$  114.62 (C-11), 149.69 (C-10), 129.36 (C-12a), 114.89 (C-8a) and 97.52 (C-13), the methine proton at  $\delta$  6.08 (H-13) correlated to  $\delta$  129.36 (C-12a), 114.89 (C-8a), 137.60 (C-13a) and 118.62 (C-12).

The methylene protons appearing at  $\delta$  5.93 (H-14) correlated to  $\delta$  146.76 (C-2) and 147.48 (C-3), the methylene protons appearing at  $\delta$  2.73 and 3.33 (H-5) correlated to  $\delta$  51.61 (C-6), 128.73 (C-4a), 125.07 (C-13b) and 108.15 (C-4), similarly the methylene protons at  $\delta$  3.71 and 3.86 (H-6) showed correlations with  $\delta$  137.60 (C-13a), 73.53 (C-8), 128.73 (C-4a) and 30.52 (C-5) and two methoxy peaks at  $\delta$  3.92 (9-OMe) and 3.86 (10-OMe) correlated to  $\delta$  146.41 (C-9) and 149.69 (C-10) respectively.

The HMBC spectra of compound **128** were in full agreement with those for the alkaloidal isolation artifact 8-trichloromethyldihydroberberine which confirmed compound **128** to be 8-trichloromethyldihydroberberine.<sup>32</sup>

### 2.10.2. Pseudopalmatine (129).<sup>31</sup>

Brownish yellow needles (MeOH), m.p.: 202-204 °C.

Molecular formula: C<sub>21</sub>H<sub>22</sub>NO<sub>4</sub><sup>+</sup>

UV  $\lambda_{\max}$  (MeOH) (log  $\epsilon$ ): 346 (5.40), 432 (4.9) nm.

IR  $\bar{\nu}$ : 2997 (C-H, st. sat.), 1603, 1520 (C=C, st. aromatic.),

1501 (finger print region) cm.<sup>-1</sup>

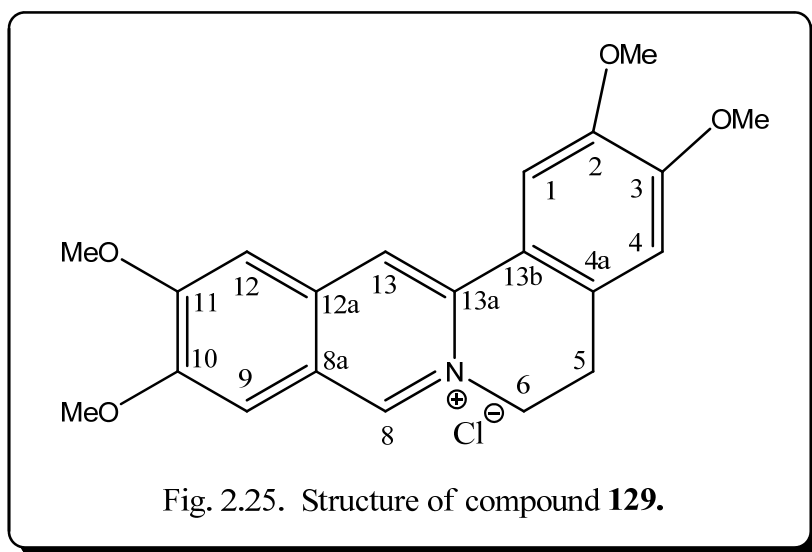
EI-MS m/z: 352 [M<sup>+</sup>] (calculated for C<sub>21</sub>H<sub>22</sub>NO<sub>4</sub><sup>+</sup>).

EI-MS  $m/z$  (rel. int. %): 352 (15,  $M^+$ ), 351 (42), 337 (100), 294 (42), 278 (34).

$^1\text{H}$ NMR: see **Table. 2.19**.

$^{13}\text{C}$ NMR: see **Table. 2.19**.

HMBC: see **Table. 2.19**.



The  $^1\text{H}$ NMR of compound **129** showed six aromatic protons at  $\delta$  7.42, (1H, s, H-1), 6.79 (1H, s, H-4), 10.51 (1H, s, H-8), 7.77 (1H, s, H-9), 7.80 (1H, s, H-12) and 8.20 (1H, s, H-13), two methylene groups at  $\delta$  3.29 (2H, t,  $J = 7.4$  Hz, H-5) and 4.75 (2H, s,  $J = 7.4$  Hz, H-6) and four methoxy peaks at  $\delta$  4.29 (3H, s, 2-OMe), 4.05 (3H, s, 3-OMe), 3.95 (3H, s, 9-OMe) and 3.83 (3H, s, 10-OMe).

$^{13}\text{C}$ NMR spectrum of compound **129** showed twenty one carbon atoms which include six methine, two methylene, four methoxy and nine quaternary carbon atoms on the basis of DEPT-q spectrum.

**Table. 2.19.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **pseudopalmatine (129)** (in DMSO- $d_6$ )

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)         |
|------|---------------------|------------------------------------|----------------------------------|
| 1    | 109.23              | 7.42, (s)                          | C-2, C-3, C-4a, C-13b, C-13a     |
| 2    | 152.43              | ---                                | ---                              |
| 3    | 151.21              | ---                                | ---                              |
| 4    | 110.57              | 6.79, (s)                          | C-2, C-3, C-13b, C-4a, C-5       |
| 4a   | 129.11              | ---                                | ---                              |
| 5    | 26.24               | 3.29, (t, 7.40)                    | C-6, C-4a, C-13b, C-4            |
| 6    | 55.01               | 4.75, (t, 7.40)                    | C-13a, C-8, C-4a, C-5            |
| 8    | 144.31              | 10.51, (s)                         | C-13a, C-6, C-8a, C-12a, C-9     |
| 8a   | 121.42              | ---                                | ---                              |
| 9    | 127.73              | 7.77, (s)                          | C-10, C-8a, C-12a, C-11, C-8     |
| 10   | 143.66              | ---                                | ---                              |
| 11   | 149.11              | ---                                | ---                              |
| 12   | 124.13              | 7.80, (s)                          | C-11, C-10, C-12a,<br>C-8a, C-13 |
| 12a  | 133.66              | ---                                | ---                              |
| 13   | 120.87              | 8.20, (s)                          | C-12a, C-8a, C-13a, C-12         |
| 13a  | 137.19              | ---                                | ---                              |

Continue

| C.No   | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|--------|------------|---------------------------|--------------------------|
| 13b    | 119.92     | ---                       | ---                      |
| 2-OMe  | 61.34      | 4.29, (s)                 | C-2                      |
| 3-OMe  | 57.21      | 4.05, (s)                 | C-3                      |
| 9-OMe  | 56.11      | 3.95, (s)                 | C-9                      |
| 10-OMe | 55.53      | 3.83, (s)                 | C-10                     |

In the HMBC spectra of compound **129** the methine proton at  $\delta$  7.72 (H-1) correlated to  $\delta$  152.43 (C-2), 151.21 (C-3), 129.11 (C-4a), 119.92 (C-13b) and 137.19 (C-13a), the methine proton appearing at  $\delta$  6.79 (H-4) showed correlations with  $\delta$  152.43 (C-2), 151.21 (C-3), 129.11 (C-4a), 119.92 (C-13b) and 26.24 (C-5), the methine proton at  $\delta$  10.51 (H-8) showed correlations with  $\delta$  137.19 (C-13a), 55.01 (C-6), 121.42 (C-8a), 133.66 (C-12a) and 127.73 (C-9), the methine proton chemical shift at  $\delta$  7.77 (H-9) correlated to  $\delta$  143.66 (C-10), 121.42 (C-8a), 133.66 (C-12a), 149.11 (C-11) and 144.31 (C-8), the methine proton appearing at  $\delta$  7.80 (H-12) showed correlations with  $\delta$  127.73 (C-11), 143.66 (C-10), 133.66 (C-12a), 121.42 (C-8a) and 120.87 (C-13), the methine proton at  $\delta$  8.20 (H-13) correlated to  $\delta$  133.66 (C-12a), 121.42 (C-8a), 137.19 (C-13a) and 124.13 (C-12).

The methylene protons appearing at  $\delta$  3.29 (H-5) correlated to  $\delta$  55.01 (C-6), 129.11 (C-4a), 119.92 (C-13b) and 110.57 (C-4). Similarly the methylene protons at  $\delta$  4.75 (H-6) showed correlations with  $\delta$  137.19 (C-13a), 144.31 (C-8), 129.11 (C-4a) and 26.24 (C-5)

and the four methoxy peaks at  $\delta$  4.29 (2-OMe), 4.05 (3-OMe), 3.95 (10-OMe) and 3.86 (11-OMe) correlated to  $\delta$  152.43 (C-2), 151.21 (C-3), 143.66 (C-10) and 149.11 (C-11) respectively.

The HMBC data for compound **129** was similar to that of pseudopalmitine and therefore compound **129** was confirmed to be pseudopalmitine.<sup>31</sup>

## 2.11. New source non-alkaloids isolated from *Berberis glaucocarpa* Stapf stem.

### 2.11.1. Syringic acid (**130**).<sup>41</sup>

White semi crystalline solid (CHCl<sub>3</sub>), m.p.: 205-207 °C (Lit: 203-205°C).<sup>41</sup>

Molecular formula: C<sub>9</sub>H<sub>10</sub>O<sub>5</sub>

UV  $\lambda_{\max}$  (MeOH) (log  $\epsilon$ ): 272.5 (5.29) nm.

IR  $\bar{\nu}$ : 3380 (O-H, st.), 1675 (C=O, st.), 1564 (C=C, st. aromatic.),

1480 (finger print region) cm.<sup>-1</sup>

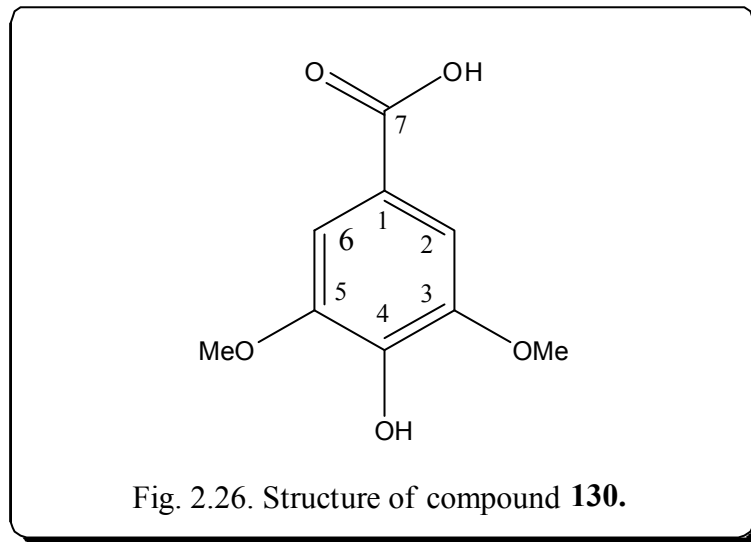
EI-MS m/z: 198 [M<sup>+</sup>] (calculated for C<sub>9</sub>H<sub>10</sub>O<sub>5</sub>).

EI-MS m/z (rel. int. %): 198 (100, M<sup>+</sup>), 183 (34), 167 (17), 153 (11), 127 (19), 97 (27), 83 (36), 71 (34), 69 (41).

<sup>1</sup>HNMR: see **Table. 2.20**.

<sup>13</sup>CNMR: see **Table. 2.20**.

HMBC: see **Table. 2.20**.



The  $^1\text{H}$ NMR of compound **130** showed only one aromatic proton peak at  $\delta$  7.34 (2H, s, H-2, 6) and one methoxy peak at  $\delta$  3.90 (3H, s, 3-OMe and 5-OMe). Both these peaks were considered for double number of what they represent.

The DEPT-q spectrum of compound **130** showed nine carbon atoms which included one methine (equal for two), one methoxy (equal for two) and five quaternary carbon atoms.

In the HMBC spectrum of compound **130** the proton at  $\delta$  7.34 showed correlations with  $\delta$  120.77 (C-1), 140.55 (C-3), 147.34 (C-4), 168.93 (C-7) and 106.77 (C-6), the methoxy peak at  $\delta$  3.90 correlated to  $\delta$  140.55 (C-3, 5)

From the above spectral data and by comparison with the reported values compound **130** was confirmed as syringic acid.<sup>41</sup>

**Table. 2.20.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **syringic acid (130)** (in  $\text{CDCl}_3$ )

| C.No  | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|-------|---------------------|------------------------------------|--------------------------|
| 1     | 120.77              | ---                                | ---                      |
| 2     | 106.77              | 7.34, (s)                          | C-1, C-3, C-4, C-7, C-6  |
| 3     | 140.55              | ---                                | ---                      |
| 4     | 147.34              | ---                                | ---                      |
| 5     | 140.55              | ---                                | ---                      |
| 6     | 106.77              | 7.34, (s)                          | C-1, C-3, C-4, C-7, C-6  |
| 7     | 168.93              | ---                                | ---                      |
| 3-OMe | 55.52               | 3.90, (s)                          | C-3                      |
| 5-OMe | 55.52               | 3.90, (s)                          | C-5                      |

**2.11.2. Syringaresinol (131).**<sup>42</sup>

Colorless crystals (MeOH), m.p.: 183-185 °C (Lit: 183-184°C).<sup>42</sup>

Molecular formula:  $\text{C}_{22}\text{H}_{26}\text{O}_8$

UV  $\lambda_{\text{max}}$  (MeOH) (log  $\epsilon$ ): 240 (5.61), 276 (5.22) nm.

IR  $\bar{\nu}$ : 3325 (O-H, st.), 1612 (C=C, st. aromatic.), 1520 (C=C, st. aromatic.), 1456, 1378, 1320 (finger print region)  $\text{cm}^{-1}$

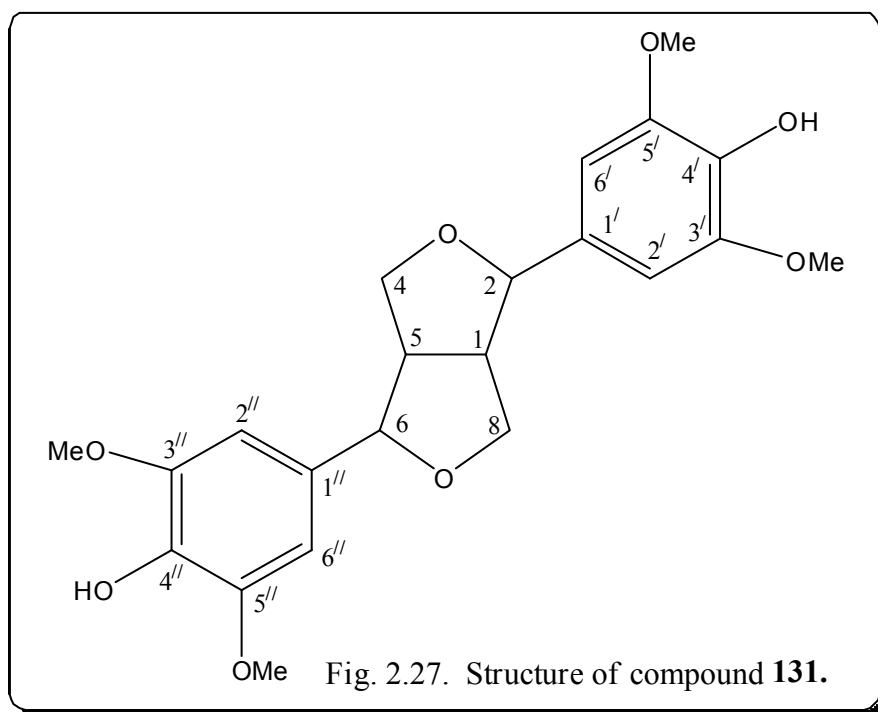
EI-MS m/z: 418 [ $\text{M}^+$ ] (calculated for  $\text{C}_{22}\text{H}_{26}\text{O}_8$ ).

EI-MS  $m/z$  (rel. int. %): 418 (100,  $M^+$ ), 210 (20), 193 (26), 181 (99), 167 (70).

$^1\text{H}$ NMR: see **Table 2.21**.

$^{13}\text{C}$ NMR: see **Table 2.21**.

HMBC: see **Table 2.21**.



The  $^1\text{H}$ NMR of compound **131** showed a resonance at  $\delta$  6.60 (4H, s, H-2', H-6', H-2'' and H-6''), 5.52 (2H, s, 4' -OH and 4'' - OH), 4.76 (2H, d,  $J$  = 4.0 Hz, H-2 and H-6), 4.31 (2H, dd,  $J$  = 9.2, 6.8 Hz, H-4 and H-8), 3.94 (2H, m, H-4 and H-8), 3.12 (2H, m, H-1 and H-6) and a methoxy peak at  $\delta$  3.92 (3H, s, 3' -OCH<sub>3</sub>, 5' -OCH<sub>3</sub>, 3'' -OCH<sub>3</sub> and 5'' -OCH<sub>3</sub>).

**Table. 2.21.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **syringaresinol (131)** (in  $\text{CDCl}_3$ )

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C)        |
|------|---------------------|------------------------------------|---------------------------------|
| 1    | 54.40               | 3.12, (m)                          | C-5, C-1'                       |
| 2    | 86.09               | 4.76, (d, 4.0)                     | C-1, C-4, C-2', C-1'            |
| 4    | 71.84               | 4.31, (dd, 9.2 and 6.8)            | C-1, C-2                        |
|      |                     | 3.94, (m)                          | C-1, C-2                        |
| 5    | 54.40               | 3.12, (m)                          | C-1, C-1''                      |
| 6    | 86.09               | 4.76, (d, 4.0)                     | C-5, C-8, C-1'', C-2''          |
| 8    | 71.84               | 4.31, (dd, 9.2 and 6.8)            | C-5, C-6                        |
|      |                     | 3.94, (m)                          | C-5, C-6                        |
| 1'   | 132.13              | ---                                | ---                             |
| 2'   | 102.76              | 6.60, (s)                          | C-2, C-6', C-4', C-3', C-1'     |
| 3'   | 147.18              | ---                                | ---                             |
| 4'   | 134.36              | ---                                | ---                             |
| 5'   | 147.18              | ---                                | ---                             |
| 6'   | 102.76              | 6.60, (s)                          | C-2', C-2, C-1', C-5', C-4'     |
| 1''  | 132.13              | ---                                | ---                             |
| 2''  | 102.76              | 6.60, (s)                          | C-6, C-6'', C-1'', C-4'', C-3'' |
| 3''  | 147.18              | ---                                | ---                             |
| 4''  | 134.36              | ---                                | ---                             |

Continue

| C.No                              | $\delta_C$ | $\delta_H$ (mult, J Hz) | HMBC (H $\rightarrow$ C)                                                           |
|-----------------------------------|------------|-------------------------|------------------------------------------------------------------------------------|
| 5 <sup>//</sup>                   | 147.18     | ---                     | ---                                                                                |
| 6 <sup>//</sup>                   | 102.76     | 6.60, (s)               | C-6, C-2 <sup>//</sup> , C-1 <sup>//</sup> , C-5 <sup>//</sup> , C-4 <sup>//</sup> |
| 3'-OCH <sub>3</sub>               | 56.40      | 3.92, (s)               | C-3'                                                                               |
| 4'-OH                             | ---        | 5.52, (s)               | C-3', C-5'                                                                         |
| 5'-OCH <sub>3</sub>               | 56.40      | 3.92, (s)               | C-5'                                                                               |
| 3 <sup>//</sup> -OCH <sub>3</sub> | 56.40      | 3.92, (s)               | C-3 <sup>//</sup>                                                                  |
| 4 <sup>//</sup> -OH               | ---        | 5.52, (s)               | C-3 <sup>//</sup> , C-5 <sup>//</sup>                                              |
| 5 <sup>//</sup> -OCH <sub>3</sub> | 56.40      | 3.92, (s)               | C-5 <sup>//</sup>                                                                  |

<sup>13</sup>CNMR spectrum of compound **131** contained a total of eight peaks for twenty two carbon atoms which included three quaternary carbons at  $\delta$  147.18 (C-3', C-5', C-3<sup>//</sup> and C-5<sup>//</sup>), 134.36 (C-4' and C-4<sup>//</sup>) and 132.13 (C-1' and C-1<sup>//</sup>), one methylene carbon at  $\delta$  71.84 (C-4, C-8), and three methine carbon atoms at  $\delta$  102.76 (C-2', C-6', C-2<sup>//</sup>, C-6<sup>//</sup>), 86.09 (C-2, C-6) and 54.40 (C-1, C-5) and one methoxy carbon peak at  $\delta$  56.40 (3' - OCH<sub>3</sub>, 5' -OCH<sub>3</sub>, 3<sup>//</sup> -OCH<sub>3</sub> and 5<sup>//</sup> -OCH<sub>3</sub>).

In the HMBC spectrum of compound **131** the resonance at  $\delta$  6.60 showed correlations with  $\delta$  86.09 (C-2, C-6), 102.76, 132.13 (C-1' and C-1<sup>//</sup>), 134.36 (C-4' and C-4<sup>//</sup>) and 147.18 (C-3', C-5', C-3<sup>//</sup> and C-5<sup>//</sup>), the proton appearing at  $\delta$  5.52 correlated to  $\delta$  134.36 (C-4', C-4<sup>//</sup>) and 147.18 (C-3', C-5', C-3<sup>//</sup>, C-5<sup>//</sup>), the doublet at  $\delta$  4.76 correlated to  $\delta$  54.40 (C-1, C-5), 71.84 (C-4, C-8), 102.76 (C-2', C-2<sup>//</sup>), 132.13 (C-1', C-1<sup>//</sup>), the doublet

of doublet appearing at  $\delta$  4.31 correlated to  $\delta$  54.40 (C-1, C-5) and 86.09 (C-2, C-6), the multiplet at  $\delta$  3.94 also correlated to  $\delta$  54.40 (C-1, C-5) and 86.09 (C-2, C-6), the multiplet appearing at  $\delta$  3.12 correlated to  $\delta$  54.40 (C-1, C-5) and 132.13 (C-1', C-1'') similarly the methoxy peak appearing at  $\delta$  correlated to  $\delta$  147.18 (C-3', C-5', C-3'', C-5'').

The 1D and 2D data of compound **131** was in complete agreement with the reported values for syringaresinol confirming the literature.<sup>42</sup>

### 2.11.3. Stigmasterol (132).<sup>43</sup>

Physical state: white powder (CHCl<sub>3</sub>).

Melting point: 173-174 °C (174-176 °C).<sup>43</sup>

Molecular formula: C<sub>29</sub>H<sub>48</sub>O

IR  $\bar{\nu}$ : 3376 (O-H, st.), 2931 (C-H, st. sat.), 1636 (C=C, st. olefinic.), 1463, 1442, 1375 (finger print region) cm.<sup>-1</sup>

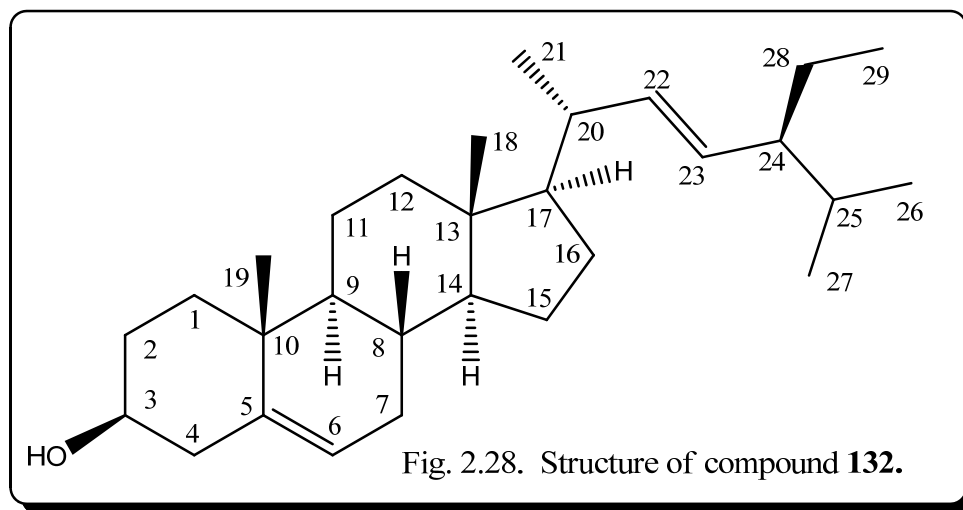
EI-MS m/z: 412 [M<sup>+</sup>] (calculated for C<sub>29</sub>H<sub>48</sub>O).

EI-MS m/z (rel. int. %): 412 (37, M<sup>+</sup>), 396 (87), 381 (44), 329 (74), 301 (80), 273 (53), 255 (72), 213 (62), 161 (55), 145 (45), 107(61), 81(35), 55 (36).

<sup>1</sup>HNMR: see **Table. 2.22.**

<sup>13</sup>CNMR: see **Table. 2.22.**

HMBC: see **Table. 2.22.**



The  $^1\text{H}$ NMR of compound **132** indicated the presence of two methyl groups singlets at  $\delta$  0.66 (3H, s, H-18) and  $\delta$  0.98 (3H, s, H-19), three methyl group doublets at  $\delta$  0.89 (d,  $J$  = 6.4 Hz, H-21), 0.79 (d,  $J$  = 8.4 Hz, H-26) and 0.82 (d,  $J$  = 8.4 Hz, H-27), one methyl triplet at  $\delta$  0.80 (t,  $J$  = 8.4 Hz, H-29), an olefinic proton broad doublet at  $\delta$  5.35 (1H, d,  $J$  = 2.5 Hz, H-6) and a multiplet at  $\delta$  3.51 (H-3).

The  $^{13}\text{C}$ NMR of compound **132** showed the presence of twenty nine carbon atoms. Six methyl carbons resonating at  $\delta$  11.87 (C-18), 19.42 (C-19), 18.81 (C-21), 19.05 (C-26), 19.81 (C-27) and 11.94 (C-29), eleven methylene carbon atoms at  $\delta$  37.25 (C-1), 31.64 (C-2), 39.81 (C-4), 31.95 (C-7), 21.08 (C-11), 42.34 (C-12), 24.30 (C-15), 28.24 (C-16), 138.43 (C-22), 130.04 (C-23), 23.07 (C-28), nine methine carbon atoms at  $\delta$  71.80 (C-3), 121.70 (C-6), 33.71 (C-8), 50.19 (C-9), 56.73 (C-14), 56.06 (C-17), 36.14 (C-20), 45.83 (C-24) and 11.94.17 (C-29) and three quaternary carbons at  $\delta$  140.73 (C-5), 36.51 (C-10) and 38.80 (C-13) according to DEPT-135 and DEPT-90 spectra.

**Table. 2.22.**  $^{13}\text{C}$ ,  $^1\text{H}$  and HMBC data of **stigmasterol (132)** (in  $\text{CDCl}_3$ )

| C.No | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|------|---------------------|------------------------------------|--------------------------|
| 1    | 37.25               | 1.06, (m)                          | ---                      |
| 2    | 31.64               | 1.22 and 1.47, (m)                 | ---                      |
| 3    | 71.80               | 3.51, (m)                          | ---                      |
| 4    | 39.81               | 1.99, (br.t, 12.4)                 | C-2, C-3, C-5, C-6, C-10 |
| 5    | 140.73              | ---                                | ---                      |
| 6    | 121.70              | 5.35, (1H, d, 2.5)                 | C-4, C-8, C-10           |
| 7    | 31.95               | 1.43 and 1.93, (m)                 | ---                      |
| 8    | 33.71               | 1.42, (m)                          | ---                      |
| 9    | 50.19               | 0.85, (m)                          | ---                      |
| 10   | 36.51               | ---                                | ---                      |
| 11   | 21.08               | 1.48, (m)                          | ---                      |
| 12   | 42.34               | 2.20, (m)                          | ---                      |
| 13   | 38.80               | ---                                | ---                      |
| 14   | 56.73               | 0.95, (br.s)                       | ---                      |
| 15   | 24.30               | 1.52, (m)                          | ---                      |
| 16   | 28.24               | 1.19, (m)                          | ---                      |
| 17   | 56.06               | 1.06, (m)                          | ---                      |

Continue

| C.No | $\delta_C$ | $\delta_H$ (mult, $J$ Hz) | HMBC (H $\rightarrow$ C) |
|------|------------|---------------------------|--------------------------|
| 18   | 11.87      | 0.66, (s)                 | C-12, C-13, C-14, C-17   |
| 19   | 19.42      | 0.98, (s)                 | C-1, C-9, C-10           |
| 20   | 36.14      | 1.33, (m)                 | ---                      |
| 21   | 18.81      | 0.89, (d, 6.4)            | C-17, C-20, C-22         |
| 22   | 138.43     | 5.03, (m)                 | ---                      |
| 23   | 130.04     | 5.12, (m)                 | ---                      |
| 24   | 45.83      | 0.90, (m)                 | ---                      |
| 25   | 29.12      | 1.24, (m)                 | ---                      |
| 26   | 19.05      | 0.79, (d, 8.4)            | C-24, C-25, C-27         |
| 27   | 19.81      | 0.82, (d, 8.4)            | C-24, C-25, C-26         |
| 28   | 23.07      | 1.19 and 1.23, (m)        | ---                      |
| 29   | 11.94      | 0.80, (t, 8.4)            | C-24, C-28               |

The  $^1\text{H}$ NMR,  $^{13}\text{C}$ NMR and EIMS data for compound **132** was compared with the literature which confirmed compound **132** to be stigmasterol.<sup>43</sup>

### **3.1. General experimental.**

#### **3.1.1. Melting points.**

Melting points were determined on Stuart digital melting point apparatus (SMP 10) and were uncorrected.

#### **3.1.2. UV-Vis spectra.**

Ultraviolet spectra were recorded on Thermo Spectronic Unicam UV-300 using methanol as a solvent for most of the samples. Dichloromethane and pyridine were used for UV spectrum of one compound.

#### **3.1.3. Infrared spectra.**

Infrared spectra were recorded on JASCO FTIR- 4200A and Thermo Scientific FTIR Nicolet-380.

#### **3.1.4. Mass spectra.**

Electron impact ionization mass spectrometry (EIMS) was carried out with JEOL MSRoute instrument. High resolution Electrospray ionization mass spectrometry (HR-ESIMS) was performed on Thermo Scientific Exactive LCQ Fleet instrument and Fast atom bombardment (FAB) spectra were collected with JEOL JMS HX 110 mass instrument.

### **3.1.5. Nuclear Magnetic Resonance (NMR) spectra.**

$^1\text{H}$ NMR,  $^{13}\text{C}$ NMR and 2D NMR were recorded on Bruker Avance DRX-400 and 500 MHz and JEOL 400 MHz instruments.

### **3.1.6. Solvents.**

Commercial grade solvents after distillation were used for chromatography and purification of compounds while deuterated solvents were used for NMR spectral analysis.

## **3.2. Techniques used for the isolation and purification of compounds.**

### **3.2.1. Column Chromatography (CC).**

Merck Kieselgel silica gel 60 PF<sub>254</sub> (70-230 mesh ASTM) and Sephadex LH-20 were used for column chromatography.

### **3.2.2. Thin layer and preparative thin layer chromatography (TLC and PTLC).**

Thin layer chromatography was carried out on silica gel 60 F<sub>254</sub> and preparative thin layer chromatography was done on silica gel 60 PF<sub>254</sub> on 20 cm x 20 cm glass plates.

## **3.3. TLC Spray reagents.**

### **(a) Ceric sulphate.**

The TLC plates were visualized by spraying with ceric sulphate which was prepared by dissolving 0.26 g of ceric sulphate in 10 ml of sulphuric acid, diluted to 100 ml with water.

**(b) Anisaldehyde.**

Anisaldehyde spray reagent used for the visualization of TLC was prepared by mixing glacial acetic acid, anisaldehyde and concentrated sulphuric acid (98:1:1).

**(c) Dragendorff's reagent.**

Dragendorff's reagent was used for the visualization of alkaloids on TLC. It was prepared by the following method.

**Solution A.** It was prepared by dissolving 0.85 g of bismuth nitrate in a mixture of acetic acid (10 ml) and distilled water (40 ml).

**Solution B.** It was prepared by dissolving 8.0 g of potassium iodide in 20 ml of distilled water.

**Stock solution.** Equal volumes of solution A and solution B were mixed to be stored as stock solution.

**Spray reagent.** Spray reagent for TLC was prepared by mixing 1.0 ml of stock solution with 1.0 ml of acetic acid and 10.0 ml of distilled water.

**3.4. Plant Material.**

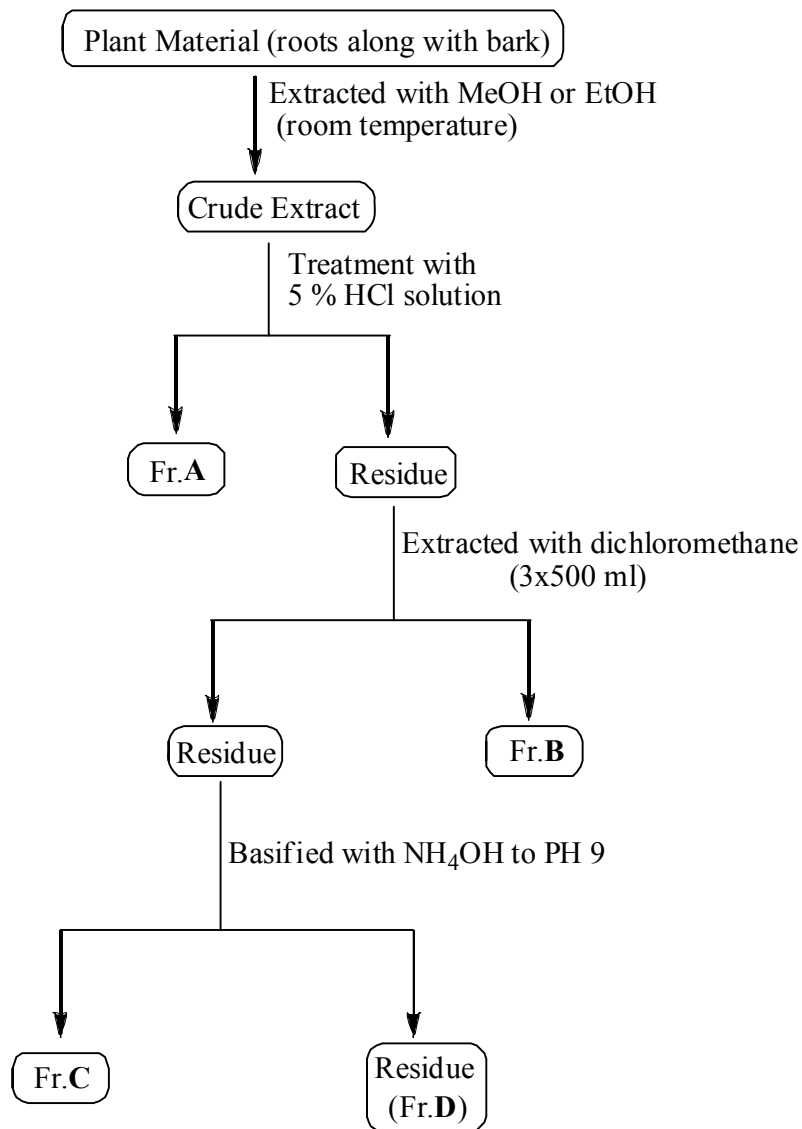
*Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf were collected from Azad Kashmir, Pakistan in July 2009 and were identified by Prof Dr. Tanveer Akhtar (Chairperson Botany Department University of Azad Jammu and

Kashmir). Voucher specimen number 9615-B and 9615-A respectively were deposited in the herbarium of the Botany Department, University of Peshawar.

#### **3.4.1. General scheme of fractionation for *Berberis jaeschkeana* and *Berberis glaucocarpa* Stapf.**

The air dried roots (with bark) and stem (with bark) of *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf were soaked in commercial grade methanol and ethanol respectively for ten days at room temperature. The extract after filtration was concentrated under reduced pressure using rotary evaporator. The concentrated crude extract was dissolved/suspended in sufficient amount of distilled water, acidified with 5 % HCl solution and allowed to stand overnight, followed by filtration to afford Fraction **A**. The residue was then repeatedly extracted with dichloromethane, using separating funnel to afford Fraction **B**. After this the residue was basified with ammonia (NH<sub>4</sub>OH) to PH 9 and then extracted with ethyl acetate to afford Fraction **C**. The remainder residue was termed as Fraction **D**.

The general scheme of extraction and isolation from *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf is shown in **Scheme. 3.1**.



Fr.A = Fraction A (acidic fraction)      Fr.B = Fraction B (dichloromethane fraction)

Fr.C = Fraction C (basic fraction)      Fr.D = (Remaider aqueous fraction)

**Scheme. 3.1. General fractionaion scheme for *Berberisjaeschkeana* Schneid and *Berberis glaucocarpa* Stapf.**

### 3.4.2. Extraction and isolation of compounds from the roots of *Berberis jaeschkeana* Schneid var. *jaeschkeana*.

*Berberis jaeschkeana* Schneid var. *jaeschkeana* roots (with bark 8 Kg) was powdered with grinder and then soaked in commercial grade methanol for ten days. The methanolic extract so obtained was concentrated under reduced pressure using rotary evaporator. The gummy crude extract obtained was suspended in sufficient amount of distilled water and then fractionated according to the fractionation scheme for alkaloids (**Scheme. 3.1**).

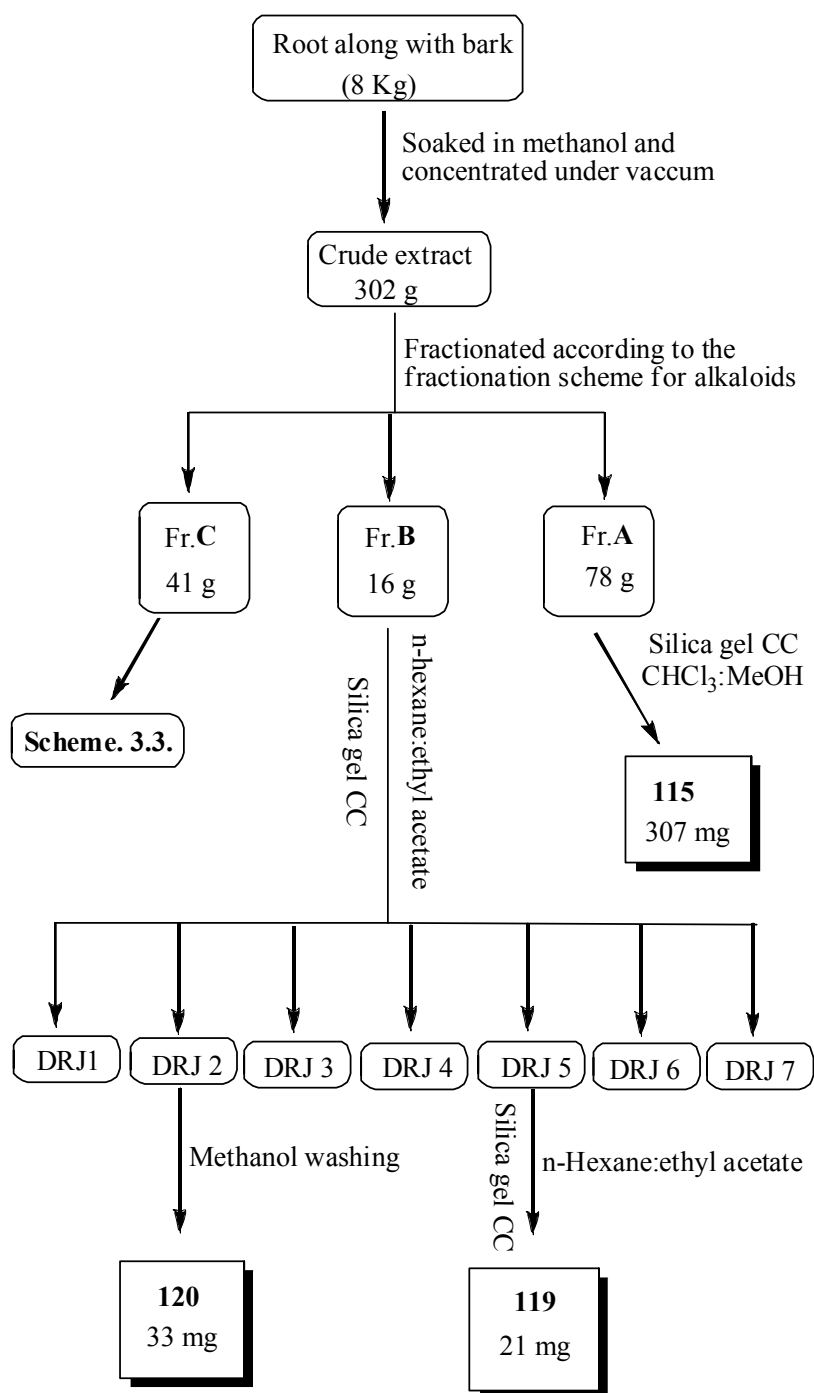
Fraction **A** consisted entirely of berberine. It was subjected to silica gel column chromatography and the column was eluted with chloroform:methanol with increasing the polarity gradually to afford compound **115**. Fraction **B** was also subjected to silica gel column chromatography and the column was eluted with n-hexane:ethyl acetate first and then with chloroform:methanol with increasing order of polarity to afford 147 fractions which were combined on the basis of comparative TLC into seven sub-fractions (**DRJ1-DRJ7**). Sub-fraction **DRJ 2** on subsequent washing with methanol resulted in compound **120**, similarly sub-fraction **DRJ 5** on subsequent column chromatography over silica gel and elution with n-hexane:ethyl acetate with increasing order of polarity resulted in compound **119**.

Fraction **C** was subjected to silica gel column chromatography and eluting the column with n-hexane:ethyl acetate (99:1 to 1:99) and then with chloroform:methanol with gradual increase in polarity to afford a total of 183 fractions, which were combined into twelve sub-fractions (**EJ1-EJ12**).

Sub-fraction **EJ3** was further subjected to column chromatography over silica gel and eluting the column with n-hexane: ethyl acetate (99:1 to 1:99) and chloroform:methanol with increasing order of polarity to afford ten sub-fractions (**EJ301-EJ310**), Sub-fraction **EJ307** on column chromatography over sephadex LH-20 and eluting the column with chloroform:methanol (40:60). Repeated column chromatography over sephadex LH-20 afforded compound **111**.

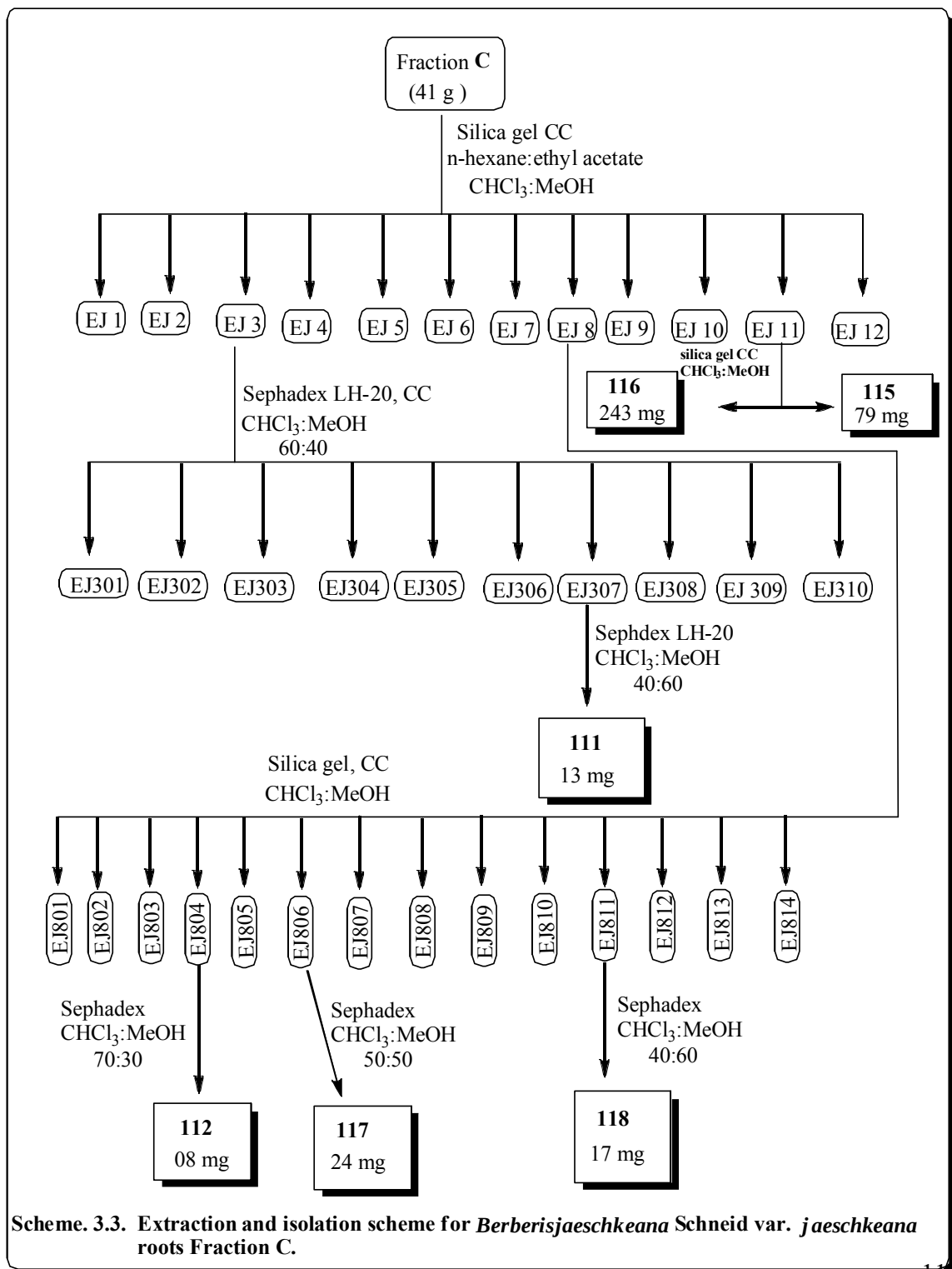
Sub-fraction **EJ8** was also subjected to column chromatography and eluting the column with n-hexane:acetone and dichloromethane:methanol with gradual increase in polarity to give fourteen sub-fractions (**EJ801-EJ814**). Sub-fraction **EJ804** was subjected to column chromatography over sephadex-LH 20 and the column was eluted with chloroform:methanol (60:40 first and then 50:50, 40:60 and 30:70). Repeated sephadex column chromatography resulted in compound **112**. Sub-fractions **EJ806** and **EJ 811** upon sephadex column chromatography and elution with chloroform:methanol (50:50) afforded compounds **117** and **118** respectively. While **EJ 11** upon repeated silica gel column chromatography and elution with chloroform:methanol with gradually increasing the polarity resulted in compounds **115** and **116**.

The scheme of extraction and isolation from *Berberis jaeschkeana* Schneid var. *jaeschkeana* roots Fraction **A** and **B** is shown in **Scheme. 3.2**.



**Scheme. 3.2.** Fractionation, extraction and isolation scheme for Fraction B of *Berberisjaeschkeana* Schneid var. *jaeschkeana* roots.

The scheme of extraction and isolation from *Berberis jaeschkeana* Schneid var. *jaeschkeana* roots Fraction C has been shown in **Scheme. 3.3**.



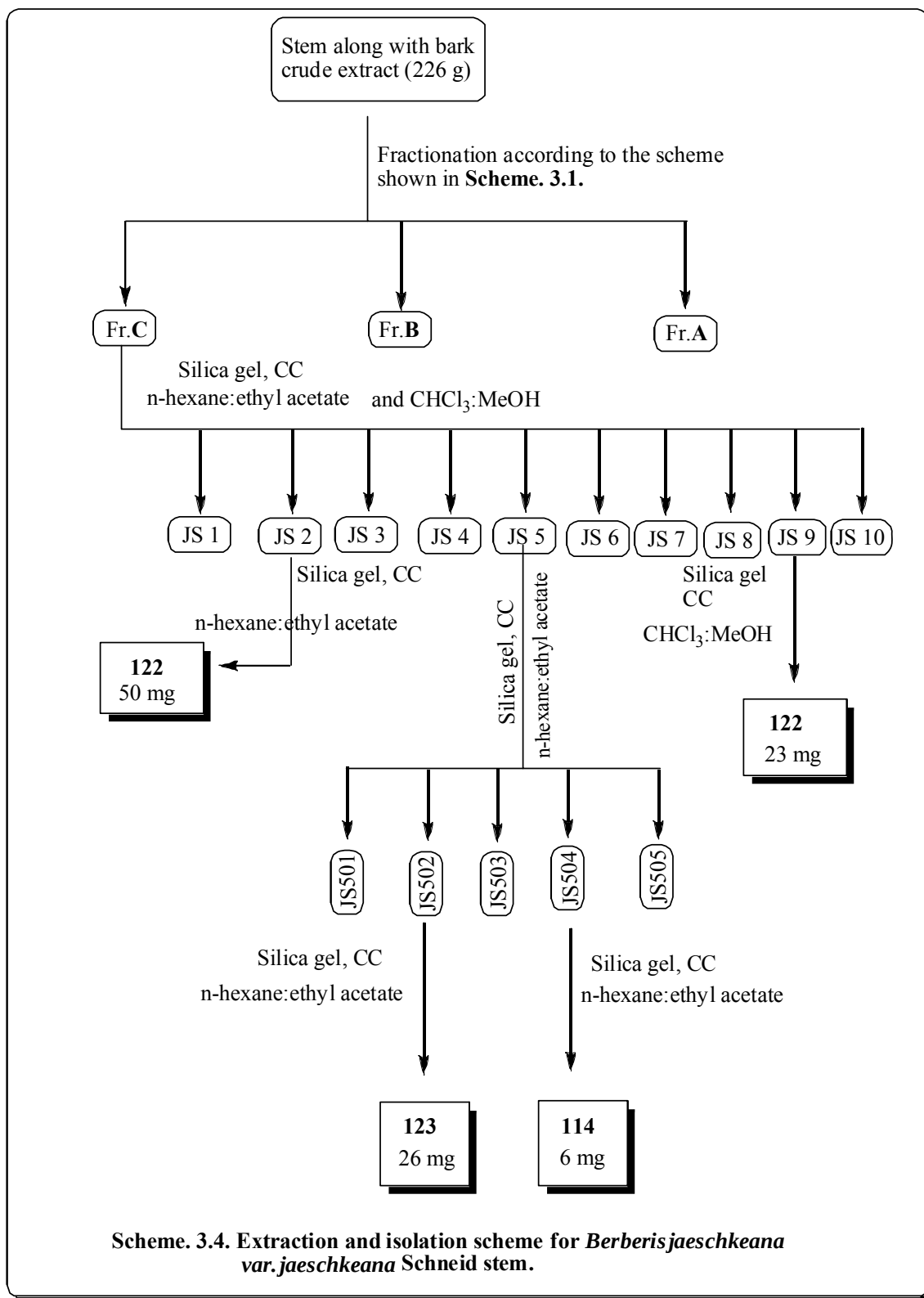
### 3.4.3. Extraction and isolation of compounds from the stem of *Berberis jaeschkeana* Schneid var. *jaeschkeana*.

*Berberis jaeschkeana* Schneid var. *jaeschkeana* stem (with bark 5 Kg) was first grinded to powder and then soaked in commercial grade methanol for one week. The extract so obtained was concentrated under reduced pressure using rotary evaporator. The gummy crude extract (226 g) was fractionated according to the fractionation scheme shown in **Scheme. 3.1.**

Fraction **C** was subjected to CC over silica gel and the column was eluted with a mixture of n-hexane:ethyl acetate (99:1 to 1:99) and chloroform: methanol with increasing order of polarity to afford 152 fractions which were combined (based on TLC) to ten sub-fractions (**JSC 1- JSC 10**). Sub-fraction **JSC 9** was subjected to silica gel column chromatography and the column was first eluted with chloroform and then gradually increasing the polarity with methanol followed by preparative TLC over silica gel using chloroform:methanol (15:85) with two drops of diethyl amine to afford compound **121**.

Sub-fraction **JSC 2** was subjected to column chromatography over silica gel using n-hexane and ethyl acetate (80:20) to afford compound **122**.

Sub-fraction **JSC 5** was subjected to CC over silica gel and eluted with a mixture of n-hexane:ethyl acetate and gradually increasing the polarity with ethyl acetate to afford five fractions (**JS501- JS505**). Fraction **JS 502** was subjected to column chromatography over silica gel and eluted with a mixture of n-hexane:ethyl acetate with gradual increase in polarity to afford compound **123**.



Fraction **JS 504** was subjected to silica gel column chromatography and eluted with n-hexane:ethyl acetate (65:35) to afford compound **114**.

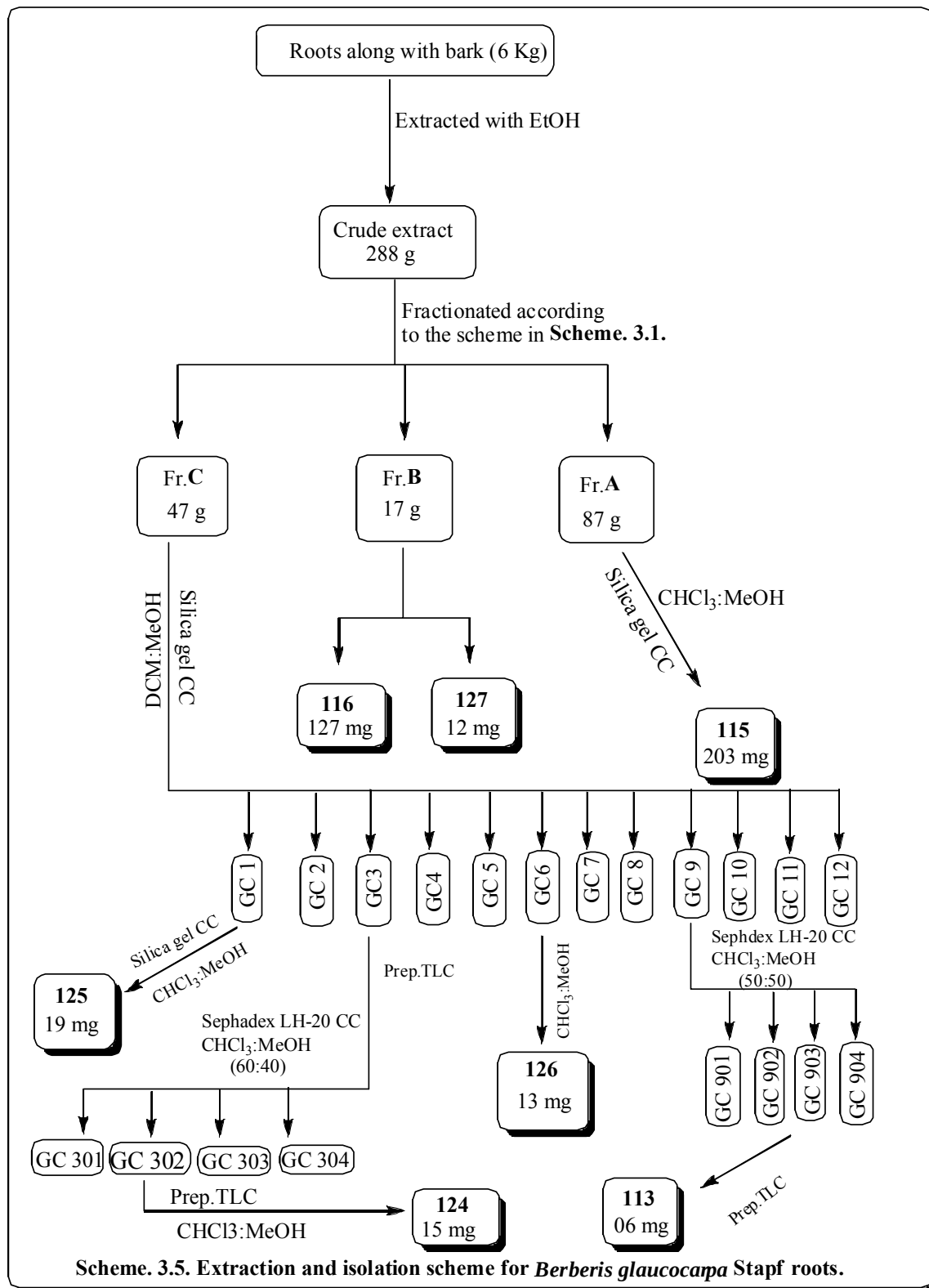
The scheme for extraction and isolation from *Berberis jaeschkeana* Schneid var. *jaeschkeana* stem has been shown in **Scheme. 3.4**.

#### **3.4.4. Extraction and isolation of compounds from the roots along with roots bark of *Berberis glaucocarpa* Stapf**

*Berberis glaucocarpa* Stapf roots (with bark 6 Kg) was grinded to powder and then soaked in commercial grade ethanol for ten days. The crude extract obtained after filtration was concentrated on a rotary evaporator to yield crude extract (288 g), which was fractionated according to the fractionation scheme shown in **Scheme. 3.1**.

Fraction **A** was berberine only. It was subjected to column chromatography using CHCl<sub>3</sub>:MeOH mixture as eluting solvent and gradually increasing the polarity with methanol to afford compound **115** (already obtained from *Berberis jaeschkeana*). Fraction **B** was subjected to silica gel CC and eluted with n-hexane:ethyl acetate first (99:1 to 1:99) and then with CHCl<sub>3</sub>:MeOH with gradual increase in polarity to afford compounds **120** (already obtained from *Berberis jaeschkeana*) and **127**.

Fraction **C** was subjected to CC and eluted with n-hexane:ethyl acetate and then CH<sub>2</sub>Cl<sub>2</sub>:MeOH by gradually increasing the polarity with ethyl acetate and methanol respectively to afford 193 fractions which were combined (based on TLC) to twelve sub-fractions (**GC 1 – GC12**). Sub-fraction **GC 1** was subjected to silica gel CC, eluted with CHCl<sub>3</sub>:MeOH with gradual increase in polarity with methanol to afford compound **125**.



Sub-fraction **GC 3** was subjected to column chromatography over sephadex LH-20 using  $\text{CHCl}_3$ :MeOH to afford four sub-fractions (**GC 301-GC 304**). The sub-fraction **GC 302** was subjected to preparative TLC on silica gel using  $\text{CHCl}_3$ :MeOH (93:07) as solvent system to afford compound **124**. Sub-fraction **GC 6** was also subjected to preparative TLC on silica gel using  $\text{CHCl}_3$ :MeOH (80:20) and few drops of diethyl amine to afford compound **126**.

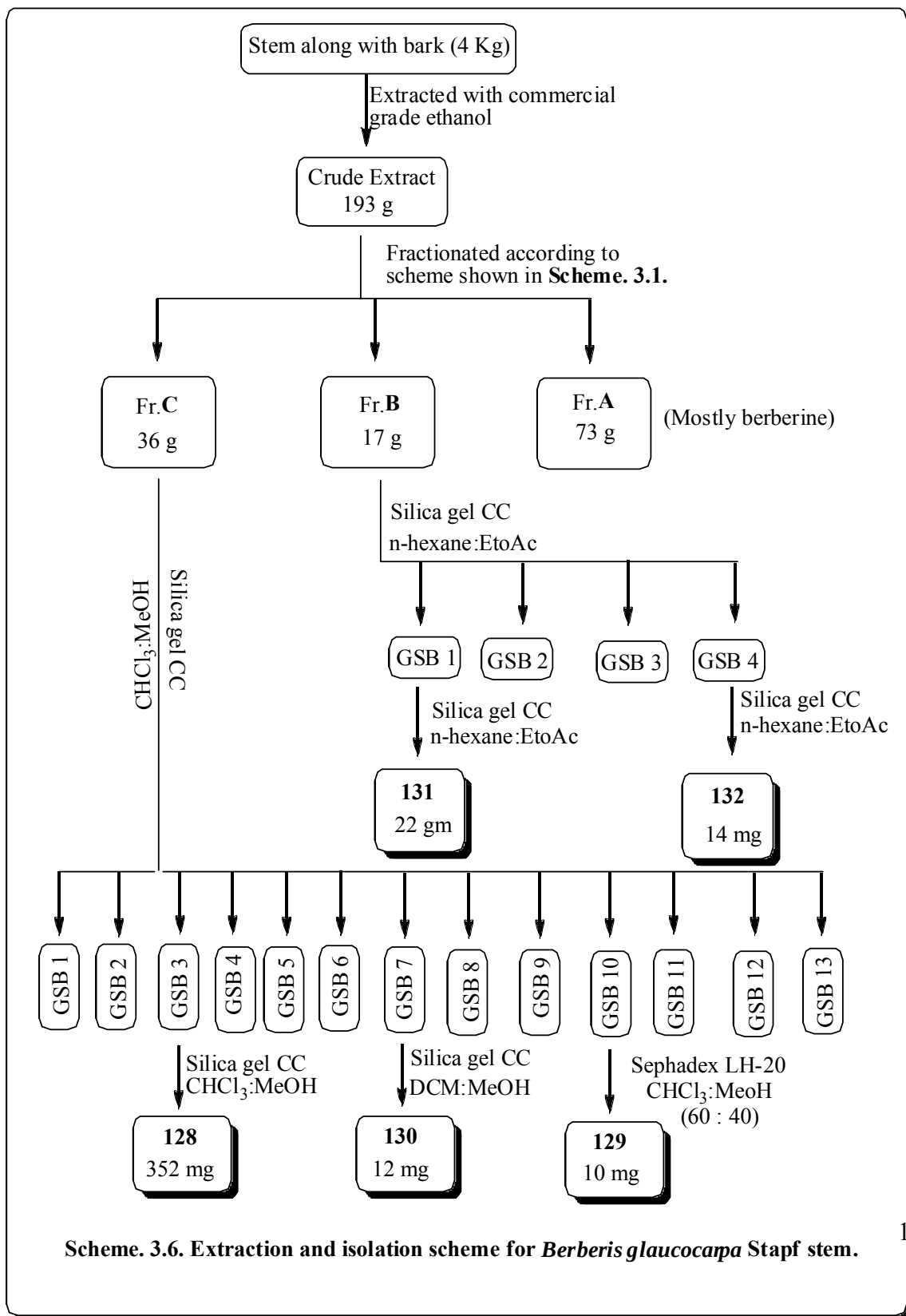
Sub-fraction **GC 9** was subjected to CC over sephadex LH-20 and elution with  $\text{CHCl}_3$ :MeOH (50:50) afforded three sub-fractions (**GC 901-GC 903**). Sub-fraction **GC 903** on subsequent preparative TLC on silica gel and using  $\text{CHCl}_3$ :MeOH (92: 08) as solvent system afforded compound **113**.

#### **3.4.5. Extraction and isolation of compounds from the stem including the bark of *Berberis glaucocarpa* Stapf.**

*Berberis glaucocarpa* Stapf stem along with the bark (4 Kg) was dried at room temperature and then grinded to powder with a grinder. The powdered material was then soaked in commercial grade ethanol for one week. The filtered extract was concentrated under reduced pressure using rotary evaporator. The concentrated crude extract (193 g) was fractionated according to the scheme shown in **Scheme. 3.1**.

Fraction **A** was almost entirely berberine (**115**), Fraction **B** was subjected to silica gel CC and eluted with n-hexane:ethyl acetate with gradually increasing order of polarity to afford four sub-fractions (**GSB1- GSB4**). Sub-fractions **GSB1** and **GSB 4** on subsequent

CC on silica gel and elution with n-hexane:ethyl acetate and gradually increasing the order of polarity afforded compounds **131** and **132** respectively.



Scheme. 3.6. Extraction and isolation scheme for *Berberis glaucocarpa* Stapf stem.

Fraction **C** was subjected to silica gel CC and eluted first with a mixtures of n-hexane:ethyl acetate (99:1 to 1:99) and then with  $\text{CHCl}_3$ :MeOH mixture with increasing order of polarity to afford 148 fractions which were combined on the basis of comparative TLC to give thirteen sub-fractions (**GSB 1–GSB 13**). The Sub-fraction **GSB 3** was then subjected to CC and eluted with a mixture of  $\text{CHCl}_3$ :MeOH (85:15) to afford compound **128**.

Sub-fraction **GSB 7** was subjected to CC and eluted with  $\text{CH}_2\text{Cl}_2$ :MeOH mixture with increasing order of polarity to afford compound **130**. Sub-fraction **GSB 10** was subjected to cc over sephadex LH-20 using  $\text{CHCl}_3$ :MeOH (50:50) followed by preparative TLC on silica gel using  $\text{CHCl}_3$ :MeOH (80:20) mixture as the solvent system to afford compound **129**.

**3.5. Experimental data of compounds from *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf.**

**3.5.1. Experimental data of new alkaloids from *Berberis jaeschkeana* Schneid var. *jaeschkeana* roots.**

**3.5.1.1. 10-Hydroxy chondrofoline (111).**

Physical state: off white amorphous powder (CHCl<sub>3</sub>)

M.p.: 195-197 °C

Molecular formula: C<sub>37</sub>H<sub>40</sub>N<sub>2</sub>O<sub>7</sub>

HR-ESIMS: 623.2772 [M-H]<sup>+</sup> (Calculated 623.2757).

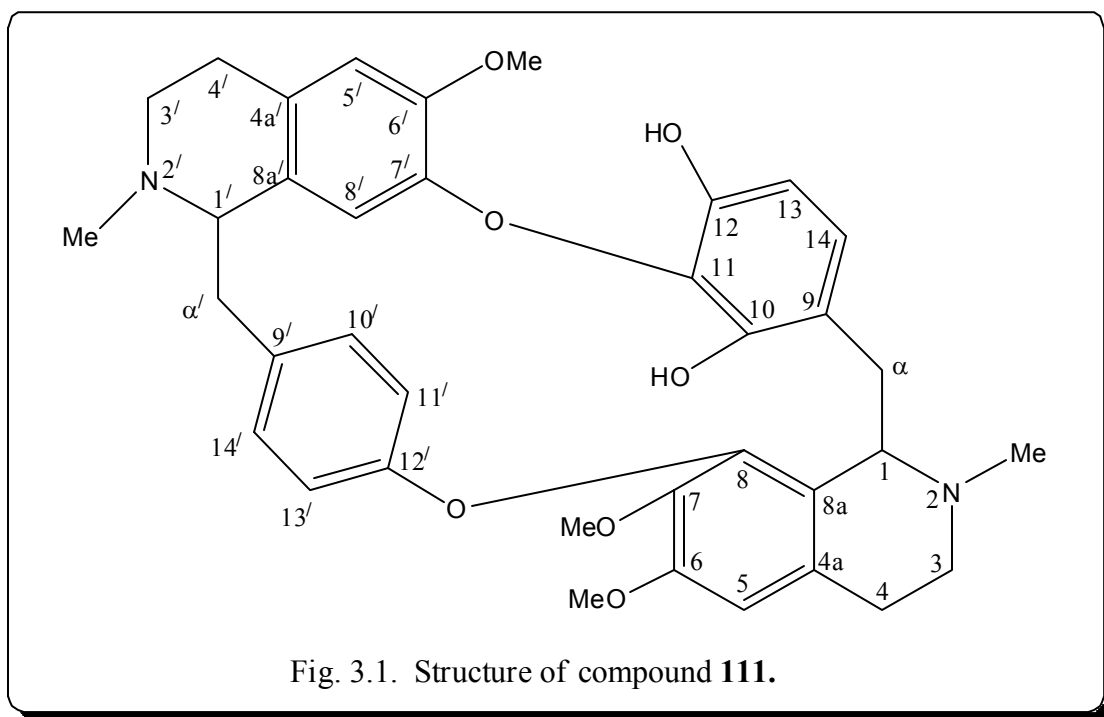
UV (MeOH) λ<sub>max</sub> (log ε): 282 (4.27) nm

IR  $\bar{\nu}$ : 3337 (O-H, st.), 2930 (C-H, st. sat.), 2840 (C-H, st. N-methyl), 1601, 1559 (C=C, st. aromatic.), 1030 cm.<sup>-1</sup>

<sup>1</sup>HNMR: see **Table. 2.1.** (Page 17)

<sup>13</sup>CNMR: see **Table. 2.1.** (Page 17)

HMBC: see **Table. 2.1.** (Page 17)



### 3.5.1.2. 8,12,12a-Trihydroxy-11,13a-berberin-dione (**112**).

Physical state: amorphous orange yellow sticky solid and decomposes to brown on exposure to light (CHCl<sub>3</sub>).

Molecular formula: C<sub>18</sub>H<sub>15</sub>NO<sub>8</sub>

HR-ESIMS: 374.0996 [M+H]<sup>+</sup> (Calculated for C<sub>18</sub>H<sub>16</sub>NO<sub>8</sub>: 374.0878).

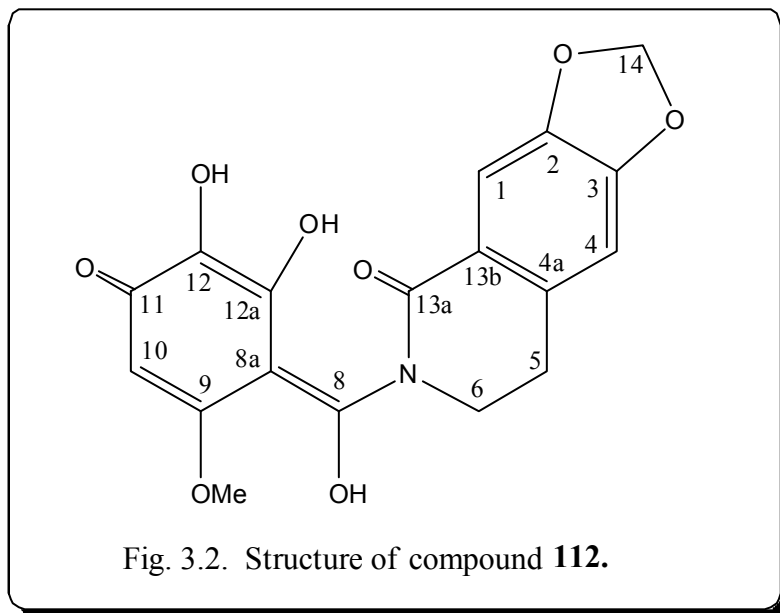
UV (MeOH) λ<sub>max</sub> (log ε): 300 (5.84) nm, 260 (5.87) nm, 230 (5.96) nm

IR ν̄: 3341 (O-H, st.), 1723, 1720 (C=O, st.) cm.<sup>-1</sup>

<sup>1</sup>HNMR: see **Table. 2.2.** (Page 23)

<sup>13</sup>CNMR: see **Table. 2.2.** (Page 23)

HMBC: see **Table. 2.2.** (Page 23)



### 3.6. Experimental data of a new alkaloid from *Berberis glaucocarpa* Stapf roots.

#### 3.6.1. 10-Methoxy chondrofoline (113).

Physical state: white amorphous solid (MeOH).

M.p.: 186-188 °C

Molecular formula: C<sub>38</sub>H<sub>42</sub>N<sub>2</sub>O<sub>7</sub>

HR-ESIMS: 639.2702 [M+H]<sup>+</sup> (Calculated 639.2992).

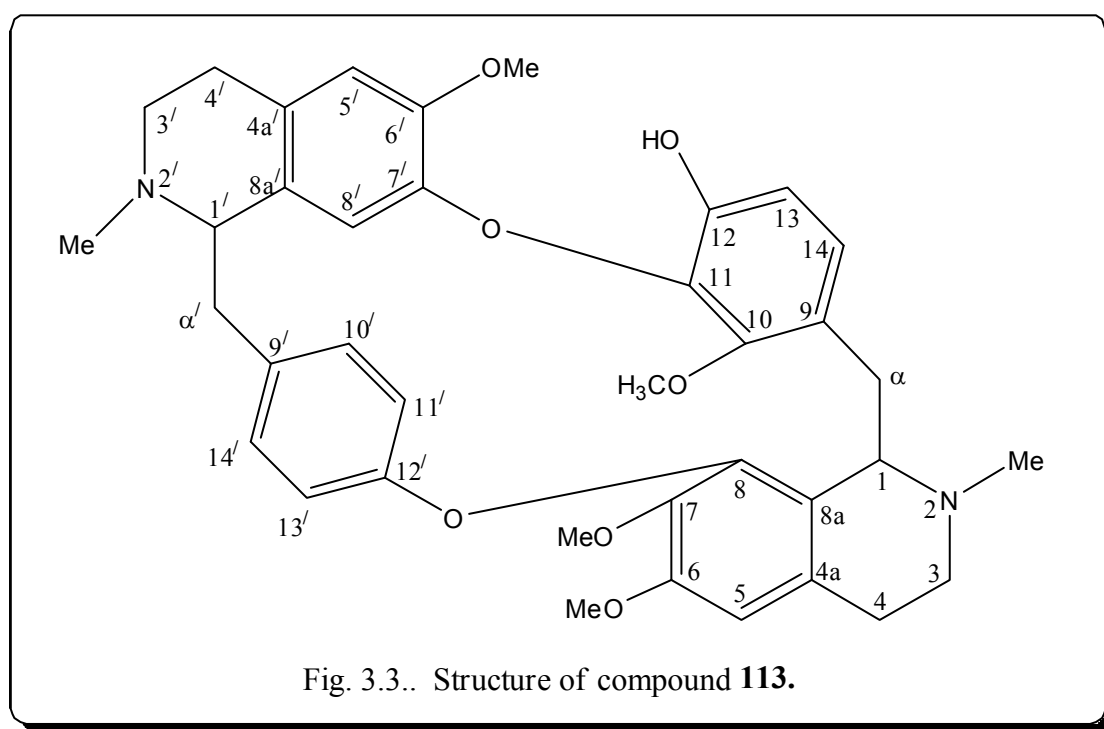
UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 281 (5.28) nm.

IR  $\bar{\nu}$ : 3351 (O-H, st.), 2987 (C-H, st. sat.), 2386 (C-H, st. N-methyl), 1600, 1501 (C=C, st. aromatic.), 1030  $\text{cm}^{-1}$

$^1\text{H}$ NMR: see **Table. 2.3.** (Page 27)

$^{13}\text{C}$ NMR: see **Table. 2.3.** (Page 27)

HMBC: see **Table. 2.3.** (Page 27)



### 3.7. Experimental data of a new non-alkaloid from *Berberis jaeschkeana*

Schneid var. *jaeschkeana* stem.

#### 3.7.1. Docosyl -2-hydroxytetracosanoate (114).

Physical state: white powder ( $\text{CHCl}_3$ ).

M.p.: 83-84 °C

Molecular formula: C<sub>46</sub>H<sub>92</sub>O<sub>3</sub>

UV (CH<sub>2</sub>Cl<sub>2</sub>) λ<sub>max</sub> (log ε): 237 (5.58) nm.

IR  $\bar{\nu}$ : 3400 (O-H, st.), 2915 (C-H, st. sat.), 1729 (C=O, st.), 1462, 1219, 1082, 729

(finger print region) cm.<sup>-1</sup>

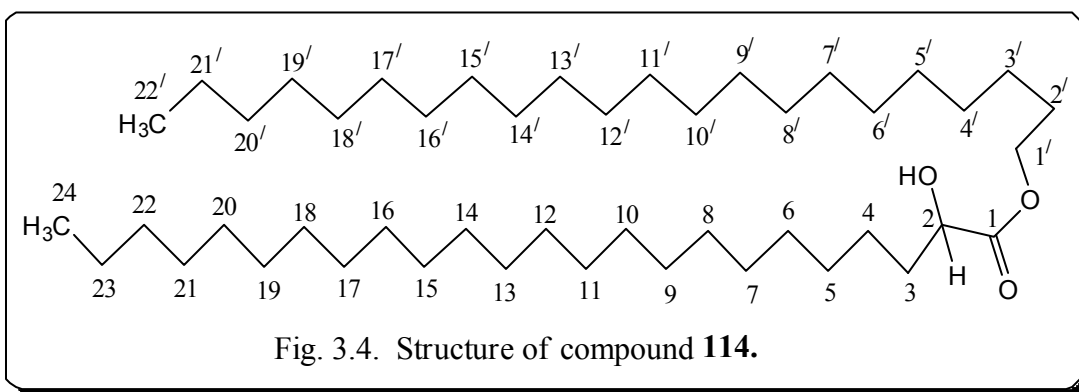
EI-MS m/z: 692 [M<sup>+</sup>] (calculated for C<sub>46</sub>H<sub>92</sub>O<sub>3</sub>)

EI-MS m/z (rel. int. %): 692 (38, M<sup>+</sup>), 678 (18), 664 (23), 398 (13), 384 (75), 370 (36), 356 (37), 337 (61), 311(25), 309 (22) 292 (7), 181 (5), 153 (11), 125 (35), 111 (69), 97 (100), 83 (85), 71 (68), 57 (95).

<sup>1</sup>HNMR: see **Table. 2.4.** (Page 35)

<sup>13</sup>CNMR: see **Table. 2.4.** (Page 35)

HMBC: see **Table. 2.4.** (Page 35)



**3.8. Experimental data of the new source alkaloids from *Berberis jaeschkeana* Schneid var. *jaeschkeana* roots.**

**3.8.1. Berberine (115).**

Physical state: yellow needles (MeOH).

M.p.: 207-208 °C (Lit 206-208 °C).<sup>33</sup>

Molecular formula: C<sub>20</sub>H<sub>18</sub>NO<sub>4</sub><sup>+</sup>

UV λ<sub>max</sub> (MeOH) (log ε): 266 (5.39), 349 (5.37), 427 (4.82) nm.

IR  $\bar{\nu}$ : 3020 (C-H, st. aromatic.), 1634 (C=C, st. aromatic.), 1601 (C=C, st. aromatic.), 1458 (finger print region) cm.<sup>-1</sup>

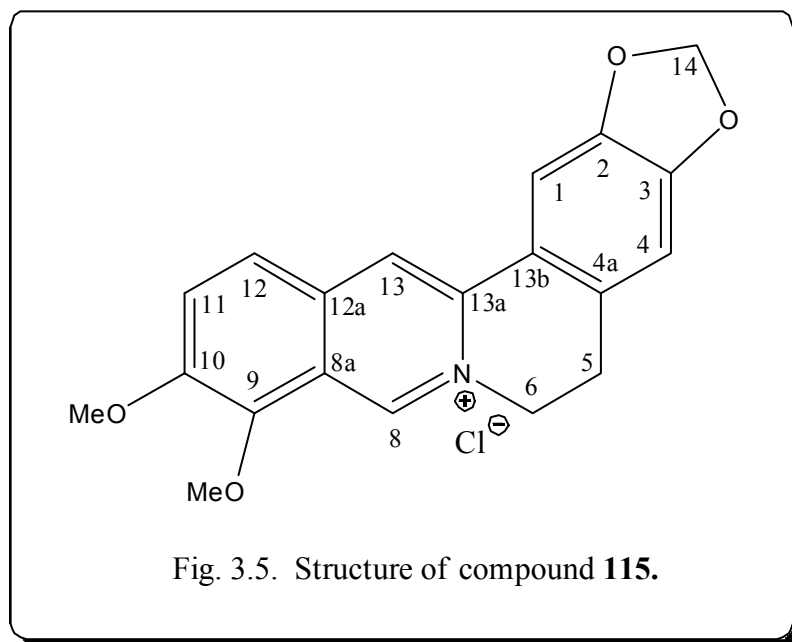
EI-MS m/z: 336 [M<sup>+</sup>] (calculated for C<sub>20</sub>H<sub>18</sub>NO<sub>4</sub><sup>+</sup>).

EI-MS m/z (rel. int. %): 336 (6, M<sup>+</sup>), 335 (7), 321 (100), 306 (38), 292 (32), 278 (75), 263 (10), 248 (6), 219 (7), 191(7), 138(7), 95(3), 44(11).

<sup>1</sup>HNMR: see **Table. 2.5.** (Page 40)

<sup>13</sup>CNMR: see **Table. 2.5.** (Page 40)

HMBC: see **Table. 2.5.** (Page 40)



### 3.8.2. Palmatine (116).

Physical state: brownish yellow needles (MeOH).

M.p.: 202-203°C (Lit: 202-204°C).<sup>33</sup>

Molecular formula: C<sub>21</sub>H<sub>22</sub>NO<sub>4</sub><sup>+</sup>

UV λ<sub>max</sub> (MeOH) (log ε): 347(5.42), 430 (4.88) nm.

IR ν̄: 2992 (C-H, st. sat.) 1624, 1522 (C=C, st. aromatic.), 1390,

1240 (finger print region) cm.<sup>-1</sup>

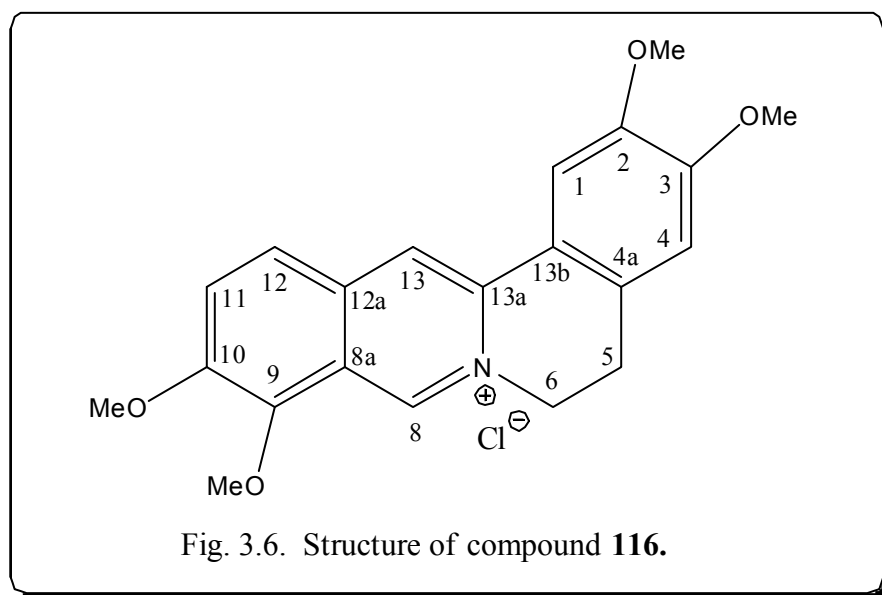
EI-MS m/z: 352 [M<sup>+</sup>] (calculated for C<sub>21</sub>H<sub>22</sub>NO<sub>4</sub><sup>+</sup>).

EI-MS  $m/z$  (rel. int. %): 352 (11,  $M^+$ ), 351 (35), 337 (100), 294 (46), 278 (34), 219 (7), 190(8), 138(5), 94(5), 43(10).

$^1\text{H}$ NMR: see **Table. 2.6.** (Page 44)

$^{13}\text{C}$ NMR: see **Table. 2.6.** (Page 44)

HMBC: see **Table. 2.6.** (Page 44)



### 3.8.3. Columbamine (**117**).

Physical state: yellow needle like crystals (MeOH).

M.p.: 281-283 °C (Lit: 280-282 °C).<sup>33</sup>

Molecular formula:  $\text{C}_{20}\text{H}_{20}\text{NO}_4^+$

UV  $\lambda_{\text{max}}$  (MeOH) (log  $\epsilon$ ): 276 (5.30), 349 (5.43) nm.

IR  $\bar{\nu}$ : 3310 (O-H, st.), 1605, 1510 (C=C, st. aromatic.), 1456,

1316 (finger print region)  $\text{cm}^{-1}$

EI-MS:  $m/z$  338 [ $M^+$ ] (calculated for  $C_{21}H_{22}NO_4^+$ ).

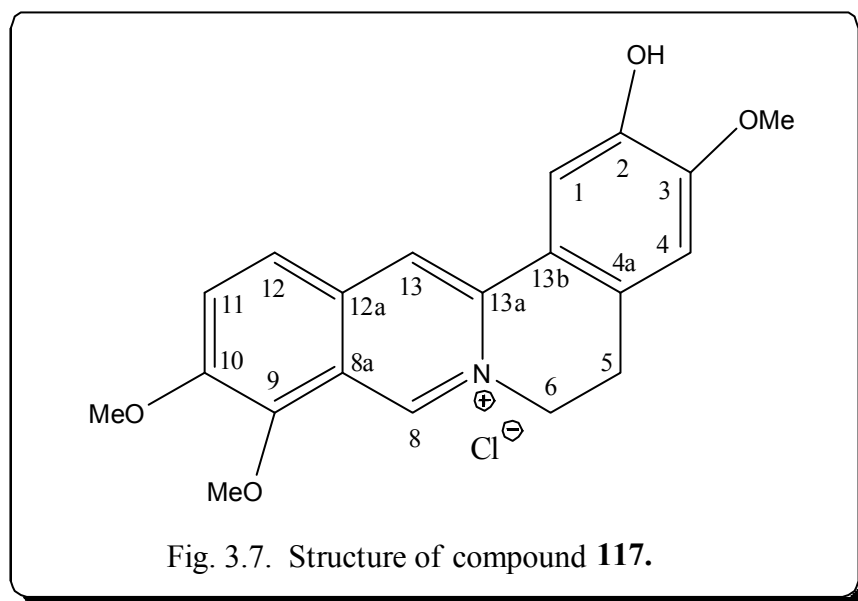
EI-MS:  $m/z$  (rel. int. %): 338 (7,  $M^+$ ), 323 (100), 305 (35), 291 (30), 278 (73), 264 (10),

217 (6), 191(8), 139 (5), 94(5), 44(8).

$^1\text{H}$ NMR: see **Table. 2.7.** (Page 47)

$^{13}\text{C}$ NMR: see **Table. 2.7.** (Page 47)

HMBC: see **Table. 2.7.** (Page 47)



#### 3.8.4. Pseudoberberine (**118**).

Physical state: yellow needles (MeOH).

M.p.: 206-208 °C.

Molecular formula:  $C_{20}H_{18}NO_4^+$

UV  $\lambda_{max}$  (MeOH) ( $\log \epsilon$ ): 347 (5.48), 429 (5.07) nm.

IR  $\bar{\nu}$ : 3037 (C-H, st. aromatic.), 1630, 1601 (C=C, st. aromatic.),

1458 (finger print region)  $cm^{-1}$

EI-MS m/z: 336 [ $M^+$ ] (calculated for  $C_{20}H_{18}NO_4^+$ ).

EI-MS m/z (rel. int. %): 336 (10,  $M^+$ ), 335 (6), 321 (100), 306 (36), 292 (37), 278 (71), 263 (10), 248 (8), 218 (6), 192(9), 138(5), 96(3), 44(10).

$^1H$ NMR: see **Table. 2.8.** (Page 51)

$^{13}C$ NMR: see **Table. 2.8.** (Page 51)

HMBC: see **Table. 2.8.** (Page 51)

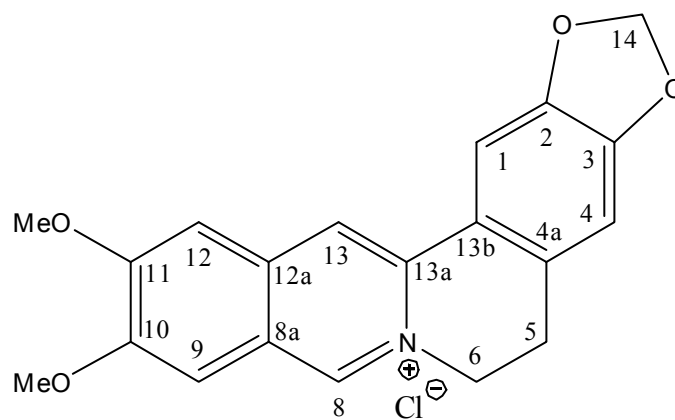


Fig. 3.8. Structure of compound **118**.

**3.9. Experimental data of new source non-alkaloids from *Berberis jaeschkeana* Schneid var. *jaeschkeana* roots.**

**3.9.1. Gallic acid (119).**

Physical state: white crystalline powder (MeOH).

M.p.: 249-251 °C (Lit: 250-252 °C).<sup>34</sup>

Molecular formula: C<sub>7</sub>H<sub>6</sub>O<sub>5</sub>

UV  $\lambda_{\text{max}}$  (MeOH) (log  $\epsilon$ ): 273 (4.83) nm.

IR  $\bar{\nu}$ : 3370 (O-H, st.), 2996 (C-H, st. sat.), 1715 (C=O, st.) cm.<sup>-1</sup>

EI-MS m/z: 170 [M<sup>+</sup>] (calculated for C<sub>7</sub>H<sub>6</sub>O<sub>5</sub>).

EI-MS m/z (rel. int. %): 170 (98, M<sup>+</sup>), 154 (94), 127 (15), 106 (7), 79 (18).

<sup>1</sup>HNMR: see **Table. 2.9.** (Page 54)

<sup>13</sup>CNMR: see **Table. 2.9.** (Page 54)

HMBC: see **Table. 2.9.** (Page 54)

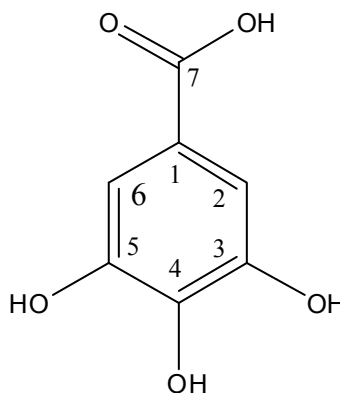


Fig. 3.9. Structure of compound **119**.

**3.9.2.  $\beta$ -Sitosterol (120).**

Physical state: white powder ( $\text{CHCl}_3$ ).

M.p.: 134-136 °C (Lit: 134-135 °C).<sup>35</sup>

Molecular formula:  $\text{C}_{29}\text{H}_{50}\text{O}$

UV  $\lambda_{\text{max}}$  (dichloromethane) ( $\log \epsilon$ ): 240.5 (5.22) nm.

IR  $\bar{\nu}$ : 3376 (O-H, st.), 2931 (C-H, st. sat.), 1636 (C=C, st. olefinic.), 1463, 1442, 1375 (finger print region)  $\text{cm}^{-1}$

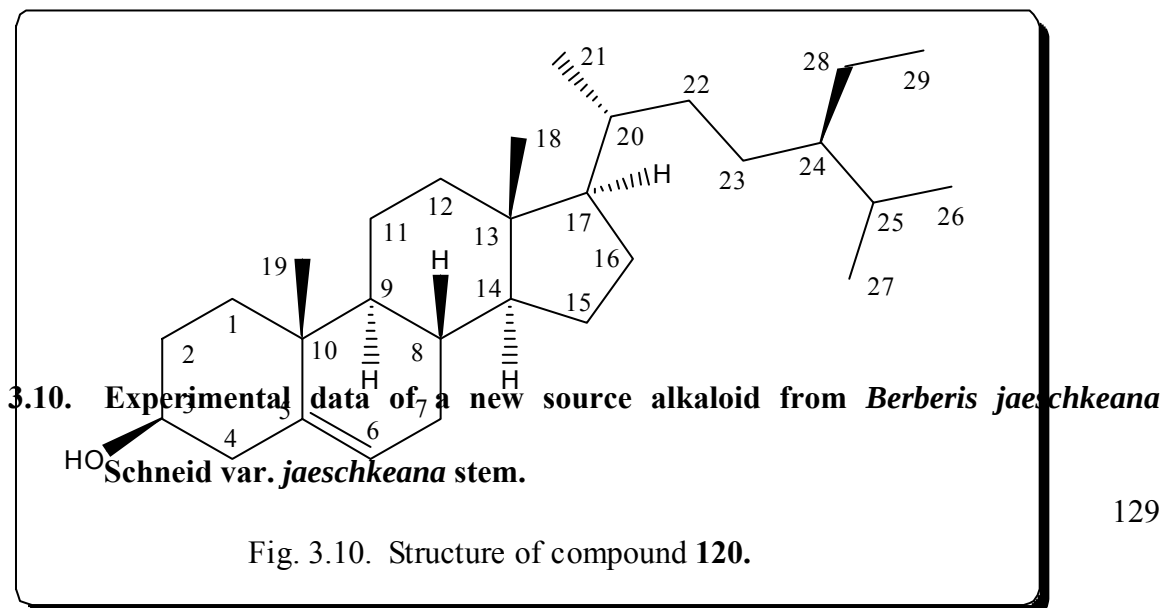
EI-MS:  $m/z$  414 [ $\text{M}^+$ ] (calculated for  $\text{C}_{29}\text{H}_{50}\text{O}$ ).

EI-MS:  $m/z$  (rel. int. %): 414 (100,  $\text{M}^+$ ), 396 (89), 382 (45), 381 (71), 329 (72), 303 (81), 255 (72), 213 (76), 161 (57), 145 (65), 107 (65), 81 (55), 55 (56).

$^1\text{H}$ NMR: see **Table. 2.10.** (Page 56)

$^{13}\text{C}$ NMR: see **Table. 2.10.** (Page 56)

HMBC: see **Table. 2.10.** (Page 56)



**3.10.1. 8-Hydroxy-7, 8-dihydroberberine (121).**

Physical state: yellow amorphous powder (MeOH).

M.p.: 145-147 °C (Lit: 144-149 °C).<sup>40</sup>

Molecular formula: C<sub>20</sub>H<sub>19</sub>NO<sub>5</sub>

UV  $\lambda_{\text{max}}$  (MeOH) (log  $\epsilon$ ): 254, 340 (5.32) nm.

IR  $\bar{\nu}$ : 3356 (O-H, st.), 2998 (C-H, st. sat.), 1574 (C=C, st. aromatic.),

1431 (finger print region) cm.<sup>-1</sup>

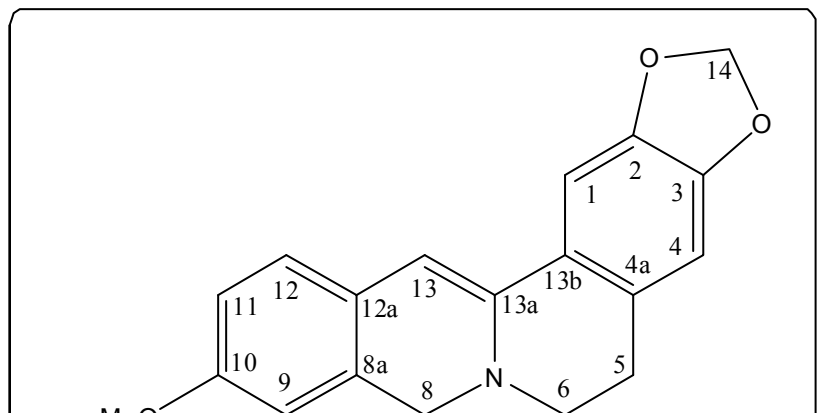
EI-MS m/z: 353 [M<sup>+</sup>] (calculated for C<sub>20</sub>H<sub>19</sub>NO<sub>5</sub>).

EI-MS m/z (rel. int. %): 353 (19, M<sup>+</sup>), 352 (7) 336 (98), 321 (76), 306 (33), 291 (26), 276 (40), 254 (11), 190(7), 138(6), 95(2).

<sup>1</sup>HNMR: see **Table. 2.11.** (Page 60)

<sup>13</sup>CNMR: see **Table. 2.11.** (Page 60)

HMBC: see **Table. 2.11.** (Page 60)



**3.11. Experimental data of new source non-alkaloids from *Berberis jaeschkeana* Schneid var. *jaeschkeana* stem.**

**3.11.1. Docosanoic acid (122).**

Physical state: white amorphous powder (CHCl<sub>3</sub>)

M.p.: 70-71 °C (Lit: 70-72 °C).<sup>36</sup>

Molecular formula: C<sub>22</sub>H<sub>44</sub>O<sub>2</sub>

UV (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{\max}$  (log  $\epsilon$ ): 235 (5.13) nm.

IR  $\bar{\nu}$ : 3452 (O-H, st.), 2928 (C-H, st. sat.), 1691 (C=O, st.) cm.<sup>-1</sup>

EI-MS m/z: 340 [M<sup>+</sup>] (calculated for C<sub>29</sub>H<sub>50</sub>O).

EI-MS m/z (rel. int. %): 340 (84, M<sup>+</sup>), 313 (12), 297 (30), 280 (14), 257 (100), 241 (21), 227 (12), 213 (19), 185 (27), 171 (15), 147 (22), 129 (51), 111 (38), 97 (63), 83 (65), 57 (88), 43 (77).

$^1\text{H}$ NMR: see **Table. 2.12.** (Page 64)

$^{13}\text{C}$ NMR: see **Table. 2.12.** (Page 64)

HMBC: see **Table. 2.12.** (Page 64)

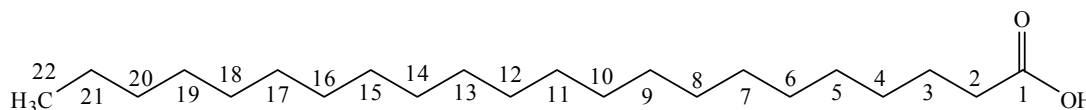


Fig. 3.12. Structure of compound **122.**

### 3.11.2. Methyl gallate (**123**).

Physical state: off white crystalline powder (MeOH).

M.p.: 202-204 °C (Lit: 203).<sup>37</sup>

Molecular formula:  $\text{C}_8\text{H}_8\text{O}_5$

UV  $\lambda_{\text{max}}$  (MeOH) (log  $\epsilon$ ): 273 (4.96) nm.

IR  $\bar{\nu}$ : 3412 (O-H, st.), 2988 (C-H, st. sat.), 1736 (C=O, st.)  $\text{cm}^{-1}$

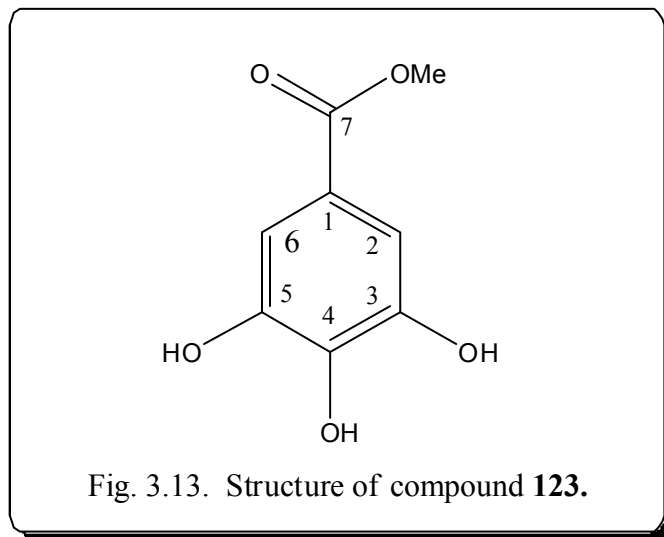
EI-MS m/z: 184 [ $\text{M}^+$ ] (calculated for  $\text{C}_8\text{H}_8\text{O}_5$ ).

EI-MS m/z (rel. int. %): 184 (41,  $\text{M}^+$ ), 154 (100), 127 (33), 107 (12), 79 (24).

$^1\text{H}$ NMR: see **Table. 2.13.** (Page 67)

$^{13}\text{C}$ NMR: see **Table. 2.13.** (Page 67)

HMBC: see **Table. 2.13.** (Page 67)



### 3.12. Experimental data for the new source alkaloids from *Berberis glaucocarpa* Stapf roots.

#### 3.12.1. Oxycanthine (124).

Physical state: off white powder (CHCl<sub>3</sub>).

M.p.: 214-216 °C (Lit: 216-217 °C).<sup>77</sup>

Molecular formula: C<sub>37</sub>H<sub>40</sub>N<sub>2</sub>O<sub>6</sub>

HR-ESIMS = 609.2958 [M+H]<sup>+</sup> (calculated for C<sub>37</sub>H<sub>40</sub>N<sub>2</sub>O<sub>6</sub> = 608.2886).

UV (MeOH) λ<sub>max</sub> (log ε): 281 (5.38) nm.

IR  $\bar{\nu}$ : 3313 (O-H, st.), 2921 (C-H, st. sat.), 2831 (C-H, st. N-methyl), 1607, 1516 (C=C, st. aromatic)  $\text{cm}^{-1}$

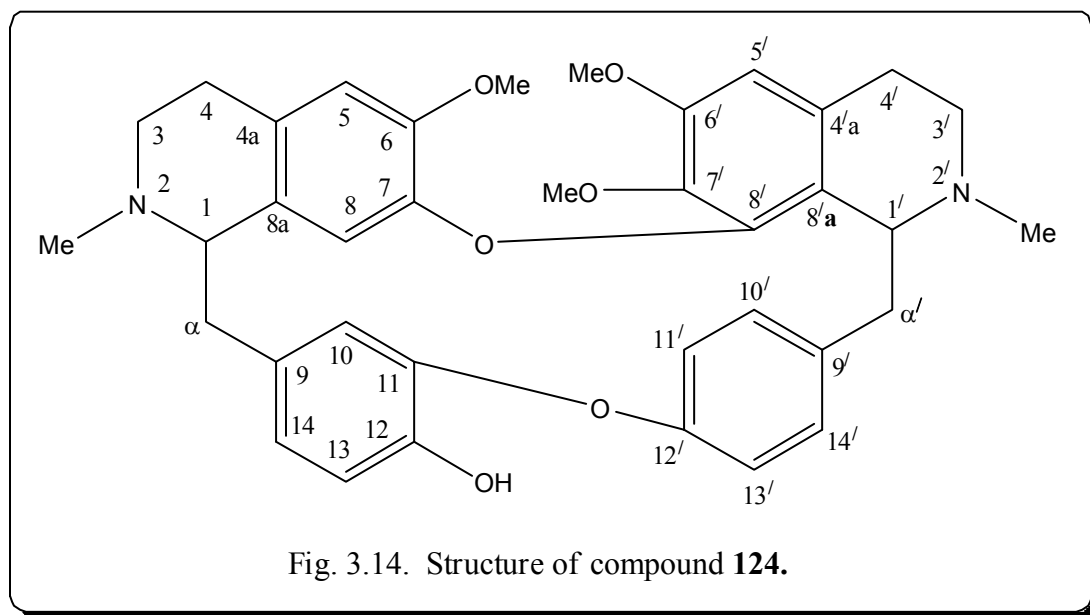
EI-MS:  $m/z$  608 [ $M^+$ ] (calculated for  $C_{37}H_{40}N_2O_6$ ).

EI-MS:  $m/z$  (rel. int. %): 608 ( $M^+$ , 100), 593 (29), 577 (6), 416 (12), 395 (81), 381(69), 379(28), 365(10), 335(6), 198(51), 192(17), 174(31), 146(8).

$^1\text{H}$ NMR: see **Table. 2.14.** (Page 69)

$^{13}\text{C}$ NMR: see **Table. 2.14.** (Page 69)

HMBC: see **Table. 2.14.** (Page 69)



### 3.12.2. Jatrorrhizine (125).

Physical state: yellow needles (MeOH).

M.p.: 280-281 °C (Lit: 280-282 °C).<sup>33</sup>

Molecular formula: C<sub>20</sub>H<sub>20</sub>NO<sub>4</sub><sup>+</sup>

UV  $\lambda_{\max}$  (MeOH) (log  $\epsilon$ ): 281(5.60), 349 (5.13) nm.

IR  $\bar{\nu}$ : 3310 (O-H, st.), 3031 (C-H, st. aromatic.), 1610 (C=C, st. aromatic.), 1465 (finger print region) cm<sup>-1</sup>

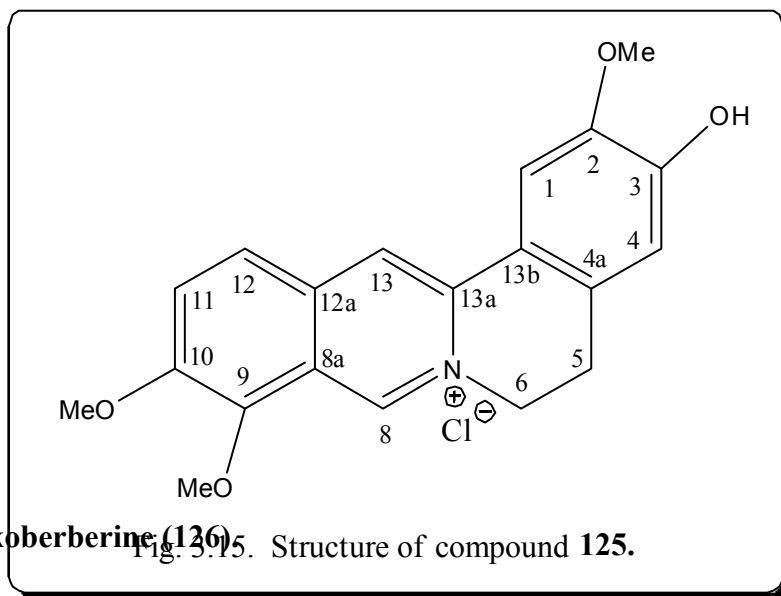
EI-MS m/z: 338 [M<sup>+</sup>] (calculated for C<sub>20</sub>H<sub>20</sub>NO<sub>4</sub><sup>+</sup>).

EI-MS m/z (rel. int. %): 338 (24, M<sup>+</sup>), 323 (100), 307 (37), 294 (39), 278 (70), 265 (11).

<sup>1</sup>HNMR: see **Table. 2.15.** (Page 75)

<sup>13</sup>CNMR: see **Table. 2.15.** (Page 75)

HMBC: see **Table. 2.15.** (Page 75)



**3.12.3. 8-Oxoerberberine (126)** Fig. 3.15. Structure of compound **125**.

Physical state: yellow needles (MeOH).

M.p.: 191-192 °C (Lit: 190-192°C).<sup>33</sup>

Molecular formula: C<sub>20</sub>H<sub>17</sub>NO<sub>5</sub>

UV  $\lambda_{\max}$  (MeOH) (log  $\epsilon$ ): 315 (5.5), 370 (5.32) nm.

IR  $\bar{\nu}$ : 3020 (C-H, st. aromatic.), 1643 (C=O, st.), 1501 (C=C, st. aromatic.), 1293, 1240 (finger print region) cm.<sup>-1</sup>

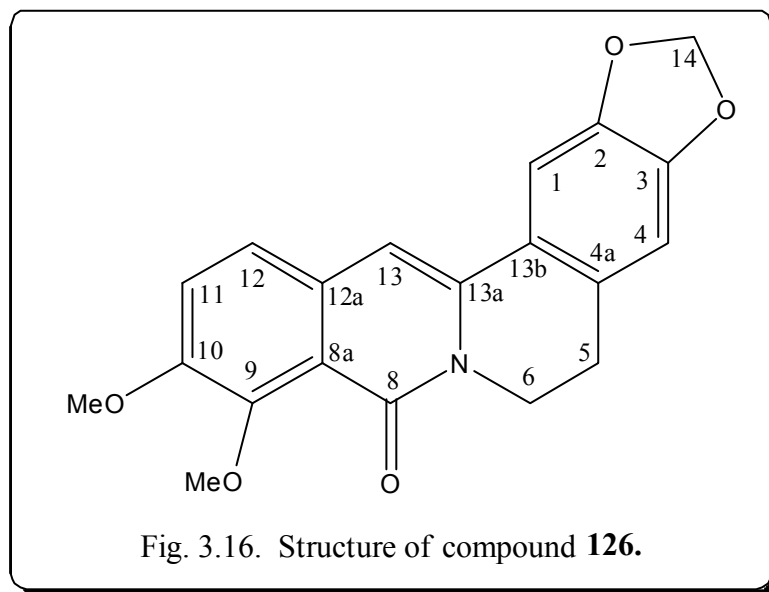
EI-MS m/z: 351 [M<sup>+</sup>] (calculated for C<sub>20</sub>H<sub>17</sub>NO<sub>5</sub>).

EI-MS m/z (rel. int. %): 351 (100, M<sup>+</sup>), 336 (71), 322 (45), 292 (37), 160 (24).

<sup>1</sup>HNMR: see **Table. 2.16.** (Page 79)

<sup>13</sup>CNMR: see **Table. 2.16.** (Page 79)

HMBC: see **Table. 2.16.** (Page 79)



### 3.13. Experimental data for a new source non-alkaloid from *Berberis glaucocarpa* Stapf roots.

#### 3.13.1 $\beta$ -Sitosterol glucoside (127).

Physical state: colorless amorphous powder (C<sub>35</sub>H<sub>50</sub>N).

M.p.: 270-272 °C (Lit: 272-274 °C).<sup>37</sup>

Molecular formula: C<sub>35</sub>H<sub>60</sub>NO<sub>6</sub>

FABMS [M<sup>+</sup>] 576 (calculated for C<sub>35</sub>H<sub>50</sub>O 576.4390).

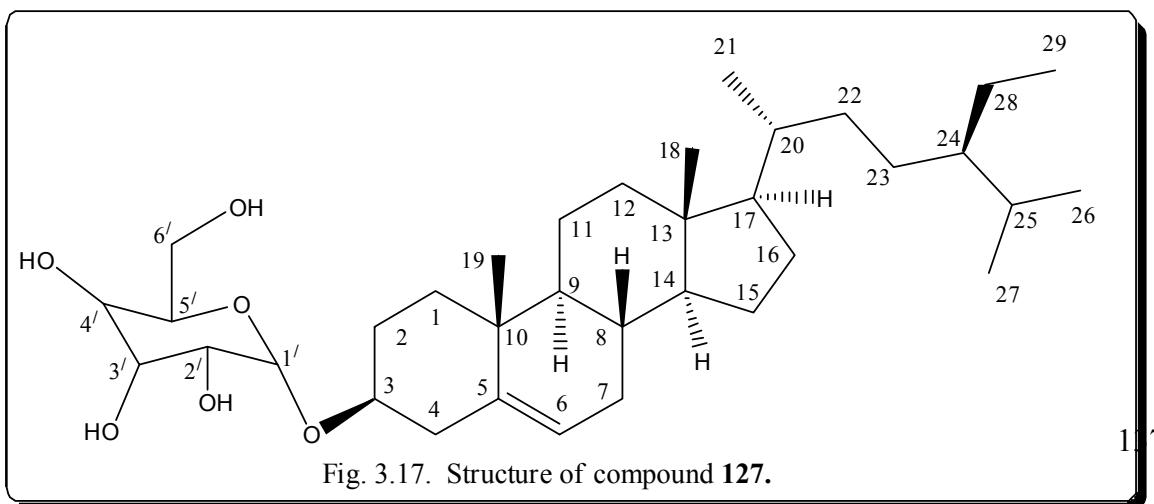
IR  $\bar{\nu}$ : 3483(O-H, st.), 2928 (C-H, st. sat.), 1014 cm<sup>-1</sup>

EI-MS: m/z (rel. int. %): 414 (28), 396 (100), 382 (88), 367 (11), 354 (8), 329 (14), 303(17), 288(24), 275 (30), 255(48), 229 (12), 213 (37), 201(12), 175 (18), 161 (42), 147 (59), 121 (34), 105 (31), 81 (39), 69 (22), 43(29).

<sup>1</sup>HNMR: see **Table. 2.17.** (Page 82)

<sup>13</sup>CNMR: see **Table. 2.17.** (Page 82)

HMBC: see **Table. 2.17.** (Page 82)



### 3.14. Experimental data for new source alkaloids from *Berberis glaucocarpa* Stapf stem.

#### 3.14.1. 8-Trichloromethyldihydroberberine (128).

Physical state: light yellow crystals (CHCl<sub>3</sub>).

M.p.: 186-187 °C (Lit: 186-188 °C).<sup>32</sup>

Molecular formula: C<sub>21</sub>H<sub>18</sub>Cl<sub>3</sub>NO<sub>4</sub>

UV  $\lambda_{\max}$  (MeOH) (log  $\epsilon$ ): 231(5.95), 285 (5.69), 381 (5.83) nm.

IR  $\bar{\nu}$ : 2980 (C-H, st. sat.), 1610 (C=C, st. aromatic.), 1501(C=C, st. aromatic.), 1290, 1240 (finger print region) cm.<sup>-1</sup>

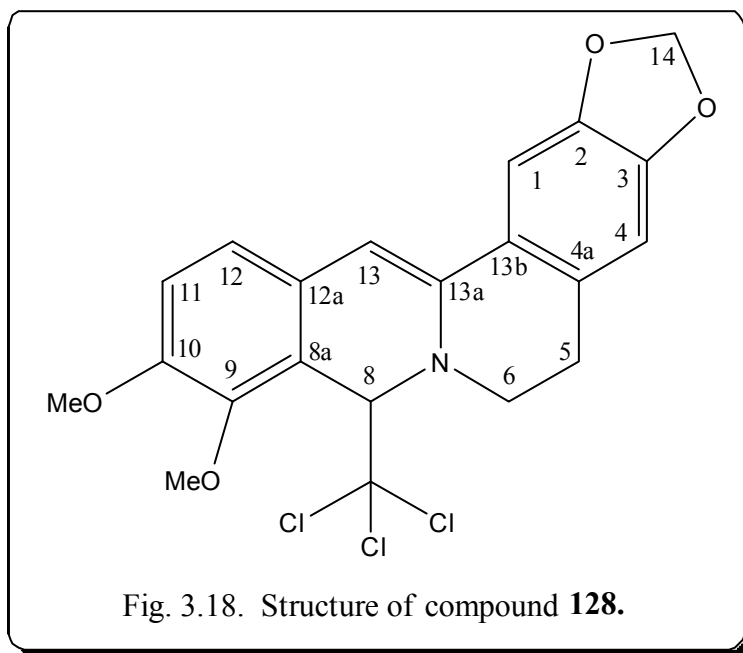
ESI-MS: 454.0313 [M+H]<sup>+</sup> (calculated for C<sub>21</sub>H<sub>19</sub>Cl<sub>3</sub>NO<sub>4</sub> = 454.0381).

EI-MS m/z (rel. int. %): 420 (78), 383 (82), 368 (75), 336 (100), 321 (26), 292 (24), 278 (27).

<sup>1</sup>HNMR: see **Table. 2.18.** (Page 86)

<sup>13</sup>CNMR: see **Table. 2.18.** (Page 86)

HMBC: see **Table. 2.18.** (Page 86)



### 3.14.2. Pseudopalmatine (**129**).

Physical state: Brownish yellow needles (MeOH).

M.p.: 202-204 °C.

Molecular formula:  $C_{21}H_{22}NO_4^+$

UV  $\lambda_{\max}$  (MeOH) (log  $\epsilon$ ): 346 (5.40), 432 (4.9) nm.

IR  $\bar{\nu}$ : 2997 (C-H, st. sat.), 1603, 1520 (C=C, st. aromatic.),

1501 (finger print region)  $cm^{-1}$

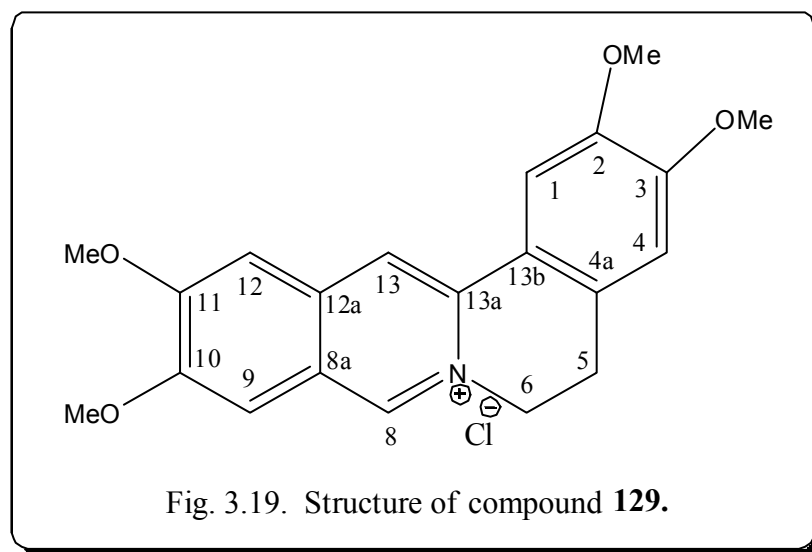
EI-MS m/z: 352 [ $M^+$ ] (calculated for  $C_{21}H_{22}NO_4^+$ ).

EI-MS m/z (rel. int. %): 352 (15,  $M^+$ ), 351 (42), 337 (100), 294 (42), 278 (34).

$^1H$ NMR: see **Table. 2.19**. (Page 90)

$^{13}\text{C}$ NMR: see **Table. 2.19.** (Page 90)

HMBC: see **Table. 2.19.** (Page 90)



### 3.15. Experimental data for new source non-alkaloids from *Berberis glaucocarpa*

#### Stapf stem.

##### 3.15.1. Syringic acid (130).

Physical state: White semi crystalline solid ( $\text{CHCl}_3$ ).

M.p.: 205-207 °C (Lit: 203-205°C).<sup>41</sup>

Molecular formula:  $\text{C}_9\text{H}_{10}\text{O}_5$

UV  $\lambda_{\text{max}}$  (MeOH) (log  $\epsilon$ ): 272.5 (5.29) nm.

IR  $\bar{\nu}$ : 3380 (O-H, st.), 1675 (C=O, st.), 1564 (C=C, st. aromatic.), 1480 (finger print region)  $\text{cm}^{-1}$

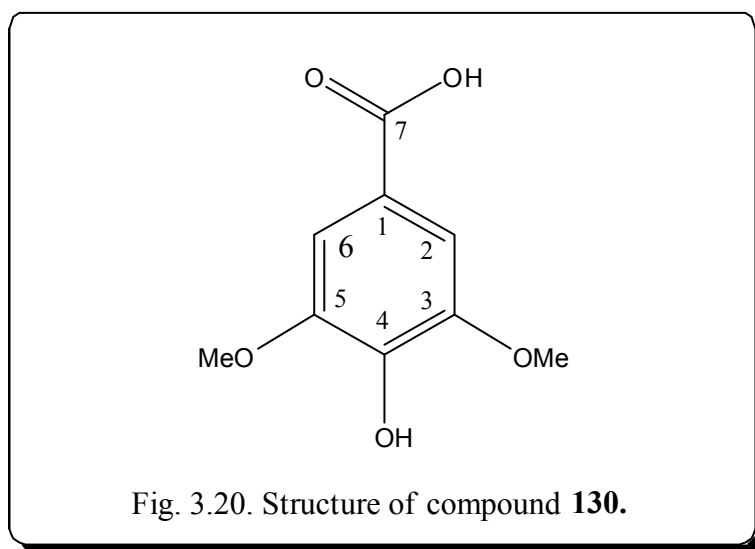
EI-MS m/z: 198 [ $M^+$ ] (calculated for  $C_9H_{10}O_5$ ).

EI-MS m/z (rel. int. %): 198 (100,  $M^+$ ), 183 (34), 167 (17), 153 (11), 127 (19), 97 (27), 83 (36), 71 (34), 69 (41).

$^1H$ NMR: see **Table. 2.20.** (Page 94)

$^{13}C$ NMR: see **Table. 2.20.** (Page 94)

HMBC: see **Table. 2.20.** (Page 94)



### 3.15.2. Syringaresinol (131).

Physical state: colorless crystals (MeOH).

M.p.: 183-185 °C (Lit: 183-184°C).<sup>42</sup>

Molecular formula:  $C_{22}H_{26}O_8$

UV  $\lambda_{\text{max}}$  (MeOH) (log  $\epsilon$ ): 240 (5.61), 276 (5.22) nm.

IR  $\bar{\nu}$ : 3325 (O-H, st.), 1612 (C=C, st. aromatic.), 1520 (C=C, st. aromatic.), 1456, 1378, 1320 (finger print region)  $\text{cm}^{-1}$

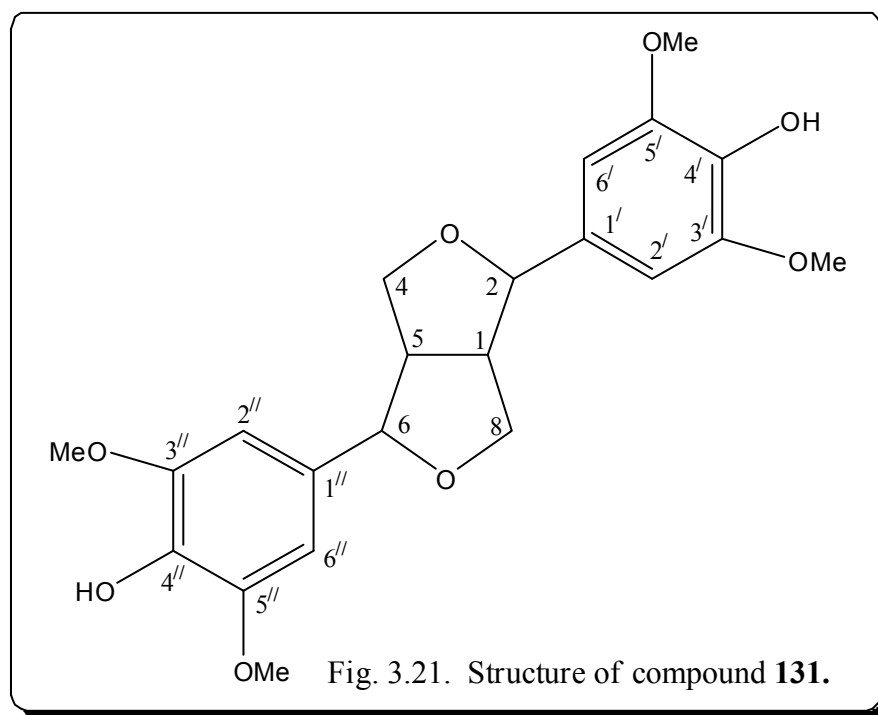
EI-MS  $m/z$ : 418 [ $M^+$ ] (calculated for  $\text{C}_{22}\text{H}_{26}\text{O}_8$ ).

EI-MS  $m/z$  (rel. int. %): 418 (100,  $M^+$ ), 210 (20), 193 (26), 181 (99), 167 (70).

$^1\text{H}$ NMR: see **Table. 2.21.** (Page 96)

$^{13}\text{C}$ NMR: see **Table. 2.21.** (Page 96)

HMBC: see **Table. 2.21.** (Page 96)



**3.15.3. Stigmasterol (132).**

Physical state: white powder (CHCl<sub>3</sub>).

M.p.: 173-174 °C (174-176 °C).<sup>43</sup>

Molecular formula: C<sub>29</sub>H<sub>48</sub>O

IR  $\bar{\nu}$ : 3376 (O-H, st.), 2931 (C-H, st. sat. ), 1636 (C=C, st. olefinic.), 1463, 1442, 1375 (finger print region) cm.<sup>-1</sup>

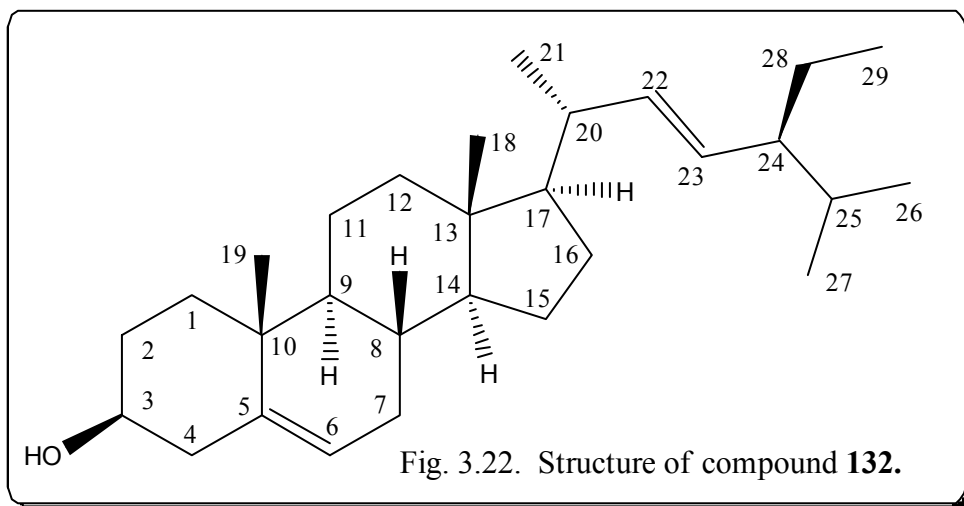
EI-MS m/z: 412 [M<sup>+</sup>] (calculated for C<sub>29</sub>H<sub>48</sub>O).

EI-MS m/z (rel. int. %): 412 (37, M<sup>+</sup>), 396 (87), 381 (44), 329 (74), 301 (80), 273 (53), 255 (72), 213 (62), 161 (55), 145 (45), 107(61), 81(35), 55 (36).

<sup>1</sup>HNMR: see **Table. 2.22.** (Page 100)

<sup>13</sup>CNMR: see **Table. 2.22.** (Page 100)

HMBC: see **Table. 2.22.** (Page 100)



#### 4.1. Introduction.

Plants are the major source of biologically active compounds that can be used as medicines. In recent years the interest in the use of medicinal plants have increased rapidly due to the resistance developed by the pathogens against the existing anti-biotics.<sup>1</sup> Onion extracts have been found to have anti-oxidant properties. Onion prevents cancer, coronary heart diseases, cataract and diabetes. Fresh onion juice have been found to have analgesic and anti-inflammatory properties.<sup>44</sup>

*Peganum. harmala* L. extracts have been found to have anti-viral, anti-diabetic, anti-fungal, anti-oxidant, anti-septic, anti-tumor, insecticidal, cytotoxic, hepatoprotective and anti-leishmanial properties.<sup>45</sup> *Berberis* L (the subject of the current study) is a member of the family Berberidaceae. The genus *Berberis* L. has 500 species around the world. They are mostly shrubs having 3-5 branched spines and have simple leaves.<sup>15</sup>

*Berberis* species are medicinal plants and are extensively used in traditional medicines. The fruits of *Berberis* are used as food additives.<sup>46</sup> Different *Berberis* species are important folk medicines in the treatment of allergies, fever, metabolic disorders, eye diseases, osteoporosis, joint pains and menopause problems.<sup>47</sup> Berberine is one of the major and most important isoquinoline alkaloid found in all the *Berberis* species and has a wide range of pharmacological uses.<sup>47</sup> The alcoholic extracts of *Berberis* spp have isoquinoline and bisbenzylisoquinoline alkaloids. The alkaloids are mainly responsible for the bioactivities of *Berberis* species.<sup>48</sup>

Phytochemical investigations carried out on the genus *Berberis* have shown that alkaloids having isoquinoline nucleus are the major constituents of this genus.<sup>49</sup>

#### **4.2. Biological activities review of the genus *Berberis*.**

All members of the genus *Berberis* are very important medicinal plants having wide range of traditional and pharmacological uses. Traditionally *Berberis* spp are used for the treatment of metabolic disorders, allergies, eye disorders, osteoporosis and many other diseases.<sup>47</sup>

A brief account of the various pharmacological activities of the genus *Berberis* is given under the following headings.

##### **4.2.1. Cardiovascular effects.**

###### **A. Vasorelaxant effects.**

Traditionally *Berberis* species are used for the treatment of several heart diseases including hypertension and arrhythmia. Aqueous extract of *Berberis* spp (0.05-1.0 mg/100 g of the body weight of rat) significantly reduces arterial blood pressure and relaxes the rings of descending aorta and mesenteric artery.<sup>50</sup> Both *in-vivo* and *in-vitro* studies of berberine, an isoquinoline alkaloid from *Berberis*, have confirmed its vasorelaxant and blood pressure lowering properties.<sup>51</sup>

###### **B. Inotropic and protective effects**

Berberine has protective effects against hypertrophy, ischemia and severe congestive heart failure. Berberine is also used for the treatment of congestive heart failure. Berberine decreases the frequency of premature ventricular complexes and increases the left ventricular ejection fraction (EF) in patients having severe congestive heart failure.<sup>52,53</sup>

### **C. Anti-platelet effects**

Berberine has anti-platelet effects and that is why it prevents thrombus formation in arteries and berberine has been successfully used for patients having high aggregability of platelets.<sup>54</sup> Berberine has inhibitory effects on the metabolism of arachidonic acid and the influx of calcium.<sup>55,56</sup>

#### **4.2.2. Immune system effects.**

##### **A. Anti-inflammatory effects.**

In many countries around the world extracts obtained from *Berberis* species are used for the treatment of different anti-inflammatory diseases and to reduce fever. Many studies have confirmed the anti-inflammatory effects of *Berberis* extracts and berberine but their exact mode of action is still unknown. In Pakistan powdered roots of *Berberis lyceum* is used along with milk for the treatment of rheumatism and muscular pains. Similarly in different areas of Pakistan extracts of different *Berberis* species are used for the treatment of gastric and duodenal ulcers.<sup>57</sup>

**B. Immunomodulation.**

Berberine showed immunosuppressive properties in tubulointerstitial nephritis model. This model is an analogue of various human kidney autoimmune diseases.<sup>58</sup>

In another study berberine was used against adjuvant-induced arthritis in mice.<sup>49</sup>

**4.2.3. Hepatoprotective activity.**

After evaluating experimental amoebic liver abscess in golden hamsters and in Immunomodulation studies it was found that *Berberis aristata* reduce the rate of infection of hepatic amoebiasis.<sup>59</sup> *Berberis* spp have been tested against hepato biliary disorders and they were found to reduce acetaminophen induced rise in serum alkaline phosphate, glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT).<sup>60</sup> Aqueous and methanolic crude extract of *Berberis aristata* fruits have shown hepatoprotective activity by microsomal drug metabolizing enzymes (MDME) inhibitory action.<sup>61</sup>

**4.2.4. Anti-diarrheal activity.**

Both *in-vivo* and *in-vitro* evaluations of *Berberis aristata* extracts have confirmed its anti-diarrheal activity.<sup>62</sup> The active component of *Berberis* called berberine inhibits intestinal secretory response of *Vibrio cholerae* and *Escherichia coli* enterotoxins and that is why it is used for the treatment of acute diarrheal diseases.<sup>63</sup>

#### 4.2.5. Anti-cancer activity.

Methanolic extracts of *Berberis* have shown anti-cancer activity against H29 colon cancer cells.<sup>64</sup> Butanolic fraction of *Berberis aristata* has shown anti-cancer properties against prostate cancer.<sup>65</sup> Berberine has also shown anti-cancer and anti-tumor properties.<sup>66</sup>

#### 4.2.6. Ophthalmic activity.

Some formulations of eye drops and capsules containing *Berberis aristata* are used for the treatment of conjunctivitis and cataract.<sup>67,68</sup>

#### 4.2.7. Activities related to central nervous system (CNS).

A tablet known as Geriforte, used for the physical and mental incompetence of old people, contains *Berberis aristata* extract.<sup>69</sup> Similarly berberine can be used for the treatment of schizophrenia, Alzheimer's disease, anxiety and mental depression.<sup>70</sup>

The number of patents on the various types of pharmacological activities of the genus *Berberis* is given in **Fig.4.1**.

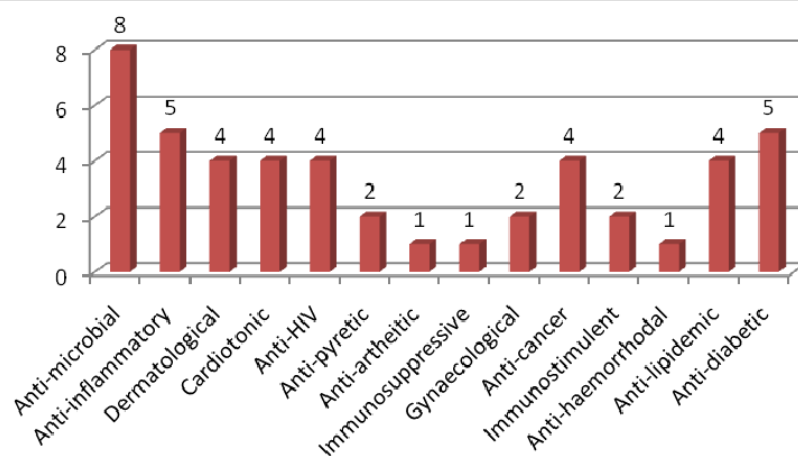


**Fig. 4.1. No. of patents on the genus *Berberis***

### 5.1. Phytochemical screening of *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf.

Phytochemical tests were carried out on the crude ethanolic extract, Fraction **A**, **B** and **C** to identify the chemical constituents.<sup>71,72</sup>

**Alkaloids** About 0.2 g of crude extract and each fraction was warmed with 2 % H<sub>2</sub>SO<sub>4</sub> for two minutes. After filtration of the reaction mixture, a few drops of Dragendorff's reagent was added to each filtered fraction. Orange red precipitate indicated the presence of alkaloids.

**Glycosides** A small amount of crude extract and each fraction was first acidified with dilute HCl and then neutralized with NaOH solution. Then a few drops of Fehling's solution **A** and **B** were added to each mixture. Formation of red precipitate indicated the presence of glycosides.

**Flavonoids** To a small amount of the aqueous filtered solution of each fraction 5 ml of dilute ammonia solution was added followed by the addition of conc. H<sub>2</sub>SO<sub>4</sub>. The appearance of a yellow colour indicated the presence of flavonoids. The yellow colour disappeared after some time.

**Steroids** About 2 ml of acetic anhydride was added to 0.5 g ethanolic extract of each fraction followed by adding 2 ml H<sub>2</sub>SO<sub>4</sub>. The colour changed from violet to blue or green indicated the presence of steroids.

**Saponins** About 0.2 g of the powdered sample was heated in 20 mL of distilled water in a boiling water bath and filtered. 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously. The appearance of frothing indicated the presence of saponins.

**Reducing sugars** Each sample was shaken with distilled water first and then filtered. To each filtrate a few drops of Fehling's solution **A** and **B** were added and boiled for few minutes. The appearance of an orange red precipitate confirmed the presence of reducing sugars.

**Terpenoids** About 0.2 g of the sample was mixed with 2 ml of chloroform first and then 3 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added to the mixture. The formation of a reddish brown coloration at the interface indicated the presence of terpenoids.

## **5.2. Anti-microbial studies.**

### **5.2.1. Anti-microbial studies (agar well diffusion method).**

Modified agar-well diffusion method was used to test the anti-bacterial properties of the crude extract, the fractions and the compounds against five strains of bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Staphylococcus epidermidis*). Muller-hinton agar was used as medium. The cultures were taken in triplicates at incubation temperature of 37 °C from 24 to 72 hrs. The broth culture (0.6 mL) of the test organism was placed in a sterile petri-dish and 20 mL of the sterile molten MHA was added. Holes were bored in the medium using 0.2 mL of the extract,

fractions and the compounds. Streptomycin (2 mg/mL) was used as a standard. Incubation at 37 °C was for 24 hrs and the diameters of the zone of inhibition of microbial growth were measured in millimeters. Results are given in **Table 5.1**.

**Table. 5.1. Anti-bacterial results of *Berberis jaeschkeana* Schneid var. *jaeschkeana* (agar well diffusion method).**

| Sample | <i>S. aureus</i> | <i>E. coli</i> | <i>S. epidermidis</i> | <i>K. pneumoniae</i> | <i>B. subtilis</i> | Streptomycin<br>(2 mg/mL) |
|--------|------------------|----------------|-----------------------|----------------------|--------------------|---------------------------|
| RBJ- C | 16               | 18             | 16                    | 14                   | 18                 | 28                        |
| RBJ- B | 14               | 14             | 16                    | 14                   | 16                 | 30                        |
| RBJ- A | 16               | 18             | 18                    | 16                   | 18                 | 28                        |
| Eth.Cr | 14               | 12             | 16                    | 14                   | 16                 | 30                        |
| Brb    | 14               | 16             | 18                    | 18                   | 20                 | 26                        |
| Col    | 20               | 18             | 14                    | 22                   | 20                 | 28                        |
| Syn.Ac | 20               | 18             | 16                    | 20                   | 18                 | 28                        |
| B.Chlo | 16               | 20             | 18                    | 14                   | 18                 | 28                        |
| Jat    | 16               | 20             | 18                    | 12                   | 20                 | 26                        |

Streptomycin (standard), RBJ-C = Basic fraction, RBJ-B = Dichloromethane fraction, RBJ-A = Acidic fraction, Eth.Cr = Ethanolic crude, Brb= Berberine, Jat = Jatrorrhizine  
Col= Columbamine, Syn.Ac = Syringic acid,  
B.Chlo = 8-trichloromethyldihydroberberine.

### 5.2.2. Anti-trypanosomal and anti-microbial studies

#### (Alamar blue™ 96 well plate assay)

Some of the isolated compounds and crude fractions were tested for their anti-bacterial and anti-trypanosomal properties using Alamar blue™ 96 well micro plate assays. Four strains of bacteria namely *Sporadic methicilin resistant Staphalococcus aureus* (SMRSA)

**Table. 5.2. Anti-bacterial studies of *Berberis jaeschkeana* Schneid var.**

*jaeschkeana* and *Berberis glaucocarpa* Stapf (Alamar blue™ 96 well plate assay).

| Sample       | <i>M. marinum</i><br>(% of c) | SMRSA<br>(% of c) | SMRSA<br>MIC | EMRSA<br>(% of c) | EMRSA<br>MIC | <i>E. coli</i><br>(% of c) | <i>E. coli</i><br>MIC |
|--------------|-------------------------------|-------------------|--------------|-------------------|--------------|----------------------------|-----------------------|
|              | 100 µg/mL                     | 200<br>µg/mL      | µg/mL        | 200 µg/mL         | µg/mL        | 500<br>µg/mL               | µg/mL                 |
| <b>RB-10</b> | - 2.9                         | - 0.1             | 12.5         | 72.0              | NC           | 51.3                       | NC                    |
| <b>RB-11</b> | 0.6                           | 50.2              | 25           | 70.5              | NC           | 19.4                       | NC                    |
| <b>RB-21</b> | 12.2                          | - 0.6             | 12.5         | 84.9              | NC           | 52.3                       | NC                    |
| <b>RBJFA</b> | 43.3                          | 92.5              | NC           | 93.3              | NC           | 57.5                       | NC                    |
| <b>4Me</b>   | 138.0                         | 90.3              | NC           | 91.7              | NC           | 68.6                       | NC                    |
| <b>BJSET</b> | 15.5                          | 1.4               | 50           | 86.5              | NC           | 56.5                       | NC                    |
| <b>RBJD</b>  | 140.4                         | 97.5              | NC           | 91.3              | NC           | 68.5                       | NC                    |
| <b>BGFD</b>  | 138.0                         | 89.7              | NC           | 85.6              | NC           | 59.4                       | NC                    |
| <b>GS-2</b>  | 161.3                         | 88.0              | NC           | 85.5              | NC           | 60.4                       | NC                    |
| <b>SWJC</b>  | 42.8                          | 62.2              | NC           | 91.2              | NC           | 58.3                       | NC                    |
| <b>BGD</b>   | 111.7                         | 91.4              | NC           | 97.3              | NC           | 80.0                       | NC                    |
| <b>BGSD</b>  | 12.2                          | - 2.2             | 25           | 14.9              | NC           | 54.0                       | NC                    |
| <b>BGA</b>   | 4.0                           | 2.0               | 50           | 78.8              | NC           | 49.7                       | NC                    |
| <b>BJSC</b>  | 41.6                          | 85.9              | NC           | 87.9              | NC           | 65.2                       | NC                    |
| <b>GR-8</b>  | 78.1                          | 71.8              | NC           | 75.3              | NC           | 52.0                       | NC                    |
| <b>L.Y</b>   | 89.9                          | 86.5              | NC           | 94.5              | NC           | 66.8                       | NC                    |

% of c = percent of control, NC = not calculated, RB-10 = Berberine, RB-11 = 8-trichloromethyldihydroberberine, RB-21 = Syringaresinol, RBJFA = *Berberis jaeschkeana* roots Fraction A, 4Me = 10-Methoxy chondrofoline, BJSET = *Berberis jaeschkeana* stem Fraction C, RBJD = *Berberis jaeschkeana* roots Fraction D, BGFD = *Berberis glaucocarpa* Fraction D, GS-2 =  $\beta$ -sitosterol, SWJC = *Berberis jaeschkeana* Fraction C, BGD = *Berberis glaucocarpa* Fraction B, BGSD = *Berberis glaucocarpa* stem Fraction D, BGA = *Berberis glaucocarpa* Fraction A, BJSC = *Berberis jaeschkeana* stem Fraction C, GR-8 = oxyacanthine, L.Y = 10-Hydroxy chondrofoline.

(200  $\mu\text{g}/\text{mL}$ ), *Endemic methicilin resistant Staphalococcus aureus (EMRSA)* (200  $\mu\text{g}/\text{mL}$ ), *Mycobacterium marinum* (100  $\mu\text{g}/\text{mL}$ ) and *Escherichia.coli* (500  $\mu\text{g}/\text{mL}$ ) and one strain of *Trypanosoma* namely *Trypanosoma brucei. brucei* (20, 10 and 5  $\mu\text{g}/\text{mL}$ ) was used for assays. Compounds and fractions having a % D control value of 10 or below were considered to be active. One of the prominent clinical uses of *Berberis* spp is in bacterial infection such as bacterial diarrhea and parasitic intestinal and ocular infections.<sup>73</sup> Berberine is a major alkaloid of all the *Berberis* species.

Berberine has shown significant antimicrobial properties against various strains of bacteria such as *Staphylococcus aureus* and *Candida* spp.<sup>74</sup> Sub-fractions containing berberine and syringaresinol were found to have good activity and berberine (MIC = 12.5  $\mu\text{g}/\text{mL}$ ), 8-trichloromethyldihydroberberine (MIC = 25  $\mu\text{g}/\text{mL}$ ) and syringaresinol (12.5  $\mu\text{g}/\text{mL}$ ) were found to be active against *SMRSA* and *Mycobacterium marinum*, hence it can be said that berberine and syringaresinol are mainly responsible for the antibacterial activities of the sub-fractions. Minimum

inhibitory concentration (MIC) were only calculated for the active compounds. Results are summarized in **Table. 5.2**

**Table. 5.3. Anti-trypanosomal studies of *Berberis jaeschkeana* Schneid var.**

***jaeschkeana* and *Berberis glaucocarpa* Stapf (Alamar blue™ 96 well plate assay).**

| Sample | <i>T.b. brucei</i> S427<br>(% of control) | <i>T.b. brucei</i> S427<br>(% of control) | <i>T.b. brucei</i> S427<br>(% of control) | <i>T.b. brucei</i> S427<br>(MIC) |
|--------|-------------------------------------------|-------------------------------------------|-------------------------------------------|----------------------------------|
|        | 20 µg/ml                                  | 10 µg/ml                                  | 5 µg/ml                                   | µg/ml                            |
| RB-10  | 1.8                                       | 2.1                                       | 54.3                                      | 12.5                             |
| RB-11  | - 1.1                                     | 1.9                                       | 86.8                                      | 12.5                             |
| RB-21  | 23.7                                      | 83.4                                      | 98.4                                      | NC                               |
| RBJFA  | 30.7                                      | 93.8                                      | 94.6                                      | NC                               |
| RBJC   | 66.4                                      | 102.5                                     | 101.6                                     | NC                               |
| BJSET  | 18.7                                      | 69.2                                      | 89.7                                      | NC                               |
| GR-05  | 97.5                                      | 118.3                                     | 106.2                                     | NC                               |
| BGFC   | 86.3                                      | 117.9                                     | 99.1                                      | NC                               |
| GR-06  | 85.0                                      | 97.0                                      | 93.0                                      | NC                               |
| RBJ-15 | 8.5                                       | 83.2                                      | 86.8                                      | NC                               |
| BFET   | 94.7                                      | 109.7                                     | 102.6                                     | NC                               |
| BGSD   | 21.6                                      | 78.5                                      | 93.4                                      | NC                               |
| BGA    | 21.0                                      | 82.0                                      | 109.2                                     | NC                               |
| BJSC   | 37.9                                      | 88.9                                      | 92.5                                      | NC                               |
| RB-8   | 85.0                                      | 97.0                                      | 93.0                                      | NC                               |
| L.Y    | 0.4                                       | 1.8                                       | 79.8                                      | 25                               |

NC = not calculated, RB-10 = Berberine, RB-11 = 8-trichloromethyldihydroberberine, RB-21= Syringaresinol, RBJFA = *Berberis jaeschkeana* roots Fraction A, RBJC = *Berberis jaeschkeana* roots Fraction C, BJSET = *Berberis jaeschkeana* stem Fraction C, GR-05= Pseudopalmatine, BGFC = *Berberis glaucocarpa* Fraction C, GR-06 = Columbamine, RBJ-15 = Oxoberberine, BFET = *Berberis glaucocarpa* Fraction C, BGSD = *Berberis glaucocarpa* stem Fraction D, BGA = *Berberis glaucocarpa* Fraction A, BJSC = *Berberis jaeschkeana* stem Fraction C, RBJ-8 = Palmatine, L.Y= 10-Hydroxy chondrofoline.

Berberine, 8-Trichloromethyldihydroberberine, oxoberberine, 10-hydroxy chondrofoline (111) and their respective sub-fractions showed good anti-trypanosomal properties which could be due to the alkaloidal nature.

### 5.3. Anti-diabetic studies of *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf (PTP1B enzyme assay).

Most of the compounds and fractions were tested against enzyme Protein Tyrosine Phosphatase 1B (PTP1B) to check their anti-diabetic properties.

**Table. 5.4. Anti-diabetic results of *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf.**

| Sample     | Umbelliferone (Counts) | Minus Background | % Control |
|------------|------------------------|------------------|-----------|
| Background | 3233                   | 3233             | 48        |
| GS-2       | 5159                   | 5159             | 77        |
| RB-14      | 6390                   | 6390             | 96        |
| RB-18      | 14291                  | 14291            | 214       |
| RB-10      | 7709                   | 7709             | 115       |
| RB-8       | 7163                   | 7163             | 107       |

|              |      |      |     |
|--------------|------|------|-----|
| <b>GS-09</b> | 4989 | 4989 | 75  |
| <b>GS-12</b> | 6865 | 6865 | 103 |
| <b>L.Y</b>   | 7392 | 7392 | 111 |
| <b>4-Me</b>  | 7065 | 7065 | 106 |
| <b>GR-8</b>  | 7143 | 7143 | 107 |
| <b>RBJ-C</b> | 4329 | 4329 | 65  |
| <b>BGS-E</b> | 4342 | 4342 | 65  |
| <b>RBJ-D</b> | 4331 | 4331 | 65  |
| <b>BGF-C</b> | 2807 | 2807 | 42  |
| <b>BGE-A</b> | 3198 | 3198 | 48  |
| <b>SWJ-C</b> | 4578 | 4578 | 68  |
| <b>BF-ET</b> | 7645 | 7645 | 114 |
| <b>BGS-D</b> | 5501 | 5501 | 82  |
| <b>HPT</b>   | 7935 | 7935 | 119 |

GS-2 =  $\beta$ -Sitosterol, RB-14 = 8-Hydroxy-7,8-dihydroberberine, RB-18 = Docosanoic acid, Rb-10 = Berberine, RB-8 = Palmatine, GS-09 = Jatrorrhizine, GS-12 = Syringic acid, L.Y = 10-Hydroxy chondrofoline, 4-Me = 10-Methoxy chondrofoline, GR-8 = Oxyacanthine, RBJ-C = *Berberis jaeschkeana* roots Fraction **C**, BGS-E = *Berberis glaucocarpa* stem Fraction **C**, RBJ-D = *Berberis jaeschkeana* roots Fraction **D**, BGFC = *Berberis glaucocarpa* roots Fraction **C**, BGE-A = *Berberis glaucocarpa* roots Fraction **A**, SWJ-C = *Berberis jaeschkeana* stem Fraction **C**, BF-ET = *Berberis glaucocarpa* stem Fraction **B**, BGS-D = *Berberis glaucocarpa* stem Fraction **D**, HPT = mixture from which 10-Hydroxy chondrofoline was obtained.

Phosphorylation and dephosphorylation of protein can stimulate or deactivate protein activity and therefore balance between protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) is of great importance. Deregulation of a single enzyme can

result in some serious diseases in human being for example diabetes is caused due to deregulation of insulin signaling. PTP1B enzyme is a negative regulator for insulin signaling can be used for the treatment of diabetes type II.<sup>75</sup> PTP1B lowers blood glucose level and improve insulin response in normal and diabetic mice by increasing insulin receptor signaling in the peripheral tissues. Thus inhibiting the activity of PTP1B could be used to treat diabetes.<sup>76</sup>

In the above studies (**Table. 5.4.**) the samples having a 40 % or less were considered to be active. Only some of the tested samples were found to be moderately active and none of them were very active.

#### **5.4. Anti-fungal studies of compounds from *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf.**

Some of the compounds were tested for their anti-fungal properties using agar tube dilution method. None of the compound showed significant anti-fungal activity. The results of anti-fungal studies are summarized in **Table. 5.5.**

**Table. 5.5. Anti-fungal results of *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf.**

| Sample | A.s. | A.n. | A.f. | T.h. |
|--------|------|------|------|------|
| GR-06  | 110  | 105  | 100  | 105  |
| 4-Me   | 115  | 115  | 115  | 115  |
| L.Y    | 95   | 100  | 100  | 105  |
| GS-2   | 115  | 115  | 115  | 115  |
| BUV    | 100  | 100  | 105  | 110  |
| RB-8   | 85   | 98   | 100  | 80   |
| B-10   | 110  | 110  | 105  | 100  |
| GS-12  | 95   | 65   | 100  | 70   |
| DMSO   | 115  | 115  | 115  | 115  |

A.s.= *Alternaria solani*, A.n.= *Aspergillus niger*, A.f. = *Aspergillus flavus*, T.h. = *Trichoderma harzianum*, 4-Me = 10-Methoxy chondrofoline, L.Y = 10-Hydroxy chondrofoline, GS-2 =  $\beta$ -Sitosterol, BUV = 8,12,12a-Trihydroxy-11,13a-berberin-dione, RB-8 = Palmatine, B-10 = Berberine, GS-12 = Syringic acid.

### 6.1. Plant Material.

*Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf were collected from Azad Kashmir Pakistan during July 2009 and were identified by Prof Dr. Tanveer Akhtar (Chairperson Botany Department University of Azad Jammu and Kashmir). A voucher specimen numbers 9615-B and 9615-A respectively were deposited in the herbarium of the Botany Department, University of Peshawar.

### 6.2. Extraction and fractionation

The extraction and fractionation of *Berberis jaeschkeana* Schneid var. *jaeschkeana* and *Berberis glaucocarpa* Stapf was performed according to the scheme shown in **Scheme**.

#### 3.1. (Page 106, Ch 3)

### 6.3. Bioassay studies on *Berberis jaeschkeana* and *Berberis glaucocarpa* Stapf.

#### 6.3.1. Anti-microbial studies (agar-well diffusion method).<sup>78</sup>

Modified agar-well diffusion method was used to test the anti-bacterial properties of the crude extract, fractions and the compounds. Muller-hinton agar (MHA) was used as medium. The cultures were taken in triplicates at incubation temperature of 37 °C for 24 to 72 hrs. The broth culture (0.6 mL) of the test organism was placed in a sterile petri-dish and 20 mL of the sterile molten MHA was added. Wells were bored in the medium using 0.2 mL of the extract, fractions and compounds. Streptomycin was used as a standard anti-microbial agent at a concentration of 2 mg/mL. Incubation at 37 °C, for 24-48 hrs, the diameters of the zone of inhibition was measured in millimeters.

### 6.3.1.1. Microbial culture preparation.

Five strains of bacteria namely *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Staphylococcus epidermidis* were used for the anti-microbial activity. The bacterial strains were collected from the stock culture of Phytopharmaceutical and Nutraceutical Research Laboratories (PNRL), Institute of Chemical Sciences, University of Peshawar Pakistan. These organisms were placed in muller-hinton agar in the refrigerator at 4 °C prior to subculture.

### 6.3.2. Anti-fungal studies.<sup>79</sup>

Agar tube dilution method was used to test the anti-fungal properties of the crude extracts, fractions and the compounds. Plant extract (24 mg) was dissolve in one mL DMSO. The media was prepared by dissolving 6.5 g of sabouraud dextrose agar (MERCK) in 100 ml of distilled water. Then agar was poured into screwed capped test tubes and was then autoclaved. The test tubes containing agar were allowed to cool down to 50 °C, then samples were added to each test tube and allowed to solidify in a slanting position. After solidification each slant was inoculated with 4 mm diameter piece of fungal strains using DMSO as a negative control. The test tubes were then incubated at 28 °C for one week. Readings were measured in millimeters and the growth inhibition was calculated with reference to negative control. Percent inhibition was calculated by the following formula.

$$\% \text{ Inhibition of fungal growth} = 100 - \frac{\text{Linear growth in test tubes (mm)}}{\text{Linear growth in control (mm)}} \times 100$$

### 6.3.3. Anti-trypanosomal and anti bacterial studies.<sup>80, 81</sup>

The anti-trypanosomal and anti-bacterial properties of the fractions and the compounds were determined by using the REDOX indicator Alamar blue.<sup>TM</sup> the active component was resazurin, which has blue color and in the presence of live parasites or cells it is reduced to resorufin, which has bright pink fluorescence. The fluorescence values for the test plates were measured with microplate reader in the fluorescence mode with excitation wavelength of 560 nm and emission wavelength of 590 nm. Wells containing active compounds remain blue in colour and have background level fluorescence. All the samples were initially screened at a single concentration and then MICs were determined for the active samples. Positive and negative control and sterility checks were also included in all the assays.

#### 6.3.3.1. Anti-trypanosomal procedure.

Samples were tested against bloodstream form of *Trypanosoma brucei brucei* (*T.b. brucei*) S427. The activities of all the samples were determined using Alamar blue <sup>TM</sup> 96 well microplate assay. All samples were first screened at 20 µg/ mL for crude extracts and 20 µM for pure compounds. The concentration of DMSO was 0.5 % in the initial screening.

Samples for testing were prepared by dissolving 10 mg/mL or 10 mM stock solutions in 100 % DMSO and were then diluted with HMI-9 medium to a concentration of 1mg/mL or 1mM. 4µL of the test sample was added to the assay well and then 96 µL medium to give a final assay concentration, after dilution, of 20 µg/mL.

**Table. 6.1. Anti-trypanosomal assay plate layout.**

|          | 1                 | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12                       |
|----------|-------------------|----|----|----|----|----|----|----|----|----|----|--------------------------|
| <b>A</b> | Sterility Control | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 1.0 $\mu$ M<br>Suramin   |
| <b>B</b> | Control           | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 0.5 $\mu$ M<br>Suramin   |
| <b>C</b> | Control           | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 0.25 $\mu$ M<br>Suramin  |
| <b>D</b> | Control           | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 0.125 $\mu$ M<br>Suramin |
| <b>E</b> | DMSO Control      | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 0.062 $\mu$ M<br>Suramin |
| <b>F</b> | DMSO Control      | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 0.031 $\mu$ M<br>Suramin |
| <b>G</b> | DMSO Control      | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 0.015 $\mu$ M<br>Suramin |
| <b>H</b> | DMSO Control      | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 0.008 $\mu$ M<br>Suramin |

The screening plate was arranged as shown in **Table. 6.1**. Sterility control and DMSO control were placed in column 1, test samples in 2 to 11 and suramin having a concentration range of 1.0  $\mu$ M to 0.008  $\mu$ M was used as positive control. *Trypanosomes* were counted with haemocytometer and were diluted to concentration of  $3 \times 10^4$  trypanosomes/mL, 100  $\mu$ L of this suspension was added to each well except the well having sterility control (**A1**). The whole assay plate was incubated at 37 °C in 5 % CO<sub>2</sub> atmosphere for 48 hours. The 20  $\mu$ L Alamar blue was added and then incubated for

another 24 hours. Wallac Victor microplate reader (excitation wavelength 530 nm and emission wavelength 590 nm) was used for the determination of the fluorescence. The results were calculated as % of the DMSO control. Samples having a value of less than 10 % of the DMSO control were active. Minimum inhibitory concentrations (MICs) were determined for the active samples. MICs were determined in duplicates. Results are tabulated in **Table 5.2. Page 147.**

#### **6.3.4. Anti-diabetic studies using Protein Tyrosine Phosphatase 1B**

**(PTP1B enzyme assay).<sup>82</sup>**

##### **6.3.4.1. Buffer preparation.**

The buffer solution consists of 2.975 g of Hepes (25mM, Sigma), 1.461 g of sodium chloride (50mM, Sigma ), 0.32 g of dithiothreitol (2mM, Sigma ), 0.365 g of ethylenediamine-tetra acetic acid (EDTA 2.5mM, Sigma) and 5 mg of bovine serum albumin (BSA 0.01mg/ml, Sigma A2153), dissolved in 500 ml of distilled water and the pH was adjusted to 7.2.

##### **6.3.4.2 . Plant extracts preparation.**

The stock solutions of all the samples were prepared by dissolving 10 mg/ml and then all samples were screened at 100 µg/ml, 1.2µl of each sample was added to 98 µl buffer in a U-shape plate having 96 round and clear bottom wells (Greiner bio-one, Germany)

**6.3.4.3. Enzyme preparation.**

The enzyme, Protein Tyrosine Phosphatase 1B (Mol.Wt 37400), 100 $\mu$ l was added to 25 mL of the buffer and then aliquoted into 250  $\mu$ l (sufficient for one plate) and stored at -80 °C.

**6.3.4.4. Substrate preparation.**

6,8-Difluoro-4-methylumbelliferyl phosphate (DiFMUP) (invitrogen) dissolved in DMSO and stored at -20 °C. Then 7.0  $\mu$ l of the stock(10mM) were added to 1.75 mL of the buffer solution.

**6.3.4.5. Inhibitor preparation.**

Bis-(4-trifluoromethylsulfonamidophenyl)-1,4-diisopropylbenzine (TFMS, Calbiochem) was dissolved in DMSO (10 mg in 1.64ml), then for TFMS (concentration range 0.0003-3 $\mu$ M) displacement curve was drawn. Km for TFMS was 6 $\mu$ M.

**6.4. Assay assembly method.**

In a 96 half well flat bottom black plate (Costar®), 10  $\mu$ l of standard (TFMS) or samples were added to the plate, then 20  $\mu$ l of PTP1B enzyme was added to each well, and incubated for 30 min at 37 °C and 5 % CO<sub>2</sub> in air. Then 10  $\mu$ l of the substrate was added to the wells and incubated for 10 min. The plate was then screened on Wallac Victor™, using Umbelliferone (355 nm/460 nm).

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# APPENDICES

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**Appendix I.** Natural products derived drugs launched from 1998 to 2004, disease area and lead compound.

| <b>Year</b> | <b>Trade Name</b>                                                         | <b>Disease Area</b>       | <b>Lead Compound</b>               |
|-------------|---------------------------------------------------------------------------|---------------------------|------------------------------------|
| 1998        | Cefoselis (Wincef <sup>®</sup> )                                          | Antibacterial             | Cephalosporin                      |
| 1998        | Orlistat (Xenical <sup>®</sup> )                                          | Antiobesity               | Lipstain                           |
| 1999        | Valrubicin (Valstar <sup>®</sup> )                                        | Oncology                  | Doxorubicin                        |
| 1999        | Colforsin daropate<br>(Adele, Adehl <sup>®</sup> )                        | Cardiotonic               | Forskolin                          |
| 1999        | Dalfopristin and quinupristin<br>(70:30 mixture) (Synercid <sup>®</sup> ) | Antibacterial             | Streptogramin B<br>Streptogramin A |
| 2000        | Arteether (Artemotil <sup>®</sup> )                                       | Antimalarial              | Artemisinin                        |
| 2001        | Telithromycin (Ketek <sup>®</sup> )                                       | Antibacterial             | Erythromycin                       |
| 2001        | Ertapenem (Invanz <sup>™</sup> )                                          | Antibacterial             | Thienamycin                        |
| 2001        | Pimecrolimus (Elidel <sup>®</sup> )                                       | Atopic dermatitis         | Ascomycin                          |
| 2001        | Caspofungin (Cancidas <sup>®</sup> )                                      | Antifungal                | Pneumocandin B                     |
| 2002        | Galantamine (Reminyl <sup>®</sup> )                                       | Alzheimer's Disease       | Galantamine                        |
| 2002        | Biapenem (Omegacin <sup>®</sup> )                                         | Antibacterial             | Thienamycin                        |
| 2002        | Amrubicin<br>hydrochloride(Calsed <sup>®</sup> )                          | Oncology                  | Doxorubicin                        |
| 2002        | Nitisinone (Orfadin <sup>®</sup> )                                        | Antityrosinaemia          | Leptospermone                      |
| 2002        | Micafungin (Funguard <sup>®</sup> )                                       | Antifungal                | FR 901379                          |
| 2003        | Daptomycin (Cubicin <sup>®</sup> )                                        | Antibacterial             | Daptomycin                         |
| 2003        | Rosuvastatin (Crestor <sup>®</sup> )                                      | Dyslipidemia              | Mevastatin                         |
| 2003        | Mycophenolate sodium<br>(Myfortic <sup>®</sup> )                          | Immunosuppression         | Mycophenolic acid                  |
| 2003        | Miglustat (Zavesca <sup>®</sup> )                                         | Type 1 Gaucher<br>disease | 1-Deoxynojirimycin                 |
| 2003        | Pitavastatin (Livalo <sup>®</sup> )                                       | Dyslipidemia              | Mevastatin                         |
| 2004        | Everolimus (Certican <sup>™</sup> )                                       | Immuno-suppression        | Sirolimus                          |

**Appendix II.** Natural products derived drugs launched from 2005 to 2010, disease area and lead compound.

| <b>Year</b> | <b>Trade Name</b>                                          | <b>Disease area</b>    | <b>Lead Compound</b> | <b>Year</b> |
|-------------|------------------------------------------------------------|------------------------|----------------------|-------------|
| 2005        | Ziconotide (Prialt <sup>®</sup> )                          | Pain                   | Ziconotide           | 2005        |
| 2005        | Fumagillin (Flisint <sup>®</sup> )                         | Antiparasitic          | Fumagillin           | 2005        |
| 2005        | Tigecycline (Tygacil <sup>®</sup> )                        | Antibacterial          | Tetracycline         | 2005        |
| 2005        | Doripenem (Finibax <sup>®</sup> / Doribax <sup>®</sup> )   | Antibacterial          | Thienamycin          | 2005        |
| 2005        | Dronabinol / Cannabidiol (Sativex <sup>®</sup> )           | Pain                   | Dronabinol           | 2005        |
| 2005        | Zotarolimus (Endeavor <sup>™</sup> stent)                  | Cardiovascular surgery | Sirolimus            | 2005        |
| 2006        | Exenatide (Byetta <sup>®</sup> )                           | Diabetes               | Exenatide-4          | 2006        |
| 2006        | Anidulafungin (Eraxis <sup>™</sup> / Ecalta <sup>™</sup> ) | Antifungal             | Echinocandin B       | 2006        |
| 2007        | Temsirolimus (Torisel <sup>™</sup> )                       | Oncology               | Sirolimus            | 2007        |
| 2007        | Ixabepilone (Ixempra <sup>™</sup> )                        | Oncology               | Epothilone           | 2007        |
| 2007        | Retapamulin (Altabax <sup>™</sup> / Altargo <sup>™</sup> ) | Antibacterial          | Pleuromutilin        | 2007        |
| 2007        | Lisdexamfetamine (Vyvanse <sup>™</sup> )                   | ADHD                   | Amphetamine          | 2007        |
| 2007        | Trabectedin (Yondelis <sup>™</sup> )                       | Oncology               | Trabectedin          | 2007        |
| 2008        | Methylnaltrexone (Relistor <sup>®</sup> )                  | Pain                   | Naltrexone           | 2008        |
| 2009        | Capsaicin (Qutenza <sup>®</sup> )                          | Pain                   | Capsaicin            | 2009        |
| 2009        | Everolimus (Afinitor <sup>®</sup> )                        | Oncology               | Sirolimus            | 2009        |
| 2009        | Telavancin (Vibativ <sup>®</sup> )                         | Antibacterial          | Vancomycin           | 2009        |
| 2009        | Romidepsin (Isodax <sup>®</sup> )                          | Oncology               | Romidepsin           | 2009        |
| 2010        | Monobactam aztreonam (Cayston <sup>™</sup> )               | Antibacterial          | Aztreonam            | 2010        |

**Appendix III.** Compounds isolated from the genus *Berberis*.<sup>18-24</sup>

(\* Structures given in appendix iv-vii)

| Alkaloids |                         |                                                               |                                                                       |
|-----------|-------------------------|---------------------------------------------------------------|-----------------------------------------------------------------------|
| S.No      | Name of compound        | M. Formula                                                    | Source. <sup>Ref</sup>                                                |
| 1         | Berberine *             | C <sub>20</sub> H <sub>18</sub> NO <sub>4</sub> <sup>+</sup>  | <i>B. vulgaris</i> . <sup>18</sup>                                    |
| 2         | Berbamine *             | C <sub>37</sub> H <sub>40</sub> N <sub>2</sub> O <sub>6</sub> | <i>B. asiatica</i> . <sup>18</sup>                                    |
| 3         | Punjabine *             | C <sub>35</sub> H <sub>32</sub> N <sub>2</sub> O <sub>7</sub> | <i>B. lyceum</i> . <sup>18</sup>                                      |
| 4         | Penduline *             | C <sub>37</sub> H <sub>40</sub> N <sub>2</sub> O <sub>6</sub> | <i>B. coriaria</i> . <sup>18</sup>                                    |
| 5         | Baluchistanamine *      | C <sub>37</sub> H <sub>38</sub> N <sub>2</sub> O <sub>8</sub> | <i>B. lyceum</i> . <sup>18</sup>                                      |
| 6         | Taxilamine              | C <sub>20</sub> H <sub>19</sub> NO <sub>6</sub>               | <i>B. lyceum</i> . <sup>18</sup>                                      |
| 7         | Oblongine               | C <sub>19</sub> H <sub>24</sub> NO <sub>3</sub> <sup>+</sup>  | <i>B. oblonga</i> . <sup>18</sup>                                     |
| 8         | Baluchistine            | C <sub>36</sub> H <sub>38</sub> N <sub>2</sub> O <sub>6</sub> | <i>B. lyceum</i> . <sup>18</sup>                                      |
| 9         | Oxyberberine            | C <sub>20</sub> H <sub>17</sub> NO <sub>5</sub>               | <i>B. asiatica</i> . <sup>18</sup>                                    |
| 10        | Jatrorrhizine           | C <sub>20</sub> H <sub>20</sub> NO <sub>4</sub> <sup>+</sup>  | <i>B. vulgaris</i> , <sup>18</sup> <i>B. asiatica</i> . <sup>18</sup> |
| 11        | Pakistanamine           | C <sub>38</sub> H <sub>42</sub> N <sub>2</sub> O <sub>6</sub> | <i>B. baluchistanica</i> . <sup>18</sup>                              |
| 12        | Pseudoberberinechloride | C <sub>20</sub> H <sub>18</sub> NO <sub>4</sub> <sup>+</sup>  | <i>B. aristata</i> . <sup>18</sup>                                    |
| 13        | Lambertine              | C <sub>20</sub> H <sub>19</sub> NO <sub>4</sub>               | <i>B. lambertii</i> . <sup>18</sup>                                   |
| 14        | Turconidine             | C <sub>23</sub> H <sub>30</sub> N <sub>2</sub> O <sub>4</sub> | <i>B. turcomanica</i> . <sup>18</sup>                                 |
| 15        | Natalinine              | C <sub>25</sub> H <sub>23</sub> NO <sub>5</sub>               | <i>B. empetrifolia</i> . <sup>18</sup>                                |
| 16        | Bernumicine             | C <sub>20</sub> H <sub>25</sub> NO <sub>4</sub>               | <i>B. nummularia</i> . <sup>18</sup>                                  |
| 17        | Calafatine              | C <sub>39</sub> H <sub>44</sub> N <sub>2</sub> O <sub>7</sub> | <i>B. buxifolia</i> . <sup>18</sup>                                   |
| 18        | Aconcaguine             | C <sub>20</sub> H <sub>23</sub> NO <sub>6</sub>               | <i>B. actinacantha</i> . <sup>18</sup>                                |
| 19        | Hediamine               | C <sub>21</sub> H <sub>19</sub> NO <sub>5</sub>               | <i>B. actinacantha</i> . <sup>18</sup>                                |
| 20        | Kalashine               | C <sub>37</sub> H <sub>40</sub> N <sub>2</sub> O <sub>6</sub> | <i>B. orthobotrys</i> . <sup>18</sup>                                 |
| 21        | 8-Oxoberberrubine       | C <sub>19</sub> H <sub>15</sub> NO <sub>5</sub>               | <i>B. heteropoda</i> . <sup>18</sup>                                  |
| 22        | Columbamine             | C <sub>20</sub> H <sub>20</sub> NO <sub>4</sub> <sup>+</sup>  | <i>B. vulgaris</i> , <sup>18</sup> <i>B. asiatica</i> . <sup>18</sup> |
| 23        | Sindamine               | C <sub>37</sub> H <sub>38</sub> N <sub>2</sub> O <sub>8</sub> | <i>B. lyceum</i> . <sup>18</sup>                                      |
| 24        | Jhelumine               | C <sub>36</sub> H <sub>38</sub> N <sub>2</sub> O <sub>7</sub> | <i>B. lyceum</i> . <sup>18</sup>                                      |
| 25        | Pakistanine*            | C <sub>37</sub> H <sub>40</sub> N <sub>2</sub> O <sub>6</sub> | <i>B. baluchistanica</i> . <sup>18</sup>                              |

|    |                           |                                                                            |                                                                      |
|----|---------------------------|----------------------------------------------------------------------------|----------------------------------------------------------------------|
| 26 | Obaberine                 | C <sub>38</sub> H <sub>42</sub> N <sub>2</sub> O <sub>6</sub>              | <i>B. tschonoskiana</i> . <sup>18</sup>                              |
| 27 | Densinine                 | C <sub>14</sub> H <sub>19</sub> NO <sub>4</sub>                            | <i>B. densiflora</i> . <sup>18</sup>                                 |
| 28 | Berpodine                 | C <sub>40</sub> H <sub>38</sub> N <sub>2</sub> O <sub>13</sub>             | <i>B. heteropoda</i> . <sup>18</sup>                                 |
| 29 | Palmatine                 | C <sub>21</sub> H <sub>22</sub> NO <sub>4</sub> <sup>+</sup>               | <i>B. vulgaris</i> . <sup>18</sup>                                   |
| 30 | Oblongamine               | C <sub>38</sub> H <sub>43</sub> N <sub>2</sub> O <sub>6</sub> <sup>+</sup> | <i>B. oblonga</i> . <sup>18</sup>                                    |
| 31 | Oxyacanthine              | C <sub>37</sub> H <sub>40</sub> N <sub>2</sub> O <sub>6</sub>              | <i>B. asiatica</i> , <sup>18</sup> <i>B. koreana</i> . <sup>18</sup> |
| 32 | Quettamine                | C <sub>19</sub> H <sub>22</sub> NO <sub>3</sub> <sup>+</sup>               | <i>B. baluchistanica</i> . <sup>18</sup>                             |
| 33 | Waziristanine             | C <sub>37</sub> H <sub>40</sub> N <sub>2</sub> O <sub>6</sub>              | <i>B. waziristanica</i> . <sup>18</sup>                              |
| 34 | (+)-Epiberbivaldine       | C <sub>36</sub> H <sub>38</sub> N <sub>2</sub> O <sub>6</sub>              | <i>B. actinantha</i> . <sup>18</sup>                                 |
| 35 | Puntarenine               | C <sub>21</sub> H <sub>19</sub> NO <sub>6</sub>                            | <i>B. empetrifolia</i> . <sup>18</sup>                               |
| 36 | Intebrinine               | C <sub>21</sub> H <sub>19</sub> NO <sub>4</sub>                            | <i>B. integerrima</i> . <sup>18</sup>                                |
| 37 | Tetrandrine               | C <sub>38</sub> H <sub>42</sub> N <sub>2</sub> O <sub>6</sub>              | <i>B. petiolaris</i> . <sup>18</sup>                                 |
| 38 | 1-O-Methylpakistanine     | C <sub>38</sub> H <sub>42</sub> N <sub>2</sub> O <sub>6</sub>              | <i>B. aristata</i> . <sup>18</sup>                                   |
| 39 | Pseudopalmatine           | C <sub>21</sub> H <sub>22</sub> NO <sub>4</sub> <sup>+</sup>               | <i>B. aristata</i> . <sup>18</sup>                                   |
| 40 | Chitraline                | C <sub>36</sub> H <sub>38</sub> N <sub>2</sub> O <sub>6</sub>              | <i>B. orthobotrys</i> . <sup>18</sup>                                |
| 41 | Aromoline                 | C <sub>36</sub> H <sub>38</sub> N <sub>2</sub> O <sub>6</sub>              | <i>B. aristata</i> . <sup>18</sup>                                   |
| 42 | Tetrahydropalmatine       | C <sub>21</sub> H <sub>25</sub> NO <sub>4</sub>                            | <i>B. asiatica</i> . <sup>18</sup>                                   |
| 43 | O-Methylcorydine-N-oxide  | C <sub>21</sub> H <sub>25</sub> NO <sub>5</sub>                            | <i>B. chitria</i> . <sup>18</sup>                                    |
| 44 | Gilgitine                 | C <sub>36</sub> H <sub>34</sub> N <sub>2</sub> O <sub>8</sub>              | <i>B. lyceum</i> . <sup>18</sup>                                     |
| 45 | (+)-N-Methylisococlaurine | C <sub>18</sub> H <sub>21</sub> NO <sub>3</sub>                            | <i>B. laurina</i> . <sup>18</sup>                                    |
| 46 | (-)-O-Methylarmepaine     | C <sub>20</sub> H <sub>25</sub> NO <sub>3</sub>                            | <i>B. laurina</i> . <sup>18</sup>                                    |
| 47 | Gandharamine *            | C <sub>19</sub> H <sub>18</sub> NO <sub>4</sub> <sup>+</sup>               | <i>B. baluchistanica</i> . <sup>18</sup>                             |
| 48 | N-Methyl dihydroberberine | C <sub>21</sub> H <sub>22</sub> NO <sub>4</sub> <sup>+</sup>               | <i>B. heteropoda</i> . <sup>18</sup>                                 |
| 49 | Isotetrandine             | C <sub>38</sub> H <sub>42</sub> N <sub>2</sub> O <sub>6</sub>              | <i>B. morrisonensis</i> . <sup>18</sup>                              |
| 50 | Magnoflorine              | C <sub>20</sub> H <sub>24</sub> NO <sub>4</sub> <sup>+</sup>               | <i>B. morrisonensis</i> . <sup>18</sup>                              |
| 51 | Densiberine               | C <sub>21</sub> H <sub>24</sub> NO <sub>4</sub> <sup>+</sup>               | <i>B. densiflora</i> . <sup>18</sup>                                 |
| 52 | Illicifoline              | C <sub>42</sub> H <sub>40</sub> N <sub>2</sub> O <sub>10</sub>             | <i>B. illicifolia</i> . <sup>18</sup>                                |
| 53 | Santiagoamine*            | C <sub>19</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub>              | <i>B. darwinii</i> . <sup>18</sup>                                   |
| 54 | Dehydropuntarenine        | C <sub>21</sub> H <sub>17</sub> NO <sub>6</sub>                            | <i>B. actinacantha</i> . <sup>18</sup>                               |

|    |                              |                                                               |                                        |
|----|------------------------------|---------------------------------------------------------------|----------------------------------------|
| 55 | Dehydrosaulatine             | C <sub>22</sub> H <sub>21</sub> NO <sub>6</sub>               | <i>B. actinacantha</i> . <sup>18</sup> |
| 56 | Khyberine                    | C <sub>36</sub> H <sub>38</sub> N <sub>2</sub> O <sub>6</sub> | <i>B. calliobotrys</i> . <sup>18</sup> |
| 57 | Intebrimine                  | C <sub>20</sub> H <sub>25</sub> NO <sub>4</sub>               | <i>B. integerrima</i> . <sup>18</sup>  |
| 58 | Chenabine                    | C <sub>37</sub> H <sub>40</sub> N <sub>2</sub> O <sub>7</sub> | <i>B. lyceum</i> . <sup>18</sup>       |
| 59 | Espinine                     | C <sub>36</sub> H <sub>40</sub> N <sub>2</sub> O <sub>6</sub> | <i>B. laurina</i> . <sup>18</sup>      |
| 60 | Berberilaurine               | C <sub>36</sub> H <sub>38</sub> N <sub>2</sub> O <sub>6</sub> | <i>B. laurina</i> . <sup>18</sup>      |
| 61 | 7-O-Dimethylisothaliceberine | C <sub>38</sub> H <sub>40</sub> N <sub>2</sub> O <sub>6</sub> | <i>B. chilensis</i> . <sup>18</sup>    |
| 62 | O-Methylisothaliceberine     | C <sub>38</sub> H <sub>42</sub> N <sub>2</sub> O <sub>6</sub> | <i>B. chilensis</i> . <sup>18</sup>    |
| 63 | Lauberine                    | C <sub>37</sub> H <sub>40</sub> N <sub>2</sub> O <sub>6</sub> | <i>B. laurina</i> . <sup>18</sup>      |
| 64 | Coyhaiquine                  | C <sub>26</sub> H <sub>27</sub> NO <sub>5</sub>               | <i>B. empetrifolia</i> . <sup>18</sup> |
| 65 | Bernumidine                  | C <sub>20</sub> H <sub>23</sub> NO <sub>4</sub>               | <i>B. nummularia</i> . <sup>18</sup>   |
| 66 | Karachine                    | C <sub>26</sub> H <sub>27</sub> NO <sub>5</sub>               | <i>B. aristata</i> . <sup>18</sup>     |
| 67 | Espinidine                   | C <sub>37</sub> H <sub>42</sub> N <sub>2</sub> O <sub>6</sub> | <i>B. laurina</i> . <sup>18</sup>      |
| 68 | Calafatimine                 | C <sub>38</sub> H <sub>40</sub> N <sub>2</sub> O <sub>7</sub> | <i>B. buxifolia</i> . <sup>18</sup>    |
| 69 | Berbidine                    | C <sub>23</sub> H <sub>28</sub> N <sub>2</sub> O <sub>5</sub> | <i>B. brandisiana</i> . <sup>19</sup>  |
| 70 | Polyberbine                  | C <sub>20</sub> H <sub>19</sub> NO <sub>6</sub>               | <i>B. valdiviana</i> . <sup>20</sup>   |
| 71 | Chillanamine                 | C <sub>37</sub> H <sub>42</sub> N <sub>2</sub> O <sub>7</sub> | <i>B. buxifolia</i> . <sup>21</sup>    |
| 72 | Pronuciferine N-Oxide        | C <sub>19</sub> H <sub>22</sub> NO <sub>4</sub> <sup>+</sup>  | <i>B. coletoides</i> . <sup>22</sup>   |
| 73 | Corydinemethine              | C <sub>21</sub> H <sub>25</sub> NO <sub>4</sub>               | <i>B. cretica</i> . <sup>23</sup>      |
| 74 | (-)-Osornine                 | C <sub>38</sub> H <sub>42</sub> N <sub>2</sub> O <sub>7</sub> | <i>B. buxifolia</i> . <sup>21</sup>    |

### Anthocynins

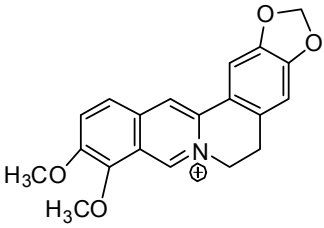
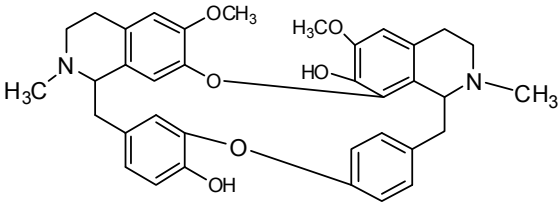
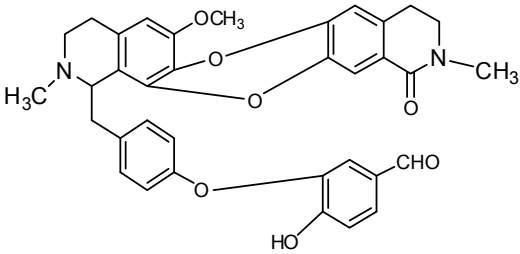
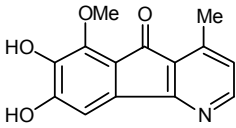
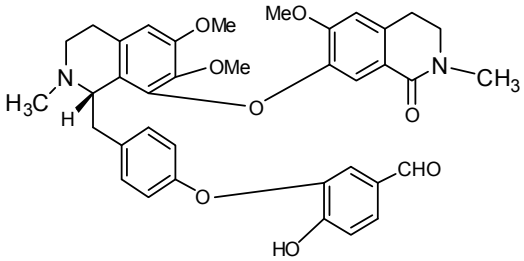
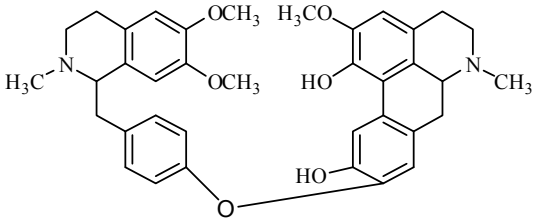
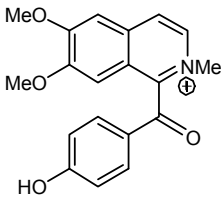
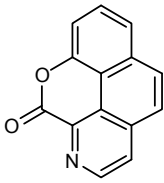
| S.No | Name of compound        | M. Formula                                                   | Source <sup>Ref</sup>                                                 |
|------|-------------------------|--------------------------------------------------------------|-----------------------------------------------------------------------|
| 1    | Cyanidin-3-digluocoside | C <sub>21</sub> H <sub>21</sub> O <sub>11</sub> <sup>+</sup> | <i>B. oblonga</i> . <sup>18</sup>                                     |
| 2    | Delphinidin             | C <sub>15</sub> H <sub>11</sub> O <sub>7</sub>               | <i>B. vulgaris</i> . <sup>18</sup>                                    |
| 3    | Cyanidin *              | C <sub>15</sub> H <sub>11</sub> O <sub>6</sub> <sup>+</sup>  | <i>B. vulgaris</i> . <sup>18</sup>                                    |
| 4    | Cyanidin-3-glucoside *  | C <sub>21</sub> H <sub>21</sub> O <sub>11</sub> <sup>+</sup> | <i>B. thunbergii</i> . <sup>18</sup>                                  |
| 5    | Peonodin *              | C <sub>16</sub> H <sub>13</sub> O <sub>6</sub> <sup>+</sup>  | <i>B. vulgaris</i> . <sup>18</sup>                                    |
| 6    | Delphinidin-3-glucoside | C <sub>21</sub> H <sub>21</sub> O <sub>12</sub> <sup>+</sup> | <i>B. oblonga</i> . <sup>18</sup> <i>B. buxifolia</i> . <sup>18</sup> |

| 7                               | Delphinidin-3-diglucoside *          | C <sub>27</sub> H <sub>31</sub> O <sub>17</sub> <sup>+</sup> | <i>B. oblonga</i> . <sup>18</sup>                                          |
|---------------------------------|--------------------------------------|--------------------------------------------------------------|----------------------------------------------------------------------------|
| 8                               | Malvidin-3-glucoside                 | C <sub>23</sub> H <sub>25</sub> O <sub>12</sub>              | <i>B. buxifolia</i> , <sup>18</sup> , <i>B. darwinii</i> . <sup>18</sup>   |
| 9                               | Delphinidin-3-glucoside              | C <sub>21</sub> H <sub>21</sub> O <sub>12</sub> <sup>+</sup> | <i>B. buxifolia</i> , <sup>18</sup> , <i>B. darwinii</i> . <sup>18</sup>   |
| 10                              | Petunidin-3-rutinoside               | C <sub>28</sub> H <sub>33</sub> O <sub>16</sub> <sup>+</sup> | <i>B. buxifolia</i> , <sup>18</sup> , <i>B. darwinii</i> . <sup>18</sup>   |
| 11                              | Petunidin-3-glucoside                | C <sub>22</sub> H <sub>23</sub> O <sub>12</sub> <sup>+</sup> | <i>B. buxifolia</i> , <sup>18</sup> <i>B. darwinii</i> . <sup>18</sup>     |
| 12                              | Petunidin-3-rutinoside-5-glucoside * | C <sub>34</sub> H <sub>43</sub> O <sub>21</sub> <sup>+</sup> | <i>B. buxifolia</i> . <sup>18</sup>                                        |
| 13                              | Pelargonidin-3-glucoside             | C <sub>21</sub> H <sub>21</sub> O <sub>10</sub>              | <i>B. thunbergii</i> , <sup>18</sup> <i>B. oblonga</i> . <sup>18</sup>     |
| 14                              | Peonodin-3-glucoside                 | C <sub>22</sub> H <sub>23</sub> O <sub>11</sub> <sup>+</sup> | <i>B. darwinii</i> . <sup>18</sup>                                         |
| 15                              | Malvidin-3-rutinoside *              | C <sub>29</sub> H <sub>35</sub> O <sub>16</sub> <sup>+</sup> | <i>B. buxifolia</i> . <sup>18</sup>                                        |
| <b>Carotenoids and vitamins</b> |                                      |                                                              |                                                                            |
| S.No                            | Name of compound                     | M. Formula                                                   | Source <sup>Ref</sup>                                                      |
| 1                               | α-Carotene *                         | C <sub>40</sub> H <sub>56</sub>                              | <i>B. vulgaris</i> , <sup>18</sup> <i>B. spp. fruits</i> . <sup>18</sup>   |
| 2                               | Lutein *                             | C <sub>40</sub> H <sub>56</sub> O <sub>2</sub>               | <i>B. vulgaris</i> , <sup>18</sup> <i>B. spp. fruits</i> . <sup>18</sup>   |
| 3                               | Capsanthin*                          | C <sub>40</sub> H <sub>56</sub> O <sub>3</sub>               | <i>B. vulgaris</i> , <sup>18</sup> <i>B. spp. fruits</i> . <sup>18</sup>   |
| 4                               | cis-Mutatoxanthin*                   | C <sub>40</sub> H <sub>56</sub> O <sub>3</sub>               | <i>B. nummularia</i> . <sup>18</sup>                                       |
| 5                               | Phytofluene *                        | C <sub>40</sub> H <sub>62</sub>                              | <i>B. spp. fruits</i> . <sup>18</sup>                                      |
| 6                               | Flavoxanthin *                       | C <sub>40</sub> H <sub>56</sub> O <sub>3</sub>               | <i>B. vulgaris</i> . <sup>18</sup> <i>B. spp. fruits</i> . <sup>18</sup>   |
| 7                               | β-Carotene                           | C <sub>40</sub> H <sub>56</sub>                              | <i>B. vulgaris</i> . <sup>18</sup> <i>B. spp. fruits</i> . <sup>18</sup>   |
| 8                               | Zeaxanthin                           | C <sub>40</sub> H <sub>56</sub> O <sub>2</sub>               | <i>B. vulgaris</i> . <sup>18</sup> , <i>B. spp. fruits</i> . <sup>18</sup> |
| 9                               | Antheraxanthin                       | C <sub>40</sub> H <sub>56</sub> O <sub>3</sub>               | <i>B. spp. fruits</i> . <sup>18</sup>                                      |
| 10                              | Auroxanthin                          | C <sub>39</sub> H <sub>52</sub> O <sub>5</sub>               | <i>B. vulgaris</i> . <sup>18</sup>                                         |
| 11                              | Chrynantheraxanthin                  | C <sub>40</sub> H <sub>56</sub> O <sub>3</sub>               | <i>B. vulgaris</i> . <sup>18</sup>                                         |
| 12                              | Ascorbic acid                        | C <sub>6</sub> H <sub>8</sub> O <sub>6</sub>                 | <i>B. oblonga</i> . <sup>18</sup> <i>B. vulgaris</i> . <sup>18</sup>       |

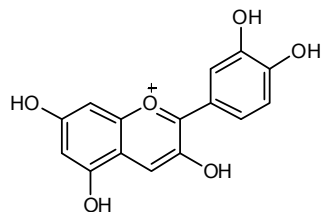
| Phenolic Compounds |                                |                                                               |                                      |
|--------------------|--------------------------------|---------------------------------------------------------------|--------------------------------------|
| S.No               | Name of Compound               | M.Formula                                                     | Source <sup>Ref</sup>                |
| 1                  | Cannabisin G *                 | C <sub>36</sub> H <sub>38</sub> N <sub>2</sub> O <sub>8</sub> | <i>B. vulgaris</i> . <sup>18</sup>   |
| 2                  | ( ± )-Lyoniresinol *           | C <sub>22</sub> H <sub>28</sub> O <sub>8</sub>                | <i>B. vulgaris</i> . <sup>18</sup>   |
| 3                  | N-(p-trans-coumaroyl) tyramine | C <sub>17</sub> H <sub>17</sub> NO <sub>3</sub>               | <i>B. vulgaris</i> . <sup>18</sup>   |
| 4                  | Berbekorin A                   | C <sub>24</sub> H <sub>26</sub> O <sub>8</sub>                | <i>B. koreana</i> . <sup>24</sup>    |
| Flavonoids         |                                |                                                               |                                      |
| S.No               | Name of compound               | M. Formula                                                    | Source <sup>Ref</sup>                |
| 1F                 | Luteolin *                     | C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>                | <i>B. gagnepaini</i> . <sup>18</sup> |
| 2F                 | Rutin                          | C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>               | <i>B. gagnepaini</i> . <sup>18</sup> |
| 3F                 | Hyperoside *                   | C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>               | <i>B. gagnepaini</i> . <sup>18</sup> |
| 4F                 | Quercitoside *                 | C <sub>21</sub> H <sub>21</sub> O <sub>12</sub>               | <i>B. gagnepaini</i> . <sup>18</sup> |
| 5F                 | 3-isorhamnetin galactoside     | C <sub>22</sub> H <sub>22</sub> O <sub>12</sub>               | <i>B. gagnepaini</i> . <sup>18</sup> |

\* Structures given in appendix IV-VII

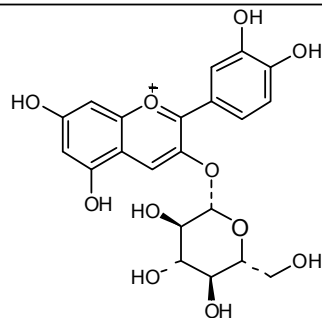
**Appendix IV.** Structures of some of the alkaloids isolated from the genus *Berberis*.

|                                                                                                                                     |                                                                                                                                      |
|-------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|
|  <p><b>Berberine [1]<sup>18</sup></b></p>          |  <p><b>Berbamine [2]<sup>18</sup></b></p>          |
|  <p><b>Punjabine [3]<sup>18</sup></b></p>          |  <p><b>Penduline [4]<sup>18</sup></b></p>         |
|  <p><b>Baluchistanamine [5]<sup>18</sup></b></p> |  <p><b>Pakistanine [25]<sup>18</sup></b></p>     |
|  <p><b>Gandharamine [47]<sup>18</sup></b></p>    |  <p><b>Santiagonamine [53]<sup>18</sup></b></p> |

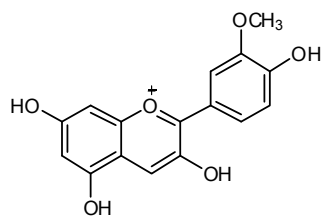
**Appendix V.** Structures of some of the Anthocynins isolated from the genus *Berberis*.



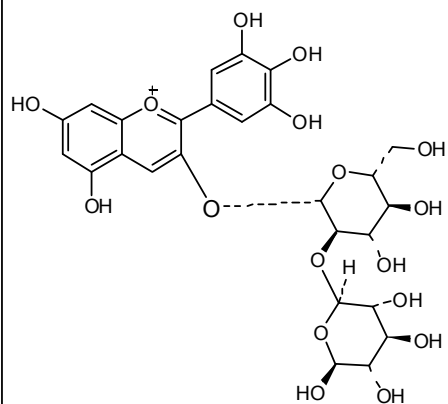
**Cyanidin [3]<sup>18</sup>**



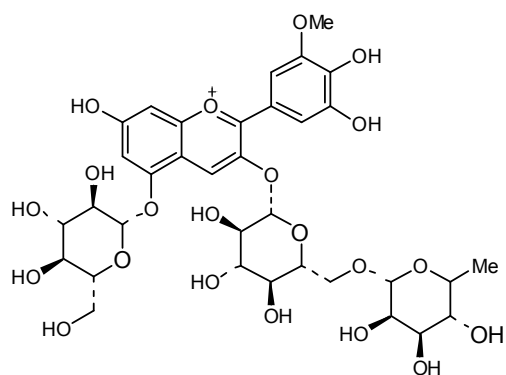
**Cyanidin-3-glucoside [4]<sup>18</sup>**



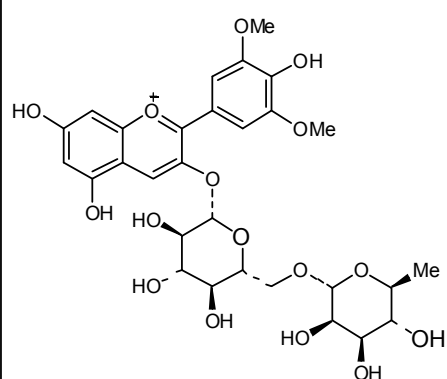
**Peonodin [5]<sup>18</sup>**



**Delphinidin-3-diglucoside [7]<sup>18</sup>**

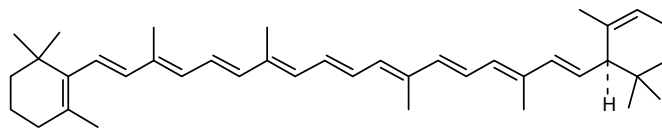


**Petunidin-3-rutinoside-5-glucoside [12]<sup>18</sup>**

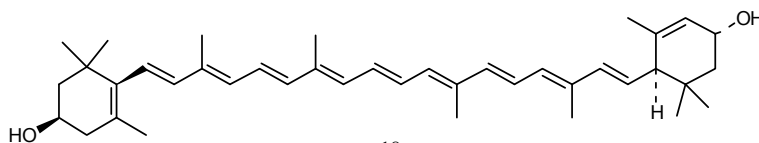


**Malvidin-3-rutinoside [15]<sup>18</sup>**

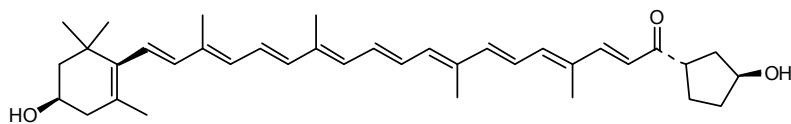
**Appendix VI.** Structures of some of the carotenoids isolated from the genus *Berberis*.



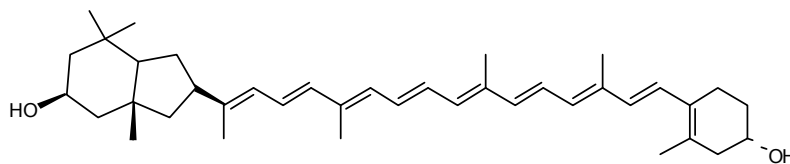
**$\alpha$ -Carotene [1]<sup>18</sup>**



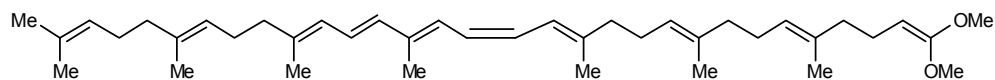
**Lutein [2]<sup>18</sup>**



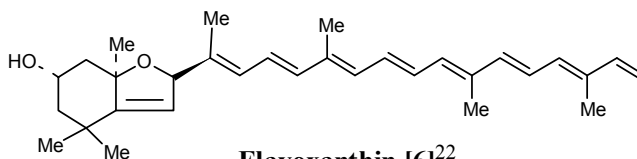
**Capsanthin [3]<sup>18</sup>**



**Cis-Mutatoxanthin [4]<sup>18</sup>**

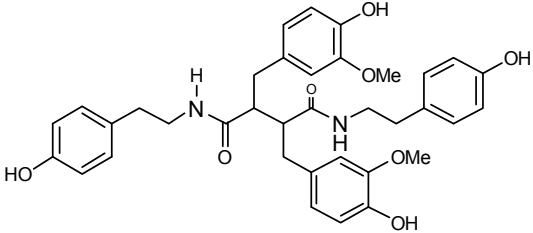
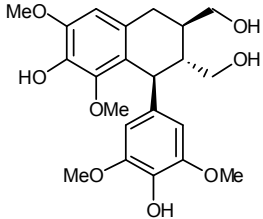
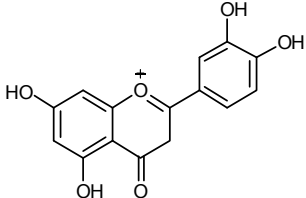
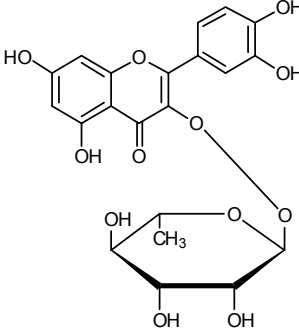
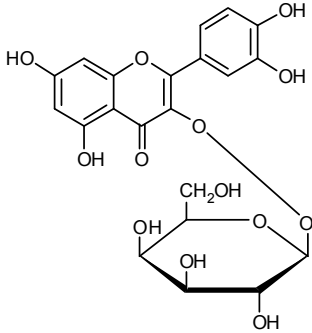
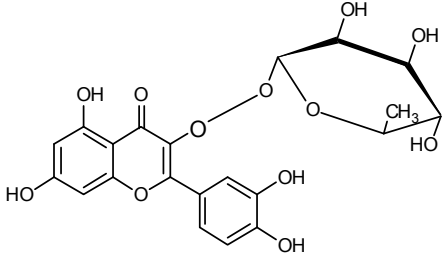


**Phytofluene [5]<sup>18</sup>**



**Flavoxanthin [6]<sup>22</sup>**

**Appendix VII.** Structures of some flavonoids and phenolic compounds isolated from  
the genus *Berberis*.

|                                                                                                                                |                                                                                                                                    |
|--------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|
|  <p><b>Cannabisin G [1]<sup>18</sup></b></p>  |  <p><b>(±)-Lyoniresinol [2]<sup>18</sup></b></p> |
|  <p><b>Luteolin [1F]<sup>18</sup></b></p>    |  <p><b>Rutin [2F]<sup>18</sup></b></p>          |
|  <p><b>Hyperoside [3F]<sup>18</sup></b></p> |  <p><b>Quercitoside [4F]<sup>18</sup></b></p>  |

## Appendix VIII. List of publications

1. Antimicrobial properties of extracts and compounds isolated from *Berberis jaeschkeana*  
**Muhammad Alamzeb**, M. Rafiullah Khan, Saqib Ali, Syed Qaiser Shah and Mamoon-Ur-Rashid, *Bangladesh J Pharmacol.* 2013; 8: 107-109.  
DOI: 10.3329/bjp.v8i2.13551.
2. Bioassay guided isolation and characterization of antimicrobial and anti-trypanosomal agents from *Berberis glaucocarpa* Stapf.  
**Muhammad Alamzeb**, M. Rafiullah Khan, Saqib Ali, Mamoon-Ur-Rashid and Ashfaq Ahmad Khan. *African Journal of Pharmacy and Pharmacology.* 2013; 7(29): 2065-2071. DOI 10.5897/AJPP2013.3444.
3. Biological screening of ethyl acetate extract of *Hedera nepalensis* stem.  
Ghias Uddin, Ashfaq Ahmad Khan, **Muhammad Alamzeb**, Saqib Ali, Mamoon-Ur-Rashid , Anwar Sadat, Muhammad Alam, Abdul Rauf and Wali Ullah. *African Journal of Pharmacy and Pharmacology.* 2012; 6(42): 2934-2937.  
DOI: 10.5897/AJPP12.828
4. Antidiabetic and antimicrobial activities of fractions and compounds isolated from *Berberis brevissima* Jafri and *Berberis parkeriana* Schneid.  
Saqib Ali, John Igoli, Carol Clements, Dima Semaan, **Muhammad Alamzeb**, Mamoon-Ur-Rashid, Syed Qaisar Shah, Valerie A. Ferro, Alexander I. Gray and M. Rafiullah Khan. *Bangladesh J Pharmacol.* 2013; 8: 336-342.  
DOI: 10.3329/bjp.v8i3.13888.