PHYTOCHEMICAL INVESTIGATION ON THE CHEMICAL CONSTITUENTS OF SARCOCOCCA SALIGNA, FICUS RELIGIOSA AND RELATED MEDICINAL PLANTS AND SYNTHETIC STUDIES TOWARDS INTERESTING NEW HETEROCYCLIC COMPOUNDS

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Karachi, 2004
SUMMARY

This Ph. D. dissertation comprises three parts A-C:

Part A

Bioassay-guided isolation of cholinesterase inhibiting pregnane-type steroidal alkaloids from *Sarcococca saligna* (D. Don.) Muell., afforded nine new and ten known pregnane-type steroidal alkaloids from the chloroform extract of the plant. The cholinesterase inhibiting studies of these compounds were carried out by Mr. Sarfraz Ahmad Nawaz in the Enzyme Inhibition Laboratory of the Institute.

All compounds were found to possess a varying degree of cholinesterase inhibitory potential in a concentration-dependent manner with the IC$_{50}$ values ranging from 12.5-200 μM against acetylcholinesterase and 1.25-25.0 μM against butyrylcholinesterase.

New Alkaloids Isolated from *Sarcococca saligna*
$\text{N-Methylformamidesaloinine-B (87)}$

$\text{Salenine-C (77)}$

$\text{Saligaarine-F (90)}$


$\text{5,14-Dehydro-}N_x\text{-demethylsaranacine (95)}$

$\text{2,3-Dehydrosarsaligione (82)}$

$\text{14-Dehydro-}N_x\text{-demethylsaranacine (98)}$

$\text{16-Dehydrosaranacine (101)}$

*Steroids, 2004 (in press)*
Known Alkaloids Isolated from *Sarcococca saligna*

- **Salignarine-C (104)**
- **Saracodine (105)**
- **Vaganine-A (106)**
- **Alkaloid-C (107)**
- **Salignamine-A (108)**
- **2-Hydroxysalignamine-A (109)**
- **Axillarine F (110)**
- **Saracovagaine-C (111)**
PART B

The part B of the thesis contains details of the bioassay-guided isolation of five antileishmanial constituents from *Ficus religiosa* Linn., for the first time from this species.

Known Compounds Isolated from *Ficus religiosa*

\[ \text{Saracocine (112)} \quad \text{Saracorine (113)} \]

\[ \text{Piperine (114)} \quad \text{Piperlonguminine (115)} \]

\[ \text{Dihydropiperlonguminine (116)} \quad \text{N-Isobutyl eicosa-trans-2-trans-4-dienamide (117)} \]

\[ \text{Methyl piperate (118)} \]

Subfractions of the plant and pure compounds were evaluated for antileishmanial activity by Mrs. Farhana Kaukab in the bioassay section of the Institute.

PART C

Part C focuses on the synthesis of the fused heterocyclic ring with the quinoxaline and benzimidazole moieties, accomplished on the basis of our previously reported rearrangement of anthranilopapavarine and β-carboline systems, either by thermolysis or pyrolysis.
The structures of compounds isolated from the *Sarcococca saligna* and *Ficus religiosa* were elucidated through spectroscopic methods using UV, IR, $^1$H- and $^{13}$C-NMR and 2D-NMR techniques (COSY, NOESY, NOE, HMBC, HMQC). Spectroscopic techniques were also employed for the structural identification of synthetic compounds.
خلاصہ

پہلے کہ ہمیشہ منتقل احوان "سازوگن کا کلک"، تحقیق ریلیجیو سیلور اور مائیکل ہیرب کے کمیٹی انجینئر کی کیمیاء کی یادگار کے لئے وارنیم ہمیشہ تحقیق کی کیمیاء کے متعلق۔

حکم 1: مسلسل

سازوگن کا سیلور کا کورونا وائرس نئے سے 9 سے اورہا آج بہت مخصوص ہے کہ مسلسل شدہ کوئی نئے سے ایمپریورل افیڈوؤس کے سے ان تکم کرکے کی نئے گیעס کی حیات کو ان کے خلاف دھوپ ہو چکی ہے۔ ان کی جناب ہنر آزاد ان کے مابین

حکم 2: مثال کا حکام

مثلاً کے حکام کہن امرشل ریلیجیو سیلور سے افسوس ہے کہ مراکز کی سعی کو کئی تفصیل بیان کرنا ہے۔ بکارا مراکز کا کھڑپہ سے

حکم 3: بیہر

پопер کے کہ کبھی اورکی شعرا مراکز کی تفصیل سے دو باہر ہے۔ اورہا کہ کوئی متر محلدار کہ ہے اور کہ

حکم 4: دیک

حکم دیکہ کہ نئے پیڈیا ریلیجیو اورکیتکن آلیاں کے حالات زیادہ ادارے ہے اورکیا کو تحقیق کو فقط باہر ہے۔ بکارا مراکز کا کھڑپہ سے

فاتح کے متعلق کہ کہ ہمیشہ اس طرح استعمال کیا گیا۔
1.0 GENERAL INTRODUCTION

Pakistan is the ninth most populous country in the world where traditional medicines system is practiced for primary health care. The system most widely practiced is called Unani system (Greek medicine) of medicine. The Arab scholars, Rhazes, Avicenna, Al-idrisi, Ibn Al-Baital, Ali Ibn-Rabban and Ibn Al-Nafis have been the major contributors in the development of herbal medicines (Usmanghani, 1997). A survey of the naturally occurring plant wealth of Pakistan shows that medicinal plants grow in abundance in Hazara, Malak and Kuram Agencies, Murree Hill, Azad Kashmir, Northern areas and Baluchistan or are cultivated on farmlands of Punjab, Sindh, Baluchistan, North West Frontier Province and Kashmir. It has been found that various parts of over 300 plants are used by the local people as sources of medicines (Maheshwari, 1993).

Although the value of medicinal plants has been widely recognized worldwide and herbal medicines are the world's primary therapeutics arsenal to fight diseases, a large proportion of floral resources has remained unexplored. Only 35,000-70,000 plant species, out of over 400,000 higher plants species, have been used as medicines.

The isolation of active components from the plant in the last 50 years has afforded a large number of important natural products, which include vinblastine and vincristine which are used for cancer therapy, ajmaline used for the treatment of cardiac arrhythmia, and taxol for the treatment of breast and ovarian cancers, etc.

Moreover, the active components serve as templates both in the synthetic and semi-synthetic (both chemical and biotransformation) preparations of related compounds,
which are potential drugs in their own right and can help establish the basis for understanding the molecular mechanism of active components and structure-activity relationships of the drugs. There are several natural products of plant origin which are currently used in modern medicines (Table-1.1).

<table>
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<tr>
<th>Acetylidigoxin</th>
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<th>Hemsleyadin</th>
<th>Papocarpine</th>
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<td>Curcumin</td>
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<td>Picropotoxin</td>
<td>Scillaren A &amp; B</td>
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<tr>
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<td>α-Lobeline</td>
<td>Quinidine</td>
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<tr>
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<td>Morphine</td>
<td>Rotundine</td>
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<td>Ouabain</td>
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<td>Xanthotoxin</td>
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<td>Peonol</td>
<td>Scopolamine</td>
<td>Yohimbine</td>
</tr>
<tr>
<td>Capalline</td>
<td>Hydrastine</td>
<td>Physostigmine</td>
<td>Sensosides A &amp; B</td>
<td>--</td>
</tr>
</tbody>
</table>

Table 1.1: Some plant-derived drugs used in modern medicines.

The medicinal uses of plants are generally based on centuries of experimentation and trials and errors. In certain cases, accurate data of therapeutic dosage and other pharmacopeial information is available. However, in order to effectively utilize this
echanobotanic information for common good, the Western science needs to understand the botanical and cultural problems inherent in the traditional medicines (Croom, 1983). Herbal medicines are not necessarily safe for use, and some of them exhibited cytotoxicity, carcinogenicity and hepatotoxicity. For these purposes bioassay-guided isolation of active constituents of medicinal plants should be carried out and their active usage and safety profile must be proven by the clinical studies. A bioassay-guided isolation from plant extract, identification of active principle sand clinical trials need multidisciplinary approaches.

A number of bioassays have been established by our research group, which include antibacterial, antifungal, insecticidal, nematocidal, cytotoxic, phytotoxic and assays for enzyme inhibition activities (polypeptidase, chymotrypsin, trypsin phosphodiesterase, α-glucosidase, thrombin). These assays are now being used routinely to direct the isolation of active components from medicinal plants and other natural products. As a result, a large number of biologically active components have been isolated from the medicinally important plants of Pakistan and other countries (Atta-ur-Rahman et al., 2002, 2003).

The work described in this thesis is based on the bioassay-guided isolation of compounds from two medicinally important plants of Pakistan: Sarcococca saligna and Ficus religiosa.
PART A

2.0 Studies on the Chemical Constituents of

*Sarcococca saligna*
2.1 INTRODUCTION

ALKALOIDS

Alkaloids are those compounds, which are:

i) Alkali like,

ii) Heterocycles containing nitrogen,

iii) Derived from amino acids,

iv) Originated from living organisms.

However, several alkaloids do not satisfy these conditions and violate one or more requirements. Morphine (1) is a good example of alkaloids. On the basis of their cyclic structure, they can be classified into the following:

a) Mono cyclic Alkaloids: Conine (2) is the best representative of monocyclic alkaloids with a structure based on piperidine. It has been isolated from hemlock and is highly toxic in nature.

b) Bicyclic Alkaloids: The tropane alkaloids have a 1,4-nitrogen bridge cycloheptane as basic skeleton. Tropine (3) was isolated from Belladona species.

c) Polycyclic Alkaloids: The indole alkaloids are the best example of polycyclic alkaloids. Reserpine (4) and vinblastine (5) are among the most important members of this class of alkaloids.

2.1.1 STEROIDAL ALKALOIDS

Steroidal alkaloids possess a cyclopentanophenanthrene skeleton containing nitrogen in the ring or in a side chain. They can be defined as alkamines, instead of pure
alkaloids, due to their biosynthesis as they are derived from mevalonic acid and not from amino acids. Steroidal alkaloids are mainly present in plants of Apocynaceae, Liliaceae and Solanaceae families, as well as in amphibians and marine invertebrates.

On the basis of structural variations, and source organisms steroidal alkaloids can be classified into the following nine groups:

1. **SAMANINE-TYPE ALKALOIDS**

   Samanine-type alkaloids are mainly found in the skins of *Salamandra* species and have a basic skeleton 6. In these alkaloids, rings A and B are joined together in a cis manner. They can be further divided into two subclasses:

   a) With an isoxazolidine system

   b) Without an isoxazolidine system
a) With an Isoxazolidine System

Samandarine (7), isolated from the skin extract of Salamandra laeviata (Shimazu, 1976), is an example of this type. The isoxazolidine ring is formed by the insertion of a nitrogen, followed by the cleavage of C-2/C-3 bond.

b) Without an Isoxazolidine System

Cycloneosamadion (8), found in the skin of Salamandra maculosa (Habermehl, 1971), is an example of this type.

2. Jerveratrum-Type Alkaloids

Jerveratrum-type alkaloids are mostly found in different species of Veratrum and Fritillaria and have the basic skeleton 9 (Atta-ur-Rahman, 1990). They contain a tetracyclic subunit, which is bound to a piperidine ring system. They usually occur as free amines or as monoglycosides. Jervine (10) is the most abundant jerveratrum-type base, found in the Veratrum species (Shakirov and Yunusov, 1971). Jervinone (11), is another example which was isolated from the rhizomes of Veratrum album (Atta-ur-Rahman et al., 1991a).
3. CERVERATRUM-TYPE ALKALOIDS

Cerveratum-type alkaloids consist of highly oxygenated six rings. They incorporate nine oxygen atoms and are usually found in *Veratum* and *Fritillaria* species. Imperialine (12) was isolated from various species of *Fritillaria* (Masterova et al., 1982).

Veratroylzygadenine (13) is another example of a complex and highly oxygenated canine-type base found in *Veratum* species (Vassova and Tomka, 1975). Verabanzoamine (14) and 6-hydroxyverabanzoamine (15) were isolated from the rhizomes of *Veratum album* (Atta-ur-Rahman et al., 1991b).

4. CONANINE-TYPE ALKALOIDS

Conanine-type steroidal alkaloids have pentacyclic pyrrolidine skeleton 16, and are mainly found in the Apocynaceae family. Conensine (17) represents this class, and has been isolated from the *Holarrhena* and *Funtumia* species (Einhorn et al., 1972; Tolela and Focche, 1979).
5. SPIROSOLANE-TYPE ALKALOIDS

Spirosolane-type alkaloids have been isolated from plants of family Solanaceae. These alkaloids contain an oxazaspirane unit, which is formed by joining α-position of methyl piperidine to C-20 of steroidal moiety. Vermaine (18) was isolated from the Veratrum species (Tomko and Vassova, 1964; Bondarenko, 1984), while tomatidine (19), another example of this class, was extracted from the juice of tomato plant and other Solanum species (Brink and Folker, 1951).

6. SOLANIDINE-TYPE ALKALOIDS

Solanidine-type alkaloids are mostly found in plants of family Liliaceae. Solanidine (20) is an example of this type of alkaloids, isolated from the Veratrum species (Shakirov and Yunusov, 1975).

![Chemical structures of Vermaine (18) and Solanidine (20)]

7. SECO-SOLANIDINE-TYPE ALKALOIDS

Secosolanidine-type alkaloids are derived from the plants of Solanaceae family such as in Veratrum and Fritillaria. Verarine (21) is an example of this class, isolated from Veratrum album (Tomko and Bœur, 1964).
8. **BUXUS ALKALOIDS**

Phytochemical investigation on *Buxus* species have afforded more than 150 steroidal alkaloids. *Buxus* alkaloids are monobasic and dibasic due to the presence of one or two nitrogen atoms as primary, secondary or tertiary amines. In monobasic alkaloids, nitrogen is attached either at C-3 or C-20 positions, while dibasic alkaloids have nitrogen both at the C-3 and C-20 positions. These alkaloids are generally classified into two subclasses:

i) Derivatives of 9β, 19-cyclo-4, 4, 14α-trimethyl-5α-pregnane

ii) Derivatives of abeo-9(9→19)-4,4,14α-trimethyl-5α-pregnane

**i) Derivatives of 9β,19-cyclo-4,4,14α-trimethyl-5α-pregnane:** They have a basic skeleton 22 with a cyclopropane ring between C-9 and C-19, e.g. cyclorolfeine (23) (Laine, 1965) and cyclobuxine-D (24) (Vassova et al., 1980).

**ii) Derivatives of abeo-9(9→19)-4,4,14α-trimethyl-5α-pregnane:** They have a basic skeleton 25 containing a 7-membered ring B resulting from the opening of 9β, 19-cyclopropane system. Buxpistine-K (26) (Točko et al., 1966) and buxamine-E (27) are examples of this class (Huong et al., 1981).
9. PREGNANE-TYPE ALKALOIDS

These alkaloids have a pregnane skeleton (4,4-14-tri nor) containing amino substituents either at C-3 or at C-20 position or at both positions. They are mainly found in the Funtumia, Sarcococca and Holarrhena species.

Buxaprogestine (28) (Choudhary et al., 1988) and ferhidamine (29) (Goutarel et al., 1967) are examples of this class of alkaloids.
2.1.2 GENUS SARCOOCOCA

*Sarcococca saligna* (Buxaceae, syn. *S. pruniformis*) (D. Don.) Muell. is a dicotyledonous evergreen shrub, widely distributed throughout the Northern areas of Pakistan at altitudes of 5,000-9,000 feet (Nasir, 1972).

The genus *Sarcococca* is also found in South-East Asia extending from Afghanistan through the Himalayas to South-East Tibet, Assam, upper Burma and China, India, Sri Lanka, Thailand, Java, Sumatra and Taiwan. The genus comprises 16 species.

2.1.3 PHARMACOLOGICAL IMPORTANCE OF GENUS SARCOOCOCA

The genus *Sarcococca* is known to contain alkaloids of 3, 20-diamino-5α-pregnane or 3/20-amino-5α-pregnane skeleton. The genus enjoys considerable reputation as a remedy for different diseases as a popular medicine. The extract of *Sarcococca ruscifolia* is reported to have antiulcer, antigastritis and antitumor activities (Minghua et al., 1994). A tertiary alkaloid, salignine, isolated from the leaves of *Sarcococca saligna*, potentiates the contractile effect of endogenous acetylcholine on the isolated rat diaphragm by reversibly inhibiting acetylcholinesterase activity (Kiamuddin and Hye, 1970). The extracts also exhibit ganglion-blocking activity by decreasing or abolishing the effects of nicotine on blood pressure and the smooth muscles of isolated guinea pig ileum, and by partially blocking the response in cat. The acute LD₅₀ value of this alkaloid (mouse s. c.) was 40 mg/kg, which was less toxic than pysostigmine or neostigmine (Kiamuddin and Hye, 1970).

A number of other steroidal alkaloids isolated from this genus induce a non-recoverable fall in the blood pressure in dogs and are toxic to paramecia, as well as
exhibit other activities. Recently Atta-ur-Rahman et al. have reported various compounds from *S. saligna* which are active against human pathogenic bacteria and fungi and some of them possess potent anticholinesterase activity (Atta-ur-Rahman et al., 2002).
2.2 BIOSYNTHESIS OF *Sarcococca* ALKALOIDS

*Sarcococca* alkaloids are pregnane-type steroidal alkaloids, which follow the same biosynthetic pathway as in steroids with the following sequence.

\[
\begin{align*}
\text{Acetate} & \quad \downarrow \\
\text{Cholesterol} & \quad \downarrow \\
\text{Pregnenolone} & \quad \downarrow \\
\text{Steroidal alkaloids} & 
\end{align*}
\]

2.2.1 Cholesterol Biosynthesis

Cholesterol biosynthesis begins from condensation of acetyl-CoA, followed by the enzymatic cyclization and rearrangement reactions. The biosynthesis of cholesterol can be divided into four steps (Makin, 1984).

1. **Formation of mevalonic acid** (C_6 compound) from three molecules of acetyl-CoA,
2. **Conversion of mevalonic acid into squalene**, 
3. **Lanosterol formation**, 
4. **Conversion of lanosterol to cholesterol**, 

**Overall Reaction Pathway**

\[
\begin{align*}
6 \text{ Acetyl-CoA} + 6 \text{ Acetoacetyl-CoA} + 14 \text{ NADPH} & \rightarrow \text{ Lanosterol} + 14 \text{ NADP}^+ + 12 \text{ Co-A-S-H} \\
+ 14 H^+ + 5H_2O + 18 \text{ ATP} + \text{O}_2 & \rightarrow + 18 \text{ ADP} + 6 \text{ P}_i + 4 \text{ PP}_i + 6 \text{ CO}_2
\end{align*}
\]
1) **Formation of Mevalonic Acid (33)**

Formation of mevalonic acid involves the conversion of acetyl-Co-A into farnesyl pyrophosphate. Two molecules of acetyl-CoA (30), derived from carbohydrate or fat metabolism, are condensed to give acetoacetyl-CoA (31) in the presence of the enzyme ketothiolase. Compound 31 further condenses with a third molecule of acetyl-CoA in the presence of enzyme hydroxymethylglutaryl-CoA synthetase, which on reduction gives mevalonic acid (33), catalyzed by methyl glutaryl-CoA reductase (Scheme-2.2.1) (Beytia and Porter, 1976).

![Scheme-2.2.1: Formation of Mevalonic Acid (33).](image)

2) **Formation of Squalene (41)**

Squalene formation involves the coupling of six molecules of mevalonic acid (33), followed by a series of phosphorylated intermediates. Subsequent phosphorylation of mevalonic acid is catalyzed by the enzyme mevalonate kinase that affords mevalonate diphosphate (35). Decarboxylation and dehydration of mevalonate diphosphate in the presence of MPP anhydrotetroxylase gives isopenyl pyrophosphate (36). Concerted trans elimination of phosphate from IPP, catalyzed by enzyme isomerase, gives dimethyl
allyl pyrophosphate (37) (Conforth et al., 1966).

Asymmetric condensation of isopentyl pyrophosphate (36) and dimethyl allyl diphosphate (37), in the presence of geranyl transferase, gives rise to geranyl diphosphate (38). The coupling between GPP and a molecule of IPP, in the same manner, gives farnesyl pyrophosphate (39) in the presence of isoprenyl transferase (Scheme-2.2.2) (Lynen et al., 1959).

Scheme-2.2.2: Formation of Farnesyl Pyrophosphate (39).
Two molecules of FPP are coupled together in head-to-head manner, catalyzed by enzyme microsomal squalene synthetase, to give a new intermediate presqualene pyrophosphate (40). Subsequent conversion of PSSP to squalene (41) occurs in the presence of NADPH (Scheme-2.2.3) (Hollyway and Popjak, 1968).

Scheme-2.2.3: Formation of Squalene (41).
3. Formation of Lanosterol (44)

The epoxidation of squalene begins with the incorporation of a molecular oxygen, which is catalyzed by squalene epoxidase giving rise to 2,3-epoxysqualene (42). Proton addition, from the acidic function of the enzyme, opens the epoxide ring and initiates cyclization of the squalene chain, followed by the series of 1,2-trans migration of hydrogen atoms and methyl groups, yielding the lanosterol (44) molecule (Scheme-2.2.4) (Shishibori, 1973).

Scheme-2.2.4: Formation of Lanosterol (44).
4. **Formation of Cholesterol (48)**

The formation of cholesterol (48) from the molecule lanosterol (44) involves the removal of three methyl groups, reduction of the double bond in the side chain and rearrangement of the double bond in ring B.

![Diagram of cholesterol formation](image)

**Scheme 2.2.5: Formation of Cholesterol (48).**
5. **Biosynthesis of Pregnenolone (50)**

   It is generally accepted that pregnenolone (50) is formed enzymatically from unesterified cholesterol (48) by the cleavage of the side chain between C-20 and C-22. This conversion involves a series of mixed function oxidase catalyzed reactions requiring NADPH and molecular oxygen. Burstein et al., have studied the biosynthesis of pregnenolone (50) by the hydroxylation of cholesterol into 20α-hydroxy cholesterol (49). Oxidative cleavage then results in the formation of pregnenolone (50) (Scheme-2.2.6) (Burstein et. al., 1970).

![Scheme-2.2.6: Formation of Pregnenolone (50).]

6. **Biosynthesis of Sarcococca Alkaloids**

   In comparison to other groups of alkaloids, relatively little effort has been directed towards investigating the biosynthesis of steroidal alkaloids. Habermehl and Haaf have demonstrated that the nitrogen atom(s) are introduced into the steroid molecule at a later stage of the biosynthesis (Habermehl and Haaf, 1968). It is proposed that simple pregnane-type steroidal alkaloids can be derived quite easily by a reductive amination of the corresponding steroidal ketone i.e. pregnenolone (50).
Sarcococca saligna

Pregnenolone (50) may undergo enzymatic oxidation and reduction, affording an oxidized product, progesterone (51) and a reduced product 52, respectively, which on subsequent reductive amination can yield different alkaline products like 53, 54, 55 and 56 (Scheme-2.2.7).
2.3 SPECTRAL GENERALIZATION OF PREGNANE-
TYPE STEROIDAL ALKALOIDS

The pregnane-type steroidal alkaloids exhibit characteristic spectral behavior, which help in their characterization.

2.3.1 Ultraviolet and Infrared Spectroscopy

Pregnane-type steroidal alkaloids have a basic steroidal skeleton with amino substituents at C-3 and/or C-20 along with various oxygen functions at various carbons of the main skeleton. Their UV and IR spectra provide valuable information about the functional groups such as carbonyl, hydroxyl and ester groups. For example, the α,β-unsaturated carbonyl moieties in compounds 57 and 58 exhibited the UV absorptions at 242 and 254 nm and their IR absorptions 1654 and 1710 cm⁻¹, respectively.

![Structural formulas of Sarcovagenine-C (57) and Z-Salignone (58)](image)

2.3.2 Mass Spectrometry

The mass fragmentation in pregnane-type steroidal alkaloids is largely based on the nature of nitrogen functions either at C-3 or C-20.
Following are some generalizations based on the observed mass fragmentations in various types of pregnane-type steroidal alkaloids.

a) 20-Aminopregnan Derivatives

In 20-aminopregnan derivatives, nitrogen is present either as amino, methylamino or dimethylamino functions at C-20 of steroidal skeleton, such as in compounds 59-61. In the absence of C-16/C-17 double bond, the cleavage of C-17/C-20 bond leads to an immonium ions as base peaks at m/z 44, 58 and 72, depending on amino substituents.

A characteristic α-cleavage of C-20/C-21 bond affords the mass fragment M⁺ -15. This fragment appears as a base peak in the mass spectra of compounds containing a double bond in ring D between C-16/C-17 (Yu et al., 1997).

b) 3-Aminopregnane Derivatives

3-Aminopregnane derivatives contain a methyl amino group at C-3, as in compound 62 or a dimethylamino group as in compound 63. They display fragments at
m/z 84 and 110, respectively. The cleavages of C-3/C-4 and C-1/C-10 bond afford the fragment of m/z 84, while the mass fragment m/z 110 results from the cleavage of C-2/C-3 and C-7/C-8 bonds as shown below:

Some pregnane-type steroidal alkaloids may contain amide functionalities at C-3. They can range from acetamide group to tigloyl and a senecioyl amide groups as present in compounds 64 and 65, respectively, or may be a benzamide moiety such as in compound 66. They display the mass fragment at m/z 84 and 105, respectively, in their mass spectra, resulting from the cleavage of NH/C=O bond.
2.3.3 $^1$H-NMR Spectroscopy

The pregnane-type steroidal alkaloids have a basic cyclopentanophenanthrene skeleton 67, with nitrogen substituents at C-3 or C-20 positions. They show characteristic signals in their $^1$H-NMR spectra. The two tertiary methyl groups resonate as singlets between $\delta$ 0.7-1.40.

The $N$-methyl group resonates between $\delta$ 2.2-2.8 as a singlet, while in some cases $N$-methyl groups resonate at different chemical shifts due to restricted rotation of C-20/$N_b$ bond because of H-bonding. Similarly when C-20 has an $N$-acetamide group, the amidic $N$-methyl protons appear between $\delta$ 2.6-2.8 as split singlets, because of the rotamerism in acetamide group, as observed in sarcedine (68). The methyl protons of the acetyl group generally resonate between $\delta$ 1.90-2.12. The methine protons geminal to $-\text{NH}$, $-\text{OH}$ or $-$
OCH₃ resonate comparatively downfield between δ 3.3-4.3. The position of these substituents i.e., at C-2, C-3 or C-4, can be inferred from the coupling constants of their signals. The geminal protons of ester substituent appeared rather downfield around δ 5.11-5.33.

The C-20 methine proton, geminal to the N-methyl group, resonates between δ 2.8-4.1 as a multiplet, depending on the nature of the C-20 amino substituents. If the double bond is present between C-16/C-17 in ring D, the C-20 methine proton resonates as a quartet ($J_{20,21}= 6.5$ Hz) e.g., in sarcovagamine-C (57) (Yu et al., 1997). The olefinic protons at C-4, C-5, C-14, C-16 and C-3' appear around δ 5.33-6.4.

![Spectral Generalization Diagram](image)

The presence of tigloyl and senecioyl groups at C-3 N can be inferred from the chemical shift and the splitting pattern of their methyl signals in ¹H-NMR spectrum. The C-4 methyl of tigloyl group, as in compound 64, appeared as a doublet at δ 1.77 ($J = 6.8$ Hz), while C-5' methyl resonated as a singlet at δ 1.81 and olefinic proton resonated at δ 6.3-6.5 as quartet with $J = 6.7$ Hz. The methyl group of senecioyl moiety in compound 65 appeared as a singlet between δ 1.8-2.2, while the olefinic proton appeared between δ 5.3-5.7.
2.4 Phytochemical Investigation on *Sarcoceca saligna*

1. Sarcocurine-B
   - Chemical Structure
   - Molecular Weight: 343
   - Altar-ur-Rahman et al., 1991

2. E. salignone
   - Chemical Structure
   - Molecular Weight: 343
   - Altar-ur-Rahman et al., 1998

3. Z. salignone
   - Chemical Structure
   - Molecular Weight: 343
   - Altar-ur-Rahman et al., 1998

4. Sarcocurine-D
   - Chemical Structure
   - Molecular Weight: 347
   - Altar-ur-Rahman et al., 1999

5. Sarcanidine
   - Chemical Structure
   - Molecular Weight: 356
   - Altar-ur-Rahman et al., 1997

6. Alkaloid-C
   - Chemical Structure
   - Molecular Weight: 439
   - Kdall et al., 1971

7. Sarcocurine-A
   - Chemical Structure
   - Molecular Weight: 370
   - Minghsa et al., 1994

8. Saliagmine
   - Chemical Structure
   - Molecular Weight: 384
   - Minghsa et al., 1960

9. Iso-N-Formylchonemorphine
   - Chemical Structure
   - Molecular Weight: 370
   - Jayasinghe et al., 1998

10. Sarogidine
    - Chemical Structure
    - Molecular Weight: 372
    - Truong-Ho, M. et al., 1953

11. *N*-Formylchonemorphine
    - Chemical Structure
    - Molecular Weight: 384
    - Cauny et al., 1959

12. Sarcocurine-A
    - Chemical Structure
    - Molecular Weight: 384
    - Minghsa et al., 1960

13. Sarcocurine-A_1
    - Chemical Structure
    - Molecular Weight: 384
    - Minghsa et al., 1960

14. Sarcorine
    - Chemical Structure
    - Molecular Weight: 388
    - Altar-ur-Rahman et al., 1997

15. *N*-Demethylsarscadine
    - Chemical Structure
    - Molecular Weight: 384
    - Altar-ur-Rahman et al., 1997
2.5 RESULTS AND DISCUSSION

2.5.1 New Steroidal Alkaloids from *Sarcococca saligna*

2.5.1.1 Salonine-A (69)

The alkaloidal fraction of the plant *Sarcococca saligna* afforded a sub-fraction C-7 (Experimental Section, Scheme-5.4, page 157), after repeated column chromatography by using increasing polarities of pet. ether: acetone: diethylamine as an eluent. This subfraction was again subjected to column chromatography on flash silica gel to obtain a pure alkaloid, salonine-A (69), as a yellow gum.

![Salonine-A (69)](image)

The EI-MS showed the molecular ion at \( m/z \) 458, which was confirmed with the FD-MS. The compound 69 has a 20-dimethylamino pregnane skeleton as inferred from the base peak at \( m/z \) 72 (Budzikiewicz, 1964). The peak at \( m/z \) 83 indicated the presence of a tigloyl group (Atta-ur-Rahman et al., 1990). The HREI-MS of 69 showed the \( M^+ \) at \( m/z \) 458.3489 corresponding to the formula \( C_{26}H_{46}N_2O_3 \) (calcld. 458.3508). Salonine-A
(69) has a mass fragmentation pattern characteristic of steroidal alkaloids (Budzikiewicz, 1964).

![Diagram of compound 69 with mass fragmentations labeled](image)

**Scheme-2.5.1: Mass fragmentation of saloline-A (69).**

The specific rotation of compound 69 was found to be $60^\circ$ ($c = 0.03$, MeOH), which indicated the presence of chirality in the molecule. The UV spectrum exhibited absorption at 212 nm. Its IR spectrum showed absorptions at 3650 (NH), 3328 (OH), 2910 (–CH), 1623 (C=O) and 1559 (C=CH) cm$^{-1}$.

The $^1$H-NMR spectrum (CDCl$_3$) of compound 69 showed two 3H singlets at δ 0.87 and 1.07, corresponding to the C-18 and C-19 tertiary methyls protons, respectively. A 6H singlet at δ 2.21 was due to the $N_b$-dimethyl protons. A 3H doublet at δ 1.09 ($J_{21,20} = 6.6$ Hz) was ascribed to C-21 secondary methyl protons. A 1H multiplet at δ 4.11 was assigned to the methine H-3α geminal to the amidic function.

A downfield broad singlet at δ 5.53 was assigned to the olefinic H-15. The $^1$H-
NMR spectrum showed characteristic resonances for a tigloyl group at δ 6.49 (q, 1H, $J_{3,4} =$ 6.9 Hz), 1.75 (d, 3H, $J_{4,3} =$ 6.8 Hz) and 1.81 (s, 3H) corresponding to the H-3', H-4' and H-5', respectively. The presence of two hydroxyl groups in the skeleton was inferred from two broad methine singlets at δ 4.04 and 4.17, and their attached carbons resonated at δ 69.8 and 66.7, respectively. The $^{13}$C-NMR spectra (broad-band decoupled, DEPT) displayed resonances for 28 carbon atoms, with seven methyl, six methylene, ten methine and five quaternary carbons.

Three partial structures a-c were identified on the basis of 2D-NMR techniques, COSY-45° and HMQC spectra. These sub-structures joined together with the help of long-range proton-proton shift correlations (HOHAHA) and long-range heteronuclear correlations (HMBC) experiments. Stereocchemical assignments in compound 69 are based on NOESY and ROESY spectra.

The spectroscopic data of salamine-A 69 closely resembled with the data sarcovagine A, previously isolated from Sarcococca vagans (Yu et al., 1997).

The partial structure a was deduced on the basis of the COSY-45°, HMQC and HMBC experiments. Vicinal couplings between H-3′ (δ 6.49), H-5′ (δ 1.81) and H-4′ (δ 1.75) were visible in COSY-45° spectra. The HMBC spectrum displayed interaction of the C-4′ methyl carbon (δ 12.4) with downfield H-3′ (δ 6.49). Similarly HMBC interaction of C-5′ methyl carbon (δ 13.9) with 3H singlet at δ 1.75 was also observed. These spectroscopic observations supported the presence of a tigloyl moiety in compound 69.
The partial structure b was deduced on the basis of vicinal couplings of C-2 methine proton (δ 4.04) with one of the C-1 methylene protons (δ 1.2), as well as with the C-3 methine proton (δ 4.11). The C-3 methine proton also showed connectivities with the C-4 proton (δ 4.17) and with the NH (δ 5.96). The C-4 methine proton on the other hand showed a weak "W" coupling with one of the C-6 methylene protons (δ 1.50). Moreover, C-6 methylene protons were coupled with the C-7 methylene protons (δ 0.92), which were in turn coupled with the C-8 methine proton (δ 1.82) in the COSY-45° spectrum. The C-9 methine proton was coupled with one of the C-11 methylene protons at (δ 1.30), while a C-11 methylene proton (δ 2.10) was coupled with one of the C-12 methylene protons (δ 1.58). The spectral connectivity information led to the identification of fragment b, which comprises rings A, B and C of the steroidal skeleton.

The partial structure c consists of ring D of the compound 69. Homonuclear \(^1\)H-\(^1\)H coupling was observed between C-15 methine proton (δ 5.53) and one of the C-16 methylene protons (δ 2.12). Similarly the C-17 methine proton (δ 1.29) was found to be coupled with the C-16 methylene protons (δ 2.12 and 1.86). The COSY-45° spectrum also showed a cross-peak between the C-20 methine proton (δ 2.81) and the C-21 methyl protons (δ 1.09).
The cross peaks in HMBC spectrum (Fig. 2.5.1) were used to join various fragments together and construct a tentative structure, which was later supported by the HOHAHA spectrum showing couplings within each spin system.

![Key HMBC interactions in salolone-A (69).](image)

The HMBC correlations between the C-3 methine proton (δH 4.11) and C-1' carbonyl carbon (δC 170.8) was observed. This connectivity helped in joining fragment a, with C-3 of ring A. The HMBC interactions of the C-19 methyl protons (δH 1.07) with the C-10 quaternary carbon (δC 36.8) and the C-9 methine carbon (δC 56.5), helped in the joining of rings A and B.

The HMBC interactions of C-8 methine proton (δH 1.82) with the C-14 quaternary carbon (δC 157.1), the interactions of the C-18 methyl protons (δH 0.87) with C-13 (δC 46.6) and quaternary C-14 (δC 157.1), and the interaction of the C-15 methine proton (δH 5.53) with the quaternary C-14, indicating the presence of a double bond between C-14.
and C-15. The HMBC coupling of C-17 methine proton (δ 1.29) with C-13 quaternary carbon (δC 46.6), and the coupling of C-15 olefinic methine proton (δH 5.53) with the C-17 methine carbon (δC 57.5), further confirmed the presence of the C-14/C-15 double bond.

The $E$ stereochemistry of the 2-methyl-2-butene moiety at C-3 was inferred from the interaction of the C-4' methyl protons (δ 1.75) with the C-5' methyl protons (δ 1.81) in the ROESY spectrum (Fig. 2.5.2).

![Fig. 2.5.2: Key ROESY interactions in salonine-A (69).](image)

The β-stereochemistry of the hydroxyl groups at C-2 and C-4 was deduced from multiplets resonating at δ 4.04 ($W_{1/2} = 5.5$ Hz) and 4.17 ($W_{1/2} = 5.5$ Hz), respectively. The smaller $W_{1/2}$ values for H-2 and H-4 signals supported axially oriented –OH functionalities with equatorially oriented geminal H-2 and H-4 (Atta-ur-Rahman et al., 1998).

From the aforementioned data, the structure of salonine-A (69) was deduced as (20S)-20-(N,N-dimethylamino)-3β-(tigloylamino)-5α-pregn-14-en-2β,4β-diol.
Table 2.5.1: $^1$H- (400 MHz) and $^{13}$C-NMR (100 MHz) Data of Salanine-A (69) in CDCl$_3$.  

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>$^1$H-NMR ($\delta, J = \text{Hz}$)</th>
<th>$^{13}$C-NMR ($\delta$)</th>
<th>Multiplicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2, 1.18 m</td>
<td>39.8</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>2</td>
<td>4.04 m</td>
<td>69.8</td>
<td>CH</td>
</tr>
<tr>
<td>3</td>
<td>4.11 m</td>
<td>48.0</td>
<td>CH</td>
</tr>
<tr>
<td>4</td>
<td>4.17 m</td>
<td>66.7</td>
<td>CH</td>
</tr>
<tr>
<td>5</td>
<td>1.65</td>
<td>47.8</td>
<td>CH</td>
</tr>
<tr>
<td>6</td>
<td>1.45, 1.50 m</td>
<td>22.2</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>7</td>
<td>1.62, 0.92 m</td>
<td>31.3</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>8</td>
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<td>33.1</td>
<td>CH</td>
</tr>
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<td>9</td>
<td>0.88</td>
<td>56.5</td>
<td>CH</td>
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<td>10</td>
<td>--</td>
<td>36.8</td>
<td>C</td>
</tr>
<tr>
<td>11</td>
<td>2.10, 1.30 m</td>
<td>20.6</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>12</td>
<td>1.40, 1.58 m</td>
<td>34.6</td>
<td>CH$_2$</td>
</tr>
<tr>
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<td>C</td>
</tr>
<tr>
<td>15</td>
<td>5.53 br s</td>
<td>123.7</td>
<td>CH</td>
</tr>
<tr>
<td>16</td>
<td>2.12, 1.86 m</td>
<td>30.9</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>17</td>
<td>1.29</td>
<td>57.5</td>
<td>CH</td>
</tr>
<tr>
<td>18</td>
<td>0.87 s</td>
<td>15.7</td>
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</tr>
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<td>19</td>
<td>1.07 s</td>
<td>16.2</td>
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<tr>
<td>20</td>
<td>2.81 m</td>
<td>59.2</td>
<td>CH</td>
</tr>
<tr>
<td>21</td>
<td>1.09 d ($J_{20,21} = 6.6$)</td>
<td>16.1</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>N$_2$(CH$_3$)$_2$</td>
<td>2.21 s</td>
<td>42.5</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>1'</td>
<td>--</td>
<td>170.8</td>
<td>C</td>
</tr>
<tr>
<td>2'</td>
<td>--</td>
<td>131.1</td>
<td>C</td>
</tr>
<tr>
<td>3'</td>
<td>6.49 q ($J_{3',4'} = 6.9$)</td>
<td>131.4</td>
<td>CH</td>
</tr>
<tr>
<td>4'</td>
<td>1.75 d ($J_{3',5'} = 6.8$)</td>
<td>12.4</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>5'</td>
<td>1.81 s</td>
<td>13.9</td>
<td>CH$_3$</td>
</tr>
</tbody>
</table>
Biogenesis of Salonine-A (69)

The biogenesis of salonine-A (69) has been described in Scheme-2.5.2. N-acetylation of compound 70 with a tigloyl amide 71 followed by enzymatic N-methylation of the base 53 at C-20 afforded the amide 72. Oxidation of amide 72 at positions C-2, C-4 and C-14, followed by the enzymatic dehydration to a double bond C-14/C-15, yielded salonine-A (69).

Scheme-2.5.2: Proposed biogenesis of salonine-A (69).
2.5.1.2 Salonine-B (74)

The alkaloidal fraction B (Experimental Section, Scheme-5.1, page 150) was subjected to column chromatography with the solvent gradients of pet. ether: acetone: diethylamine. This yielded a subfraction B-1, which was again subjected to column chromatography to afford a new alkaloid 74 (6.3 mg), along with a known base alkaloid-C (107) (Kohli, et al., 1970). Both compounds showed positive Dragendorff's test.

![Salonine-B (74)]

The optical rotation of compound 74 was found to be \( \theta = -116^\circ \) (\( c = 0.04, \text{MeOH} \)), which indicated the presence of chirality in the molecule. Its IR spectrum showed absorptions at 3650 (NH) and 2910 (C-H) cm\(^{-1}\). The UV spectrum of 74 exhibited only terminal absorptions.

The compound 74 was isolated as a colorless amorphous solid. The EJ-MS showed the \( M^+ \) at \( m/z \) 357, which was further confirmed by FD-MS and FAB-MS (+ve) (\( m/z \) 358) mass spectrometric techniques. The HREI-MS of 74 showed the exact mass at \( m/z \) 357.2972, corresponding to the formula \( C_{24}H_{39}NO \) (calcd. 357.3031). The peaks at \( m/z \) 342 and 326 arose due to the loss of methyl and methoxy groups from the \( M^+ \),
respectively. The presence of an $N, N$-dimethylaminoethane substituent at C-17 was deduced from the characteristic ion at $m/z$ 72 (Budzikiewicz, 1964). However, the lower abundance of this ion was due to the presence of a double bond between C-16 and C-17 (Yu et al., 1997). This inference was also supported by an olefinic proton signal at $\delta$ 5.53 in the $^1$H-NMR spectrum, and a pair of olefinic carbons resonated at $\delta$ 154.6 and 125.7 in the $^{13}$C-NMR spectrum.

Scheme 2.5.3: Mass fragmentation of salonicne-B (74).

The $^1$H-NMR spectrum of compound 74 showed two singlets at $\delta$ 0.85 and 1.01, corresponding to the C-18 and C-19 angular methyls, respectively. Two additional 3H
singlets at δ 2.20 and 3.33 were due to the N(CH₃)₂ and methoxy protons, respectively. A 3H doublet at δ 1.09 (J₁₂₃ = 6.6 Hz) was due to CH₃-21 secondary methyl protons. A downfield multiplet at δ 3.04 (W₁/₂ = 17.7 Hz) was attributed to C-3 methine proton geminal to OCH₃ group. The coupling pattern of H-3 signal supported a β configuration (equitorial) of geminal OCH₃. Two mutually uncoupled olefinic signals at δ 5.33 (br s), and 5.53 (m) indicated the presence of at least two double bonds in the molecule. The ¹³C-NMR spectra (broad-band decoupled, DEPT) of 74 displayed resonances for 24 carbons, with six methyl, seven methylene, seven methine and four quaternary carbons. Downfield signals at δ 121.3 (CH) and 125.7 (CH) further indicated the presence of two trisubstituted double bonds, while a signal at δ 80.3 indicated a methine carbon bearing an OCH₃ group.

Compound 74 consists of three different spin systems i.e. a, b and c, which were deduced on the basis of COSY-45° and HMQC spectra and joined together on the basis of HMBC technique.

The spin system a was deduced with the help of COSY-45° and HMQC interactions. The methylene proton of C-1 (δ 1.90) showed interaction with C-2 methylene protons (δ 1.80), which in turn showed couplings with C-3 methine proton (δ 3.04) in COSY-45° spectrum. The presence of OCH₃ group at C-3 was also inferred from the HMBC interaction of the C-3 methine proton with methoxy carbon (δ 55.5). The HMBC spectrum also showed correlation between C-3 proton and C-4 (δ 32.5).  

![Diagram](image-url)
The isolated spin system b was deduced on the basis of 2D-NMR techniques. The interactions of olefinic H-6 (δ 5.33) with C-5 (δ 141.0), C-8 (δ 32.3) and C-10 (δ 35.5) in HMBC spectrum were observed. The C-8 methine proton (δ 1.48) showed homonuclear coupling with the C-9 methine proton (δ 1.35) and C-14 methine proton (δ 1.75). The C-9 methine proton also showed COSY–45° interactions with one of the C-11 methylene protons (δ 1.99), which in turn were coupled with the C-12 methylene proton (δ 1.53). The C-12 protons also showed HMBC interactions with C-13 (δ 47.5). The C-18 methyl protons (δH 0.85) showed interactions with quaternary C-13 (δC 47.5) in the HMBC spectrum. These observations led to the identification of rings B and C of the molecule.

The spin system c was deduced on the basis of mass fragmentation pattern and 2D-NMR techniques. The double bond in ring D was placed between C-16/C-17 on the basis of mass fragmentation pattern which showed the base peak at m/z 342, which can arose due to the loss of a methyl. The lower abundance of ion at m/z 72 indicated the presence of a double bond between C-16/C-17. The C-20 methine proton (δH 2.81) showed HMBC interactions with the C-17 olefinic carbon (δC 154.6). Moreover, C-14 (δ 57.5) exhibited couplings with the C-15 methylene protons (δH 1.60), which were in turn coupled with the C-16 olefinic proton (δ 5.53). The C-20 methine proton (δ 2.81) showed vicinal coupling with the C-21 methyl protons (δ 1.09).
These spin systems were joined together with the help of HMBC and HOHAHA experiments. The fragments a and b were joined together through C-4/C-5, as the C-4 proton showed coupling with the C-5 olefinic carbon (δc 141.0). The C-6 olefinic proton (δh 5.33) also displayed HMBC correlations with quaternary C-5 (δc 141.0) and C-10 (δc 35.5). Similarly, the C-1 methylene protons (δ 1.90) were also coupled with the C-10 quaternary carbon in HMBC spectrum (Fig. 2.5.3).

**Fig. 2.5.3: Key HMBC interactions in salamine-B (74).**

Furthermore fragment c was connected with fragment b on the basis of HMBC interactions of the C-16 methylene protons (δh 5.53) with C-13 (δc 47.5) and C-14 (δc 57.5).

The stereochemistry at different chiral centers in compound 74 was assigned on the basis of biogenetic consideration and NMR data. The β-orientations of (axial) H-8, CH3-18 and CH3-19 in compound 74 were inferred from the ROESY interactions of H-8.
Table 2.5.2: $^1$H- (400 MHz) and $^{13}$C-NMR (100 MHz) Data of Salomine-B (74) in CDCl₃.

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>$^1$H-NMR (δ, J = Hz)</th>
<th>$^{13}$C-NMR (δ)</th>
<th>Multiplicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.90, 1.35 m</td>
<td>34.0</td>
<td>CH₂</td>
</tr>
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<td>2</td>
<td>1.80, 1.75 m</td>
<td>29.6</td>
<td>CH₂</td>
</tr>
<tr>
<td>3</td>
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<td>80.3</td>
<td>CH</td>
</tr>
<tr>
<td>4</td>
<td>2.14, 2.39 m</td>
<td>32.5</td>
<td>CH₂</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>141.0</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
<td>5.33 br s</td>
<td>121.3</td>
<td>CH</td>
</tr>
<tr>
<td>7</td>
<td>1.5, 2.30 m</td>
<td>33.3</td>
<td>CH₂</td>
</tr>
<tr>
<td>8</td>
<td>1.48 m</td>
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<tr>
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<td>1.7, 1.99 m</td>
<td>26.9</td>
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<tr>
<td>12</td>
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<td>31.7</td>
<td>CH₂</td>
</tr>
<tr>
<td>13</td>
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<td>C</td>
</tr>
<tr>
<td>14</td>
<td>1.75 m</td>
<td>57.5</td>
<td>CH</td>
</tr>
<tr>
<td>15</td>
<td>2.0, 1.60 m</td>
<td>31.2</td>
<td>CH₂</td>
</tr>
<tr>
<td>16</td>
<td>5.53 m</td>
<td>125.7</td>
<td>CH</td>
</tr>
<tr>
<td>17</td>
<td>--</td>
<td>154.6</td>
<td>C</td>
</tr>
<tr>
<td>18</td>
<td>0.85 s</td>
<td>18.3</td>
<td>CH₃</td>
</tr>
<tr>
<td>19</td>
<td>1.01 s</td>
<td>19.2</td>
<td>CH₃</td>
</tr>
<tr>
<td>20</td>
<td>2.81 q ($J_{19,21} = 6.2$)</td>
<td>59.4</td>
<td>CH</td>
</tr>
<tr>
<td>21</td>
<td>1.09 d ($J_{19,21} = 6.6$)</td>
<td>15.9</td>
<td>CH₃</td>
</tr>
<tr>
<td>$\text{Me (CH₃)}$₂</td>
<td>2.20 s</td>
<td>42.3</td>
<td>CH₃</td>
</tr>
<tr>
<td>$\text{-OCH₃}$</td>
<td>3.33 s</td>
<td>55.5</td>
<td>CH₃</td>
</tr>
</tbody>
</table>
(δ 1.48) with CH₃-18 (δ 0.85) and CH₃-19 (δ 1.01). While the H-18 methyl protons (δ 0.85) also showed dipolar coupling with H-20 (δ 2.81) (Fig. 2.5.4). The equatorial orientation of the methoxy group at C-3 was deduced on the basis of \(W'_{1/2}\) value of geminal H-3 signal (\(W'_{1/2} = 17.7\) Hz).

![Key ROESY interactions in salonine-B (74).](image)

From the aforementioned data, structure of the salonine-B (74) was deduced to be (20S)-20-(N,N-dimethylamino)-3β-methoxy-pregn-5,16-diene.

**Biogenesis of Salonine-B (74)**

The proposed biogenesis of compound 74 is presented in Scheme-2.5.4. O-methylation at C-3 in pregnenolone (50), followed by the reductive amination at C-20 and the subsequent methylation could yield the steroidal base 75. Enzymatic oxidation at C-17 can be followed by dehydration can afford salonine-B (74).
Scheme 2.5.4: Proposed biogenesis of saloline-B (74).
2.5.1.3 Salonine-C (77)

Salonine-C (77) was purified as a colourless amorphous solid from the subfractioin C-5-5 (Experimental Section, Scheme-5.4, page 157). Thus sub-fraction was subjected to column chromatography and eluted with the solvent system pet. ether: acetone along with a few drops of diethylamine. Compound 77 was finally purified as a colourless solid by preparative TLC by using the same solvent system.

The El-MS of compound 77 exhibited the M⁺ at m/z 424, which was further confirmed by FD-MS, while the HREI-MS displayed the M⁺ at m/z 424.3489 corresponding to the formula C₂₃H₄₄N₂O (calcd. 424.3508) with eight degrees of unsaturation. The mass fragments at m/z 72 and 83 were characteristic of C-20 dimethylamino and C-3 tigloyl groups. The UV spectrum exhibited an absorption at 206 nm. The IR spectrum showed absorptions at 3650 (NH), 3391 (CH), 2810 and 1601 (C=O) cm⁻¹. The specific rotation -120° (c = 0.048, MeOH) showed the chiral nature of the compound 77. The ¹H-NMR spectrum of compound 77 showed three angular methyl singlets at δ 0.86, 1.06 and 1.78 for C-18, C-19 and C-5' methyis, respectively. A doublet
of secondary methyl protons resonated at $\delta$ 1.59 ($J_{2\alpha,2\beta} = 6.5$ Hz) ascribed to CH$_3$-21. A downfield 6H singlet at $\delta$ 2.91 was assigned to the NMe$_2$ group.

**Scheme-2.5.5:** Mass fragmentation of salonine-C (77).

The presence of a tigloyl moiety was inferred from the appearance of a 3H singlet at $\delta$ 1.78, a 3H doublet at $\delta$ 1.72 ($J_{F,3} = 6.9$ Hz) and a 1H quartet at $\delta$ 6.34 ($J_{3\alpha,4\beta} = 6.9$ Hz) assigned C-5', C-4' and C-3' protons of the tigloyl group, respectively. This was further confirmed by the HMBC and COSY-45° interactions. Two 2H downfield olefinic singlets at $\delta$ 5.41 and 5.78 indicated the presence of two tri-substituted olefinic moieties in the molecule. The $^{13}$C-NMR spectra (broad-band decoupled, DEPT) displayed resonances for 28 carbons, with seven methyl, seven methylene, eight methine and six quaternary carbons.

Spectroscopic analysis of compound 77 revealed the presence of four distinct spin
systems a-d, which were joined together with the help of $^1$H-, $^{13}$C- and 2D-NMR techniques.

Spin system a consists of a N-tigloyl group that was inferred on the basis of vicinal coupling of the C-3’ olefinic proton (δ 6.34) with the C-4’ methyl protons (δ 1.72). The later methyl protons showed a homoallylic coupling with the C-5’ methyl protons (δ 1.78). These observations led to the deduction of spin system a.

Another isolated spin system b was deduced from the COSY-45° interactions of the C-2 methine proton (δ 1.94) with one of the C-1 methylene protons (δ 1.69), as well as with the C-3 methine proton (δ 4.15). The C-3 methine proton also showed connectivity with the C-4 olefinic proton (δ 5.78). Fragment b was thus deduced.

The largest spin system c was constructed similarly with the help of COSY-45° spectrum. The vicinal coupling of C-6 methylene protons (δ 2.15) with C-7 methylene protons (δ 1.88), which in turn was coupled with the C-8 methine proton (δ 1.55). The C-8 methine proton showed COSY 45° connectivity with the C-9 methine proton (δ 0.88), whereas the C-9 methine proton showed interaction with one of the C-11 methylene protons (δ 1.39). The C-11 methylene protons exhibited coupling with one of the C-12 methylene protons (δ 1.35). These spectral observations led to the deduction of fragment c.
The determination of spin system \( d \) was based on the COSY-45° interactions of C-15 methine proton (\( \delta \ 5.41 \)) with C-16 methylene protons (\( \delta \ 2.06 \)), which was in turn coupled with the C-17 methine proton (\( \delta \ 1.10 \)). Moreover, the C-17 methine proton was also coupled with the C-20 methine proton (\( \delta \ 2.81 \)). Finally, the C-20 methine proton exhibited a cross peaks with the C-21 methyl protons (\( \delta \ 1.59 \)). These COSY-45° interactions led to fragment \( d \).

After the deduction of all spin systems separately, they were joined together with the help of HMBC and HOHAHA experiments. The H-3 methine proton (\( \delta \ 4.15 \)) of fragment \( b \) showed cross peak with the C-1' (\( \delta \ 168.3 \)) of the fragment \( a \) in the HMBC spectrum (Fig. 2.5.5). This observation helped to join the side chain with C-3 of ring A of the steroidal skeleton. One of the C-6 methylene protons (\( \delta_H \ 2.15 \)) of fragment \( c \) showed HMBC interaction with the quaternary C-5 (\( \delta_C \ 149.4 \)) in fragment \( b \). This quaternary carbon showed coupling with the C-19 methyl protons (\( \delta_H \ 1.06 \)). Hence, the presence of a double bond between the C-4 and C-5 in ring A of the steroidal skeleton was inferred. This was further supported by the coupling of C-3 methine carbon (\( \delta_C \ 45.5 \)) with the C-4 olefinic proton (\( \delta_H \ 5.78 \)) in the HMBC spectrum.

Fragments \( c \) and \( d \) were joined together based on the HMBC interaction between H-8 (\( \delta_H \ 1.55 \)) and the quaternary C-14 (\( \delta_C \ 154.6 \)). The presence of a second double bond, between C-14 and C-15 was also inferred from spectroscopic data. Other important HMBC correlations are shown in Fig. 2.5.5.
Table-2.5.3: $^1$H- (400 MHz) and $^{13}$C-NMR (100 MHz) Data of Salonine-C (77) in CDCl$_3$.

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>$^1$H-NMR ($\delta, J = \text{Hz}$)</th>
<th>$^{13}$C-NMR ($\delta$)</th>
<th>Multiplicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.59, 1.15 m</td>
<td>34.5</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>2</td>
<td>2.60, 1.94 m</td>
<td>37.4</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>3</td>
<td>4.15 m</td>
<td>45.5</td>
<td>CH</td>
</tr>
<tr>
<td>4</td>
<td>5.78 br s</td>
<td>126.4</td>
<td>CH</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>149.4</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
<td>1.49, 2.15 m</td>
<td>30.3</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>7</td>
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<td>1.55 m</td>
<td>33.5</td>
<td>CH</td>
</tr>
<tr>
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<td>C</td>
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<td>5.41 br s</td>
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</tr>
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<td>1.10 m</td>
<td>52.3</td>
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<td>2.81 m</td>
<td>59.5</td>
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</tr>
<tr>
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<td>1.59 d ($J_{20,21} = 6.6$)</td>
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<td>$CH_3$</td>
<td>2.91 s</td>
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</tr>
<tr>
<td>2'</td>
<td>--</td>
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</tr>
<tr>
<td>3'</td>
<td>6.34 q ($J_{3',4'} = 6.8$)</td>
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<td>CH</td>
</tr>
<tr>
<td>4'</td>
<td>1.72 d ($J_{4',5'} = 6.9$)</td>
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<td>CH$_3$</td>
</tr>
<tr>
<td>5'</td>
<td>1.78 s</td>
<td>13.9</td>
<td>CH$_3$</td>
</tr>
</tbody>
</table>
Fig. 2.5.5: Key HMBC interactions in salonine-C (77).

The β-orientations of H-8, CH₃-18 and CH₃-19 were assigned on the basis of ROESY spectrum and biogenetic considerations. The dipolar couplings of H-8 (δ 1.55), with H-18 (δ 0.86) and H-19 (δ 1.06) in the ROESY spectrum indicated their axial orientation.

Finally, on the basis of the aforementioned spectral studies (HREI-MS, UV, IR, ¹H- and ¹³C-NMR), the structure of the compound was deduced as (20S)-20-(N,N-di methylamino)-3β-(tigloylamino) pregn-4,14-diene.

Biogenesis of Salonine-C (77)

Salonine-C (77) may be produced in nature from the methylation of 53, which can afford the amide 80 via the esterification of base 78. Selective oxidation of 80 at C-5 and C-14 may give rise to compound 81 which on dehydration can yield compound 77.
Scheme 2.5.6: Proposed biogenesis of saloline-C (77).
2.5.1.4 2,3-Dehydrosarsalagine (82)

A fraction C-5 (2.1 g) was obtained after repeated column chromatography of chloroform extract C on silica gel by using the increasing polarities of pet. ether: acetone: diethylamine (Experimental Section, Scheme-5.4, page 157). The fraction C-5 was subjected to further column chromatography on flash silica gel which yielded a semi-impure alkaloidal mixture which was finally purified on preparative TLC plates, using pet. ether: acetone: diethylamine as a solvent system, affording two white amorphous compounds, 2,3-dehydrosarsalagine (82) and salonine-C (77). Both compounds showed positive Dragendorff's test, which indicated their alkaloidal nature.

![Diagram of 2,3-Dehydrosarsalagine (82)](image)

The EI-MS spectrum of compound 82 showed the molecular ion at $m/z$ 438, which was also confirmed by FD-MS. The HREI-MS showed the $M^+$ at $m/z$ 438.3284 corresponding to the formula $C_{26}H_{42}N_2O_2$ (caled. 438.3246). The fragments at $m/z$ 72 and 83 indicated the presence of an $N,N$-dimethylamino substituent at C-20, and a tigloyl group at C-3, respectively.
The optical rotation of compound 82 was found to be $-38^\circ$ ($c = 0.048$, MeOH), which indicated the presence of chirality in the molecule. The UV spectrum exhibited an absorption at 234 nm. Its IR spectrum showed absorptions at 3390 (NH), 2929 ($\equiv$CH), 1723 ($\alpha,\beta$-unsaturated ketonic group), 1664 (C=O) and 1511 (C=C) cm$^{-1}$. The compound 82 was found to be distinctly similar to the known alkaloid, sarcovaginine-A, previously isolated from the Sarcococca vagans (Yu et al., 1997).

Scheme-2.5.7: Mass fragmentation of 2,3-dehydrosaragine (82).

The $^1$H-NMR spectrum of 2,3-dehydrosaragine (82) showed three tertiary methyl singlets at $\delta$ 0.83, 0.89 and 1.86, assigned to the C-18, C-19 and C-5', respectively. Two 3H doublets of secondary methyl groups resonated at $\delta$ 1.54 ($J_{21,20} = 6.5$ Hz) and 1.75 ($J_{4',5} = 6.9$ Hz) due to C-21 and C-4' methyls, respectively. A 6H singlet resonating at $\delta$ 2.83 was assigned to the $\text{N}(\text{CH}_3)_2$ group. The downfield signals resonating
at δ 6.45 and 7.65 were assigned to olefinic H-2 and H-6, respectively, conjugated with the unsaturated amide and α,β-unsaturated ketonic carbonyl groups, respectively. The appearance of an NH proton as a singlet at δ 8.15 indicated the presence of a double bond at vicinal C-3 (Yu et al., 1997). The \(^1\)H-NMR spectrum showed characteristic resonances for a tigloyl group i.e. δ 6.45 (q, 1H, \(J_{5',4'} = 6.8\) Hz), 1.75 (d, 3H, \(J_{4',3'} = 6.9\) Hz) and 1.86 (s, 3H), corresponding to the H-3', H-4' and H-5', respectively. The \(^{13}\)C-NMR spectra (broad-band decoupled, DEPT) of compound 82 displayed resonances for 28 carbons, with seven methyl, six methylene, eight methine and seven quaternary carbons.

Three partial fragments a–c (sub-structures) in compound 82 were deduced by 2D-NMR spectroscopic techniques. The partial structure a comprises a tigloyl group, which was inferred from the mass fragment m/z 83, and from the vicinal coupling of olefinic H-3' (δ 6.45) with C-4' methyl protons (δ 1.75). The C-4' protons were also found to be coupled with the C-5' methyl protons (δ 1.86) in the COSY-45° spectrum.

Partial structure b was deduced in the same way on the basis of COSY-45° spectrum, which showed cross-peaks between C-1 methylene protons (δ 2.51) and a downfield olefinic H-2 (δ 7.65). The latter olefinic proton also showed HMBC interaction with quaternary carbon C-3 (δ 132.2).

The large partial structure c that comprised rings A, B, C and D of steroidal skeleton was similarly deduced on the basis of COSY-45° and HMBC spectra. The
vicinal interaction of the C-6 olefinic proton (δ 6.43) with the C-7 methylene protons (δ 2.23) was also observed. The C-8 methine proton (δ 1.45) showed interaction with the C-7 methylene protons and with the C-9 methine proton (δ 1.08), which in turn showed coupling with one of the C-11 methylene protons (δ 1.99). Moreover, the C-8 methylene protons also showed an HMBC interaction with the methine C-14 (δC 55.4). The C-11 methylene protons showed vicinal couplings with one of the C-12 methylene protons (δ 1.53). The placement of a double bond between C-5 and C-6 was made from the HMBC interaction of H-6 olefinic proton (δH 6.43) with the quaternary C-10 (δC 41.9) as well as from the interaction of C-19 methyl protons (δH 0.89) with the quaternary C-5 (δC 149.9).

The isolated spin system e, which comprised ring D, was also deduced on the basis of COSY-45° spectrum. The H-15 proton (δ 1.30) showed couplings with C-16 methylene protons (δ 1.61, 1.20), which were coupled with the C-17 methine proton (δ 1.38). The C-20 methine proton (δ 2.93) showed COSY-45° interaction with C-21 methyl protons (δ 1.54) and C-17 methine proton.

After the deduction of three fragments (a-e), they were joined together on the basis of HMBC (Fig. 2.5.6) and HOHAHA interactions.

The HMBC interactions of NH proton (δH 8.15) with quaternary C-4 (δC 196.4) and C-2 (δC 125.7) in HMBC spectrum (Fig. 2.5.6) indicated the substitution of a tigloyl amine moiety at C-3 of ring A.
Fig. 2.5.6: Key HMBC interactions in 2,3-dehydrosarsalignone (82).

The *E*-stereochemistry of the 2-methyl-2-buten moiety at C-3 was inferred from the interaction of the C-4′ methyl protons (δ 1.75) with the C-5′ methyl group (δ 1.86) in the ROESY spectrum (Fig. 2.5.7).

Fig. 2.5.7: Key ROESY interactions in 2,3-dehydrosarsalignone (82).
Table 2.5.4: $^1$H- (400 MHz) and $^{13}$C-NMR Data (100 MHz) of 2,3-Dehydrosalsalignone (82) in CDCl$_3$.

<table>
<thead>
<tr>
<th>Carbon No.</th>
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<th>$^{13}$C-NMR ($\delta$)</th>
<th>Multiplicity</th>
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</tr>
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<td>7.65 dd ($J_{1,2} = 6.8, 2.6$ Hz)</td>
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<td>6.43 br s</td>
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</tr>
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Results and Discussion

The axial orientations of CH$_3$-18, H-8 and CH$_3$-19 was deduced on the basis of ROESY interactions of H-8 ($\delta$ 1.45) with CH$_3$-19 ($\delta$ 0.89), while CH$_3$-18 methyl protons ($\delta$ 0.83) showed dipolar coupling with H-20 ($\delta$ 2.93).

From the aforementioned data, the structure of 2,3-dehydrosarsalignone (82) was deduced as (20S)-20-($N,N$-dimethylamino)-3$\beta$-(tigloylamino)-5-pregn-2,5-diene-4-one.

**Biogenesis of 2,3-Dehydrosarsalignone (82)**

Compound 82 may arise in nature from the $N$-methylation of alkamine 53, affording base 83, which on esterification with tigloyl acid 84 can yield amide 85. Subsequent selective oxidation and dehydration 85 can afford compound 82.

![Scheme 2.5.8: Proposed biogenesis of 2,3-dehydrosarsalignone (82).](image-url)
2.5.1.5 \textit{N}-Methylformamidesaloline-B (87)

The subfraction C-1, obtained from alkaloidal fraction C (Experimental Section, Scheme-5.3, page 154), was subjected to column chromatography (silica gel) by using the solvent gradients of pet. ether: acetone: diethylamine as eluent, yielding compound 87 as a yellowish gum, which gives an orange colored with Dragendorff's reagent spray, indicating its alkaloidal nature.

![N-Methylformamidesaloline-B (87)](image)

The specific rotation of compound 87 was found to be $-21^\circ$ ($c = 0.075$, MeOH), which indicated the presence of chirality in the molecule. Its IR spectrum showed absorptions at 3374 (CH), 2928 (C=C) and 1627 (C=O) cm$^{-1}$. The UV spectrum of compound 87 exhibited only terminal absorptions.

The EI-MS of compound 87 showed the M$^+$ at m/z 371, which was further confirmed by FD-MS and FAB $^{+ve}$ (m/z 372) mass spectrometric techniques. The HREI-MS showed the M$^+$ at m/z 371.2980, corresponding to the formula C$_{24}$H$_{37}$NO$_2$ (caled. 371.2982). The base peak at m/z 356 was due to the loss of a methyl group, while the ion at m/z 340 arose due to the loss of a methoxy group from the M$^+$. The presence of an \textit{N}-
methylformamide moiety at C-17 was deduced from the characteristic fragment at m/z 86. However, the more abundant ion m/z 356 was attributed to the presence of a double bond between C-16 and C-17 (Yu et al., 1997). This inference was also supported by the HMBC interactions between the C-16 olefinic proton (δ 5.78) and olefinic carbons at δ 153.5 and 125.8.

Scheme-2.5.9: Mass fragmentation of N-methylformamidesalonine-B (87).

The ¹H-NMR spectrum of compound 87 exhibited two 3H singlets at δ 0.75 and 1.01 for the C-18 and C-19 methyl protons, respectively, along with a 3H doublet resonating at δ 1.31 (J₂₁,₂₀ = 6.5 Hz) for C-21 secondary methyl protons. Two 1H downfield signals at δ 5.34 and 5.78 were assigned to olefinic H-6 and H-16, respectively. Two downfield 1H multiplets at δ 3.03 and 4.19 were assigned to methine
protons geminal to the $OCH_3$ and $N$-methyiformamide groups, respectively. A 3H split singlet at $\delta$ 2.64/2.72 was due to an $N$-methyl group, while a 1H split singlet was due to an aldehyde proton at $\delta$ 8.02/8.19. The splitting of peaks was attributed to the rotamerism that exists in $N$-methyiformamide group at C-20. The presence of a methoxy group was inferred from a 3H singlet which resonated at $\delta$ 3.33. The $^{13}$C-NMR spectra (broad-band decoupled, DEPT) displayed resonances for 24 carbons, with five methyl, seven methylene, eight methine and (by difference from the broad-band decoupled spectrum) four quaternary carbons. The $\beta$-orientation of an $OCH_3$ group at C-3 was inferred from the coupling constants of the multiplet which resonated at $\delta$ 3.03 ($W_{1/2}$ = 17.7 Hz).

The two partial structures a and b of compound 87 were deduced on the basis of $^1$H-NMR, $^{13}$C-NMR, HMQC and COSY-45° spectra. These two partial structures were joined together with the help of HMBC and HOHAHA NMR spectroscopic techniques.

The partial structure a was determined from the vicinal couplings of the C-1 methylene protons ($\delta$ 1.90) with the C-2 methylene protons ($\delta$ 1.75), which in turn were coupled with the C-3 methine proton ($\delta$ 3.03). The presence of a methoxy group at C-3 was inferred from the HMBC interaction of the C-3 methine proton with a methoxy carbon ($\delta$ 55.5). The C-3 methine proton also showed vicinal coupling with one of the C-4 methylene protons ($\delta$ 2.39) in the COSY-45° spectrum.

The large partial structure b was deduced on the basis of COSY-45° and HMQC spectra. The olefinic H-6 ($\delta$ 5.34) showed vicinal coupling with one of the C-7 methylene
protons (δ 2.30), which in turn showed interaction with the C-8 methine proton (δ 1.48). The C-8 methine proton also showed vicinal coupling with the C-9 methine proton (δ 1.35). Moreover, the interaction of one of the C-11 methylene protons (δ 1.99) with C-9 and C-12 methylene protons (δ 1.53) was observed in the COSY-45° spectrum. The C-12 methylene protons were coupled with C-13 (δ 47.5) in the HMBC spectrum. This sub-structure thus comprised on rings B and C. The ring D of compound 87 was also deduced by COSY-45°, HMQC and mass spectra. The vicinal coupling of the C-14 methine proton (δ 1.75) with one of the C-15 methylene protons (δ 1.60) was appeared in COSY-45° spectrum. The presence of a double bond between C-16 and C-17 was inferred from the appearance of the base peak at m/z 356 [M-15] (Yu, et al., 1997). The C-16 olefinic proton (δH 5.78) showed HMBC interaction with C-15 (δ 31.2), while the olefinic C-17 (δC 153.4) showed interaction with the C-18 methyl protons (δ 0.75). The C-20 methine proton (δ 4.19) showed interaction with the C-21 methyl protons (δ 1.31). The N-methyl group, resonating at δ 2.64/2.72, showed HMBC interaction with the carbonyl carbon of the amide group (δ 169.6). The aldehydic proton singlet appeared at δ 8.02/8.19. The doubling of these signals can be ascribed on the basis of rotamerism that exists in the N-methylformamide group.

These two structural fragments were joined together with the help of HMBC connectivities. The fragment a was joined with fragment b through C-4/C-5 and C-1/C-10 on the basis of HMBC interactions of one of the C-4 methylene protons (δH 2.39) with
the quaternary C-5 (\(\delta_C 141.1\)), which in turn was coupled with C-6 olefinic proton (\(\delta_H 5.34\)).

![Chemical structure diagram](image)

**Fig. 2.5.8: Key HMBC interactions in \(N\)-methylformamidesalonine-B (87).**

The presence of a double bond between C-5 and C-6 was also inferred from the HMBC interaction of olefinic H-6 (\(\delta_H 5.34\)) with C-10 (\(\delta_C 35.5\)) and quaternary C-5 (\(\delta_C 141.1\)). The C-1 methylene protons (\(\delta_H 1.90\)) also showed couplings with the C-10 quaternary carbon in HMBC spectrum.

The stereochemistry of the methoxy group at C-3 in compound 87 was inferred from the coupling constant of H-3 signal and its cross-peaks in ROESY spectrum (Fig. 2.5.9). The C-3 proton resonating at \(\delta 3.03\) showed \(W_{1/2} = 17.7\) Hz indicating a pseudo axial orientation. The ROESY spectrum of compound 87 showed the pseudo axial-pseudo axial interaction of the C-3\(\alpha\) methine proton with the C-2\(\beta\) methylene protons, pseudo equatorial-pseudo axial interaction with the C-2\(\alpha\) methine proton and pseudo equatorial pseudo axial interaction with the axially oriented C-4\(\alpha\) methine proton.
Table 2.5.5: $^1$H- (400 MHz) and $^{13}$C-NMR (100 MHz) Data of N-Methylformamide-saline-B (87) in CDCl$_3$.

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<th>Carbon No.</th>
<th>$^1$H-NMR ($\delta, J$ = Hz)</th>
<th>$^{13}$C-NMR ($\delta$)</th>
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<td>35.5</td>
<td>C</td>
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<td>1.7, 1.99 m</td>
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Finally on the basis of the aforementioned spectral studies, the structure of compound 87 was deduced as (20S)-20-(N-methylformamide)-3β-methoxy-pregn-5,16-diene.

Biogenesis of \(N\)-Methylformamidesalolone-B (87)

The proposed biogenesis of 87, (Scheme-2.5.10), may involve the O-methylation of pregnenolone (50), followed by a reductive amination at C-20, and then the subsequent methylation which can afford the base 88, which on selective formylation and hydroxylation at C-17, can finally yield compound 87 after dehydration.

Scheme-2.5.10: Proposed biogenesis of \(N\)-methylformamidesalolone-B (87).
2.5.1.6 Salignarine-F (90)

Salignarine-F (90) was obtained as yellowish gum from fraction C-6 after repeated column chromatography on silica gel (Experimental Section, Scheme-5.4, page 157). The El-MS spectrum of 90 showed the molecular ion at m/z 442. The HREl-MS showed the M⁺ at m/z 442.3577 (C₂₈H₄₆N₂O₂, calcd. 442.3559). The mass fragments at m/z 72 and 84 indicated the presence of N,N-dimethyl and tigloyl groups at C-20 and C-3, respectively. The fragments at m/z 427 and 425 were due to the loss of methyl and hydroxyl groups, respectively, from the M⁺.

The specific rotation of compound 90 was found to be -71° (c = 0.014, MeOH), which indicated the presence of a chiral center in the molecule. The UV spectrum exhibited absorption at 203 nm. Its IR spectrum showed absorptions at 3650 (NH), 3328 (OH), 2810 (=CH), 1601 (C=O) and 1450 (C=CH) cm⁻¹.

The ¹H-NMR spectrum of compound 90 indicated the presence of three methyl singlets at δ 0.82, δ 1.04, and δ 1.83 due to C-18, C-19 and C-5' methyls, respectively, along with a 3H doublet for C-21 methyl at δ 1.38 (J₁₂₃₄ = 6.5 Hz). The N,N-dimethyl
protons resonated at $\delta\ 2.23$ as a singlet. Two $^{1}$H broad singlets resonating at $\delta\ 3.95$ and 3.99 were ascribed to H-3 (geminal to amidic group) and H-4 (geminal to $-\text{OH}$ group), respectively. A $^{1}$H olefinic signal resonated at $\delta\ 5.78$. A rather downfield $^{1}$H multiplet resonating at $\delta\ 2.80$ was due to the C-20 methine proton. The $^{13}$C-NMR spectra (broadband decoupled, DEPT) displayed resonances for 28 carbons, with seven methyl, seven methylene, nine methine and five quaternary carbons.

**Scheme-2.5.11: Mass fragmentation of salignarine-F (90).**

The compound 90 possesses three different spin systems a, b and c, which were deduced on the basis of 2D-NMR spectroscopic techniques.

The deduction of fragment a started from the vicinal interaction of downfield C-3' olefinic proton ($\delta\ 6.34$) with the C-4' methyl protons ($\delta\ 1.73$) in the COSY-45° spectrum. The one-bond heteronuclear interactions were observed between
protons and their attached carbons including C-3' (δ 130.8), H-4'' (δ 6.34) and C-5' (δ 13.1), CH₃-5' (δ 1.83) in HMQC spectrum. These studies led to the identification of a tigloyl group in compound 90.

The other spin system b, comprising ring A of the steroidal skeleton, was deduced with the help of vicinal coupling between the C-1 methylene protons (δ 1.85) with the C-2 methylene proton (δ 1.61), which was in turn coupled with the C-3 methine proton (δ 3.95). The C-4 methine proton (δ 3.99) geminal to the OH group, showed vicinal coupling with the C-3 methine proton.

The COSY-45° correlations were also used to deduce the larger spin system c of compound 90. The interactions in spin system "c" started from the interaction of the olefinic H-6 (δ 5.78) with one of the methylene protons of C-7 (δ 2.51), which was in turn coupled with the C-8 methine. Moreover, the C-11 methylene protons also showed interaction with the C-12 methylene protons (δ 1.20). One of the C-15 methylene protons (δ 1.92) showed vicinal coupling with the C-14 methine proton (δ 1.32) and C-16 methylene protons (δ 1.63).

The latter showed interaction with the C-17 methine proton (δ 1.22).

After the deduction of the three isolated spin systems, a-c, they were joined together on the basis of HMBC and HOHAHA spectra.
The fragment a was linked with fragment b at C-3 position of ring A based on the HMBC interactions of the C-3 methine proton (δ_H 3.95) with quaternary C-1' (δ_C 168.3) (Fig. 2.5.10).

![Diagram of Sarcococca saligna](image)

**Fig-2.5.10: Key HMBC interactions in salignarine-F (90).**

Further connection with the large fragment c was supported by the HMBC interaction of the C-4 methine proton (δ_H 3.99) with quaternary C-5 (δ_C 140.5), which also showed interaction with olefinic H-6 (δ_H 5.78).

These interactions also supported the presence of a hydroxyl group at C-4 and a double bond between C-5 and C-6. Another important HMBC interaction of olefinic H-6 (δ_H 5.78) was observed with C-10 (δ_C 39.5), which in turn displayed interaction with the C-19 methyl protons (δ_H 1.04). These spectroscopic studies helped in linking fragments a, b and c together, leading to the complete structure of salignarine-F (90).
Table 2.5.6: $^1$H- (400 MHz) and $^{13}$C-NMR (100 MHz) Data of Salignarine-F (90) in CDCl₃.

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<th>$^{13}$C-NMR ($\delta$)</th>
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<td>CH₂</td>
</tr>
<tr>
<td>13</td>
<td>--</td>
<td>45.6</td>
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<td>14</td>
<td>1.32 m</td>
<td>55.5</td>
<td>CH</td>
</tr>
<tr>
<td>15</td>
<td>1.70, 1.92 m</td>
<td>34.4</td>
<td>CH₂</td>
</tr>
<tr>
<td>16</td>
<td>1.63, 1.73 m</td>
<td>32.7</td>
<td>CH₂</td>
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<tr>
<td>17</td>
<td>1.22 m</td>
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<td>CH</td>
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<tr>
<td>18</td>
<td>0.82 s</td>
<td>15.8</td>
<td>CH₂</td>
</tr>
<tr>
<td>19</td>
<td>1.04 s</td>
<td>18.7</td>
<td>CH₂</td>
</tr>
<tr>
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<td>2.80 m</td>
<td>37.9</td>
<td>CH</td>
</tr>
<tr>
<td>21</td>
<td>1.38 d ($J_{21,31} = 6.5$)</td>
<td>19.3</td>
<td>CH₃</td>
</tr>
<tr>
<td>$\alpha$ (CH₃)$_2$</td>
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<td>42.5</td>
<td>CH₃</td>
</tr>
<tr>
<td>1′</td>
<td>--</td>
<td>168.3</td>
<td>C</td>
</tr>
<tr>
<td>2′</td>
<td>--</td>
<td>131.3</td>
<td>C</td>
</tr>
<tr>
<td>3′</td>
<td>6.34 q ($J_{3′,4′} = 6.9$)</td>
<td>130.8</td>
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</tr>
<tr>
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<td>1.73 d ($J_{3′,4′} = 6.8$)</td>
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</tr>
<tr>
<td>5′</td>
<td>1.83 s</td>
<td>13.1</td>
<td>CH₃</td>
</tr>
</tbody>
</table>
Biogenesis of Salignarine-F (90)

The biogenesis of salignarine-F (90) has been proposed in Scheme-2.5.12. The esterification of 91 with a tigloyl ester 92 afforded an amide 93. Oxidations of amide 93 at C-4 and C-5 positions, followed by the enzymatic dehydration at C-5/C-6, can yield salignarine-F (90).

Scheme-2.5.12: Proposed biogenesis of salignarine-F (90).
2.5.1.7 5,14-Dehydro-$N_\alpha$-demethylsaracodine (95)

Compound 95 was isolated as a yellowish gum from the sub-fraction C-2 (Experimental Section, Scheme-5.3, page 154), which was obtained from the chloroform extract (pH = 9) after repeated column chromatography using different polarities of solvent system, pet. ether: acetone: diethylamine. The compound 95 gave an orange colored test with Dragendorff’s spray, indicating its alkaloidal nature.

The HREI-MS showed the $M^+$ at $m/z$ 384.3158 corresponding to the formula $C_{25}H_{44}N_2O$ (clad. 384.3140), having six degrees of unsaturation. The mass fragments at $m/z$ 100 and 70 indicated the presence of $N_\alpha$-methylacetamide and $N_\alpha$-methyl at C-20 and C-3, respectively (Budzikiewicz, 1964).

The specific rotation of compound 95 was found to be -23° ($c = 0.23$, MeOH). Its IR spectrum showed absorptions at 3407 (NH), 2934 (=CH) and 1632 (C=O) cm$^{-1}$. The UV spectrum of compound 95 exhibited absorptions at 213 nm.
Scheme-2.5.13: Mass fragmentation of 3,14-dehydro-N\textsubscript{\alpha}-demethylsaracodine (95).

The \textsuperscript{1}H-NMR spectrum (CDCl\textsubscript{3}, 400 MHz) of 95 indicated 3H singlets at \(\delta\) 0.69/0.75 and \(\delta\) 0.99/1.01 for C-18 and C-19 methyls, respectively, along with a 3H doublet for the C-21 methyl protons resonating at \(\delta\) 1.12/1.24 \((J_{21,20} = 6.5\ \text{Hz})\). Two 3H split singlets appeared at \(\delta\) 2.64/2.67 and 2.02/2.14 for the \(N_{\beta}\)- and acetyl methyls, respectively. The doubling of peaks was because of the rotamerism existed in \(N_{\beta}\)-acetamide group. A 3H singlet resonating at \(\delta\) 2.32 corresponding to the \(N_{\alpha}\)-methyl group. Two 1H downfield olefinic signals resonated at \(\delta\) 5.42 and 5.63. A rather downfield 1H multiplet resonating at \(\delta\) 2.78 indicated the NH-bearing methine proton, and multiplets at \(\delta\) 4.35/4.37 were ascribed to the C-20 methine proton. The \textsuperscript{13}C-NMR spectra (broad-band decoupled, DEPT) displayed resonances for 25 carbons, with six methyl, seven methylene, seven methine and five quaternary carbons.

The spectral data of compound 95 was distinctly similar to the one reported for
compound \textit{N\textsubscript{a}}-demethylisaracodine (Atta-ur-Rahman \textit{et al.}, 1998). A notable difference between two compounds included the two downfield olefinic signals at \(\delta \) 5.63 and 5.42 assigned to the H-6 and H-15, respectively, in the \(\textsuperscript{1}H\)-NMR spectrum of compound 95.

The \(\textsuperscript{13}C\)-NMR spectrum also showed the signals for two sets of trisubstituted carbons at \(\delta \) 127.5, 147.4, 154.6 and 125.3 assigned to C-5, C-6, C-14 and C-15, respectively.

The structure of compound 95 was established by deducing three isolated structural fragments \textit{a}, \textit{b} and \textit{c}, based on \(\textsuperscript{1}H\), \(\textsuperscript{13}C\) and 2D-NMR spectroscopic techniques.

The cross-peaks in HMBC spectrum were used to join various fragments together. The C-19 methyl protons (\(\delta\textsubscript{H} \) 0.99/1.01) displayed HMBC interactions with the quaternary C-10 (\(\delta\textsubscript{C} \) 37.5). Similarly, the C-1 methylene protons (\(\delta\textsubscript{H} \) 1.95) were also found to be coupled with the same quaternary carbon (C-10) in the HMBC spectrum (Fig. 2.5.11). HMBC interactions were also observed between C-4 methylene protons (\(\delta\textsubscript{H} \) 1.73) and quaternary C-5 (\(\delta\textsubscript{C} \) 147.4). The quaternary C-5 also showed HMBC interactions with C-19 methyl protons (\(\delta\textsubscript{H} \) 0.99/1.01). The olefinic H-6 (\(\delta\textsubscript{H} \) 5.63) was coupled with C-10.
(δc 37.5). These observations led to the construction of rings A and B of the steroidal skeleton and supported the presence of a double bond between C-5 and C-6 in ring B.

Fig. 2.5.11: Key HMBC interactions in 5,14-dehydro-\(N_\alpha\)-demethylsarcodine (95).

The information about rings C and D was inferred from the HMBC interactions of the C-18 methyl protons (δH 0.69/0.75) with quaternary C-13 (δc 46.8). A double bond between C-14 and C-15 was inferred from the HMBC interaction of quaternary carbon C-14 (δc 154.6) with the C-18 methyl protons (δH 0.69/0.75).

Based on the biogenetic considerations and ROESY spectrum (Fig. 2.5.12) (Atta-ur-Rahman et al., 1998), compound 95 was suggested to have a stereochemistry corresponding to that found in \(N_\alpha\)-demethylsarcodine, where substituents at C-3 and C-17 and the methyl groups at C-10 and C-13 possess a \(\beta\)-stereochemistry.

Based on the above data, the structure of compound 95 was deduced as 3\(\beta\)-\(N_\alpha\)-methyl-20S-\(N_\beta\)-acetyl-\(N_\delta\)-methylamino-pregn-5,14-diene.
Results and Discussion

Table 2.5.7: $^1$H- (400 MHz) and $^{13}$C-NMR (100 MHz) Data of 5,14-Dehydro-$N_d$-demethylsarcodine (95) in CDCl$_3$.

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>$^1$H-NMR (δ, J = Hz)</th>
<th>$^{13}$C-NMR (δ)</th>
<th>Multiplicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.95, 1.75 m</td>
<td>33.5</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>2</td>
<td>1.25, 1.80 m</td>
<td>28.6</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>3</td>
<td>2.78 m</td>
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<tr>
<td>4</td>
<td>1.90, 1.73 m</td>
<td>31.3</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>147.4</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
<td>5.63 br s</td>
<td>127.5</td>
<td>CH</td>
</tr>
<tr>
<td>7</td>
<td>2.12, 20.5 m</td>
<td>32.5</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>8</td>
<td>1.45 m</td>
<td>45.2</td>
<td>CH</td>
</tr>
<tr>
<td>9</td>
<td>1.35 m</td>
<td>56.5</td>
<td>CH</td>
</tr>
<tr>
<td>10</td>
<td>--</td>
<td>37.5</td>
<td>C</td>
</tr>
<tr>
<td>11</td>
<td>1.65, 1.99 m</td>
<td>20.9</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>12</td>
<td>1.53, 1.22 m</td>
<td>34.9</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>13</td>
<td>--</td>
<td>46.8</td>
<td>C</td>
</tr>
<tr>
<td>14</td>
<td>--</td>
<td>154.6</td>
<td>C</td>
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<tr>
<td>15</td>
<td>5.42 m</td>
<td>125.3</td>
<td>CH</td>
</tr>
<tr>
<td>16</td>
<td>2.55, 1.99 m</td>
<td>31.2</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>17</td>
<td>1.23 m</td>
<td>53.5</td>
<td>CH</td>
</tr>
<tr>
<td>18</td>
<td>0.69/0.75 s</td>
<td>15.8/16.0</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>19</td>
<td>0.99/1.01 s</td>
<td>18.9/19.3</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>20</td>
<td>4.35/4.37 m</td>
<td>56.5</td>
<td>CH</td>
</tr>
<tr>
<td>21</td>
<td>1.12/1.24 d (J$_{20,21}$ = 6.5)</td>
<td>16.4/17.4</td>
<td>CH$_3$</td>
</tr>
<tr>
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<td>29.7/31.8</td>
<td>CH$_3$</td>
</tr>
<tr>
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<td>21.6/22.6</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>$N_d$COCH$_3$</td>
<td>--</td>
<td>179.1</td>
<td>C</td>
</tr>
<tr>
<td>$N_a$CH$_3$</td>
<td>2.32 s</td>
<td>34.96</td>
<td>CH$_3$</td>
</tr>
</tbody>
</table>
Biogenesis of 5,14-Dehydro-$N_d$-demethylsaracodine (95)

Compound 95 may arise in nature by the enzymatic $N$-methylation of alkamine 55 to give (96), which on selective $N$-acetylation and then hydroxylation can afford the base 97. Finally the enzymatic dehydration of 97 may give rise to compound 95 (Scheme-2.5.14).
2.5.1.8 14-Dehydro-N_d-demethyIsaracodine (98)

Compound 98 was isolated as yellowish gum from a sub-fraction C-2 (Experimental Section, Scheme-5.3, page 154). It exhibited a positive test with Diagendorff's reagent, indicating an alkaloidal nature.

![14-Dehydro-N_d-demethyIsaracodine (98)](image)

The EI-MS showed the molecular ion at m/z 386, which was further confirmed by FD-MS. The HREI-MS showed the M⁺ at m/z 386.3489, corresponding to the formula C_{25}H_{42}N_{2}O (C_{25}H_{42}N_{2}O, calcd. 386.3559) with five degrees of unsaturation. The mass fragments m/z 100 and 70 indicated the presence of N_d-methylacetamide and N_d-methyl at C-20 and C-3, respectively.

The specific rotation of compound 98 was found to be -16° (c = 0.13, MeOH), while its IR spectrum showed absorptions at 3421 (NH), 2923 (=CH) and 1636 (C=O) cm⁻¹. The UV spectrum of compound 98 exhibited absorption at 212 nm.

The ¹H-NMR spectrum (CDCl₃, 400 MHz) of 98 showed two 3H singlets δ 0.85 and 1.23 for C-18 and C-19 methyls respectively, along with a 3H doublet for C-21 methyl at δ 1.51 (J_{21,20} = 6.5 Hz). Two 3H split singlets appeared at δ 2.76/2.77 a
Scheme 2.5.15: Mass fragmentation of 14-dehydro-\(N_6\)-demethylsaracodine (98).

1.97/2.19 which were due to \(N_6\)-methyl and acetyl methyls, respectively. The doubling of peaks was due to the rotamerism in \(N_6\)-acetamide group. A 3H singlet at \(\delta\) 2.82 corresponded to the \(N_6\)-methyl group. The C-16 olefinic proton resonated at \(\delta\) 5.65. A downfield 1H multiplet at \(\delta\) 2.72 was due to C-3 methine proton geminal to \(N_6\)-methyl group, while the signal at \(\delta\) 3.37/3.45 was ascribed to the C-20 methine proton. The \(^{13}\)C-NMR spectra (broad-band decoupled, DEPT) displayed resonances for 25 carbons, with six methyl, eight methylene, seven methine and by difference from broad-band decoupled spectrum, four quaternary carbons. The spectral
data for compound 98 was found to be distinctly similar to that of the reported compound, \( N_d \)-demethylsaracodine (Atta-ur-Rahman et al., 1998).

Two different spin systems \( a \) and \( b \), were established on the basis of \(^1\text{H}-\), \(^{13}\text{C}-\) and 2D-NMR spectroscopic techniques.

The HMBC spectrum was used to join these fragments together. The C-19 methyl proton singlet at \( \delta_H 1.23 \) displayed HMBC interactions with quaternary C-10 (\( \delta_C 37.5 \)). Similarly, the C-1 methylene protons (\( \delta_H 1.15 \)) were also coupled with the same quaternary carbon (C-10) in the HMBC spectrum (Fig. 2.5.13).

Another HMBC interaction was observed between the C-4 methylene protons (\( \delta_H 1.73 \)) and C-5 (\( \delta_C 54.3 \)). The C-5 also showed HMBC interactions with the C-19 methyl protons (\( \delta_H 1.23 \)). H-6 resonating at \( \delta_H 1.23 \) was found to be coupled with C-10 (\( \delta_C 37.5 \)). These observations led to the construction of rings A and B of the steroidal skeleton. The information about rings C and D came from the HMBC interactions of the C-18 methyl protons (\( \delta_H 0.85 \)) and C-17 methine proton (\( \delta_H 1.25 \)) with the quaternary carbon C-13 (\( \delta_C 47.5 \)). The placement of a double bond between C-14 and C-15 was
**Table 2.5.8**: $^1$H- (400 MHz) and $^{13}$C-NMR (100 MHz) Data of 14-Dehydro-$N_o$-demethylisarcodine (98) in CDCl$_3$.

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>$^1$H-NMR ($\delta$, $J$ = Hz)</th>
<th>$^{13}$C-NMR ($\delta$)</th>
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</tr>
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<td>1.63, 1.79 m</td>
<td>28.4</td>
<td>CH$_2$</td>
</tr>
<tr>
<td>3</td>
<td>2.72 br s</td>
<td>54.9</td>
<td>CH</td>
</tr>
<tr>
<td>4</td>
<td>1.73, 1.91 m</td>
<td>31.2</td>
<td>CH$_2$</td>
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<td>1.79</td>
<td>54.3</td>
<td>CH</td>
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</tr>
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<td>CH$_2$</td>
</tr>
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<td>1.35, 1.23 m</td>
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<td>CH$_2$</td>
</tr>
<tr>
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<td>--</td>
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<tr>
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<td>--</td>
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<td>CH</td>
</tr>
<tr>
<td>15</td>
<td>5.65 br s</td>
<td>127.9</td>
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<td>2.52, 1.65 m</td>
<td>31.4</td>
<td>CH</td>
</tr>
<tr>
<td>17</td>
<td>1.25 m</td>
<td>53.6</td>
<td>C</td>
</tr>
<tr>
<td>18</td>
<td>0.85 s</td>
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<td>1.23 s</td>
<td>18.8</td>
<td>CH$_3$</td>
</tr>
<tr>
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<td>3.37/3.45 m</td>
<td>56.9</td>
<td>CH</td>
</tr>
<tr>
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<td>1.51 d, ($J_{20,21} = 6.5$ Hz)</td>
<td>19.7</td>
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<tr>
<td>$N_o$CH$_3$</td>
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<td>29.7/30.1</td>
<td>CH$_3$</td>
</tr>
<tr>
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<td>CH$_3$</td>
</tr>
<tr>
<td>$N_o$COCH$_3$</td>
<td>--</td>
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<td>C</td>
</tr>
<tr>
<td>$N_o$CH$_3$</td>
<td>2.82 s</td>
<td>34.5</td>
<td>CH$_3$</td>
</tr>
</tbody>
</table>

83
on the HMBC interaction of the quaternary olefinic C-14 (δC 153.5) with the C-18 methyl protons (δH 1.23).

Fig. 2.5.13: Key HMBC interactions in 14-dehydro-Nα-demethylsaracodine (98).

Based on biogenetic considerations, and the ROESY spectrum (Fig. 2.5.14), it was concluded that compound 98 has similar stereochemistry as found in Nα-demethyl saracodine, where substituents at C-3 and C-17 and methyl groups at C-10 and C-13 all have a β- stereochemistry.

Fig. 2.5.14: Key ROESY interactions in 14-dehydro-Nα-demethylsaracodine (98).
Based on the above data, the structure of compound was deduced to be 3β-\(N_a\)-methyl-20S-\(N_b\)-acetyl-\(N_b\)-methylamino-5α-pregn-14-ene (98).

**Biogenesis of 14-Dehydro-\(N_a\)-demethylsaracodine (98)**

The biogenesis of compound 98 may based on the enzymatic \(N\)-methylation of alkamine 53 to yield 99, which upon selective \(N\)-acetylation and then hydroxylation can afford the base 100. Finally the enzymatic dehydration can give rise to compound 98 (Scheme-2.5.16).

*Scheme-2.5.16: Proposed biogenesis of 14-dehydro-\(N_a\)-demethylsaracodine (98).*
2.5.1.9  16-Dehydrosarcorine (101)

The sub-fraction C-4 (Experimental Section, Scheme-5.3, page 154) was subjected to column chromatography on silica gel using different polarities of solvent system, pet. ether: acetone: diethylamine, affording compound 101 as a yellowish gum. It gave an orange colored test with Dragendorff’s reagent, indicating its alkaloidal nature.

The HREI-MS of 101 showed the $M^+$ at $m/z$ 386.3060, corresponding to the formula $C_{25}H_{42}N_2O$ (calcd. 386.2994). The peak at $m/z$ 371 arose due to the loss of a methyl group from the $M^+$. The presence of an $N,N$-dimethylaminomethyl substituent at C-17 was deduced from an ion at $m/z$ 72 (Budzikiewicz et al., 1964). However, the lower abundance of this ion, was attributed to the presence of a double bond between C-16 and C-17 (Yu, et al., 1997). Another important fragment at $m/z$ 58 indicated the presence of an NH-acetyl group (Budzikiewicz et al., 1964).

The optical rotation of compound 101 was found to be -58° ($c = 0.05$, MeOH). The IR spectrum showed absorptions at 3350 (NH), 2993 (=CH) and 1665 (C=O) cm$^{-1}$, while the UV spectrum showed only a terminal absorption.
Scheme-2.5.1.17: Mass fragmentation of 16-dehydrosarcorine (101).

The $^1$H-NMR spectrum of compound 101 showed two 3H singlets at $\delta$ 0.82 and 1.23 corresponding to the C-18 and C-19 angular methyls, respectively. A 3H singlet resonating at $\delta$ 1.92/1.96 was due to the $N_o$-acetyl methyl protons. Another 6H singlet resonating at $\delta$ 2.20 was due to the $N,N$-dimethyl protons. A 3H doublet at $\delta$ 1.19 ($J_{2,3} = 6.5$ Hz) was assigned to the CH$_3$-21 secondary methyl protons. A downfield 1H broad singlet at $\delta$ 4.08 ($W_{1/2} = 17.7$ Hz) was attributed to the C-3 methine proton, geminal to $N_o$-acetyl group. The coupling pattern of the H-3 signal supported a $\beta$ configuration (equatorial) of its geminal NHCOCH$_3$ at this position. An olefinic signal at $\delta$ 5.65 (br s) was due to trisubstituted double bonds in the molecule. The $^{13}$C-NMR spectra (broad-band decoupled, DEPT) of 101 displayed resonances for 25 carbons, with six methyl,
eight methylene, seven methine and four quaternary carbons.

A downfield quaternary carbon signal at δ 153.4 and an olefinic methine signal at δ 125.8 indicated the presence of a tri-substituted double bond in the molecule. A methine carbon appearing at δ 62.3 revealed the presence of a $N_d$COCH$_3$ carbon bearing.

These spectral observations suggested that the compound 101 has a sarcorine-type pregnane-type steroidal skeleton with $N$H-acetyl and $N,N$-dimethylamino substituents at C-3 and C-20, respectively. The $^{13}$C-NMR chemical shift assignments were made by comparing the $^{13}$C-NMR data with that reported for some pregnane-type steroidal alkaloids (Table-2.5.9). Two-dimensional $^1$H-NMR experiments (vicinal COSY-45°, HMQC, HMBC and ROESY spectra) fully agreed with the proposed structure 101 for 16-dehydrosarcorine. The placement of a double bond between C-16 and C-17 was based on HMBC interactions of C-20 methine proton (δ 2.83) and the C-16 methine proton (δ 5.65) with the C-17 olefinic carbon (δ 153.4) (Fig. 2.5.15).

**Fig. 2.5.15:** Key HMBC interactions in 16-dehydrosarcorine (101).
Table 2.5.9: $^1$H- (400 MHz) and $^{13}$C-NMR (100 MHz) Data of 16-Dehydroascorcorine (101) in CDCl₃.

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>$^1$H-NMR ($\delta$, J= Hz)</th>
<th>$^{13}$C-NMR ($\delta$)</th>
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<tr>
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<td>1.90, 1.35 m</td>
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<td>8</td>
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<td>2.83 q ($\text{J}_{20,21} = 6.6$)</td>
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<td>21</td>
<td>1.19 d ($\text{J}_{11,20} = 6.5$)</td>
<td>18.3</td>
<td>CH₃</td>
</tr>
<tr>
<td>N₅(CH₃)₂</td>
<td>2.20 s</td>
<td>42.5</td>
<td>CH₃</td>
</tr>
<tr>
<td>N₂COCH₃</td>
<td>--</td>
<td>168.6</td>
<td>C</td>
</tr>
<tr>
<td>N₆COCH₃</td>
<td>1.92/1.96 s</td>
<td>23.6</td>
<td>CH₃</td>
</tr>
</tbody>
</table>
The stereochemistry at asymmetric centers in compound **101** was assigned on the basis of biogenetic considerations and ROESY spectrum. The β-orientations of H-8 (axial), CH₃-18 (axial) and CH₃-19 (axial) were deduced from the ROESY spectrum, as H-8 (δ 1.43) showed cross-peaks with H-19 (δ 1.23), while H-18 methyl protons (δ 0.82) also showed correlations with H-8 and H-20 (Fig. 2.5.16).

![Important ROESY interactions in 16-dehydrosarcorine (101)](image)

**Fig. 2.5.16: Important ROESY interactions in 16-dehydrosarcorine (101)**

From the aforementioned data, the structure was deduced to be (20S)-20-(N,N-dimethylamino)-3β-(Nₐ-acetylamido)-5α-pregn-16-enec (101), and given the trivial name 16-dehydrosarcorine.

**Biogenesis of 16-Dehydrosarcorine (101)**

The biogenesis of 16-dehydrosarcorine (101) may start from hydrogenation and selective N-methylation of alkaline **55** to afford **102**, which upon subsequent hydroxylation and selective N-acetylation may give rise to compound **101** (Scheme-2.5.18).
Scheme-2.5.18: Proposed biogenesis of 16-dehydroascorine (101).
2.5.2 Known Alkaloids from Sarcococca saligna

2.5.2.1 Salignarine-C (104)

The subfraction C-6-4-1 (Experimental Section, Scheme-5.4, page 157) was subjected to column chromatography using different polarities of solvent system, pet. ether: acetone: diethylamine, affording compound 104 as a yellowish gum.

![Salignarine-C (104)](image)

The HREI-MS of salignarine-C showed the molecular ion at m/z 442.3556, in agreement with the formula C\(_{25}\)H\(_{38}\)N\(_2\)O\(_2\). Its IR spectrum displayed absorptions at 3345 (NH), 3330 (OH) and 1645 (C=O) cm\(^{-1}\). The \(^1\)H-NMR spectrum (CDCl\(_3\), 400 MHz) showed resonances for two tertiary methyls at \(\delta\) 0.78 and 0.96 corresponding to C-18 and C-19 methyl groups, respectively; while a secondary methyl group, CH\(_3\)-21, resonated as a doublet at \(\delta\) 1.23 (\(J_{20,21}\) = 6.5 Hz). A 6H singlet of \(N_6\) (CH\(_3\))\(_2\) protons resonated at \(\delta\) 2.38. Two singlets resonating at \(\delta\) 1.80 and 1.85, corresponding to CH\(_3\)-4' and CH\(_3\)-5', respectively.

Compound 104 was identified as the known compound, salignarine-C (104), by
comparing its spectral data with the previously reported literature values (Atta-ur-Rahman et al., 1996).

2.5.2.2 Saracodine (105)

Saracodine (105) was isolated from the fraction C-3 (Experimental Section, Scheme-5.3, page 154) as a white amorphous powder. The HRMS of 105 showed the molecular ion at m/z 402.3579 corresponding to the formula C\textsubscript{26}H\textsubscript{46}N\textsubscript{2}O (caled. 402.3609), with five degrees of unsaturation.

![Saracodine (105)](image)

The optical rotation (14.4°, c = 0.92, CHCl\textsubscript{3}) indicated the presence of chiral centers in the molecule. The UV spectrum showed absorption at 203 nm.

The \textsuperscript{1}H-NMR spectrum (CDCl\textsubscript{3}, 500 MHz) of 105 displayed split singlets for the angular tertiary methyls at δ 0.69/0.72 (CH\textsubscript{3}-18) and 0.79/0.80 (CH\textsubscript{3}-19). Split 3H doublets resonating at δ 1.04/1.14 (d, J\textsubscript{20,21} = 6.5 Hz) were due to the CH\textsubscript{3}-21 secondary methyl group. A 6H singlet, resonating at δ 2.21, was due to the N,N-dimethyl protons. A 3H split singlet resonating at δ 2.01/2.07 was due to a N-acetyl methyl, while another 3H-split singlet resonating at δ 2.71/2.75 was due to a N\textsubscript{0}-methyl group. A split methine
signal resonating at δ 3.58/4.61 was assigned to the C-20 methine protons. The splitting of the singlet was due to the rotamerism that existed in Nβ-acetamide group, substituted at C-20.

Compound 105 was identified as saracodine, by comparing its spectral data with the previously reported data. This compound was previously isolated from Sarcococca saligna (Kohli et al, 1967).

2.5.2.3 Vaganine-A (106)

Vaganine-A (106) was isolated from fraction C-7 (Experimental Section, Scheme-5.4, page 157) as a white amorphous powder, which gave positive test with Dragendorff's reagent. The optical rotation (115°, c = 0.21, MeOH) indicated the chiral nature of the molecule. The UV spectrum showed only terminal absorption at 202 (1.32) nm, while the IR spectrum showed absorptions at 3475 (NH), 1692 (C=O) and 1596 (NH) cm⁻¹.

The HREI-MS of compound 106 showed the M⁺ at m/z 486.3808 corresponding to the formula C₃₀H₅₀N₂O₃ (caled. 486.3821), indicating seven degrees of unsaturation.

The ¹H-NMR spectrum of compound 106 showed four 3H singlets at δ 0.84
(CH$_3$-18), 0.94 (CH$_3$-19), 1.79 (CH$_3$-4') and 2.16 (CH$_3$-5'), along with a 3H doublet at δ 1.33 ($J_{20,21} = 6.5$ Hz, H-21). A downfield 6H broad singlet appearing at δ 2.61 was due to the $N$(CH$_3$)$_2$ protons. The presence of an acetyl group was also inferred from the $^1$H-NMR signal at δ 2.07 and corresponding to the $^{13}$C-NMR signals at δ 171.0 and 21.1.

Based on spectral studies (UV, IR, MS, NMR) and comparison with the literature values, compound 106 was identified as vaganine-A, previously isolated from $S$. vaganus (Minghua et al, 1993).

2.5.2.4 Alkaloid-C (107)

![Alkaloid-C (107)](image)

Compound 107 was isolated from fraction B-1 (Experimental Section, Scheme-5.2, page 151) as a white amorphous powder. The optical rotation (-29°, c= 0.015, CHCl$_3$) indicated the presence of chirality in the molecule. The UV spectrum showed only terminal absorptions.

The EI-MS of the compound 107 showed the molecular ion at m/z 359 and its HR-EI-MS was found to be m/z 359.3125 (caled. 359.3161), corresponding to the
formula C$_{24}$H$_{41}$NO with five degrees of unsaturation. The molecular ion was further confirmed by FD-MS.

The $^1$H-NMR spectrum (CDCl$_3$, 400 MHz) of 107 exhibited two 3H singlets at δ 0.65 (H-18) and 0.86 (H-19), due to the two angular methyl groups. A 3H doublet resonating at δ 0.97 ($J_{20,21} = 6.5$ Hz) was consistent with the presence of a secondary methyl group in the compound. A 6H singlet at δ 2.14 was due to the $N,N$-dimethyl group, while another three-proton singlet at δ 3.33 was assigned to the $OCH_3$ protons. Three methine signals resonated at δ 2.39 (m, H-20), 3.03 (m, H-3) and 5.34 (br s, H-6).

Compound 107 was identified as the known compound alkaloid-C, by comparing its physical and spectroscopic data with the literature values. This compound has previously been isolated from Sarcococca saligna (Kohli et al, 1971).

2.5.2.5 Salignamine-A (108)

The chloroform fraction B-3 (Experimental Section, Scheme 5.2, page 151) was subjected to repeated column chromatography using different polarities of solvent
system, pet. ether: acetone: diethyl amine as eluent to afford the compound 108 as a yellowish gum. The optical rotation of 108 was found to be -28° (c = 0.23, CHCl₃), which indicated the presence of chirality in the molecule. The IR spectrum of 108 showed absorptions at 3303 (-NH) and 1645 (C=O) cm⁻¹. The UV spectrum showed absorptions at 202 and 194 nm.

The EI mass spectrum of compound 108 showed the M⁺ at m/z 343, which was further confirmed by FD (m/z 343) and FAB +ve (m/z 344) mass spectrometric techniques. The HREI-MS of 108 showed the M⁺ at m/z 343.2880, corresponding to the molecular formula C₂₄H₃₀NO (calcd. 343.2875), having six degrees of unsaturation.

The ¹H-NMR spectrum (CDCl₃, 400 MHz) of compound 108 displayed signals for two angular methyl groups at δ 0.84 (CH₃-18) and 1.02 (CH₃-19). A 3H doublet resonating at δ 1.17 (J₁₁,₂₀ = 6.5 Hz) was due to the C-21 secondary methyl group. A 3H downfield singlet resonating at δ 3.34 was assigned to the OCH₃, and another 3H singlet resonating at δ 2.34 was due to the N₆CH₃ protons. Two methine signals that appeared at δ 2.94 as a quartet (J₂₉,₂₁ = 6.6 Hz) and at δ 3.03 as multiplet were due to the H-20 and H-3, geminal to OCH₃ group. Two olefinic protons resonated downfield at δ 5.32 and 5.72.

The spectral data of compound 108 was identified as salignamine-A by comparison with the literature data (Atta-ur-Rahman, et al., 2004).

2.5.2.6 2-Hydroxysalignamine-A (109)

The chloroform fraction B-4-1 (Experimental Section, Scheme-5.2, page 151) afforded compound 109 after repeated column chromatography using different polarities.
of pet. ether: acetone: diethylamine mixture, as a white amorphous solid. Its specific rotation was found to be 20° (c = 0.01, CHCl₃), which indicated the presence of chirality in the molecule. The IR spectrum of compound 109 showed absorptions at 3375 (OH) and 1638 (C= C) cm⁻¹. The UV spectrum was featureless, apart from end absorptions at 204 and 198 nm.

The EI-MS showed the M⁺ at m/z 373, which was further confirmed by FD (m/z 373) and FAB +ve (m/z 374) mass spectrometric techniques. The HREI-MS showed the M⁺ at m/z 373.2982, corresponding to the formula C₂₆H₃₉NO₂ (calcd. 373.2980) having six degrees of unsaturation.

The ¹H-NMR spectrum (CDCl₃, 500 MHz) of compound 109 displayed signals for two angular methyls resonating at δ 0.84 (CH₃-18) and 1.18 (CH₃-19). A 3H doublet resonated at δ 1.26 (3H, d, J₂₁,₂₀ = 6.5 Hz, CH₃-21). A three-proton downfield singlet resonating at δ 3.36 and a six-proton downfield singlet resonating at δ 2.17, were assigned to the OCH₃ and N₆(CH₃)₂ protons, respectively. A quartet at δ 2.90 (J₂₀,₂₁ = 6.6 Hz) integrating for one-proton was due to H-20 vicinal to the C-21 methyl group. Two
multiplets at δ 4.14 and 3.03, and two broad singlets at δ 5.37 and 5.69 were due to the methine H-2, H-3 (geminal to OH) and vinylic H-6, H-16, respectively.

By comparing the spectral data with the literature values, the compound was identified as 2-hydroxysalignamine-A (109) (Atta-ur-Rahman, et al., 2004).

2.5.2.7 Axillarline-F (110)

Axillarline-F (110) was isolated from the subfraction C-8 (Experimental Section, Scheme-5.4, page 157) of chloroform extract after repeated column chromatography using different polarities of the solvent system pet. ether: acetone: diethylamine.

The optical rotation of compound 110 was observed at 24.4° (c = 0.02, CHCl₃), indicating the presence of chirality in the molecule. The IR spectrum displayed absorptions at 3490 (NH), 3330 (OH), 2932 (=CH) and 1652 (C=O) cm⁻¹.

The EI-MS spectrum of axillarline-F (110) showed the molecular ion at m/z 502, which further was confirmed by the FD-MS. The ¹H-NMR spectrum (CDCl₃, 400 MHz) of 110 showed resonances of two angular methyls at δ 0.66 and δ 1.21, corresponding to
CH$_3$-18 and -19, respectively. A 3H doublet for the secondary methyl group, CH$_3$-21, resonated at $\delta$ 1.43 ($J_{20,21} = 6.5$ Hz). A 6H singlet of $N_b$ (CH$_3$)$_2$ protons resonated at $\delta$ 2.16. The H-20 resonated as a multiplet at $\delta$ 2.56. Three methine protons resonated comparatively downfield at $\delta$ 4.03 (geminal to -OH), 4.08 (geminal to -NH) and 5.33 (geminal to -OAc). The methyl group of acetyl resonated at $\delta$ 2.07. The presence of a tigloyl group was inferred from the signals at $\delta$ 6.35 (1H, q, $J_{3',4'} = 6.9$ Hz, H-3'), 1.73 (3H, d, $J_{4',3'} = 6.8$ Hz, CH$_3$-4') and 1.79 (3H, s, CH$_3$-5').

Compound 110 was identified as axillarine-F (110), by comparing its spectral data with the literature values of the previously reported compounds from Sarcococca saligna (Atta-ur-Rahman et al., 1996).

2.5.2.8 Saracovagamine-C (111)

The subfraction C-8 (Experimental Section, Scheme-5.4, page 157) afforded a white amorphous powder after repeated column chromatography on silica gel using increasing polarities of solvent system pet. ether: acetone: diethylamine, followed by the
use of preparative TLC plates for final purification of compound 111.

The optical rotation of compound 111 was 41° (c = 0.023, MeOH), indicating the presence of chirality in the molecule. The IR spectrum displayed absorptions at 3345 (NH), 2939 (=CH) and 1642 (C=O) cm⁻¹. The UV spectrum showed absorption at 212 nm.

The EI-MS spectrum showed the molecular ion at m/z 486, which further was confirmed by the FD-MS. The ¹H-NMR spectrum (CDCl₃, 400 MHz) showed two singlets for tertiary methyls at δ 0.67 and 0.94, corresponding to CH₃-18 and CH₃-19, respectively. A doublet for the secondary methyl CH₃-21 resonated at δ 1.30 (J₂₀₂₁ = 6.5 Hz). A 6H singlet of N₀ (CH₃)₂ protons resonated at δ 2.62. H-20 resonated as a multiplet at δ 3.12. Two methine protons resonated comparatively downfield at δ 3.99 (geminal to -NH) and 5.28 (geminal to -OAc). The methyl group of the acetyl resonated at δ 2.07. The presence of a tigloyl group was inferred from the signals at δ 6.30 (1H, q, J₆₅₄' = 6.9 Hz, H-3'), 1.70 (3H, d, J₄₃₄' = 6.8 Hz, CH₃-4') and 1.81 (3H, s, CH₃-5'). Compound 111 was identified as saracovaganine-C (111) by comparing its spectral data with literature values (Yu, et al., 1997).

2.5.2.9 Saracocine (112)

Saracocine (112) was isolated from the subfraction C-4-1 (Experimental Section, Scheme-5.3, page 154) as a white amorphous solid after repeated column chromatography using different polarities of the solvent mixture pet. ether: acetone: diethylamine as eluent.

The optical rotation of compound 112 was 56° (c = 0.23, MeOH),
indicating the presence of chirality in the molecule. The IR spectrum displayed absorptions at 3490 (NH), 2929 (=CH) and 1642 (C=O) cm\(^{-1}\).

![Saracocine (112)](image)

The EI-MS spectrum of saracocine (112) showed the molecular ion at m/z 400, which further was confirmed by the FD-MS. The \(^1\)H-NMR spectrum (CDCl\(_3\), 500 MHz) of 112 displayed split singlets of the angular tertiary methyls at \(\delta\) 0.69/0.72 (CH\(_3\)-18) and 0.79/0.80 (CH\(_3\)-19). Split 3H doublets resonating at \(\delta\) 1.04/1.14 (d, \(J_{20,21} = 6.5\) Hz) were due to the CH\(_3\)-21 secondary methyl group. A 6H singlet, resonating at \(\delta\) 2.21, was due to the \(N,N\)-dimethyl protons. A 3H split singlet resonating at \(\delta\) 2.01/2.07 was due to an \(N\)-acetyl methyl groups, and another 3H split singlet resonating at \(\delta\) 2.71/2.75 was due to the \(N\)-methyl proton. A split methine signal resonating at \(\delta\) 3.58/4.61 was assigned to the C-20 methine protons. The splitting of signals was due to the rotamerism that existed in \(N\)-acetamide group substituted at C-20.

Compound 112 was identified as saracocine by comparing its spectral data with the previously reported data from the same plant (Kohli et al, 1967).
Compound 113 was isolated from fraction C-4-1 (Experimental Section, Scheme-5.3, page 154) as a white amorphous powder, giving positive alkaloidal test. The optical rotation (49°, c = 0.815, CHCl₃) indicated the presence of chirality in the molecule. The UV spectrum showed absorptions at 202 nm and 194 nm, which indicated the absence of a chromophore in the molecule. The IR spectrum showed intense absorption bands at 3650 (NH), and 1658 (amidic carbonyl) cm⁻¹.

The HREI MS of 113 showed the M⁺ ion at m/z 388.3446 for the molecular formula C₂₅H₄₄N₂O (calcd. 388.3453), indicating five degrees of unsaturation. The molecular ion was further confirmed by FDMS.

The ¹H-NMR spectrum (CDCl₃, 500 MHz) of 113 displayed 3H singlets at δ 0.63 (CH₃-18) and 0.73 (CH₃-19), which were ascribed to the two angular methyl groups. Another 3H singlet at δ 1.96 indicated the presence of an acetyl or allylic methyl. A 3H
doublet resonating at δ 0.90 \((J_{20,21} = 6.5 \text{ Hz})\) was consistent with the presence of a secondary methyl group in the compound. A 6H singlet, resonating at δ 2.23, was due to the \(N'-(\text{CH}_3)_2\) protons, while a one-proton multiplet at δ 4.09 was due to the H-3α.

The signals for 25 carbons were observed in the \(^{13}\text{C}-\text{NMR}\) (broad-band decoupled) spectrum, which corresponded to the molecular formula. The DEPT spectra revealed that the compound contained six methyl, nine methylene, seven methine and three quaternary carbons, characteristic of a C-21 pregnane-type steroidal alkaloid skeleton.

Compound 113 was identified as the known compound sarcorine, by comparing its spectral data with the literature values previously reported by Atta-ur-Rahman et al., 1967.
2.5.3 Cholinesterase Inhibition Activity of Alkaloids Isolated from *Sarcococca saligna*

2.5.3.1 What is Cholinesterase?

Cholinesterase is one of the many important enzymes needed for the proper functioning of the nervous system of humans, other vertebrates and insects. The enzyme system, responsible for the termination of acetylcholine (ACh) at cholinergic synapses, was first discovered by Dale in 1914. In 1932, Stedman *et al.* proposed the term “cholinesterases” (ChE) to describe the enzymes which catalyse the hydrolysis of ACh and other choline esters at a faster rate than noncholine esters. Other esterases occurring in animal tissues were found incapable of hydrolyzing ACh.

Cholinesterases from different species were found to differ in their substrate specificity and susceptibility to inhibitors. This provoked numerous schemes for naming the various cholinesterases. They now constitute a family of enzymes, which fall broadly into two types, depending on their substrate preference (Silver, 1974). This division is not absolute and holds true more in mammalian than in non-mammalian species. Those enzymes which preferentially hydrolyze acetyl esters such as ACh are called acetylcholinesterase (AChE) or acetylcholine acetylhydrolase (EC 3.1.1.7), and those which prefer other types of esters such as butyrylcholine are termed butyrylcholinesterase (BChE) or acetylcholine acylhydrolase (EC 3.1.1.8).

AChE is found in all excitable tissues, whether nerve or muscle, central or peripheral, cholinergic or adrenergic, motor or sensory, in most erythrocytes and in
placental tissues. BCHE is found much more widely in the central and peripheral nervous system and in many other tissues especially the liver tissues, and in the plasma.

Both AChE and BCHE show different biochemical properties. AChE shows high affinity for ACh. It also shows a high turnover number and substrate inhibition at high concentrations of ACh with low affinity for noncholine esters. The specific substrate for AChE is acetyl-[beta]-methylcholine. BCHE, on the other hand, has a lower affinity for ACh. The hydrolysis rate of the aliphatic choline esters increases with increasing length of the acyl chain up to n-butyryl.

2.5.3.2 How Does Cholinesterase Work?

Electrical switching centers, called 'synapses', are found throughout the nervous systems of humans, other vertebrates and insects. Muscles, glands and nerve fibers called 'neurons' are stimulated or inhibited by the constant firing of signals across these synapses. Stimulating signals are usually carried by a chemical called 'acetylcholine'. Stimulating signals are discontinued by a specific type of cholinesterase enzyme, acetylcholinesterase, which breaks down the acetylcholine. These important chemical reactions usually take place all the time at a very fast rate, with acetylcholine causing stimulation and acetylcholinesterase ending the signal. If cholinesterase-affecting chemical agents (insecticides, nerve gases etc.) are present in the synapses, this situation is thrown out of balance. The presence of cholinesterase inhibiting chemicals prevents the breakdown of acetylcholine, which can then build up, causing a "jam" in the nervous system. Thus, when a person's body is greatly exposed to cholinesterase inhibiting compounds, it is unable to break down the acetylcholine.
2.5.3.3 Natural Cholinesterase Inhibitors

Natural products continued to be viewed as one of the few de novo sources of drug discovery, yielding unorthodox and often unanticipated chemical structures that offer novel points of departure for molecular modifications leading to new therapies.

However, it is worth mentioning that since the discovery of physostigmine as the first natural ChE inhibitor, only a few natural inhibitors have attracted the attention of neuropharmacologists. Table 2.5.3.1. lists some of the most important natural ChEs inhibitors, while Figure 2.5.3.1 shows the structures of some of these natural inhibitors.

2.5.3.4 Alzheimer's Disease

A role for the cholinergic system in human memory was suggested in the early 1970s, by demonstrating that the cholinergic antagonist scopolamine (hyosine) impaired learning in man (Crow and Grove-White, 1973; Drachman and Leavitt, 1974). The AChE inhibitor physostigmine was found to increase long-term memory processes (Davis et al., 1978). About the same time it was discovered that post mortem AD brain tissue showed reduction in the cholinergic neuronal markers, choline acetyltransferase and AChE (Davies and Maloney, 1976). Loss of AChE in AD has been more recently refined to selective loss of membrane bound 

These findings led to the formulation of a "cholinergic hypothesis", linking abnormalities in the cholinergic system to certain functional and pathological changes in AD (Perry and Perry, 1980). Since then abnormalities have been found in other parts of the cholinergic system: ACh synthesis (Sims et al., 1980), choline uptake (Rylett et al., 1983), and in some cases muscarinic receptors (Wood et al., 1983).
Table 2.5.3.1: Names and Sources of Some Natural Cholinesterase Inhibitors.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Inhibitor</th>
<th>Source</th>
<th>Ref.</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AnatoxineAS</td>
<td>Strains <em>Cyanobacteria anabaena</em> flos-aquae</td>
<td>Henriksen, 1997</td>
<td>Toxine; potent nicotinic acetylcholine receptor agonist</td>
</tr>
<tr>
<td>2</td>
<td>ArisugacinA</td>
<td>Culture of fungus <em>Penicillium</em> sp. FO-4259 growing on rice</td>
<td>Otoguro, 1997</td>
<td>Mycotoxin</td>
</tr>
<tr>
<td>3</td>
<td>ArisugacinB</td>
<td>Culture of fungus <em>Penicillium</em> sp. FO-4259 growing on rice</td>
<td>Otoguro, 1997</td>
<td>Mycotoxin</td>
</tr>
<tr>
<td>4</td>
<td>Atropine</td>
<td><em>Atropa belladonna, Datura stramonium</em></td>
<td>Seto, Y., 1998</td>
<td>Anticholinergic (muscarinic) competitive antagonist at central and peripheral cholinergic synapses</td>
</tr>
<tr>
<td>5</td>
<td>CGA-134-735</td>
<td>Cultures of <em>Streptomyces antibioticus</em> strain DSM 1951</td>
<td>Neumann, 1987</td>
<td>Natural organophosphate inhibitor of AChE</td>
</tr>
<tr>
<td>6</td>
<td>CGA-134-736</td>
<td>Cultures of <em>Streptomyces antibioticus</em> strain DSM 1951</td>
<td>Neumann, 1987</td>
<td>Natural organophosphate inhibitor of AChE</td>
</tr>
<tr>
<td>7</td>
<td>Cocaine</td>
<td></td>
<td>Berkman, 1997</td>
<td>$K_i = 3900 \mu M$ (AChE); $K_i = 390$ and $30 \mu M$ (BChE)</td>
</tr>
<tr>
<td>8</td>
<td>D-Tubocurarine</td>
<td><em>Chondodendron tomentosum</em> (Menispermaceae)</td>
<td>Zorko, 1986</td>
<td>Acts as skeletal muscle relaxant Nicotinic</td>
</tr>
<tr>
<td>9</td>
<td>Galanthamine</td>
<td>Common snowdrop <em>Galanthus nivalis</em></td>
<td>Greenbalt, 1999</td>
<td>Approved for AD treatment in Austria; specific competitive and reversible AChE inhibitor; allosteric modulator at nicotinic cholinergic receptor sites potentiates cholinergic nicotinic neurotransmission</td>
</tr>
<tr>
<td>10</td>
<td>HuperzineA</td>
<td><em>Huperzia serrata</em> (Thunb.) Trev.</td>
<td>Cheng, 1994</td>
<td>Agent in Chinese herbal medicine; used for dementia; potential for use in AD, $K_i = 16 &amp; 1$ nM (human AChE)</td>
</tr>
<tr>
<td>11</td>
<td>Onchidal</td>
<td><em>Onchidella blumei, Onchidella borealis</em></td>
<td>Abramson, 1983</td>
<td>Lipophilic acetate ester</td>
</tr>
<tr>
<td>12</td>
<td>Physostigmine (Eserint)</td>
<td>Calabar bean: <em>Phytostrigma venenosum</em></td>
<td>Singh, 1998</td>
<td>Carbamate alkaloid, $IC_{50} = 14$ nM (human AChE)</td>
</tr>
<tr>
<td>13</td>
<td>Selagine</td>
<td>Stems and leaves of <em>Lycopodium selago</em> L.</td>
<td>Valenta, 1960</td>
<td>Powdered plant is used as insecticide in Poland</td>
</tr>
<tr>
<td>14</td>
<td>TerrilremA</td>
<td>Culture of fungus <em>Aspergillus terreus</em> 23-1 growing on rice</td>
<td>Ling, 1998</td>
<td>Mycotoxin</td>
</tr>
</tbody>
</table>
Fig. 2.5.3.1: Structures of Some Natural Inhibitors of Cholinesterases.
The importance of AChE in AD was studied by Smith and Cuello (1984), suggesting that different cell groups, where lesions occur in AD share a common feature, i.e. they all contain AChE.

Cholinesterases are found in senile plaques (Friede, 1965), even at the initial stages of their formation (Morán et al., 1993), and in neurofibrillary tangles (Mesulam and Morán, 1987). Mesulam and co-workers have reported that AChE in plaques and tangles shows a lower pH optimum; a reduced sensitivity to the inhibitors, BW284c51 (Geula and Mesulam, 1989), taerine and physostigmine (Mesulam et al., 1987); and an increased sensitivity to indoleamine inhibitors (Wright et al., 1993). The characteristic substrate inhibition of AChE is altered in plaques and tangles, leading to an altered form of the enzyme (Schätz et al., 1990). NPs and NFTs present an unnatural hydrophobic environment for AChE, and therefore change in the enzyme's kinetics should not be surprising. Navaratnam et al., in 1991, reported an anomalous form of AChE present in the CSF in the life of patients later who were confirmed at post mortem to have been suffering from AD. Since this anomalous form represents only a small fraction of the AChE in CSF, it has not been possible to produce any kinetic data.

Current therapeutic strategies for the treatment of AD aim mainly to alleviate cognitive deficit by activating defective cholinergic transmission. Work has been mainly centred on inhibitors of AChE (Nordberg and Winblad, 1993). The only drug currently licensed in the USA for the management of AD is tacrine, under the brand name cognex.
### Table 4.1: Chemical Structures of Steroidal Alkaloids 1-19.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compound Name</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>R⁴</th>
<th>Unsaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Salonine-A</td>
<td>OH</td>
<td>HN-Tigloy</td>
<td>OH</td>
<td>CH₃</td>
<td>Δ⁵,6</td>
</tr>
<tr>
<td>2.</td>
<td>Salonine-B</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
<td>CH₃</td>
<td>Δ⁵,6 &amp; Δ¹⁶,17</td>
</tr>
<tr>
<td>3.</td>
<td>Salonine-C</td>
<td>H</td>
<td>HN-Tigloy</td>
<td>H</td>
<td>CH₃</td>
<td>Δ⁴,5 &amp; Δ¹⁴,15</td>
</tr>
<tr>
<td>4.</td>
<td>1,2-Dehydroseringone</td>
<td>H</td>
<td>HN-Tigloy</td>
<td>O</td>
<td>CH₃</td>
<td>Δ²,3 &amp; Δ⁵,6</td>
</tr>
<tr>
<td>5.</td>
<td>N-Methylformamidesalonine-B</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
<td>CHO</td>
<td>Δ⁵,6 &amp; Δ¹⁶,17</td>
</tr>
<tr>
<td>6.</td>
<td>Salignarne-F</td>
<td>H</td>
<td>HN-Tigloy</td>
<td>OH</td>
<td>CH₃</td>
<td>Δ⁵,6</td>
</tr>
<tr>
<td>7.</td>
<td>5,14-Dehydro-4'-methylsacaridine</td>
<td>H</td>
<td>NHCH₃</td>
<td>H</td>
<td>COCH₃</td>
<td>Δ⁵,6 &amp; Δ¹⁴,15</td>
</tr>
<tr>
<td>8.</td>
<td>14-Dehydro-4'-methylsacaridine</td>
<td>H</td>
<td>NHCH₃</td>
<td>H</td>
<td>COCH₃</td>
<td>Δ¹⁴,15</td>
</tr>
<tr>
<td>9.</td>
<td>16-Dehydrosercorine</td>
<td>H</td>
<td>NaCOCH₃</td>
<td>H</td>
<td>CH₃</td>
<td>Δ⁵,6 &amp; Δ¹⁶,17</td>
</tr>
<tr>
<td>10.</td>
<td>Alkaidol-C</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
<td>CH₃</td>
<td>Δ⁵,6</td>
</tr>
<tr>
<td>11.</td>
<td>Salignamine</td>
<td>H</td>
<td>OCH₃</td>
<td>H</td>
<td>H</td>
<td>Δ⁵,6 &amp; Δ¹⁶,17</td>
</tr>
<tr>
<td>12.</td>
<td>2-Hydroxysalignamine</td>
<td>OH</td>
<td>OCH₃</td>
<td>H</td>
<td>CH₃</td>
<td>Δ⁵,6 &amp; Δ¹⁶,17</td>
</tr>
<tr>
<td>13.</td>
<td>Saracosome</td>
<td>H</td>
<td>N(CH₃)₂</td>
<td>H</td>
<td>OAC</td>
<td>Δ⁵,6</td>
</tr>
<tr>
<td>14.</td>
<td>Saracodine</td>
<td>H</td>
<td>NCH₃</td>
<td>H</td>
<td>OAC</td>
<td>--</td>
</tr>
<tr>
<td>15.</td>
<td>Sarcorine</td>
<td>H</td>
<td>NHAc</td>
<td>H</td>
<td>CH₃</td>
<td>--</td>
</tr>
<tr>
<td>16.</td>
<td>Vagamine-A</td>
<td>H</td>
<td>HN-Senecioyl</td>
<td>OAc</td>
<td>CH₃</td>
<td>--</td>
</tr>
<tr>
<td>17.</td>
<td>Axillarine-F</td>
<td>OH</td>
<td>HN-Tigloy</td>
<td>OAc</td>
<td>CH₃</td>
<td>Δ⁵,6 &amp; Δ¹⁴,15</td>
</tr>
<tr>
<td>18.</td>
<td>Sarcovagaine-C</td>
<td>H</td>
<td>HN-Tigloy</td>
<td>OAc</td>
<td>CH₃</td>
<td>--</td>
</tr>
<tr>
<td>19.</td>
<td>Salignarne-C</td>
<td>OH</td>
<td>HN-Senecioyl</td>
<td>H</td>
<td>CH₃</td>
<td>Δ⁵,6</td>
</tr>
</tbody>
</table>
2.5.3.5 Cholinesterase Inhibition Studies on Steroidal Alkaloids

The majority of compounds isolated during this study, as shown in Table-2.5.3.1, exhibited selective inhibitory activities against butyrylcholinesterase as compared to acetylcholinesterase. The IC$_{50}$ values (Table-2.5.3.2) revealed clearly that C-3 and C-20 amino groups are the most important structural features that significant effect the inhibitory potency of these steroidal alkaloids.

The role of amino nitrogen in this class of compounds may be due to their protonation at physiological pH and mimicking the quaternary nitrogen of the potent quaternary inhibitors such as propdium.

This argument was supported by the fact that structurally identical compounds, lacking amino groups at C-3 and/or C-20 positions, such as compounds 5, 6, 10, 11 and 12 showed very low inhibitory potency against ACh. This decrease in the activity could be due to the absence of C-3 amino group and the resulting decrease in the positive charge on these compounds. Moreover, AChE and BChE inhibitory activities further support the prediction that ring A of the steroidal skeleton of these compounds always enters the active site first.

The most active members of this pregnane-type steroidal alkaloids series were found to be compounds 4, 6, 16, 17 and 18. These compounds have carbonyl, hydroxyl or acetoxy substituents at C-4. This suggests a role of these substituents in the activity of these compounds.

It has been observed that the presence of electron withdrawing substituents such as acetamide group at C-3 of compounds 9 and 15 enhances their activities.
against AChE. The presence of similar substituents at C-20 results in a considerable decrease in the activity of the compounds 8, 9, 13 and 14 against AChE.

Table 2.5.3.2: Summary of the *In vitro* Anticholinesterase Activities of Steroidal Alkaloids 1-19.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Compound Name</th>
<th>Acetylcholinesterase IC₅₀ [µM] (mean ± SEM)</th>
<th>Butyrylcholinesterase IC₅₀ [µM] (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Salolone-A</td>
<td>33.4 ± 3.2</td>
<td>32.7 ± 1.2</td>
</tr>
<tr>
<td>2</td>
<td>Salolone-B</td>
<td>inactive</td>
<td>4.5 ± 0.072</td>
</tr>
<tr>
<td>3</td>
<td>Salolone-C</td>
<td>63.1 ± 4.51</td>
<td>15.6 ± 1.5</td>
</tr>
<tr>
<td>4</td>
<td>1,2-Dehydroconaline</td>
<td>7.0 ± 0.1</td>
<td>32.2 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>N-Methylformamidesalolone-B</td>
<td>100.2 ± 2.02</td>
<td>27.0 ± 0.51</td>
</tr>
<tr>
<td>6</td>
<td>Salignamine-I</td>
<td>7.8 ± 0.5</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>7</td>
<td>5,14-Dehydro-N₄-methylsarcodine</td>
<td>184.0 ± 2.6</td>
<td>10.1 ± 0.15</td>
</tr>
<tr>
<td>8</td>
<td>14-Dehydro-N₄-methylsarcodine</td>
<td>&gt;500</td>
<td>25.0 ± 0.6</td>
</tr>
<tr>
<td>9</td>
<td>16-Dehydroxysarcodine</td>
<td>12.5 ± 0.02</td>
<td>3.95 ± 0.5</td>
</tr>
<tr>
<td>10</td>
<td>Alkaloid-C</td>
<td>40.8 ± 9.07</td>
<td>4.3 ± 0.02</td>
</tr>
<tr>
<td>11</td>
<td>Salignamine</td>
<td>249.0 ± 10.30</td>
<td>25.7 ± 0.25</td>
</tr>
<tr>
<td>12</td>
<td>2-Hydroxysalignamine</td>
<td>82.5 ± 1.01</td>
<td>20.9 ± 3.2</td>
</tr>
<tr>
<td>13</td>
<td>Saracoline</td>
<td>65.9 ± 1.6</td>
<td>10.3 ± 0.21</td>
</tr>
<tr>
<td>14</td>
<td>Saracodine</td>
<td>49.7 ± 1.26</td>
<td>18.3 ± 0.74</td>
</tr>
<tr>
<td>15</td>
<td>Sarcocine</td>
<td>26.3 ± 1.30</td>
<td>3.86 ± 0.01</td>
</tr>
<tr>
<td>16</td>
<td>Vagamine-A</td>
<td>8.59 ± 0.11</td>
<td>2.32 ± 0.2</td>
</tr>
<tr>
<td>17</td>
<td>Axillarine-G</td>
<td>227.9 ± 8.67</td>
<td>17.9 ± 0.22</td>
</tr>
<tr>
<td>18</td>
<td>Sarcovagamine-C</td>
<td>187.8 ± 0.71</td>
<td>1.5 ± 0.02</td>
</tr>
<tr>
<td>19</td>
<td>Salignamine-C</td>
<td>19.7 ± 0.05</td>
<td>1.25 ± 0.01</td>
</tr>
<tr>
<td>20</td>
<td>Galanthamine</td>
<td>0.5 ± 0.01</td>
<td>8.1 ± 1.01</td>
</tr>
</tbody>
</table>
PART B

3.0 Studies on the Chemical Constituents of

Ficus religiosa
3.1 INTRODUCTION

3.1.1 Genus *Ficus*

Genus *Ficus* belongs to the family Moraceae, comprising, 1,000 species found in tropics and subtropics regions, especially in Indonesia and Polynesia. In Pakistan, 24 species are found, in which eleven are native (Nasir, 1974). *Ficus religiosa* is a large glabrous, evergreen tree, commonly known as pipal, found in Pakistan, India, Bangladesh, Ceylon, China, Burma and Thailand.

*Ficus* sp. are monoecious or functionally dioecious trees, shrubs or root climbers, twiners and hemi-epiphytes, often with adventitious roots (Nasir, 1974). The dense rounded and graceful branches of weeping *Ficus* made them quite popular as landscape trees. *Ficus* is one of the most loved bonsai for many reasons. It is an excellent tree for beginners, as most of the *Ficus* species are fast growing, tolerant of most soil and light variations. Genus *Ficus* exhibited several biological activities including antibacterial, antifungal and antitumor activities.

3.1.2 Pharmacological Importance of *Ficus religiosa*

*Ficus religiosa* exhibits several biological activities and it is effectively used for the treatment of different diseases. The barks of the plant are used as astringent and for curing gonorrhea. For the treatment of scabies, the infusion of the barks is used intensively. In inflammatory swelling, the paste of the powdered bark is used as absorbent. The leaves and young shoots of *Ficus religiosa* are effectively used in skin diseases. Fruits of *F. religiosa* is purgative, laxative, alterative and cooling and is used for digestion (Nasir, 1974).
3.2 RESULTS AND DISCUSSION

The fruits of *Ficus religiosa* Linn. were collected from the suburban areas of Karachi city in July 1999. The concentrated ethanolic extract of the fruits was subjected to vacuum liquid column chromatography (VLC), which yielded ten subfractions (Frfr-1- Frfr-10) by increasing polarities of CHCl₃: pet. ether/pet. ether: ETOAc/MeOH (Experimental Section, Scheme-5.5, page 177). The subfractions Frfr-3-Frfr-7 were then further subjected to repeated column chromatography, affording five known compounds: piperine (114), piperlonguminine (115), dihydropiperlonguminine (116), N-isobutyleicosa-trans-2-trans-4 dienamide (117), and methyl piperate (118); they were isolated for the first time from this plant. Their structures were elucidated with the help of extensive spectroscopic techniques.

3.2.1 Known Compounds Isolated from *Ficus religiosa*

3.2.1.1 Piperine (114)

The crystals of piperine amide (114) were isolated from the fraction Frfr-7 (Experimental Section, Scheme-5.5, page 177) by using repeated column chromatography (silica gel). This amide was previously isolated from the *Piper longum* (Okogun, J. I., 1974).

The presence of a conjugated aromatic system was supported by the UV absorptions at 310 and 340 nm, indicating the presence of a 3,4-methylenedioxy-trans-styryl moiety in the molecule (Scott, 1964). The IR spectrum showed absorption at 1630 cm⁻¹, for an α, β-unsaturated amide group.
The EI-MS spectrum of 114 showed the M⁺ at m/z 285, which was confirmed by FD-MS. The fragment ion at m/z 84 indicated the presence of a piperidine ring in the molecule (Bina, S. S., 2002). The HREI-MS showed the molecular ion at m/z 285.3420, corresponding to the formula C₁₇H₁₆NO₃ (calcd. 285.3413).

The ¹H-NMR spectrum of 114 (CDCl₃, 400 MHz) showed signals for olefinic protons of an α,β,γ,δ-conjugated system at δ 6.45, 7.35 (ddd, J = 14.6 Hz, J = 8.7 Hz, J = 1.5 Hz), 6.73 and 6.75 (Raina, M. L., 1976). The methylenedioxy protons resonated as singlet at δ 5.95. The methylene protons of the piperidine ring resonated at δ 3.15 (4H, d, J = 16.3 Hz, C-1'/C-5') and 1.63 (6H, m, C-2''/C-3''/C-4'').

The ¹³C-NMR spectrum indicated the presence of 17 carbons, including six methylene, seven methine and four quaternary carbons.

Comparison of spectral data of 114 with the literature values (Okogun, J. I., 1974) indicated its identity as piperine.

3.2.1.2 Piperlongumine (115)

The amide 115 was isolated as a white solid from fraction Frf-4 (Scheme-5.5, page 177). This amide was earlier isolated from Piper longum (Wanbyr, T., 1983).
The IR spectrum of amide 116 showed absorptions at 3340 (NH), 1645 (C=O) and 1545 (C=C) cm\(^{-1}\). The UV spectrum showed absorptions at 339, 290 and 240 nm.

The El-MS spectrum of 115 showed the molecular ion at \(m/z\) 273, which was confirmed by the FD-MS. The \(^1\)H-NMR spectrum (300 MHz, CDCl\(_3\)) of 115 showed two methyl signals as a doublet at \(\delta\) 0.95 \((J = 6.6\) Hz\). The N-CH\(_2\) protons of \(N\)-isobutyl amide moiety which resonated as a triplet at \(\delta\) 3.15 \((J = 7.0\) Hz\), while the methine proton resonated as a multiplet at \(\delta\) 1.76. The olefinic protons of an \(\alpha,\beta,\gamma,\delta\)-conjugated system appeared at \(\delta\) 6.56, 7.30 \((\text{dd}, J = 14.6\) Hz, \(J = 8.7\) Hz, \(J = 1.5\) Hz\), 6.72 and 6.74. The methylenedioxy protons resonated as a singlet at \(\delta\) 5.95. The aromatic protons resonated at \(\delta\) 6.78 \((s, H-2')\), 6.86 \((\text{dd}, J = 8.1\) Hz, \(J = 1.6\) Hz, H-6') and 6.96 \((d, J = 1.6\) Hz, H-5').

The amide 115 was identified as piperlongumine by comparing its spectral data with the literature values (Chatterjee, A., 1967).

### 3.2.1.3 Dihydropiperlongumine (116)

Another amide 116 was isolated as a colorless amorphous solid, along with the amide 115, from fraction Frf-4 (Experimental Section, Scheme-5.5, page 177). This
amide was isolated first time from *Ficus religiosa* and was previously from the *Piper longum* (Wnabyr, T., 1983).

The IR spectrum of amide 117 showed absorptions at 3343 (NH), 1651 (C=O) and 1541 (C=C) cm$^{-1}$. The UV spectrum showed absorptions at 335, 287 and 243 nm. The EI-MS spectrum of 116 showed the molecular ion at $m/z$ 275, which was confirmed by the FD-MS.

![Dihydropiperlonguminine (116)](image)

The $^1$H-NMR spectrum (300 MHz, CDCl$_3$) of compound 116 was similar to the compound 115. It showed signals for two methyl protons at $\delta$ 0.97 (d, $J = 6.6$ Hz). The N-CH$_2$ protons of *N*-isobutyl amide moiety resonated as a triplet at $\delta$ 3.13 ($J = 7.0$ Hz), while the C-O methine proton resonated as a multiplet at $\delta$ 1.77. The olefinic protons of $\alpha,\beta$-conjugated system appeared at $\delta$ 6.66, 7.33 (dd, $J = 14.6$ Hz, $J = 1.5$ Hz). The methylenedioxy protons resonated as a singlet at $\delta$ 5.96. The aromatic protons resonated at $\delta$ 6.76 (s, H-2'), 6.87 (dd, $J = 8.1$ Hz, $J = 1.6$ Hz, H-6') and 6.93 (d, $J = 1.6$ Hz, H-5').

The structure of amide was identified as dihydropiperlonguminine 116 by comparing its spectral data with the literature values (Chatterjee, A., 1967).
3.2.1.4 \textit{N-Isobutyl eicosa-trans-2-trans-4 dienamide (117)}

Another amidic compound 117 was isolated from the fraction Frf-5 as a white amorphous solid, after repeated column chromatography (Experimental Section, Scheme-5.5, page 177). The IR spectrum of 117 showed the absorptions at 3295 (NH), 1634 (C=O) and 1545 (C=C) cm\(^{-1}\), while the UV spectrum showed absorption at 260 nm. The El-MS spectrum of 117 showed the molecular ion at m/z 363, which was confirmed by the FD-MS.

![Structural formula of N-Isobutyl eicosa-trans-2-trans-4 dienamide (117)]

The \(^1\)H-NMR spectrum (400 MHz, CDCl\(_3\)) of 117 showed a characteristic N-CH\(_2\) signal of \textit{N-isobutylamide moiety} as a triplet at \(\delta 3.14\) (\(J = 7.0\) Hz). A doublet of three methyl protons resonated at \(\delta 0.95\) (\(J = 6.6\) Hz). The methylene protons resonated as a complex multiplet at \(\delta 1.25\). The olefinic protons of \(\alpha,\beta,\gamma,\delta\)-conjugated system resonated at \(\delta 5.71\) (1H, d, \(J = 15.0\) Hz), 7.14 (1H, dd, \(J = 8.1\) Hz, \(J = 1.6\) Hz), and 6.02-6.13 (2H, m).

The structure of amide 117 was identified as \textit{N-isobutyl eicosa-trans-2-trans-4 dienamide} by comparing its spectral data with the literature values (Riana, 1976).
3.2.1.5 Methyl piperate (118)

The ester analog 118 of piperic acid was isolated from the fraction Frf-3 (Experimental Section, Scheme-5.5, page 177) as a colorless amorphous compound. Previously this ester was isolated from the *Piper officinarum* (Gupta, et al., 1972).

The presence of a conjugated system was inferred from the UV absorptions at 312 and 340 nm, indicating the presence of 3,4-methylenedioxy-trans-styryl moiety in the molecule. The IR spectrum showed absorption at 1734 cm\(^{-1}\) for an ester group.

The EI-MS of 118 showed the M\(^+\) at \(m/z\) 232, which was confirmed by the FD-MS. The mass fragment at \(m/z\) 173 [M\(^+\)-59] indicated the presence of an ester group. The HREI-MS showed the molecular ion at \(m/z\) 232.0742, corresponding to the formula C\(_{13}\)H\(_{12}\)O\(_4\).

![Methyl Piperate (118)](image)

The \(^1\)H-NMR spectrum of 118 contained a singlet of methylenedioxy protons at \(\delta\) 5.96, while the methyl protons of the ester group appeared as a singlet at \(\delta\) 3.76. The signals for olefinic protons of an \(\alpha,\beta,\gamma,\delta\)-conjugated system of a 2E, 4E-dienamide appeared at \(\delta\) 6.66, 7.38 (ddd, \(J = 14.6\) Hz, \(J = 8.7\) Hz, \(J = 1.5\) Hz), 6.68 and 6.72. The aromatic protons resonated at \(\delta\) 6.78 (s, H-2'), 6.89 (dd, \(J = 8.1\) Hz, \(J = 1.6\) Hz, H-6') and
6.98 (d, J = 1.6 Hz, H-5'). The $^{13}$C-NMR spectrum of 118 showed the presence of 13 carbons, with one methyl, one methylene, seven methine and four quaternary signals.

Comparing the spectral data of 118 with the literature values, the compound was inferred as methyl piperate (Banerji, A. et al., 1984).
3.3 Antileishmanial Activity of Compounds

Isolated from *Ficus religiosa*

3.3.1 Leishmaniasis: An Overview

Most common pathogens for tropical diseases in man and domestic animals are protozoan parasites. Leishmaniasis is caused by a protozoa parasite of genus *Leishmania*. Members of the genus *Leishmania* are a biologically diverse group of flagellate parasites of the *Trypanosomatidae* family, which can be differentiated by genetic, biochemical and immunological studies.

Leishmaniasis is widely distributed in the old world around the Mediterranean sea, in East and West Africa, Afghanistan, India and China. In the new world, this disease mostly prevails from the southern part of the United States to the northern part of Argentina and Paraguay (Beaver, P. C., 1986).

The domestic and wild animals are main reservoirs of *Leishmania* parasites, while the female flying insects of the genus *Phlebotomus* and *Lutzomya* are vectors of leishmaniasis.

On the basis of various manifestations of the disease, leishmaniasis has been classified in three clinical forms:

a) Visceral leishmaniasis
b) Mucocutaneous leishmaniasis
c) Cutaneous leishmaniasis
a) Visceral Leishmaniasis: Visceral leishmaniasis is caused by the *Leishmania donovani*. It affects internal organs, such as liver, spleen and bone marrow. Visceral leishmaniasis is the most severe clinical forms of the disease and delay in the treatment can be fatal.

b) Mucocutaneous Leishmaniasis: Mucocutaneous leishmaniasis can cause facial disfiguration due to erosion in the mucocutaneous sites of the mouth and nose.

c) Cutaneous Leishmaniasis: Cutaneous leishmaniasis is an autolimited infection and the least severe form of the leishmaniasis. *Leishmania maxicana* is responsible for cutaneous leishmaniasis.

During the biological cycle of *Leishmania* parasites in different hosts, the protozoan parasite exists in two forms: a) promastigote, and b) amastigote.

Promastigote is a flagellated extracellular form that infects both man and other vertebrates. Amastigote is the intercellular form which is located inside the host’s macrophages.

### 3.3.2 Use of Medicinal Plants in the Treatment of Leishmaniasis

The secondary metabolites of plants such as alkaloids, quinone and terpenes have been used to cure protozoan parasitic diseases. Quinine and emetine, which were isolated from the genus *Cinchona* and *Cephaelis*, respectively, are best examples for the treatment of protozoan parasite diseases, malaria and amoebiasis,

Based on the effective use of these natural products for curing protozoan parasite diseases, the search for plant derived substances with leishmanicidal activity has been extensively conducted, resulting in the identification of several promising leads. Among
them diospyrin (119), isolated from the *Diospyros mantana*, was found to be active against *L. donovani* (Hazra, B. *et al.*, 1987); berberine (120) was affective against cutaneous leishmaniasis in rats; and harmaline (121), isolated from the *Peganum harmala*, showed antiprotozoal action (Wright, C. W., 1990 and Evans, 1987). Steroidal alkaloids, holamine (122) and hydroxyi holamine (123), isolated from the *Holarrhena curtisii*, also exhibited leishmanicidal activity against *L. donavani* (Kam, T., 1998). An alkaloid, benzoxazol-2(3H)-one (124) from *Acanthus ilicifolius* also showed leishmanicidal activity (Kapil, A., 1993 and Kapil, A., 1994).

Another group of plant metabolites with significant leishmanicidal activity are the iridoids, monoterpenoids and glycosides with the cyclopenta [c]-pyran skeleton. Arbortristosides A (125), B (126) and C (127), which have been isolated from the seeds

Antileishmanial Activity of the Compounds of *Ficus religiosa*

Bioassay-guided isolation of leishmanicidal constituents from *Ficus religiosa* was carried out. The methanolic extract of the plant showed antileishmanial activity at 6.25 μg/mL. All fractions of the crude extract were obtained by vacuum liquid chromatography (VLC) by using different polarities of solvent system (CHCl₃: pet. ether/pet. ether: ETOAc/MeOH) (Experimental Section, Scheme-5.5, page 178) evaluated for antileishmanial activity (Scheme-3.3.1, page 137). The main aim of the study was to isolate the active constituents from the plant. This bioassay work was conducted by one of our colleagues, Mrs. Farhana Kaukab in the Bioassay and Plant Screening Section of the H. E. J. Research Institute of Chemistry.
**Ficus religiosa**

Crude Extract (6.25 µg/mL)

Vaccum liquid chromatography (VLC)

- Pet. ether
- P.E.: CHCl₃ 8:2
- P.E.: CHCl₃ 6:4
- P.E.: CHCl₃ 4:6
- P.E.: CHCl₃ 2:8
- CHCl₃
- P.E.: ETOAc 5:5
- ETOAc
- MeOH
- Water

FrF1

FrF2 (50 µg/mL)

FrF3

FrF4 (50 µg/mL)

FrF5 (12-25 µg/mL)

FrF6

FrF7 (50 µg/mL)

FrF8 (25 µg/mL)

FrF9

FrF10

Repeated column chromatography

Piperic ester (118)

Piperlonguminine (115)

Dihydropiperlonguminine (116) 53.1 µg/mL

N-Isobutyl eicoso-trans-2-trans-4 dienamide (117) 12 µg/mL

Scheme-3.3.1
The IC₅₀ values for compounds 114-118 are shown in Table-3.3.1. Compound 117 was found to be the most active among the series, while compounds 114, 115, and 116 showed moderate activity.

Table-3.3.1: In vitro antileishmanial activities of compounds 114-118 against promastigotes of Leishmania major.

<table>
<thead>
<tr>
<th>S No.</th>
<th>Compound Name</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IC₅₀ [µg/ml]</td>
</tr>
<tr>
<td>1</td>
<td>Piperine (114)</td>
<td>50.1</td>
</tr>
<tr>
<td>2</td>
<td>Piperlongumine (115)</td>
<td>51.1</td>
</tr>
<tr>
<td>3</td>
<td>Dihydropiperlongumine (116)</td>
<td>53.3</td>
</tr>
<tr>
<td>4</td>
<td>4-Isobutyl eicoso-trans-2-trans-4-dienamide (117)</td>
<td>12.5</td>
</tr>
<tr>
<td>5</td>
<td>Methyl piperate (118)</td>
<td>inactive</td>
</tr>
</tbody>
</table>

The crude extract of Ficus religiosa (fruits) showed antileishmanial activity at 6.25 µg/mL against L. major; while the most active pure constituent 4-isobutyl eicoso-trans-2-trans-4 dienamide (117), isolated through a bioassay-guided fractionation, was active at 12.5 µg/mL (Table-3.3.1). The pure compound exhibited lower antileishmanial activity as compared to the crude extract. This may be due to the additive effect of constituents present in the crude extract.
PART C

4.0 Synthesis and Rearrangement of Heterocyclic Compounds
4.1 INTRODUCTION

Heterocyclic compounds play an important role in the metabolism of living cell. Many of the heterocyclic compounds have pharmacological activity and clinical uses. Quinoxaline and its derivatives are known to possess various biological actions.

Quinoxaline

Quinoxaline (128) is a weak, basic, bicyclic compound commonly called “1,4-diazanaphthalene” or “benzopyrazine” containing a fused benzene and a pyrazine ring.

Natural products containing quinoxaline ring are rare, and can be easily synthesized. Quinoxaline (128) has been synthesized by the condensation of o-phenylene diamine with glyoxal sodium bisulfite in 85-90% yield (Jones, R. G., 1950). It can also be prepared in an excellent yield by the use of 30% aqueous glyoxal in the presence of sodium carbonate (Zmijdzin, A., 1974).
Numerous quinoxaline derivatives have been synthesized by using the substituted o-phenylenediamine and by suitable α-dicarbonyl compounds. Alkyl and aryl quinoxalines, quinoxalinones and quinoxaline carboxylic acids have also been prepared.

Pharmacological Importance

Quinoxaline and its derivatives have attracted interest from chemists because of their interesting chemistry and pharmacological uses. Quinoxaline and its derivatives exhibited interesting biological activities, such as antibacterial, antifungal, anticancer, antidepressant and anti-inflammatory properties (Kaneko, 1988; Sarges, 1990; Kinashi, 1988). It also exhibits a DNA interacting behavior (Nallas, 1996; Milkevitch, 1966; Molnar, 1994). Some quinoxaline derivatives act as antidiabetic (El-Bandray, 1996), as antiviral agents (Keeble, 2001) and as NMDA receptor antagonist (Lin, S.K., 1996).

Among a wide variety of quinoxaline derivatives, imidazoquinoxaline ribonucleosides were found to have potent antiviral activity (Zhu, Z., 1988), while pyrazolquinoxaline showed antibacterial activity against Bacillus licheniformis and Cellulomonas sp. (Makino, K., 1988a, 1988b). The N-oxide derivative of quinoxaline has been used for the treatment of tuberculosis (Sainz, Y., 1994). Pyrimidol [4,5-b] quinoxaline is effectively used for the treatment of hypertension; it also acts as a blood platelet antiaggregating agent (Mange, A., 1989). Some quinoxaline derivatives exert cytotoxic effects on human cancer cell lines (Yoo, H. W., 1998, Gozyo, S., 1988). Quinoxaline derivatives also have commercial importance as agrochemicals (Kaneko, 1988) and herbicides (Hiramatsu, T., 1988). They can be used in conjugated polymer synthesis due to their electron withdrawing properties (Finar, I.L., 1955). In addition, the
fluorescence characteristic of 6,7-dimethoxyquinoxalines and their potential as fluroinophores (Wolfe, J. F., 1974).

**Benzimidazole**

Benzimidazole is a fused benzene and imidazole rings, with a basic skeleton 129. It is also called "beniminazol" or "benzyglyoxalines" is rare in nature.

![Benzimidazole Structure](image)

It can be synthesized using benzene derivatives, (containing nitrogen function at ortho position) and acids. Several benzimidazole derivatives were synthesized by using different amino substituted and carbonyl containing reagents.

**Pharmacological Importance**

Benzimidazole and its derivatives possess a wide range of biological activities and are known for their relatively non-toxic nature. They also have little effect on the blood pressure.

![Benzimidazole Synthesis Reaction](image)
Benzimidazole derivatives are used as antimalarial agents (Chatterjee, 1929) as well as anaesthetic agents, such as 2-diethylaminopropyl-5-phenoxy benzimidazole (Putzer, B., 1932). A benzimidazole derivative, 5-ethoxy-2-phenoxy methyl benzimidazole, was found to possess antipyretic activity (Bryer and Co.). Benzimidazoles exhibit anticonvulsant activity at large doses (Bywater, W. G., 1945 and Toman, J. E. P., 1946). Benzimidazole derivatives have also been reported to have antibacterial activity (Martin, G. J., 1949). Some of them are reported to reduce skeletal muscle tone by acting on the central nervous system (Goodman, L., 1943 and Goodman, L., 1944). Benzimidazoles are also reported to have some fungicidal properties (Fischer, O., 1889). A large number of benzimidazole derivatives are used in the textile industry as wetting, emulsifying, foaming or softening agents or as dispersant for use in dyeing. Several other benzimidazoles are used in the photographic industry. Benzimidazole derivatives also protect the skin by absorbing the ultraviolet rays, therefore they can be potentially used in the preparation of sunburn preventative.

Anthranils/ Benzisoxazoles

Isoxazoles (130) contain nitrogen and oxygen atoms in a 1,2 relationship. Two types of benzo-fused analog have been found: benzoisoxazole (131) and benzisoxazole or anthranil (132).
Rearrangement of Anthranils or Benzisoxazoles

Interesting rearrangement reactions of anthranils on thermolysis and pyrolysis have been reported previously (Ning, et al., 1974; Coe, et al., 1966; Kwok, et al., 1968; Davis, 1968). Cava et al., have reported the rearrangement of nitropapavarine into azaberbinone N-oxide (Cava, et al., 1970). Another interesting rearrangement reaction of nitropapavrine involving the benzisoxazole was reported by Kametani et al., (Kametani, et al., 1970). Keeping in view the interesting chemistry involved in these type of rearrangement reactions, we carried out benzisoxazole rearrangement reaction on different heterocyclic compounds. For example, anthranilopapavrine (133) was subjected to thermolysis and pyrolysis to obtain the rearranged products isoquino [1,2-b]-quinazoline (134) and azaberbinone (135), respectively (Atta-ur-Rahman et al., 1987).

![Chemical structures](image)

In another study, involved the tryptamine isoxazole derivative 136 was subjected to thermal and photochemical rearrangement reactions to obtain the cinnoline derivative 137. This represents a new method for the synthesis of cinnoline derivatives (Atta-ur-Rahman et al., 1996).
Keeping in view the biological importance of quinoxaline derivatives, we directed our efforts towards synthesizing quinoxaline derivative and studying the previously reported rearrangement.
4.2 RESULTS AND DISCUSSION

4.2.1 Synthesis of Quinoxaline and Benzimidazole Derivatives

For the synthesis of target molecules, condensation reaction of o-phenylene diamine (138) with o-nitrophenyl pyruvic acid (139) was carried out in toluene and a few drops of HCl were added as a catalyst, by using Dean and Stark apparatus. This yielded a major product, 3-(2-nitrobenzyl)-2(1H)-quinoxalinone (140) in a 47% yield, along with a minor benzimidazole derivative, 2-(2-nitrobenzyl)-1H-benzimidazole (141) in 13% yield (Scheme-4.2.1, page 137).

This condensation reaction is a reversible reaction and formation of water is a rate-limiting step; removal of water during the reaction directed the reaction towards the right side and the targeted product was obtained. Different other solvents were also used in the reaction medium, such as methanol, water and THF but no product was obtained. This may be due to the generation of water, which is miscible in methanol and THF.

The condensed product was purified by silica gel column chromatography with the use of solvent system, dichloromethane : methanol (9.5 : 0.5).

4.2.1.1 3-(2-Nitrobenzyl)-2(1H)-quinoxalinone (140)

The quinoxaline derivative was identified as 3-(2-nitrobenzyl)-2(1H)-quinoxalinone (138). Its EI-MS showed the molecular ion at m/z 281, which was confirmed by the FAB-MS (+ve) and FD-MS that showed M⁺ at m/z 282 and 281, respectively. The IR spectrum showed absorptions at 3345 (NH), 1665 (C=O), 1515 and 1345 (NO₂) cm⁻¹, while the UV spectrum exhibited absorptions at 317, 299 and 193 nm.
The $^1$H-NMR spectrum (CDCl$_3$, 400 MHz) of 140 showed signals for C-5 and C-8 aromatic protons of quinoxaline moiety resonating as a double doublet at $\delta$ 7.28 ($J_{6,6,8,7} = 11.9$ Hz, $J_{5,7,8,6} = 3.4$ Hz), while the C-6 and C-7 protons collectively appeared as a multiplet at $\delta$ 7.63. A three-proton multiplet resonating at $\delta$ 7.50 was ascribed to H-5', H-
6' and H-7'. The H-4' appeared as a doublet at δ 8.01 (J₄',₅ = 8.1 Hz). The C-1' methine proton resonated as a singlet at δ 4.59.

These spectroscopic studies led to the identification of the main reaction product as 3-(2-nitrobenzyl)-2(1H)-quinoxalinone (140).

![Chemical structure of 3-(2-nitrobenzyl)-2(1H)-quinoxalinone (140)]

**Table 4.2.1: **$^1$H-NMR Data of 3-(2-Nitrobenzyl)-2(1H)-quinoxalinone (140).

<table>
<thead>
<tr>
<th>Hydrogen</th>
<th>(δ, J = Hz)</th>
<th>Hydrogen</th>
<th>(δ, J =Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>--</td>
<td>8a</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>--</td>
<td>1'</td>
<td>4.59 s</td>
</tr>
<tr>
<td>3</td>
<td>--</td>
<td>2'</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>--</td>
<td>3'</td>
<td>--</td>
</tr>
<tr>
<td>4a</td>
<td>--</td>
<td>4'</td>
<td>8.01 d (J₄',₅ = 8.1)</td>
</tr>
<tr>
<td>5</td>
<td>7.28 dd (J₅,₆ = 11.9, J₅,₇ = 3.4)</td>
<td>5'</td>
<td>7.50 m</td>
</tr>
<tr>
<td>6</td>
<td>7.63 m</td>
<td>6'</td>
<td>7.50 m</td>
</tr>
<tr>
<td>7</td>
<td>7.63 m</td>
<td>7'</td>
<td>7.50 m</td>
</tr>
<tr>
<td>8</td>
<td>7.28 dd (J₈,₇ = 11.9, J₈,₆ = 3.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.2.1.2 2-(2-Nitrobenzyl)-1H-benzimidazole (141)

The minor condensed product 141 was identified as 2-(2-nitrobenzyl)-1H-benzimidazole (141). Its EI-MS showed the molecular ion at m/z 253, which was confirmed from the FD-MS. The IR spectrum showed absorptions at 3350 cm\(^{-1}\) (NH), 1517 and 1320 cm\(^{-1}\) (NO\(_2\)). The UV showed absorptions at 377, 330, 274, 219 and 193 nm.

![2-(2-Nitrobenzyl)-1H-benzimidazole (141)](image)

The \(^1\)H-NMR spectrum (CDCl\(_3\), 400 MHz) showed a methine proton singlet at \(\delta\) 4.41. The aromatic H-4 and H-7 together resonated at \(\delta\) 7.45 (dd, \(J_{4,5} = 15.1\) Hz, \(J_{4,6} = 7.4\) Hz, \(J_{4,7} = 1.4\) Hz), while H-5 and H-6 together resonated as a doublet of double doublet at \(\delta\) 7.05 (\(J_{7,6} = 13.3\) Hz, \(J_{7,5} = 7.2\) Hz, \(J_{7,4} = 4.1\) Hz). The aromatic H-5', -6' and -7' of ring C containing nitro group collectively appeared as a multiplet at \(\delta\) 7.28, while the C-4' proton resonated as a double doublet at \(\delta\) 7.88 (\(J_{4',5'} = 8.3\) Hz, \(J_{4',6'} = 1.2\) Hz).

These spectral studies led to the structure determination of benzimidazole derivative 141 as 2-(2-nitrobenzyl)-1H-benzimidazole.
Table-4.2.2: $^1$H-NMR Data of 2-(2-nitrobenzyl)-1H-benzimidazole (141).

<table>
<thead>
<tr>
<th>Hydrogen</th>
<th>(δ, $J$=Hz)</th>
<th>Hydrogen</th>
<th>(δ, $J$=Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>--</td>
<td>7a</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>--</td>
<td>1'</td>
<td>4.41 s</td>
</tr>
<tr>
<td>3</td>
<td>--</td>
<td>2'</td>
<td>--</td>
</tr>
<tr>
<td>3a</td>
<td>--</td>
<td>3'</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>7.45 ddd ($J=15.1, 7.4, 1.4$)</td>
<td>4'</td>
<td>7.88 dd, ($J_{r,s}=8.3, J_{r,0}=1.2$)</td>
</tr>
<tr>
<td>5</td>
<td>7.05 ddd ($J=13.3, 7.2, 4.1$)</td>
<td>5'</td>
<td>7.28 m</td>
</tr>
<tr>
<td>6</td>
<td>7.05 ddd ($J=13.3, 7.1, 4.1$)</td>
<td>6'</td>
<td>7.28 m</td>
</tr>
<tr>
<td>7</td>
<td>7.45 ddd ($J=15.1, 7.4, 1.4$)</td>
<td>7'</td>
<td>7.28 m</td>
</tr>
</tbody>
</table>

4.2.2 Synthesis of Benzisoxazole Derivatives

The synthesis of benzisoxazole of quinoxaline and benzimidazole derivatives was accomplished after the dehydration of compounds 140 and 141 in 10% methanolic solution of potassium hydroxide for two and a half hours, affording the corresponding benzisoxazoles 142 and 143 in 51% and 53% yields, respectively (Scheme-4.2.1, page 137).

4.2.2.1 3-(1,2-Benzisoxazole-3-yl)-2(1H)-quinoxalinone (142)

The corresponding benzisoxazole of quinoxaline derivative, 3-(1,2-benzisoxazole-3-yl)-2(1H)-quinoxalinone (142), showed the molecular ion at m/z 263 (EI-MS), which was confirmed by FD-MS (m/z 263). The IR spectrum showed the absence of any NO$_2$ group as compared to the starting quinoxaline (140), while absorptions at 3230 (NH),
1667 (C=O) and 1563 cm\(^{-1}\) were observed. The UV spectrum of 142 showed absorptions at 387, 334 and 271 nm.

The \(^1\)H-NMR spectrum (CDCl\(_3\), 400 MHz) of compound 142 showed a multiplet for the aromatic H-8 and H-5 of quinoxaline ring at \(\delta\) 7.34, while the aromatic H-6 and H-7 resonated at \(\delta\) 7.58 also as a multiplet. The aromatic H-4\(^\prime\) and H-7\(^\prime\) of benzisoxazole nucleus appeared as doublets at \(\delta\) 8.34 (\(J_{4',5'} = 8.9\) Hz) and 8.01 (\(J_{7',6'} = 8.2\) Hz), respectively. The remaining protons (H-6\(^\prime\) and H-5\(^\prime\)) appeared as a multiplet at \(\delta\) 7.81.

![Chemical Structure](image)

**Table-4.2.3:** \(^1\)H-NMR Data of 3-(1,2-benzisoxazole-3-yl)-2(1H)-quinoxalinone (142).

<table>
<thead>
<tr>
<th>Hydrogen</th>
<th>((\delta, J = Hz))</th>
<th>Hydrogen</th>
<th>((\delta, J = Hz))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>--</td>
<td>1(^\prime)</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>--</td>
<td>2(^\prime)</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>--</td>
<td>3(^\prime)</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>--</td>
<td>3(^\prime)(^a)</td>
<td>--</td>
</tr>
<tr>
<td>4(^a)</td>
<td>--</td>
<td>4(^\prime)</td>
<td>8.34 d ((J_{4',5'} = 8.9))</td>
</tr>
<tr>
<td>5</td>
<td>7.34 m</td>
<td>5(^\prime)</td>
<td>7.81 m</td>
</tr>
<tr>
<td>6</td>
<td>7.58 m</td>
<td>6(^\prime)</td>
<td>7.81 m</td>
</tr>
<tr>
<td>7</td>
<td>7.58 m</td>
<td>7(^\prime)</td>
<td>8.01 d ((J_{7',6'} = 8.2))</td>
</tr>
<tr>
<td>8</td>
<td>7.34, m</td>
<td>7(^a)</td>
<td>--</td>
</tr>
<tr>
<td>8(^a)</td>
<td>--</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
These spectroscopic studies led to the deduction of structure 142 as 3-(1,2-benzisoxazole-3-yl)-2(1H)-quinoxalinone.

4.2.2.2 3-(1H-Benzimidazole-2-yl)-2,1-benzisoxazole (143)

The benzimidazole benzisoxazole derivative 143 was identified as 3-(1H-benzimidazole-2-yl)-2,1-benzisoxazol. Compound 143 showed the M⁺ at m/z 235, which was further confirmed by FD-MS. The IR spectrum showed NH absorption at 3335, while the nitro group absorption was not observed in the IR spectrum, indicating the formation of a benzisoxazole ring. The UV spectrum showed absorptions at 367, 324, 265 and 194 nm.

The ¹H-NMR spectrum (CDCl₃, 400 MHz) of compound 143 showed signals for aromatic H-4 and H-7 at δ 7.90 (dt, J = 7.1 Hz) and 7.65 (dt, J = 9.1 Hz), respectively. The H-5 and H-6 appeared as multiplets at δ 7.35 and 7.41, respectively. Aromatic proton H-7' of benzisoxazole nucleus resonated as a doublet at δ 7.60 (J₇',₆ = 7.2 Hz), while H-5' and H-6' together resonated as a doublet of double doublet at δ 7.21 (J = 14.1, 8.3, 2.1 Hz). The remaining proton (H-4') appeared at δ 8.39 (dt, J = 8.9 Hz).
Table-4.2.3: $^1$H-NMR Data of 3-(1H-benzimidazole-2-yl)-2,1-benzisoxazole (143).

<table>
<thead>
<tr>
<th>Hydrogen</th>
<th>(δ, J = Hz)</th>
<th>Hydrogen</th>
<th>(δ, J = Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>--</td>
<td>1'</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>--</td>
<td>2'</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>--</td>
<td>3'</td>
<td>--</td>
</tr>
<tr>
<td>3a</td>
<td>--</td>
<td>3'a</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>7.90 dt (J = 7.1)</td>
<td>4'</td>
<td>8.39 dt (J = 8.9)</td>
</tr>
<tr>
<td>5</td>
<td>7.35 m</td>
<td>5'</td>
<td>7.21 ddd (J = 14.1, 8.3, 2.1)</td>
</tr>
<tr>
<td>6</td>
<td>7.41 m</td>
<td>6'</td>
<td>7.21 ddd (J = 14.1, 8.3, 2.1)</td>
</tr>
<tr>
<td>7</td>
<td>7.65 dt (J = 9.1)</td>
<td>7'</td>
<td>7.60 d (J $\gamma$ = 7.2)</td>
</tr>
<tr>
<td>7a</td>
<td>--</td>
<td>7'a</td>
<td>--</td>
</tr>
</tbody>
</table>

4.2.3 5H-Quinoxalino[2,1-b] quinazolin-6,12-dione (144)

Thermal and photochemical rearrangements of benzisoxazole derivative 5 afforded a single isolable rearranged product 144 in 24% and 63% yields, respectively.

![5H-Quinoxalino[2,1-b] quinazolin-6,12-dione (144)](image)

The corresponding benzisoxazole of quinoxaline 142, furnished the quinazolinone derivative 144 on thermal rearrangement via a ketene intermediate (path b, Scheme-4.2.2, page-144), as a white amorphous powder in a 24% yield.
The rearranged product 144 showed the $M^+$ at $m/z$ 263.0186 in HREI-MS corresponding to the molecular formula $\text{C}_{16}\text{H}_9\text{N}_3\text{O}_2$ (calcld. 263.0119). The UV spectrum showed absorptions at 387, 271 and 219 nm. The IR spectrum exhibited absorptions at 335 (NH) and 1695 (C=O) cm$^{-1}$.

The proposed structure 144 was deduced from the $^1$H- and $^{13}$C-NMR spectra. The $^1$H-NMR spectrum showed signals for eight aromatic protons. Two doublets at $\delta$ 8.22 ($J_{1,2} = 1.0$ Hz) and 8.97 ($J_{11,10} = 1.1$ Hz) were assigned to H-1 and H-11, respectively. The aromatic protons H-4 and H-8 resonated as double doublets at $\delta$ 7.69 ($J_{4,3} = 8.2$ Hz, $J_{4,2} = 1.5$ Hz) and 7.46 ($J_{8,9} = 7.1$ Hz, $J_{8,10} = 1.7$ Hz), respectively. The $^{13}$C-NMR spectrum indicated the presence of 15 carbons in the molecule. The carbonyl carbons resonated at $\delta$ 164.7 (C-6) and 169.75 (C-12), respectively.
Table-4.2.4: $^1$H-NMR data of 5H-quinoxalino[2,1-b] quinazolin-6,12-dione (144).

<table>
<thead>
<tr>
<th>Hydrogen</th>
<th>$\delta, J$ (Hz)</th>
<th>Hydrogen</th>
<th>$\delta, J$ (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.22 d ($J_{1,2} = 1.0$)</td>
<td>7a</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>7.04 m</td>
<td>8</td>
<td>7.46 dd ($J_{8,9} = 7.1, J_{8,10} = 1.7$)</td>
</tr>
<tr>
<td>3</td>
<td>7.19 m</td>
<td>9</td>
<td>7.81 m</td>
</tr>
<tr>
<td>4</td>
<td>7.69 dd, ($J_{4,1} = 8.2, J_{4,2} =1.5$)</td>
<td>10</td>
<td>7.08 m</td>
</tr>
<tr>
<td>4a</td>
<td>--</td>
<td>11</td>
<td>8.97 d ($J_{11,10} = 1.1$)</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>11a</td>
<td>--</td>
</tr>
<tr>
<td>6</td>
<td>--</td>
<td>12</td>
<td>--</td>
</tr>
<tr>
<td>6a</td>
<td>--</td>
<td>13</td>
<td>--</td>
</tr>
<tr>
<td>7</td>
<td>--</td>
<td>13a</td>
<td>--</td>
</tr>
</tbody>
</table>

The corresponding benzisoxazole derivative of quinoxaline 142 on photochemical rearrangement afforded the same rearranged product, obtained through thermal rearrangement reaction in 63%, yield. The rearranged products were identified collectively on the basis of mass and $^1$H- and $^{13}$C-NMR spectroscopic techniques.

4.2.4 5a,12a-Dihydrobenzimidazo[2,1-b] quinazolin-12(6H)-one (145)

Similarly the corresponding benzisoxazole derivative of benzimidazole 143 was subjected to thermal and photochemical rearrangement reactions (path b) and an isolable rearranged product 145 in 33% and 53% yields, respectively, was obtained.

The rearranged product 145 showed the M$^+$ at m/z 235.0730 in HREI-MS, corresponding to the formula C$_{14}$H$_9$N$_3$O (calcd. 235.0728). The UV spectrum showed absorptions at 393, 290 and 223 nm, while the IR spectrum 145 exhibited absorptions at 3343 (NH) and 1675 (C=O) cm$^{-1}$.
The structure of the thermal rearranged product 145 was inferred from the $^1$H- and $^{13}$C-NMR spectroscopic studies. The $^1$H-NMR spectrum of 145 showed signals for eight aromatic protons. Two double doublets at $\delta$ 7.69 ($J_{7.8} = 8.2$ Hz, $J_{7.9} = 1.5$ Hz) and 8.22 ($J_{10.9} = 8.6$ Hz, $J_{10.8} = 1.1$ Hz) were assigned to H-7 and H-10, respectively. The aromatic protons H-8 and H-9 resonated as multiplets at $\delta$ 7.19 and 7.04, respectively. Other aromatic protons, H-2 and H-3, appeared as multiplets at $\delta$ 7.80 and 7.08 while H-1 and H-4 resonated at $\delta$ 7.34 (dd, $J_{1.2} = 7.1$ Hz, $J_{1.3} = 1.2$ Hz) and 8.97 (br s), respectively. The $^{13}$C-NMR spectrum indicated the presence of 14 carbons in the molecule including a carbonyl carbon at $\delta$ 174.7.

The corresponding benzisoxazole derivative of compound 142 was also subjected to photochemical rearrangement, which afforded the same rearranged product 145 in a 63% yield. The rearranged product was identified on the basis of mass spectroscopy, and $^1$H- and $^{13}$C-NMR spectroscopic techniques.
Table 4.2.6: $^1$H-NMR Data of 5a,12e-dihydrobenzimidazo[2,1-b]quinazolin-12 (6H)-one (8).

<table>
<thead>
<tr>
<th>Hydrogen</th>
<th>$\delta, J = \text{Hz}$</th>
<th>Hydrogen</th>
<th>$\delta, J = \text{Hz}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.34 dd ($J_{1,7} = 7.1, J_{1,3} = 1.2$)</td>
<td>7</td>
<td>7.69 dd ($J_{7,8} = 8.2, J_{7,9} = 1.5$)</td>
</tr>
<tr>
<td>2</td>
<td>7.80 m</td>
<td>8</td>
<td>7.19 m</td>
</tr>
<tr>
<td>3</td>
<td>7.08 m</td>
<td>9</td>
<td>7.04 m</td>
</tr>
<tr>
<td>4</td>
<td>8.97 br s</td>
<td>10</td>
<td>8.22 dd ($J_{10,9} = 8.6, J_{10,8} = 1.1$)</td>
</tr>
<tr>
<td>4a</td>
<td>--</td>
<td>10a</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>11</td>
<td>--</td>
</tr>
<tr>
<td>5a</td>
<td>--</td>
<td>12</td>
<td>--</td>
</tr>
<tr>
<td>6</td>
<td>--</td>
<td>12a</td>
<td>--</td>
</tr>
<tr>
<td>6a</td>
<td>--</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.0 EXPERIMENTAL

5.1 General Experimental Condition

5.1.1 Instrumentation

The ultraviolet absorption spectra were measured on a Hitachi U3200 spectrophotometer, using spectroscopic grade methanol. Infrared spectra were recorded on a Shimadzu FTIR-460 or on a JASCO AR-320 IR spectrophotometers. Optical rotations were measured on a JASCO DIP-360 digital polarimeter in methanol. The melting points were determined in glass capillary tubes using a Buchi 535 melting point apparatus. Mass spectra were recorded on a double focusing, Varian MAT 312 mass spectrometer. The FAB and HREI-MS were measured on Jeol JMS 600 and HX 110 mass spectrometers. The $^1$H-NMR spectra were recorded on Bruker AM 400 and AMX 500 NMR spectrometers using UNIX data system at 300 and 500 MHz, respectively, while the $^{13}$C- NMR spectra were recorded at 75 and 125 MHz, respectively on the same instruments using CDCl$_3$.

5.1.2 Chromatography

Column chromatography (CC) was performed on silica gel (E. Merck, type-60, 70-230 mesh). Preparative thin-layer chromatography was performed using precoated silica gel glass plates (GF-254, 20 $\times$ 20 cm) (E. Merck, 0.2 mm thickness). Thin layer chromatography was carried out using solvent system, pet. ether : acetone and a few drops of diethylamine. The sample purity was checked on TLC plates under UV light (254 and 366 nm). Dragendorff’s spraying reagent was used to stain alkaloidal compounds while
saturated solution of ceric sulphate in 60% sulphuric acid and iodine crystals were used for the detection of non-alkaloidal constituents.

5.2 Sarcococca saligna

5.2.1 Plant Material

5.2.1.1 Collection and Identification of Plant Material

The whole plants of Sarcococca saligna (D. Don.) Muell (30 kg) were collected in October 2000 from the District Bagh of Azad Kashmir, (Pakistan) by Dr. M. Riaz Khan, Assistant Professor Government College Bagh and it was identified by Mr. Tahir Ali, Taxonomist, Department of Botany, University of Karachi, Pakistan. A voucher specimen was deposited in the Herbarium of the University of Karachi (KU # 19290).

5.2.1.2 Extraction and Isolation

The air-dried whole plant (30 Kg) was crushed and dissolved in 50 Lit. of ethanol:water mixture (8:2) for 20 days. The ethanolic extract (1.78 Kg) was filtered and concentrated under vacuum. The concentrated EtOH extract was then dissolved in water and defatted with pet. ether (251 g). After the removal of fatty material, aqueous layer was extracted with CHCl₃ at pH 5 and pH 9 to afford two fractions B (24.5 g) and C (70.54 g), respectively. The remaining aqueous layer was then extracted with AcOEt (385.6 g) and finally with butanol (260.7 g) (Scheme-5.1). The crude extract showed anti-cholinesterase activity. All fractions obtained from the solvent-solvent extraction were evaluated for anticholinesterase activity against both enzymes: acetylcholinesterase and butyrylcholinesterase; only the CHCl₃ fractions B and C exhibited activity against this enzyme. The CHCl₃ extract B was subjected to flash column chromatography on silica
**Scheme-5.1:** Extraction and Fractionation of *Sarcococca saligna*. 

*General Experimental*

**Sarcococca saligna**  
(Whole plant)  
30 kg

Extracted with EtOH-H₂O (8 : 2)  
50 lit. (20 days) and evaporated under vacuum

Crude EtOH extract  
(1.786 Kg)

Dissolved in dist. water (5.0 lit.)

Soluble

Insoluble

Extraction with Pet. ether (20 lit.)

Pet. ether extract  
(251 g)

Aqueous extract

Extraction with Chloroform at pH 5 and 9 (20 lit.)

Chloroform extract  
(24.5 g) B

Chloroform extract  
(70.5 g) C

Aqueous extract

Extraction with AcOEt (20 lit.)

Aqueous extract

Extraction with Butanol (20 lit.)

Aqueous extract

Ethyl acetate extract  
(385.6 g)

Butanol extract  
(260.7 g)
gel by using increasing polarities of pet. ether: acetone: diethylamine to afford subfractions B1-B4, which showed significant inhibition. The purification of compounds from subfractions B1-B4 was carried out by flash column chromatography followed by purification on prep. TLC (precoated silica gel glass plates) with increasing polarities of solvent mixture, pet. ether: acetone: diethylamine (Scheme-5.2, page 151). Fraction C was similarly fractionated and eight subfractions C1-C8 were obtained which on purification by repeated column chromatography or preparative TLC yielded the pure active components (Schemes-5.3 and -5.4).

Scheme-5.2: Isolation of Alkaloids from Chloroform Extract (pH 5) of Sarcococca saligna.
5.2.2 Spectral Data of New Steroidal Alkaloids from *Sarcococca saligna*

5.2.2.1 Salonine-A (69)

The fraction C (70.54 g) obtained from the extraction of aqueous extract of *Sarcococca saligna* with chloroform at pH 9 (Scheme-5.1, page 150), was subjected to repeated column chromatography (silica gel) by using increasing polarities of pet. ether: acetone (60:40) with a few drops of diethylamine. Several subfractions were thus obtained. Subfraction C-7 was subjected to column chromatography on (silica gel), afford the pure alkaloid 69 as a yellowish gum (Scheme-5.4, page 157).

![Molecular structure of Salonine-A](image)

**IUPAC Name:** (20S)-20-(N,N-Dimethylamino)-3β-(tigloylamine)-5α-pregn-14-en-2β,4β-diol

**State:** Yellowish gum

**Yield:** 21.03 mg, 1.50 × 10⁻⁴%

**Rf:** 0.345 [pet. ether: acetone (7:3), 4 drops of diethylamine in 10 mL]

[α]D²⁰: 60° (c = 0.03, MeOH)

**UV (MeOH) λmax nm (log ε):** 212 (2.66), λmax nm (log ε): 194 (1.72), 365 (0.76)

**IR (CHCl₃) νmax cm⁻¹:** 3650 (NH), 3328 (OH), 2910 (=CH), 1623 (C=O)

**EI-MS m/z (rel. int. %):** 458 (5.6) [M⁺], 443 (70) [M⁺-15], 100 (24.1), 84 (37), 72 (100)

**FD-MS:** m/z 458 (C₂₆H₄₆N₂O₃)
HR ESI-MS  $m/z$ (formula, calc'd): 458.3489 (C$_{28}$H$_{46}$N$_{2}$O$_{3}$, 458.3508), 443.3233 (C$_{27}$H$_{43}$N$_{2}$O$_{3}$, 443.3273), 83.0504 (C$_{3}$H$_{7}$NO, 83.0496), 72.081 1 (C$_{4}$H$_{10}$N, 72.0813)

$^1$H-NMR (CDCl$_3$): $\delta$ see Table-2.5.1

$^{13}$C-NMR (CDCl$_3$): $\delta$ see Table-2.5.1

5.2.2.2 Salonine-B (74)

The chloroform fraction B obtained from the extraction of the aqueous extract with chloroform at pH 5 (Scheme-5.1, page 150), was subjected to repeated column chromatography on silica gel by using increasing polarities of pet. ether: acetone: diethylamine, to afford several subfractions. The subfraction B-1, when subjected to column chromatography, afforded the pure alkaloid 74 as a colorless amorphous powder (Scheme-5.2, page 151).

![Salonine-B chemical structure]

**IUPAC Name:** (20S)-20-(N,N-Dimethylamino)-3β-methoxy-pregn-5,16-diene

**State:** Colorless amorphous powder

**Yield:** 6.3 mg, 3.53 $\times$ 10$^{-4}$ %

$R_f$: 0.64 [pet. ether: acetone (9:1), 3 drops of diethylamine in 10 mL]

$[\alpha]$$_{D}^20$: -116° ($c = 0.04$, MeOH)

**UV (MeOH)** $\lambda_{max}$ nm (log $\varepsilon$): 206 (2.12), $\lambda_{min}$ nm (log $\varepsilon$): 196 (1.78), 359 (0.91)
IR (CHCl₃) ν_max cm⁻¹: 3650 (NH), 2910 (=CH)

EI-MS m/z (rel. int. %): 357 (11.6) [M⁺], 342 (100) [M⁺-15], 72 (74.7)

FD-MS: m/z 357 (C₂₄H₃₉NO)

HREI-MS m/z (formula, calcd.): 357.2972 (C₂₄H₃₉NO, 357.3031), 342.2741 (C₂₃H₃₀NO, 342.2796), 72.0811 (C₄H₁₀N, 72.0813)

¹H-NMR (CDCl₃): δ see Table-2.5.2

¹³C-NMR (CDCl₃): δ see Table-2.5.2

Scheme-5.3: Isolation of Alkaloids from Chloroform Extract (pH 5) of Sarcocoea saligna
5.2.2.3 Salonine-C (77)

The fraction C-5 (Scheme-5.4, page 157) obtained from the chloroform extract of the plant afforded several subfractions after repeated column chromatography (silica gel) by using increasing polarities of pet. ether: acetone and a few drops of diethylamine. One of the subfractions, C-5-5 yielded two colourless amorphous compounds, 77 and 82, when subjected to preparative thin layer chromatography.

![Chemical structure of Salonine-C](image)

**IUPAC Name:** (20S),20-(N,N-Dimethylamino)-3β-(tigloylamino)-pregn-4,14-diene

**State:** Colourless amorphous powder

**Yield:** 12.5 mg, 7.02 × 10⁻⁴ %

**Rf:** 0.54 [pet. ether: acetone (8.3:1.7), 4 drops of diethylamine in 10 mL]

**M.P.:** 142-144°C

**[α]D²⁰:** -120° (c = 0.048, MeOH)

**UV (MeOH) λmax nm (log ε):** 206 (2.23), λmin nm (log ε): 192 (1.34), 364 (0.67)

**IR (CHCl₃) νmax cm⁻¹:** 3650 (NH), 3391 (CH), 2810 (=CH), 1601 (C=O)

**El-MS m/z (rel. int. %):** 424 (3.0) [M⁺], 409 (25) [M⁺-15], 100 (7.6), 83 (39.2), 72 (100)

**FD-MS:** m/z 424 (C₂₉H₃₄N₂O)
HREI-MS \( m/z \) (formula, calcd.): 424.33489 (C\(_{28}\)H\(_{44}\)N\(_2\)O, 424.3508), 409.3233 (C\(_{27}\)H\(_{41}\)N\(_2\)O, 409.3273), 72.0811 (C\(_{4}\)H\(_{10}\)N, 72.0813)

\(^1\)H-NMR (CDCl\(_3\)): \( \delta \) see Table-2.5.3

\(^{13}\)C-NMR (CDCl\(_3\)): \( \delta \) see Table-2.5.3

5.2.2.4 2,3-Dehydrorsalsigennone (82)

The chloroform extract C, obtained by extraction of the aqueous extract with chloroform at pH 9 (Scheme-5.1, page 154), which afforded several subfractions after column chromatography (silica gel) using increasing polarities of solvent system pet. ether: acetone. Subfraction C-5 was subjected to repeated column chromatography, afforded subfraction C-5-5, yielded the compound 82 along with the compound 77, by using preparative thin layer chromatographic technique (Scheme-5.4, page 157).

IUPAC Name: (20S)-20-(N,N-Dimethylamino)-3\(\beta\)-(tigloylamino)-pregn-2,5-diene-4-one

State: White amorphous solid

Yield: 16.8 mg, 9.43 \( \times \) 10\(^{-4}\) %

\( R_f \): 0.63 [pet. ether: acetone (8.5:1.5), 4 drops of diethylamine in 10 mL]

M.P.: 162-163° C

\([\alpha]\)\(^{23}\)\(\text{D} \): -38° (c = 0.048, MeOH)

UV (MeOH) \( \lambda_{\text{max}} \) nm (log \( \varepsilon \)): 234 (2.32), \( \lambda_{\text{min}} \) nm (log \( \varepsilon \)): 201 (1.32), 368 (0.67)
Sarcococca saligna

IR (CHCl₃) ν_max cm⁻¹: 3390 (NH), 2929 (=CH), 1723 (α,β-unsaturated ketonic group), 1664 (C=O), 1511 (C=CH)

EI-MS m/z (rel. int. %): 438 (6.1) [M⁺], 423 (48.9) [M⁺-15], 83 (39.2), 72 (100)

FD-MS: m/z 438 (C₂₈H₄₂N₂O₂)

HREI-MS m/z (formula, calcld.): 438.3284 (C₂₈H₄₂N₂O₂, 438.3246), 423.3042 (C₂₇H₃₉N₂O₂, 423.3011), 72.0706 (C₄H₁₀N, 72.0734)

¹H-NMR (CDCl₃): δ see Table-2.5.4

¹³C-NMR (CDCl₃): δ see Table-2.5.4

**Scheme-5.4: Isolation of Alkaloids from Chloroform Extract (pH 9) of Sarcococca saligna**
5.2.2.5 \textit{N-Methylformamidesaloline-B} (87)

The chloroform extract (fraction C) at pH 9 (Scheme-5.1, page 150) afforded several subfractions after repeated column chromatography (silica gel), subfraction C-1 obtained by using pet. ether: acetone (85:5), with a few drops of diethylamine. Subfraction C-1 (Scheme-5.3, page 154) was subjected to column chromatography to afford a pure alkaloid 87 as a yellowish gum.

\begin{center}
\begin{tikzpicture}

\end{tikzpicture}
\end{center}

\textbf{IUPAC Name:} (20S)-20-(\textit{N-Methylformamide})-3\beta\textit{-methoxy-pregn-5,16-diene}

State: Yellowish gum

Yield: 4.8 mg, 2.69 $\times$ 10^{-4} %

$R_s$: 0.645 [pet. ether: acetone (7:6:2.4), 3 drops of diethylamine in 10 mL]

[$\alpha$]$_{D}^{20}$: -21° ($c = 0.075$, MeOH)

\textbf{UV (MeOH)} $\lambda_{\text{max}}$ nm (log $\varepsilon$): 205 (3.32), $\lambda_{\text{min}}$ nm (log $\varepsilon$): 193 (2.19), 363 (1.11)

\textbf{IR (CHCl$_3$)} $\nu_{\text{max}}$ cm$^{-1}$: 3374 (CH), 2928 (CH), 1601 (C=O)

\textbf{EI-MS \textit{m/z} (rel. int. %)}: 371 (3) [M$^+$], 356 (100) [M$^+$-15], 58 (14)

\textbf{FD-MS: }\textit{m/z} 371 (C$_{24}$H$_{37}$NO$_2$)

\textbf{HREI-MS \textit{m/z} (formula, calcd.):} 371.2980 (C$_{24}$H$_{33}$NO$_2$, 371.2982), 356.3233 (C$_{23}$H$_{34}$NO$_2$, 356.3273), 58.0706 (C$_{22}$H$_{34}$NO, 58.0734)

$^1$H-NMR (CDCl$_3$): δ see Table-2.5.5

$^{13}$C-NMR (CDCl$_3$): δ see Table-2.5.5
5.2.2.6 Salignurine-F (90)

The subfraction C-6 obtained after repeated column chromatography of chloroform extract C (pH 9) (silica gel) by using solvent system pet. ether: acetone (70: 30) as eluent (Scheme-5.4, page 157) was further subjected to column chromatography to afford a pure alkaloid 90, as a yellowish gum.

\[ \text{UPAC Name: } (2\text{S})-20-(N,N-\text{Dimethylamino})-3\beta-(\text{tigloylamino})-\text{pregn}-4\beta-\text{hydroxy}-5\text{-ene} \]

State: Yellowish gum

Yield: 5.8 mg, 3.25 \times 10^{-4} \%

Rf: 0.345 [pet. ether: acetone (7:3), 5 drops of diethylamine in 10 mL]

[\alpha]^{20}_D: -71^\circ (c = 0.014, \text{MeOH})

\textbf{UV (MeOH)} \lambda_{\text{max}} \text{ nm (log e): } 203 (2.61), \lambda_{\text{min}} \text{ nm (log e): } 195 (1.32), 359 (0.67)

\textbf{IR (CHCl}_3) \nu_{\text{max}} \text{ cm}^{-1}: 3650 (\text{NH}), 3328 (\text{OH}), 2931 (=\text{CH}), 1612 (C=O), 1450 (C=CH)

\textbf{EI-MS} \text{ m/z (rel. int. %): } 442 (6.1) [M^+] , 427 (10) [M^+-15], 83 (57), 72 (100)

\textbf{FD-MS:} \text{ m/z 442 (C}_{28}\text{H}_{40}\text{N}_{2}\text{O}_2)

\textbf{HREI-MS} \text{ m/z (formula, calc'd): } 442.3577 \text{ (C}_{28}\text{H}_{46}\text{N}_{2}\text{O}_2), 442.3559, 427.3233 \text{ (C}_{27}\text{H}_{43}\text{N}_{2}\text{O}_2, 427.3273), 72.0811 \text{ (C}_{4}\text{H}_{10}\text{N}, 72.0734)
$^1$H-NMR (CDCl$_3$): δ see Table-2.5.6

$^{13}$C-NMR (CDCl$_3$): δ see Table-2.5.6

5.2.2.7 5,14-Dehydro-$N_d$-demethylsaracoline (95)

The chloroform extract C (70.54 g) was subjected to column chromatography on silica gel by using increasing polarities of solvent system, pet. ether: acetone; diethylamine, to afford several subfractions. Subfraction C-2 obtain from chloroform extract C after column chromatography (silica gel) using pet. ether: acetone (90:10) as eluent, was subjected to further repeated column chromatography afforded a pure alkaloid 95 as a yellowish gum.

[Image of the molecule]

IUPAC Name: 3β-(N$_d$-Methylamino)-20S-(N$_d$-acetyl-N$_d$-methylamino)-pregn-5,14-diene

State: Yellowish gum

Yield: 10.8 mg, 6.06 × 10$^{-4}$%

R$_f$: 0.64 [pet. ether: acetone (8.5:1.5), 3 drops of diethylamine in 10 mL]

[$\alpha$]$^{20}_{D}$: -23° ($c = 0.23$, MeOH)

UV (MeOH) $\lambda_{max}$ nm (log ε): 213 (2.32), $\lambda_{min}$ nm (log ε): 196 (1.56), 367 (0.97)

IR (CHCl$_3$) $\nu_{max}$ cm$^{-1}$: 3407 (NH), 2934 (–CH), 1632 (C=O), 1446 (C=CH)

EI-MS $m/z$ (rel. int. %): 384 (40) [M$^+$], 369 (18) [M$^+$-15], 341 (12), 100 (33.5), 70 (100)

FD-MS: $m/z$ 384 (C$_{25}$H$_{40}$N$_2$O)
IREI-MS *m/z* (formula, calcd.): 384.3158 (C_{23}H_{40}N_{2}O, 384.3140), 369.3004 (C_{24}H_{37}N_{2}O, 369.3062), 70.0713 (C_{4}H_{8}N, 70.0656)

$^1$H-NMR (CDCl$_3$): δ see Table-2.5.7

$^{13}$C-NMR (CDCl$_3$): δ see Table-2.5.7

5.2.2.8 14-Dehydro-$N_8$-demethylsarcodine (98)

The aqueous extract was extracted with chloroform at pH 9, afforded chloroform fraction C (Scheme-5.1, page 150). The fraction C afforded several subfractions after subjected to column chromatography (silica gel) using increasing polarities of solvent system pet. ether: acetone. Subfraction C-2 afforded a pure alkaloid 98 as a yellowish gum along with the compound 95 after repeated column chromatography on silica gel by using increasing polarities of pet. ether: acetone: diethylamine (Scheme-5.3, page 154).

![Chemical structure](image)

IUPAC Name: 3β-($N_6$-Methylamino)-20$\delta$-($N_6$-acetyl-$N_8$-methylamino)-5α-pregn-14-ene

State: Yellowish gum

Yield: 13.2 mg, 7.41 × 10$^{-4}$%

$R_f$: 0.343 [pet. ether: acetone (8:2), 4 drops of diethylamine in 10 mL]

$[\alpha]^{20}D$: -16° (c = 0.13, MeOH)

UV (MeOH) $\lambda_{max}$ nm (log ε): 212 (2.02), $\lambda_{min}$ nm (log ε): 193 (1.63), 361 (1.01)

IR (CHCl$_3$) $v_{max}$ cm$^{-1}$: 3421 (NH), 2923 (–CH$_3$), 1640 (C=O), 1456 (C=CH)
EI-MS $m/z$ (rel. int. %): 386 (35) [M$^+$], 371 (13) [M$^+$-15], 100 (33.5), 70 (100)

FD-MS: $m/z$ 386 ($C_{25}H_{42}N_2O$)

HREI-MS $m/z$ (formula, calc.): 386.3489 ($C_{25}H_{42}N_2O$, 384.3559), 371.3104 ($C_{24}H_{39}N_2O$, 371.3152), 70.0713 ($C_4H_8N$, 70.0656)

$^1$H-NMR (CDCl$_3$): $\delta$ see Table-2.5.8

$^{13}$C-NMR (CDCl$_3$): $\delta$ see Table-2.5.8

5.2.2.9 16-Dehydrosaracorine (101)

Extraction of the aqueous layer with chloroform at pH 9 afforded chloroform extract which was subjected to column chromatography on silica gel by using increasing polarities of solvent system pet. ether: acetone: diethylamine yield several subfractions. Subfraction C-4 was subjected to column chromatography on silica gel using pet. ether: acetone as eluent to obtain a pure alkaloid 101 as a yellowish gum.

![Chemical structure](image)

IUPAC Name: (20S)-20-(N,N-Dimethylamino)-3β-(N$_\gamma$-acetylamido)-5α-pregn-16-ene

State: Yellowish gum

Yield: 9.6 mg, 5.39 x 10^{-4} %

$R_f$: 0.46 [pet. ether: acetone (8:2), 3 drops of diethylamine in 10 mL]

$[\alpha]^{20}_D$: -58° ($c = 0.05$, MeOH)

UV (MeOH) $\lambda_{max}$ nm (log $\varepsilon$): 202 (1.65), $\lambda_{min}$ nm (log $\varepsilon$): 191 (1.01), 365 (0.56)
IR (CHCl₃) νmax cm⁻¹: 3350 (NH), 2993 (=CH), 1665 (C=O), 1546 (C=CH)
EI-MS m/z (rel. int. %): 386 (25) [M⁺], 371 (100) [M⁺-15], 326 (13), 100 (33.5), 72 (67)
FD-MS: m/z 386 (C₂₅H₄₂N₂O)
HREI-MS m/z (formula, calcld.): 386.3060 (C₂₅H₄₀N₂O₂, 386.2994), 371.3114 (C₂₁H₂₆N₂O, 371.3009), 72.0713 (C₅H₁₀N, 72.0656)
¹H-NMR (CDCl₃): δ see Table-2.5.9
¹³C-NMR (CDCl₃): δ see Table-2.5.9

5.2.3 Spectral Data of Known Steroidal Alkaloids from Sarcococca saligna

5.2.3.1 Salignarine-C (104)

The sub-fraction C-6-4-1 obtained by repeated column chromatography of chloroform extract C (pH 9) (Scheme-5.4, page 157) yielded compound 104 as a white amorphous material after repeated column chromatography on silica gel by using increasing polarities of solvent system, pet ether: acetone (70:30), with a few drops of diethylamine.

IUPAC Name: (20S)-20-(N,N-Dimethylamino)-3β-(seneclidylamino)-pregn-2β-hydroxy-5-ene
State: White amorphous powder

Yield: 5.6 mg, $3.14 \times 10^{-4}$ %

**Rf** 0.28 [pet. ether: acetone (7.5:2.5), 4 drops of diethyl amine in 10 mL] [Lit. value: 0.36 (pet. ether: acetone: diethylamine 14:5:1)]

$[\alpha]^{20}_D = -15^\circ (c = 1.16, \text{MeOH}),$ [Lit. value: $-12^\circ (c = 0.6, \text{CHCl}_3)]$

**UV (MeOH)** $\lambda_{\text{max}} \text{nm (log } e) = 205 (2.52), \lambda_{\text{min}} \text{nm (log } e) = 195 (1.98), 361 (0.87)$

**IR (CHCl}_3\text{) } v_{\text{max}} \text{cm}^{-1}: 3345 (\text{NH}), 3330 (\text{OH}), 2931 (\text{=CH}), 1645 (\text{C=O})$

**EI-MS** $m/z$ (rel. int. %): 442 (5.6) $[M^+]$, 428 (70) $[M^+-15]$, 72 (100)

**FD-MS:** $m/z$ 442 (C$_{28}$H$_{46}$N$_2$O$_2$)

**HREI-MS** $m/z$ (formula, calcd.): 442.3556 (C$_{28}$H$_{46}$N$_2$O$_2$, 442.3566)

**$^1$H-NMR (CDCl$_3$):** 8 0.78 (3H, s, CH$_3$-18), 0.96 (3H, s, CH$_3$-19), 1.23 (3H, d, $J_{20,21} = 6.5$ Hz, CH$_3$-21)$_2$, 1.80 (3H, d, $J_{4',5'} = 1.0$ Hz, CH$_3$-4'), 1.85 (3H, d, $J_{7',8'} = 1.0$ Hz, CH$_3$-5'), 2.38 (6H, s, N(CH$_3$)$_2$), 2.82 (1H, m, H-20), 3.62 (1H, m, H-3), 4.13 (1H, m, H-4), 5.32 (1H, brs, H-6), 5.53 (1H, m, NH), 5.74 (1H, brs, H-2').

### 5.2.3.2 Saracodine (105)

The aqueous layer of crude extract was extracted with chloroform at pH 9 afford chloroform extract C (Scheme-5.1, page 150) when subjected to the repeated column chromatography on silica gel, by using solvent system, pet.ether: acetone (85:15) with a
few drops of diethylamine, afforded subfraction C-3. The subfraction C-3 yielded a white amorphous compound 105 after repeated column chromatography on silica gel using solvent system pet. ether : acetone as eluent (Scheme-5.3, page 154).

**IUPAC Name:** 3β-(Nα-Methylamino)-20S-(Nβ-acetyl-Nβ-methylamino)-5α-pregnane

**State:** White amorphous material

**Yield:** 130.5 mg, 7.3 × 10⁻³ %

**Rf:** 0.234 [pet. ether: acetone (8:2), 4 drops of diethylamine in 10 mL]

**M.P.:** 240-241° C, [Lit. value: 245-246° C]

**[α]_D^{20}** : -14.4° (c = 0.02, CHCl₃), [Lit. value: -14° (MeOH)]

**UV (MeOH)** λ_max nm (log ε): 203 (1.12), λ_min nm (log ε): 195 (1.00), 364 (0.34)

**IR (CHCl₃) ν_max cm⁻¹:** 1632 (C=O)

**EI-MS m/z (rel. int. %):** 402 (29), [M⁺], 387 (2) [M⁺-15], 310 (62), 100 (15), 84 (100), 72 (22)

**FD-MS: m/z 402 (C₂₅H₄₆N₂O)***

**HREI-MS m/z (formula, calcd.):** 402.3579 (C₂₅H₄₆N₂O), 402.3609

**¹H-NMR (CDCl₃):** δ 0.69/0.72 (3H, s, CH₃-18), 0.79/0.80 (3H, s, CH₃-19), 1.04/1.14 (3H, d, J₂₀₂₁ = 6.5 Hz, CH₃-21), 2.01/2.07 (3H, s, COCH₃), 2.21 (6H, s, N(CH₃)₂), 2.71/2.75 (3H, s, Nβ-CH₃), 3.58/4.61 (1H, m, H-20).

**5.2.3.3 Vaganine-A (106)**

The fraction C-7 (Scheme-5.4, page 157) obtained from the chloroform extract C, after repeated column chromatography on silica gel, afforded a white amorphous compound 106 after repeated column chromatography on silica gel by using pet. ether:
acetone (65:35) with a few drops of diethylamine.

**IUPAC Name:** (20S)-20-(N,N-Dimethylamino)-3β-(senecioylamino)-4β-acetyl-5α-pregnane

**State:** White amorphous material

**Yield:** 10.5 mg, 5.8 x 10⁻⁴%

**Rf:** 0.65 [pet. ether: acetone (8:2), 4 drops of diethylamine in 10 mL]

**[α]D:** 115° (c = 0.21, MeOH), [Lit. value : 119° (c = 0.2 MeOH]

**UV (MeOH)** λ<sub>max</sub> nm (log ε): 202 (2.53), λ<sub>min</sub> nm (log ε): 192 (1.32), 355 (0.73)

**IR (CHCl₃)** ν<sub>max</sub> cm⁻¹: 3475 (NH), 3391 (CH), 2932 (=CCH), 1692(C=O)

**EI-MS m/z (rel. int. %):** 486 (18) [M⁺], 471 (60) [M⁺-15], 449 (17), 300 (20), 83 (86), 72 (100)

**FD-MS:** m/z 486 (C₃₀H₅₀N₂O₃)

**HREI-MS m/z (formula, calcd.):** 486.3808 (C₃₀H₅₀N₂O₃, 486.3821)

**¹H-NMR (CDCl₃):** δ 0.84 (3H, s, CH₃-18), 0.94 (3H, s, CH₃-19), 1.33 (3H, d, J<sub>20,21</sub> = 6.5 Hz, CH₃-21), 1.79 (3H, d, J<sub>4,5</sub> = 1.0 Hz, CH₃-4'), 2.07 (3H, s, COCH₃), 2.16 (3H, d, J<sub>4,5</sub> = 1.0 Hz, CH₃-5'), 2.61 (6H, s, N(CH₃)₂), 5.13 (1H, dd, J = 6.1, 5.9 Hz, H-4).
5.2.3.4 Alkaloid-C (107)

The fraction B obtain as a result of extraction of aqueous layer of crude extract with chloroform at pH 5, was subjected to column chromatography afforded several subfraction. The subfraction B-1 was obtained as a result of repeated column chromatography of chloroform extract B (pH 5) (Scheme-5.1, page 150). The fraction B-1 yielded compound 107 along with the compound 74, by column chromatography on (silica gel) using solvent system pet. ether: acetone and few drops of diethylamine as eluent (Scheme-5.2, page 151).

![Chemical structure of 107](image)

**IUPAC Name:** (20S)-20-(N,N-Dimethylamino)-3β-methoxy-pregn-5-ene

**State:** White amorphous powder

**Yield:** 21.8 mg, 1.2 x 10⁻³ %

**Rf:** 0.345 [pet. ether: acetone (8.5:1.5), 3 drops of diethylamine in 10 mL]

**M.P.:** 156-157°C, [Lit. value: 152-153°C]

**[α]_D^25:** -29° (c = 0.015, CHCl₃), [Lit. value: -32° (Acetone)]

**UV (MeOH)**

\[ \lambda_{max} \text{ nm (log } e) = 214 (2.82), \lambda_{min} \text{ nm (log } e) = 193 (1.69), 361 (1.01) \]

**IR (CHCl₃)**

\[ \nu_{max} \text{ cm}^{-1}: 2931 (\equiv \text{CH}), 1511 (C=\text{CH}) \]

**EI-MS m/z (rel. int. %):** 359 (34) [M⁺], 344 (48.9) [M⁺-15], 83 (39.2), 72 (100)

**FD-MS:** m/z 359 (C₂₉H₄₁NO)
HREI-MS m/z (formula, calcd.): 359.3125 (C_{23}H_{38}NO, 359.3161)

$^{1}$H-NMR (CDCl$_3$):  δ 0.65 (3H, s, CH$_3$-18), 0.86 (3H, s, CH$_3$-19), 0.97 (3H, d, $J_{20,21}$ = 6.5 Hz, CH$_3$-21), 2.14 (6H, s, N(CH$_3$)$_2$), 2.39 (1H, m, H-20), 3.03 (1H, m, H-3), 3.33 (3H, s, OCH$_3$), 5.34 (1H, brs, H-6).

5.2.3.5 Salignamine-A (108)

The fraction B obtained from the extraction of aqueous extract of plant with chloroform at pH 5 (Scheme-5.1, page 150), subjected to column chromatography on silica gel afforded several subfractions. The subfraction B-3, afforded compound 108 as a yellowish gum after repeated column chromatography on silica gel by using solvent system, pet. ether: acetone (85:15) with a few drops of diethylamine.

![Chemical Structure](Image)

IUPAC Name: (20S)-20-(N,N-Dimethylamino)-3β-methoxy-pregn-5,16-diene

State: Yellowish gum

Yield: 3.8 mg, 4.9 x 10^{-4} %

Rf: 0.32 [pet. ether: acetone (9:1), 3 drops of diethylamine in 10 mL]

M. P.: 219-221°C, [Lit. value: 223-224°C]

$[\alpha]^{20}_D$: -28° (c = 0.23, CHCl$_3$), [Lit. value: -23° (c = 0.12, CHCl$_3$)]

UV (MeOH) $\lambda_{max}$ nm (log e): 202 (2.30), $\lambda_{max}$ nm (log e): 194 (1.32), 351 (0.63)

IR (CHCl$_3$) $\nu_{max}$ cm$^{-1}$: 2927 (C=CH)
EI-MS m/z (rel. int. %): 343 (27) [M⁺], 328 (100) [M⁺-15], 72 (70)
FD-MS: m/z 343 (C₂₄H₃₉NO)
HREI-MS m/z (formula, calcd.): 343.2880 (C₂₂H₂₇NO, 343.2875)

¹H-NMR (CDCl₃): δ 0.84 (3H, s, CH₃-18), 1.02 (3H, s, CH₃-19), 1.17 (3H, d, J₂₀,₂₁ = 6.5 Hz, CH₃-21), 2.34 (3H, s, NCH₃), 2.94 (1H, q, J₂₁,₂₀ = 6.6 Hz, H-20), 3.03 (1H, m, H-3), 3.34 (3H, s, OCH₃), 5.32 (1H, brs, H-6), 5.72 (1H, br s, H-16).

5.3.2.6 2-Hydroxysalignamine-A (109)

The chloroform extract B obtained from the extraction of aqueous extract with chloroform at pH 5, afforded several subfractions by column chromatography on silica gel using increasing polarity of solvents system p.t. ether: acetone. The subfraction B-4-1, obtained from repeated column chromatography of chloroform extract B (Scheme-5.2, page 151) yielded a white amorphous compound 109 along with compound 108 after column chromatography on silica gel using solvent system, p.t. ether: acetone (80:20) with a few drops of diethylamine.

IUPAC Name: (20S)-30-(N,N-Dimethylamino)-2β-hydroxy-3β-methoxy-pregn-5,16-diene
State: White amorphous material
Yield: 10.8 mg, 6.06 x 10^{-4} %

Rf: 0.55 (pet. ether: acetone (9:1), 4 drops of diethylamine in 10 mL)


[α]^{20}_D: 20° (c = 0.01, CHCl₃), [Lit. value: 26° (c = 0.02, CHCl₃)]

UV (MeOH) λ_{max} nm (log ε): 204 (2.45), λ_{min} nm (log ε): 194 (1.34), 368 (0.98)

IR (CHCl₃) ν_{max} cm^{-1}: 3375 (OH), 1545 (C=C)

EI-MS m/z (rel. int. %): 373 (6.1) [M^+] , 358 (100) [M^+ -15], 83 (57), 72 (75)

FD-MS: m/z 373 (C_{24}H_{30}NO₂)

HREI-MS m/z (formula, calcd.): 373.2982 (C_{24}H_{30}NO₂, 373.2980)

¹H-NMR (CDCl₃): δ 0.84 (3H, s, CH₃-18), 1.18 (3H, s, CH₃-19), 1.26 (3H, d, J_{20,21} = 6.5 Hz, CH₃-21), 2.17 (6H, s, N(CH₃)₂), 2.90 (1H, q, J_{21,20} = 6.6 Hz, H-20), 3.03 (1H, m, H-3), 3.36 (3H, s, OCH₃), 4.14 (1H, m, H-2), 5.37 (1H, brs, H-6), 5.69 (1H, brs, H-16)

3.2.3.7 Axillarine-F (110)

The fraction C-8 obtain from the repeated column chromatography of chloroform extract C at pH 9 (Scheme-5.4, page 157), afforded compound 110 as a white amorphous material after repeated column chromatography on silica gel by using solvent system, pet. ether: acetone (60:40) with a few drops of diethylamine.
IUPAC Name: (20S)-20-(N,N-Dimethylamino)-3β-(tigloylamine)-5α-pregn-2β-hydroxy-4β-acetyl

State: White amorphous material

Yield: 5.8 mg, 3.2 x 10⁻⁴ %

Rf: 0.63 [pet. ether: acetone (7:3), 4 drops of diethylamine in 10 mL]

M.P.: 245-246° C, [Lit. value: 241-244°C]

[α]²⁰ D: 24.4° (c = 0.02, CHCl₃), [Lit. value: 29.5° (c = 0.398, CHCl₃)]

UV (MeOH) λ_max nm (log ε): 232 (2.31), λ_min nm (log ε): 194 (1.32), 360 (0.56)

IR (CHCl₃) ν_max cm⁻¹: 3490 (NH), 3330 (OH), 2932 (=CH), 1652 (C=O), 1446 (C=CH)

EI-MS m/z (rel. int. %): 502 (14) [M⁺], 487 (18) [M⁺-15], 416 (44), 100 (33.5), 72 (100)

FD-MS: m/z 502 (C₃₀H₅₀N₂O₄)

HREI-MS m/z (formula, calcd.): 502.3158 (C₃₀H₅₀N₂O₄, 502.3140)

¹H-NMR (CDCl₃): 8 0.66 (3H, s, CH₃-18), 1.21 (3H, s, CH₃-19), 1.43 (3H, d, J₂₀,₂₁ = 6.5 Hz, CH₃-21), 1.73 (3H, d, J₂₅,₂₆ = 6.8 Hz, CH₃-4'), 1.79 (3H, s, CH₃-5'), 2.07 (3H, s, COCH₃), 2.16 (6H, s, N(CH₃)₂), 2.56 (1H, m, H-20), 4.03 (1H, m, H-2), 4.08 (1H, m, H-3), 5.13 (1H, m, H-4), 6.35 (1H, q, J₄,₅ ≈ 6.9 Hz, H-5').

5.2.3.8 Saracovagamine-C (111)
The subfraction C-8 (Scheme-5.4, page 157) gave the white amorphous compound 111 after a repeated column chromatography on silica gel by using solvent system, pet. ether: acetone (60:40) with a few drops of diethylamine.

**IUPAC Name:** (20S)-20-(N,N-Dimethylamino)-3β-(tigloylamine)-5α-pregn-4β-acetyl

**State:** White amorphous

**Yield:** 13.2 mg, 7.4 x 10⁻⁴ %

**Rf:** 0.45 [pet. ether: acetone (7.5:2.5), 4 drops of diethylamine in 10 mL]

**M.P.:** 190-191° C, [Lit. value: 192-194° C]

\[ \alpha^2 \text{L} L: 41° (c = 0.023, MeOH), [\text{Lit. value:} -9° (c = 0.12, CHCl}_3) \]

**UV (MeOH) \lambda_{\text{max}} \text{nm (log e):} 212 (2.78), \lambda_{\text{min}} \text{nm (log e):} 192 (1.18), 359 (0.98)

**IR (CHCl}_3 \nu_{\text{max}} \text{cm}^{-1}: 3345 (\text{NH}), 3333 (\text{OH}), 2939 (\text{CH}), 1642 (\text{C=O}), 1446 (\text{C=CH})

**EI-MS m/z (rel. int. %): 486 (35) [M⁺], 471 (13) [M⁺-15], 100 (33.5), 72 (100)

**FD-MS:** m/z 486 (C₂₇H₄₀N₂O₂)

**HREI-MS m/z (formula, calcd.):** 486.3489 (C₂₇H₅₀N₂O₄, 486.3559)

**¹H-NMR (CDCl}_3):** δ 0.67 (3H, s, CH₃-18), 0.94 (3H, s, CH₃-19), 1.30 (3H, d, J₂₀,₂₁ = 6.5 Hz, CH₃-21), 1.70 (3H, d, J₄₋₅ = 6.8 Hz, CH₃-4'), 1.81 (3H, s, CH₃-5'), 2.07 (3H, s, COCH₃), 2.62 (6H, s, N(CH₃)₂), 3.12 (1H, m, H-29), 3.99 (1H, m, H-3), 5.28 (1H, m, H-4), 6.30 (1H, q, J₄₋₅ = 6.9 Hz, H-3').

5.2.3.9 Saracocine (112)

The chloroform extract C obtain by extraction of the aqueous extract with chloroform at pH 9 (Scheme-5.1, page 150), yielded several subfractions after repeated column chromatography on silica gel by using increasing polarities of solvent system pet.
ether: acetone: diethylamine. Subfraction C-3 thus obtained was subjected to column chromatography to afford a pure alkaloid 112, as a yellowish gum.

**IUPAC Name:** 3β-(Nα-Methylamino)-20S-(Nβ-acetyl-Nα-methylamino)-pregn-5-ene

**State:** Yellowish gum

**Yield:** 9.6 mg, 5.3 x 10^{-4} %

**Rf:** 0.45 [pet ether: acetone (8.5:1.5), 3 drops of diethylamine in 10 mL]

**M.p.:** 226-228° C, [Lit. value: 232-233° C]

**[α]_20^20:** 56° (c = 0.23, MeOH), [Lit. value: -56.4° (MeOH)]

**UV (MeOH) **λ_{max} nm (log ε): 219 (2.7 l), λ_{min} nm (log ε): 193 (1.31), 366 (0.76)

**IR (CHCl₃) **ν_{max} cm⁻¹: 3490 (NH), 2929 (=CH), 1642 (C=O), 1546 (C=CH)

**EI-MS m/z (rel. int. %):** 400 (25) [M⁺], 385 (40) [M⁺-15], 100 (33.5), 84 (100)

**FD-MS:** m/z 400 (C_{26}H_{44}N_{2}O)

**HREI-MS m/z (formula, calcd.):** 400.3471 (C_{26}H_{44}N_{2}O, 400.3453)

**¹H-NMR:** δ 8 0.69/0.72 (3H, s, CH₃-18), 0.79/0.80 (3H, s, CH₃-19), 1.04/1.14 (3H, d, J_{20,21} = 6.5 Hz, CH₃-21), 2.01/2.07 (3H, s, COCH₃), 2.21 (6H, s, N(CH₃)₂), 2.79/2.75 (3H, s, Nα-CH₃), 3.58/4.61 (1H, m, H-20), 5.32 (1H, brs, H-6).
5.2.3.10 Sarcorine (113)

The fraction C (70.54 g) after the extraction of aqueous layer with chloroform at pH 9 (Scheme-5.1, page 150) yielded several subfractions after repeated column chromatography on silica gel by using increasing polarities of solvent system pet.ether: acetone with a few drops of diethylamine. Subfraction C-4 was subjected to column chromatography, affording a pure alkaloid 113, as white amorphous material (Scheme-5.3, page 151).

![Chemical Structure](image)

IUPAC Name: (20S)-20-(N,N-Dimethylamino)-3β-(N-acetylamido)-5α-pregnane

State: White amorphous material

Yield: 12.8 mg, 9.14 x 10⁻⁴ %

Rf: 0.436 [pet. ether: acetone (7.5:2.5), with few drops of diethyl amine in 10 mL]

[α]²⁰°: 49° (c = 0.81, CDCl₃)

UV (MeOH) λₘₐₓ nm (log ε): 202 (2.30), λₘᵦₙ nm (log ε): 192 (1.90), 366 (0.58)

IR (CHCl₃) νₘₐₓ cm⁻¹: 3650 (NH), 1658 (C=O)

EI-MS m/z (rel. int. %): 388 (16) [M⁺], 373 (20) [M⁺-15], 84 (18), 72 (100), 58 (19)

FD-MS: m/z 388 (C₂₅H₄₄N₂O)

HREI-MS m/z (formula, caled.): 388.3446 (C₂₅H₄₄N₂O, 388.3453), 373.3217 (C₂₃H₄₁N₂O, 373.3218), 72.0803 (C₄H₁₀N, 72.0813)
$^1$H-NMR (CDCl$_3$): $\delta$ 0.63 (3H, s, 18-CH$_3$), 0.73 (3H, s, 19-CH$_3$), 0.96 (3H, d, $J_{20,21} = 6.5$ Hz, 21-CH$_3$), 1.96 (3H, s, COCH$_3$), 2.23 (6H, s, N$_2$(CH$_3$)$_2$), 2.69 (1H, m, 20-CH), 4.09 (1H, m, 3-CH).
5.3 *Ficus religiosa*

5.3.1 Plant Material

5.4.1.1 Collection of Plant Material

The dried fruits of *Ficus religiosa* Linn. (1 kg) were collected in July 1999.

5.4.1.2 Extraction and Isolation

The air-dried fruits (1.0 kg) were grind and soaked in 5.0 Lit. of MeOH for 20 days. The methanolic extract (85.34 g) of the fruits was subjected to vacuum liquid chromatography (VLC) on silica gel using increasing polarities of different solvents (pet. ether/ CHCl₃/ EtOAc/ MeOH) to afford several sub-fractions (Frfr1-Frfr10) (Scheme-5.5, page 179). The crude extract of plants (fruit) showed antileishmanial activity at 6.25 µg/mL against *Leishmania major*. All fractions resulting from the vacuum liquid chromatography were evaluated for antileishmanial activity against *Leishmania major*. Some of them were inactive or showed moderate activity while a few of them showed good activity (Scheme-3.3.1, page 130). The sub-fraction Frf5, which showed significant antileishmanial activity (12.5 µg/mL), was subjected to column chromatography with the use of pet. ether: acetone (7.5: 2.5) to afford several subfractions which were further subjected to repeated column chromatography by using solvent system, pet. ether: acetone (7.5: 2.5) to obtain a pure active constituent, *N*-isobutyl eicosa-trans-2-trans-4 dienamide (117) (Scheme-5.5, page 179).
**Ficus religiosa**

Dried Fruits (1kg)

Extracted with MeOH 5.0 lit. (15 days)
and evaporated under vacuum

Crude Extract
(85.34 g)

Vacuum liquid chromatography (VLC)

- P.E.: CHCl₃ 8.7
  - FrF1 0.56 g
    - Methyl piperate ester (118) 105 mg
    - Piperlonguminine (115) 6.0 mg
    - Dihydropiperlonguminine (116) 4.5 mg
  - FrF2 1.56 g
  - FrF3 2.78 g
  - FrF4 7.06 g

- P.E.: CHCl₃ 6.8
  - FrF5 2.34 g
  - FrF6 1.30 g

- P.E.: CHCl₃ 2.8
  - FrF7 10.56 g
  - FrF8 3.53 g
  - FrF9 4.26 g
  - FrF10 6.16 g

- P.E.: ETOAc 5:5
  - Piperine (114) 600 mg

- MeOH

- Water

Scheme-5.5: Extraction and Isolation of Compounds from *Ficus religiosa*
5.3.2 Spectral Data of Known Compounds from *Ficus religiosa*

5.3.2.1 Piperine (114)

The subfraction Frf7 obtained after vacuum liquid chromatography of crude extract by using solvent system pet. ether: EtOAc (5:5) was subjected to repeated column chromatography by using increasing polarities of pet. ether: ethylacetate mixture, affording an amide 114 as a yellowish gum.

![Chemical Structure]

**Yield:** 630 mg, $7.0 \times 10^{-1}$%  

**UV (MeOH)** $\lambda_{\text{max}}$ nm ($\log \epsilon$): 342 (3.33), 310 (2.98), 262 (1.33), 203 (1.67), $\lambda_{\text{min}}$ nm ($\log \epsilon$): 314 (1.35), 271 (2.18), 233 (2.05), 195 (0.33).

**IR (CHCl$_3$)** $\nu_{\text{max}}$ cm$^{-1}$: 1630 (C=O), 1546 (C=CH), 935 (O-CH$_2$-O)

**EI-MS** $m/z$ (rel. int. %): 285 (33) [M$^+$], 201 (100), 173 (82), 143 (56), 115 (93), 84 (76), 55 (13)

**FD-MS:** $m/z$ 285 (C$_{17}$H$_{19}$NO$_2$)

**HREI-MS** $m/z$ (formula, calcd.): 285.3420 (C$_{17}$H$_{19}$NO$_2$, 285.3413)

**$^1$H-NMR (CDCl$_3$):** $\delta$ 1.63 (3H, m, H$_{2\text{-3',4'}}$), 3.15 (4H, d, $J = 16.3$ Hz, H$_{1\text{-2'}}$), 5.95 (2H, s, O-CH$_2$-O), 6.45 (1H, s, H-2), 6.73 (1H, d, $J = 6.5$ Hz, H-4), 6.75 (1H, s, H-2'), 6.86 (1H, dd, $J = 8.1$, 1.7 Hz, H-6'), 6.96 (1H, d, $J = 1.7$ Hz, H-5'), 7.35 (1H, ddd, $J = 14.6$ Hz, $J = 8.7$ Hz, $J = 1.5$ Hz, H-3').
5.3.2.2 Piperlonguminine (115)

The crude extract of plant was subjected to vacuum liquid chromatography by using different polarities of a solvent mixture (pet. ether/CHCl₃/ethylacetate/MeOH), yielding several subfractions. Subfraction Frf4 was subjected to repeated column chromatography (silica gel) that afforded amidic compound 115 as a white solid (Scheme-5.5, page 179).

Yield: 6.0 mg, 7.0 \times 10^{-3} \% 

**UV (MeOH)** \( \lambda_{\text{max}} \) nm (log e): 339 (3.24), 290 (2.65), 240 (1.33), \( \lambda_{\text{min}} \) nm (log e): 298 (2.12), 263 (1.67), 203 (1.32).

**IR (CHCl₃)** \( \nu_{\text{max}} \) cm\(^{-1}\): 3340 (NH), 1645 (C=O), 1545 (C=C)

**EI-MS m/z (rel. int. %):** 273 (33) [M\(^+\)], 201 (100), 173 (52), 135 (36)

**FD-MS: m/z** 273 (C\(_{16}\)H\(_{19}\)NO\(_3\))

\(^1\text{H-NMR (CDCl₃)}\): \( \delta \) 0.95 (6H, d, J = 6.6 Hz, CH\(_3\)-3\(^\prime\)), 3.15 (2H, t, J = 7.0 Hz, H-1\(^\prime\)), 1.76 (1H, m, H-2\(^\prime\)), 6.56 (1H, br s, H-3), 7.30 (1H, ddd, J = 14.6 Hz, J = 11.5 Hz, J = 8.7 Hz, H-3), 6.72 (1H, br s, H-4), 6.74 (1H, s, H-5), 5.95 (2H, s, O-CH\(_2\)-O), 6.78 (1H, s, H-5\(^\prime\)), 6.86 (1H, dd, J = 8.1 Hz, J = 1.6 Hz, H-6\(^\prime\)), 6.96 (1H, d, J = 1.6 Hz, H-5\(^\prime\))
5.3.2.3 Dihydropiperlonguminine (116)

The subfraction Frf4, which was obtained as a result of vacuum liquid chromatography of crude extract by using solvent system pet. ether: chloroform (4:6), was subjected to repeated column chromatography (silica gel) by using increasing polarities of solvent system, pet. ether: ethylacetate, to obtain a dihydro derivative of compound 116 as a white solid (Scheme-5.5, page 179).

![Chemical structure of Dihydropiperlonguminine (116)]

Yield: 4.56 mg, 5.2 × 10⁻³ %

**UV (MeOH)** \( \lambda_{\text{max}} \) nm (log ε): 335 (3.56), 287 (2.67), 243 (1.56), \( \lambda_{\text{min}} \) nm (log ε): 303 (2.01), 266 (1.67), 210 (0.35).

**IR (CHCl₃)** \( \nu_{\text{max}} \) cm⁻¹: 3343 (NH), 1651 (C=O) and 1541 (C=C).

**EI-MS** \( m/z \) (rel. int. %): 275 (13) \([\text{M}^+]\), 203 (100), 175 (43), 137 (36)

**FD-MS** \( m/z \): 275 (C₁₆H₁₉NO₃)

\(^1\text{H-NMR (CDCl₃)}\): δ 0.97 (6H, d, \( J = 6.6 \) Hz, CH₃-3", CH₃-4"), 3.13 (2H, t, \( J = 7.0 \) Hz, H-1"), 1.77 (1H, m, H-2"), 7.33 (1H, ddd, \( J = 14.6, J = 11.5 \) Hz, \( J = 8.7 \) Hz, H-3), 6.78 (1H, s, H-5), 5.96 (2H, s, O-CH₂-O), 6.87 (1H, s, H-2'), 6.86 (1H, dd, \( J = 8.1 \) Hz, \( J = 1.6 \) Hz, H-6'), 6.93 (1H, d, \( J = 1.6 \) Hz, H-5')}
5.3.2.4 N-Isobutyl eicosa-trans-2-trans-4 dienamide (117)

The crude extract of plant was subjected to vacuum liquid chromatography by using different polarities of a solvent mixture (pet. ether/CHCl\textsubscript{3}/ethylacetate/MeOH), yielding several subfractions. The subfraction Frf-5 was subjected to repeated column chromatography by using increasing polarities of solvent system pet. ether: EtOAc yields an amideic compound 117 as a white amorphous solid (Scheme-5.5, Page 179).

Yield: 5.0 mg, 5.8 x 10\textsuperscript{-3} %

UV (MeOH) \( \lambda_{\text{max}} \) nm (log \( \varepsilon \)): 337 (3.56), 260 (2.78), 219 (2.01), \( \lambda_{\text{min}} \) nm (log \( \varepsilon \)): 287 (2.01), 223 (1.67), 197 (0.67).

IR (CHCl\textsubscript{3}) \( \nu_{\text{max}} \) cm\textsuperscript{-1}: 3295 (NH), 1634 (C=O), 1545 (C=C)

EI-MS \( m/z \) (rel. int. %): 363 (21) [M\textsuperscript{+}], 335, 291, 279, 265, 180, 154

FD-MS: \( m/z \) 363 (C\textsubscript{24}H\textsubscript{45}NO)

\textsuperscript{1}H-NMR (CDCl\textsubscript{3}): \( \delta \) 0.95 (9H, d, \( J = 6.6 \text{ Hz}, \text{CH}_3-3', \text{CH}_3-4' , \text{CH}_3.20 \)), 1.25 (26 H, m), 1.79-2.20 (3H, m), 3.14 (2H, t, \( J = 7.0 \text{ Hz}, \text{H-1} ' \)), 5.71 (1H, d, \( J = 15.0 \text{ Hz} \)), 7.14 (1H, d, \( J = 8.1 \)), 7.1 (1H, d, \( J = 1.6 \text{ Hz} \)), 6.0-6.13 (2H, m)
5.3.1.4 Methyl piperate (118)

The crude extract of plant was subjected to vacuum liquid chromatography by using different polarities of a solvent mixture (pet. ether/CHCl₃/ethylacetate/MeOH), yielding several subfractions. The fraction Frf-3 afforded the ester analog 118 of piperic acid as a colorless amorphous solid, after repeated column chromatography by using solvent system, pet. ether: ethylacetate (7:3) (Scheme-5.5, page 179).

![Chemical Structure Image]

**Yield:** 105 mg, $1.2 \times 10^{-1}\%$

**UV** (MeOH) $\lambda_{\text{max}}$ nm (log $\varepsilon$): 340 (3.56), 287 (2.78), 243 (1.98), $\lambda_{\text{min}}$ nm (log $\varepsilon$): 310 (2.01), 259 (1.34), 206 (0.45).

**IR** (CHCl₃) $\nu_{\text{max}}$ cm⁻¹: 1734 (C=O) and 1541 (C=C).

**EI-MS** $m/z$ (rel. int. %): 232 (13) [M⁺], 201 (27), 173 (100), 143 (44), 115 (64), 100 (12)

**FD-MS:** $m/z$ 232 (C₁₃H₁₂O₄)

**¹H-NMR** (CDCl₃): 8 3.76 (3H, s, COCH₃), 6.66 (1H, br s, H-2), 7.38 (1H, ddd, $J = 14.6$ Hz, $J = 8.7$ Hz, $J = 1.5$ Hz, H-3), 6.68 (1H, br s, H-4), 6.72 (1H, s, H-5), 5.96 (2H, s, O-CH₂-O), 6.78 (1H, s, H-2'), 6.89 (1H, dd, $J = 8.1$ Hz, $J = 1.6$ Hz, H-6'), 6.98 (1H, d, $J = 1.6$ Hz, H-5').
5.4 *Synthesis and Rearrangement of Heterocyclic Compounds*

3-(2-Nitrobenzyl)-2(1H)-quinoxalinone (140)

 o-Nitrophenyl pyruvic acid (300 mg), o-phenylene diamine (300 mg) and a few drops of HCl were added in toluene (250 mL) and refluxed on a heating mantle for 5 h, by using Dean and Stark apparatus. Purification of product mixture on silica gel column with increasing polarities of chloroform and methanol furnished a major product 140 in 233.5 mg, 47% yield.

**UV (MeOH)** $\lambda_{\text{max}}$ nm ($\log e$): 317 (3.23), $\lambda_{\text{min}}$ nm ($\log e$): 299 (2.58), 193 (0.23)

**I.R. (KBr)** $v_{\text{max}}$ cm$^{-1}$: 3345 (N-H), 1665 (C=O), 1515, 1345 (-NO$_2$)

**EI-MS (m/z):** 281, 265, 235, 205, 180, 145

**HREI-MS (m/z):** 281.0819 (C$_{15}$H$_{11}$NO$_3$, 281.0778)

$^1$H-NMR: δ see Table-4.2.1.

2-(2-Nitrobenzyl)-1H-benzimidazole (141)

 o-Nitrophenyl pyruvic acid (300 mg), o-phenylene diamine (300 mg) and a few drops of HCl were added in toluene and refluxed on a heating mantle for 5 h, by using Dean and Stark apparatus. Purification on silica gel column with increasing polarities of chloroform and methanol furnished a minor product 141 in 156.5 mg, 13% yield.

**UV (MeOH)** $\lambda_{\text{max}}$ nm ($\log e$): 330 (3.56), 219 (2.68), $\lambda_{\text{min}}$ nm ($\log e$): 377 (1.33), 274 (1.33), 195 (0.23).

**I.R. (KBr)** $v_{\text{max}}$ cm$^{-1}$: 3350 (N-H), 1517, 1320 (-NO$_2$)

**EI-MS (m/z):** 253 (M$^+$), 235, 223, 108, 194
HREI-MS (m/z): 253.0846 (C_{14}H_{11}N_{3}O_{3}, 253.0851)

\(^1^H\)-NMR; \(\delta\) see Table-4.2.2.

3-(1,2-Benzisoxazole-3-yl)-2(1H)-quinoxalinone (142)

To a solution of KOH (0.5 g, 1.75 mmol) in methanol (50 mL), compound 140 (100 mg) was added and the solution was refluxed on a water bath for 2.5 hr, cooled and diluted with dist. water (20 mL). The aqueous solution was extracted with CHCl\(_3\) (3 \times 20 mL) and the organic layer was washed twice with water and dried over anhydrous Na\(_2\)SO\(_4\), filtered and the solvent was removed in vacuo. The resulting residue was purified by preparative TLC plates using chloroform as a solvent to give compound 142 in 51.5 mg, 51 % yield.

UV (MeOH) \(\lambda_{\text{max}}\) nm (log \(\varepsilon\): 387 (3.39), 334 (2.89), 271 (2.45), 219 (2.33), \(\lambda_{\text{min}}\) nm (log \(\varepsilon\): 380 (2.33), 296 (1.33), 256 (0.36), 205 (0.67), 193 (0.13)

I.R. (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 3200 (NH\(_2\)), 1660 (C=O), 1560 (C=C) cm\(^{-1}\)

EI-MS (m/z): 263, 213,145, 81, 55

HREI-MS (m/z): 263.0501 (C_{15}H_{10}N_{3}O_{2}, 263.0483)

\(^1^H\)-NMR; \(\delta\) see Table-4.2.3.

3-(1H-Benzimidazole-2-yl)-2,1-benzisoxazole (143)

To a solution of KOH (0.5 g, 1.75 mmol) in methanol (50 mL), compound 141 (100 mg) was added and the solution was refluxed on a water bath for 2.5 hr, cooled and diluted with dist. water (20 mL). The aqueous solution was extracted with CHCl\(_3\) (3 \times 20 mL) and the organic layer was washed twice with water and dried over anhydrous Na\(_2\)SO\(_4\), filtered and the solvent was removed in vacuo. The resulting residue was
separated by preparative TLC plates using chloroform as a solvent to give compound 143 in 52.5 mg, 53 % yield.

**UV (MeOH) \( \lambda_{max} \text{nm (log } \varepsilon) \):** 367 (3.12), 324 (2.99), 265 (1.67), \( \lambda_{min} \text{nm (log } \varepsilon) \): 354 (2.33), 293 (0.63), 198 (0.13)

**I.R. (KBr) \( v_{max} \text{ cm}^{-1} \):** 3335 (NH), 1560

**EI-MS \( m/z \):** 235 (M'), 201, 135, 72

**HREI-MS \( m/z \):** 235.0737 (C\(_{14}\)H\(_9\)N\(_3\)O, 235.0745)

\(^1\)H-NMR: δ see Table-4.2.4.

**5H-Quinoxalino[2,1-b] quinazolin-6,12-dione (144)**

The benzisoxazole (142) (20 mg) was heated under \( \text{N}_2 \) at 260-270° C in a metal bath for 25 min. The resulting residue was dissolved in CH\(_2\)Cl\(_2\) and separated on TLC preparative plates. The rearranged compound 144 was obtained in 5.8 mg, 24 % yield.

**UV (MeOH) \( \lambda_{max} \text{nm (log } \varepsilon) \):** 387 (3.56), 271 (2.86), 219 (1.33), \( \lambda_{min} \text{nm (log } \varepsilon) \): 367 (2.03), 283 (1.43), 229 (1.93 0.23)

**I.R. (KBr) \( v_{max} \text{ cm}^{-1} \):** 3335 (NH), 1695 (C=O), 1560 (C=C) cm\(^{-1}\)

**EI-MS \( m/z \):** 263, 213, 145, 81, 55

**HREI-MS \( m/z \):** 263.0186 (C\(_{15}\)H\(_{9}\)N\(_3\)O\(_2\), 263.0119)

\(^1\)H-NMR: δ see Table-4.2.5.

**5a,12a-Dihydrobenzimidazo[2,1-b] quinazolin-12(6H) -one (145)**

The benzisoxazole (143) (30 mg) was heated under \( \text{N}_2 \) at 260-270° C in a metal bath for 5 min. The resulting residue was dissolved in CH\(_2\)Cl\(_2\) and separated on TLC preparative plates. The rearranged compound 145 was obtained in 7.8 mg, 33 % yield.
UV (MeOH) \( \lambda_{\text{max}} \) nm (log \( \varepsilon \)): 396 (3.24), 313 (2.68), 253 (1.34), \( \lambda_{\text{min}} \) nm (log \( \varepsilon \)): 373 (2.33), 290 (1.03), 223 (0.66), 193 (0.23)

**I.R. (KBr)** \( \nu_{\text{max}} \) cm\(^{-1}\): 3340 (NH), 1670 (C=O), 1540 (C=C) cm\(^{-1}\)

**EI-MS (m/z):** 235

**HREI-MS (m/z):** 235.0730 (C\(_{14}\)H\(_9\)N\(_3\)O, 235.0728)

**\(^1\)H-NMR:** \( \delta \) see Table-4.2.6.
5.5 Biological Activities

5.5.1 Cholinesterase Inhibition

*In Vitro Cholinesterase Inhibition Assay*

Electric-eel acetylcholinesterase (EC 3.1.1.7), horse-serum butyrylcholinesterase (EC 3.1.1.8), acetylthiocholine iodide, butyrylthiocholine chloride, 5,5′-dithiobis [2-nitrobenzoic acid] (DTNB) and galanthamine were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade. Acetylcholinesterase and butyrylcholinesterase inhibiting activities were measured by the spectrophotometric method developed by Ellman *et al.*, 1961. Acetylthiocholine iodide and butyrylthiocholine chloride were used as substrates to assay acetylcholinesterase and butyrylcholinesterase activities, respectively. The reaction mixture contained 150 µL of (100 mM) sodium phosphate buffer (pH 8.0), 10 µL of DTNB, 10 µL of test-compound solution and 20 µL of acetylcholinesterase or butyrylcholinesterase solution, which were mixed and incubated for 15 minutes (25°C). The reaction was then initiated by the addition of 10 µL acetylthiocholine or butyrylthiocholine, respectively. The hydrolysis of acetylthiocholine and butyrylthiocholine were monitored by the formation of a yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine and butyrylthiocholine, respectively, at a wavelength of 412 nm (15 min). Test compounds and the control were dissolved in EtOH. All the reactions were performed in triplicate in 96-well micro-plate in *SpectraMax 340* (Molecular Devices, USA). The percentage (%) inhibition was
calculated as follows: \( \frac{E - S}{E} \times 100 \); where \( E \) is the activity of the enzyme without test compound and \( S \) is the activity of enzyme with test compound.

**Estimation of IC\(_{50}\) values**

The concentrations of the test compound that inhibit the hydrolysis of substrates (acetyltiococholine and butyryltiococholine) by 50\% (IC\(_{50}\)) were determined by monitoring the effect of increasing concentrations of these compounds on the inhibition values. The IC\(_{50}\) values were then calculated using the EZ-Fit Enzyme Kinetics program (*Perrella Scientific Inc., Amherst, USA*).

**5.5.2 Antileishmanial Activity**

**In Vitro Screening Systems**

In *vitro* cultivation of the causative organisms probably plays an important role in the study and treatment of the leishmaniasis than in any other group of the diseases caused by protozoa.

Basically two bioassay systems are currently available i.e. extra-cellular promastigote (axenic amastigotes) system, and intra-macrophagic amastigote systems. Nevertheless, these axenic amastigotes are reported to be morphologically closer to the intramacrophagic amastigotes which are present in the infected humans.

**Maintenance of Leishmania Parasite Culture**

Various methods of maintenance of live parasites are available such as culturing, cryopreservation and freeze-drying lyopholization. At present *Leishmania* parasites are maintained in culture media in the Institute, which is much easier and cheaper than other methods, but need regular passage.
In Vitro Cultivation and Revival of Cryopreserved Parasites (Promastigotes)

The culture medium used for isolation, routine maintenance and mass production was a modified NNN medium (Ash L. R., Orithe T. C., 1987) containing brain-heart infusion 7% (w/v), 1% dextrose and 500 U/mL penicillin. Cryo-vials were thawed quickly by rolling between the palms. The contents were transferred aseptically to another sterile vial of 10 mL capacity. The promastigotes were grown in sterile screw top tubes containing 10 mL culture media at 18-22°C and subcultured weekly.

Culture Media

A wide variety of media suitable for leishmanial culture are currently available. These range from very simple blood agar based biphasic medium to expensive tissue culture medium supplemented with fetal calf serum. However we have found a traditional blood agar based biphasic medium quite adequate for the regular maintenance of leishmania promastigotes. For screening purposes, a monophasic liquid medium is preferable. It is always better that parasites are maintained in a nutritionally less rich, blood agar based medium so that overgrowth does not take place and parasites readily adapt more nutritious fetal calf serum based liquid media for screening purpose. Following is the recipe of blood agar based biphasic and fetal calf serum based liquid media.

Blood Agar Based Biphasic Medium NNN

1Plain Agar : 1.1 g
1Bacto Peptone : 0.5 g
1Beef Extract : 0.3 g
2KCl : 0.04 g
\[ \text{KH}_2\text{PO}_4 : 0.03 \text{ g} \]
\[ \text{MgSO}_4 : 0.02 \text{ g} \]
\[ \text{Na}_2\text{HPO}_4 : 0.00475 \text{ g} \]
\[ \text{NaCl} : 0.8 \text{ g} \]

During our study, the above contents (except agar) were mixed well in 75 mL of warm distilled water and pH was adjusted to 7.4 with NaOH/HCl. Agar was added and volume made up to 100 mL with distilled water. The contents were transferred to a screw-capped bottle and autoclaved at 121° C for 15 minutes. The content was allowed to cool at 45° C and defibrinated rabbit (less preferably human) blood (heat inactivated at 56° C for 30 minutes) was added to a final concentration of 15%. The contents were mixed with screw caps. The vials were kept slanted so that a slope was formed after solidification of agar. Vials were incubated at 37° C for overnight. If any bacterial growth was visible by the next day, the bottles were discarded immediately. Vials were stored between 4-6° C for not more than two weeks.

**Fetal Calf Serum Based Liquid Medium RPMI-1640**

Medium RPMI-1640: 10.0 g (Sigma R-6504)

TES : 6.0 g (Sigma T-4152)

The above contents were mixed well in 750 mL of deionized water and pH adjusted to 7.4 with NaOH/HCl. The volume was made up to 1 L with deionized water and the contents were passed through 0.22 micron (pre-washed with sterile phosphate buffered saline) between 4-6° C. Just before use, 90 mL of this “basal”
medium was mixed with 10 mL fetal calf serum (Life Technologies 16000044) (now called supplemented or complete medium) (shelf life at 4-6° C, 1 week) and used for culture purpose.

Control of Contamination

Bacterial and fungal contamination is generally not a problem if the culture is handled carefully. However, one or two drops (40 mg/mL) of gentamycin can control the contamination. If any fungal contamination occurs, the culture was discarded immediately as most antifungal substance are also antileishmanial.

Preparation of Sample

1.0 mg of experimental compound or crude extract (test sample) was dissolved in 50 μL of DMSO and the volume was made up to 1.0 mL with complete medium containing antibiotics.

96-Well Micro Titer Plate Bioassay

Leishmania promastigotes were grown in bulk, in modified NNN biphasic medium using normal saline. Parasites at log phase were centrifuged at 2000 rpm for 10 minutes, and washed three times with saline at some speed and time. Parasites were then diluted with fresh culture medium to a final density of 10⁶ cells/mL. 100 μL of culture was added in all wells except the first column, which receive 180 μL of culture. The last two wells were set aside for negative and positive controls. Controls received medium with solvent, while the control contained varying concentrations of standard antileishmanial compounds such as amphotericin B, pentaacetine.
Serial Dilution of the Test Sample

20 μL of solubilized compound was added into the first well (duplicate or triplicate) and mixed well by micropipette. 100 μL of sample was removed and added into the next well, mixed well, 100 μL removed and added into the next till 8th well is reached. Remaining 100 μL was discarded. By doing this, the first well received a final concentration of 100 μg mL⁻¹, while the last well received 0.78 μg mL⁻¹ of test compound/ or crude extract.

The plates were incubated in the dark at 25°C for 3-5 days (preferably on an orbital shaker). After 5 days of exposure, activity of the test compound was assessed microscopically using improved Neubauer chamber (ethanol can be added to a final concentration of 5/1) in order to immobilize the parasites for ease of counting.

Cell Counting Using Improved Neubauer-Counting Chamber

Using a micropipette, 10 μL of culture was removed and transferred to both chambers of the hemocytometer. Starting with chamber “O” of the hemocytometer, cells were counted in a 1 mm center and four 1 mm corner squares under 40 x objective. Neubauer of cells/mL were determined by using the following formula.

Cells per mL = the average count per square x 10⁴ eg. If the average count per large square is 45 cells x 10⁴ = 4.5 x 10³ cells/mL.

An average number of parasites was counted in general control wells. Parasites exposed to varying concentrations of drug were counted and % mortality was calculated using the following formula:
\[% \text{Mortality} = 100 - \frac{\text{Number of Parasites}_{(\text{test})} \times 100}{\text{Number of Parasites}_{(\text{ve control})}} \%
\]

For studying antileishmanial activity, an essential requirement is the complete solubility of the test compounds. DMSO and ethanol are generally the solvents of choice. However, there are occasions where a compound is not soluble in either solvent; and even after sonication only a poor suspension is obtained.


8.0 LIST OF PUBLICATIONS


7.0 Glossary

**a.m.u.:** Atomic mass units

**Base Peak:** In the mass spectrum, the most intense peak is referred to as the base peak and intensities of other ion-peaks are measured with respect to this.

**Broad-Band (BB):** It is a fully decoupled $^{13}$C-NMR spectrum.

**Biosynthesis:** The synthesis of natural products within the living organisms is termed as "Biosynthesis". Biosynthetic studies investigate the actual pathways followed by living organism for the synthesis of secondary metabolites.

**COSY–45° Spectrum:** Homonuclear shift correlation spectrum is used to determine the $^1$H–$^1$H connectivities within a spin system, both geminal or vicinal.

**Cytotoxic:** Any substance which inhibits the growth of cells.

**DEPT:** Distortionless Enhancement by Polarization Transfer is a $^{13}$C-NMR experiment used to enhance the intensities and to determine the multiplicities of carbon signals to differentiate between CH$_3$, CH$_2$ and CH signals.

**EI-MS:** The electron-impact mass spectrum is a technique, which is, base or bombardment of organic molecules with an electron beam (normally 70 eV) to produce fragments of their structures.
**FAB-MS:** Fast atom bombardment mass spectrometry (FAB MS), a soft ionization technique in mass spectrometry, is used for the identification of the molecular ion of a compound, as a positive or negative ion.

**HMBC:** It is a Heteronuclear Multiple Bond Connectivity experiment, an inverse, two-dimensional NMR technique, which is used to determine long-range, heteronuclear interactions between carbon and protons; normally $J_{CH}$, $2J_{CH}$, $3J_{CH}$.

**HMQC:** Heteronuclear Multiple Quantum Coherence NMR spectroscopy is used to determine direct $^1H/^{13}C$ one-bond shift correlations.

**HREI-MS:** High-resolution electron impact mass spectrum gives information about the elemental composition through exact mass measurements. A high resolution focussing mass spectrometer is normally used for this purpose.

**HOHAHA:** Homonuclear Hartmann-Hahn spectroscopy is used to determine long-range $^1H/^1H$ correlations within a spin system.

**IR:** Infrared rays cause changes in the vibrational and rotational movements of the molecules, which help in the detection of functional groups. Experiments are observed within the range of 400-4,000 cm$^{-1}$. 

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