بسم الله الرحمن الرحيم

In the name of Allah, the Merciful, the Beneficent

It is He who made the sun a radiance,
and the moon a light,
and determined it by stations, that you
might know the number of the years
and the reckoning.
God created that not save with the truth,
distinguishing the signs
to a people who know.

(Yunus: 5)

EXTENDED STUDIES ON THE CHEMICAL CONSTITUENTS OF COCCULUS HIRSUTUS

THESIS SUBMITTED

FOR

THE FULFILLMENT OF THE DEGREE

OF

DOCTOR OF PHILOSOPHY

BY

SHAISTA IQBAL

H.E.J. RESEARCH INSTITUTE OF CHEMISTRY

UNIVERSITY OF KARACHI

1993

Dedicated
to
My Parents

ACKNOWLEDGMENTS

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Despite the fret and fear that I have, I express my profound, heart felt and affectionate gratitude to all those from whom I sought any type of help for the accomplishment of this arduous tark.

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Words are Inadequate to convey my sincere gratitude to my parents, brothers and sisters for this persistent patience, incessant endearment, Instigation and invaluable encouragement throughout my research endeavors. I also thankful of My one year Son Haider Iqbal for his sacrifice.

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SHAISTA IQBAL

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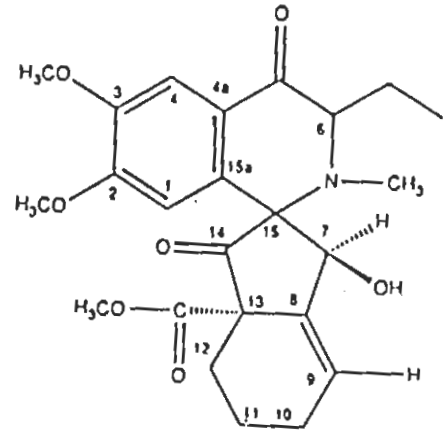
Summary

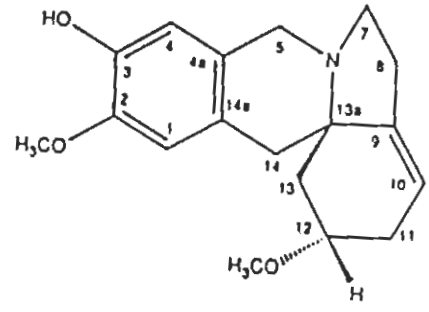
SUMMARY

This thesis describes the studies on the chemical constituents of *Cocculus hirsutus*. In the course of our studies of this plant five new compounds have been isolated and characterized.

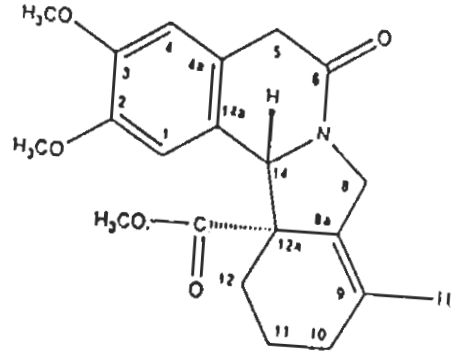
1) Cohirsitine
Fitoterapia **63**, 308 (1992)



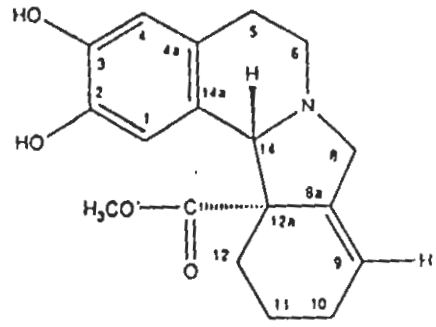
2) Cohirsitinine
J. Nat. Prod.,
55, 237-240 (1992).



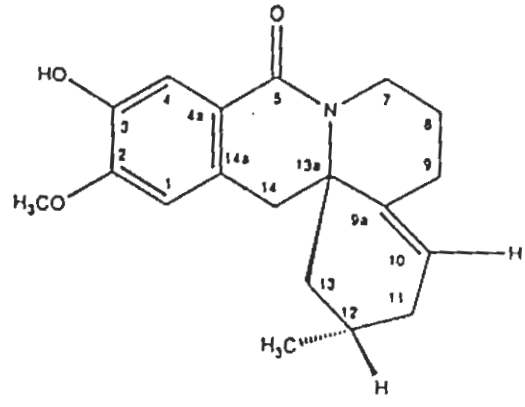
3) Jamtinine
Phytochemistry
33, 735-736 (1993)



4) Haiderine
Nat. Prod. Lett.,
2, 105-109 (1993)



5) Cohirsinine
Phytochemistry
30, 1350-1351 (1991)



The structures of all these alkaloids have been elucidated on the basis of modern spectroscopic techniques such as 1D and 2D-nmr (COSY, NOE, ^{13}C-nmr), inverse nmr experiments (HMQC, HMBC), mass spectroscopic techniques (EI, FD, FAB, HR), IR and UV spectra.

GENERAL INTRODUCTION:-

Among the innumberable gifts of nature belong the fascinating varieties of natural products which in some guise have been inseparable parts of mankind's history, since they fulfil many of our most basic requirements.

Beside animals (including man himself), plants are an important source of natural products. Thus from the very beginning of his existance, man has familiarised himself with plants and used them in a variety of ways.

Therefore some plants came to be widely used as food, while others showed beneficial effects against various human sufferings such as injuries and diseases. This relationship has grown between plants and knowledge to cure disease continues at an accelerating pace and the number of new plant-derived drugs increases likewise.

Even some vegetables like garlic were kept in homes and were considered effective in driving away evil spirits. It was not realised in those days that garlic has antibacterial, antifungal, anticancer, hypotensive and has various other useful properties. The active constituents are thioglycosides and various disulfides present in its essential oil.

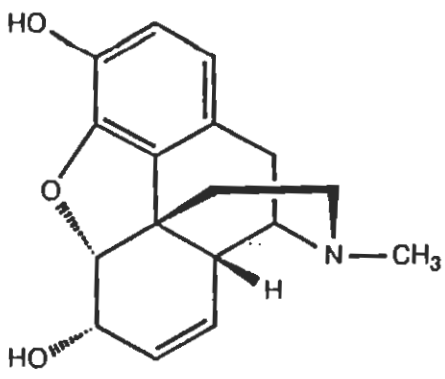
Alkaloids:-

The term alkaloid was coined in 1819 by the German pharmacist W. Meissner and meant simply, "alkali-like". The first modern definition of alkaloids by Winsterstein and Trier [1] described these substances in a broad sense as basic, nitrogen-containing compounds of either plant or animal origin.

The history of alkaloids is almost as old as civilization. Mankind has used drugs containing alkaloids in lotions, medicines, teas, poultices and poisons for 4000 years. The begining of coherent alkaloid chemistry usually is dated back about 170 years when, in 1805, F.W. Serturner isolated morphine (**1**), which was the substance responsible for the main physiological effects of opium. In France (1803), Dersone published a method for the extraction of the same alkaloid and isolated narcotine (**2**). Later, in 1810, Gomes treated an alcoholic extract of cinchona bark with alkali and obtained cinchonine (**3**). Subsequent studies (1820) by P.J. Pelletier and J.B. Caventou manifested that "cinchonine was a mixture of two new alkaloids named quinine and cinchonine". Subsequently various investigators isolated more than two dozen of other bases from species of *Cinchona* and *Remijia*. Between 1820 to 1850 a large number of alkaloids of new and varied types were isolated and charcterized. Hyoscyamine (**4**), the optically active form of atropine (**5**), piperine (**6**), an alkaloid of pepper plants, berberine (**7**), of barberry root, and nicotine (**8**), the toxic compound in tobacco, are among some of the alkaloids that have been isolated and used by mankind.

Alkaloids frequently occur as salts of plant acids such as malic acid (**9**), meconic acid (**10**), and quinic acid (**11**). Some alkaloids occur in the plants combined with sugars, e.g., solanine (**12**) from potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill.), whereas others are present as amides (e.g., piperine (**6**), from black pepper *Piper nigrum* L.) or as esters (e.g., cocaine (**13**) from coca leaves, *Erythroxylon coca* Lam.). Still other alkaloids occur as quaternary salts or as tertiary amine oxides.

More than 5500 alkaloids of a variety of structural types are known. Indeed, no other class of natural products possesses such an enormous variety of structures. Thus all steroids belong to a few skeletal types. The same holds true for triterpenes, flavonoids, or polysaccharides whereas alkaloids exhibit dozens of different skeletal types.



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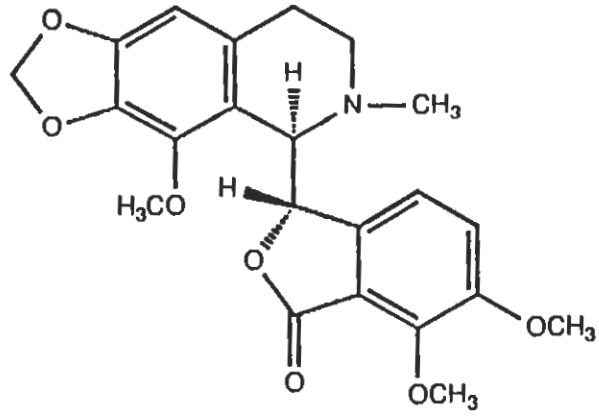

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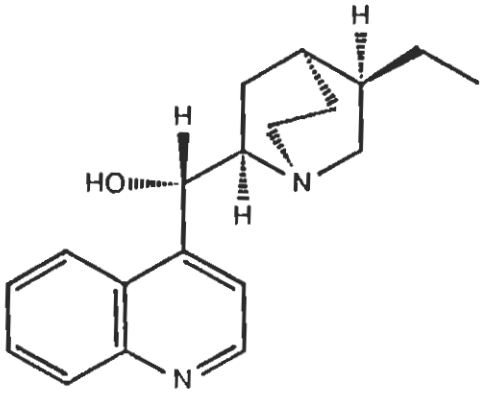

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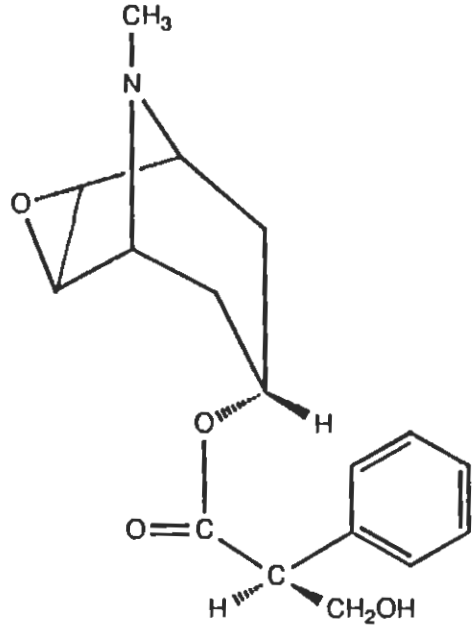

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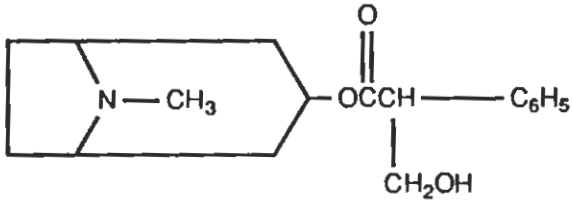

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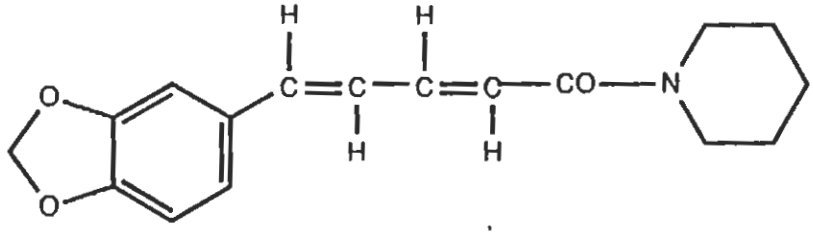

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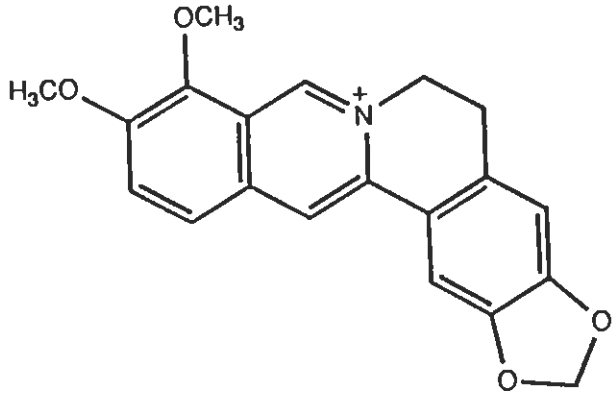

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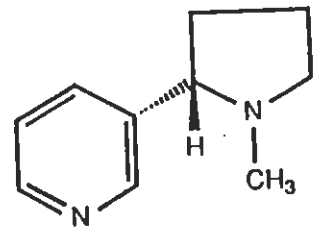

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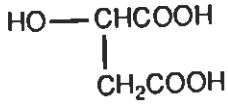

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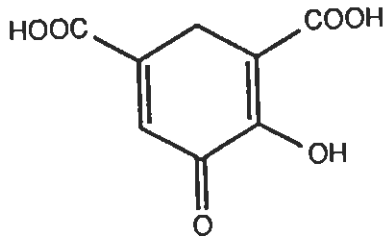

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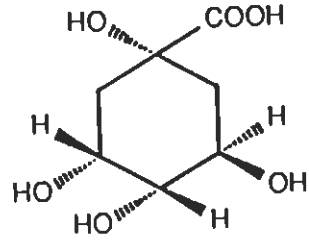

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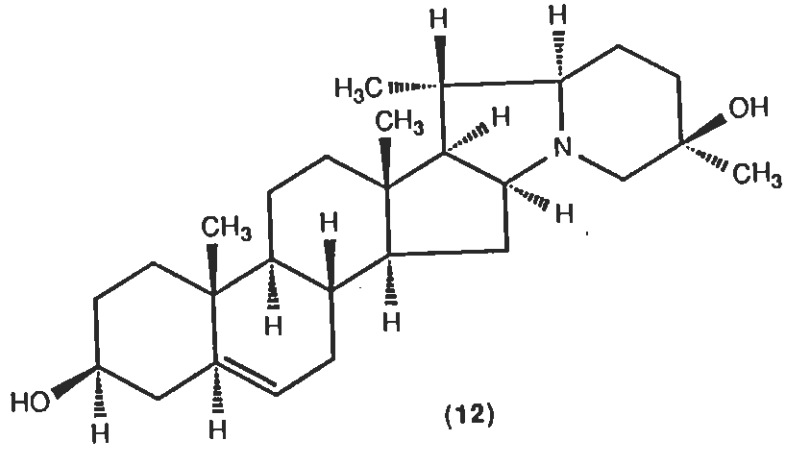

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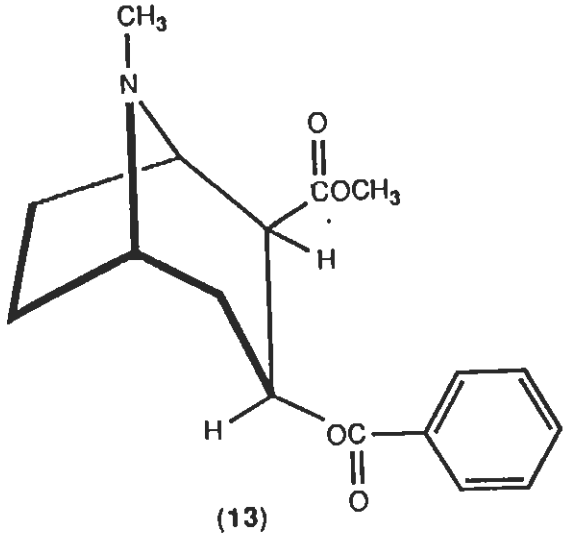

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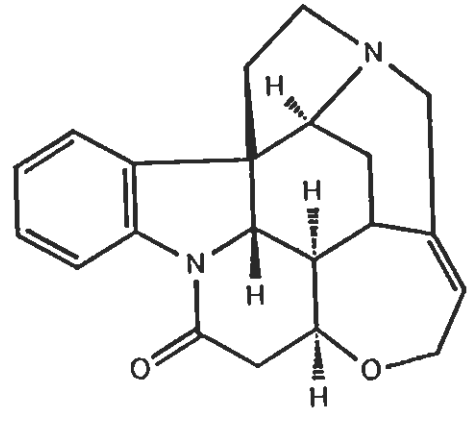


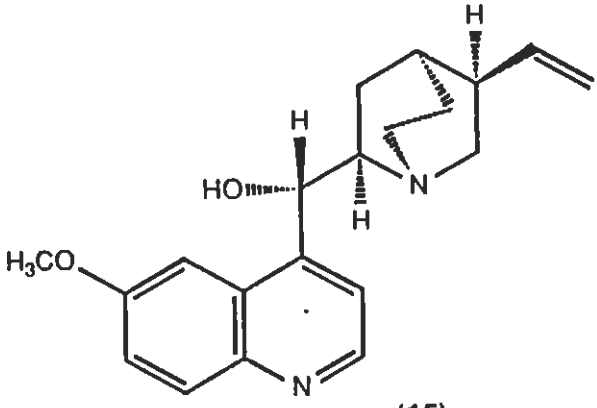
(14)

Examples of well known alkaloids are morphine (**1**, from opium poppy, *Papaver somniferum* L.), strychnine (**14**, from *Strychnos nux-vomica* L. and *S. ignatii* Berg.); quinine (**15**, from *Cinchona* bark, various *Cinchona* species); and coniine (**16**, from poison hemlock, *Conium maculatum* L.) isolated by Pelletier and Caventou in 1817, 1820 and 1826 respectively. Coniine is of special historical interest in that it was the compound responsible for the death of Socrates in 400 B.C., when he drank a cup of tea made from the poison hemlock, and it was the first alkaloid to be synthesized (Laudenburg, 1886). An example of the alkaloids of modern era is reserpine (**17**, *Rauwolfia serpentina* (L. Benth, ex Kurz.), a compound which was once widely used as an hypertensive agent and as a tranquillizer [2].

Alkaloids are restricted to certain families and genera of the plant kingdom. About 40% of all plant families contain at least one alkaloid-bearing species. Alkaloids have been reported in only 9% of over 10,000 plant genera. Among the angiosperms they occur abundantly in certain dicotyledons and particularly in the families *Apocynaceae* (dogbane, quebracho, pereira bark), *Asteraceae* = *Compositae* (ground sel, ragwort), *Berberidaceae* (European barberry), *Fabaceae* = *Leguminosae* (broom, gorse, laburnum, lupine, butterfly-shaped flowers), *Lauraceae* (rosewood tree), *Loganiaceae* (American jasmine, *Strychnos* species), *Menispermaceae* (moonseed), *Papaveraceae* (poppies, chelidonium), *Ranunculaceae* (aconite, delphinium, larkspur), *Rubiaceae* (cinchona bark, ipecac), *Rutaceae* (citrus, fagara), and *Solanaceae* (tobacco, deadly nightshade, tomato, potato, thorn apple). They are rarely found in Cryptogams (exception ergot alkaloids), Gymnosperms (monocotyledons), ferns, mosses and lower plants. In monocotyledons, the *Amaryllidaceae* (Amaryllis, Narcissus) and *Liliaceae* (meadow, saffron, veratrum) are important alkaloid bearing families.

Medicinal Value

Owing to their profound physiological effects, many alkaloids are important therapeutically. Some are widely used in the control of human malignancies including solid tumors - notable exaples are the alkaloids from the forms used are semisynthetic *Catharanthus roseus*, e.g. vinblastine (**18**), vincristine (**19**) and from *Podophyllum peltatum* (podophyllotoxin derivatives). Some are abused as narcotics and hallucinogenic drugs. The isoquinoline nucleus (**20**) and 1,2,3,4-tetrahydroisoquinoline nucleus (**21**) are abundant among alkaloids and their derivatives are often physiologically active, e.g. hallucinogens, central nervous



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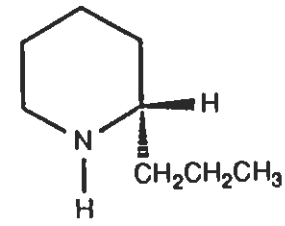

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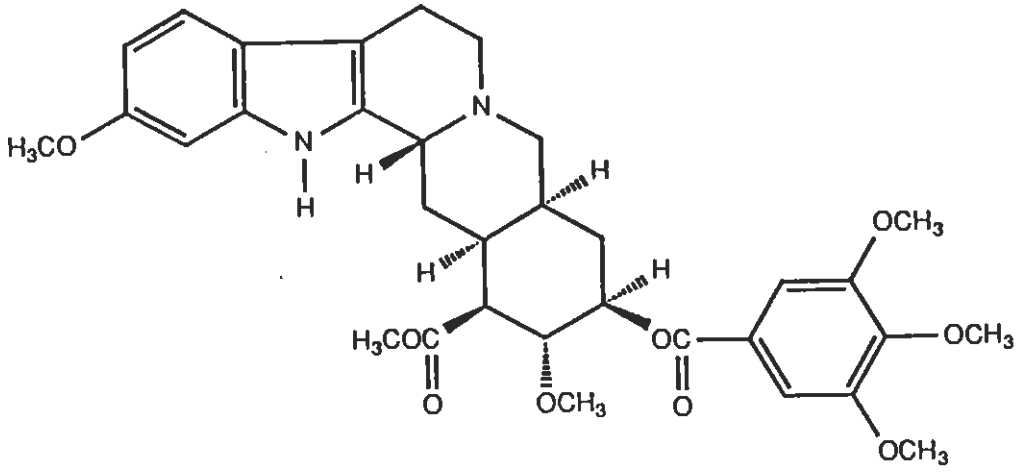

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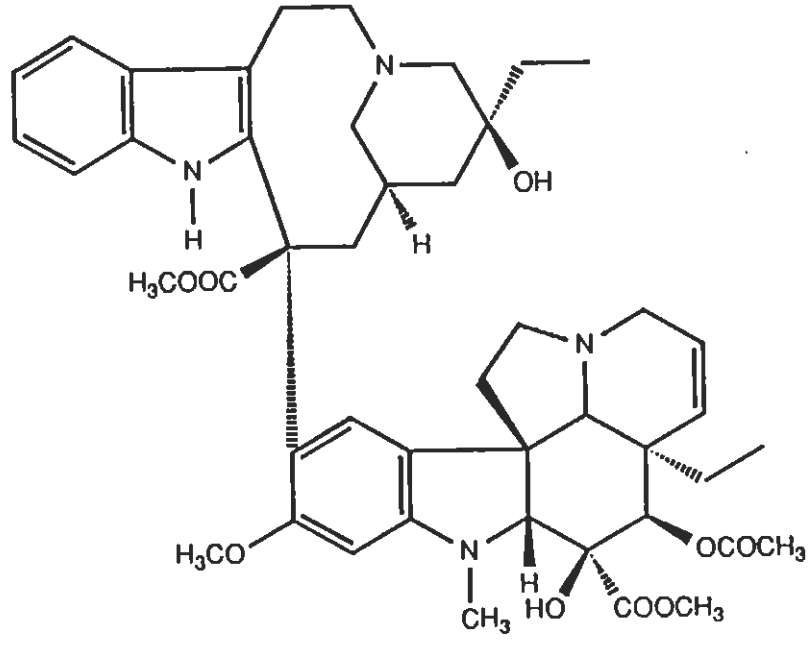

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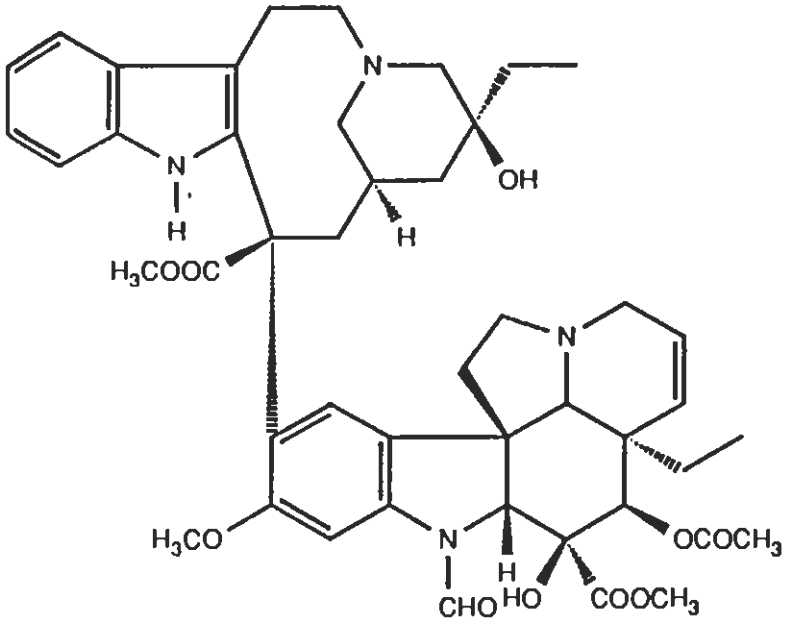
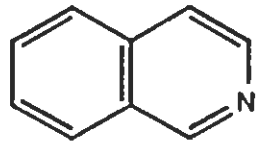

N
OH
H
N
H
H3COOC
N
H3CO
N
H
OCOCH3
CHO
HO
COOCH3

(19)


N

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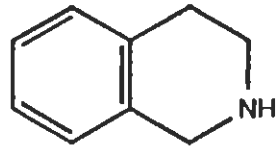
NH

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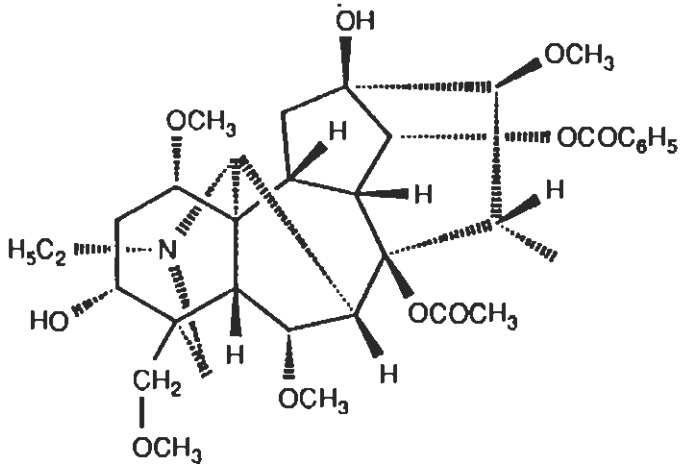
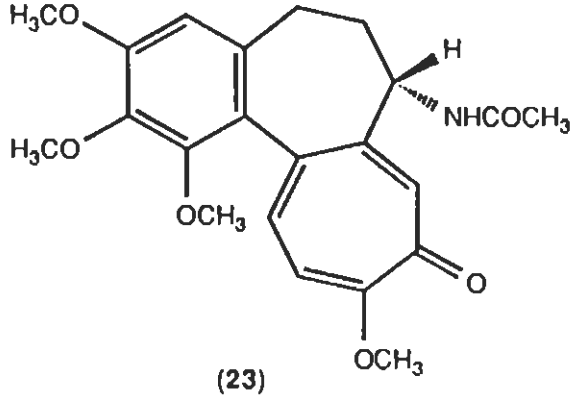
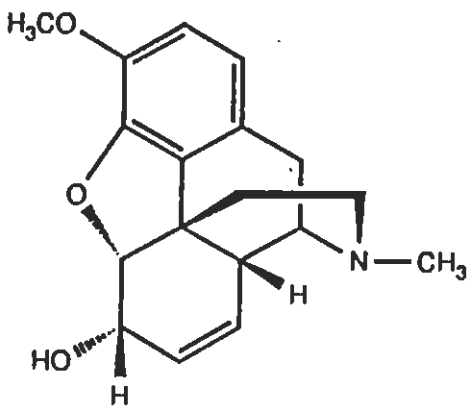

OH
OCH3
OCH3
H
OCOC6H5
H
H
H5C2
N
HO
OCOCH3
H
H
CH2
OCH3
OCH3

(22)


H3CO
H
NHCOCH3
H3CO
OCH3
O
OCH3

(23)

system agents (depressants and stimulants) or hypotensives are known. Aconitine (**22**) is one of the most toxic materials of plant origin known to man; atropine (**5**) is a powerful mydriatic agent; colchicine (**23**) is the alkaloid of the meadow saffron, which is used extensively in the treatment of *gout*; codeine (**24**) is a close relative of morphine (**1**), and is a valuable pain killer and cough repressant. Alkaloids also exhibit the following types of medicinal activity: Antiamoebic (e.g., emetine, **25**), anticholinergic (e.g., atropine, **5**; hyoscyamine **4**; scopolamine, **26**), antihypertensive (e.g., reserpine, **17**; rescinnamine, **27**; deserpidine, **28**; protoveratrine A, **29**), antimalarial (e.g., quinine, **15**), antitumor (vinblastine, **18**; vincristine, **19**; tylophorine, **30**; camptothecine, **31**), antitussive (e.g., codeine, **24**; noscapine, **32**), cardiac depressant and antiarrhythmic (e.g., quinidine, **33**), central stimulant, (e.g., caffeine, **34**), diuretic (e.g., theobromine, **35**; theophylline, **36**), emetic (e.g., emetine, **25**), gout suppressant (e.g., colchicine, **23**), local anesthetic (e.g., cocaine, **13**), narcotic analgesic (e.g., codeine, **24**; morphine, **1**), opthalmic cholinergic (e.g., physostigmine, **37**; pilocarpine, **38**), oxytocic (e.g., ergonovine, **39**), skeletal muscle relaxant (e.g., (+)-tubocurarine, **40**), smooth muscle relaxant (e.g., papaverine, **41**; theophylline, **36**), sympathomimetic (e.g., ephedrine, **42**), and tranquilizer (e.g., reserpine, **17**; deserpidine, **28**).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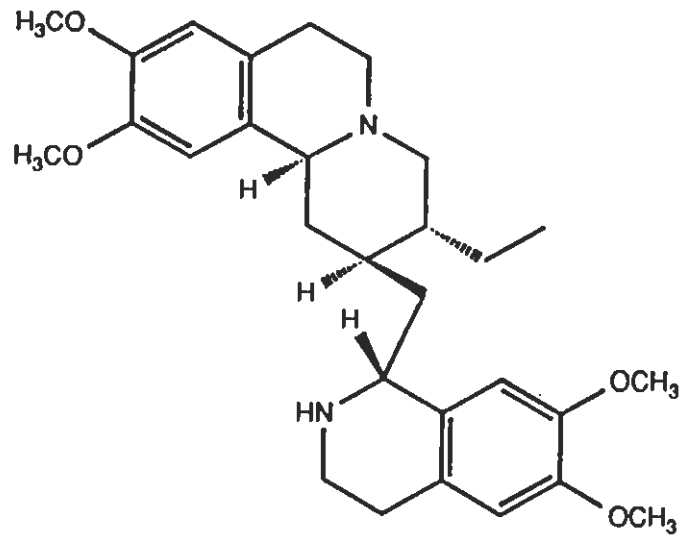
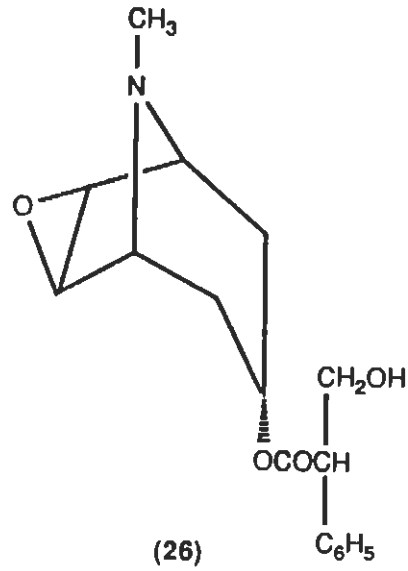
O
N—CH3
H
HO
H

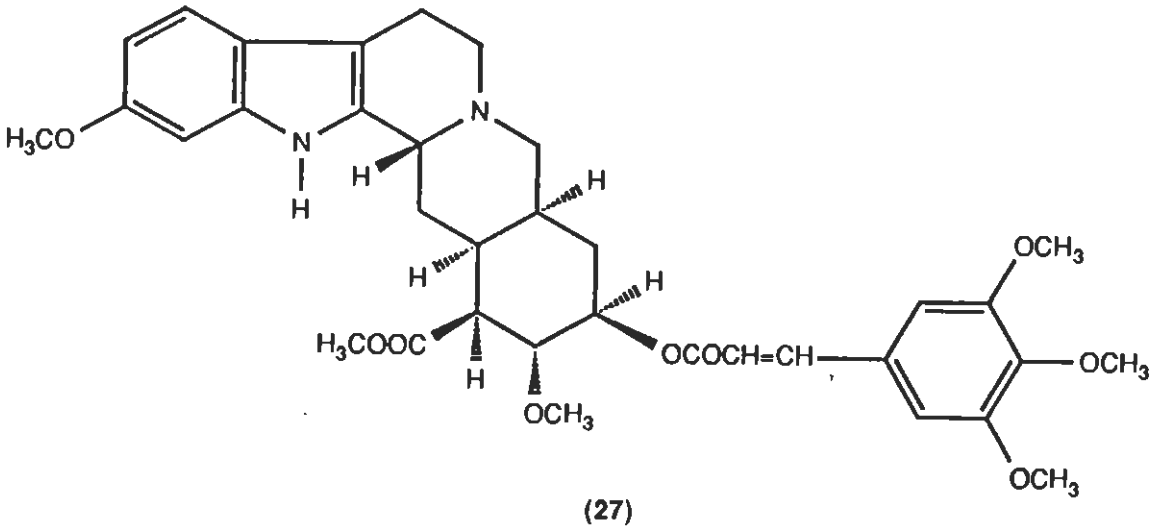
(24)


H3CO
H3CO
N
H
H
H
HN
OCH3
OCH3

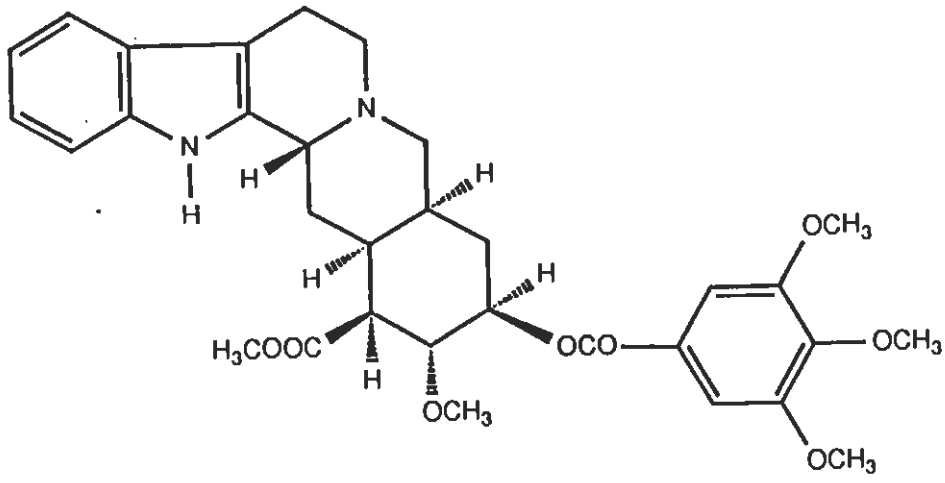
(25)


CH3
N
O
CH2OH
OCOCH
C6H5

(26)


H3CO
N
H
N
H
H
H
H
H3COOC
H
OCH3
OCOCH=CH
OCH3
OCH3
OCH3

(27)



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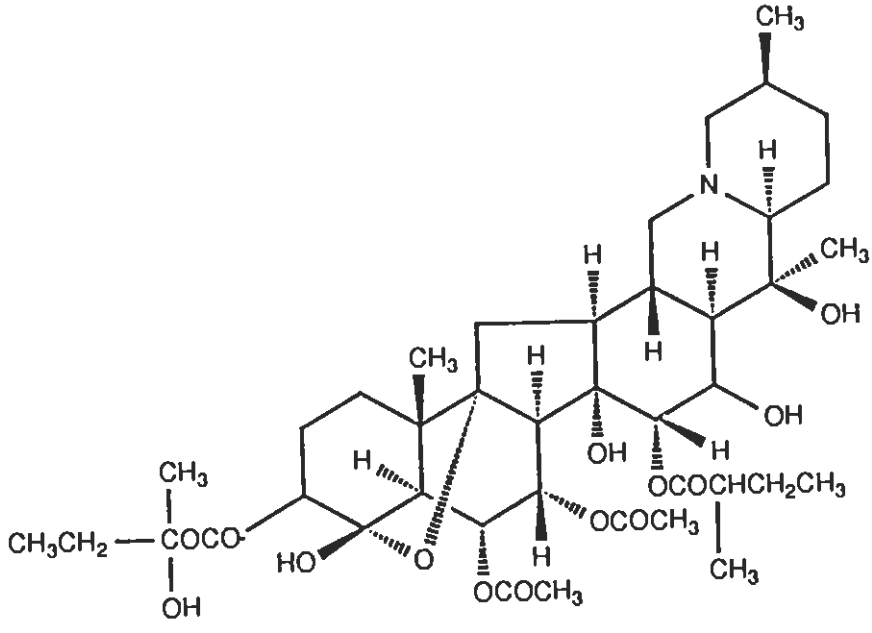

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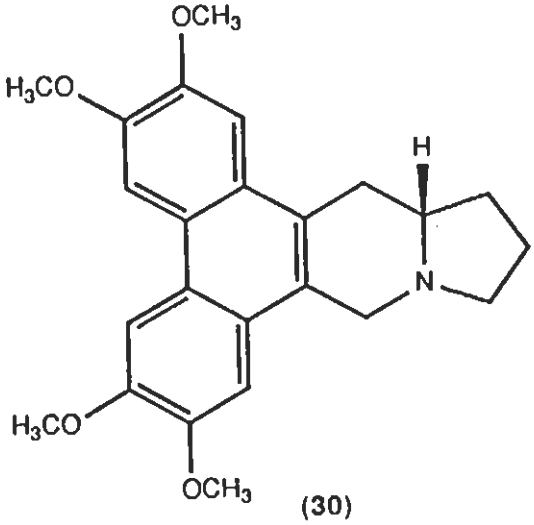

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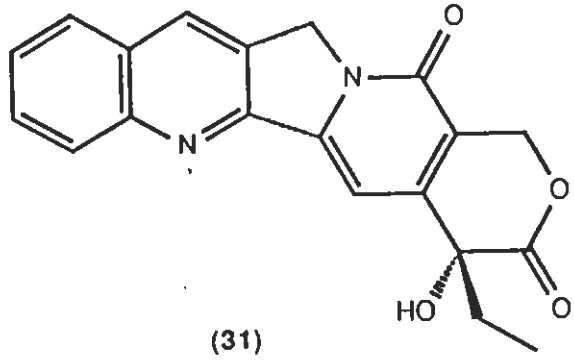

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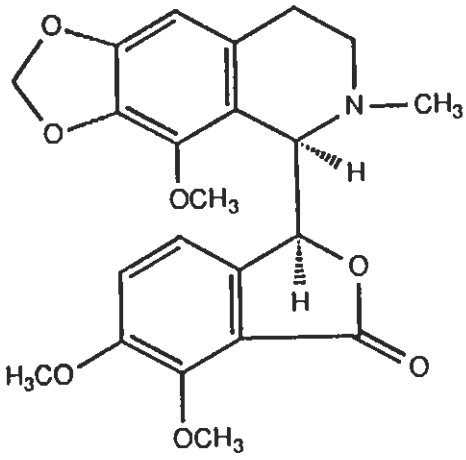

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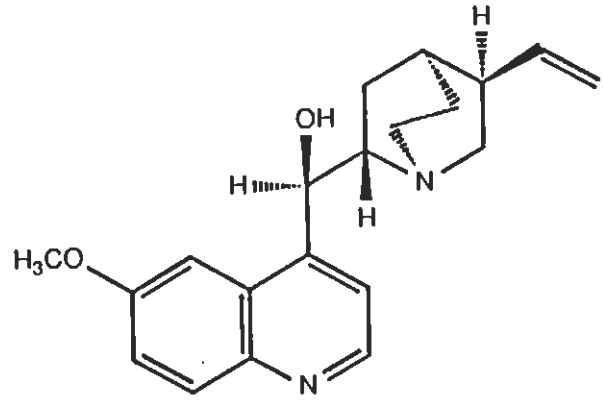

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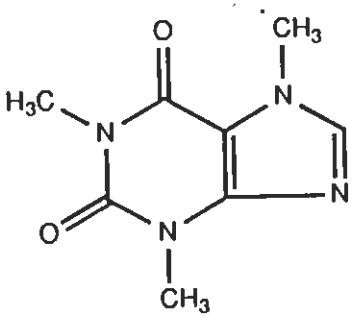

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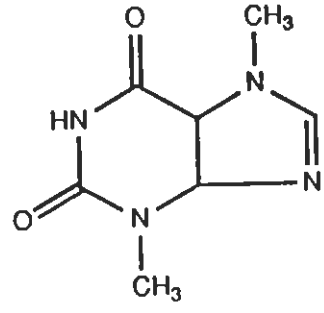

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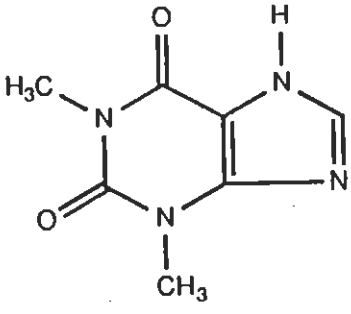

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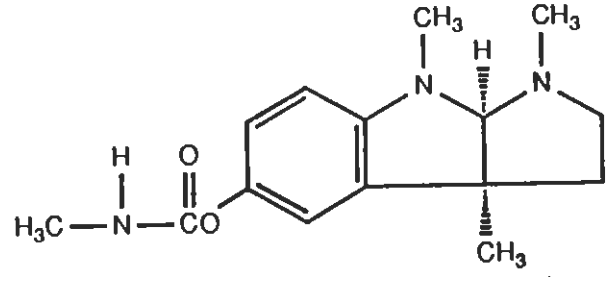

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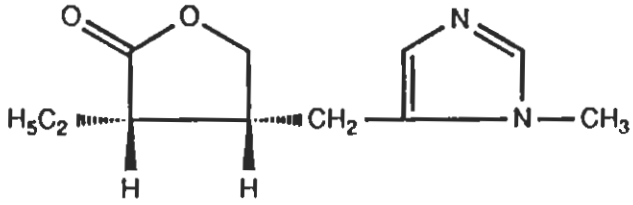

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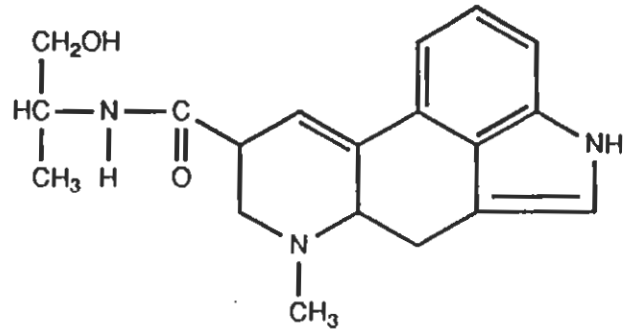

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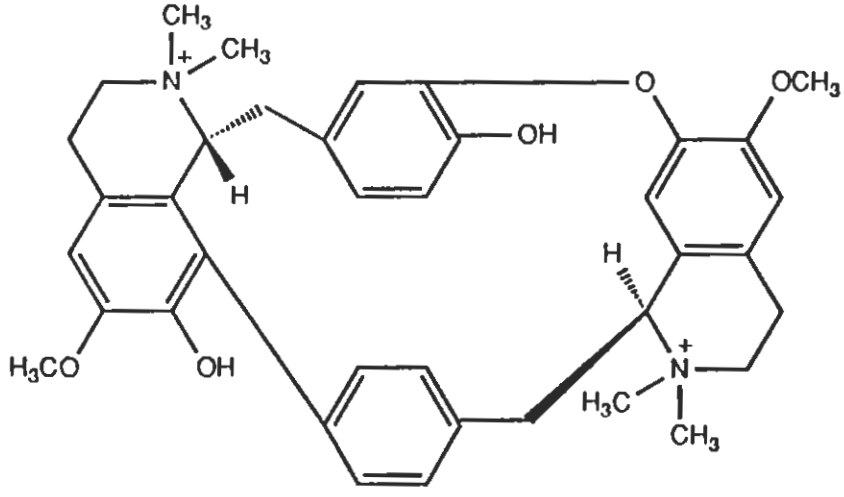

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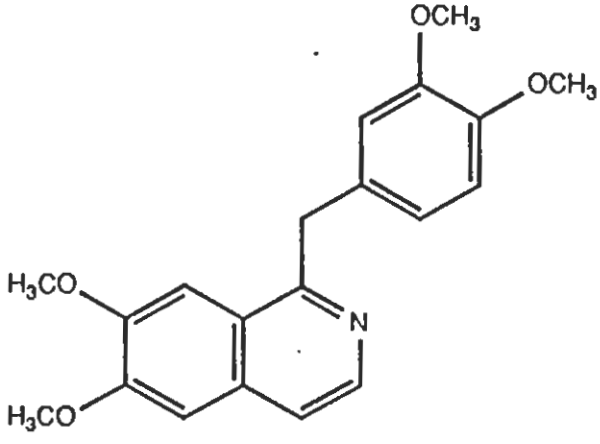

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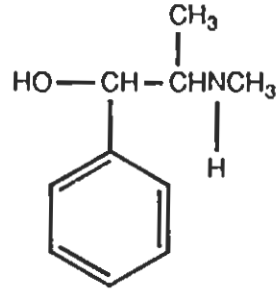

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Benzylisoquinoline Alkaloids.

Benzylisoquinoline alkaloids are a major and important class of natural products. These alkaloids exhibit facinating structural variations which are due in part to their biogenetic transformation into each other

Generally the benzylisoquinoline alkaloids are sub-divided into the following groups according to their structure or biogenesis.

These major groups are:

i) Simple isoquinoline alkaloids

ii) Benzylisoquinoline alkaloids

iii) Bisbenzylisoquinoline alkaloids

iv) Pavine and isopavine alkaloids

v) Coclaurine and related alkaloids

vi) Proaporphine alkaloids

vii) Aporphine alkaloids

viii) Protoberberine alkaloids

ix) Protopine alkaloids

x) Phthalide alkaloids

xi) Morphine and related alkaloids

xii) Hasubanonine and related alkaloids

xiii) Emetine and related alkaloids

xiv) Erythrina and related alkaloids

xv) Amaryllidaceae alkaloids

xvi) Phenethylisoquinoline and colchicine alkaloids

xvii) Tyrophorine and cryptopleurine alkaloids

xviii) Ipecac alkaloids

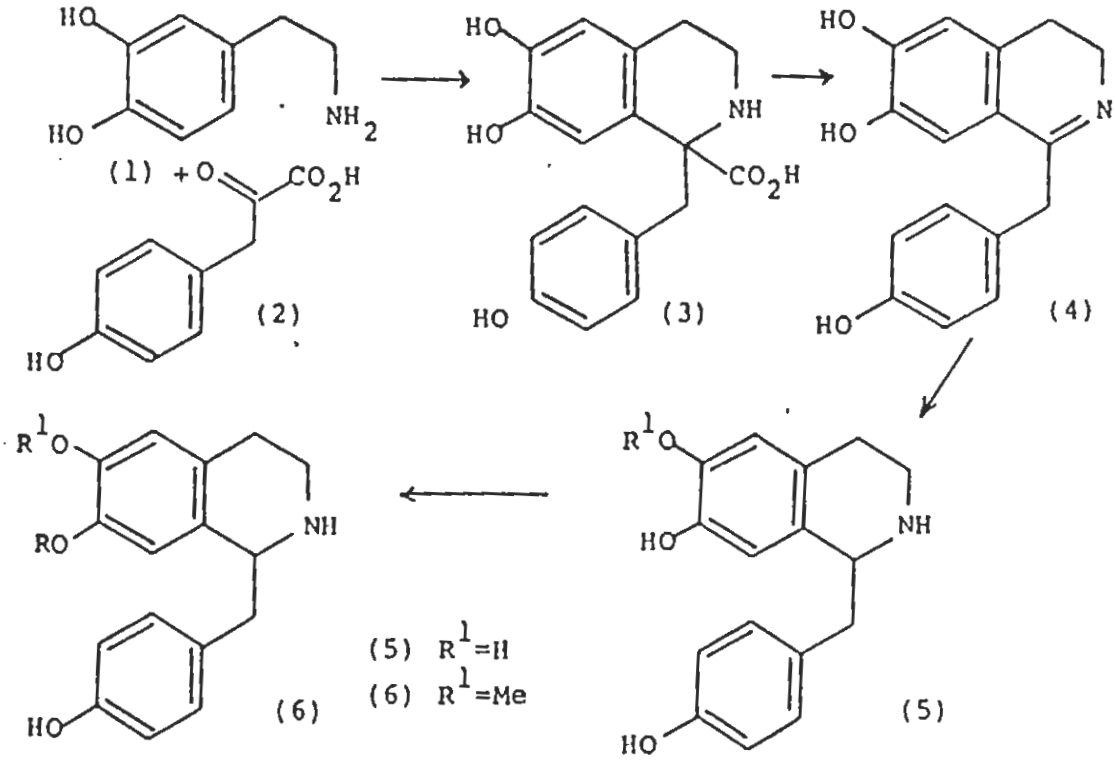
xix) Mesembrine and related alkaloids

xx) Rhoeadine alkaloids

Biosynthesis of Benzylisoquinoline Alkaloids.

Coclaurine :

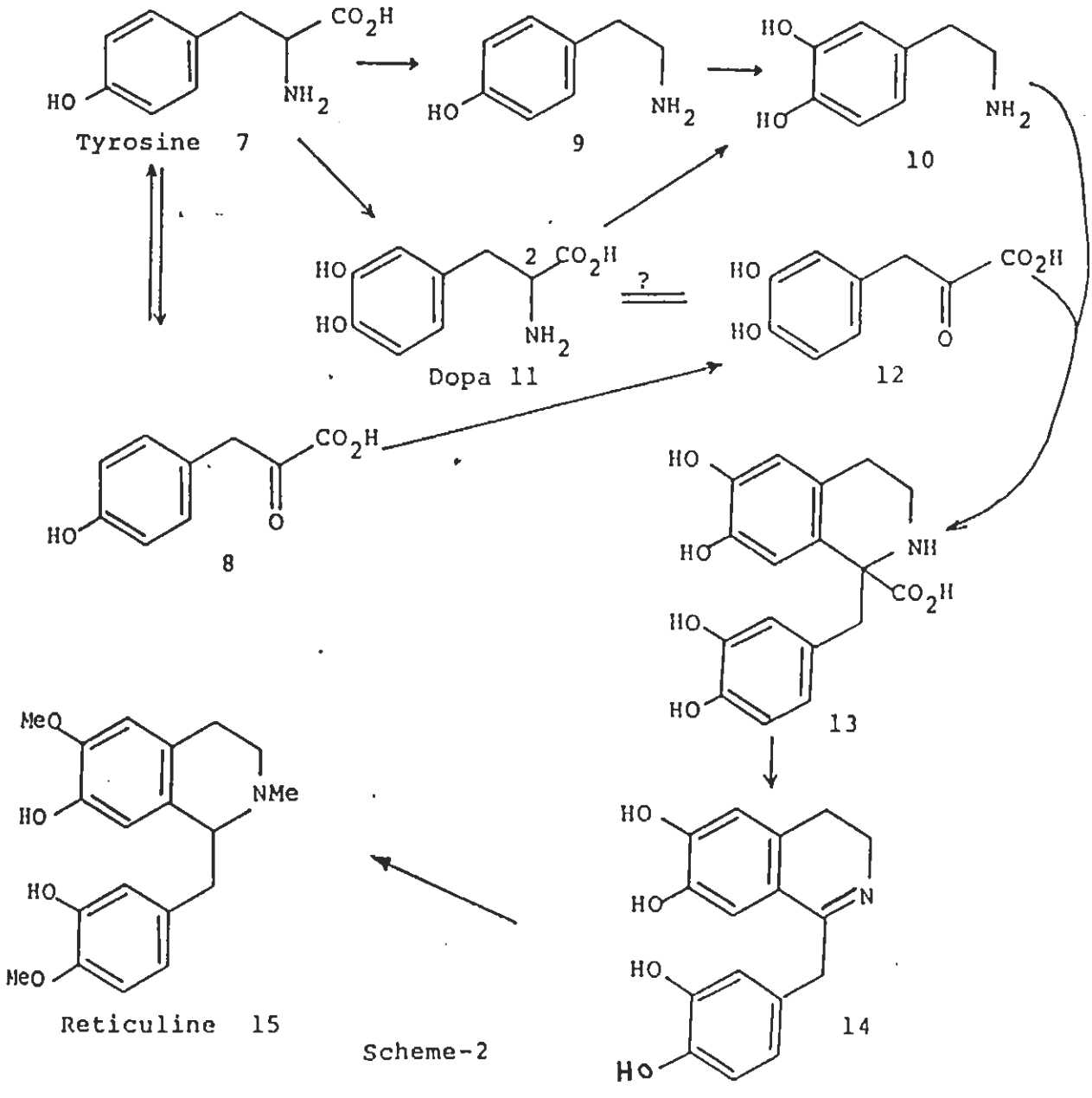
The biosynthesis of this alkaloid was investigated in *Annona reticulata* by Prakash *et al.* It was found to originate from two molecules of tyrosine [3,4,5]. Radioactive tyramine, dopa and dopamine (1) labelled the phenethylamine portion of coclaurine (6) whereas 4-hydroxyphenylpyruvic acid was incorporated into both halves.



Scheme-1

Reticuline:

Tyrosine is the precursor of the important benzylisoquinoline alkaloid reticuline (**15**). The 3,4-dihydroxyphenylpyruvic acid (**12**) formed *via* 4-hydroxyphenyl-pyruvic acid (**8**) condenses with dopamine (**10**) which, in turn, originates from dopa (**11**). The resulting precursor (**13**) gives reticuline (**15**) *via* another precursor (**14**) [6-8].

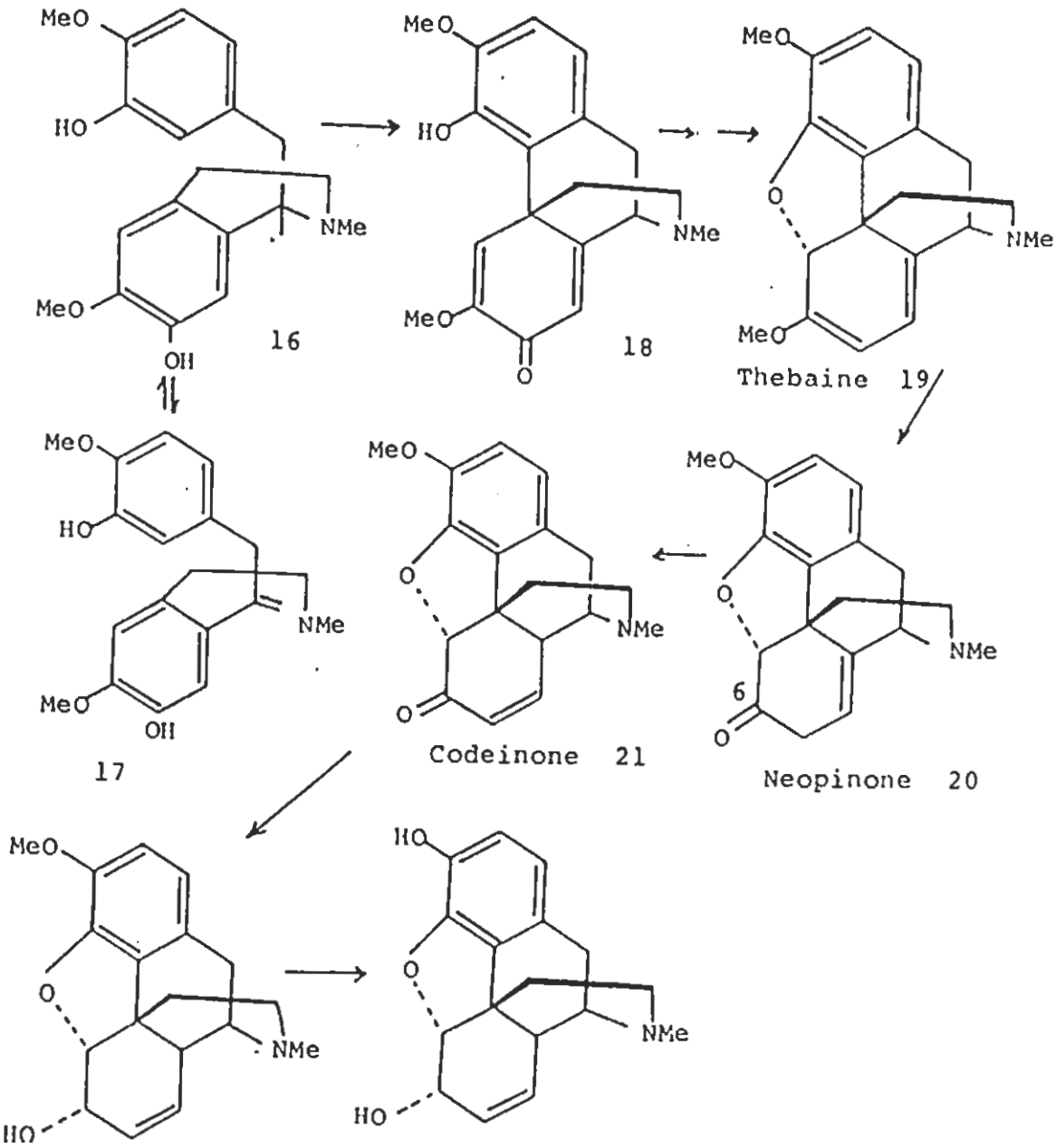


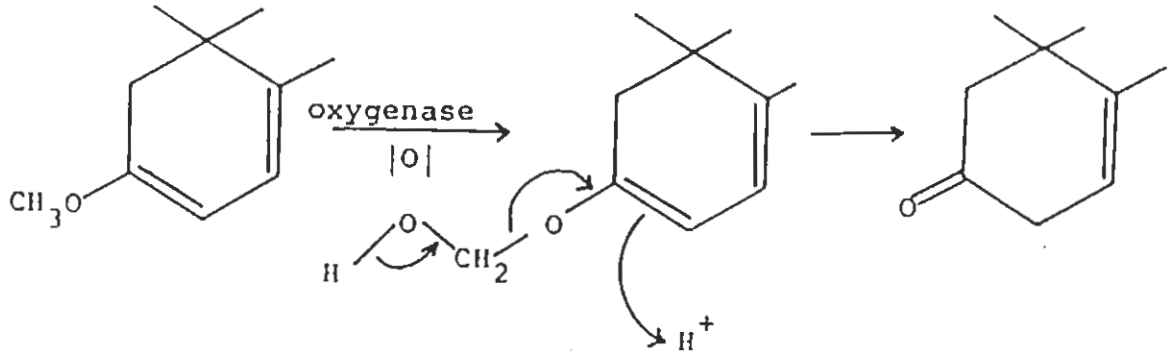
Scheme-2

Morphine:

Extensive research on the biosythesis of morphine (**23**) in *Papaver somniferum* provided the following pathway from tyrosine through reticuline (**16**), thebaine (**19**), codeine (**22**) to morphine (**23**) [9- 11].

Biotransformation of **19** into **22** takes place through neopinone (**20**) and codeinone (**21**) involving 6-O-demethylation as a first step [10,12]. It was shown through isotopic labelling that the conversion of **19** into **20** occurs without loss of the oxygen at C-6. This proved that mechanism of chemical hydrolysis of an enol ether does not operate and the following alternative mechanism was suggested (Scheme 4) [11].

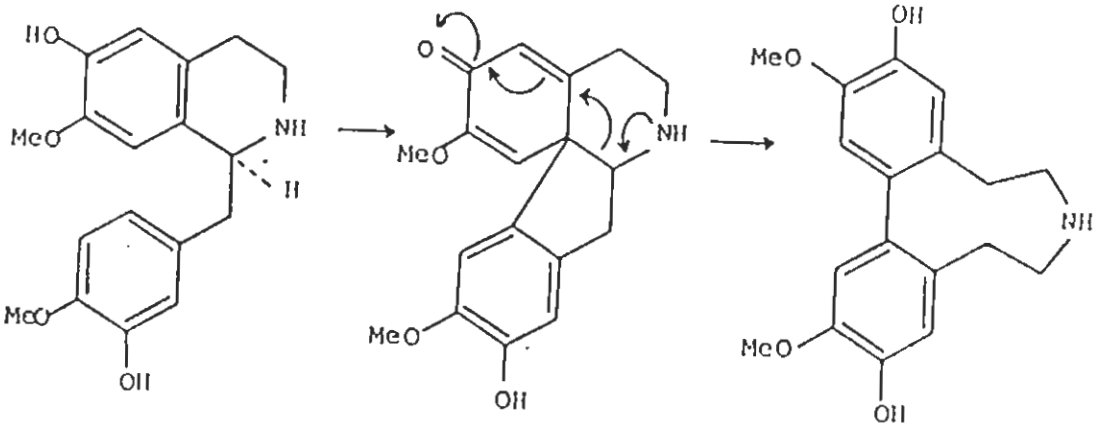




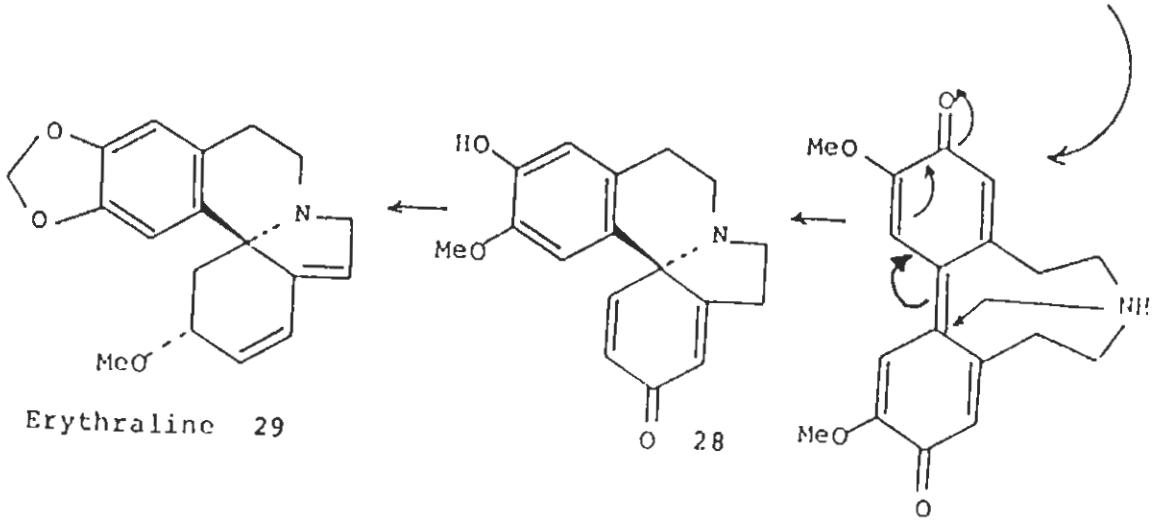
Scheme-4

Erythrina alkaloids:

Erythralline (**29**), cocculidine (**30**) and isococculidine (**31**) are formed like other erythrina alkaloids, from a key benzylisoquinoline precursor, namely (S)-N-norprotosinomenine, (**24**). The biosynthesis involves dienone **25** and dibenzazonine **26** as intermediates [13-16].

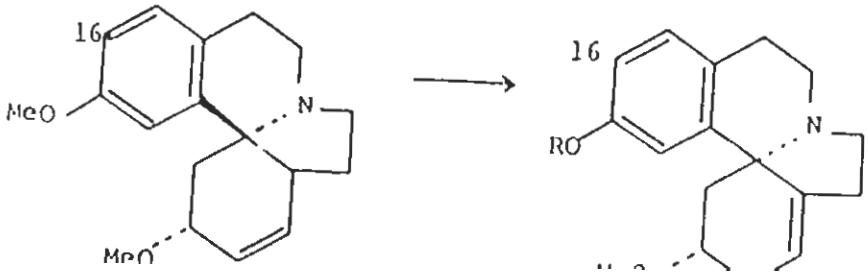


(S)-N-Norprotosinomenine 24 dienone 25 dibenzazonine 26



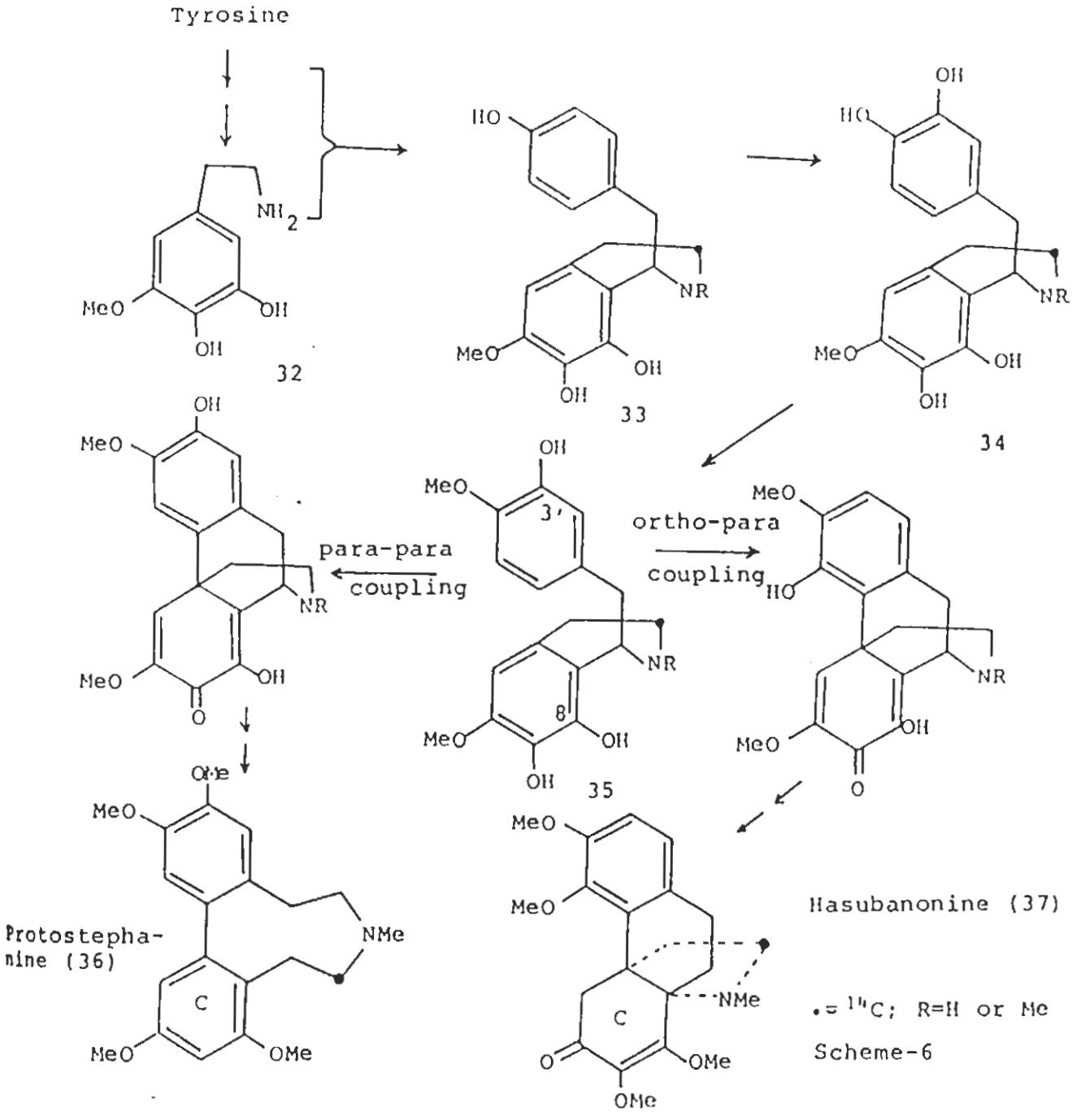
Erythraline 29 28 27

Scheme-5



Protostephanine and Hasubanonine:

Biosynthetic experiments have revealed that protostephanine (**36**) and hasubanonine (**37**), both produced by *Stephania japonica*, are constructed from two C_6-C_2 units derived from tyrosine. The unit which is the source of ring C and the attached ethanamine side chain in (**36**) and (**37**) combines, as (**32**), with the other units [17-19].



INTRODUCTION

Cocculus hirsutus Diels (locally known as Jamti-ki-bel) belongs to family Menispermanceae. It is a climbing shrub inhabiting the dry regions of Sind and Baluchistan provinces of Pakistan. The leaves of this plant are sometimes sublanceolate, retuse or obtuse, and mucronate; sometimes three-lobed, base subcordate or truncate, with young villosus on both surfaces; the petiole is half inch long [20-22]. The roots are very crooked and twisted upon themselves, keeled, seldom branched with a peculiar, acrid odour and a disagreeable and bitter taste. It is alterative, laxative, refrigerant, sudorific and demulcent [22-23]. The male flowers consist of short axillary panicles while the female flowers comprise of axillary clusters of drupe which are dark purple in colour [21].

Medicinal uses of *Cocculus* Plants:

The plants belonging to the genus *Cocculus* are reputed for their curative properties and hence played a vital role in folklore medicine for the last few centuries in the Indo-Pak sub-continent.

The juice of the leaves coagulate in water and form a mucilage which is used externally as a cooling and soothing application in prurigo, eczema and impetigo [22,24,25]. Sweetened with sugar, its juice is given in acute gonorrhoea [20-22,24] to soothe the smarting and scalding. Roxburgh says that a decoction of the root (1 in 10) mixed with long pepper and goat's milk, may be given in doses of two to four ounces in chronic rheumatism [20-25], syphiltic cachexia [24] and is considered heating, laxative and sudorific [20]. Its decoction in combination with ginger and sugar is given in cases of bilious dyspepsia and cases of fevers alongwith other bitters and aromatics. Roots rubbed with bonduc nuts in water are given to relieve stomach-ache, specially in children [20-22]. The water soluble fraction of the ammonical extract has sedative, hypotensive, bradycardiac, cardiotonic, spasmolytic and slight anticonvulsant actions [26]. The juice of the ripe berries makes a durable, bluish-purple ink [20].

The juice of the fresh fruit of *C. suberosus* or *C. indicus* has exactly the opposite effects of morphia on the blood pressure it forms a best antitode to morphia poisoning [22].

The *C. trilobus* is used as anodyne and a diuretic, a remedy for dropsy,

gonorrhoea, and fever. A decoction of the dried roots is drunk to treat cystitis, edema, bronchitis and paralysis. The roots are imported as a drug for rheumatism and such diseases as cholera and pulmonary hemorrhage [27]. The Chinese pharmacies in Vietnam receive the stem of *C. sarmentosus* (Lour) Diels cut into fine rounds; in an infusion it is prescribed to reduce swollen legs; a decoction of the roots is considered to be fibrifuge, and a remedy for epilepsy, as is that of *C. trilobus* [27].

The entire plant of *C. cardifolius* is regarded as a valuable alternative and tonic. The stem is antipurgative. In large doses the roots are a powerful emetic. The creeper, from which "Guduchi Satwam" is prepared, heads the list of valuable bitter tonics in the Ayurvedic Pharmacopoeia and is the bitterest amongst them. It is a very valuable tonic and is best given in infusion with or without milk; the tincture and extract (Guduchi Satwam), which is a starchy matter, is administered in ghee or in sugar and water, or milk. It is also a valuable nutrient when there is intestinal irritability and inability to digest any kind of food, and is largely used in (indigenous) practice, in cold fevers, seminal weakness and urinary affections; especially the extract in 5 to 10 gram doses, is useful in general and seminal debility, fever, vomiting, jaundice, torpidity of the liver, skin diseases (patches and small boils on the surface of the skin, generally in the extremities, often painful and persistent), secondary syphilis, rheumatism, acidity of urine, and urinary diseases, various forms of diabetes, some forms of dyspepsia, irritability of the stomach, splenic affections, chronic gonorrhoea, chronic diarrhoea, and in some forms of obstinate chronic dysentery [22]. A decoction of the root is also a vermifuge.

The work on the chemical constituents of *Cocculus* species started as far back as 1923 when K. Goto reported two new alkaloids namely cucoline[(1)] and dehydrocucoline from *C. diversifolius* [28]. The structure of dehydrocucoline could not be elucidated.

In 1924, H. Knodo and T. Nakzato reported the isolation of trilobine[(2)] from *C. trilobus* [29] and elucidated its structure. Later the base was found to be of general occurance and was isolated from a large number of *Cocculus* species [30-35].

H. Kondo and T. Kondo in 1925 isolated another new alkaloid coclaurine[(3)] from *C. laurifolius* [36]. Later, this alkaloid was reported from many other *Cocculus*

species [32,34,37,39]. In the same year K. Ohta reported two further new alkaloids: diversine and kukoline, respectively [40,41].

Menisarine[4] and isotirilobine[5] were isolated from *C. saramentosus* by Kondo and Tomita in the same year [42,43]. These alkaloids were also subsequently identified from other species of *Cocculus* [32-35,38].

In 1931, the same authors reported the isolation of another new alkaloid from *C. trilobus*. They named it trilobamine[6] [44]. The chemical and spectral evidence provided following structure for this compound $C_{35}H_{36}O_6N_2$ Formula number .

H. kondo and M. Tomita in 1935 isolated another new alkaloid normenisarine[7], from *C. saramentous* [45]. Two more alkaloids having molecular formulae $C_{36}H_{34}N_2O_6$ and $C_{36}H_{36}N_2O_6$ were isolated by them in the same year [46].

Calumbine and palmatine[8] were isolated from *C. leaba* by Beauquesne In 1938 [47-48]. Palmatine is represented by the following structure[8] while the structure of the calumbine could not be established.

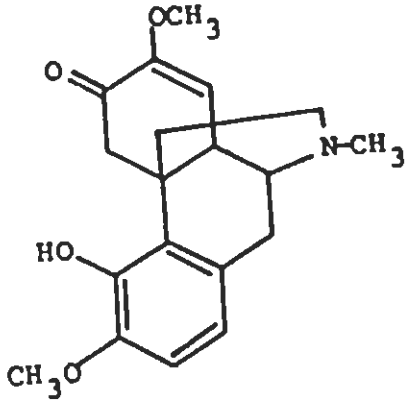
In 1950, Yunusov and co-workers communicated the isolation of cocculidine[9] and coculine[10] [49-55]. The structures **9** and **10** were assigned to these compounds on the basis of chemical and spectral studies.

M. Tomita and F. Kusuda in 1953 isolated another new alkaloid laurifoline[11] , from *C. laurifolius* [56]. Later this alkaloid was also reported from many other *Cocculus* species [57,58]. In the same year Kusuda reported a new alkaloid coclanoline[12] from *C. laurifolius* [59].

Dihydroerysodine[13] was isolated from *C. laurifolius* [60] by Tomita and Yamaguchi in 1955.

In 1956 the same authors reported the isolation of a base of unknown structure[14] from *C. laurifolius* which they named as coclamine [61]. In the same year Nakano reported a new α magnoflorine from *C. laurifolius* and *C. trilobus* [62-63] respectively. Later the base was found to be of general occurrance and was isolated from a large number of other *Cocculus* species [34,48,57,58].

A Sinha, in 1960, isolated a new alkaloid, sinactine[15] , from *C. trilobus* [38].



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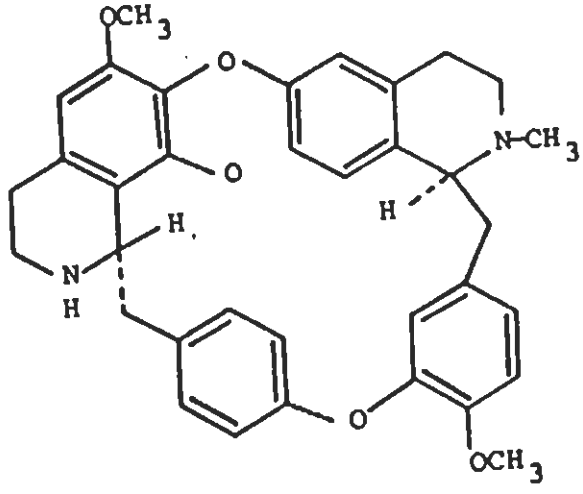

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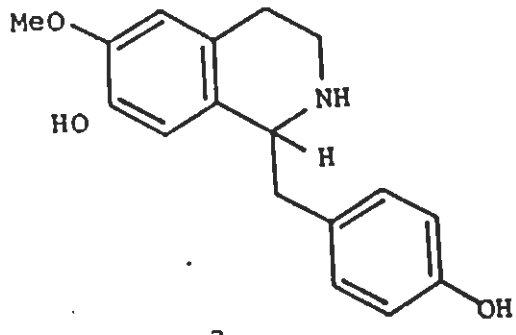

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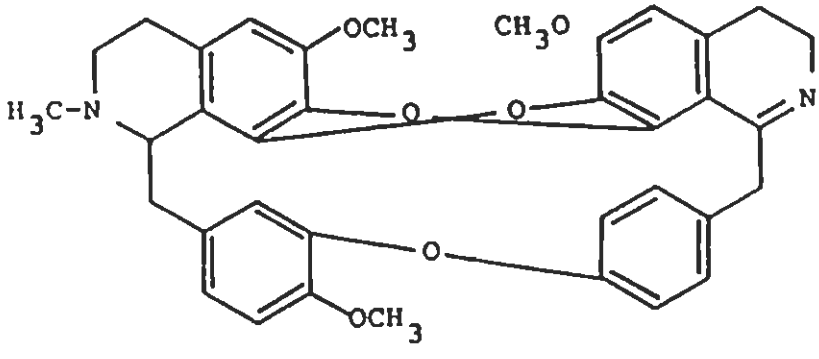

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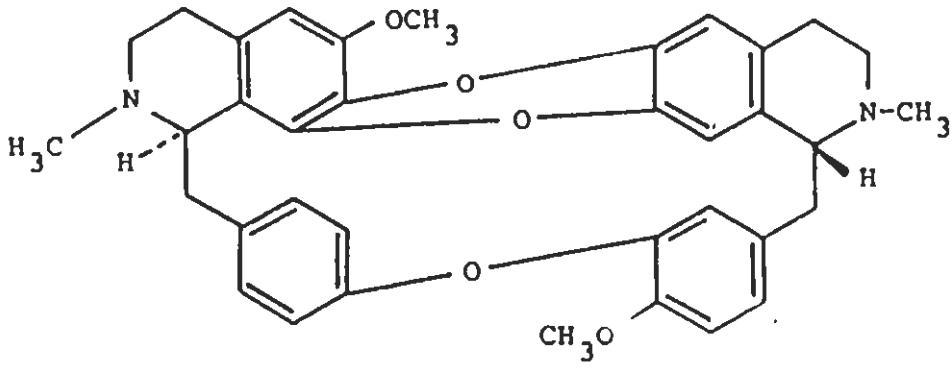

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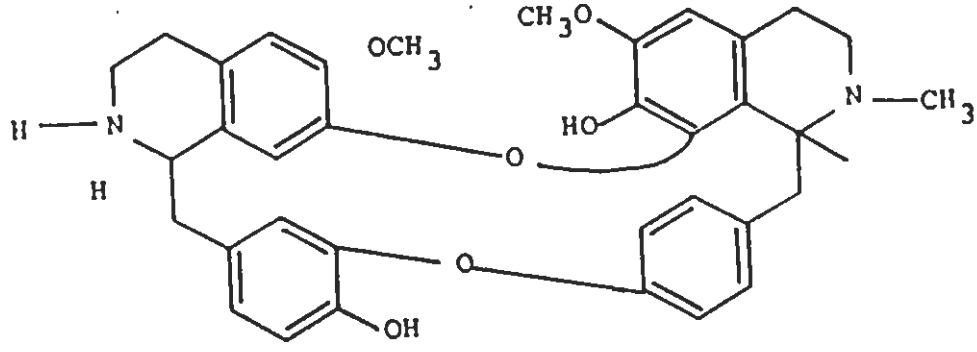

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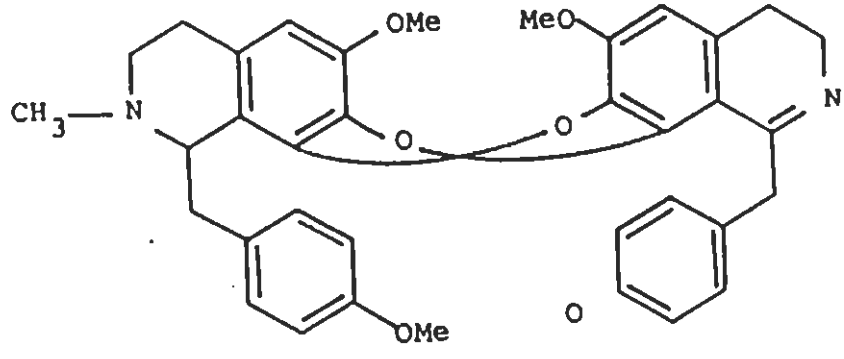

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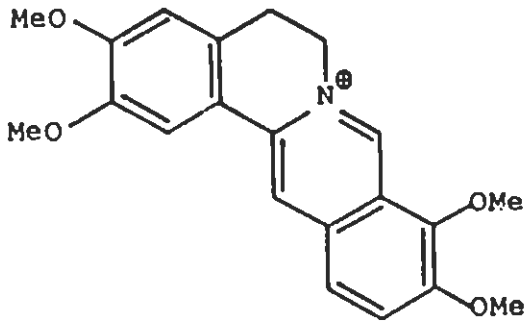
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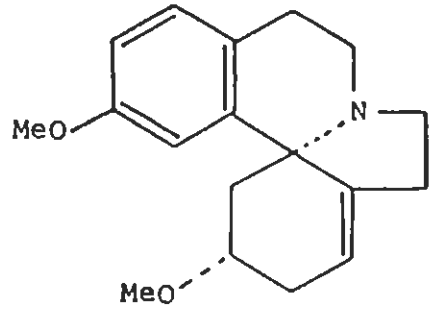
MeO
N⊕
OMe
OMe

8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N
MeO

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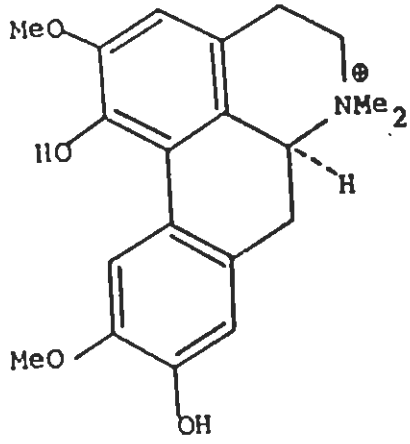
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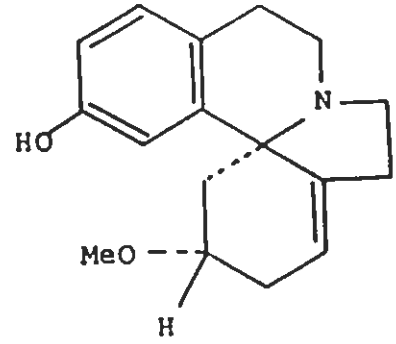
⊕
NMe2
HO
H
MeO
OH

11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N
MeO
H

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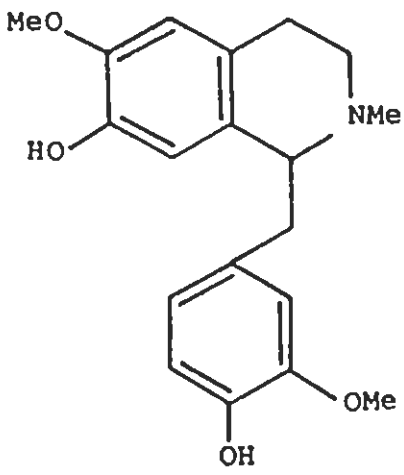
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OH

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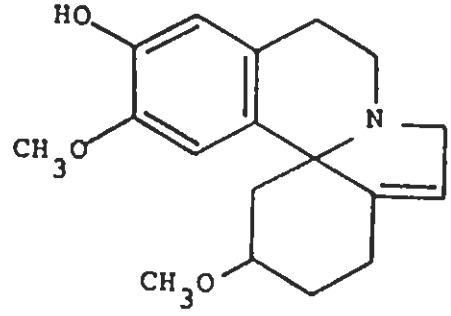
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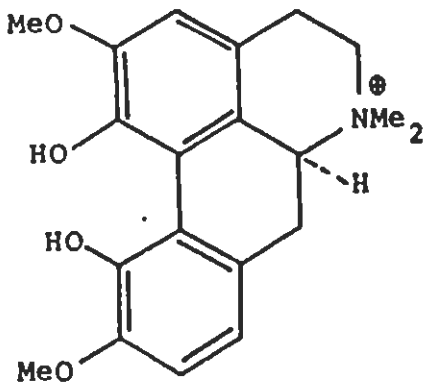
CH3O

13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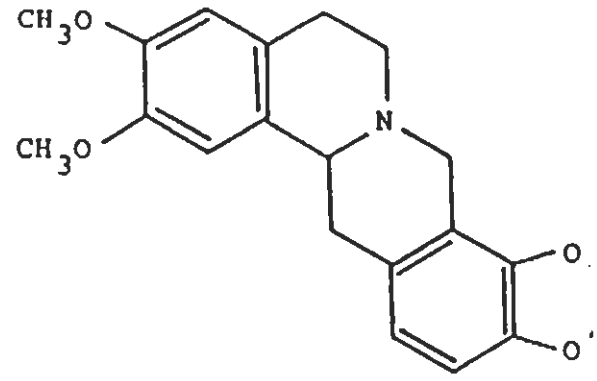
HO
HO
MeO
NMe2
H

14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CH3O
N
O
O

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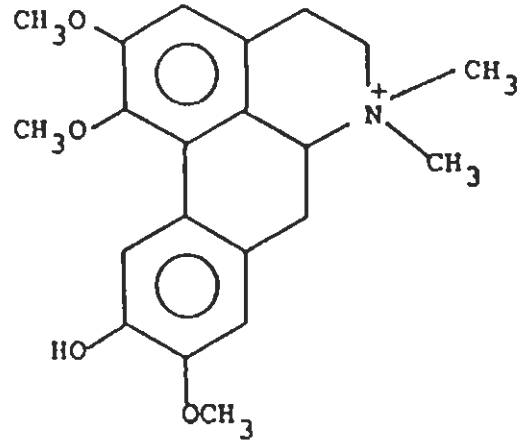
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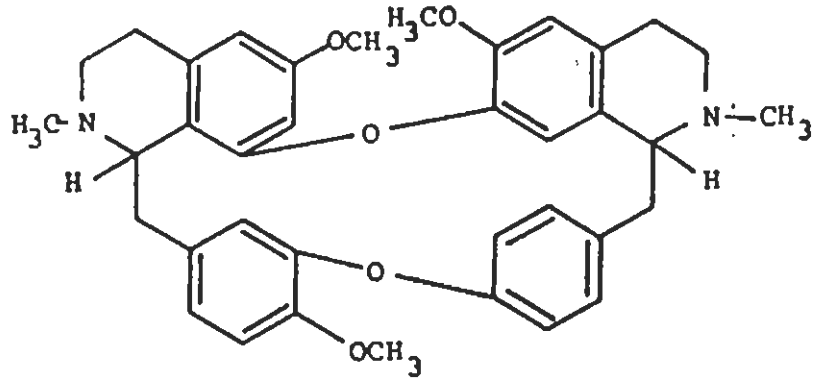
CH3O
N
CH3
CH3
HO
OCH3

16


H3CO
OCH3
H3C
N
O
N
CH3
H
H
O
OCH3

17

Cocsarmine[16] and tetrandrine[17] were isolated from *C. sarmentosus* by Tomita and Furukawa in 1963 [33].

In 1968, Wada and co-workers reported the isolation of isoboldine[18] from *C. trilobnus* [64-65] and elucidated its structure.

I. Kazuo *et. al.*, in 1969, isolated another alkaloid coclobine[19], from *C. trilobus* [66]. In the same year Yasuo *et. al.* reported another alkaloid which they named as erythroculine[20] and presented structure[20] for this compound [57,67].

Reticuline[21] was isolated from *C. laurifolius* by Yasuo *et .al.* in 1970 [57].

In 1972, Slatkin and co-workers reported the isolation of sinoacutine[22] from *C. carolinus* [48].

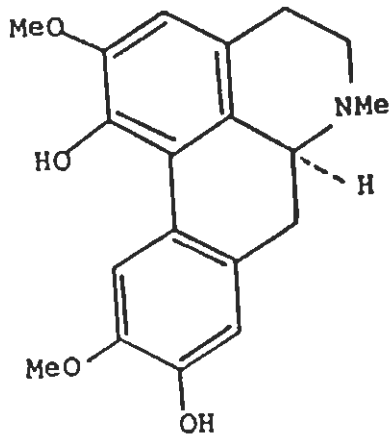
In 1974, the same authors reported the isolation of another new alkaloid from *C. carolinus* and named it carococculine[23] [67]. Chemical and spectral evidence provided the following stucture for carococculine[23].

Bhakuni and Joshi, in 1975, reported six new alkaloids, namely: cocculine[24], cocsoline[25], coculinine[26], pendine, penduline and pendulinine, from *C. pendulus* [69-73]. The structures of cocculine, cocsoline, and penduline were established by them through spectral studies. Later, cocsuline was also reported from *C. laurifolius* [70-73]. The structures of coculinine, pendine and pendulinine could not be established by them.

Laurifine[27], laurifinine[28] and laurifonine[29] were isolated from *C. laurifolius* by Uprety and Bhakuni in 1975 [74-75].

In 1976, Mc Phail *et. al.* reported the isolation of coccutrine[30] from *C. trilobus* [52,55]. In the same year, Elsohly *et. al.* reported cocculolidine[31], from *C. carolinus* [53]. Later, this alkaloid was also reported from *C. trilobus* [55,64,65,76,77].

The Bhakuni group, in 1976, isolated three new alkaloids: isococculidine[32], coccoline[33], and coccolimine[34], from *C. laurifolius* [51.78]. In the same year coccuvine was isolated from *C. laurifolius* by Singh, Pande and Bhakuni [79].



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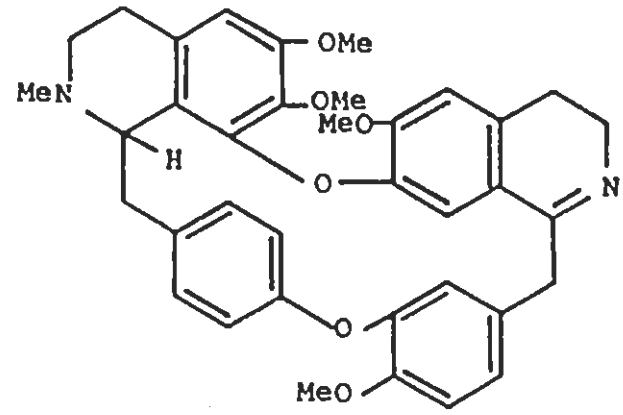

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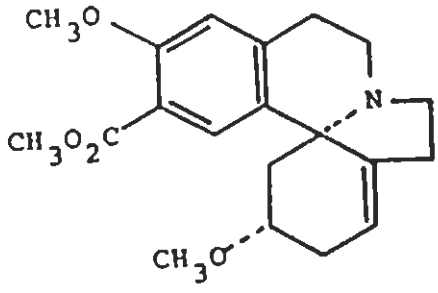

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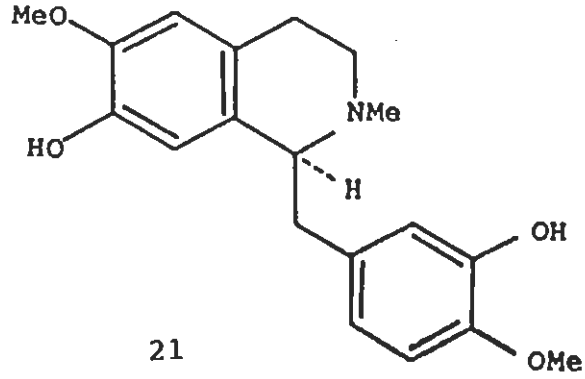

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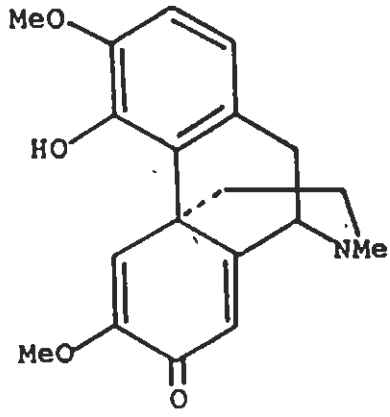

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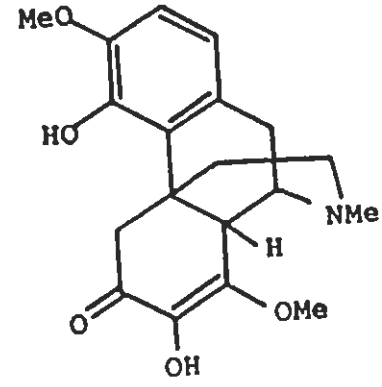

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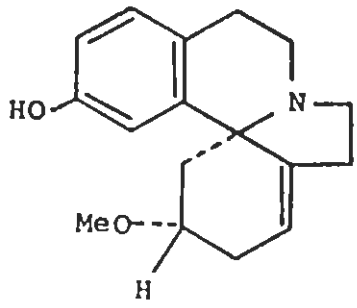
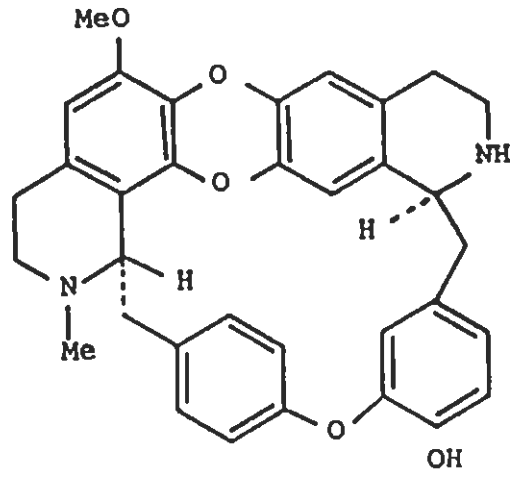
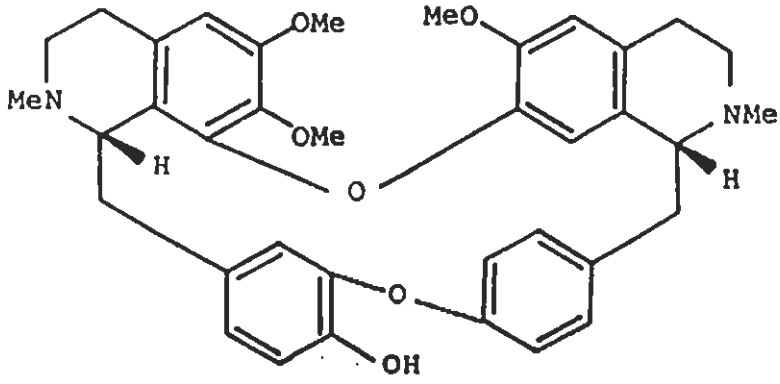

HO
N
MeO
H

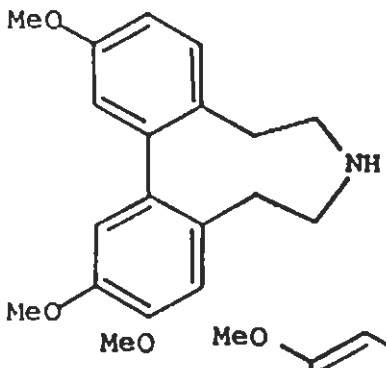
24


MeO
O
O
NH
H
N
H
Me
O
OH

25


OMe
MeO
MeN
OMe
NMe
H
H
O
O
OH

26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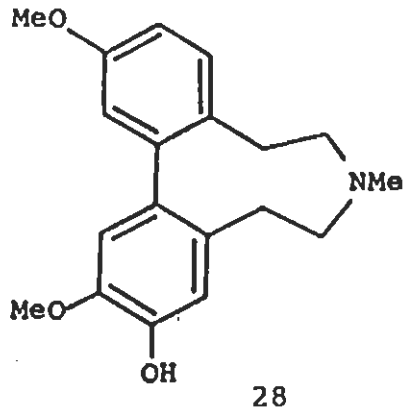
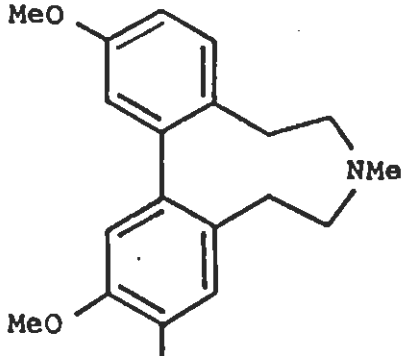
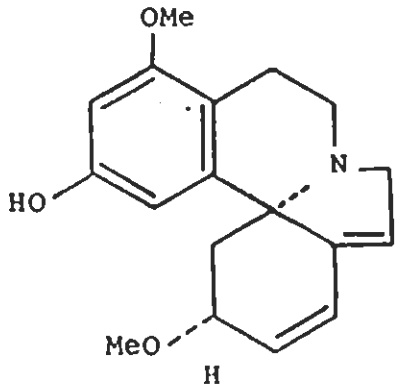
NH
MeO
MeO

27


MeO
NMe
MeO
OH

28


MeO
NMe
MeO



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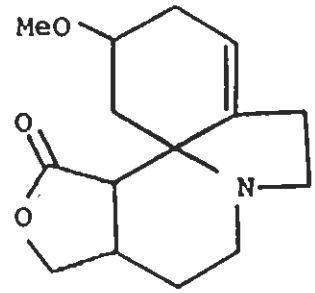

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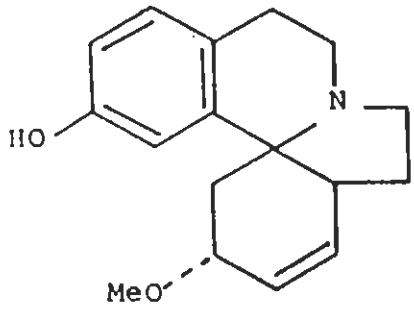

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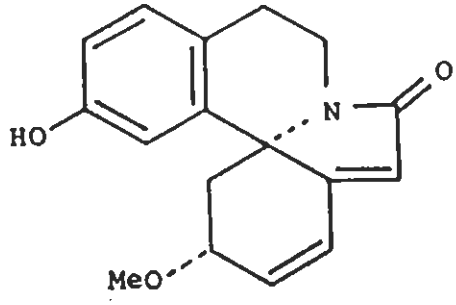

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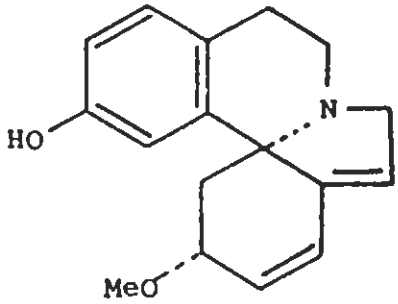

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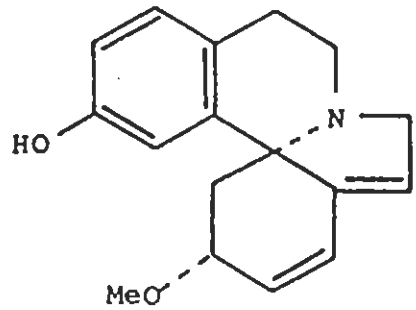

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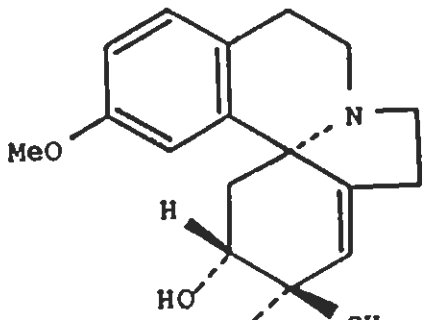
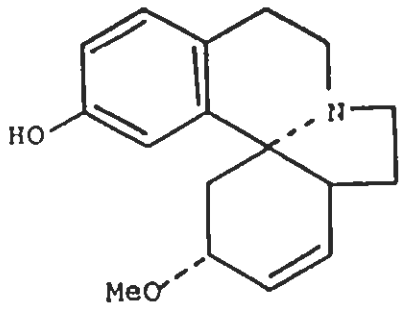
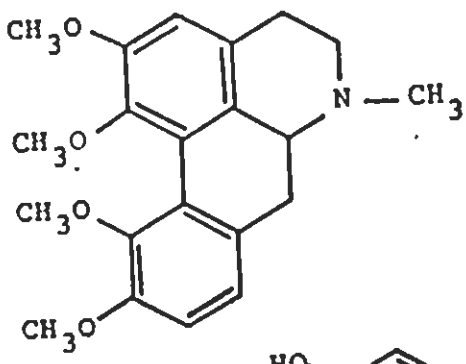
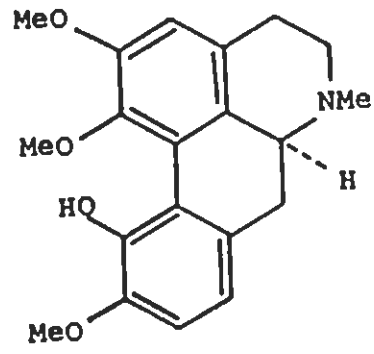



HO
N
MeO

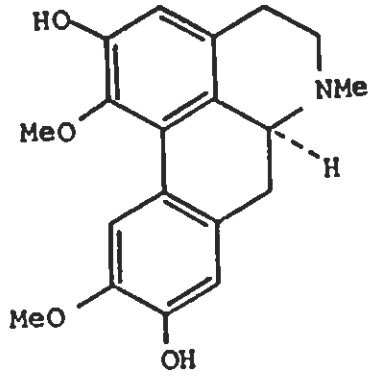
37


CH3O
CH3O
CH3O
CH3O
N — CH3

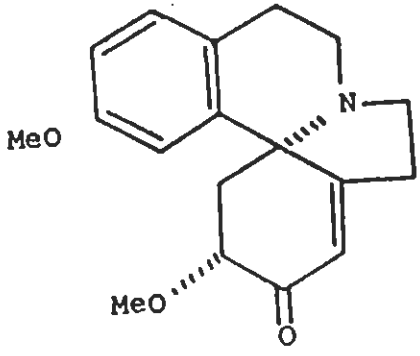
38

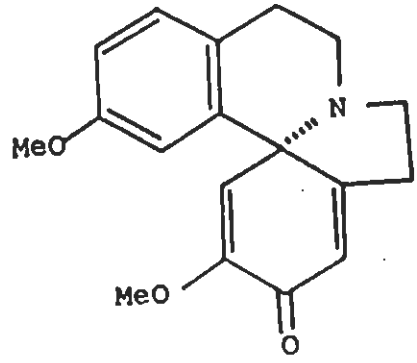

MeO
MeO
HO
MeO
NMe
H

39

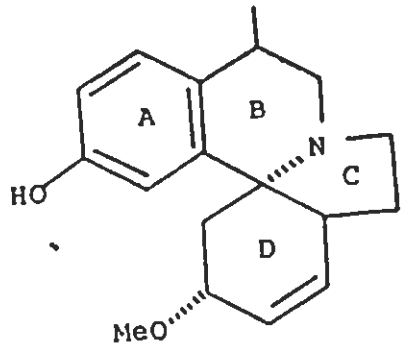

HO
MeO
MeO
OH
NMe
H

40


MeO
MeO
N
O


MeO
MeO
N
O

42



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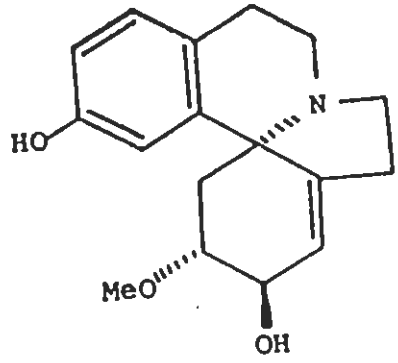

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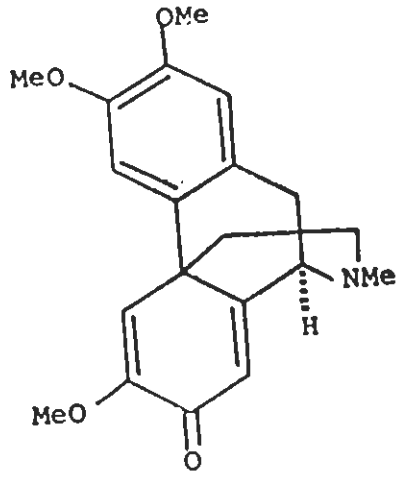

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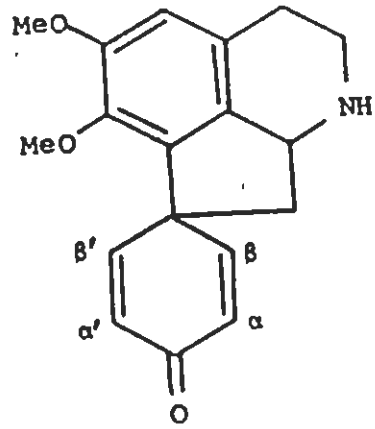

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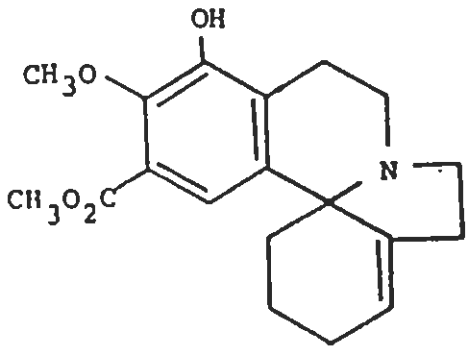

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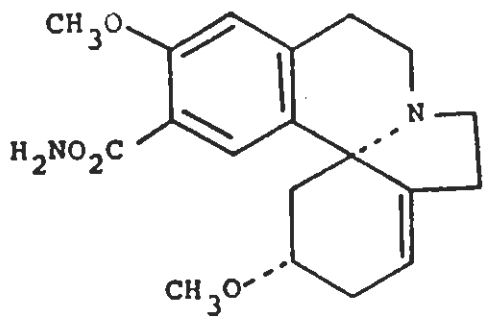

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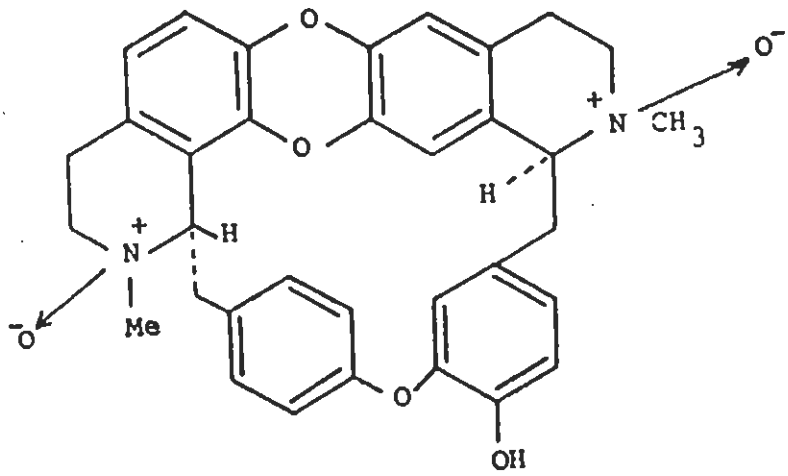

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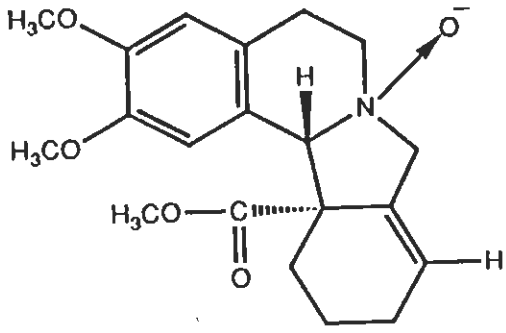

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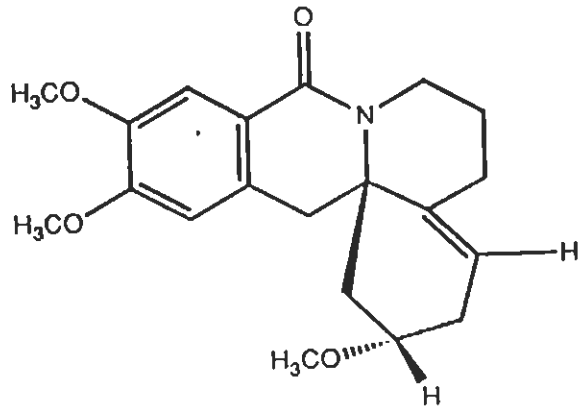

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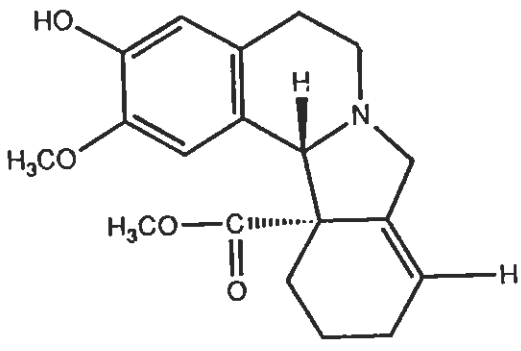

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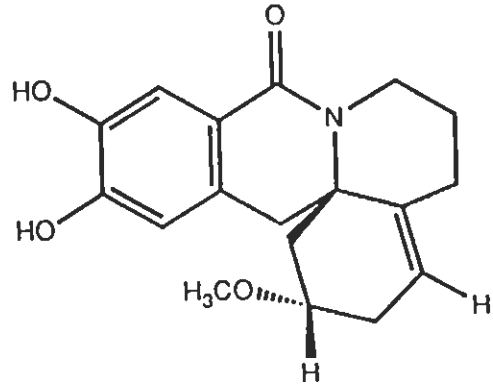

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In 1977, Singh *et. al.* reported the isolation of a new base from *C. laurifolius* and named as coccuvinine[35] and cocculitine[36] [80,81]. The chemical and spectral evidence provided structures for these compounds. In the same year Juichi *et. al.* reported dihydroerysovine, but its structure could not be elucidated [80].

Singh, Jain and Bhakuni, in 1978, isolated isococculine[37] from *C. laurifolius* [83-84].

O-methyl isocorydine[38], isocorydine[39] and boldine were isolated from *C. laurifolius* by Sexena and Bhakuni in 1979 [58].

In 1980, Bhakuni and Jain reported the isolation of six new alkaloids coculidinone[40], cocculienone[41], cocculimine[42], cocculitinine[43], sebiferine[44] and stepharine[45] from *C. laurifolius* [85].

Juichi *et. al.* (1981) isolated erythlaurine[46] and erythramide[47] from *C. laurifolius* [86,87].

In 1982, Bhakuni, Gupta and Joshi reported the isolation of ophiocarpinone[48], from *C. pendulus* [88-89].

Cocsuline N-2-oxide[49] was isolated from *C. hirsutus* by El- Shabrawy *et. al.* in 1984 [79]. It was the first N-oxide which was reported in this species. In the same year Hussain and co-authors reported four new alkaloids, cheratamine, kohatine, kurramine and norpenduline, from *C. pendulus* [90].

In 1986 V.U. Ahmad *et. al.* reported hirsudiol and nonacosan-10-ol from *C. hirsutus* [91] V.U. Ahmad *et. al.* also issued a phytochemical report of chemical constituents of *C. hirsutus* in 1986 [92]. In 1987, V.U. Ahmad *et.* al. Isolated jamtine-*N*-oxide (50) from *C. hirsutus* [93]. In the same year V.U. Ahmad *et. al.* also reported cohirsine (51) from same plant [94]. In 1991 Tahir Rasheed *et. al.* isolated hirsutine (52) from *C. hirsutus* [95]. In the same year Tahir Rasheed *et. al.* also Isolated from *C. hirsutus* shaheenine (53) [96].

Present Work

The present work is mainly concerned with the isolation of the chemical constituents of *Cocculus hirsutus* and elucidation of their structures. In order to avoid the formation of artefacts only fresh plant material was used in these studies.

The ethanolic extract of the total plant material was evaporated under reduced pressure and the residue obtained was defatted by partitioning between ethyl acetate and water. The aqueous portion was ammoniated and the crude alkaloids were extracted with chloroform. The alkaloid-containing mixture obtained on evaporation of the chloroform layers was subjected to different isolation techniques, mainly column chromatography, flash column chromatography, and a number of new and reported alkaloids were isolated. They were characterized through specteral and chemical studies.

Five new compounds were isolated from *C.hirsutus* in the present studies: They are:

1) Cohrisitine

2) Cohirsitinine

3) Jamtinine

4) Haiderine

5) Cohirsinine, and

6) Besides, yangambin [97] was also isolated for the first time from *C.hirsutus*

The compounds listed below have already been reported earlier from the same plant, but were also Isolated in the present work.

7) Jamtine-N-oxide [93]

8) Hirsudiol [91]

9) Shaheenine [96]

10) Hirsutine [95]

11) Cohirsine [94]

12) (+) Syringoresinol [92]

13) α-Oxypropiosyringon [92]

14) Nonecosan-10-ol [91]

15) Isotrilobine [92]

16) Trilobine [92] and

17) (+) Protoquaercitol [92]

Some additional compounds were also isolated, however the complete structure elucidation of these compounds awaits the collection of more quantities.

Apart from these constituents, the fatty acid component of the plant were also determined and the fatty acids identified as caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid and behneic acid. [98]

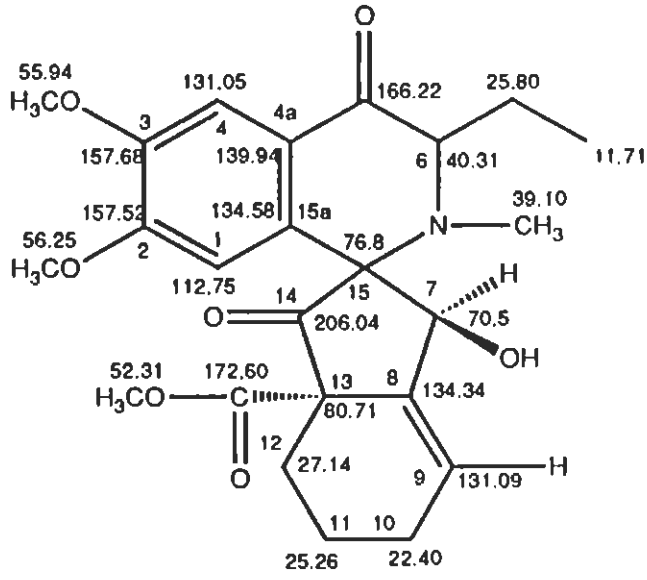
Isolation and structural study of Cohirsitine (1)

The extract was evaporated, extracted with EtOAc, the layer was basified with ammonia and the crude alkaloids were extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated and the residue was extracted with hexane, Et_2O, $CHCl_3$ and MeOH. The Et_2O soluble portion was subjected to Si-gel PTLC with $CHCl_3$-MeOH (9.5:5). The major alkaloid, cohirsitine [1] (Rf=0.40, 20 mg), $[\alpha]^{27}_D$ + 147° ($CHCl_3$), gave the characteristic orange colour reaction with Dragendroff's reagent.

The UV spectrum of the isolated alkaloid showed absorptions at 238, 289, 313 nm reflecting the isoquinoline chromophore [99]. The IR spectrum showed an intense absorption at 1670 and 1710 cm^{-1}, indicating the presence of carbonyl [100], and a hydroxyl group at 3570 cm^{-1}. High resolution mass spectroscopy (HRMS) afforded the molecular ion peak at *m/z* 445.21299 corresponding to the molecular formula $C_{24}H_{31}NO_7$. Other prominent peaks were found to occur at *m/z* 430 $(M\text{-}Me)^+$, 370 ($430\text{-}C_2H_4O_2$, base peak), 312, 308, 298, 285, 256, 228 and 208. The molecular ion peak at *m/z* 445 was confirmed by FAB mass spectroscopy[101].

The ^{1}H-NMR spectrum showed the presence of 31 protons, each of which was identified by a series of homodecoupling experiments, NOE difference measurements, COSY-45, and heterocosy experiments. A one proton singlet at δ 6.69 was assigned to the C-1 proton. The C-4 proton appeared at δ 7.74 as singlet, its downfield chemical shift reflecting the presence of a β carbonyl function [102]. The presence of two 1H singlets in the aromatic region indicated the substitution at C-2 and C-3[14]. A broad singlet at δ 5.62 was assigned to the C-9 olefinic proton, the small coupling constant indicating that this olefinic proton is gauche to the adjacent methylenic proton [103]. The rather upfield singlet at δ 3.25 was assigned to the C-13 carbomethyoxy group, which is due to the shielding influence of the aromatic nucleus [101,103]. The signal at δ 4.33 was assigned to an allylic hydrogen (H-7) geminal to a hydroxyl group resonating at δ 7.01. Evidence for the configuration at C-7 comes from the fact that H-7 is unaffected when the N-CH_3 is irradiated [103].

On the basis of NOE measurements of different groups we came to conclusion that C-15 has the relative stereochemistry indicated in structure [1]. For example, the C-7αH did not show a NOE with any other proton and this is only possible when C-15 and C-7 have an 'α' linkage. If C-7 and C-15 would be 'β' oriented



^{13}C NMR COHIRSITINE (1)

then C-7αH would have shown a NOE with N-CH_3 and C- 6βH. Similarly, the *N*-methyl showed a NOE effect with H-6β meaning that the N-C-15 bond had a β-orientation. By Dreiding model examination it was demonstrated that C-15 has the stereochemistry indicated in structure 1. Any other orientation of bonds increases the strain in ring C [105].

Two dimensional ^{1}H-NMR measurements were carried out to verify the assignments. The coupling interactions were established through correlated spectroscopy (COSY-45) spectrum while the multiplicity of overlapping proton signals was determined from the 2D-*J*-resolved spectrum [106-109]. The assignments for the C-10 protons at δ 2.69 could thus be confirmed by the COSY-45 spectrum of cohirsitine, which showed a strong cross peak with the signal at δ 5.62 for the C-9 olefinic proton. The assignments for the Nuclear Overhauser Enhancement Spectroscopy (NOESY) spectrum served to establish the spatial proximities. The stereochemistry of the ester group could be determined by the NOESY spectrum, which showed a cross peak between C-1 proton and the methoxy protons. This could only arise if the ester group possesses α stereochemistry [104]. The H-6 proton resonated as a double doublet and the downfield chemical shift is due to the presence of the adjacent nitrogen atom. The methylene protons of the ethyl group attached at C-6 resonated as a multiplet and the methyl protons of the same group at δ 1.12 as a triplet.

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) showed the presence of 24 carbon atoms in the molecule (see Table I). The multiplicity assignments were made using a DEPT pulse sequence with the last polarization pulse angle θ = 45, and 135°.

Table-(I) ^{13}C-chemical shift of Cohirsitine (1)

Carbon No.	δ	Carbon No.	δ
1	112.75	12	27.14
2	157.52	13	80.71
3	157.68	14	206.04
4	131.05	15	76.8
4a	139.94	15a	134.58
5	166.22	2-OMe	56.25
6	40.3	3-OMe	55.94
7	70.5	CO_2Me	52.31
8	134.09		
10	22.40		
11	25.26		

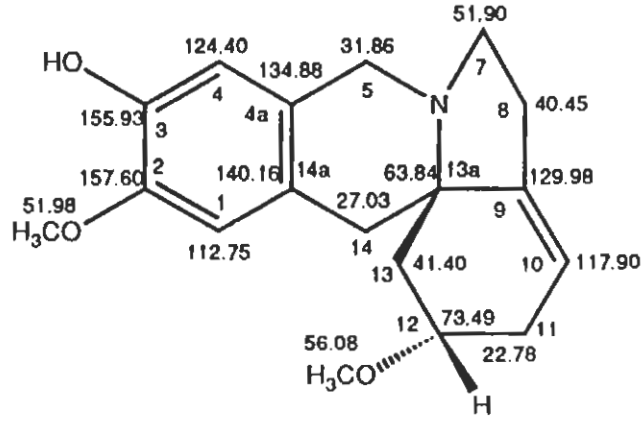
Isolation and structural study of Cohirsitinine

The aqueous layer was basified with NH_3, and the crude alkaloids were extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated, dried with anhydrous Na_2SO_4 (74 g), and subjected to cc. The fraction obtained with hexane-Me_2CO (6:4) was subjected to preparative tlc on Si gel plates with $CHCl_3$-MeOH (9:1) as the solvent system. This afforded the pure alkaloid, cohirsitinine [2]. R_f 4.5 (6 mg), $[\alpha]^{25}_D$=51° ($CHCl_3$), which gave the characteristic dark orange color reaction with Dragendorff's reagent.

The pure alkaloid, cohirsitinine [2], was isolated as a gummy material. Its uv spectrum showed absorptions at 213, 236, and 290 nm, which indicated an isoquinoline skeleton [110]. The IR spectrum showed an intense absorption at 3500 and 1230 cm^{-1}, indicating the presence of hydroxyl groups [106]. The phenolic nature of the latter was confirmed by a positive reaction with $FeCl_3$ which gave a green color. When the compound was treated with D_2O and the mass spectrum was recorded again, the $[M]^+$ peak was found to be shifted by 1 mass unit to m/z 302, suggesting the presence of one exchangeable hydrogen atom in the molecule[112]. The HRMS indicated the molecular ion peak to be at m/z 301.1663, consistent with the molecular formula $C_{18}H_{23}NO_3$ (calcd 301.1671), indicating eight degrees of unsaturation in the molecule. Other prominent peaks were found at m/z 270, 243, 226, 210, 165, 150, and 132. The peak at m/z 270.1482 ($C_{17}H_{20}NO_2$, calcd 270.1488) corresponded to the loss of an OMe group from the molecular ion.

There was a prominent peak at m/z 243.1177 ($C_{15}H_{17}NO_2$, calcd 243.1254) in the mass spectrum, which showed the loss of 58 mass units (C_3H_6O) from the molecular ion attributed as a result of retro-Diels-Alder fragmentation of ring D. The peak at m/z 226.1219 ($C_{15}H_{16}NO$, calcd 226.1227) indicated the loss of a hydroxyl group from m/z 243.1177 ($C_{15}H_{17}NO_2$).

The peak at m/z 120.0809 ($C_8H_{10}N$, calcd 120.0810) indicated the loss of 150 mass units ($C_9H_{10}O_2$) from m/z 270.1482 ($C_{17}H_{20}NO_2$, calcd 270.1488), corresponding to the RDA fragmentation process of ring B. The molecular ion was confirmed by fabms [113].



^{13}C NMR OF COHIRSITININE (**2**)

The ^{1}H-nmr spectrum of cohirsitinine [2] ($CDCl_3$, 300 MHz) showed the presence of 23 protons in the molecule, each of which was identified by a series of homodecoupling experiments, nOe difference measurements, and COSY-45 spectra. H-13β appeared as a triplet at δ 1.68 ($J_{13\beta,12}$= $J_{13\beta,13\alpha}$=13.6 Hz), and H-13α appeared as a double doublet at δ 2.26 showing geminal coupling with H-13β ($J_{13\alpha,13\beta}$=13.6 Hz) and vicinal coupling ($J_{13\alpha,13\beta}$=13.6 Hz) with H-12. The downfield chemical shift observed for H-12 indicated the presence of an OMe function at this carbon atom. The multiplets at δ 2.69 and 3.20 were assigned to H- 11α and H-11β, respectively. A broad singlet at δ 5.67 was due to the C-10 olefinic proton [114]. The H-5α and H-5β signals appeared as doublets ($J_{5\alpha,5\beta}$= $J_{5\beta,5\alpha}$=13.5 Hz) at δ 3.29 and 2.96, showing only geminal coupling and indicating the presence of a quaternary carbon α to C-4, and the downfield chemical shift of C-5 showed its direct attachment to nitrogen. Similarly, H-14α and H-14β exhibited doublets ($J_{14\alpha,14\beta}$= $J_{14\beta,14\alpha}$=12.4 Hz) at δ 2.42 and 2.40 showing only geminal coupling, thus indicating the presence of two quaternary carbons α to C-14. The H-7α proton appeared at δ 3.53 as a multiplet while another multiplet at δ 3.07 was assigned to the H-7β proton. The H-8α and β protons resonated at δ 3.54 and 2.68 as multiplets. Two 3H singlets at δ 3.27 and 3.86 were assigned to the 12-OCH_3 and 2-OCH_3 groups, respectively. The C-1 proton resonated at δ 6.66 as a singlet while another singlet at 7.81 was assigned to the C-4 proton.

The nOe and NOESY spectra served to established the spatial proximities. The signal at δ 6.66 (H-1) showed an nOe interaction with the signal at δ 3.86 (2-OCH_3). The signal at δ 2.26 (H-13α) showed an nOe interaction with the H-13β proton at δ 1.68 in the NOESY spectrum. In order to confirm the relative stereochemistry of the molecule and to record the subtle nOe effects not visible in the NOESY spectrum, nOe difference measurements were carried out. Irradiation at δ 1.68 (H-13β) resulted in a 14.1% nOe at δ 2.26 (H-13α) and 4.7% nOe at δ 3.74 (H-12). The nOe interaction between H-13β and H-12 could result only if the 12-OMe possessed α-stereochemistry. Irradiation at δ 2.26 (H-13α) caused a 11.9% nOe at δ 1.68 (H-13β) and 10.3% nOe at δ 2.40 (H-14β). The nOe interaction between H-13α and H-14β suggested that these protons lie close to each other in the preferred conformation of ring "D" and established that the C-13a/C-13 bond is β-oriented.

Irradiation at δ 3.53 (H-7α) resulted in a 6.7% nOe at δ 7.81 (H-4) and 14.9% nOe at δ 3.29 (H-5α). This established that the H-7α proton lies closer to the H-5α proton and not so close to the H-4 proton. It also suggested that the orbtal

containing the lone pair of electrons of nitrogen has a β orientation. Irradiation at δ 7.81 (H-4) and at 3.29 (H-5α) resulted in an 11.6% nOe and a 13.7% nOe at δ 3.53 (H-7α), respectively, and established the proximity of H-4 and H-5α in the preferred conformation of ring "C". Irradiation at δ 6.66 (H-1) resulted in a 10.8% nOe at δ 3.86 (2-OCH_3). Irradition at δ 7.81 (H-4) also resulted in a 11.8% nOe at δ 3.46, H-8α, establishing that H-8α lies close to H-4; the absence of an nOe effect on the OMe group clearly indicated the presence of an aromatic hydroxy group at C-3.

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) showed the presence of 18 carbon atoms in the molecule (See Table II). The multiplicity assignments were made using DEPT. The C-13a signal resonated at δ 63.84, its downfield chemical shift suggesting the α nitrogen function. The C-12 peak appeared at δ 73.49 [115] while the C-10 olefinic carbon resonated at δ 117.99. The C-8 peak appeared at δ 40.45. The C-7 carbon resonated at 51.90. The C-4 signal appeared at δ 124.40. The signal at δ 51.98 was assigned to the methoxy carbon at C-2 while the signal at δ 56.08 was assigned to the 12-OCH_3 carbon [116].

Table II. ^{13}C-NMR chemical shifts of cohirsitinine (2)

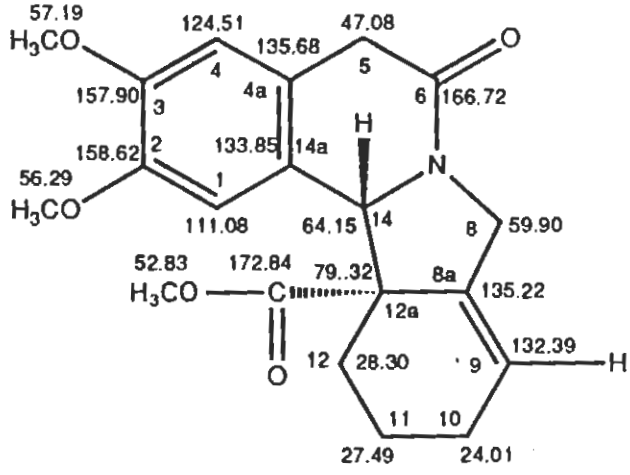
Carbon No.	δ	Carbon No.	δ
1	112.75	C-9	129.98
2	157.60	C-10	117.99
3	155.93	C-11	22.78
4	124.40	C-12	73.49
4a	134.88	C-13	41.40
5	31.86	C-13a	63.84
7	51.90	C-14	27.03
8	40.45	C-14a	140.16
		2-OMe	51.98
		12-OMe	56.08

Isolation and Structural Study of Jamtinine (3)

The EtOH extract was evaporated under reduced pressure, and the residue was partitioned between EtOAc and H_2O. The aqueous layer was basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated, dried with anhydrous Na_2SO_4 (74 gm) and subjected to CC. The fraction obtained with hexane-Me_2CO (1:0.5) was subjected to preparative TLC on si gel (GF-254) precoated plates with $CHCl_3$-MeOH (9.5:0.5) as the solvent system. This afforded the pure alkaloid jamtinine [3].

Its UV spectrum showed absorptions at 220, 240 and 302 nm. The IR spectrum showed prominent peaks at 1665 (amide) and 1720 (ester) cm^{-1}, indicating the presence of 6 membered cyclic amide in the molecule [117]. The HRMS indicated the molecular ion peak at m/z 357.1245 (calcd 357.1124) consistent with the molecular formula $C_{20}H_{23}NO_5$, indicating 10 double bond equivalents in the molecule. Other prominent peaks were found at m/z 342 and suggested the attachment of the methyl ester to a quaternary carbon [118]. The molecular ion was confirmed by FABMS [119].

The ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) showed the presence of 23 protons in the molecule, each of which was identified by a series of homo decoupling of experiments, NOE difference measurements, COSY-45 and hetero-COSY experiments. A one proton singlet at δ 6.72 was assigned to the C-1 proton. The C-4 proton appeared at δ 7.24 as singlet, Its downfield chemical shift reflecting the presence of a carbonyl group. The C-5α & C-5β protons resonated at δ 1.80 & 1.65 as a doublet. The presence of two 1H singlets in the aromatic region revealed that the substitution must be at the C-2 and C-3 positions. Two singlet (3H each) at δ 3.82 and 2.85 were assigned to the 2-OCH_3 and 3-OCH_3 groups respectively. The rather upfield singlet at 3.23 was assigned to the carbomethoxy group which position is due to the shielding influence of the aromatic nucleus [120-121]. The C-8α and C-8β protons resonated at δ 3.14 and 3.90 as a doublet.



^{13}C NMR OF JAMTININE (**3**)

The rather downfield chemical shift are due to α-amidic function [121], while the singlet at δ 4.03 was assigned to the C-14 proton. A broad singlet at δ 5.92 was assigned to the C-9 olefinic proton.

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) (see Table III) showed the presence of 20 carbon atoms in the molecule. The multiplicity assignments were amidic carbonyl carbon made by DEPT pulse sequence analysis with the last polarization pulse angle θ=45°, 90° and 135°.

Two dimensional ^{1}H-NMR mesurements were carried out to verify the assignments. The coupling interactions were established through analysis of a correlated spectroscopy (COSY-45) spectrum while the multiplicity of the overlapping proton signals was determined from the 2D-J- resolved spectrum. The assignments for the C-10 protons at δ 2.67 could thus be confirmed by its COSY-45 spectrum, which showed a strong cross peak with the signal at δ 5.92 for the C-9 olefinic proton. Nuclear Overhauser enhancement spectroscopy (NOESY) spectra served to established the spatial proximities. The stereochemistry of the ester group could be confirmed from the NOESY spectrum since it showed strong cross peaks with the signals at δ 6.72 for the C-1 proton and at 3.82 for the methoxy proton. This could only arise if the ester group possessed α-stereochemistry, which would also result in the C-14 proton having a β-configuration. The NOESY interactions between the C-14 proton and C-5β proton also confirmed the β-stereochemistry of the C-14 proton.

The methyl carbon of the ester resonated at δ 52.83 while the two methoxy carbons appeared at δ 56.29 and 57.19, respectively. The C-9 olefinic carbon resonated at δ 132.39 while the C-1 and C-4 carbons appeared at δ 111.08 and 124.51, respectively. The signal at δ 79.32 was assigned to C-12a. The rather downfield chemical shift of this carbon may be due to the α-disposition of the double bond. The C-14 carbon resonated at 64.15. The C-5 carbon signal appears at 47.08. Its downfield chemical shift reflects the influence of the

carbonyl function. The signal at δ 166.72 was assigned to the amidic carbonyl carbon.

Table-III: ^{13}C-NMR chemicalshifts of Jamtinine (3)

Carbon No.	(δ)	Carbon No.	(δ)
1	111.08	10	24.01
2	158.62	11	27.49
3	157.90	12	28.30
4	124.51	12a	79.32
4a	135.68	14	64.15
5	47.08	14a	133.85
6	166.72	2-OCH_3	56.29
8	59.90	3-OCH_3	57.19
9	132.39	C-OCH_3	172.84

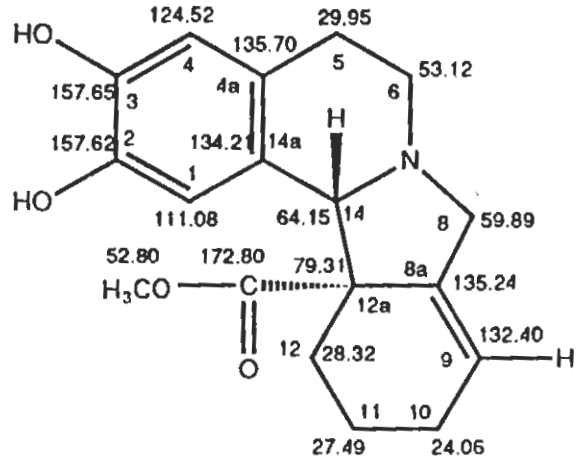
Isolation and Structural Study of Haiderine (4)

The $CHCl_3$ layer was dried with anhydrous Na_2SO_4 evaporated and subjected to chromatography on a silica gel (200 g) column. The fraction obtained with hexane-Me_2CO 1:9 (50 g) was subjected to flash column chromatography on silica gel with CH_3Cl:MeOH 9:5:0.5 as the solvent system. This afforded the pure alkaloid haiderine.

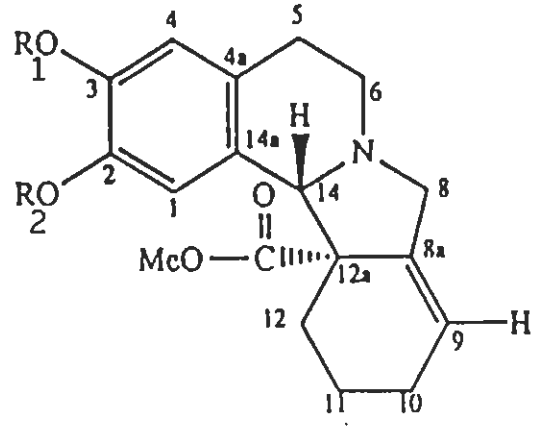
The UV spectrum of haiderine showed absorption maxima at 212, 240 and 303 nm, and its IR spectrum indicated the presence of an OH group (3500 cm^{-1}) and an ester carbonyl (1720 cm^{-1}). The high resolution MS indicated the molecular ion peak to be at m/z 315.1615 (calcd. 315.1629) consistent with the molecular formula $C_{18}H_{21}NO_4$. This showed the presence of nine double bond equivalents in the molecule. Other prominent peaks were found at m/z 285, 259, 226 and 208. The presence of two phenolic OH groups was suggested from the molecular ion peak at m/z 317 after treatment with D_2O [123]. Haiderine gave a positive color reaction for phenols with ferric chloride.

The ^{1}H-NMR spectrum of (4) ($CDCl_3$, 300 MHz) is quite similar to that of hirsutine (4a) [124]. It contained a singlet for a carbomethoxy group at δ 3.24 which is shifted upfield due to the shielding influence of the aromatic nucleus [125-126]. The H-9 olefinic proton resonated at δ 5.92 as a broad singlet and a double doublet at δ 1.89 ($J_{5\beta,5\alpha}$=11.7 Hz, $J_{5\beta,6\beta}$= 4.7 Hz) was assigned to the H-5β proton. The H-5α proton resonated as double doublet at δ 1.72 ($J_{5\alpha,5\beta}$ = 5.4 Hz). The two protons at H-6 resonated at δ 3.67-3.90 as a multiplet, while a singlet at δ 4.03 was assigned to the H-14 proton. The H-8α and H-8β protons resonated at δ 3.15 and 3.90, respectively, while the signals of aromatic protons at δ 6.82 and 7.30 as singlets are due to the 2,3-disubstituted benzene ring system.

Two dimensional ^{1}H-NMR measurements were carried out to verify the assignments. The coupling interactions were established through correlated spectroscopy (COSY-45) and the multiplicity of the overlapping proton signals was determined from the 2D J-resolved spectrum [127-130]. The assignments for the H-10 proton at δ 2.68 could thus be confirmed by its COSY-45 spectrum, which showed a strong cross peak with the signal at δ 5.92 for the H-9 olefinic proton. The assignments for the H-5β proton at δ 1.89 and C- 5α proton at δ 1.72 was also confirmed by the COSY-45 interactions with the H-6 protons at δ 3.67 and 3.90 respectively. The H-8α proton at δ 3.15 showed a strong COSY



^{13}C NMR OF HAIDERINE (**4**)



4 = R_1 = R_2 = H

4a = R_1 = H

R_2 = Me

5a = R_1 = R_2 = Me

interaction with the H-8β proton at δ 3.90.

The nuclear Overhauser enhancement spectroscopy (NOESY) spectrum served to established the spatial proximities. The stereochemistry of the ester group could be confirmed from the NOESY spectrum since it showed strong cross peaks with the signal at δ 6.82 for the H-1 proton. This could only arise if the ester group possessed α-stereochemistry and also requires the H-14 proton to have a β-configuration. The NOESY interactions between the H-14 proton and H-5β proton also confirmed the β stereochemistry of the H-14 proton.

The ^{13}C-NMR spectra (see Table-IV). Treatment of (4) with CH_2N_2 gave **4a** thus confirming that haiderine is the monodemethyl derivative of hirsutine. The product was identified by comparison of IR, MS and Rf values with hirsutine as an authenitic sample [128]. The O- methylderivative (5a) was derived from **4a** by methylation with CH_2N_2 [128].

Table-IV: ^{13}C-NMR of (4) and (4a)

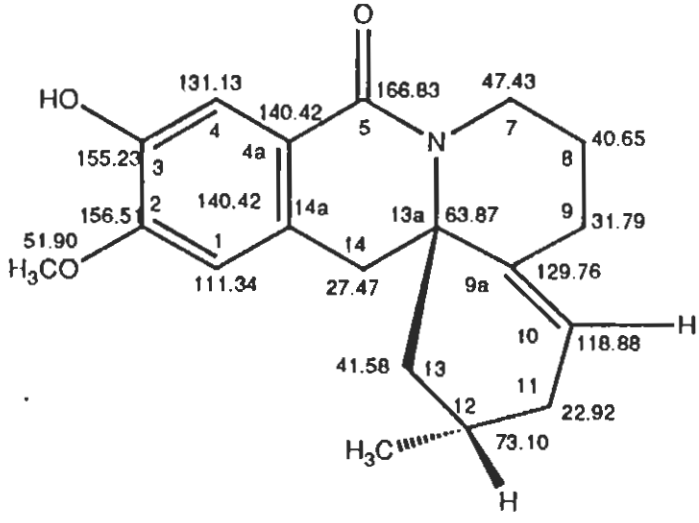
C	(4)	(4a)
1	111.08	111.08
2	157.62	157.62
3	157.65	156.36
4	124.52	124.51
4a	135.70	135.68
5	29.95	29.93
6	53.12	52.46
8	59.89	59.88
8a	135.24	135.22
9	132.40	132.39
10	24.06	24.01
11	27.49	27.49
12	28.32	28.30
12a	79.31	79.31
14	64.15	64.11
14a	134.21	133.81

Isolation and structural study of Cohirsinine (5)

The aqueous layer was basified with ammonia and extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated, dried with Na_2SO_4 (74 g) and subjected to CC. The fraction obtained with C_6H_{12}-Me_2CO (3:1) was subjected to preperative TLC on silica gel (GF-254) precoated plates with $CHCl_3$ MeOH (9:1) as the solvent system. This afford a pure alkaloid, cohirsinine [5] (Rf 0.3) (8 mg) $[\alpha]^{25}_D$ + 136° ($CHCl_3$) which gave a characteristic color reaction with Dragendorff's reagent.

The crude alkaloidal mixture obtained from the EtOH extract of *Cocculus hirsutus* was basified with ammonia and extracted with $CHCl_3$. The $CHCl_3$ extract was subjected to column chromatography. The fraction obtained with hexane-acetone (3:1) was subjected to preparative TLC on silica-gel plates to afford the alkaloid (5) as a gummy material. Its UV spectrum showed absorptions at 213, 240 and 301 nm, reflecting the isoquinolones-type or isocarbostyril chromophore [99]. The IR spectrum revealed the presence of a hydroxyl group at 3500 and the presence of a phenolic hydroxyl group in [5] as was suggested when the compound was treated with D_2O and the mass spectrum recorded again where upon M^+ was found to be shifted by 1 atomic mass unit to *m/z* 330, due to the one exchangeable hydrogen (OH group) in the molecule [112]. Intense absorptions at 1670 and 1710 cm^{-1} indicated the presence of an α,β-unsaturated 6 membered cyclic amide in the molecule [131]. The HRMS indicted the molecular ion peak at *m/z* 329.1671 consistent with the molecular formula of $C_{19}H_{23}NO_4$, requiring nine double bond equivalents to be in the molecule. Other prominent peaks were found to occur at *m/z* 298,285 $[M\text{-}OMe]^+$, 271 $[M\text{-}C_3H_6O]^+$ (base peak), 226, 194, 164 and 134.

The ^1H NMR ($CDCl_3$, 300 MHz) spectrum showed two singlets at δ 3.29 and 3.89, corresponding to the C-12 methoxy and an aromatic methoxy group respectively. The presence of two singlets at δ 6.60 and 7.81 in the aromatic region revealed that the substitutions must be at the C-2 and C-3 positions. A broad singlet at δ 5.64 can be ascribed to the C-10 olefinic proton and is gauch to the adjacent methylenic protons, due to a small coupling constant. In addition, a multiplet at δ 3.73 was assigned to the C-12 proton, the downfield chemical shift indicating the presence of a methoxy group at this carbon. The C-8α and β protons showed multiplet and downfield chemical shifts at δ 3.46 and 3.07 due to the amid functions. The C-14 protons resonated as doublets at δ 2.29 and 2.29 indicating the geminal coupling; the absence of vicinal coupling indicated that this moiety is



^{13}C NMR OF COHIRSININE (**5**)

between two quaternary carbons.

The above data of **5** closely resemble those of cohirsine which bears the methoxy groups at C-2 and C-3 respectively. The two compounds, however, differ widely in their repective optical rotation. Their IR spectra showed common features but they could not be superimposed. In the ^{13}C NMR spectrum (See Table V) the chemical shifts of the nucleus carbon atoms showed close agreement with the published spectrum of cohirsine but indicated the absence of one methoxy group. Compound **1** has, therefore, the same basic skeleton and stereochemistry as cohirsine and differs only in the substituents at C-2 and C-3. Therefore, the structure of a new alkaloid **1** was determined except for the positions of the methoxy and hydroxy groups on an aromatic ring.

The aromatic methoxy and aromatic hydroxy groups were determined to be at C-3 and C-4, respectively on the basis of the results of NOE experiments. Irradiation at δ 6.60 (H-1) resulted in a 12.4% NOE interaction at δ 3.89 (OMe-2). Irradiation at δ 7.81 (H-4) resulted in on 11.8% NOE at δ 3.46 (H-8), establishing that H-8α lies close to H-4, and the absence of an NOE effect with the methoxy group clearly indicated the absence of an aromatic methoxy group at C-3. When H-8α was irradiated, corresponding NOE interactions with H-4 (13.6%) and with H-8β, H-7α and H-7β were also observed. From the NMR spectral results, it has been shown clearly that the aromatic methoxy and hydroxy group are located at C-2 and C-3, respectively. Because of the ready determination on the position of substituents(s) on the aromatic ring, the NOE technique is confirmed to be of significant value as a tool for the determination of substituent patterns on aromatic rings of isoquinolone alkaloids.

Tbale-V ^{13}C-NMR chemical shifts of cohirsinine (5)

Carbon No.	(δ)	Carbon No.	(δ)
1	111.34	10	118.88
2	156.51	11	22.92
3	155.23	12	73.10
4	131.13	13	41.58
4a	139.67	13a	63.87
5	166.83	14	27.47
7	47.43	14a	140.42
8	40.65	2-O$\underline{C}$H$_3$	52.83
9	31.79	12-O$\underline{C}$H$_3$	56.54
9a	129.76		

Experimental

GENERAL EXPERIMENTAL CONDITIONS

Physical constants:

Melting points were determined in glass capillary tubes using a Gallenkamp melting point apparatus and were uncorrected. Optical rotations were recorded with a JASCO DIP-360 digital polarimeter using either chloroform or in methanol as solvent.

The pH values were measured by pH paper (by observing the change in colour).

Spectroscopy:

Ultra-violet (UV) spectra were recorded in methanol using Pye Unicam SP 800 G or Shimadzu UV-240 (Shimadzu Corporation Kyoto, Japan) instruments.

Infrared (IR) spectra were recorded in chloroform with a JASCO IRA-I (JASCO International Co. Ltd., Japan) or JASCO A-302 (Japan Spectroscopic Co. Ltd.) spectrophotometers.

Nuclear magnetic resonance (nmr) spectra were determined in deutero- chloroform or deuteromethanol using as an internal standard, at 300 MHz or 400 MHz (^{1}H-nmr) with Bruker AC-300, AM-300 or AM-400 nuclear magnetic resonance spectrometers with Aspect 3000 data systems at a digital resolution of 32K. The ^{13}C-nmr spectra were recorded at 75 MHz and 100 MHz with the same instruments.

Mass spectra (MS) were determined using a Finnigan (Varian MAT) 112 or Finnigan MAT 312 double focussing mass spectrometers connected to a MAT 188 data system with PDP 11/34 DEC computer system. Peak matching, field desorption (FD) and fast atom bombardment (FAB) measurements were also performed on the MAT 312 mass spectrometer. FABMS were measured in glycerol - water (1:1) in the presence of KI. Accurate mass measurements were made with the FAB source using glycerol as an internal standard.

Techniques Employed For the Purification of Alkaloids

In order to fractionate the crude alkaloidal extract and to purify the different components, different chromatographic techniques were employed.

Column Chromatography:

It is the oldest but very effective technique. Generally, in our lab. liquid solid column chromatography is utilized. It consists of two phases. One is stationary and the other is mobile. The stationary phase is usually of silica gel and the mobile phase may be of an organic solvent. This procedure is termed as adsorption chromatography. In a glass column of desired size, silica gel was introduced in the form of slurry in a suitable solvent then the column was left overnight to give it the maximum time to settle down. This is called column packing. The crude extract was then loaded either in the form of dry slurry or in solution form. This is known as loading the sample. After loading, some silica gel was poured over it so that bands coming down might not be disturbed by pouring fresh solvent into the column. Then the column was eluted with a suitable solvent or solvent mixture.

Vacuum Liquid Chromatography:

This is another technique to fractionate the crude extract. It uses reduced pressure to increase the flow rate of the mobile phase but the pressure at the head of ..... is the atmospheric pressure. A sintered glass funnel was used, dry packed with a suitable adsorbent such as silica gel of TLC grade. The packing was under vacuum to acheive maximum packing density. The sovent was poured and sucked dry under vacuum reapplied to settle the sample. A little amount of silica was added on it and a filter paper (round, of sintered funnel circumference) was placed on it. Then the column was eluted with appropriate solvent mixutre. In each attempt the solvent was sucked up to dryness.

Chromatotron:

This is another useful device to purify the compound. It consists of use of a 24 cm diameter circular glass plate placed upon an inclined rotor. For one plate coating, 36 gm of silica gel of the TLC grade (GF-254), with a few milligram of binding agent ($CaCO_3$), in 100 ml of water, was used. Masking tape was inserted on the edges of the plate which was fixed in the stand before pouring the silica gel slurry. The apparatus so prepared was kept at room

TECHNOIQUES EMPLOYED FOR THE PURIFICATION OF ALKALOIDS

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It is the oldest but very effective technique. Generally, in our lab. liquid solid column chromatography is utilized. It consists of two phases one is stationary and the other is mobile. The stationary phase is usually of silica gel and mobile one may be of an organic solvent, also termed as adsorption chromatography. In a glass column of desired size silica gel was introduced in the form of slurry in a suitble solvent thenthe column was left overnight to give it the maximum time to settle down called column packing. The crude extract was then loaded either in the form of dry slurry or in solution form, known as loading the sample. After loading some silica gel was poured over it so that bands coming down may not disturbed by pouring the solvent. Then the column was eluted with suitable solvent or solvent mixture.

Vacuum Liquid Chromatography:

It is another technique to fractionate the crude extract. It uses reduced pressure to increase the flow rate of mobile phase but the pressure at the head is the atmospheric pressure. A sintered glass funnel was used, dry packed with a suitable adsorbent such as silica gel of TLC grade. The packing was under vacuum to acheive mzximum packing density. The sovent was pured and sucked dry under vacuum reapplied to settle the sample. A little amount of silica was added on it and a filter paper (round, of sintered funnel circumference) was placed on it. Then the column was eluted with appropriate solvent mixutre. In each attempt the solvent was sucked up to dryness.

Chromatotron:

temperature for air drying and then dried in an oven. After complete drying the surface layer was removed by a scraping tool fixed to the centre of the plate. The plate was then screwed onto the hub of an electric motor and rotated. First the plate was washed to remove impurities of adsorbent by introducing solvent through a pump. Then the sample was loaded. As much as 500 mg of sample can be used at one time. Then concentric bands were collected by solvent elution through an exit tube.

Preparative thin layer chromatography

Merck-precoated, silica gel GF-254 preparative plates (20 x 20 cm) from E. Merck were used for preparative thin layer chromatrography (TLC).

Detection of alkaloids on chromatographic plates:

Plates were viewed with ultra-violet light at 254 nm for fluorescence quenching spots and at 366 nm for fluorescent spots.

Chromogenic reagents employed:

The plates were sprayed with Dragendorff's reagent solution ceric sulphate-sulphuric acid reagent. The reagents and solvents were obtained from E. Merck.

Plant Material

The plant material was collected from the Karachi region and identified by the Department of Botany, University of Karachi. A voucher specimen [no.14-12-66(KUH)] has been deposited in the herbarium of the Department of Botany, University of Karachi.

Extraction and Isolation

The 40 kg of the plant material was chopped into small pieces and exhaustively extracted with ethyl alcohol. The ethanolic extract was evaporated under reduced pressure and the residue thus obtained was partitioned between ethyl acetate (A) and water (M- Aq). The ethyl acetate layer (A) was freed of the solvent. A small portion of the residue obtained from ethyl acetate layer (A) was divided into hexane-soluble and insoluible protions. The hexane soluble porition was evaporated and the residue obtained was taken in 5% methanolic KOH and refluxed on the water bath for one hour. It was shaken out with ethyl acetate to remove unsaponifiable matter. The alkaline phase was acidified with dilute HCl and extracted with ethyl acetate. The residue obtained from ethyl acetate layer was methylated with freseh1y prepared diazomethane and the resulting methyl esters of fatty acids [98] were analysed through GC-MS. The remaining A portion was subjected to column chromatography on silica gel. Elution was carried out with a gradient of increasing polarity in the order: *n*-hexane, benzene, ethyl acetate and methanol. The compound Al was eluted with hexane:benzene (1:9). The benzene-ethyl acetate (7:8) eluate after purification through repeated recrystallization from methanol afforded hirsutdiol [91]. The benzene-ethyl acetate (95:5) eluate was repeatedly crystallized from ethyl acetate and identified as nonacosan-10-ol [91].

The main aqueous fraction (M-Aq) was basified with dilute ammonia and shaken out with chloroform to extract the crude alkaloids. The alkaline aqueous phase (Aq) was acidified with dilute HCl and kept aside for examination of its

constituents. The chloroform layer was dried with sodium sulphate (anhydrous) and freed of the solvent under reduced pressure. The residue obtained was divided into ether soluble (B) and Insoluble (C) fractions. The ether soluble fraction (B) showed two spots on TLC ($CHCl_3$: MeOH 1:1). One was characterized as jamtine-*N*-oxide [93] and other ther was cohirsitine ($CHCl_3$: MeOH 9.5 : 0.5 was used as the solvent system).

The ether-insoluble fraction (C) referred to above was again divided into chloroform - soluble (D) and chloroform - insoluble (E) portions. The chloroform soluble portion (D) was subjected to flash column chromatography using as solvent system cyclohexane - chloroform : dimethyl amine (20:70:10). This, ultimately, resulted in isolation of two known bisbenzylisoquinoline alkaloids, isotrilobine and trilobine [92].

The chloroform insoluble fraction (E) was subjected to suction column chromatography (silica gel 100) using different solvents and mixtures of increasing polarities. Fractions of one liter each were collected and the solvent was removed under reduced pressure.

The fraction obtained with hexane - acetone (8:2) was subjected to preparative TLC on silica gel plates with chloroform:methanol (9.5:0.5) as the solvent system. This offorded the pure but gummy alkaloid, cohirsine [94]. The fraction obtained with hexane-acetone (3:1) was subjected to preparative TLC on silica gel plates with chloroform: methanol (9:1) as the solvent system. This afforded a pure gummy alkaloid, cohirsinine.

The fraction obtained with hexane acetone (6:4) was subjected to preparative TLC on silica gel plates with chloroform : methanol (9:1) this afforded the pure alkaloid, cohirsitinine.

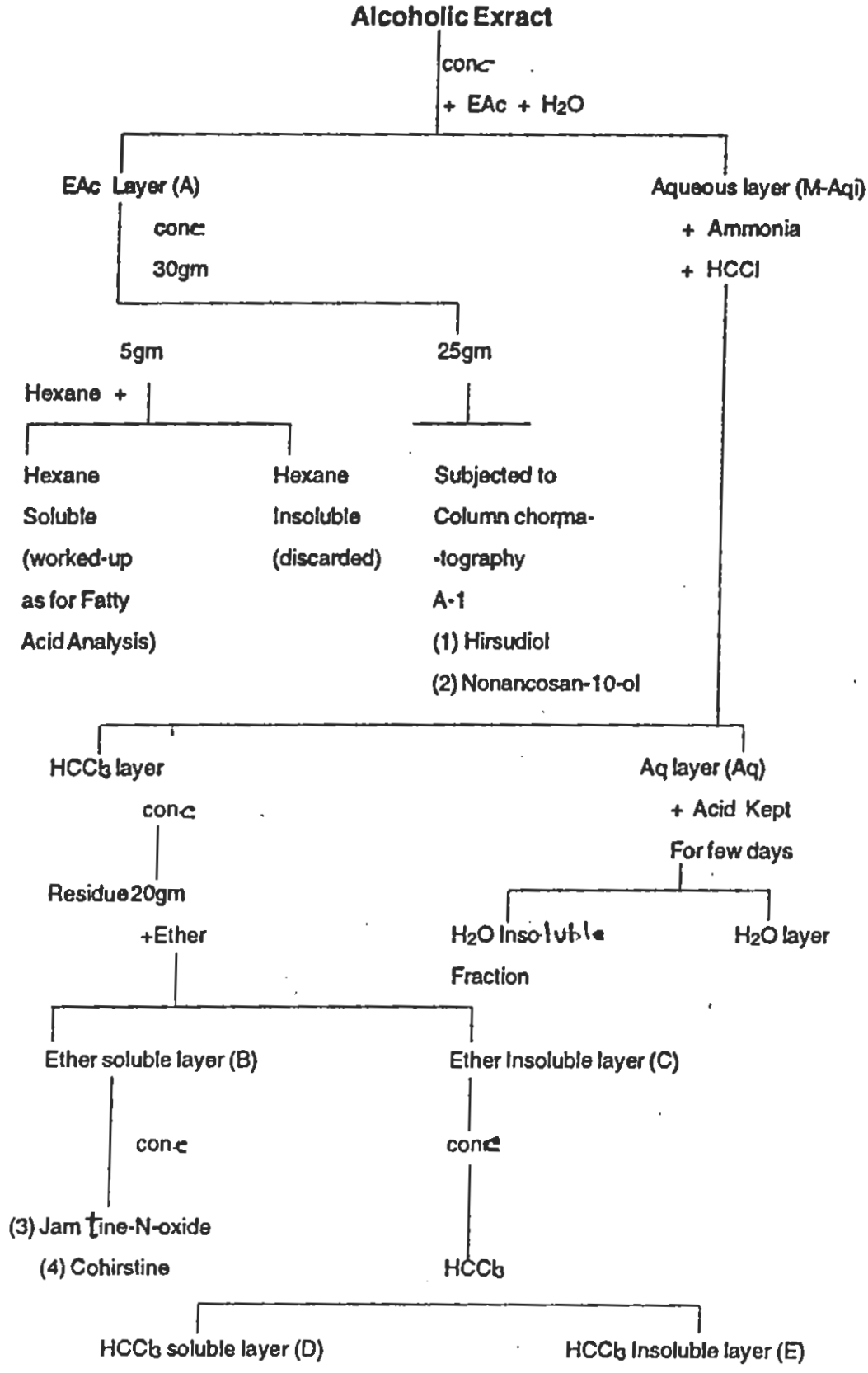
The fraction obtained with hexane-acetone (1.8:0.7) was subjected to preparative TLC silica gel plates with chloroform methanol (9.5:0.5) as the solvent system. This afforded a pure alkaloid, hirsutine [95].

The fraction obtained with (hexane-acetone 7:3) affording shaheenine [96]. The fraction obtained with (hexane-acetone (1:9) was subjected to flash chromatography on silica gel starting with chloroform - methanol (9.5:0.5) as the solvent system. This afforded a pure alkaloid, haiderine using the chloroform - methanol (98:02) as solvent and this was purified through the use of preparative TLC in the same solvent mixture. The other compound obtained was lignin, identified as (+)- syringasesinol [92].

The fraction obtained from the chloroform-methanol (95:05) eluate, after purification through preparative TLC, was another lignin. It was identified as yamgambin [93].

The fraction obtained from the chloroform-methanol (97:3) eluate, after purification through preparative TLC, afforded another lignin. It was identified as α-oxypropiosyringen [92].

The fraction obtained with hexane : acetone (1:05) was subjected to preparative TLC on silica gel precoated plates with $CHCl_3$:MeOH (9.5:0.5) as the solvent system. This afforded the pure alkaloid jamtinine.


Alcoholic Exract
conc
+ EAc + H2O
EAc Layer (A)
conc
30gm
Aqueous layer (M-Aqi)
+ Ammonia
+ HCCl
5gm
25gm
Hexane +
Hexane Soluble (worked-up as for Fatty Acid Analysis)
Hexane Insoluble (discarded)
Subjected to Column chorma--tography
A-1
(1) Hirsudiol
(2) Nonancosan-10-ol
HCCl3 layer
conc
Residue 20gm
+Ether
Aq layer (Aq)
+ Acid Kept
For few days
H2O Insoluble Fraction
H2O layer
Ether soluble layer (B)
Ether Insoluble layer (C)
conc
conc
(3) Jam tine-N-oxide
(4) Cohirstine
HCCl3
HCCl3 soluble layer (D)
HCCl3 Insoluble layer (E)

(D)

Flash column chrometography

(5) IsoTrilobine

(6) Trilobine

(7) Jamtinine

(E)

Suction column chromatography

(8) Cohirsine

(9) Cohirsinine

(10) Cohirsitinine

(11) Hirsutine

(12) Shaheenine

(13) Haiderine

(14) Syringasesinol

(15) Yamgambin

(16) α-oxypropiosyringen

Spectral Data

COHRSITINE

UV λ_{max} (MeOH): 238, 289, 313 nm:

IR ν_{max} ($CHCl_3$): 3570, 1710 and 1670 cm^{-1}:

MS m/z: 445 (4), 430 (6), 370 (100), 312 (10), 308 (19), 298 (7), 285 (10), 256 (46), 228 (35), 208 (42);

^{1}H-NMR: 2.69 (1H, m, C-10H), 3.20 (1H, m, C-10βH), 1.90 (1H, m, C-11H), 2.28 (1H, m, C-11H), 2.40 (1H, m, C-12H), 3.25 (3H, s, CO_2Me), 3.53 (1H, m, C-12H), 3.89 (6H, s, 2OMe), 5.62 (1H, br., C-9H), 6.69 (1H, s, C-1H) and 7.74 (1H, s, C-4H);

^{13}C-NMR ($CDCl_3$, 75 MHz), see Table-I.

COHIRITININE

UV (MeOH) λ_{max} 213, 236, 290 nm, min 233, 266 nm;

IR ($CHCl_3$) ν_{max} cm^{-1} 3500 (OH), 1230 2835 (CH);

HRMS $(M)^+$ 301.1663 (calcd 301.1671) ($C_{18}H_{23}NO_3$, 10%), 270.1482 (calcd 270.1488) ($C_{17}H_{20}NO_2$), 226.1219 (calcd 226.1234) $C_{15}H_{16}NO$, 20%), 210.0916 (calcd 210.0915) ($C_{14}H_{12}NO$, 13%), 165.1148 (calcd 165.1149) ($C_{10}H_{15}NO_2$, 63%), 150.0671 (calcd 150.0661) ($C_9H_{10}O$, 33%), 132.0816 (calcd 132.0729) ($C_9H_{10}O$, 29%), 120.0809 (calcd 120.0810) ($C_8H_{10}O$, 16%);

^{1}H-NMR ($CDCl_3$, 300 MHz, ppm) 1.68 (1H, t, $J_{13\beta,12}$=$J_{13\beta,13\alpha}$=13.6 Hz, H-13β), 2.68 (1H, m, H-8β), 2.26 (1H, dd, $J_{13\alpha,13\beta}$=13.6 Hz, $J_{13\alpha,12}$=6.44 Hz,H-13α), 2.40 (1H, d, $J_{14\beta,14\alpha}$=12.4 Hz, H- 14β), 2.42 (1H, d, $J_{14\alpha,14\beta}$=12.4 Hz, H- 14α), 3.46 (1H, m, H-8α), 2.69 (1H, m, H-11α), 2.96 (1H, d, $J_{5\alpha,5\beta}$=13.5 Hz, H-5β), 3.07 (3H,s, 12-OCH_3), 3.29 (1H, m, H-7α), 3.74 (1H, m, H-12β), 3.86 (3H, s, 2-OCH_3), 5.67 (1H, brs, H-10), 7.81 (1H, s, H-4), 6.66 (1H, s, H-1):

^{13}C-NMR ($CDCl_3$, 75 MHz) see Table-II.

JAMTININE

UV λ^{MeOH}_{max} 213, 240, 300.

IR ν_{max} $CHCl_3$ cm^{-1} 2835 (C-H), 1720 (ester carbonyl), 1670 (amide) and 1090 (C-O).

MS *m/z* (rel.int.) 357 (30), 342 (8), 321 (12), 285 (100), 271 (28), 226 (13), 208 (20).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.93 (1H, m, H-11), 2.38 (1H, m, H-11), 2.52 (1H, m, H-12), 2.67 (1H, m, H-10), 3.15 (1H, d, $J_{8\alpha,8\beta}$=10.1 Hz, H- 8α), 3.22 (3H, s, C-OMe), 3.83 (3H, s, $2OCH_3$), 3.85 (3H, s, 3-OCH_3), 3.80 (1H, d, $J_{8\beta,8\alpha}$=10.1 Hz, H-8β), 4.02 (1H, s, H-14), 5.91 (1H, br., H-99), 6.68 (1H, s, 1H), 7.74 (1H, s, H-4).

^{13}C-NMR ($CDCl_3$, 75 MHz) See Table-III.

HAIDERINE

UV (MeOH) λ_{max} 212, 240, 303 nm

IR ($CHCl_3$) ν_{max} cm^{-1} 3500 (OH) and (ester crboxyl) 1720

EIMS m/z (rel. int%) 315 (5) 285 (22), 259 (100), 226 (4), 208 (19)

^{1}H-NMR ($CDCl_3$): δ 1.93 (1H, m, H-11), 2.52 (1H, m, H-12), 2.68 (1H, m, H-10), 3.15 (1H, d, $J_{8\alpha,8\beta}$=10.1 Hz, H-8α), 3.24 (3H, s, -C-OMe) 4.03 (1H, s, H-14), 3.90 (1H, d, $J_{8\beta,8\alpha}$=10.1 Hz, H-8β), 5.92 (1H, br, H-9), 6.82 (1H, s, H-1), 7.30 (1H, s, H-4), 1.89 (1H, dd, H-5), 1.72 (1H, dd, H-5), 3.67 (1H, m, H-6) 3.90 (1H, m, H-6)

^{13}C-NMR : see Table-IV.

COHIRSININE

UV λ^{MeOH}_{max} 213, 240 and 303 nm IR (F 12BM), n (F255D) CH_3Cl_3 cm^{-1} 3500 (OH) 1710 (1665 (a,B-unsaturated amide) 1600 (C=O) and 1090 (C=O).

(MS m/z (rel. int.): 329 (6), 298 (10), 285 (17), 171 (100), 226 (27), 194 (7), 164 (13), 134 (20).

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.68 (1H, t,$J_{13\beta,13\alpha}$~ $J_{13\beta,12}$=12.1 Hz, H-13β), 1.14 (1H, dd, $J_{13\alpha,13\beta}$=12.1 Hz, $J_{13\alpha,12}$=6.2 Hz, H-13α), 2.40 (1H, d, $J_{14\alpha,14\beta}$=12.1 Hz, H- 14α), 2.29 (1H), d, $J_{14\beta,14\alpha}$= 12.1 Hz, H- 14β), 3.73 (1H, m, H-12), 2.61 (1H, m, H- 11α), 3.13 (1H, m, H-11β), 3.46 (1H, m, H-8α), 3.07 (1H, m, H-8β), 2.69 (1H,m, H-7α), 2.06 (1H, m, H-7β), 6.60 (1H, s, H-1), 7.74 (1H, s, H-4), 3.89 (3H, s, OMe-2), 3.29 (3H, s, OMe-12).

^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 22.92 (C-11), 27.47 (C-14), 31.79 (C-9), 40.65 (C-8), 41.58 (C-13), 47.43 (C-7), 63.87 (C-13a), 73.10 (C-12), 111.34 (C-1), 118.88 (C-10), 129.76 (C-9a), 131.13 (C-4), 139.67 (C-4a), 140.42 (C-14a), 155.23 (C-3), 156.51 (C-2), 166.83 (C-5), two methoxy groups 52.83 (OMe-20) and 56.54 (OMe-12).

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