90

STUDIES ON THE CHEMICAL CONSTITUENTS

OF

CADABA FARINOSA

BY

AZIZ-UR-REHMAN AMBER

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IN THE NAME OF ALLAH
THE MOST COMPASSIONATE
THE MOST MERCIFUL

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1 SUMMARY

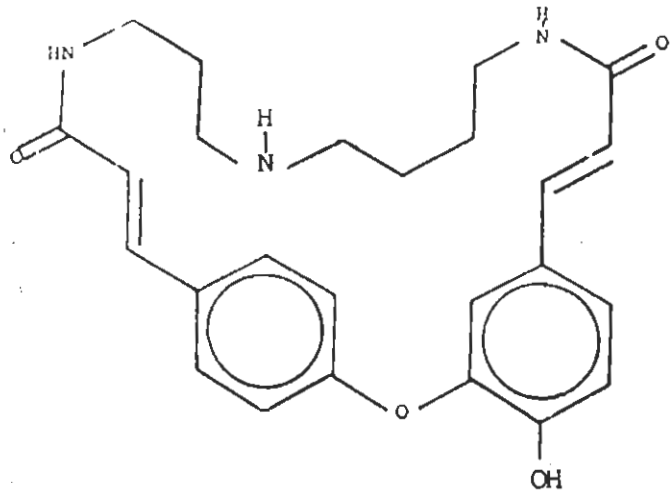
SUMMARY

The "studies on the chemical constituents of Cadaba farinosa Forssk (Syn. fruticosa, L. Druce.)" have led to the isolation of three novel spermidine kaloids, one novel sesquiterpenoid, alongwith another spermidine alkaloid , pparisine and an aromatic acid, α,β-Dihydro ferulic acid. The last two mpounds were isolated from this genus for the first time.

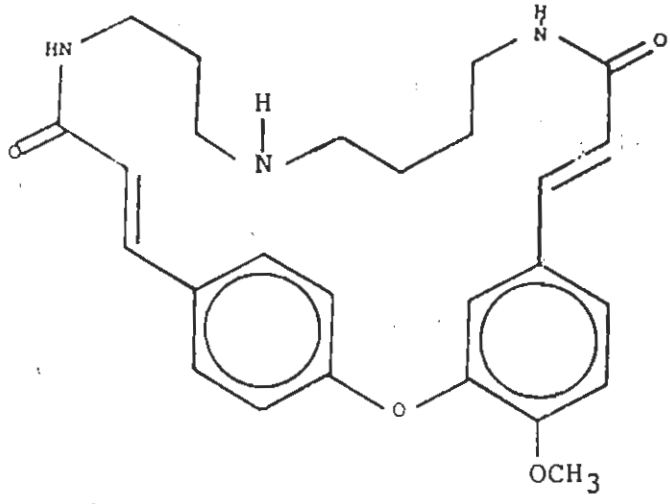
The thesis also includes the total synthesis of a spermidine alkaloid, labicine methyl ether.

The three novel spermidine alkaloids named as cadabicine, cadabicine methyl er and cadabicine diacetate, have the same skeletal system. They are rocyclic alkaloids containing, hydroxylated, methoxylated or acetylated, trans namic acid residues which are linked to each other through oxygen bridge and spermidine, through amide bonds. These type of compounds may exist in two o isomeric forms with respect to spermidine moiety. The spectral data uding, UV, IR, ^{1}H-NMR and ^{13}C-NMR cannot decide the exact structure of such ers but their structure can be confirmed by X-rays crystallography or ugh mass spectral fragmentation pattern.

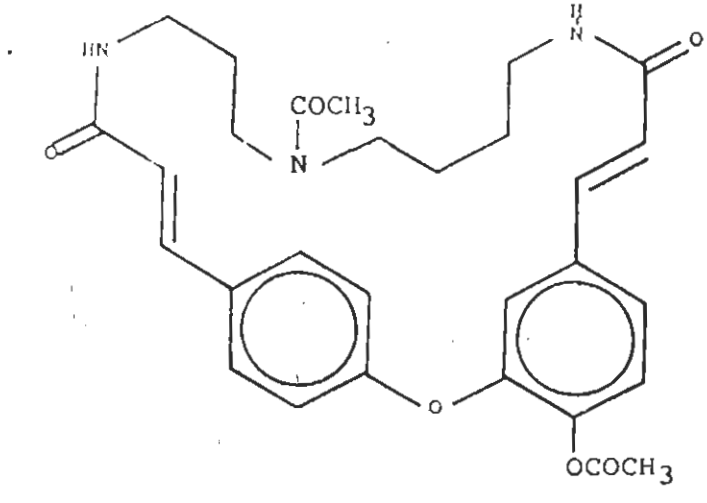
The structure of cadabicine was confirmed through X-ray analysis. The cture of cadabicine methyl ether was elucidated by mass spectral entation and comparison with cadabicine and finally confirmed by its total esis. Similarly the structure of cadabicine diacetate was confirmed through spectral fragmentation pattern, other modern spectroscopic methods and t comparison with cadabicine and its diacetyl derivative. Their structures ive below.



Cadabicine



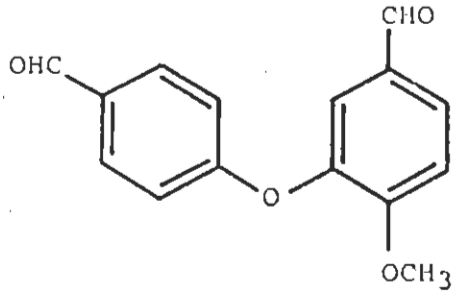
Cadabicine methyl ether



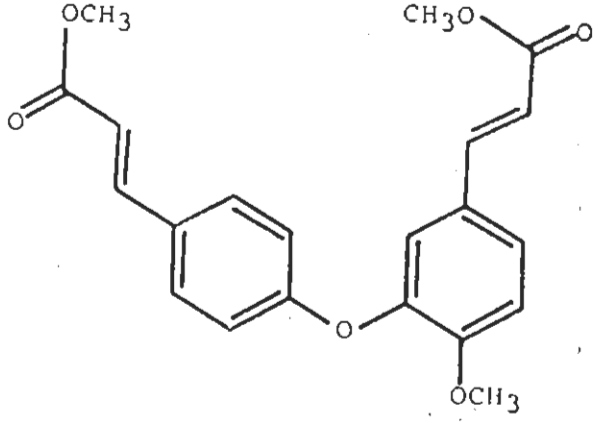
Cadabicine diacetate

We attempted to synthesize cadabicine methyl ether, utilizing a new synthetic route, but with the help of known aminolysis method as reported for codonocarpine. This synthesis consists of six steps, during which five new compounds were prepared, which are:

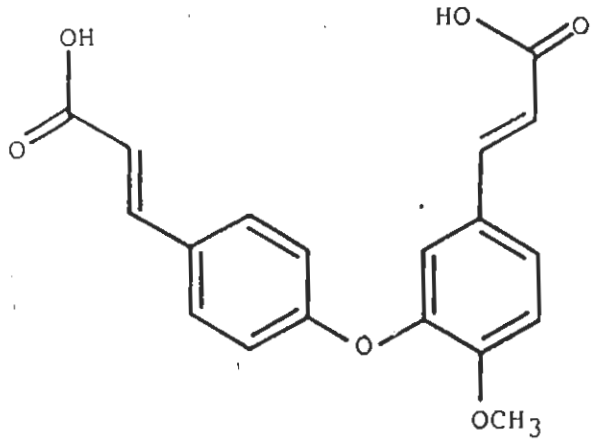
1) 3(4-formyl phenoxy)-4-methoxy benzaldehyde
2) Methyl cinnamate, methyl ferulate ether
3) Ether of -p-cinnamic acid-m-ferulic acid
4) Thiazolidine compound
5) Cadabicine methyl ether and its regio isomer



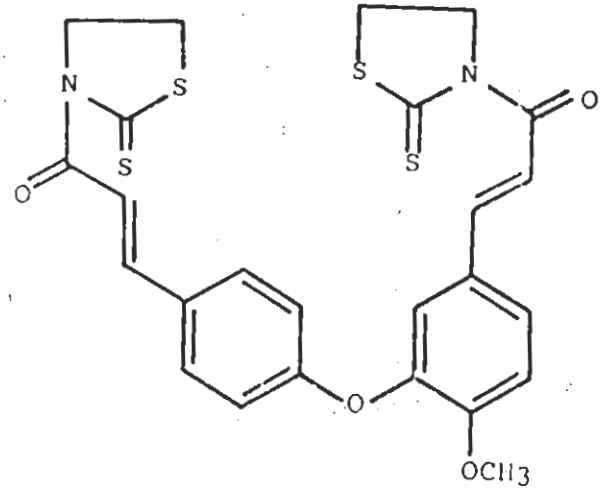
3(4-formyl phenoxy)-4-methoxy benzaldehyde



Methyl cinnamate, methyl ferulate ether

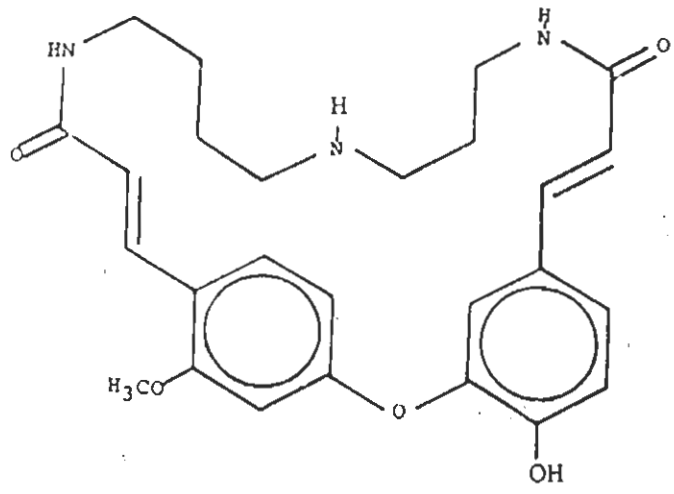


Ether of -p-cinnamic acid-m-ferulic acid



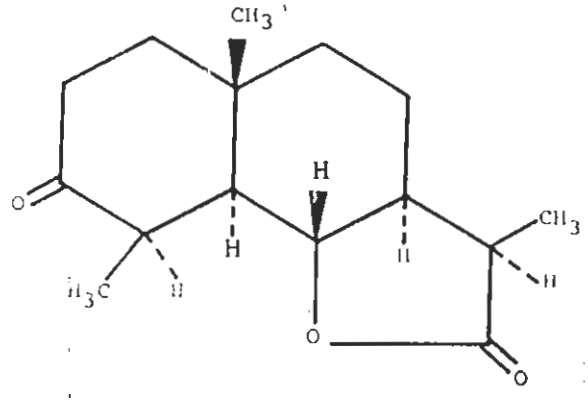
Thiazolidine compound

The alkaloid capparisine, is the methoxy derivative of regio isomer of labicine. This is the first spermidine alkaloid isolated from this plant, in ch the attachment of spermidine moiety is reversed. Its structure was firmed through mass spectral fragmentation pattern and direct comparison with spectral data of compound which has been reported earlier.



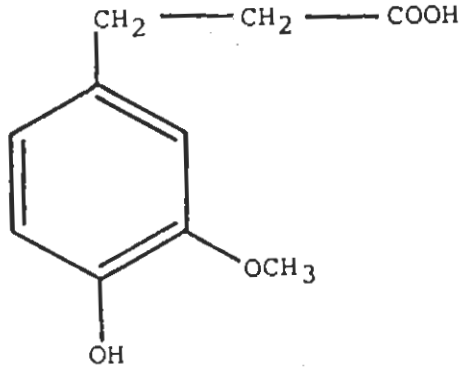
Capparisine

The isolated sesquiterpene named cadabicilone is a tricyclic lactone, longing to the eudesman series. Its structure was elucidated by modern ctroscopic methods including 2D, J-resolved, COSY-45 and Heteronuclear pling experiments.



Cadabicilone

The isolated aromatic acid has the molecular formula $C_{10}H_{12}O_4$. It has two ydrogen atoms more than that of ferulic acid ($C_{10}H_{10}O_4$). The spectral data owed that, this is an α,β-dihydro derivative of ferulic acid.



α,β-Dihydro ferulic acid

2 INTRODUCTION

2.1 General Introduction

The mysteries of nature are open to be conquered by human beings through sizeless struggle, constant observations, curious research and practicals. Human curiosity and preoccupation brought about a beneficial increment in the radiant fund of knowledge in the domain of scientific research and thus men succeded in discovering cure of a large number of ailments with herbal, animal and mineral medicines in the ancient times. Chemistry, biology, medical science and countless branches of science have now began to expand their horizon due to necessity and curiosity of human nature. To this day, the splendid voyage of knowledge and research is continued onward for the sake of sublime causes, in the name of human betterment and welfare.

The Chen Nung [1] in China and Sekhet Enanach [2] in Egypt were the first to persue studies in the medicinal uses of herbs around 3000 BC, for the treatment of various human ailments. The other earlier known references of medicine are in "Rig vedas" (4500-1600 BC) and the Ayurveda (2500-600 BC). This was followed by the classical system of chinese medicine (2500-600 BC).

There is a pharmacopoeia like compilation in Chinese tradition called "entsao" or the "Great herbal" (about 1500 BC), containing thousands of descriptions. The famous Egyptian materia "Ebers papyrus" was also written about 1500 BC. The search for informations regarding ancient medicine leads naturally from the papyri of Egypt to Hebrew literature.

The actual history of medicine and pharmacy begins from Hippocrates [3], the "Father of Medicine" (460 BC), and Theophrastus [4] (287-370 BC). In the writings of Hippocrates nearly 400 samples are named as medicinal substances. However the most significant pharmacological compilations of the Greeks was the authoritative text of Discordies [2]. After him, Pliny the Elder (23-79 AD) wrote "Natural history" in 37 volumes. Galen wrote some 30 books on pharmacology alongwith his "galenicals" preparations [5]. After the time of Galen, the works of earlier Greeks physicians were transfered to Romans and then to Muslims.

In the Muslims period of civilization we find a valuable medical knowledge. The great physicians and philosopher Avicenna (Bu-Ali Sina, 908-1037 AD), has described 700 herbal drugs, in his famous book "Qanun fial Tibb" [6], the other famous names of this period are Rhazes (930 AD), Al-Idrisi (1100-1166 AD), Rashiduddin Suri and Ibnul-Baiter.

The sources of Indian medicine are derived from Rig-Veda believed to have been compiled between 4500-1000 BC. and from Ayurveda 2500-600 BC. They mainly based on the use of drugs of plant origin. The Ayurvedic system of medicine in India is mainly attributed to Charaka [7] and Sushruta [8], who cited about 00 medicinal plants.

Due to great importance of plants as sources of medicine work began to focus on the chemical entities that are constituents of such plants. The search for new medicinal agents from plants having folkloric repute are the most promising for thorough investigation.

ALKALOIDS AS REMEDY

Even before the end of the eighteenth century, organic materials has been isolated from living organisms and their products. Friederich W. Sertürner (1783-1841) obtained morphine from opium [9] in 1805, and Pelletier and Coventou isolated strychnine, brucine, quinine, cinchonine and coffeine in the next fifteen years. Because of their chemical complexity and important physiological properties these alkaloids should be considered as the first of the typical, natural organic substances isolated by man as pure compounds.

The mankind has used the drugs containing alkaloid for the last 4000 years, for example, the major constituent of *Cinchona* species," quinine " isolated in 1639 was found to be an effective anti-malarial medicine [10]. Derosene in 1803 isolated an alkaloid, "narcotine" from crude drug opium, whereas in 1826 Pellete and Cavantou reported "Conine" an alkaloid of historical significance [11].

The herb "mahuang" (*Ephedra vulgaris*) has been used in China for atleast 000 years. The alkaloid ephedrine isolated from it, has been used even in he Western countries for treatment of asthma and similar conditions. In recent ears "reserpine", isolated from *Rouwolfia* is now frequently employed in the reatment of high blood pressure and some emotional and mental conditions. jmaline hydrochloride is being used for the treatment of cardiac arrythmias. ıe discovery of such type of compounds, has further strengthened interest ı research relating to therapeutic constituents of medicinal plants.

CLASSIFICATION OF ALKALOIDS

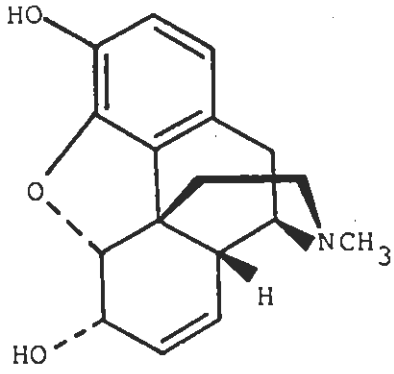
The alkaloids outclassed all the rest of the catagory of chemical compounds in showing the patent pharmacological action on animal and human subjects. It is due to this fact that alkaloid constitute the largest class of medicinal agents from plants. The term alkaloid or "alkali-like" was first proposed by the pharmacist W. Meissner in 1889. This term is drived from the fact that, they are nitrogen-containing (usually heterocylic) compounds, having basic properties and form salts with acids, but when the complexity of structure increases it becomes difficult to draw a sharp line between alkaloids and amines [12].

Many attempts are made to provide a system of classification for alkaloids. Usually they are classified on the basis of their source, chemical structures and pharmacological action. On the basis of basic skeleton, alkaloids may be divided into different classes.

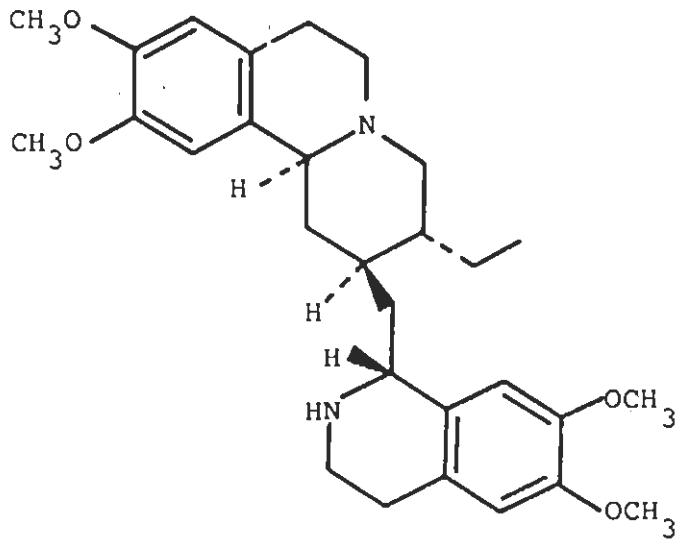
One of the most widely accepted classification system was given by Pegnauer [13]. This system divides the alkaloids into three classes.

i) True alkaloids:

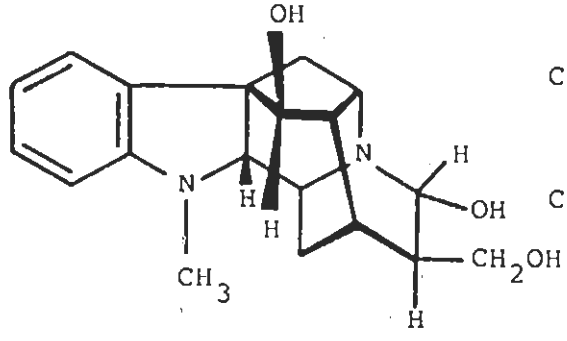
The true alkaloids are almost invariably basic, they normally contain nitrogen in a heterocyclic ring, they are derived from amino acids and they normally occur in the plants as the salt of an organic acid; examples of true


HO
O
NCH3
H
HO

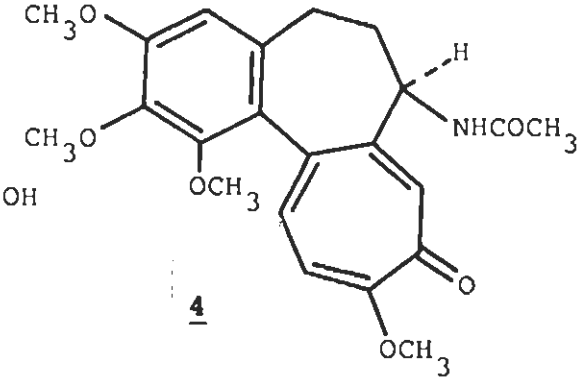
1


CH3O
CH3O
N
H
H
H
HN
OCH3
OCH3

2


OH
N
H
H
H
OH
CH2OH
N
CH3
H

3


CH3O
CH3O
OCH3
H
NHCOCH3
O
OCH3

4

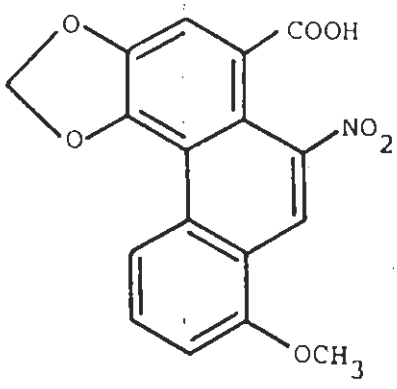
alkaloids are morphine (1), cephaeline (2), ajmaline (3) etc. Some exception to above "rules" are colchicine (4) and aristolochic acid (5), which have no heterocyclic ring and are acidic rather than basic.

(ii) Proto alkaloids:

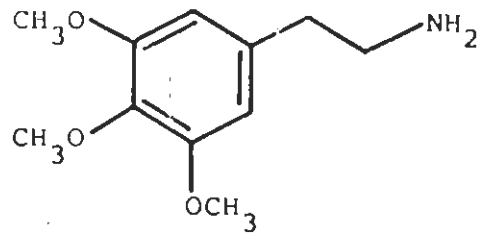
They are biosynthesized from amino acids and are basic in nature. They are relatively simple amines, and their nitrogen atoms are not in a heterocylic ring. The term "biological amines" is often used for this group of compounds, examples are mescaline (6), ephedrine (7) etc.

(iii) Pseudo alkaloids:

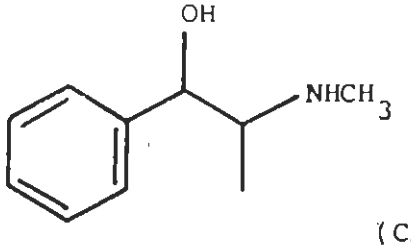
They are usually basic, but not derived from an amino acid. There are two important serieses of alkaloids in this class, the steroidal alkaloids, e.g. conessine (8) and the purines, e.g. caffeine (9).


O
O
COOH
NO2
OCH3

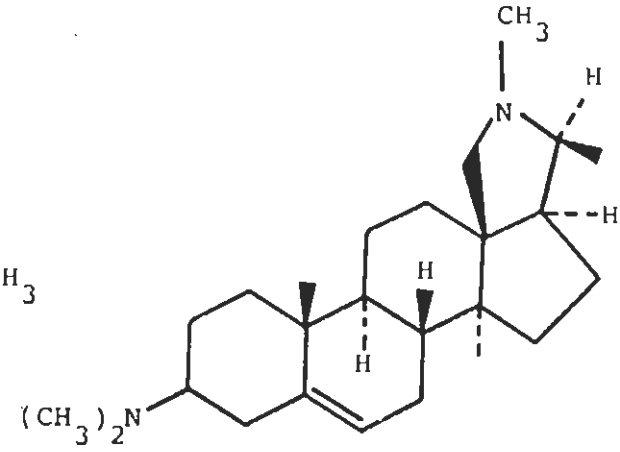
5


CH3O
CH3O
OCH3
NH2

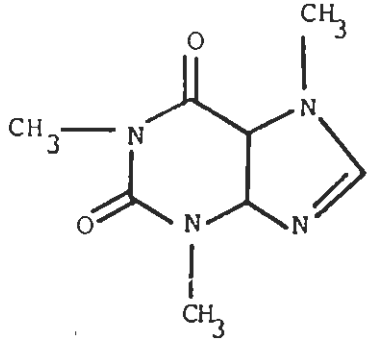
6


OH
NHCH3

7


CH3
H
N
H
H
H
(CH3)2N

8


CH3
O
N
CH3
N
O
N
N
CH3

9

2.2 Spermidine alkaloids

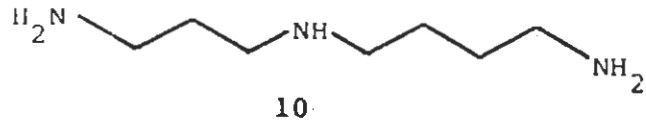
Diamines and polyamines occur in the plants and animal kingdoms as free bases (biogenic amines) as well as derivatives. Spermidine (10) is a triamino compound, in which two amino groups are primery and 3rd one is a secondary amino group. In most of the spermidine alkaloids, spermidine unit is a part of a large heterocyclic ring, and it is attached with the rest of molecule through the terminal nitrogens, as in case of codonocarpine (11) and capparidisine (12).

Structure elucidation of spermidine alkaloids:

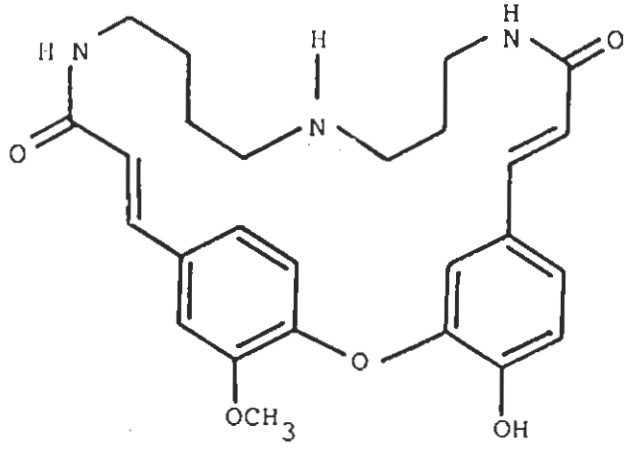
In contrast to the polycyclic indole, isoquinoline or terpene alkaloids, the di-, tri-, and poly-amine alkaloids seem to be of much simpler construction. This first impression is misleading. Special structural features render this group of alkaloids even more difficult to handle than the above mentioned ones. It should be noted that the structures of several polyamines alkaloids have had to be revised. Because of this, two main factors should be mentioned.

) The alkaloids sometimes occur as mixtures, that are very difficult to eparate.

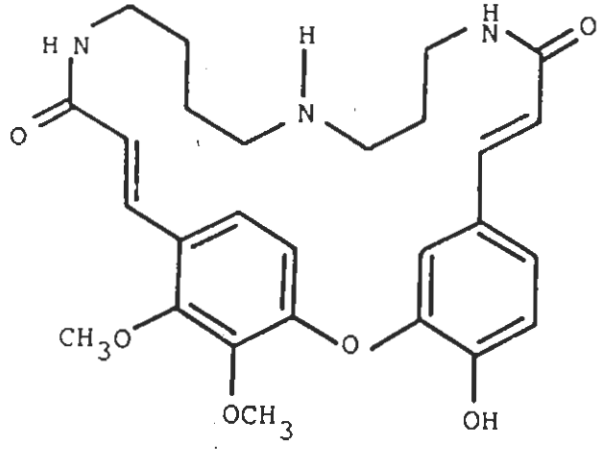
) The results from spectral or chemical analysis are usually not sufficient o distinguish between two regio isomers of a macrocylic spermidine alkaloids.



10



11

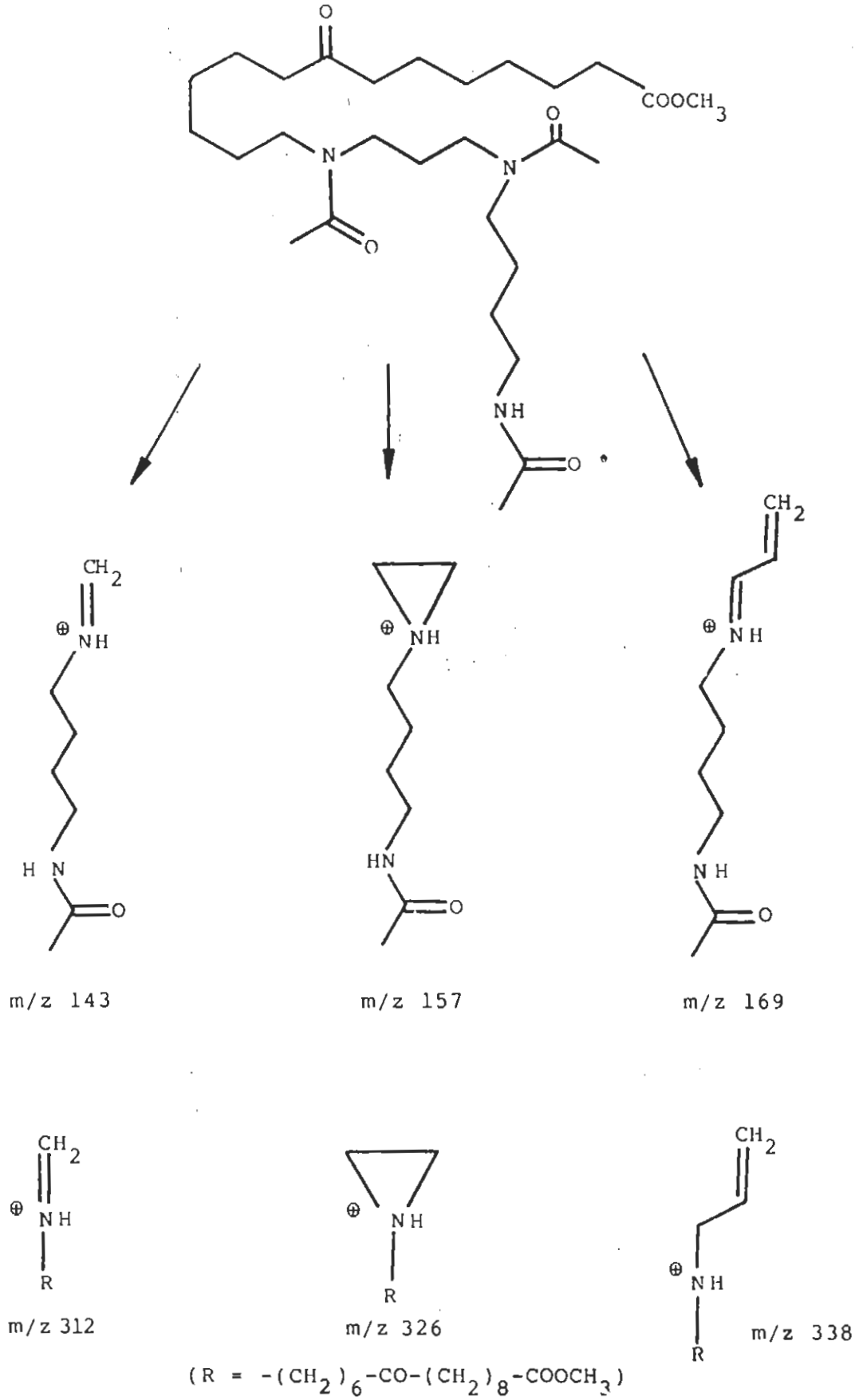


12

Some of the alkaloids contain phenolic hydroxyl groups in addition to the basic amino nitrogen, so they are very polar, strong bases. In order to isolate these materials from plants and to purify the compounds to some extent, procedures must be applied that are different from those used in "normal" alkaloid chemistry. Extraction of plant sources under strongly basic or even strongly acidic conditions is dangerous because of the possibility of transamidation reactions.

Another difficulty is the determination of the homogenity of the isolated alkaloids, for example, by different chromatographic methods and because of its spectral properties, the natural, approximate 1:1 mixture of the spermidine alkaloids, "inandenin-12-one" and "inandenin-13-one", appeared to be a pure compound. This was supported by the sharp melting point of its hydrochloride. Only after chemical degradation the nature of the mixture was determined by mass spectrometry. On the other hand, the behaviour of the pure crystalline spermidine alkaloid "aphelandrine" was that of a mixture.

Another major problem is to decide the position of exact attachment of amino nitrogen atoms of the spermidine and the substituents. In spermidine both primary amino groups are very similar with respect to their neighbouring groups, but not identical (three and four methylene groups between two amino groups, respectively). When there are different substituents on the two nitrogen atoms, chemical degradation combined with spectral analysis often, as necessary to determine the correct structure.



lass Spectral fragmentation of inandenin-12-one-derivative

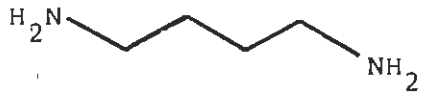
(Scheme-1)

An additional procedure for determining alkaloid structure is the mass spectral analysis of the acetylated hydrolysates prepared from the alkaloids under mild conditions. For example in mass spectral fragmentation (Scheme-1) of inandenin-12-one derivative, two sets of three peaks (each) are observed : m/z 143, 157 and 169 as well as m/z 312, 326 and 338. The first set of peaks corresponds to ions containing the unbroken 1,4-diaminobutane unit while the 1,3-diaminopropane unit is degraded. The second set of signals corresponds to ions in which the 1,3-diaminopropane unit again is fragmented, but the positive charge is located at the other nitrogen atom. In both cases the fragmentation reactions take place in the smaller unit. By combining these two sets of peaks it is possible to determine the substitution pattern of the three nitrogen atoms and by analyzing some other signals, the structure itself. A different substitution pattern at the spermidine residue will cause shifts of the peaks in above mentioned sets of signals.

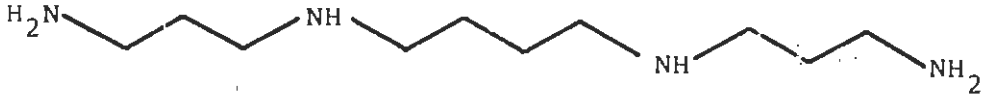
Occurrence and Biosynthesis of Spermidine alkaloids:

The triamine spermidine (10) is analogue of the diamine putrescine (13), which is produced by reductive decarboxylation of lysine. It may be regarded therefore as being derived from a core unit of putrescine, which is then substituted on "N" by propylamine residue.

Spermidine occurs in almost all animal and micro organisms and possibly most higher plants. Detections and isolations from higher plants used as foodstuffs include, cabbage leaves, tomato juice, apples and spinach as well



13



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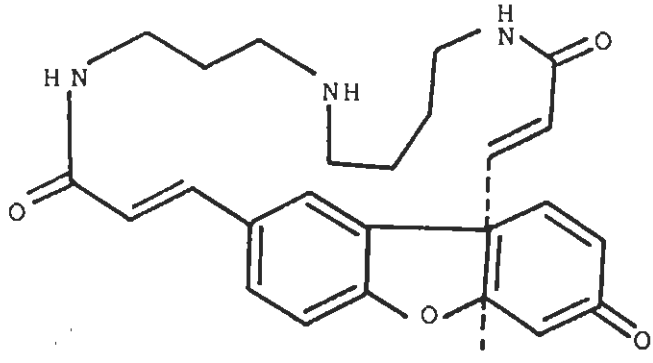


15

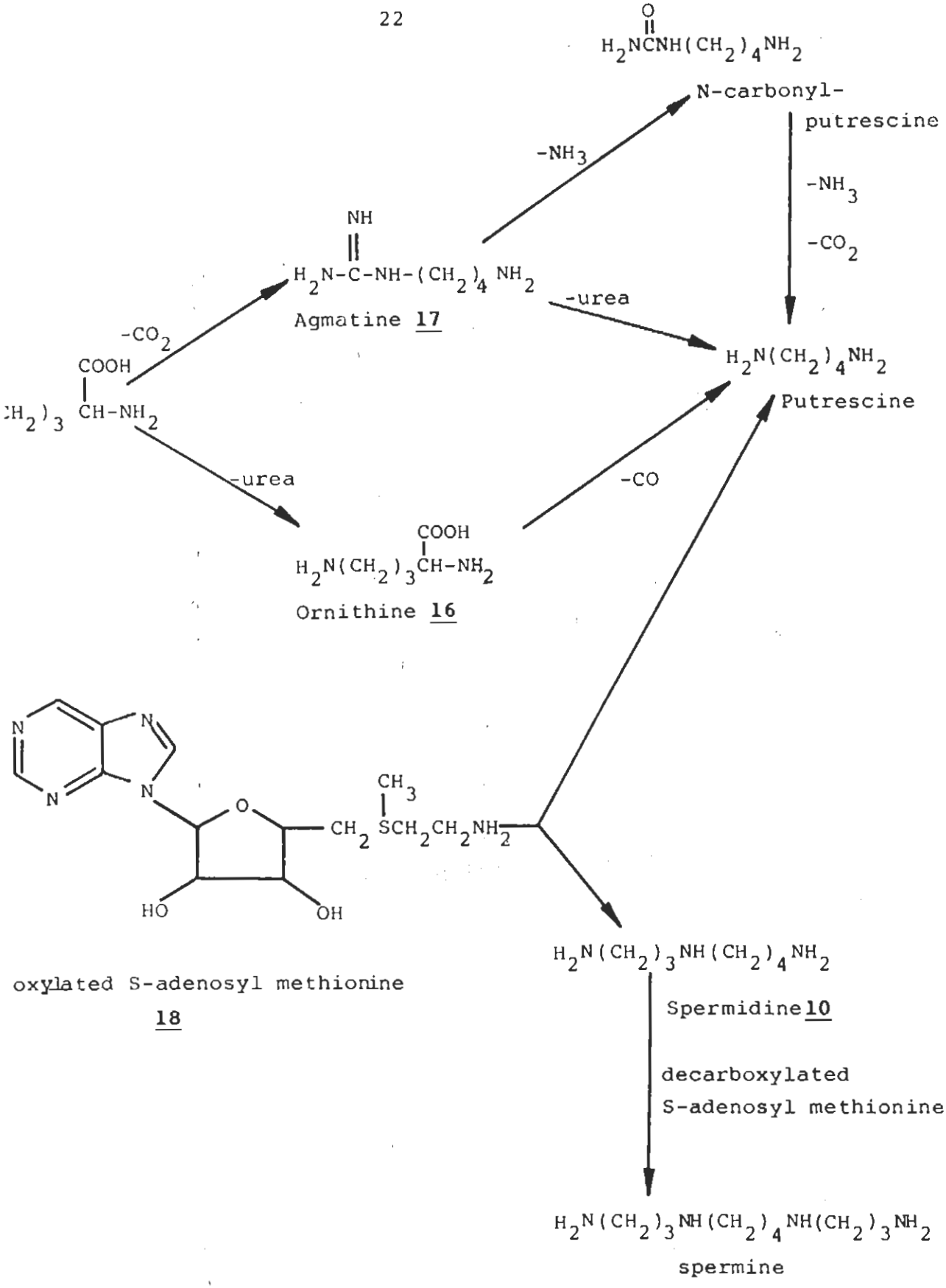
is the leaves of wheat, maize, pea, black currant and tobacco [14]. Spermidine 10) alongwith spermine (14) (12:1 ratio) also occur in human semen in high concentration (0.5-3.5 mg/5 mls). The polyamines of human semen are formed primarily in the prostrate gland, although their function remain unclear. They ay be present for their bacteriostatic effects or for stabilization of DNA 14].

The structurally most interesting spermidine alkaloids are those ontaining a macrocyclic ring as Lunarine (15), codonocarpine (11) etc. permidines substituted with cinnamic acid derivatives seem to be widely istributed in the plant kingdom.

Although spermidine is propyl amino derivative of putrescine (13), but ery little is known about the conversion of putrescine to spermidine in higher lants [14] uptil now, no experimental biosynthetic investigation in higher lants have been published [15].

In *E.coli* [14], spermidine is formed by transfer of a propylamine residue erived from decarboxylated S-adenosyl methionine to putrescine derived from nithine (16) (Scheme-2). Both putrescine and spermidine inhibit ornithine carboxylate.

Richards and Coleman [14] in 1952 proved that potassium-deficient barley ants accumulated putrescine, and this has been confirmed in many other ants. Ornithine (16) was subsequently found to be only poorly incorporated,



Biosynthesis of Putrescine, spermidine and spermine

(Scheme-2)

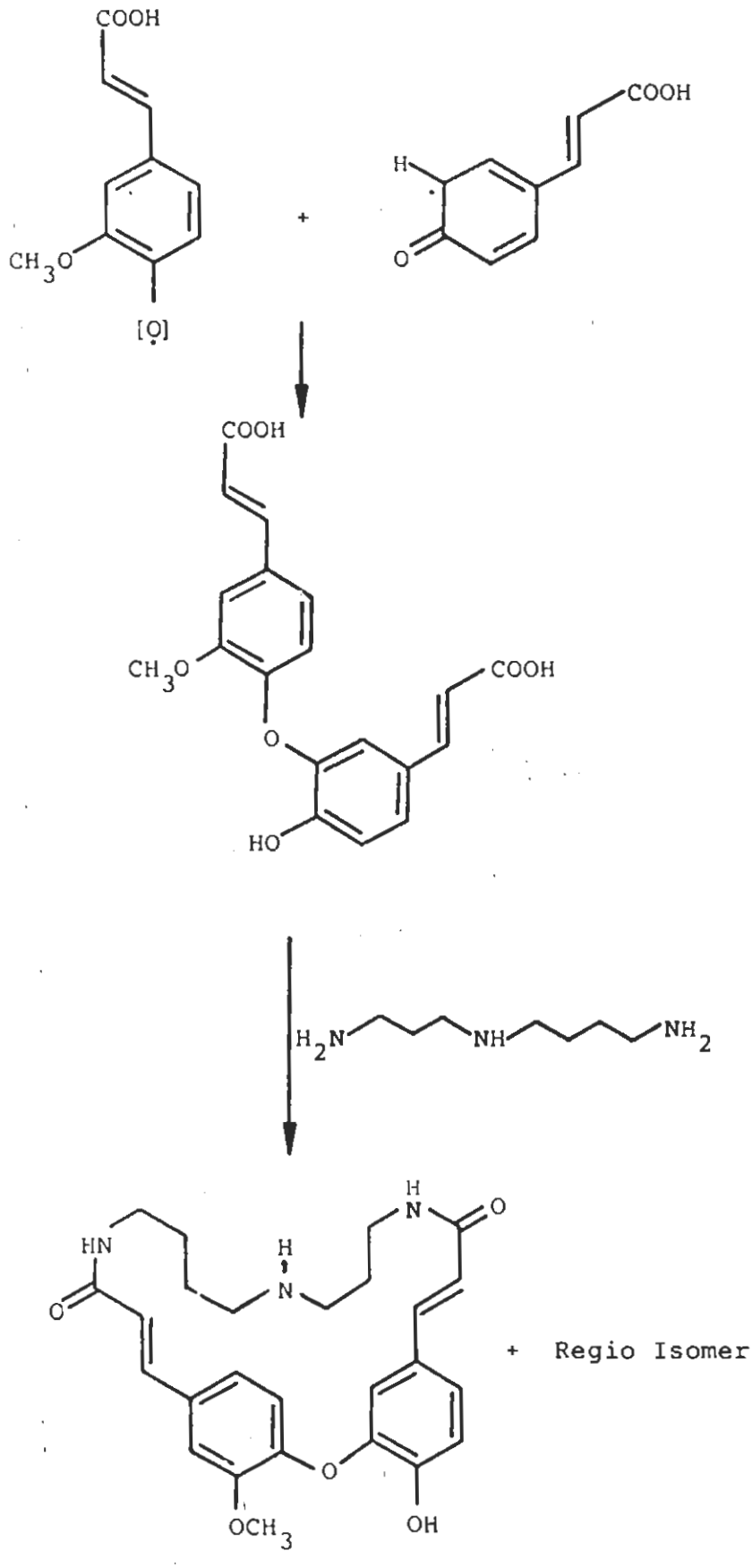
and the discovery of agmatine (17) suggested arginine decarboxylation as an early step in putrescine biosynthesis in barley. The way of conversion of putrescine to spermidine is not very well known, but it is commonly regarded that decarboxylated S-adenoxylmethionine (18) also provides the propylamine group in this case.

In spermidine alkaloids, obtained from higher plants, the non basic parts of the alkaloids are in most cases cinnamic acid and fatty acid derivatives. The cinnamic acid derivatives may contain additional aromatic hydroxyl or methoxyl groups, or two cinnamic acid residues may be condensed with each other, via phenolic coupling, or one of the cinnamic acid residues may be connected wtih a second nitrogen of the spermidine backbone (Michael reaction product) in addition to the amide linkage. In most cases, only a few chemical reactions, involved in the formation of polyamine alkaloids from their building blocks in nature, are known as amide formation, Michael reaction and phenolic coupling [15]. The proposed schematic pathway [15] for, codonocarpine, cappiridisine and cadabicine is shown in Scheme-3.

In principle, the oxidative coupling can take place at two different stages.

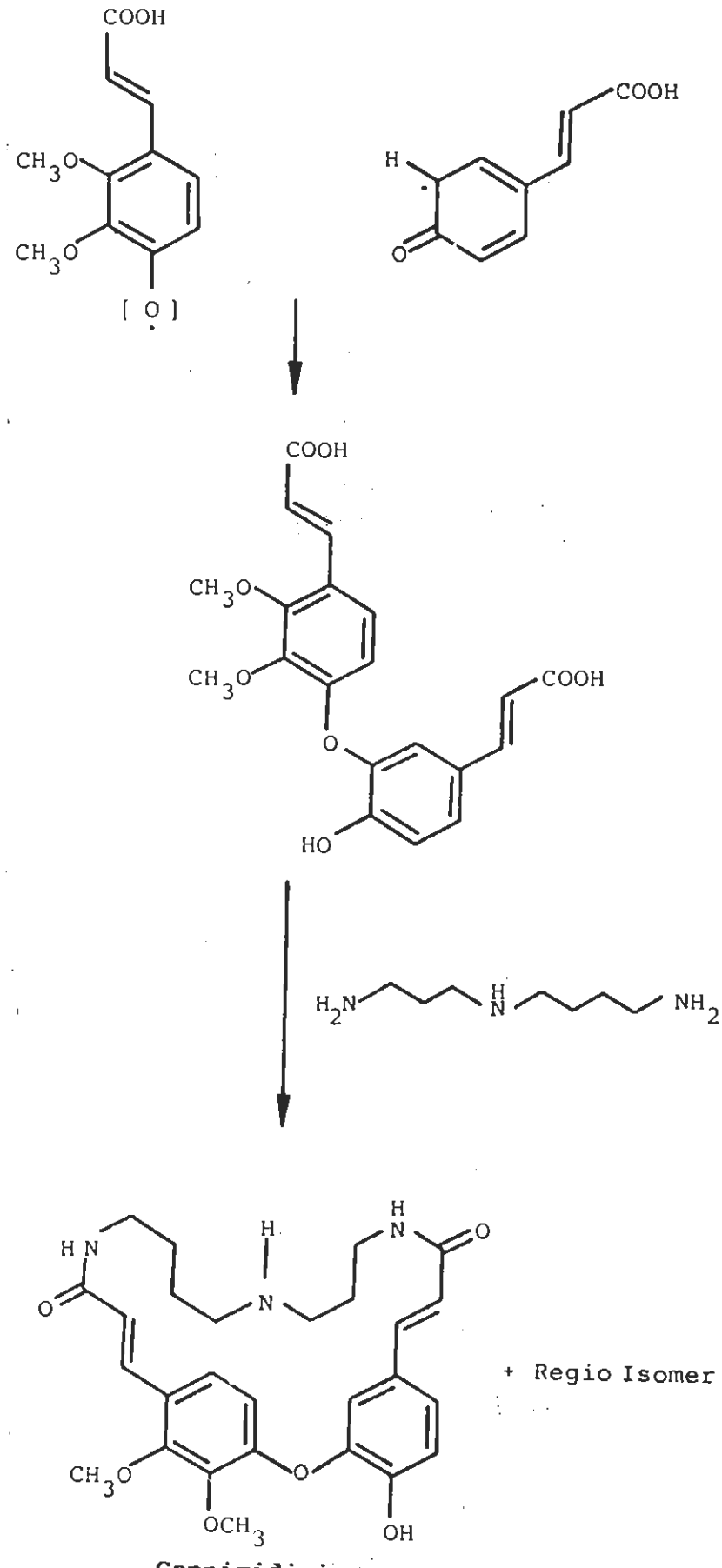
) In the first the two cinnamic acids units are coupled togather followed by amide linkage formation, with spermidine.

) The second mechanism involves an initial amide formation



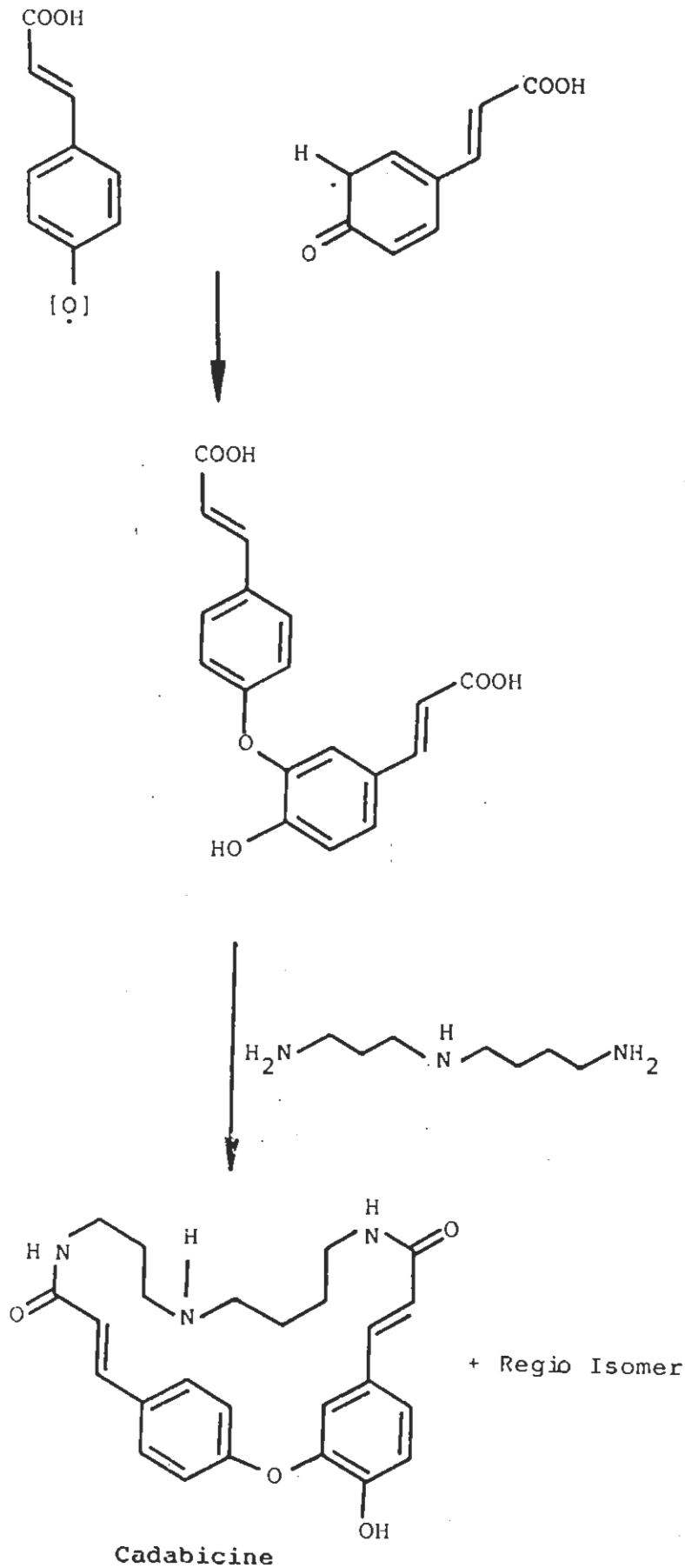
Codonocarpine

(Scheme-3)



Cappiridisine

(Scheme-3) Continued



Cadabicine

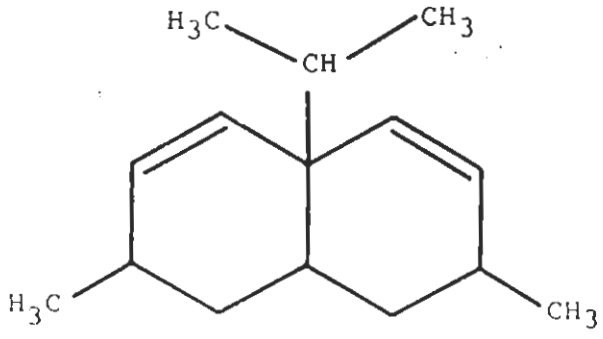
(Scheme-3) Continued

between cinnamic acid and spermidine. In a later step, the cinnamic amides, are coupled in an oxidation reaction. Such a mechanism has been proposed for formation of the hordatines from coumaroylagmatines [16].

2.3 Sesquiterpenes

The name sesquiterpene is applied to the hydrocarbon constituents of the essential oils which have the composition $C_{15}H_{24}$. There are in the literature over four hundred references to the occurrence of sesquiterpenes in nature, but in comparatively few instances, these have been characterised and shown to be homogeneous substances.

The valuable pioneering researches of Gladstone [17], Wallach was the first to suggest a general formula for the sesquiterpenes. As a guiding principle, he adopted the view that the sesquiterpenes, like the simpler terpenes, was built up of "Isoprene nuclei" and by the combination of three such nuclei, he devised the structure (19), which represented them as derivatives of a partially hydrogenated substituted naphthalene.



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This interpretation was not intended to apply to any particular hydrocarbon, but only to show their general type.

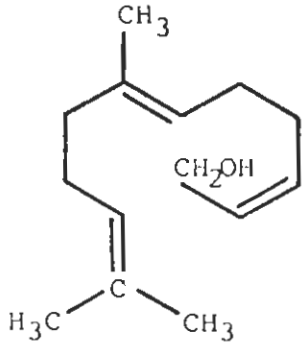
Semmler, Schreiner and Kremers, independently proposed a classification of the sesquiterpene, which was proved of great value in this field of research on the basis of this classification, the sesquiterpenes may be divided into four main classes [18-19].

i) Acyclic (as farnesol) (20)

ii) Monocyclic (as bisabolene) (21)

iii) Dicyclic (as cadinene) (22)

iv) Tricyclic (as copaene) (23)



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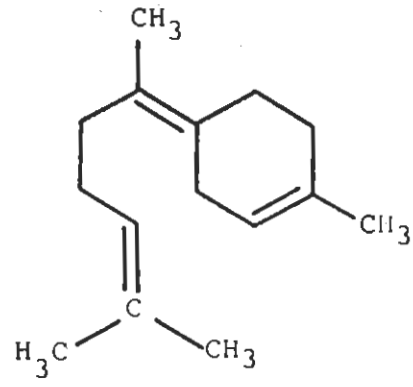

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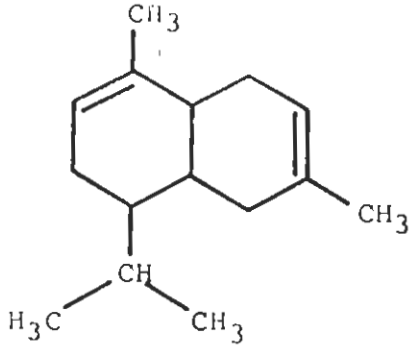

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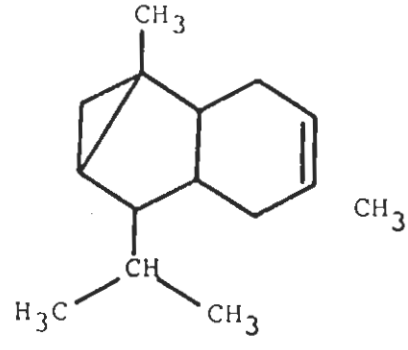


23

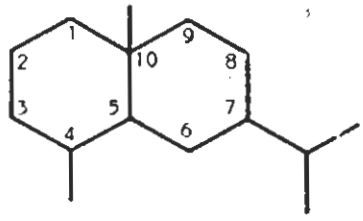
The naturally occuring sesquiterpenoids isolated from the plants can be classified on the basis of basic skeletons of sesquiterpenes. Generally they are divided into following four groups [20].

1) The eudesmane

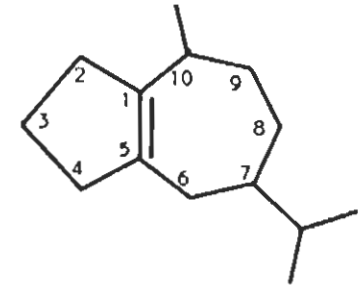
2) The guainane

3) The germacrane

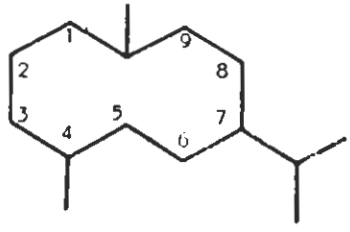
4) The drimane



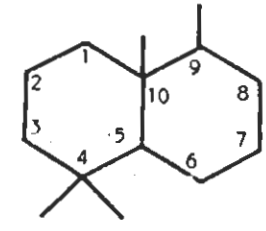
Eudesmanes



Guainanes



Germacranes



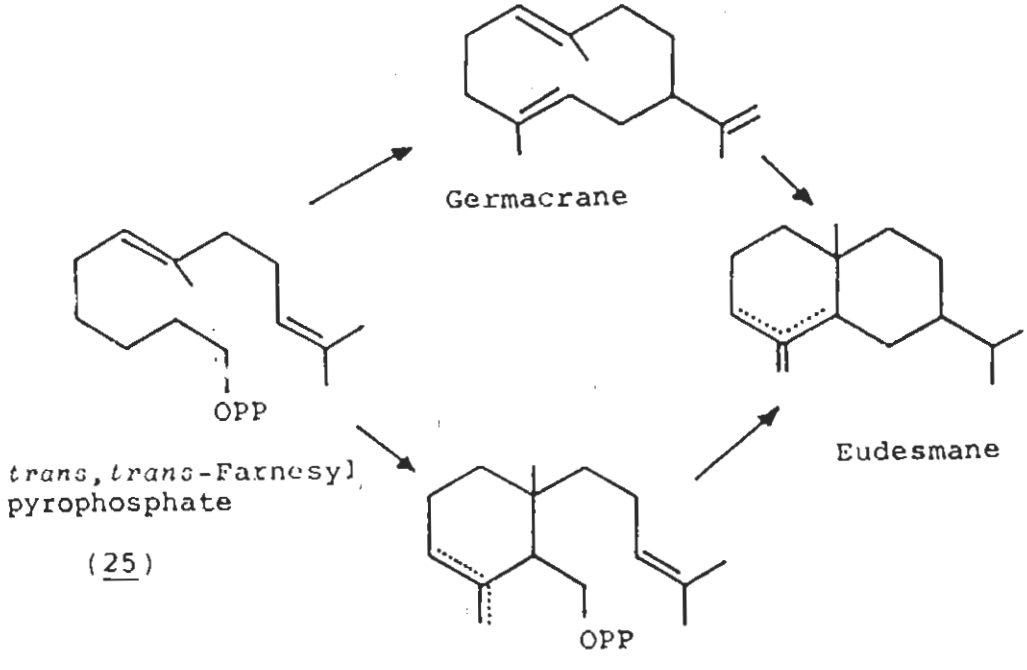
Drimanes

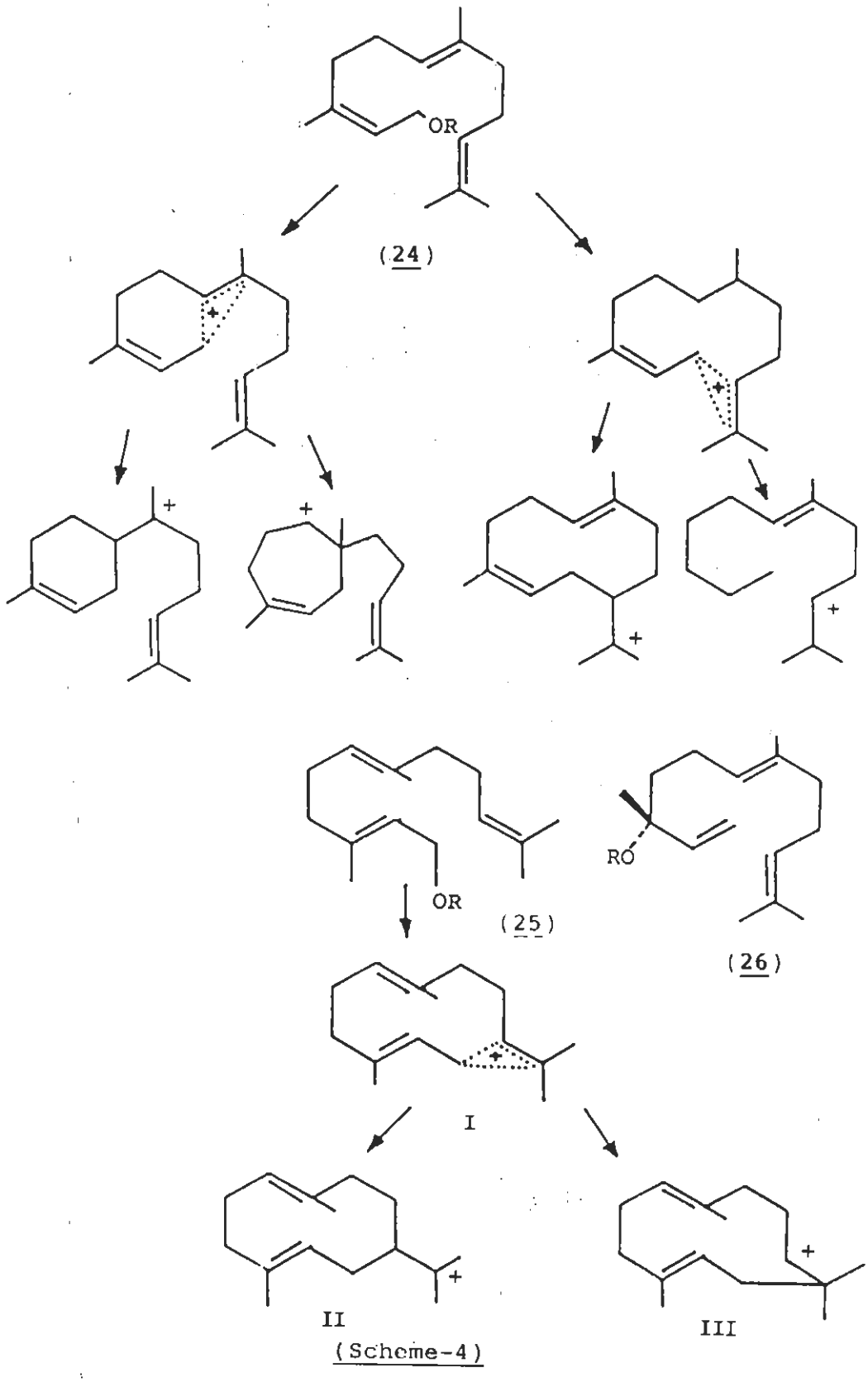
The biosynthesis of sesquiterpenoids involve the cis and trans-farnesyl pyrophosphate (24) and trans-trans-farnesyl pyrophosphate (25) as starting materials, with the proviso that nerolidyl pyrophosphate (26) can also serve as crucial building block.

During the cyclization, the pyrophosphate group acts as a leaving group and pi - electrons of a double bond are involve in ring closure. If the central double bond is involved in cyclization a six membered ring is formed and if terminal double bond is involved then a ten membered ring is formed, as represented in Scheme-4.

The eudesmane:

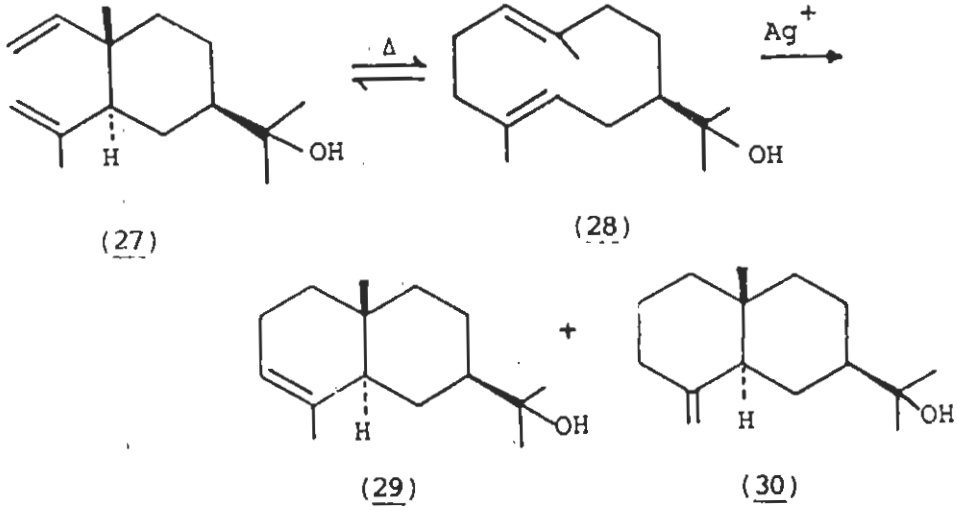
According to biosynthetic point of view the eudesmane group of sesquiterpenoids is derived by cyclization of a germacrane derivative formed from trans-trans-farnesyl pyrophosphate (25) [21]. The logical precursor of this group [22] is the cation II in Scheme-4.





(Scheme-4)

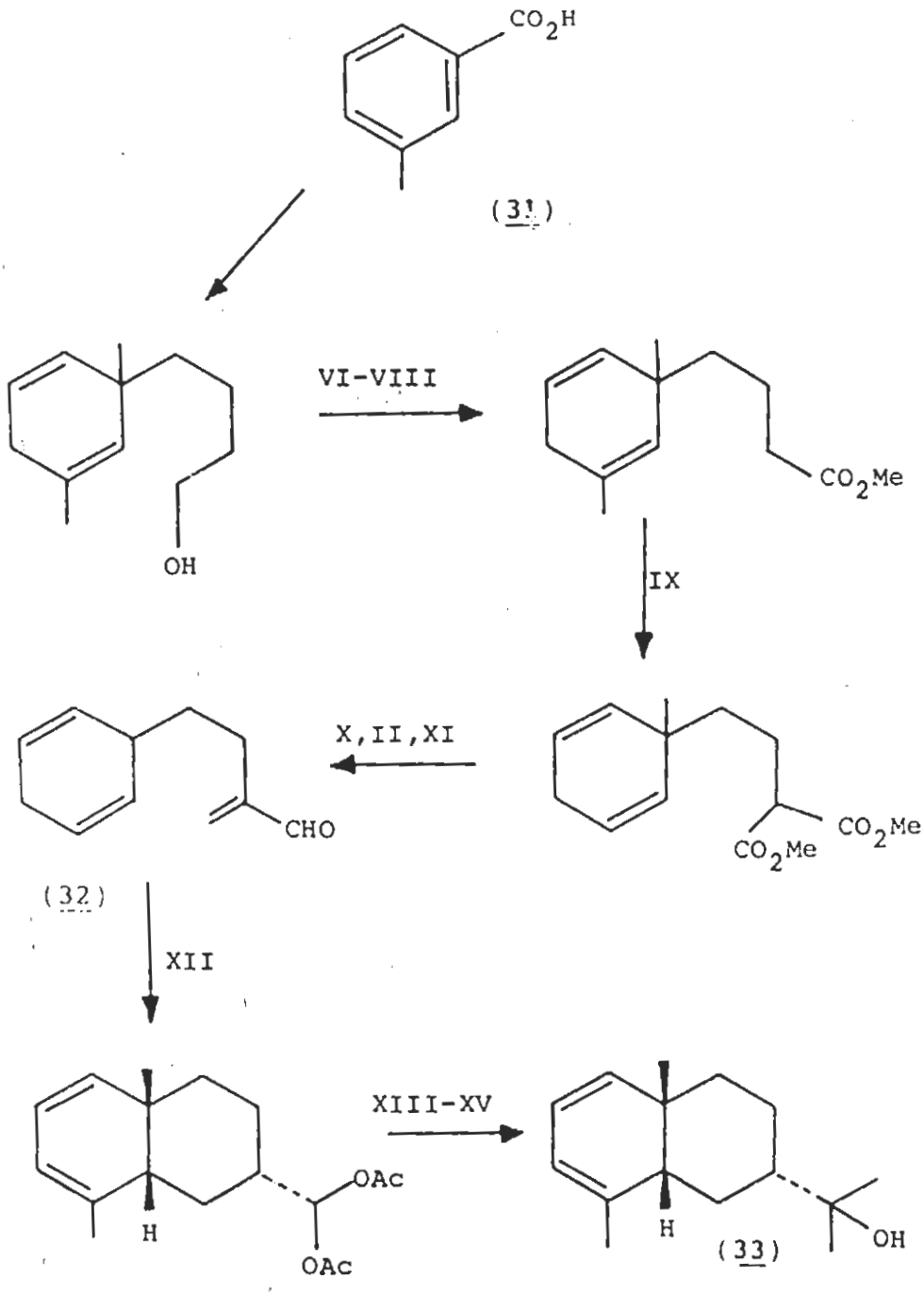
Elemol (27) thermally and reversibly cyclizes to hedycaryol (28). The mixture of (27) and (28) undergoes silver ion catalyzed rearrangement to α-eudesmane (29) and β-eudesmane (30) [23]. This biosynthetic relationship between elemol and eudesmols is proposed on the basis of the fact that, both type compounds co-occure in the citronella oil.



(27) (28)

(29) (30)

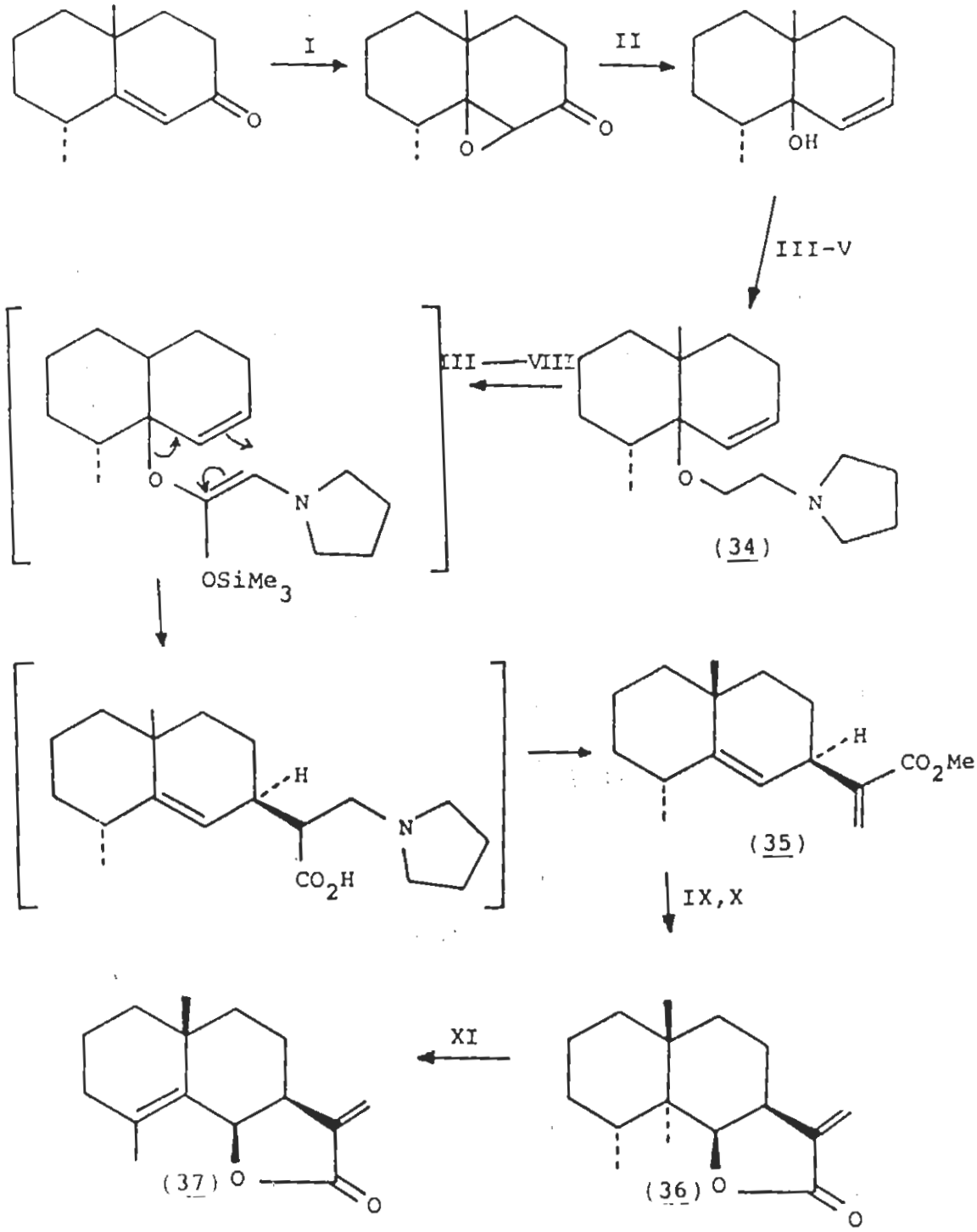
A new synthetic route to (±)-occidentalol (33) is the cyclization of a suitably substituted cyclohexadiene aldehyde (32) derived from m-toluic acid (31) [24] (Scheme-5). It seems reasonable to assume that modification of this approach would be valuable in the syntehsis of eudesmanolides and germacranolides.



gents: I,Li-NH_3-$Br(CH_2)_4OCH_2Ph$; II,$LiAlH_4$; III,Tscl-Py; IV,$LiBHEt_3$-HMPA; V,Na-NH_3-BuOH; VI,NCS-Me_2S-toluene; VII,Ag_2O; VIII,MeOH-H^+, IX,$ClCO_2$- (); X,NaH; XI,MnO_2; XII,Ac_2O-$HClO_4$;

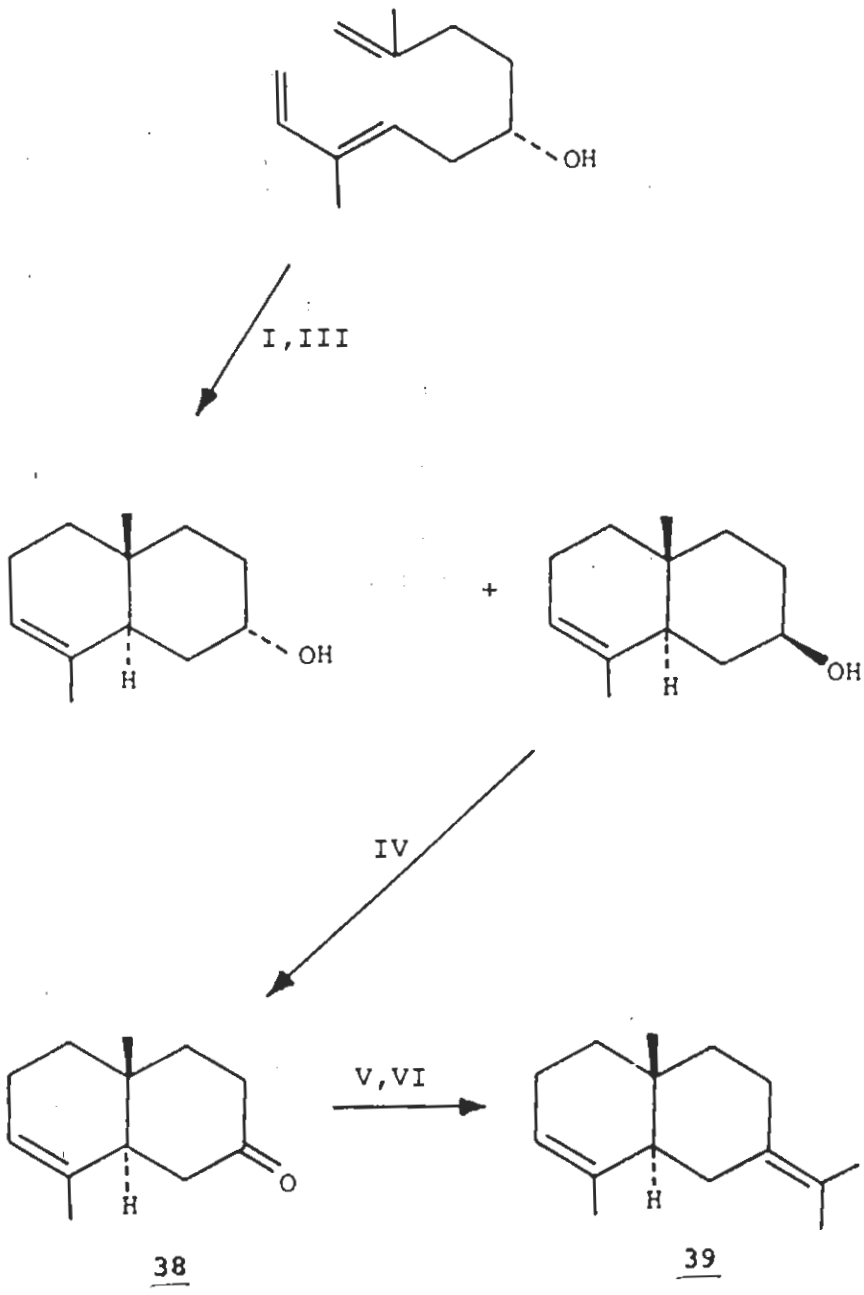
XIII,Ag_2O-NaOH; XIV,CH_2N_2;XV,MeLi.

(Scheme-5)



ents I,H_2O_2-OH;II,NH_2NH_2,H_2O-MeOH,III,$LiNPr_2$;IV,CH_2=CHCOCl;
V,pyrrolidine;VI,Et_3SiCl;VII,toluene,Δ;VIII,Me_2SO_4-K_2CO_3-
MeOH;IX,KOH-MeOH;X,KI_3-$NaHCO_3$-H_2O;XI,DBu-THF,Δ

(Scheme-6)



38 39

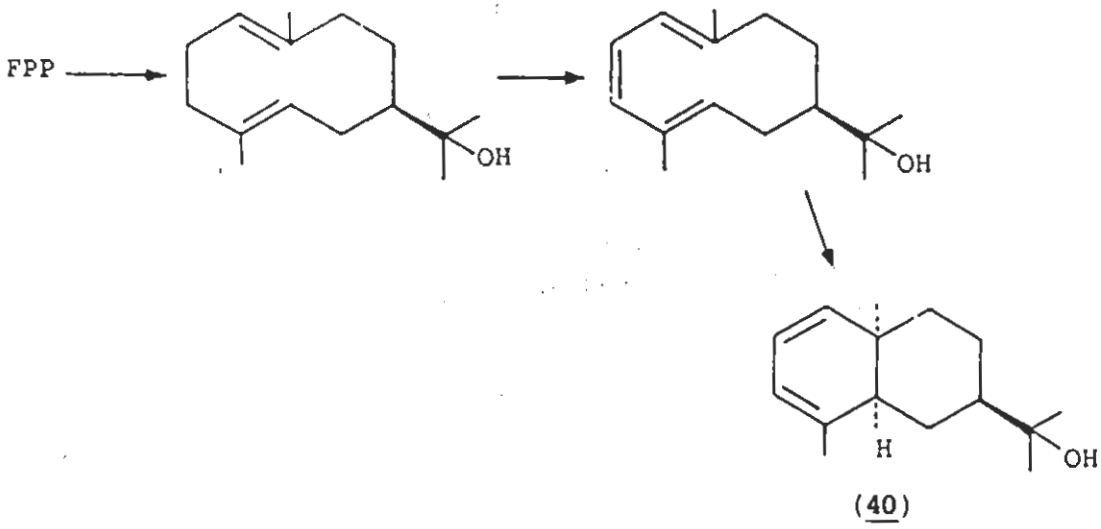
Reagents: I, Me_3SCl, Py; II, Δ; III, H_3O^+; IV, |O|; V, CBr_4-PhP; IV, Me_2CuLi

(Scheme-7)

An interesting new approach to α-substituted acrylic ester (34) has been successfully incorporated into the first total synthesis of the allergenic acid sesquiterpenoid frullanolide (36) (Scheme-6) [25]. Related studies have shown that the butenolide derivative can be used as an annulating agent in the synthesis of linear tricyclic γ-lactones (36) and (37), and it is expected that this methodology may be applied to the synthesis of naturally occuring eudesmanolides [26].

A short intramolecular Diels-Alder route has been developed for the synthesis of the ketone (38) which can be converted into selina-3,7,(11)-diene (39) (Scheme-7) [27].

The biogenetic type synthesis of occidentalol (40) a eudesmane found in Thuja occidentolis, that possesses a cis-ring junction, were achieved (Scheme-8) [28].



(40)

(Scheme-8)

2.4 Botanical description of Cadaba farinosa

Cadaba farinosa, Forssk, (Syn. *C. fruticosa*, L. Druce, *C. indica*, Lam.) belongs to family Capparidaceae (Capparaceae). It is a medium sized family with about 45 genera and nearly 600 species, which are distributed throughout the world, mostly in tropical and sub-tropical regions. It is represented by only 7 genera and 23 species in Pakistan. The capparidaceous plants are usually herbs, erect or scandent, shrubs and rarely trees [29].

The genera *Cadaba* is represented by about 30 species, out of which only 2 species are reported from Pakistan [29].

The genus derives its title from "Kadhab" [30], an Arabic name for the *Cadaba rotundifolia* of Forskal, who mentions *Cadaba farinosa* as a medicinal plant. The latter plant, under the name of "Sarah" is described by Az., from information given to him by an Arab of the desert, as a shrub with a dusty colour, with small leaves and lank branches or twigs and always growing slanting.

Cadaba farinosa is a common shrub [29] of the arid plains of Sind and Baluchistan provinces of Pakistan. It has slender to twiggy tangled branches upto 5 m high. The young twigs farinose with small white waxy sessile scales, becomes glabrous with age. The leaves are oblong-ovate or elliptic-oblong, with more or less rounded apex., which are 5-50 mm long and 3-30 mm broad. The flowers are oblong-ovate, concave, acute, covered with scales and usually

creamy yellow. The fruits are narrowly cylenerical, 20-50 mm long and 3-4 mm broad, they are many seeded, seeds are about 2.5 mm in diameter and are surrounded by orange flesh.

2.5 Medicinal Importance of *Cadaba farinosa*

Leaves of *Cadaba farinosa* are considered to be purgative, antisyphilitic, antihelmintic and antiphlogistic and also employed in preparing medicated oils [30]. Leaves are also used as a remedy for cough, fever, dysentry and as poultice in sores [31], externally leaves are used with the leaves of *Odina wodier*, to relieve rheumatic pains and as poultice to boils, to promote suppuration [30], boiled leaves are eaten as an antihelmintic, decoction with other ingredients is employed in the treatment of amenorrhoea, dysmenorrhoea and uterine obstructions, decoction of leaves with myrobalans and ginger or with senna and epsom-salts given as purgative and antiphilogistic in syphilis, scrofula and rheumastism [32].

Roots of the plant, also possess similar medicinal properties as that of leaves, the root preparation is used in anthrax [31]. Fruit is edible, ash of plant is rubbed into skin to releive general body pains, flower buds are stimulant, antiscorbutic, purgative, emmenagogue, antiphlogistic and antihelmintic (especially for round worms) [31].

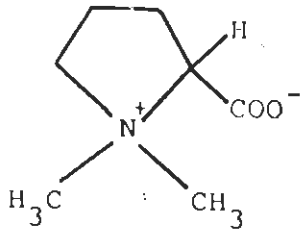
2.6 Literature Review of *Cadaba farinosa*

In pharmacographia Indica [30] by William Dymock, it is reported that, the ethereal and alcoholic extract of the leaves of *Cadaba farinosa* yielded somewhat bitter alkaloids giving crystallisable salt, when evaporated. The aqueous extract contains organic acids, from which one resembles in some of its reactions with Cathartic acid. The leaves contain a considerable quantity of nitrates, and the ash of dried leaves contains alkaline chlorides, carbonates and sulphates.

S.T. Vander walt and D.G. Steyn in 1943 reported that the genus *Cadaba* has toxicity [33].

V.U. Ahmad and A. Basha in 1971 reported the isolation of cadabine (L-stachydrine) (41) [34] from the leaves of the plant. Two years later P. Delaveau and Co-workers proved the existance of L-3-hydroxy-stachydrine (42) [35] alongwith L-stachydrine in the plant.

In a survay for alkaloids in Rajhistan (India) desert plants, S.P. Garg and R. Bhushan in 1980 reported the existance of alkaloids [36] in *Cadaba ruticosa*, but isolation and structures of alkaloides were not mentioned. The same group of workers also reported the sioaltion and structure of a dilactone, adabalone (43) [37] in 1981, from the pods of the plant. They also prepared the methyl ester and acetyl derivatives of cadabalone.



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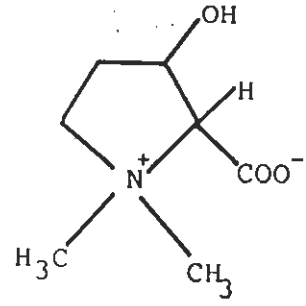

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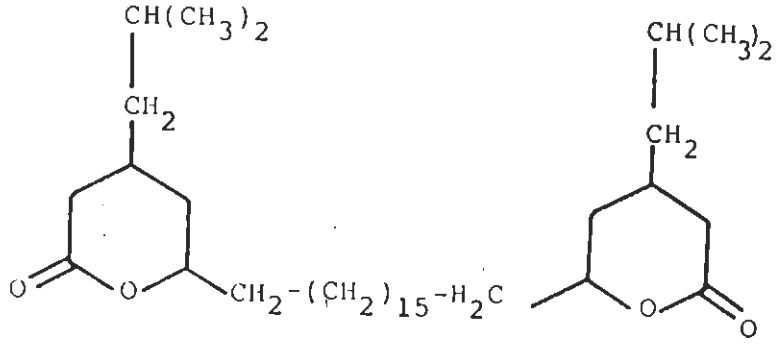

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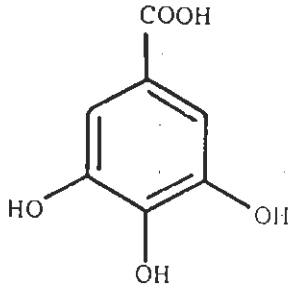
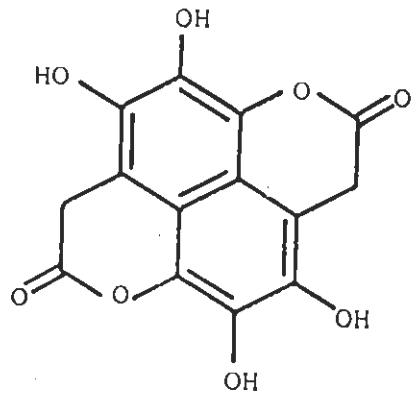
In 1983 M.H. Kittur and Co-workers reported that the seeds [38] of the plant contain 27.8% oil and the fatty acids were rich in oleic acid and linoleic acid, the seeds also contain 23.6% protein.

Reed, Jess.D. and Co-workers in 1985 described a method for the gravimetric determination soluble phenolics [39] of *Cadaba farinosa*, based on precipitation with $Yb.^{+3}$

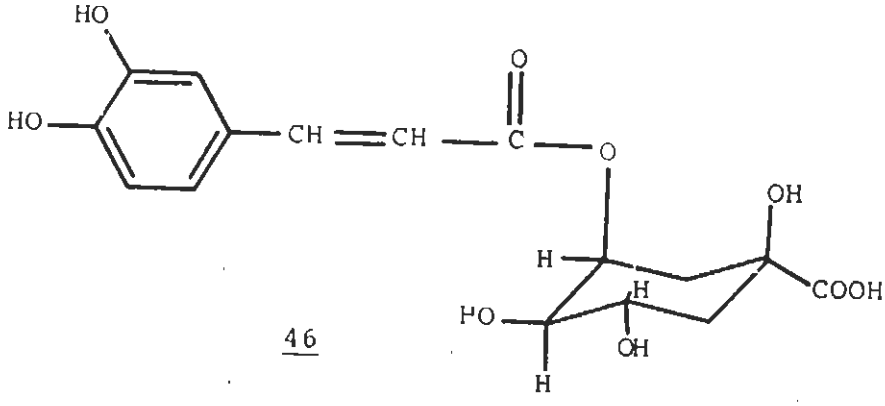
Mueller-Harvey and Co-workers in 1987, examined the acetone-water (7:3) extract of the plant for their phenolic constituents [40] by HPLC, and found that, it contains flavonols, flavonols glycosides, gallic acid (44), ellagic acid (45) and chlorogenic acid (46). Condensed and hydrolyzable tannins were also detected, trans and cis-p-coumaric acid (47), trans ferulic acid (48) were also identified after alkaline hydrolysis of the leaves.


COOH
HO
OH
OH

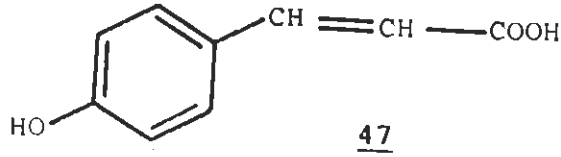
44


OH
HO
O
O
O
O
OH
OH

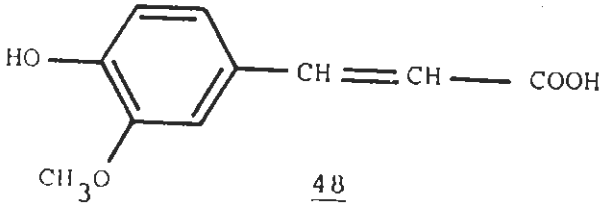
45


HO
HO
CH
CH
C
O
O
OH
H
H
COOH
HO
OH
H

46


CH
CH
COOH
HO

47


HO
CH
CH
COOH
CH3O

48

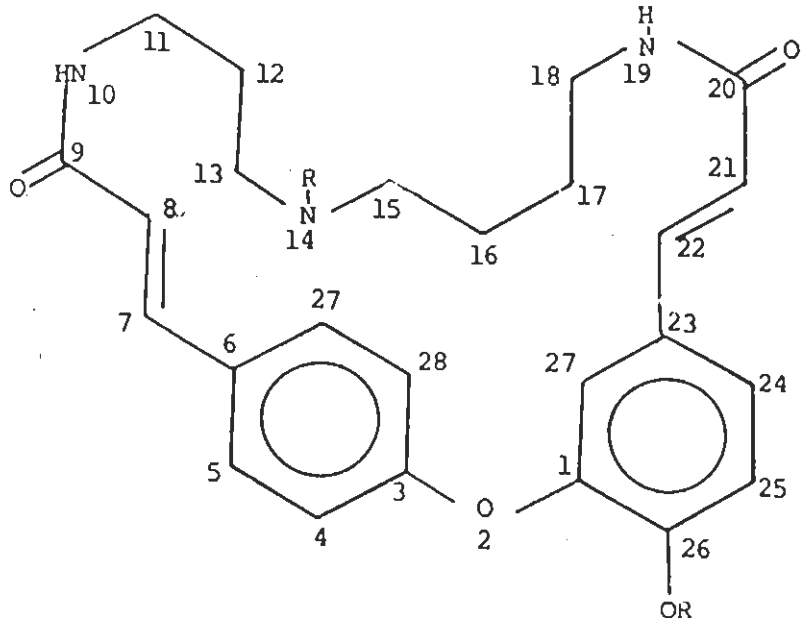
3 PRESENT WORK (Results and Discussion)

3.1 Cadabicine (1)

The stem bark of *Cadaba farinosa* was chopped into small pieces and extracted exhaustively with ethanol. The residue obtained on evaporation of the alcoholic extraction was partitioned between ethyl acetate and water. The aqueous layer was then basified with ammonia to pH10 and extracted with chloroform. The alkaloid-containing chloroform layers were combined, washed, dried and concentrated. This solution on storing at 0° deposited crystals of cadabicine (1). After recrystallization from methanol pure cadabicine was obtained.

Cadabicine (1), M.P. 270° (decomp.), gave a positive test for phenols with ferric chloride solution. High resolution mass spectrometry gave an $[M]^+$ at m/z 435.2145 corresponding to the molecular formula $C_{25}H_{29}N_3O_4$ (calcd. 435.2157). The ultra-violet spectrum displayed maxima at 217 ($\log \varepsilon = 3.55$), 284 ($\log \varepsilon = 3.76$) and a shoulder at 310 nm. These values are very close to those of codoncarpine [41]. The IR spectrum had bands at 3400-3240 (br. OH and NH), 1655 (α,β-unsaturated amide) and 1600 cm^{-1} (aromatic ring).

The ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra of cadabicine in DMSO-d_6 showed doubling of several signals which may be due to slowly interconverting Z and E conformers with respect to amide bond, and also due to the flexability of macromolecule in solution. A similar phenomenon has been reported by other authors [42-43] in amide.


11
12
HN
10
9
O
13
R
N
14
15
16
17
18
H
N
19
20
O
21
22
8
7
27
6
28
5
3
4
O
2
27
23
24
1
25
26
OR

1) R-H

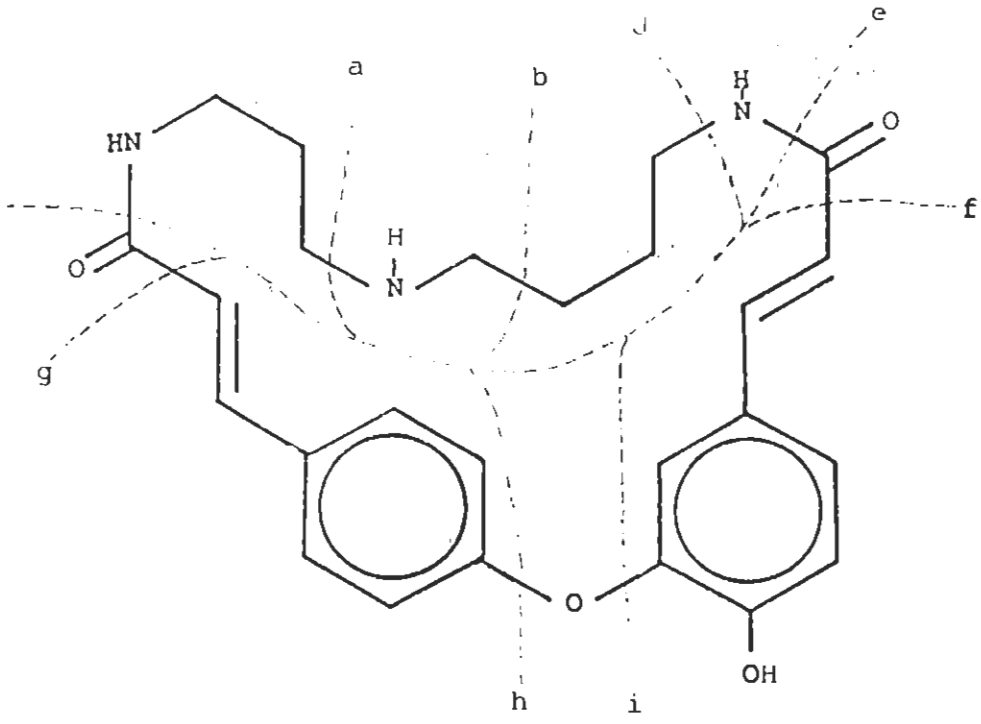
2) R-Ac

In the ^{1}H-NMR spectrum a multiplet between δ 1.22 and 1.84 (6H) is assigned to three methylene groups (2xH-12, 2xH-16 and 2xH-17), another multiplet between 2.70 and 3.12 (8H) is due to four methylene groups adjacent to nitrogens (2xH-11, 2xH-13, 2xH-15 and 2xH-18). There were four doublets (J=15.5 Hz, each 1H) at δ 5.84, 6.63, 7.12 and 7.44 from the olefinic protons of the trans, cinnamic acid residues. A doublet at δ 6.36 (J=1.8 Hz) is assigned to H-27 with meta coupling only. There are two doublets at δ 7.18 (2H) and 7.64 (2H) with ortho coupling (J=7.8 Hz) typical of a para disubstituted benzene ring. Another doublet is present at δ 7.21 (J=8.0 Hz), showing only ortho but no meta coupling, is attributed to H-25. A double doublet at δ 6.95 (J=8.0 Hz, 1.8 Hz), showing both ortho and meta coupling is due to H-24.

These assignments were confirmed by 2D correlation of proton shifts through a COSY-45 and 2D, J-resolved experiments.

The coupling interactions were established by a COSY-45 experiment.

Two doublets of H-7 at δ 7.44 and H-8 at 6.63 are coupled with each other. Similarly two doublets of H-21 at δ 5.84 and H-22 at 7.12 are coupled with each other. A doublet at δ 7.18 (for H-4 and H-28) has cross peak with a doublet at δ 7.64 (for H-5 and H-29). A double doublet of H-24 at δ 6.95 has cross peaks with H-25 at δ 7.21 and with H-27 at δ 6.36. A multiplet between δ 1.22 and 1.84 (6H) for 2xH-12, 2xH-16 and 2xH-17 is coupled with another multiplet between δ 2.70 and 3.12 (8H) for 2xH-11, 2xH-13, 2xH-15 and 2xH-18.

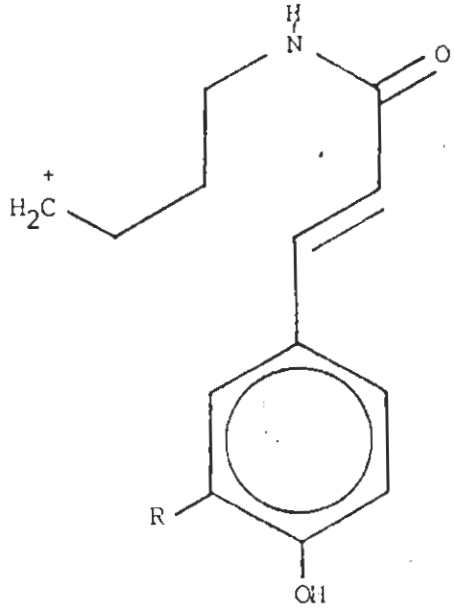


		m/z	Formula for ion
M^+	=	435	$(C_{25}H_{29}N_3O_4)$
cleavage			
(a-b)	=	407	$(C_{24}H_{27}N_2O_4)$
(b-c)	=	349	$(C_{21}H_{19}NO_4)$
(c-d)	=	307	$(C_{18}H_{13}NO_4)$
(c-e)	=	291	$(C_{18}H_{11}O_4)$
(c-f)	=	264	$(C_{17}H_{12}O_3)$
(a-h)	=	250	$(C_{13}H_{18}N_2O_3)$
(g-f)	=	235	$(C_{16}H_{11}O_2)$
(a-i)	=	234	$(C_{13}H_{18}N_2O_2)$
(h-j)	=	234	$(C_{13}H_{16}NO_3)$
(i-j)	=	218	$(C_{13}H_{16}NO_2)$
(a-i)	=	205	$(C_{12}H_{15}NO_2)$
(a-h)	=	189	$(C_{12}H_{15}NO)$
(c-i)	=	146	$(C_9H_6O_2)$
(c-h)	=	131	(C_9H_7O)

Fig.-1

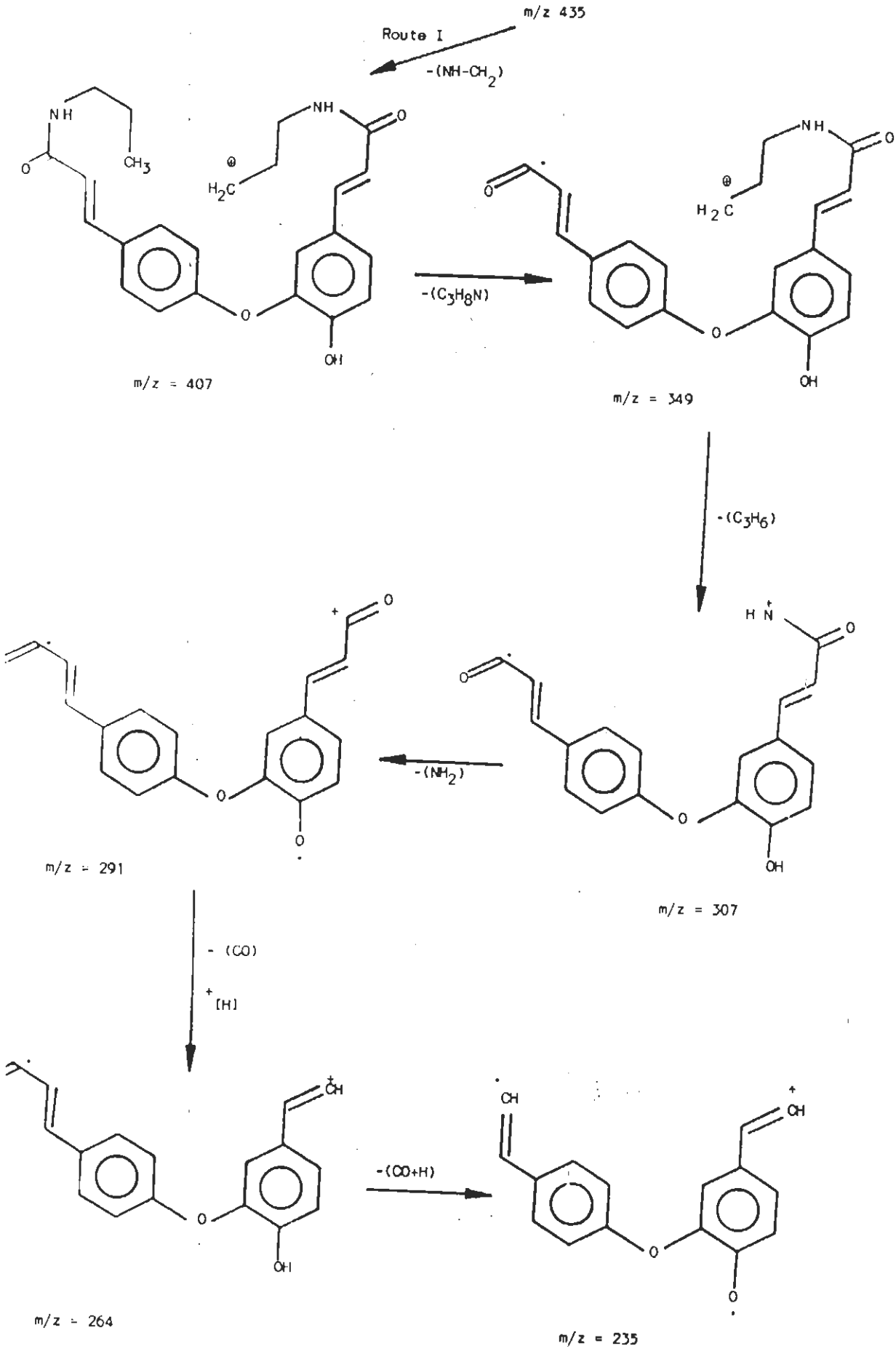
All these assignments show that the hydroxy group in this skeleton is ttached to meta disubstituted benzene ring at position 26. The exact ttachment of spermidine moiety with the rest of the molecule may be proved ith the help of mass fragmentation of compound 1 (Fig.-1, Scheme-1, Route-II nd III).

The high resolution mass spectrum of compound 1, shows several peaks that llowed the assignments of the $(CH_2)_3$ unit of spermidine to the para isubstituted benzene ring and the $(CH_2)_4$ unit of spermidine to the other ring earing hydroxy group at C-26. The peaks at m/z 218 and 234 correspond to ions and 4 and show that the $(CH_2)_4$ unit of spermidine moiety is attached with henyl ring bearing hydroxy group, otherwise there should be two peaks at /z 204 (for $C_{12}H_{14}NO_2$) and m/z 220 (for $C_{12}H_{14}NO_3$) but there were not bserved. Similarly ion 5 (m/z = 234) and ion 6 (m/z = 250) prove the same tuation.

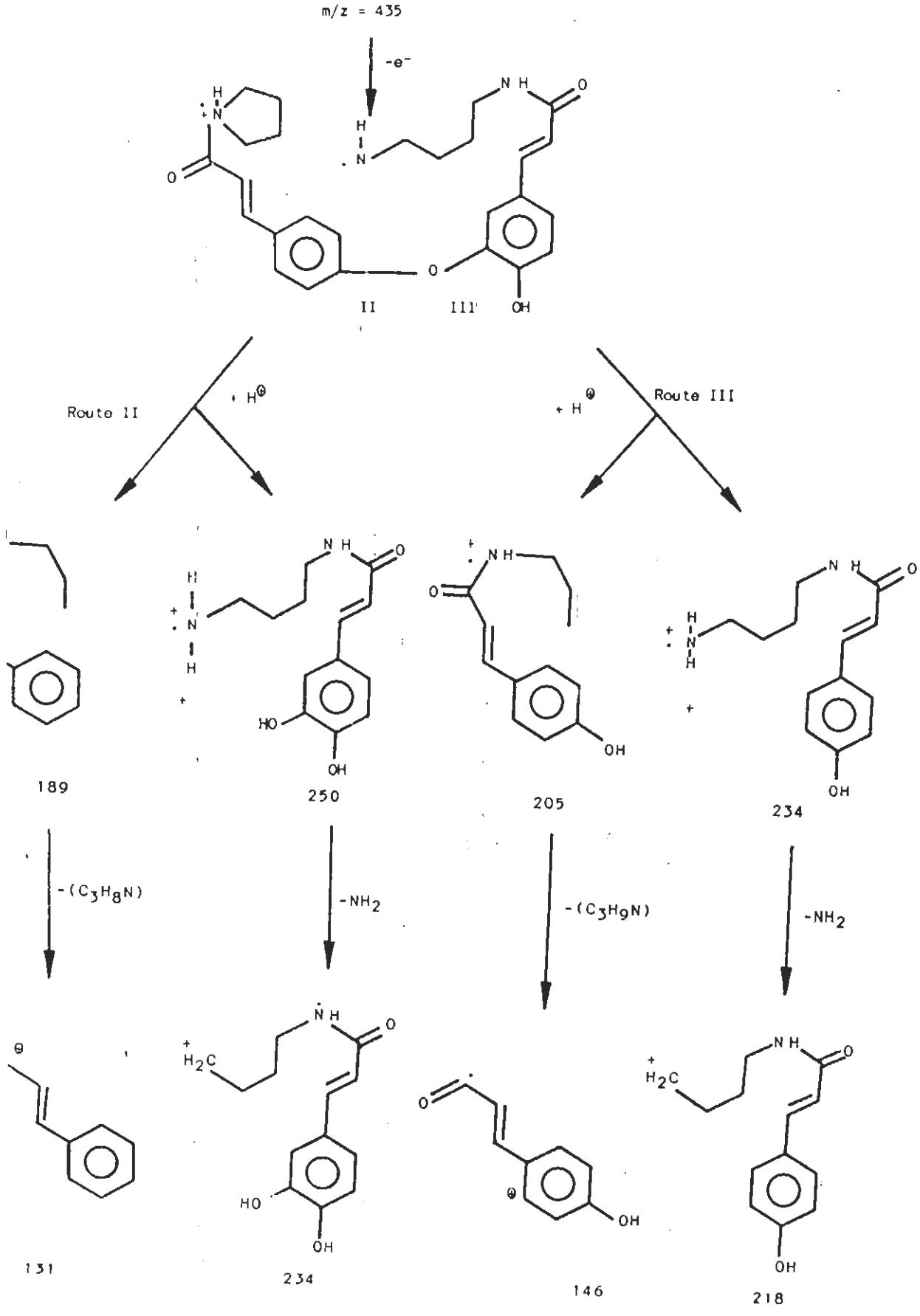


3 R-H, m/z 218.1184
(calcd. for $C_{13}H_{16}NO_2$, 218.1181)

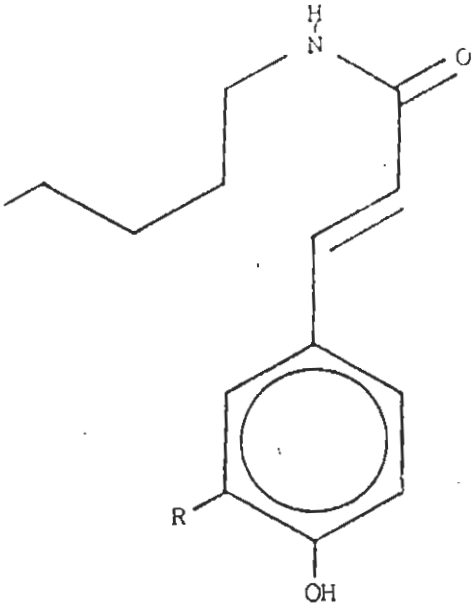
4 R-OH, m/z 234.1138
(calcd. for $C_{13}H_{16}NO_3$, 234.1130)



(Schema 11 (Route I))



(Scheme-1) (Routes II and III)



5 R=H, m/z 234.1371
(calcd. for $C_{13}H_{18}N_2O_2$, 234.1368)

6 R=OH, m/z 250.1317
(calcd. for $C_{13}H_{18}N_2O_3$, 250.1317)

ongwith mass spectral fragments, the ^{13}C-NMR spectrum (Table-1) and the DEPT periment also supported structure 1 for cadabicine.

For the confirmation of structure 1 for cadabicine a single crystal X-ray ffraction experiment was undertaken. Preliminary X-ray diffraction otographs showed monoclinic symmetry. Accurate lattice parameters of a=13.216 , b=9.495 (1), c=21.361 (2)Å and β=75.15 (1)° were calculated from a st-squares fit of fifteen diffractometer measure 2θ-values. Systematic inctions and the crystal density were uniquely accommodated by the achiral ce group $P2_1/C$ with one molecule of composition $C_{25}H_{29}N_3O_4$ forming the mmetric unit. All unique diffraction maxima with 2θ<114° were collected a computer controlled four-circle diffractometer using variable speed -scans and graphite monochromated CuKα radiation (1.54178 Å). Of the 2747

TABLE - 1

^{13}C-NMR chemical shifts of cadabicine (1)
(DMSO-d_6, 100 MHz)

Carbon	ppm	Carbon	ppm
	151.80	17	25.89
	155.15	18	42.43/42.72*
	122.33[a]	20	164.78/164.88*
	129.49	21	124.66/124.71*[d]
	133.51[b]	22	136.90/137.05*[c]
	137.62/137.70*[c]	23	133.50[b]
	124.90/124.44*[d]	24	123.89
	164.00	25	110.70
1	45.09/46.21*	26	141.10
2	27.01/27.70*	27	122.21[a]
3	38.21/38.40*	28	122.30[a]
5	35.31/35.91*	29	129.51
6	26.40/26.51*		

,b,c,d = Assignments may be interchanged.

= Observed doubling of signals due to conformers with regard to amide bond.

reflections measure in this way, 1472 (54%) were judged observed ($F_o \geq 3\sigma F_o$) after correction for Lorentz, polarization and background effects. A multi-solution sign determining procedure gave a good phasing model and 30 of the 32 nonhydrogen atoms were located in the initial E-synthesis. The structure was extended by weighted Fourier techniques to give all of the non-hydrogen atoms. The hydrogens were located in ΔF-synthesis after partial refinement of the nonhydrogen atoms. Block diagonal least-square refinements with anisotropic nonhydrogen atoms and isotropic hydrogens have converged to a conventional crystallographic residual of 0.081 for the observed reflections. A computer generated perspective drawing of the final X-ray model is presented in Fig.-2. Hydrogens are omitted for clarity. The aromatic rings are roughly orthogonal with torsional angles of 21° about the C-1-O-2 bond, and 79° about the O-2-C-3 bond. In general the molecular parameters agree well with accepted values. The fractional coordinates and thermal parameters are given in Table-2.

Acetylation of Cadabicine:

Cadabicine (20 mg) was treated with acetic anhydride (2 ml) and pyridine 0.5 ml), warmed slightly and kept overnight. Ice was added to the reaction mixture, the colourless precipitate filtered, dried and crystallized from MeOH to yield shining rods of cadabicine diacetate (M.P. 265-268°). It gave negative test for phenol with neutral ferric chloride solution.

The UV spectrum displayed maxima at 210 ($\log \varepsilon = 4.12$), 273 ($\log \varepsilon = 4.02$) and a shoulder at 305 nm.

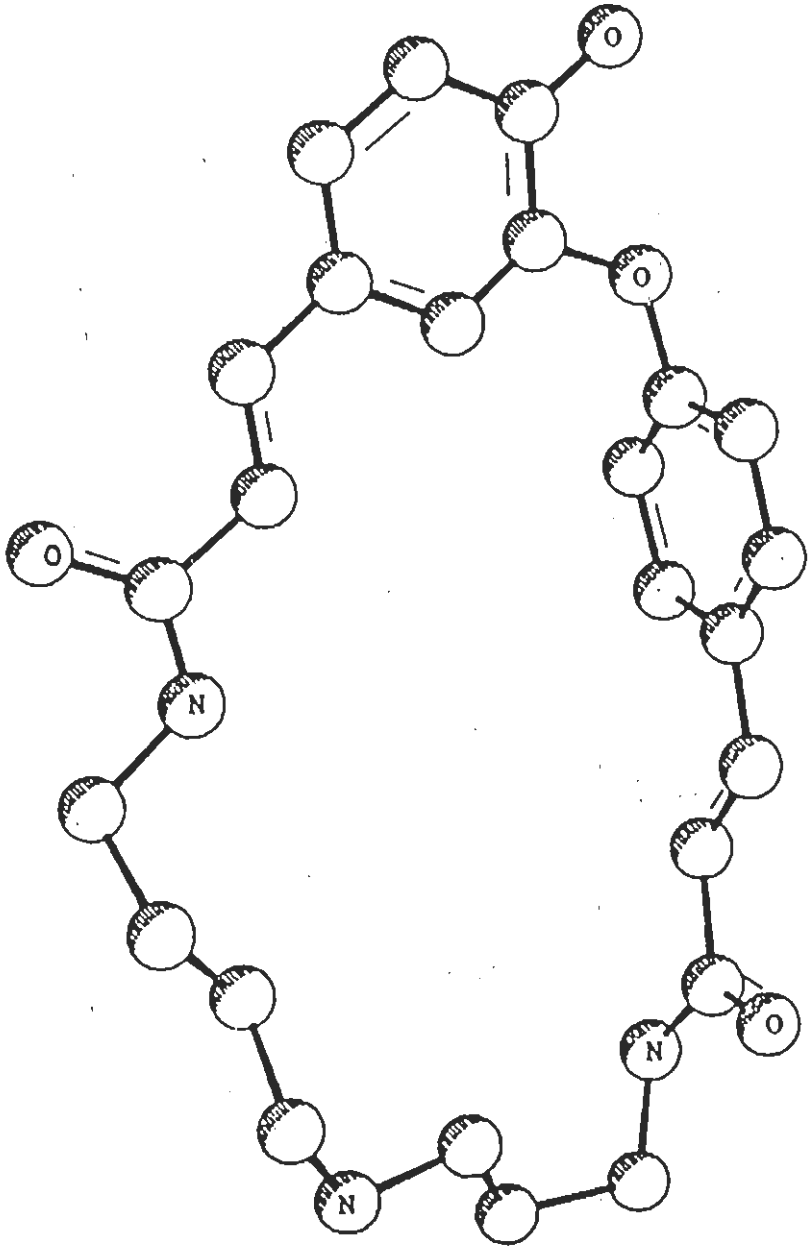


Fig.-2

Table-2

Fractional coordinates and thermal parameters
for CABABICINE

Standard deviations of the least significant figures are given in parentheses. The isotropic equivalent thermal parameter is given for anisotropic atoms (denoted by an asterisk).

Atom	x	y	z	B
C1	0.7540(10)	0.1674(15)	0.4227(6)	4.5(7)*
O2	0.6964(7)	0.1775(11)	0.3756(4)	5.3(5)*
C3	0.5871(10)	0.1936(16)	0.3994(6)	4.5(8)*
C4	0.5252(10)	0.0824(16)	0.4222(6)	5.0(8)*
C5	0.4137(10)	0.1060(15)	0.4495(6)	4.5(8)*
C6	0.3742(10)	0.2346(17)	0.4521(7)	5.4(8)*
C7	0.2657(10)	0.2589(16)	0.4831(6)	4.7(8)*
C8	0.2169(10)	0.3795(17)	0.5021(6)	4.9(8)*
C9	0.1101(11)	0.3835(16)	0.5497(7)	5.5(8)*
N10	0.0732(9)	0.5152(13)	0.5658(5)	5.4(7)*
C11	-0.0234(10)	0.5358(16)	0.6175(7)	5.1(8)*
C12	0.0008(10)	0.5875(15)	0.6813(6)	4.7(8)*
C13	0.0751(10)	0.4874(16)	0.7034(6)	4.6(7)*
N14	0.0785(8)	0.5263(12)	0.7715(5)	4.5(6)*
C15	0.1560(11)	0.4404(16)	0.7958(7)	5.4(8)*
C16	0.2693(10)	0.4773(15)	0.7616(6)	4.7(8)*
C17	0.3401(11)	0.4028(21)	0.7973(7)	6.0(9)*
C18	0.4567(11)	0.4494(20)	0.7712(7)	6.2(9)*
N19	0.5012(8)	0.3833(13)	0.7068(5)	4.8(6)*
C20	0.6038(11)	0.3570(16)	0.6883(7)	5.6(8)*
C21	0.6382(10)	0.2968(15)	0.6236(6)	4.7(8)*
C22	0.7373(10)	0.2664(14)	0.5957(6)	4.5(7)*
C23	0.7770(9)	0.2104(14)	0.5291(6)	4.0(7)*
C24	0.8763(10)	0.1524(16)	0.5078(7)	5.0(8)*
C25	0.9121(10)	0.0989(17)	0.4456(7)	5.0(8)*
C26	0.8526(10)	0.1031(15)	0.3989(7)	4.5(8)*
C27	0.7149(9)	0.2191(14)	0.4840(6)	4.1(7)*
C28	0.5488(11)	0.3312(17)	0.3977(7)	5.9(9)*
C29	0.4418(11)	0.3586(17)	0.4231(7)	5.6(9)*
O30	0.0647(8)	0.2760(11)	0.5723(5)	7.0(7)*
O31	0.6662(7)	0.3913(13)	0.7206(4)	6.3(6)*
O32	0.3867(6)	0.0551(10)	0.3403(4)	4.8(5)*
SOL1	0.8528(7)	0.3019(9)	0.7374(4)	5.0(5)*
SOL2	0.3790(8)	0.2005(12)	0.1148(5)	7.6(7)*
SOL3	0.2617(10)	0.3835(14)	0.2013(6)	4.3(8)*
H4	0.570(10)	-0.028(15)	0.426(6)	9.9(41)
H5	0.354(9)	-0.001(13)	0.473(5)	7.1(33)
H7	0.231(8)	0.156(11)	0.517(5)	5.7(30)
H8	0.267(9)	0.471(12)	0.486(5)	6.9(32)
H10	0.121(12)	0.602(18)	0.549(8)	13.3(55)
H11A	-0.075(9)	0.605(13)	0.608(6)	8.0(36)

Table-2, continued.

Atom	x	y	z	B
H11B	-0.079(10)	0.433(14)	0.640(6)	8.9(38)
H12A	0.035(7)	0.680(10)	0.675(4)	3.5(24)
H12B	-0.083(8)	0.580(12)	0.720(5)	6.2(30)
H13A	0.051(10)	0.370(14)	0.707(6)	9.3(39)
H13B	0.164(8)	0.500(12)	0.671(5)	6.0(29)
H14A	0.108(9)	0.623(12)	0.765(5)	6.9(33)
H14B	0.006(10)	0.517(14)	0.797(6)	8.3(36)
H15A	0.143(9)	0.460(13)	0.836(6)	8.2(36)
H15B	0.147(12)	0.306(17)	0.791(7)	12.6(51)
H16A	0.235(9)	0.582(13)	0.760(6)	7.8(35)
H16B	0.278(7)	0.439(10)	0.712(5)	4.3(25)
H17A	0.313(8)	0.423(12)	0.845(5)	6.9(33)
H17B	0.320(10)	0.278(16)	0.803(7)	10.4(44)
H18	0.453(12)	0.563(18)	0.765(8)	13.6(55)
H18B	0.502(9)	0.416(12)	0.802(5)	6.9(32)
H19	0.453(10)	0.380(14)	0.678(6)	9.6(41)
H21	0.588(11)	0.236(15)	0.594(7)	10.7(44)
H22	0.780(15)	0.248(21)	0.579(10)	18.7(73)
H24	0.917(9)	0.132(14)	0.540(6)	8.6(37)
H25	0.979(10)	0.069(14)	0.435(6)	8.7(38)
H27	0.645(9)	0.271(13)	0.509(6)	7.5(34)
H28	0.607(9)	0.427(13)	0.373(6)	7.5(34)
H29	0.404(8)	0.286(12)	0.446(5)	5.8(29)
OH32	0.863(13)	0.157(18)	0.298(8)	13.9(55)

The IR spectrum showed bands at 3400 (br. NH), 1760 (phenolic acetate), 1745 (N-acetate), 1655 (α,β-unsaturated amide) and 1590 cm^{-1} (aromatic ring).

Mass spectrometry gave an $[M]^+$ at 519 corresponding to the molecular formual $C_{29}H_{33}N_3O_6$. Two peaks at 477 ($M-CH_2CO$) and 434 (477-Ac) are also present. All other peaks are similar to compound 1.

The ^1H-NMR of diacetate in DMSO-d_6 (400 MHz) and ^{13}C-NMR (100 MHz) showed the doubling of several signals as observed in cadabicine. The singlets at δ 1.95 and 1.97 which togather integrated to 3H were attributed to the N-acetate. Another singlet at δ 2.34 (3H) is due to the phenolic acetate, other NMR signals were almost similar to the compound 1.

The formation of a diacetyl derivative was further confirmed by ^{13}C-NMR (Table-3). As compared to cadabicine there were four extra peaks. Two peaks at δ 168.54 and 20.41 indicated a phenolic acetate and two other peaks at δ 169.01/169.04 and 21.08/21.20 indicated the presence of N-acetate in compound.

TABLE - 3

^{13}C-NMR chemical shifts for diacetyl cadabicine (2)
(DMSO-d_6, 100 MHz)

Carbon	ppm	Carbon	ppm
	151.81	20	164.78/164.88*[e]
	155.16	21	124.66/124.70*[d]
	122.31[a]	22	136.98/137.06*[c]
	129.54	23	133.52[b]
	133.54[b]	24	124.01
	137.62/137.72*[c]	25	110.78/110.81*
	124.44/124.54*[d]	26	140.33
	164.09[e]	27	122.22[a]
1	45.00/47.42*	28	122.31[a]
2	27.04/27.73*	29	129.54
3	38.26/38.44*	$O\underline{C}OCH_3$	168.54
5	35.36/35.94*	$OCO\underline{C}H_3$	20.41
6	26.48/26.58*	$N\underline{C}OCH_3$	169.01/169.04*
7	25.89	$NCO\underline{C}H_3$	21.08/21.20*
8	42.43/43.72*		

,b,c,d,e = Assignments may be interchanged.

= Doubling of peaks due to conformers.

ne status of each carbon confirmed through DEPT experiment.

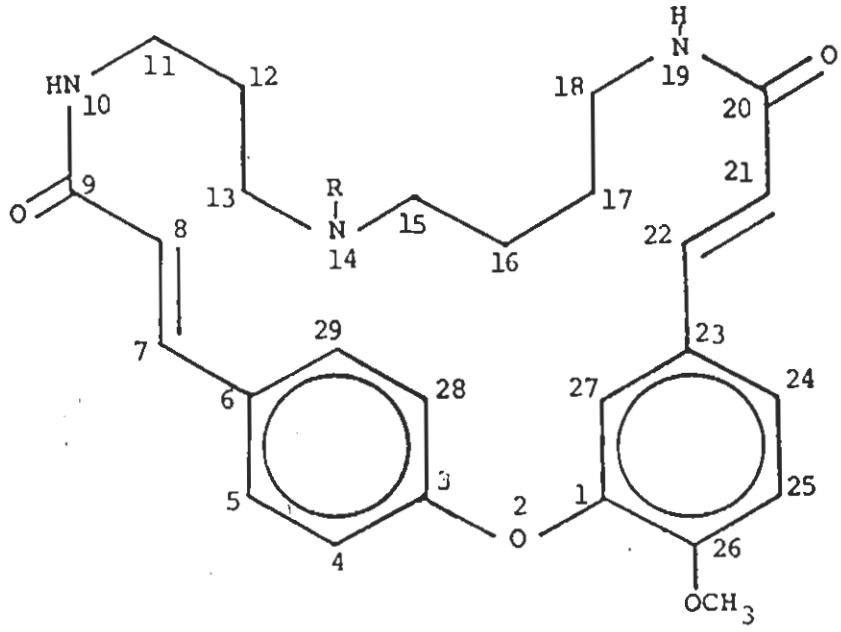
3.2 Cadabicine methyl ether (7)

Cadabicine methyl ether was isolated from *Cadaba farinosa* and its structure was determined by spectroscopic studies and confirmed by its total synthesis.

The crude alkaloidal material from the stem bark was partitioned between $_2O$ and CH_2Cl_2 at pH-11. The residue from CH_2Cl_2 layer was separated by silica el column chromatography with CH_2Cl_2, CH_3OH and NH_3 (90:9:1), which led to he isolation of yellowish brown crystals of cadabicine methyl ether (7). ecrystallization from methanol afforded pale yellow crystals of pure adabicine methyl ether, M.P. 190-192°C.

The high resolution mass spectrum, showed a molecular ion peak at m/z 19.2282 corresponding to molecular formula $C_{26}H_{31}N_3O_4$ (calcd. for 449.2314). he UV spectrum showed maxima at 220 ($\log \varepsilon$=2.84), 282 ($\log \varepsilon$=2.78) and a houlder at 310 nm. These values are very close to those of cadabicine (1) nd codonocarpine [41]. The IR spectrum has bands at 3400-3200 (br, NH), 1660 r,β-unsat. amide) and 1600 cm^{-1} (aromatic ring).

The molecular formula shows that the compound (7) has one "CH_2" unit gher than that of cadabicine ($C_{25}H_{29}N_3O_4$). The peak at δ 3.56 in ^{1}H-NMR ectrum and at δ 56.22 in ^{13}C-NMR, show the presence of a methoxy group, and gative test for phenol with $FeCl_3$ reagent indicates that the new isolated mpound may be a methyl ether of cadabicine (1).


HN
10
11
12
13
R
N
14
15
16
17
18
H
N
19
20
O
21
22
23
24
25
26
OCH3
27
28
29
O
1
2
3
4
5
6
7
8
9
O

7) R = H,

12) R = Ac,

The ^{1}H-NMR and ^{13}C-NMR (Table-4) spectra of cadabicine methyl ether showed doubling of several signals, which is due to the slowly interconverting E and Z confermers with regard to amide bonds, in solution. The same phenomenon has been observed in cadabicine.

The ^{1}H-NMR spectrum of 7 showed two multiplets appearing between δ 1.22-1.71 (6H) and δ 3.15-3.39 (8H) which are assigned to three methylene groups (2xH-12, 2xH-16 and 2xH-17) adjacent to other methylenes and four methylene groups (2xH-11, 2xH-13, 2xH-15 and 2xH-18) adjacent to nitrogen atoms respectively. A sharp singlet at δ 3.56 (3H) proved the presence of a methoxy group. The four doublets at δ 5.83, 6.59, 7.14 and 7.48 (each 1H, J=15.5 Hz), indicate the presence of four olefinic protons of the two trans-cinnamic acid moieties (H-21, H-8, H-22 and H-7 respectively). A doublet at δ 6.42 (J=2.2 Hz) with meta coupling only is assigned to H-27. The two doublets at δ 7.19 and 7.84 (each 2H, J=7.6 Hz), with ortho coupling only, are typical for a para disubstituted benzene ring. A doublet at δ 6.97 (1H, J=7.7 Hz) with ortho coupling only is attributed to H-25. The H-24 gives rise a double doublet at δ 7.02 (1H, J=7.8 and 2.0 Hz) with ortho and meta coupling.

These assignments have been confirmed through a COSY-45 and 2D, J-resolved experiments. The coupling interactions were established by a COSY-45 experiment.

Two doublets of H-7 at δ 7.48 and H-8 at 6.59 are coupled with each other.

TABLE - 4

^{13}C-NMR chemical shifts of cadabicine methyl ether (7)
(DMSO-d_6, 100 MHz)

arbon	ppm	Carbon	ppm
	148.71	17	25.63
	155.41	18	43.93/44.24*d
	123.34	20	164.94/165.08*[c]
	129.26	21	125.43/125.77*[b]
	133.72	22	137.79/138.14*[a]
	137.54/137.62*[a]	23	128.88
	125.82/125.97*[b]	24	121.76
	164.61[c]	25	112.08
1	45.05/46.16*[d]	26	140.12
2	25.96/26.13*[e]	27	118.94
3	38.63/39.40*	28	120.26
5	35.28/35.95	29	129.33/129.45*
6	22.29/22.66*	$\underline{O}CH_3$	56.22

,b,c,d,e = Assignments may be interchanged.

= Observed doubling of signals due to conformers with regards to amide bond.

ne status of each carbon confirmed through DEPT experiment.

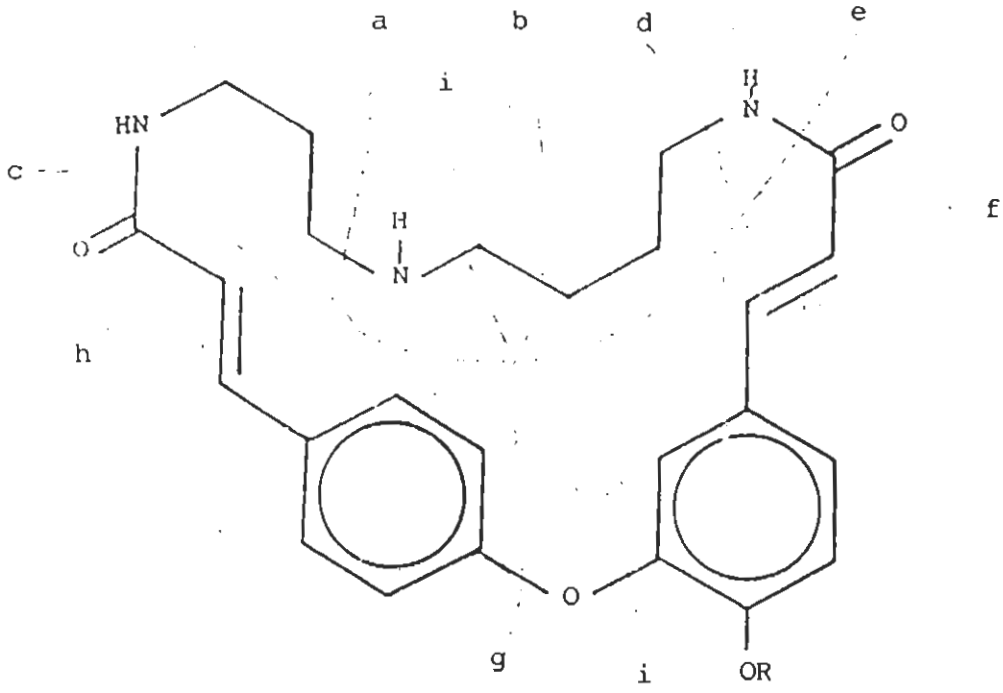
Similarly two doublets of H-21 at δ 5.83 and H-22 at δ 7.14 are coupled with each other. A doublet at δ 7.19 (for H-4 and H-28) has cross peak with a doublet at δ 7.84 (for H-5 and H-29). A double doublet of H-24 at δ 7.02 has cross peaks with H-25 at δ 6.97 and with H-27 at δ 6.42. A multiplet between δ 1.22 and 1.71 (6H) for 2xH-12, 2xH-16 and 2xH-17 is coupled with another multiplet between δ 3.05 and 3.29 (8H) for 2xH-11, 2xH-13, 2xH-15 and 2xH-18.

All the above assignments show that the methoxy group in this skeleton is attached to meta disubstituted benzene ring at C-26, just as hydroxy group in basic skeleton of cadabicine (1).

The spermidine moiety in 7 may be joined with the rest of the molecule in two different manners, but the mass fragmentation (Fig.-3 and Scheme-2) and comparison with cadabicine (1) proves that the spermidine joined in the manner as shown in structure (7).

The high resolution mass spectrum of compound (7), shows some specific peaks that allowed the assignment of secondary amino nitrogen at position 14 as in cadabicine (1).

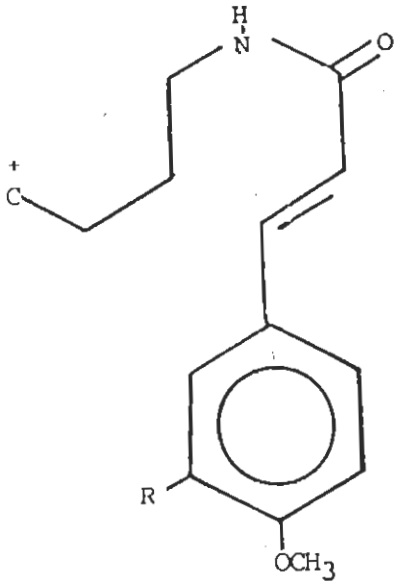
The peaks at m/z 232 and 248 correspond to ions 8 and 9 and show that the $(CH_2)_4$ unit of spermidine moiety is attached with aromatic ring bearing methoxy group, otherwise there should be two peaks at m/z 218 (for $C_{13}H_{16}NO_2$) and 234 (for $C_{13}H_{16}NO_3$) but they were not observed. Similarly two other peaks at m/z 178 (ion 10) and 264 (ion 11) also prove that, the secondary amino nitrogen



M^+	-	449	$C_{26}H_{31}N_3O_4$
M^+-CH_2O	-	419	$C_{25}H_{29}N_3O_3$

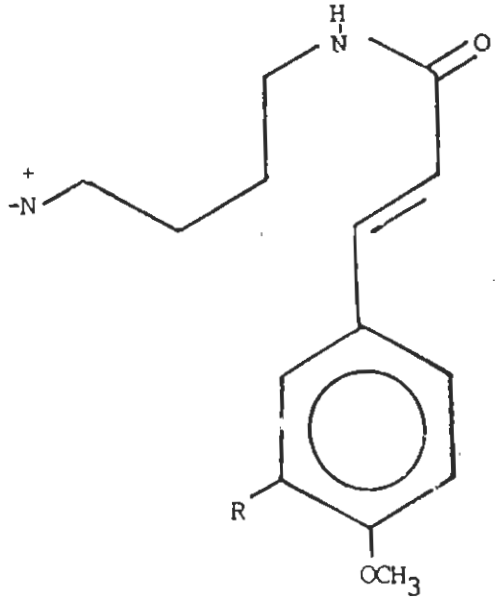
cleavage	R	m/z	Formula for fragment
(a-b)	CH_3	421	$C_{25}H_{29}N_2O_4$
	H	407	$C_{24}H_{27}N_2O_4$
(b-c)	CH_3	363	$C_{22}H_{21}NO_4$
	H	349	$C_{21}H_{19}NO_4$
(c-d)	CH_3	321	$C_{19}H_{15}NO_4$
	H	307	$C_{18}H_{13}NO_4$
(c-e)	CH_3	306	$C_{19}H_{14}O_4$
	H	292	$C_{18}H_{12}O_4$
(c-f)	CH_3	278	$C_{18}H_{14}O_3$
	H	264	$C_{17}H_{12}O_3$
(a-g)	CH_3	264	$C_{14}H_{20}N_2O_3$
(f-h)	CH_3	250	$C_{17}H_{14}O_2$
(g-i)	CH_3	248	$C_{14}H_{18}NO_3$
(a-j)	CH_3	248	$C_{14}H_{20}N_2O_2$
(f-h)	H	236	$C_{16}H_{12}O_2$
(i-j)	CH_3	232	$C_{14}H_{18}NO_2$
(a-j)	-	204	$C_{12}H_{14}NO_2$
(a-g)	-	189	$C_{12}H_{15}NO$
(c-j)	-	146	$C_9H_6O_2$
(c-g)	-	131	C_9H_7O

in present at position 14, in basic skeleton.



8 R=H, m/z 232.1341
(calcd. for $C_{14}H_{18}NO_2$, 232.1337)

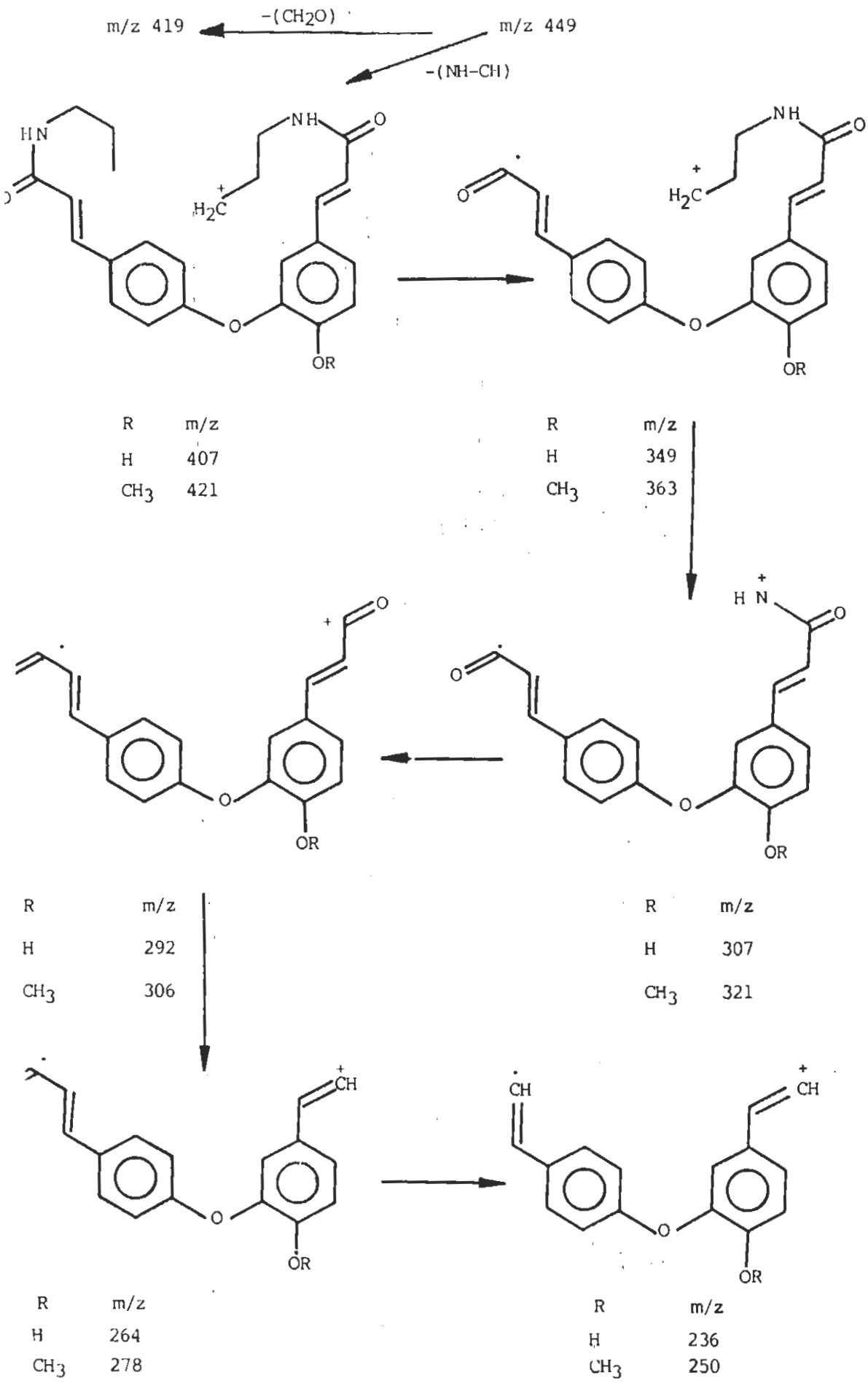
9 R=OH, m/z 248.1291
(calcd. for $C_{14}H_{18}NO_3$, 248.1286)



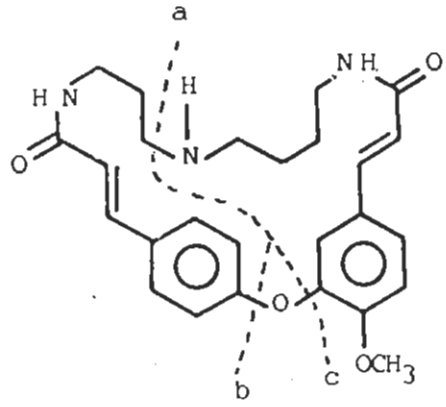
10 R=H, m/z 248.1552
(calcd. for $C_{14}H_{20}N_2O_2$, 248.1524)

11 R=OH, m/z 264.1502
(calcd. for $C_{14}H_{20}N_2O_3$, 264.1473)

ongwith mass spectral fragments, the ^{13}C-NMR spectrum (Table-4) and DEPT periment also supported structure 7 for cadabicine methyl ether.



(Scheme-2) (Route-I)



Route II
cleavage (a-b)

Route III
cleavage (a-c)

189

264

204

248

$-(C_3H_8N)$

$-NH_2$

$-(C_3H_8N)$

$-NH_2$

131

248

146

232

(Scheme-2) (Routes II and III)

Formation of N-acetyl cadabicine methyl ether (12);

Cadabicine methyl ether was dissolved in minimum amount of pydridine and allowed to react with acetic anhydride at room temperature and kept overnight. On addition of ice to the mixture a mono-acetyl derivative (12) was obtained as an amorphous solid. It was recrystallized from hot methanol. The colourless crystals melted at 180-82°C.

The electron impact and field desorption mass spectrum showed $[M]^+$ at m/z 491. The mono-acetyl derivative showed the same UV pattern as for cadabicine (1) and cadabicine methyl ether (7). The IR spectrum showed the band at 3400 (NH), 1660 (α,β-unsaturated amide), 1590 (aromatic ring). In addition to these bands there was a band at 1740 cm^{-1}, indicating the presence of N-acetate and not an O-acetate, otherwise this band should be present between 1755 to 1765 cm^{-1}. This fact alongwith the formation of a mono-acetate rather than a di-acetate proved that there is no free "OH" group in the molecule. In ^{1}H-NMR two singlets at δ 1.93 and 1.95, totally integrating for 3H (due to Z and E conformers), in ^{13}C-NMR (Table-5) two peaks at δ 170.84 (N-$\underline{C}OCH_3$) and at δ 21.82 (N-CO$\underline{C}H_3$) also proved the formation of N-acetyl derivative.

The absence of a free "OH" group, a peak at δ 3.57 in ^{1}H-NMR and a peak at δ 52.82 in ^{13}C-NMR further confirmed the presence of a methoxy group in compound 7 instead of Hydroxy group.

The high resolution mass spectrum of N-acetyl derivative, measured the

TABLE - 5

^{13}C-NMR chemical shifts for N-acetyl cadabicine methyl ether (12) (DMSO-d_6, 100 MHz)

Carbon	ppm	Carbon	ppm
	149.16	18	45.07/45.62*[d]
	153.28	20	168.24/169.13*[c]
	122.44	21	126.24/126.44*[b]
	122.23	22	140.13/140.32*[a]
	130.14	23	128.34
	140.38/140.49*[a]	24	122.84
	125.76/125.89*[b]	25	114.08
	166.62[c]	26	147.34
1	46.08/46.22*[d]	27	118.70
2	27.74/27.88*[e]	28	125.85
3	37.18/38.16*	29	129.28/129.46*
5	35.62/35.74*	$O\underline{C}H_3$	52.82
9	23.45/23.50*	$NO\underline{C}CH_3$	170.84
7	26.85[e]	$NCO\underline{C}H_3$	21.82

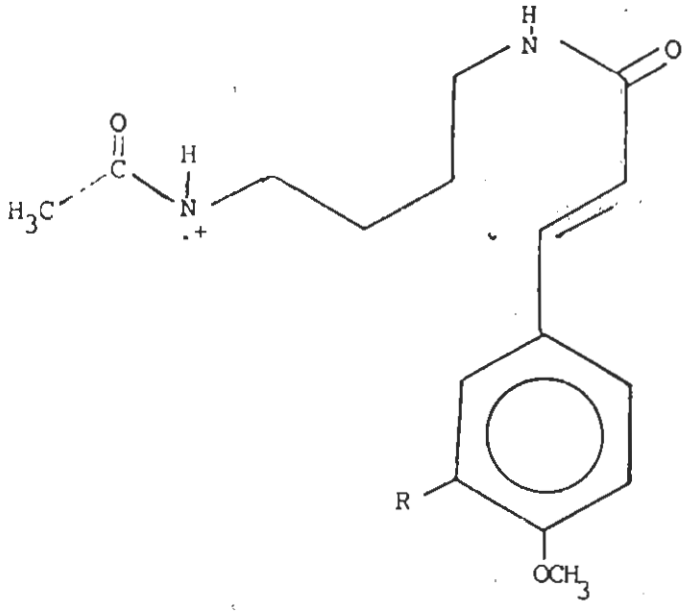
b,c,d,e = Assignments may be interchanged.

= Observed doubling of signals due to conformers with regards to amide bond.

e status of each carbon confirmed through DEPT experiment.

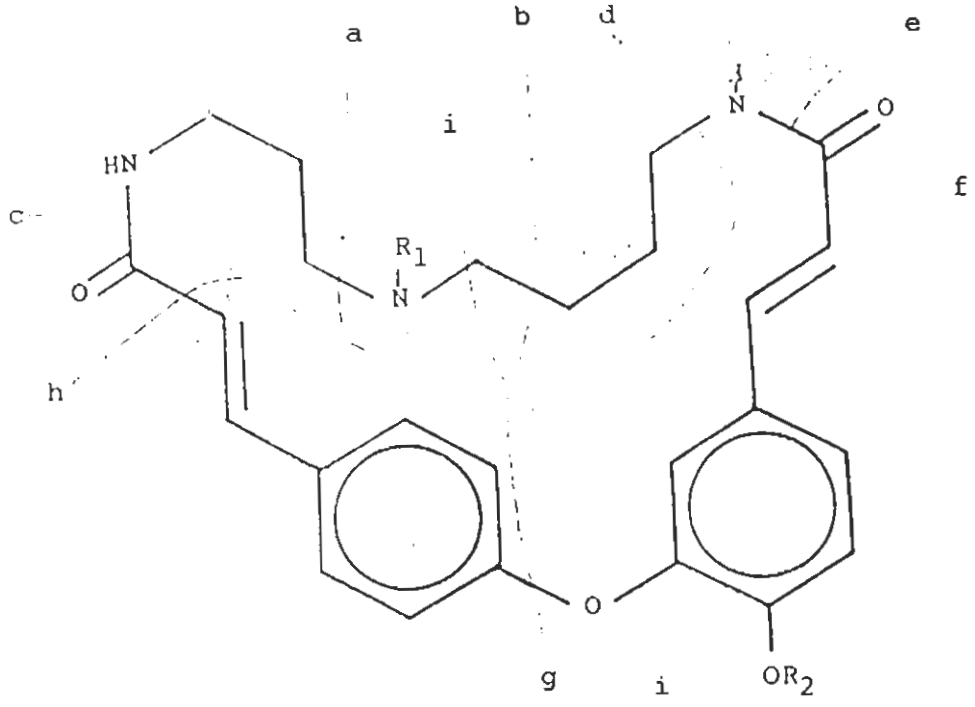
xact mass at m/z 491.2418 attributed to molecular formula $C_{28}H_{33}N_3O_5$ (calcd. 91.2420). An important peak at m/z 461 indicated the loss of "OCH_2" unit. The ass fragmentation pattern (Fig.-4) of N-acetate also confirmed the exact ttachment of spermidine moiety with the rest of molecule as represented in tructures 7 and 12.

Two peaks at m/z 290 and 306 correspond to ions 13 and 14 and show that $CH_2)_4$ unit of spermidine is attached with aromatic ring bearing methoxy group, therwise there should be two peaks at m/z 276 (for $C_{15}H_{20}N_2O_3$) and m/z 292 for $C_{15}H_{20}N_2O_4$) but they were not observed.



3 R=H, m/z 290.1626 (calcd. for $C_{16}H_{22}N_2O_3$, 290.1630)

4 R=OH, m/z 306.1574 (calcd. for $C_{16}H_{22}N_2O_4$, 306.1579)



	R_1	R_2	m/z	Formula for ion
$^+$	$COCH_3$	CH_3	491	$C_{28}H_{33}N_3O_5$
$^+$-CH_2O	$COCH_3$	-	461	$C_{27}H_{31}N_3O_4$
$^+$-$COCH_2$	H	CH_3	449	$C_{26}H_{31}N_3O_4$
$^+$-$COCH_2$-CH_2O	H	-	419	$C_{25}H_{29}N_3O_3$
leavage				
a-g)	$COCH_3$	CH_3	306	$C_{16}H_{22}N_2O_4$
	H	CH_3	264	$C_{14}H_{20}N_2O_3$
a-j)	$COCH_3$	CH_3	290	$C_{16}H_{22}N_2O_3$
	H	CH_3	248	$C_{14}H_{20}N_2O_2$

All other fragments were similar to compound 7).

Fig.-4

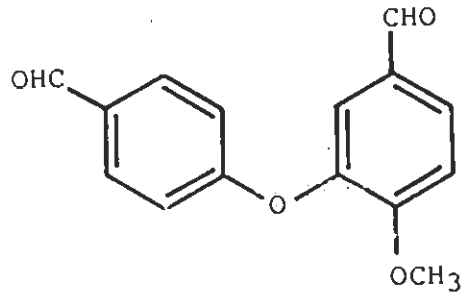
3 Synthesis of cadabicine methyl ether (7)

The structure of isolated cadabicine methyl ether was elucidated by ›ectroscopic means, and a chemical correlation was needed for its ›nfirmation. Therefore, it was considered of interest to synthesized the ›quired compound. We attempted to synthesize cadabicine methyl ether utilizing new synthetic route (Scheme-3), but with the help of aminolysis method as ›ported for codonocarpine [44]. The synthesis of cadabicine methyl ether (7) ıs carried out as shown in Scheme-3. During this synthesis five new compounds ·re prepared, which had not been prepared or isolated elsewhere.

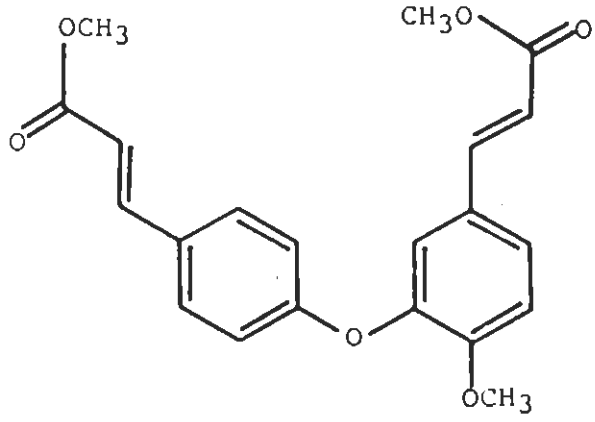
3.1 3-(4-formyl phenoxy)-4-methoxy Benzaldehyde (17):

p-Bromobenzaldehyde (15) and isovaniline (16), in 1:1 molar ratio were lowed to condense in boiling N,N-dimethyl acetamide, in the presence of Cu_2O .5 molar ratio), under nitrogen atmosphere. The mixture was heated for 72 urs contineously and then filtered off, the residue is washed with ether and ltrate was diluted with water, the ether layers were separated and evapo-ted. The material thus obtained was chromatographed on a silica gel column ich furnished pure diphenyl ether (17).

The IR spectrum of compound 17 showed two overlapped bands for 2xCHO groups 1700 cm^{-1} and a band at 1590 cm^{-1} for aromatic ring. In ^1H-NMR two peaks : two aldehydes groups were observed at δ 8.9 (1H) and 9.1 (1H). The high ›olution mass spectrum measured the exact mass at m/z 256.0756 (calcd. for



(17)



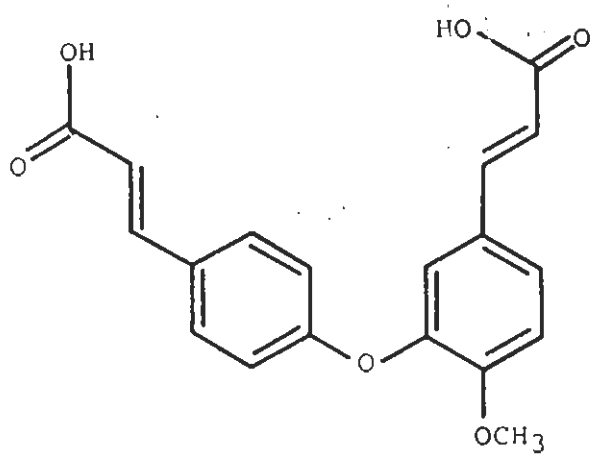
(18)

$C_{15}H_{12}O_4$, 256.0735). Two important peaks were also observed at m/z 227 (M^+-CHO) and 198 (M^+-2xCHO).

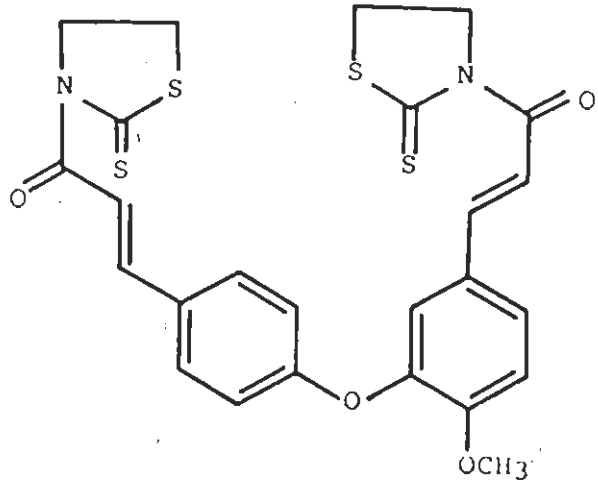
3.3.2 Methyl cinnamate, methyl ferulate ether (18)

A phosphonium ylid was prepared by the action of triphenyl phosphine on α-bromo methyl acetate in benzene. The residue thus obtained was treated with a solution of K_2CO_3 as the result of which the crystalline ylid was obtained.

Compound 17 was allowed to react with this ylid (Wittig reaction) at about 70°C. The mixture was partitioned between H_2O and ethyl acetate. The ethyl acetate layers were separated and evaporated which furnished a crystalline compound 18. The IR spectrum of compound 18 showed two bands at 1720 and 1725 cm^{-1} for two ester groups. The ^{1}H-NMR showed two singlets at δ 3.75 and 3.77 for two ester ($COOCH_3$) groups. There was also another sharp singlet for methoxy group at δ 3.82. Four doublets at δ 6.24, 6.32, 7.52 and 7.63 (each 1H, J=15.6 Hz) were also observed for four olefinic protons of two trans-cinnamic acid residues. The two doublets at δ 7.31 and 7.63 (2H, J=9.0 Hz) indicated a para disubstituted benzene ring. Three other peaks at δ 6.91 (dd, 1H, J=3.1 and 9.0 Hz) with ortho and meta coupling, δ 7.00 (d, 1H, J=9.0 Hz) with ortho coupling and δ 7.21 (d, 1H, J=3.2 Hz) with meta coupling were due to three protons of meta disubstituted benzene ring bearing a methoxy group. These assignments were confirmed by 2D-J-resolved experiment. The high resolution mass spectrum showed exact mass at m/z 368.1262 (calcd. for $C_{21}H_{20}O_6$, 368.1259). Two other important peaks were at m/z 337 (M^+-OCH_3) and 221 (M^+-OCH_3-2x$COOCH_2$).



(19)



(20)

3.3.3 Ether of -p-cinnamic acid-m-ferulic acid (19)

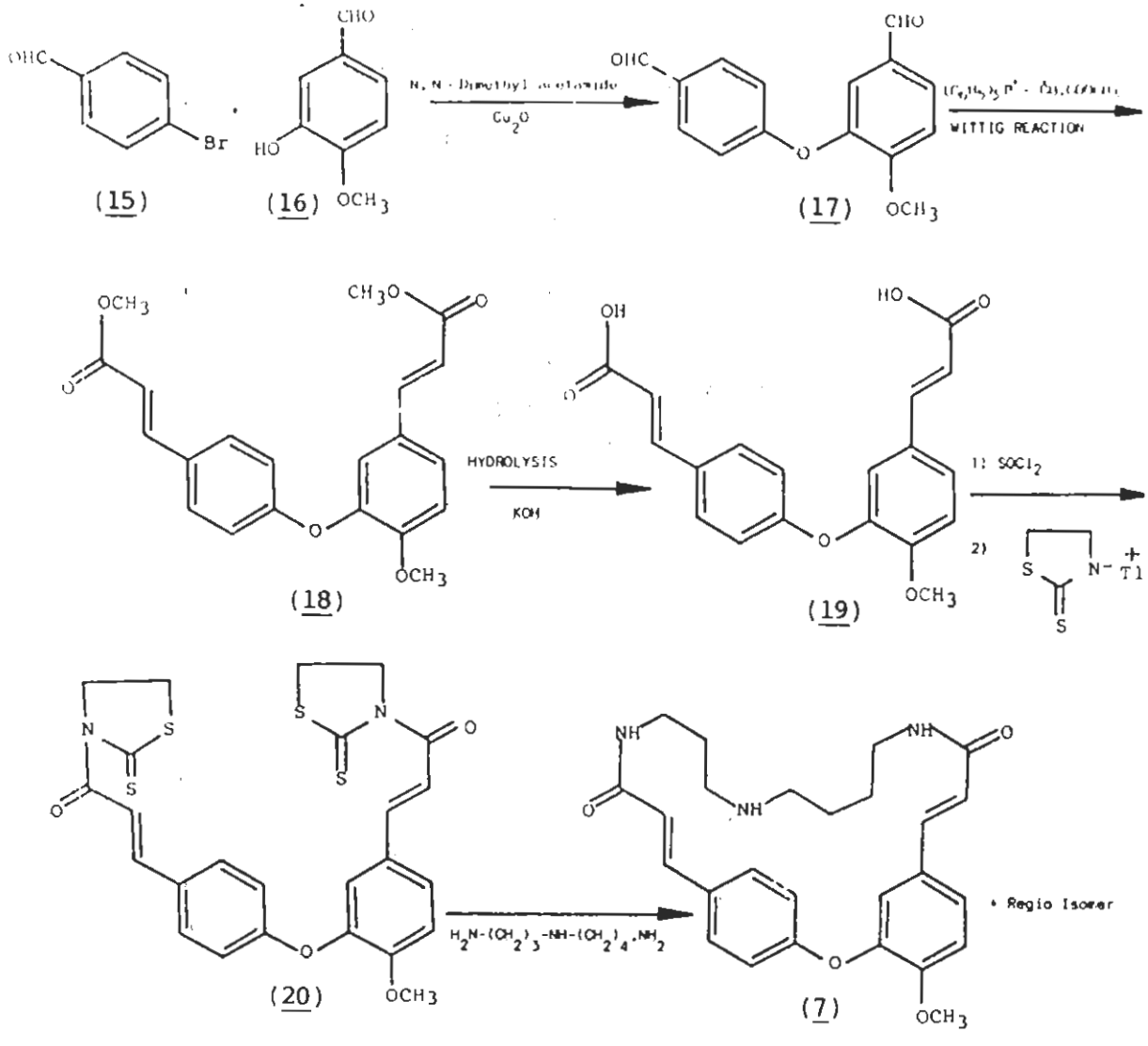
The di-ester (18) was hydrolysed with alkali on heating. The di-acid (19) thus obtained was a colourless crystalline compound but its molecular ion peak was not observed in mass spectrum. Other peaks were similar to compound 18. The IR spectrum showed two bands at 1695 and 1700 cm^{-1} for two carboxylic groups. In ^{1}H-NMR a singlet at δ 9.97, 9.99 (2xCOOH) represented two acidic protons.

3.3.4 Thiazolidine compound (20)

The compound 19 was allowed to react with thionyl chloride in benzene solvent, the obtained oily acid choride was treated with Thallium (I) salt [45] of thiazolidine-2-thione with contineous stirring in THF at room temperature under nitrogen atmosphere for about 20 hours. A dirty white solid (20) was obtained by extraction of mixture with CH_2Cl_2. It was recrystallized from $CHCl_3$.

The high resolution mass spectrum showed a molecular ion peak at m/z 542.0466 (calcd. for $C_{25}H_{22}O_4N_2S_4$, 542.0462) other important peak was observed at m/z 308 (M^+-2 thiazolidine moieties).

IR spectrum showed a band at 1680 cm^{-1} (CO-N-). In ^{1}H-NMR a sharp singlet was observed for OCH_3 group at δ 3.65. Four triplets were observed at δ 3.37, 3.56, 3.68 and 3.98 (J=7.00 Hz) for four methylenes of thiazolidine moieties.


OHC
Br
(15)
CHO
HO
OCH3
(16)
N, N - Dimethyl acetamide
Cu2O
OHC
CHO
O
OCH3
(17)
WITTIG REACTION
OCH3
CH3O
O
(18)
HYDROLYSIS
KOH
OH
HO
(19)
1) SOCl2
2)
S
N
Tl
+
N
S
O
(20)
H2N-(CH2)3-NH-(CH2)4-NH2
NH
(7)
* Regio Isomer

(Scheme-3)

The peaks at aromatic region of spectrum were similar to compounds 18.

3.3.5 Cadabicine methyl ether from thiazolidine compound (20)

The cyclization between compound 20 and spermidine was performed by the high dilution method [46]. The obtained residue was chromatographed on HPTLC, using $CHCl_3$:MeOH:H_2O:NH_3 (7:2:1.5:0.5) system. The compound thus obtained was recrystallized from hot MeOH-$CHCl_3$, mixture. The pale yellow crystals of cadabicine methyl ether M.P. 190-92°C was obtained in overall 20% yield.

The synthetic cadabicine methyl ether when treated with acetic anhydride in the presence of pyridine at room temperature afforded N-acetyl cadabicine methyl ether (12) M.P. 180-82°C.

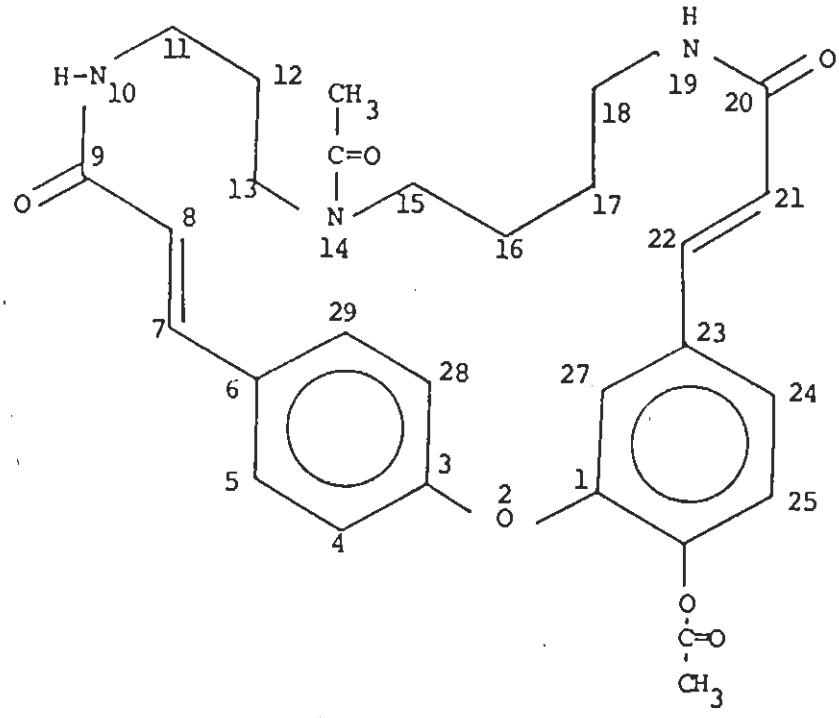
The accomplishment of this total synthesis was confirmed by the identity of melting points and spectral data between synthetic and natural cadabicne methyl ether and their N-acetyl derivatives.

3.4 Cadabicine diacetate (21)

Cadabicine diacetate was isolated as natural product from stem bark of *Cadaba farinosa*. It is the first alkaloid of spermidine series which exists naturally in acetylated form.

The ethanolic extracts of stem bark were processed according to the standard procedure. The crude alkaloidal material thus obtained was partitioned between $CHCl_3$ and H_2O at pH 7. The $CHCl_3$ layers were combined, washed with water, dried and concentrated. The material thus obtained was subjected to column chromatography using chloroform, with gradually increasing polarity of methanol. The fractions obtained furnished cadabicine diacetate (21) as yellowish brown amorphous solid, which on recrystallization from hot methanol produced colourless shining rods M.P. 265-267°. These crystals were readily soluble in hot methanol.

The high resolution mass spectrum showed the molecular ion peak at m/z 519.2684 corresponding to molecular formula $C_{29}H_{33}N_3O_6$ (calcd. for 519.2732). The ultra-violet spectrum exhibited the maxima at 210 ($\log \varepsilon$=4.12), 273 ($\log \varepsilon$=4.08) and a shoulder at 305 nm. The infra-red spectrum showed the band at 3400 (NH), 1760 (phenolic acetate), 1745 (N-acetate), 1655 (α,β-unsaturated amide) and 1590 cm^{-1} (aromatic ring). The bands at 1760 and 1745 cm^{-1} were not present in UV spectrum of cadabicine, this indicating two additional acetyl groups in basic skeleton.



(21)

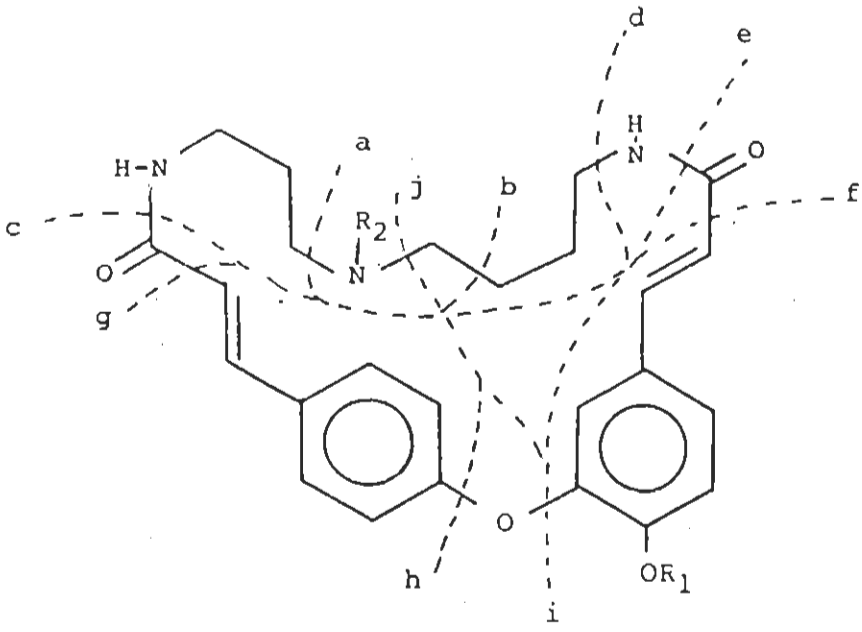
The ^{1}H-NMR of cadabicine diacetate in DMSO-d_6 (400 MHz) showed the doubling of several signals and this was due to the presence of interchangeable Z and E conformers with respect to amide bond (as mentioned in case of cadabicine 1). Two singlets which together integrated to 3H at δ 1.95 and 1.97 was attributed to the N-acetate. Another singlet at δ 2.32 (3H) is due to the phenolic acetate. The upfield position of the singlet and the presence of two singlets (due to the presence of conformers with respect to amide bond) with a total integration of 3H showed the occurrence of N-acetate. The downfield position of the singlet clearly indicating the presence of phenolic acetate. The presence of phenolic acetate was also confirmed by negative phenolic test with $FeCl_3$. A multiplet between δ 1.23 and δ 1.60 integrated for 6H was attributed to three methylene groups (2xH-12, 2xH-16 and 2xH-17) which are attached to other methylenes, and another multiplet between δ 3.00 and δ 3.35 (8H) was due to the four methylene groups (2xH-11, 2xH-13, 2xH-15 and 2xH-18) adjacent to nitrogen atoms. The seven methylene groups four of which are adjacent to nitrogen showed the presence of spermidine moiety in the alkaloid. The ^{13}C-NMR also showed the occurrence of spermidine which exhibited the peaks of seven methylenes. The chemical shifts of the seven methylene groups are very close to that of cadabicine (1). Four doublets at δ 5.98, 6.52, 7.19 and 7.46 were assigned to olefinic protons of two trans-cinnamic acid H-21, H-8, H-22 and H-7 respectively. Each doublet showed the presence of a single proton and having a coupling constant of 15.5 Hz. A doublet at δ 6.43 integrating for 1H showed only meta coupling (J=1.7 Hz). It was attributed to H-27. There are two doublets at δ 7.13 and δ 7.71 each integrating for 2H showing only ortho coupling (J=7.8 Hz) typical of a para disubstituted benzene ring. Another

doublet at δ 7.21 with integration of 1H and coupling constant of 8.1 Hz having only ortho coupling but no meta coupling is attributed to H-25. The doublet of doublet resonated at δ 7.15 with integration of 1H exhibiting a meta (J=1.7 Hz) and ortho (J=8.1 Hz) coupling was attributed to H-24.

These assignments were confirmed by 2D correlation of proton shift through a COSY-45 experiment, and the assigned coupling constants were observed through a 2D J-resolved spectrum.

The coupling interactions were established by a COSY-45 experiment. Two doublets of H-7 at δ 7.46 and H-8 at 6.52 were coupled with each other. Similarly two doublets of H-21 at δ 5.98 and H-22 at 7.19 were coupled with each other. Two doublets at δ 7.13 and 7.71 (for para disubstituted benzene ring) were also coupled with each other. A double doublet of H-24 at δ 7.15 had cross peaks with H-25 at δ 7.21 and H-27 at δ 6.43. A multiplet between δ 1.23 and 1.60 (6H) for 2xH-12, 2xH-16 and 2xH-17 was coupled with another multiplet between δ 3.00 and 3.35 (8H) for 2xH-11, 2xH-13, 2xH-15 and 2xH-18.

All the above assignments indicate that, the acetoxy group in this skeleton is attached to benzene ring at position 26, just as the hydroxy group in cadabicine (1). But there are two different possible attachments of spermidine moiety with the rest of molecule. The secondary nitrogen of spermidine may be present at position 14 as in case of cadabicine (1) and Isocodonocarpine 47] or it may be at position 15 as in case of codonocarpine (22) and apparidisine (23). The exact attachments of spermidine moiety in the skeleton



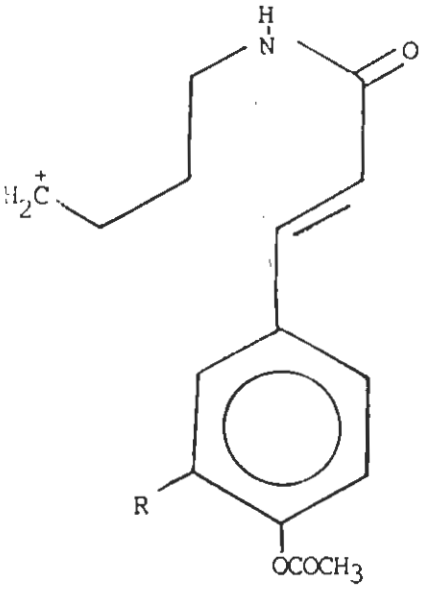
M^+		519	$C_{29}H_{33}N_3O_6$
$M^+-(COCH_2)$		477	$C_{27}H_{31}N_3O_5$
$M^+-2(COCH_2)$		435	$C_{25}H_{29}N_3O_4$

cleavage	R_1	R_2	m/z	Formula for fragment
(a-b)	H	-	407	$C_{24}H_{27}N_2O_4$
(b-c)	H	-	349	$C_{21}H_{19}O_4$
(a-h)	$COCH_3$	$COCH_3$	334	$C_{17}H_{22}N_2O_5$
(c-d)	H	-	307	$C_{18}H_{13}NO_4$
(a-h)	$COCH_3$	H	292	$C_{15}H_{20}N_2O_4$
(c-e)	H	-	291	$C_{18}H_{11}O_4$
(a-i)	$COCH_3$	H	276	$C_{15}H_{20}N_2O_3$
(h-j)	$COCH_3$	-	276	$C_{15}H_{18}NO_4$
(c-f)	H	-	264	$C_{17}H_{12}O_2$
i-j)	$COCH_3$	-	260	$C_{15}H_{18}NO_3$
a-h)	H	H	250	$C_{13}H_{18}N_2O_3$
g-f)	H	-	235	$C_{16}H_{11}O_2$
h-j)	H	-	234	$C_{13}H_{16}NO_3$
a-i)	H	H	234	$C_{13}H_{18}N_2O_2$
i-j)	H	-	218	$C_{13}H_{16}NO_2$
a-i)	-	-	205	$C_{12}H_{15}NO_2$
a-h)	H	-	189	$C_{12}H_{15}NO$
e-i)	-	-	146	$C_9H_6O_2$
c-h)	-	-	131	C_9H_7O

Fig.-5

were proved with the help of mass fragmentation of compound 21 (Fig.-5 and Scheme-4).

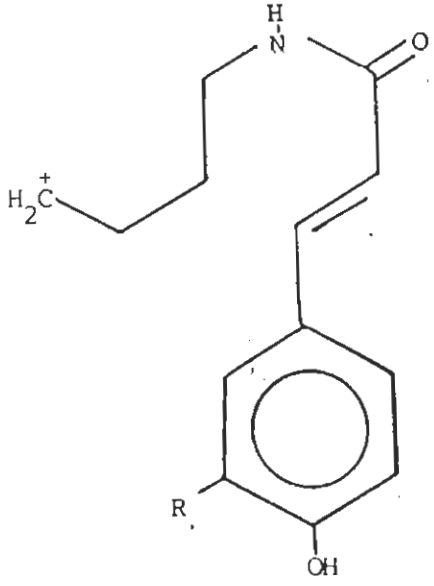
The high resolution mass spectrum of compound 21 shows several peaks that allowed the assignments of the $(CH_2)_3$ unit of spermidine to the para disubstituted benzene ring and the $(CH_2)_4$ unit, to the other ring bearing acetoxy group at C-26. The peaks at m/z 260 and 276 correspond to ions 24 and 25, and show that butyl unit of spermidine is attached with phenyl ring bearing acetoxy group, otherwise there should be two peaks at m/z 246 (for $C_{14}H_{16}NO_3$) and m/z 262 (for $C_{14}H_{16}NO_4$) but they were not observed.



24 R=H, m/z 260.1293
(calcd. for $C_{15}H_{18}NO_3$, 260.1286)

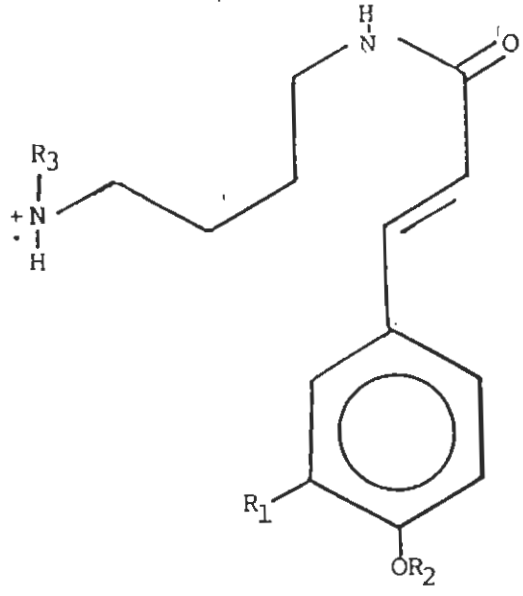
25 R=OH, m/z 276.1241
(calcd. for $C_{15}H_{18}NO_4$, 276.1235)

The peaks at 218, 234, 250, 276, 292 and 334 correspond to ions 26, 27, 28, 29, 30 and 31 respectively. These ions also proved the same attachments of spermidine.

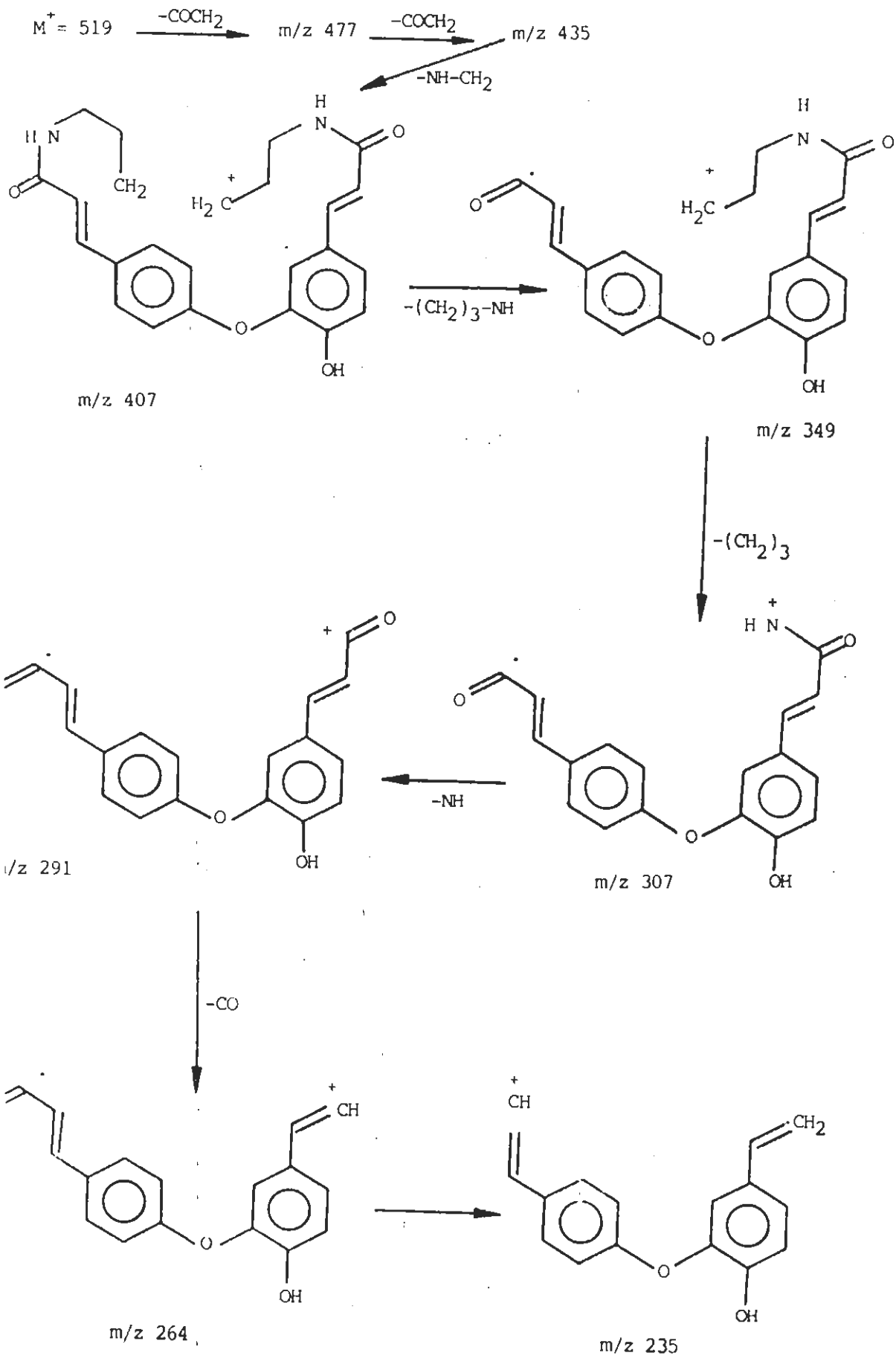


26 R=H, m/z 218.1183 (calcd. for $C_{13}H_{16}NO_2$, 218.1181)

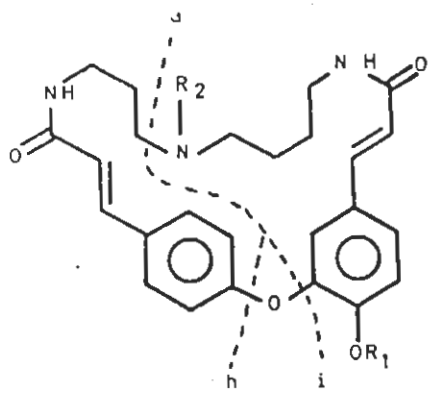
27 R-OH, m/z 234.1136 (calcd. for $C_{13}H_{16}NO_3$, 234.1130)



R_1	R_2	R_3	m/z	(calcd. for)
OH	H	H	250.1309	($C_{13}H_{18}N_2O_3$, 250.1317)
H	$COCH_3$	H	276.1481	($C_{15}H_{20}N_2O_3$, 276.1473)
OH	$COCH_3$	H	292.1409	($C_{15}H_{20}N_2O_4$, 292.1423)
OH	$COCH_3$	$COCH_3$	334.1532	($C_{17}H_{22}N_2O_5$, 334.1528)



(Scheme-4) (Route-I)



M^+ = 519 (R_1 - R_2 = $COCH_3$)

M^+-($COCH_3$) = 477 (R_1 = H, R_2 = $COCH_3$)

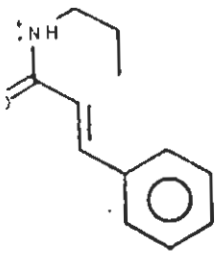
M^+-2($COCH_3$) = 435 (R_1 = R_2 = H)

Route-II

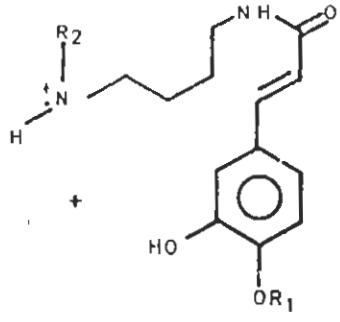
(a-h) cleavage

Route-III

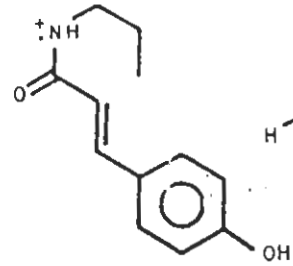
(a-i) cleavage



189



R_1, R_2 ,	m/z
H , H	250
H , $COCH_3$	292
$COCH_3$, $COCH_3$	334



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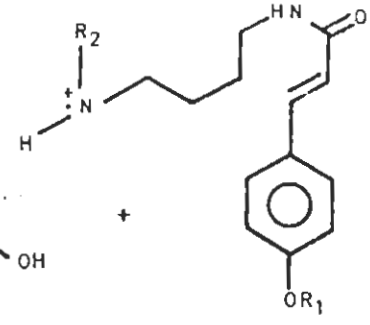
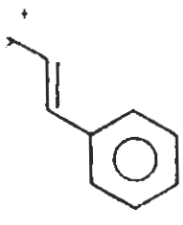
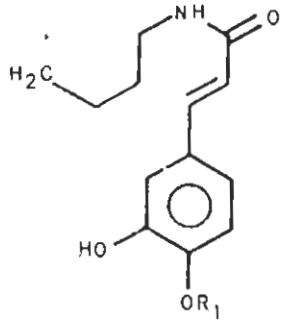


R_1, R_2	m/z
H , H	234
H , $COCH_3$	276

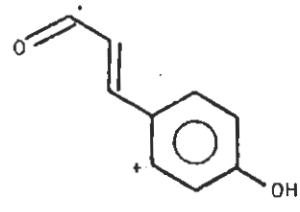


131

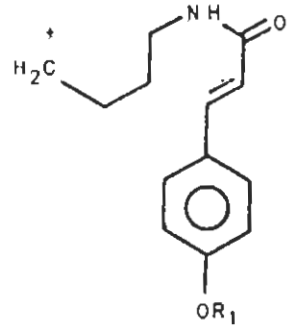


R_1 = H, 234

R = $COCH_3$, 276



146



R_1 = H, 218

R = $COCH_3$, 260

(Scheme-4) (Routes II and III)

Alongwith mass spectral fragmentation, the ^{13}C-NMR spectrum (Table-6) and the DEPT experiment also supported structure 21 for cadabicine diacetate.

It may be mentioned here that no acetic acid, acetic anhydride or any other reagent containing an acetyl group was used in the isolation procedure, therefore the diacetate is a genuine alkaloid and not an artifact.

TABLE - 6

^{13}C-NMR chemical shifts of cadabicine diacetate (21)
(DMSO-d_6, 100 MHz)

Carbon	ppm	Carbon	ppm
1	151.80	20	164.79/164.87*[e]
3	155.17	21	124.63/124.69*[d]
4	122.31[a]	22	136.97/137.06*[c]
5	129.54	23	133.50[b]
6	133.54[b]	24	124.01
7	137.62/137.70*[c]	25	110.78/110.84*
8	124.43/124.54*[d]	26	140.33
9	164.09[e]	27	122.23[a]
11	45.00/47.42*	28	122.30
12	27.04/27.71*	29	129.54
13	38.27/38.43*	$O\underline{C}OCH_3$	168.54
15	35.36/35.94*	$OCO\underline{C}H_3$	20.43
16	26.48/26.59*	$N\underline{C}OCH_3$	169.00/169.02*
17	25.90	$NCO\underline{C}H_3$	21.10/21.20*
18	42.43/43.71*		

a,b,c,d,e = Assignments may be interchanged.

* = Doubling of peaks due to conformers.

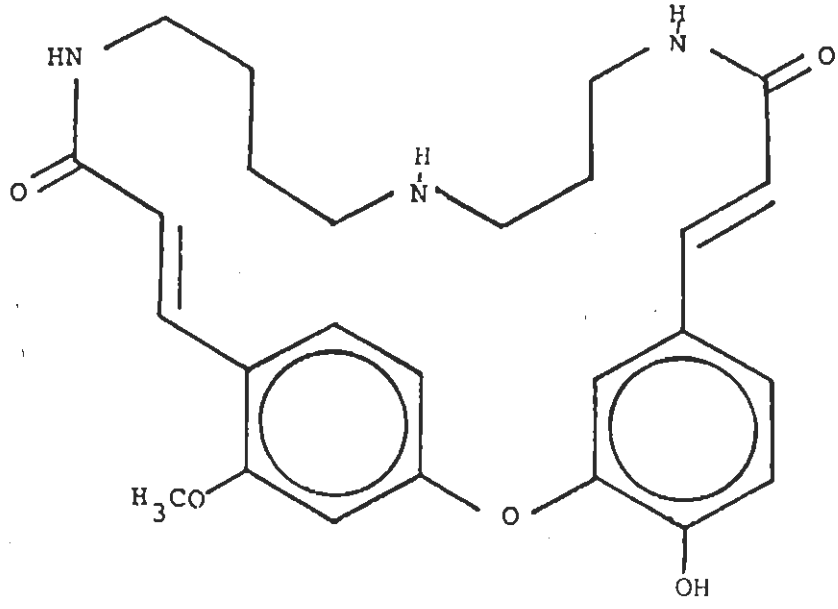
The status of each carbon confirmed through DEPT experiment.

3.5 Cappirisine (32)

Capparisine is the first spermidine alkaloid, isolated from *Cadaba farinosa* in which secondary nitrogen of spermidine is present at position-15, as in case of codonocarpine (22), whereas in all other alkaloids, isolated from this plant the secondary nitrogen of spermidine was present at position-14.

The crude alkaloidal material, obtained from the plant, was partitioned between $CHCl_3$ and water at pH-10. The $CHCl_3$ layers were combined, washed with water dried and concentrated. The concentrated material was subjected to column chromatography using $CHCl_3$ and MeOH as mobile phase. The polar fraction was again chromatograph on preparative TLC-plates (silica gel), which led to the isolation of capparisine as amorphous solid. It was recrystallized from hot methanol.

The high resolution mass spectrum showed the molecular ion peak at m/z 465.2256 corresponding to molecular formula $C_{26}H_{31}N_3O_5$ (calcd. for 465.2263). The UV and IR spectra were very close to those of capparisine [48], codonocarpine [41] and isocodonocarpine [47]. All these alkaloids has same molecular formula. The ^{1}H-NMR spectroscopic data of aromatic region was very close (almost superimposable) to capparisine an spermidine alkaloid isolated from *Capparis decidua* [48] in 1986.

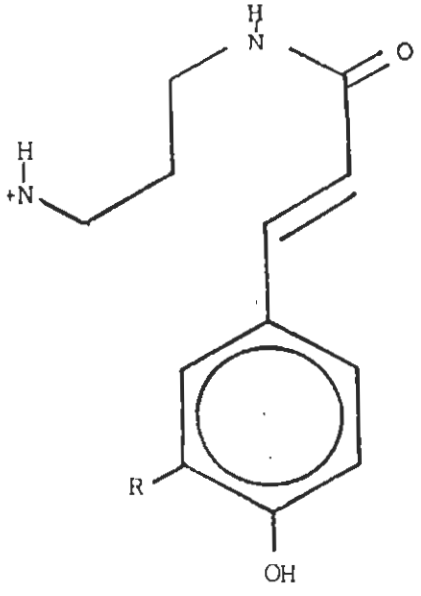


(32)

The ^{1}H-NMR and ^{13}C-NMR (Table-7) data of compound 32 cannot decide the exact attachments of spermidine with the rest of the molecule and also the positions of "OH" and "OCH_3" groups, but it can be decided on the basis of mass fragmentation pattern. Therefore high resolution mass spectrum of compound 32 was studied for the confirmation of actual structure.

The mass fragmentation pattern (Fig.-6 and Scheme-5) showed several peaks that allowed the assignments of the $(CH_2)_4$ unit of spermidine to the phenyl ring bearing methoxy group and the $(CH_2)_3$ unit of spermidine to the other ring bearing "OH" group.

The peaks at m/z 219 and 235 correspond to ions 33 and 34 respectively and show that $(CH_2)_3$ unit of spermidine is attached with phenyl ring bearing "OH" group (at C-26), otherwise there should be two peaks at m/z 233 (for $C_{13}H_{17}N_2O_2$) and m/z 249 (for $C_{13}H_{17}N_2O_3$), but they were not observed.



33 R=H, 219.1129
(calcd. for $C_{12}H_{15}N_2O_2$, 219.1133)

34 R=OH, 235.1078
(calcd. for $C_{12}H_{15}N_2O_3$, 235.1082)

TABLE - 7

^{13}C-NMR chemical shifts for capparisine (32)
(DMSO-d_6, 75.43 MHz)

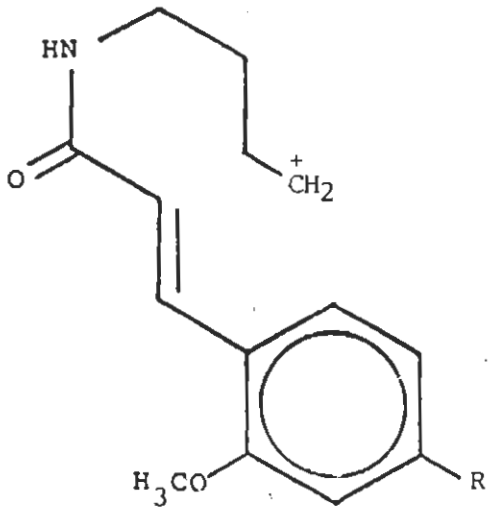
Carbon	ppm	Carbon	ppm
1	152.10	17	25.49/25.58*[b]
2	155.71	18	42.30
3	138.54	20	165.79[a]
5	148.11	21	124.11
6	134.59	22	137.81/138.22*
7	137.65	23	133.22
8	125.49/125.67*	24	123.53
9	166.01[a]	25	110.23
11	47.20	26	143.61
12	25.61[b]	27	142.90
13	39.10	28	122.05
14	37.34/37.82*	29	120.37
16	28.01/28.51*	$O\underline{C}H_3$	56.10

a,b = Assignments may be interchanged.

* = Doubling of peaks due to conformers.

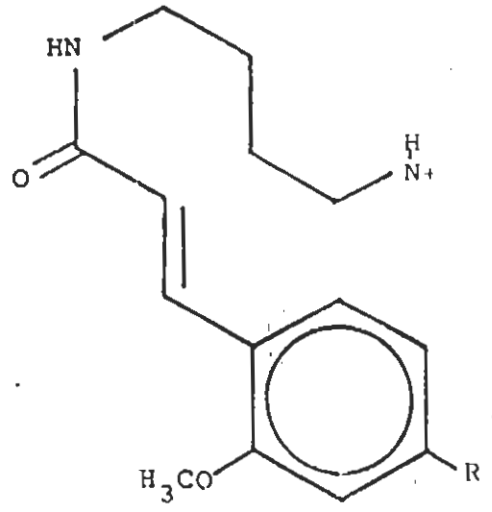
The status of each carbon confirmed through DEPT experiment.

Similarly two peaks at m/z 232 and 248 correspond to ions 35 and 36 respectively, and show that $(CH_2)_4$ unit of spermidine is attached with phenyl ring bearing "OCH_3" group (at C-5), otherwise there should be two peaks at m/z 218 (for $C_{13}H_{16}NO_2$) and m/z 234 ($C_{13}H_{16}NO_3$), but they were also not observed. The ions 37 and 38 also proved the same situation otherwise there should be two peaks at m/z 233 (for $C_{13}H_{17}N_2O_2$) and 249 (for $C_{13}H_{17}N_2O_3$).



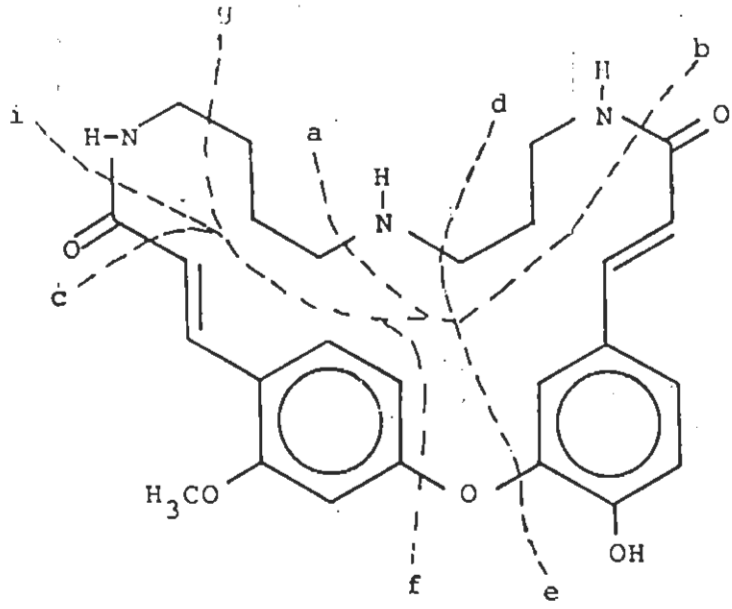
35 R=H, 232.1341
(calcd. for $C_{14}H_{18}NO_2$, 232.1337)

36 R=OH, 248.1291
(calcd. for $C_{14}H_{18}NO_3$, 248.1286)



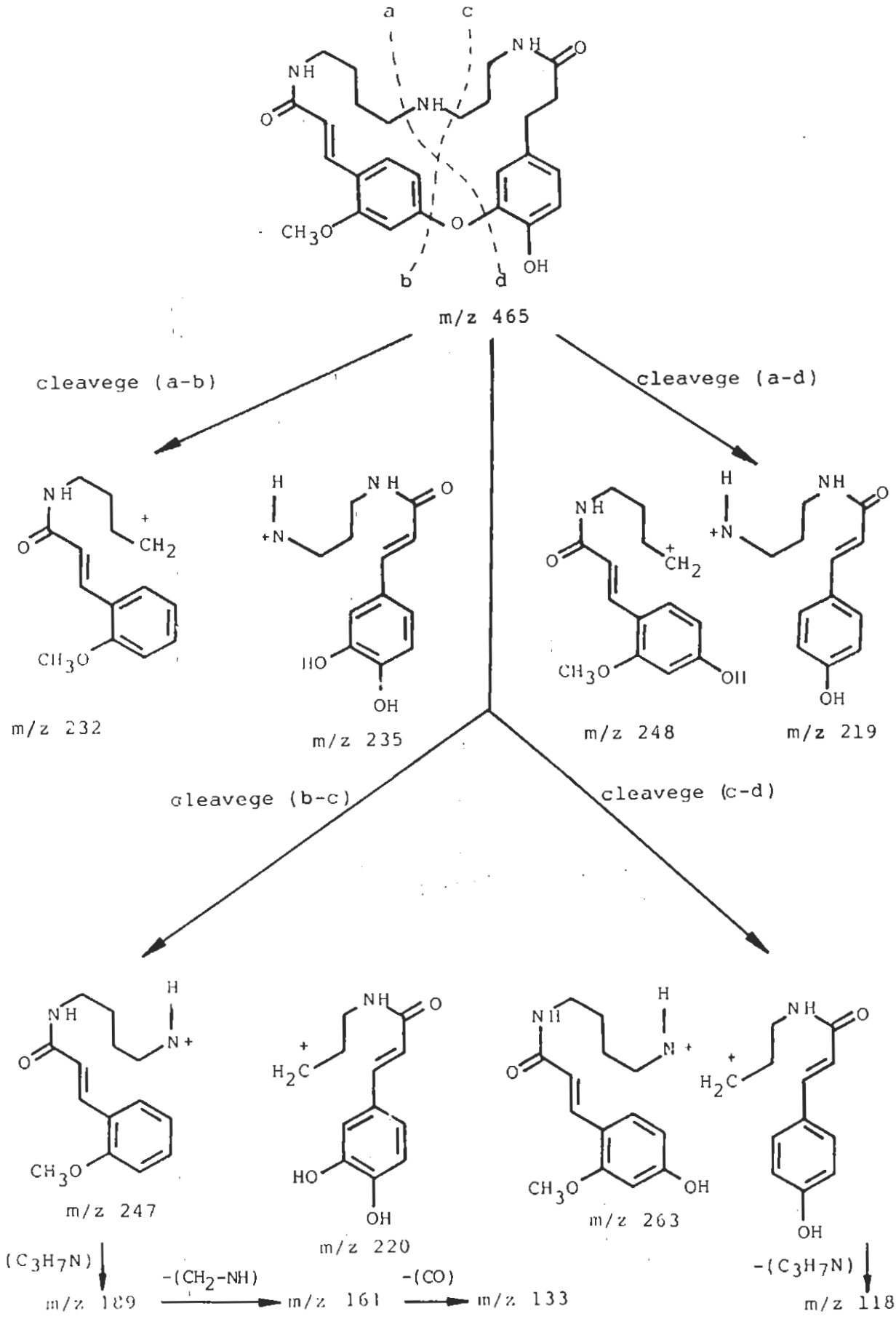
37 R=H, 247.1442
(calcd. for $C_{14}H_{19}N_2O_2$, 247.1446)

38 R=OH, 263.1391
(calcd. for $C_{14}H_{19}N_2O_3$, 263.1395)



		m/z	Formula for ion
M^+	=	465	$(C_{26}H_{31}N_3O_5)$
M^+-OCH_2	=	435	$(C_{25}H_{29}N_3O_4)$
cleavage			
(a-b)	=	366	$(C_{22}H_{24}NO_4)$
(b-c)	=	264	$(C_{17}H_{12}O_3)$
(d-e)	=	263	$(C_{14}H_{19}N_2O_3)$
(a-e)	=	248	$(C_{14}H_{18}NO_3)$
(d-f)	=	247	$(C_{14}H_{19}N_2O_2)$
(a-f)	=	235	$(C_{12}H_{15}N_2O_3)$
(a-f)	=	232	$(C_{14}H_{18}NO_2)$
(d-f)	=	220	$(C_{12}H_{14}NO_3)$
(a-e)	=	219	$(C_{12}H_{15}N_2O_2)$
(d-e)	=	204	$(C_{12}H_{13}NO_2)$
(f-g)	=	189	$(C_{11}H_{11}NO_2)$
(f-i)	=	161	$(C_{10}H_9O_2)$
(c-f)	=	133	(C_9H_9O)
(b-e)	=	118	(C_8H_6O)

Fig.-6


a
c
b
d
m/z 465
cleavege (a-b)
cleavege (a-d)
m/z 232
m/z 235
m/z 248
m/z 219
cleavege (b-c)
cleavege (c-d)
m/z 247
m/z 220
m/z 263
m/z 118
(C3H7N)
-(CH2-NH)
-(CO)
m/z 189
m/z 161
m/z 133

(Scheme-5)

All the above observation show that the isolated compound 32 is capparisine and this is 2nd report of its isolation, but from a new source.

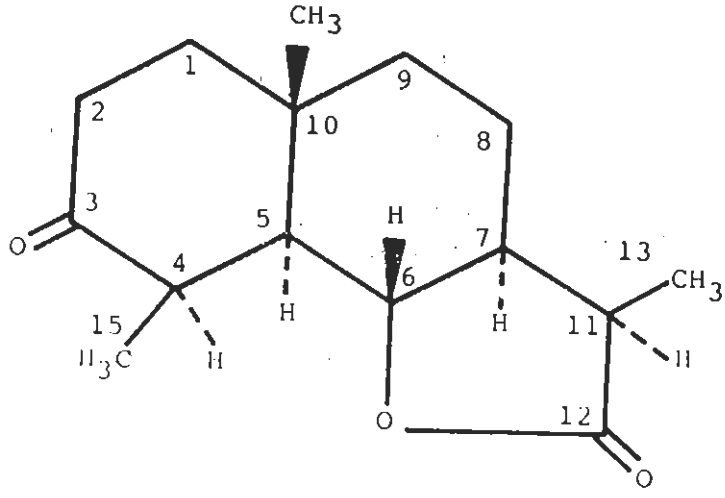
3.6 Cadabicilone (39)

A new eudensmanolide sesquiterpene lactone was isolated from the plant. Its structure was determined by spectroscopic studies including 2D-NMR, specially COSY-45 and Hetero COSY experiments.

The residue obtained on evaporation of the alcoholic extraction was partitioned between $CHCl_3$ and H_2O, at pH-6. The $CHCl_3$ layers were combined, washed with water, dried and converted into a yellowish powder. It was chromatographed on a column of silica gel. Elution with $CHCl_3$:Hexane (50:50) furnished Cadabicilone. Fractional crystallization with $CHCl_3$-ether mixture yielded pure cadabicilone (39) as white crystals. M.P. 118-120°C.

Cadabicilone (39), in high resolution mass spectrometery gave an $[M]^+$ at m/z 250.1554 corresponding to the molecular formula $C_{15}H_{22}O_3$ (calcd. 250.1568). The IR spectrum showed bands at 1760 (lactone) and 1700 cm^{-1} (ketone). In the ^{1}H-NMR, there is a singlet at δ 1.18 due to 14-CH_3. Two doublets at δ 1.20 and 1.22 (each for 3H, J=7.0 Hz), are assigned to two methyls at C-4 and C-11 respectively. There are two doublets of doublets at δ 1.58 (J=11 Hz and 5 Hz) and δ 3.90 (J=11 Hz and 12 Hz) due to H-5 and H-6 respectively. Two multiplet centered at δ 2.25 and 2.50 are assigned to H-4 and H-11 respectively.

There are two doublets of doublets of doublet at δ 2.40 and 2.49 (each for 1H, J=3.0 Hz, 5.5 Hz and 12 Hz) due to β and α hydrogen respectively at C-2, similarly another set of doublets of doublets of doublet at δ 1.62 and 1.68



39

(each for 1H, J=3.1, 5.5 and 11.5 Hz) assigned to β and α-hydrogen, respectively at C-9. Two multiplet centered at δ 1.72 and 1.85 were assigned to α,β hydrogens at C-1 and α,β hydrogens at C-8 respecitvely. A complex multiplet centered at δ 1.55 is due to H-7. These assignments are confirmed by 2D-J-resolved, COSY-45 and Hetero COSY experiments.

The coupling interactions were established by a COSY-45 experiment. Two doublets of doublet of H-5 at δ 1.58 and H-6 at δ 3.90 were coupled with each other and similarly δ 1.58 (H-5) is also coupled with δ 2.25 (H-4) and δ 3.90 (H-6) is coupled with δ 1.55 (H-7). Two ddd of β-H-2 at δ 2.40 and α-H-2 at 2.47 had cross peaks with each other and with 2xH-1 at δ 1.72. Similarly two other ddd of β-H-9 at δ 1.62 and α-H-9 at δ 1.68 are coupled with each other and with 2xH-8 at δ 1.85.

A multiplet at δ 2.25 (H-4) had cross peaks with δ 1.20 (4-CH_3) and δ 1.58 (H-5). Similarly another multiplet at δ 2.5 (H-11) had cross peaks with δ 1.22 (11-CH_3) and δ 1.55 (H-7).

In ^{13}C-NMR spectrum (Table-8), a signal at δ 211.39 shows the presence of ketonic carbon and the signal at δ 178.94 indicates the presence of a lactone moiety, which is also confirmed by mass spectral fragmentation (Fig.-7 and Scheme-6).

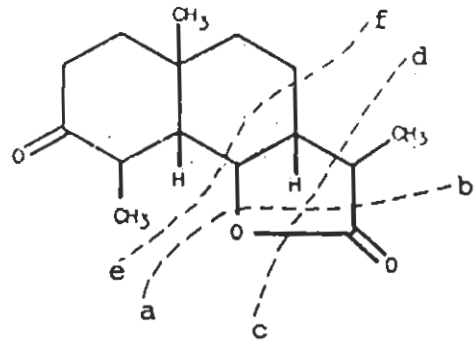
All the coupling interactions and above indications showed that, the oxygen f lactone moiety is attached with C-6 and ketonic group at C-4.

TABLE - 8

^{13}C-NMR chemical shifts for cadabicilone (39)
($CDCl_3$, 100 MHz)

Carbon	ppm	Carbon	ppm
1	40.53	9	40.10
2	37.20	10	34.21
3	211.39	11	44.86
4	40.40	12	178.94
5	52.79	13	13.81
6	83.02	14	18.29
7	53.43	15	12.35
8	23.12		

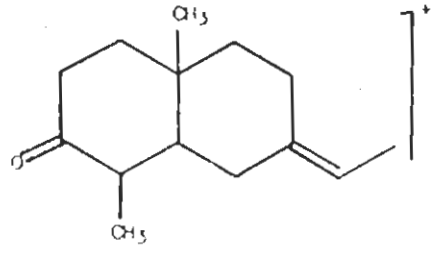
ne status of each carbon confirmed through DEPT experiment.



$M^+ = 250 \quad C_{15}H_{22}O_3$

$M^+-CH_3 = 235 \quad C_{14}H_{19}O_3$

Cleavage	m/z	Fragment	Formula
(a-b)	206	I	$C_{14}H_{22}O$
(c-d)	194	II	$C_{12}H_{18}O_2$
(a-d)	177	III	$C_{12}H_{17}O$
(e-f)	153	IV	$C_{10}H_{17}O$



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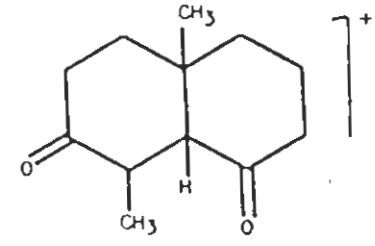
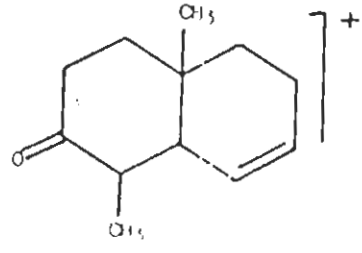
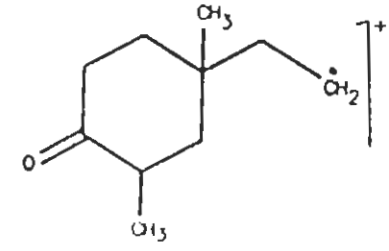


II

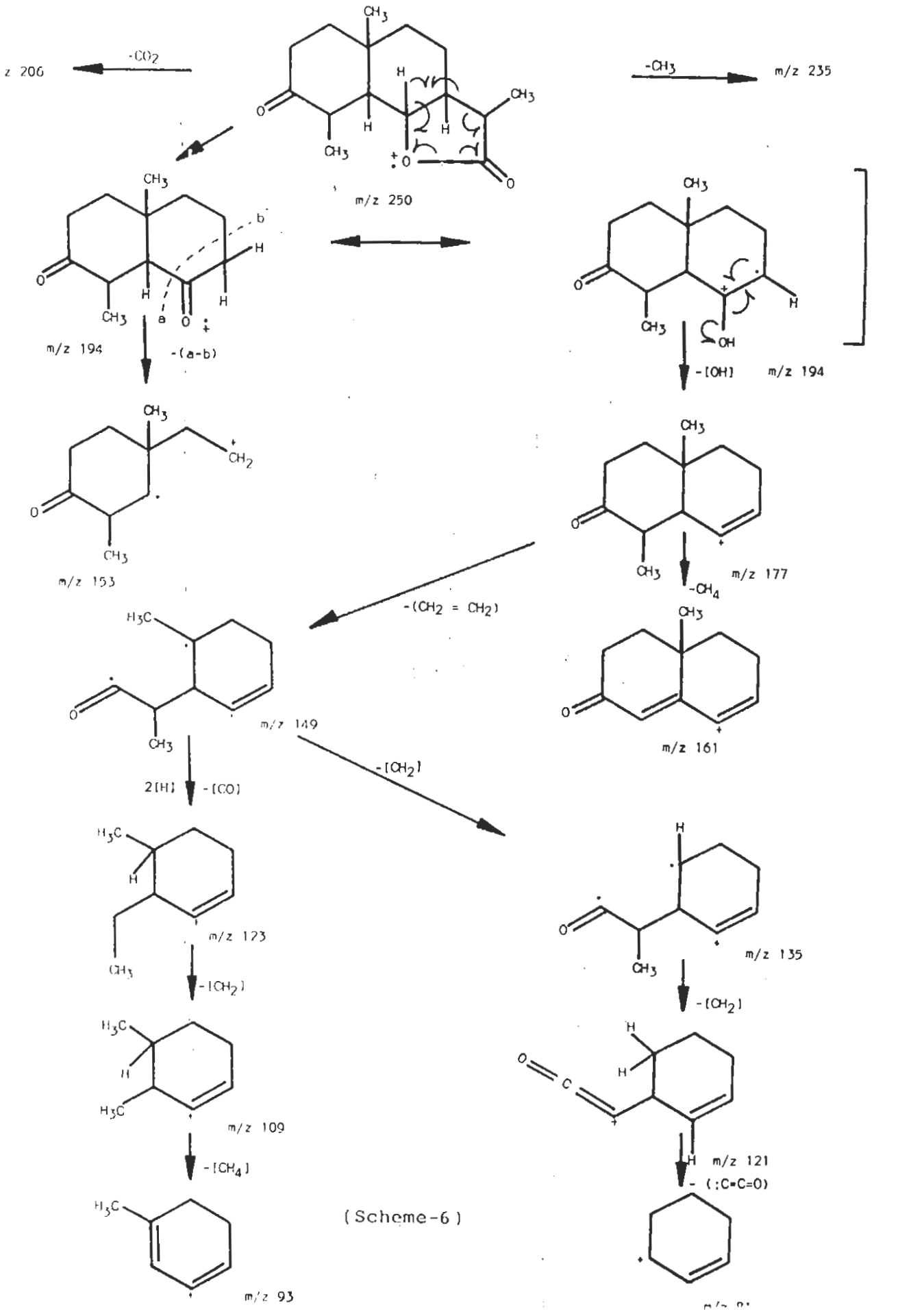


III



IV

Fig.-7


z 206
-CO2
-CH3
m/z 235
m/z 250
m/z 194
-(a-b)
-[OH]
m/z 194
m/z 153
m/z 177
-CH4
-(CH2 = CH2)
m/z 149
m/z 161
2[H]
-[CO]
-[CH2]
m/z 123
m/z 135
-[CH2]
m/z 109
-[CH4]
m/z 121
- (:C=C=O)
m/z 93

(Scheme-6)

The nature of the carbons was established by ^{13}C-DEPT and Hetero COSY experiments (Table-9 and Chart-1).

^{13}C-DEPT spectrum showed three methyl carbons at δ 12.35, 13.81 and 18.29. The four peaks for methylene carbons were present at δ 23.12, 37.20, 40.10 and 40.53. Five peaks at δ 40.40, 44.86, 52.79, 53.43 and 83.02 were present for five methyne groups. The remaining three peaks for quarternary carbons in ^{13}C-NMR spectrum were present at δ 34.21, 178.94 and 211.39.

In Hetero COSY spectrum (Chart-1), three peaks at δ 12.35, 13.81 and 18.29 (C-15, C-13 and C-14 respectively) were coupled with δ 1.20, 1.22 and 1.18 respectively (for three methyls). A peak at δ 37.20 was coupled with two peaks at δ 2.40 and 2.49 (α and β Hydrogens of C-2), similarly another peak at 40.10 was coupled with two peaks at δ 1.62 and 1.68 (α and β Hydrogens of C-9). Two peaks at δ 23.12 and 40.53 showed coupling with δ 1.85 and 1.72 respectivley (for 2xH-8 and 2xH-1 respectively). Five peaks at δ 40.40, 44.86, 52.79, 53.43 and 83.02 were coupled with δ 2.25, 2.50, 1.58, 1.55 and 3.90 respectively (for 1xH-4, 1xH-11, 1xH-5, 1xH-7 and 1xH-6 respectively). Three peaks in ^{13}C-NMR at δ 34.21, 178.94 and 211.39, which were not coupled with other peaks in Hetero COSY spectrum, indicated the three quarternary carbons in compound (39).

Table-9: ^{13}C-^{1}H Hetero nuclear coupling

Carbon No.	Nature of Carbon	^{13}C-nmr (ppm)	^{1}H-^{13}C correlation
1	CH_2	40.53	1.72
2	CH_2	37.20	2.40 and 2.49
3	C = O (Ketone)	211.39	No coupling
4	CH	40.40	2.25
5	CH	52.79	1.58
6	CH-O	83.02	3.90
7	CH	53.43	1.55
8	CH_2	23.12	1.85
9	CH_2	40.10	1.62 and 1.68
10	quart.	34.21	No coupling
11	CH	44.86	2.50
12	C = O (lactone)	178.94	No coupling
13	CH_3	13.81	1.22
14	CH_3	18.29	1.18
15	CH_3	12.35	1.20

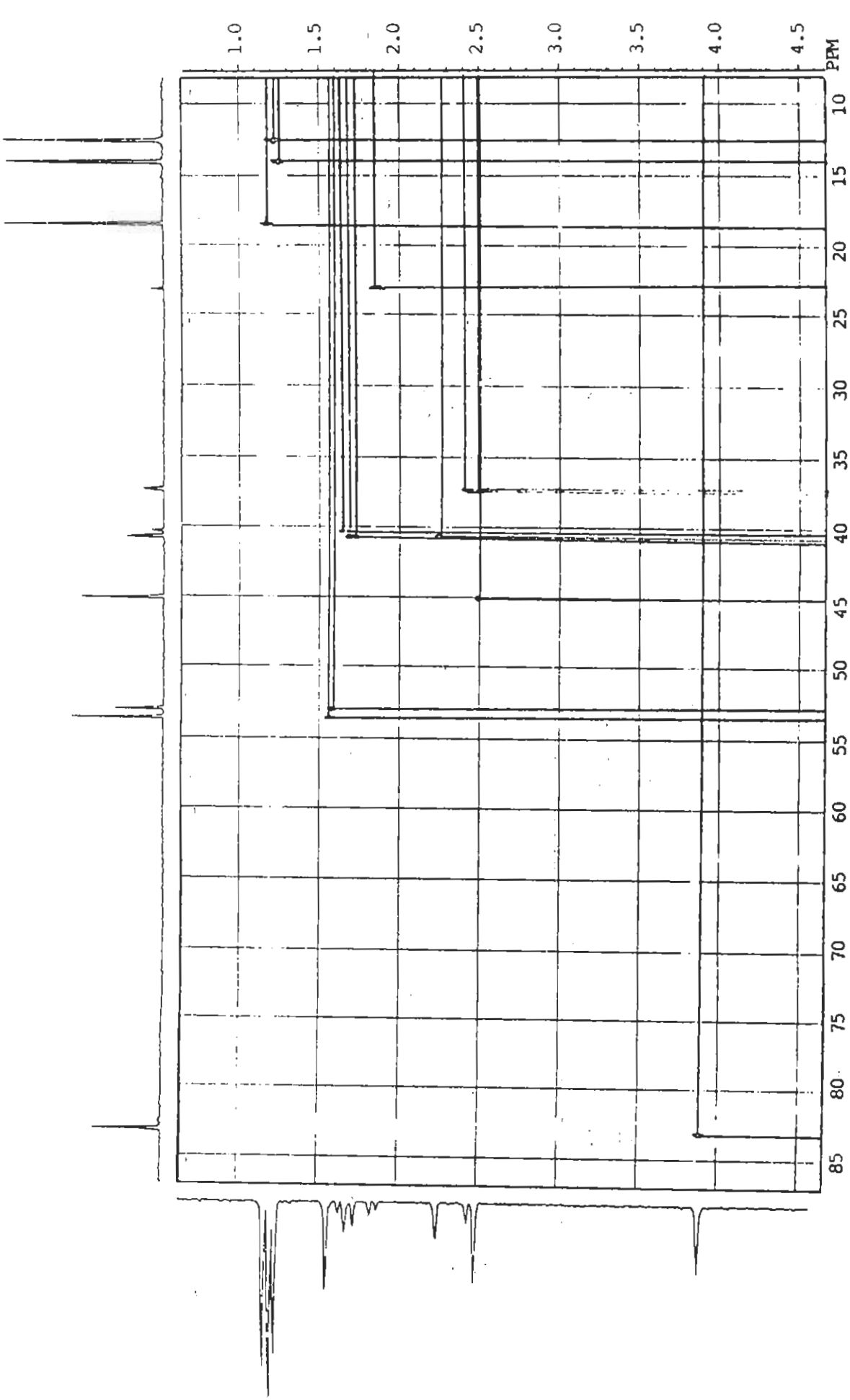


Chart-1: ^{13}C-^{1}H Hetero nuclear Coupling

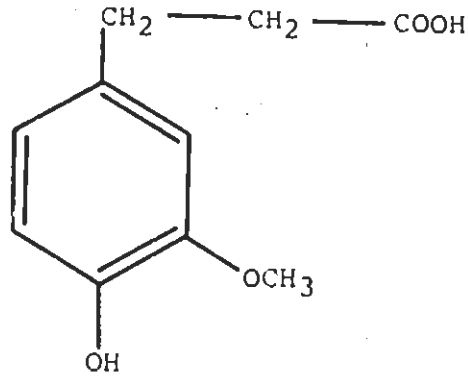
3.7 α,β-Dihydroferulic acid (40)

α,β-Dihydro ferulic acid (40) was isolated from alcoholic extracts of stem bark of the plant. Compound (40) is the α,β-dihydro, meta methoxy derivative of cinnamic acid, which is a part (fragment) of all the spermidine alkaloids isolated from this plant or from *Capparis decidua* [48,49,50], a plant of the same family.

The biosynthesis (discussed earlier) of spermidine alkaloids suggested that the different derivatives of cinnamic acid are formed at initial stage in the plant, two units of such compounds become coupled through an ether linkage and finally spermidine moiety make the amide bonds with carboxylic groups of cinnamic acids residues.

The dried ethanolic extracts of stem barks was partitioned between ethyl-acetate and water at slightly acidic pH. The ethyl-acetate layers were combined, washed with water, and concentrated. The gummy material thus obtained was allowed to solvent separation.

After separation of methanol, methanol-acetone and acetone soluble part, the material was allowed to dissolve in benzene in cold conditiones and finally in hot benzene. The hot benzene soluble part furnished a pale yellow solid, which on recrystallization from a mixture of benzene and methanol produced colourless crystals of α,β-dihydro ferulic acid (40), M.P. 32-34°C. These crystals were partially soluble in methanol and completely soluble in ether,



(**40**)

chloroform and in a mixture of benzene and methanol (1:1).

The EI and FD mass spectra showed the molecular ion peak at m/z 196. The compound (40) gave a positive test for phenol with ferric chloride reagent and for carboxylic group with sodium bicarbonate.

The ultra-violet spectrum displayed maxima at 210 and 291 nm. The IR spectrum had bands at 3300-3450 (br. OH), 1640 (carboxylic group) and 1610 cm^{-1} (aromatic ring).

A signal after δ 10.0 in ^{1}H-NMR spectrum and at δ 205.52 in ^{13}C-NMR spectrum proved the carboxylic group in the compound. Another peak at δ 3.63 in ^{1}H-NMR and at 59.69 in ^{13}C-NMR showed the presence of a methoxy group. Two triplets centered at δ 1.87 and 1.91, and the absence of signals of olefinic protons below δ 4.0 indicated that the α and β carbons of carboxylic group are saturated, it is also confirmed by the absence of signals between δ 120 to 140 and presence of two signals at δ 23.60 and δ 27.29 in the ^{13}C-NMR spectrum (Table-10).

The aromatic signals are compareable with ferulic acid. The molecular ion peak at m/z 196 in mass spectrum corresponding to formula $C_{10}H_{12}O_4$, and all other information from ^{1}H-NMR and ^{13}C-NMR spectra indicated that compound (40) is α,β-dihydro derivative of ferulic acid which has the molecular weight 194 and formula $C_{10}H_{10}O_4$. Trans ferulic acid already has been isolated [40] from this plant.

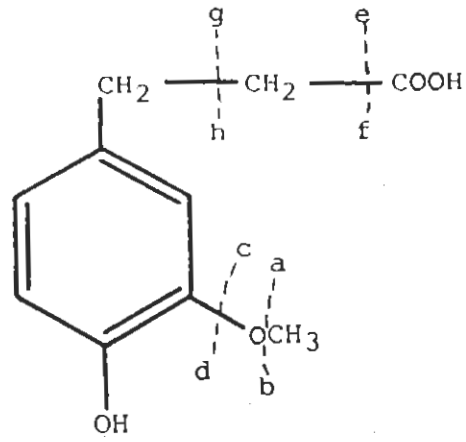
TABLE - 10

^{13}C-NMR chemical shifts for α,β-Dihydro Ferulic acid (40)
($CDCl_3$, 100 MHz)

arbon	ppm	Carbon	ppm
1	205.52	6	164.72
2	27.29	7	148.72
3	23.60	8	116.31
4	118.27	9	116.13
5	120.46	$O\underline{C}H_3$	59.69

he status of each carbon confirmed through DEPT experiment.

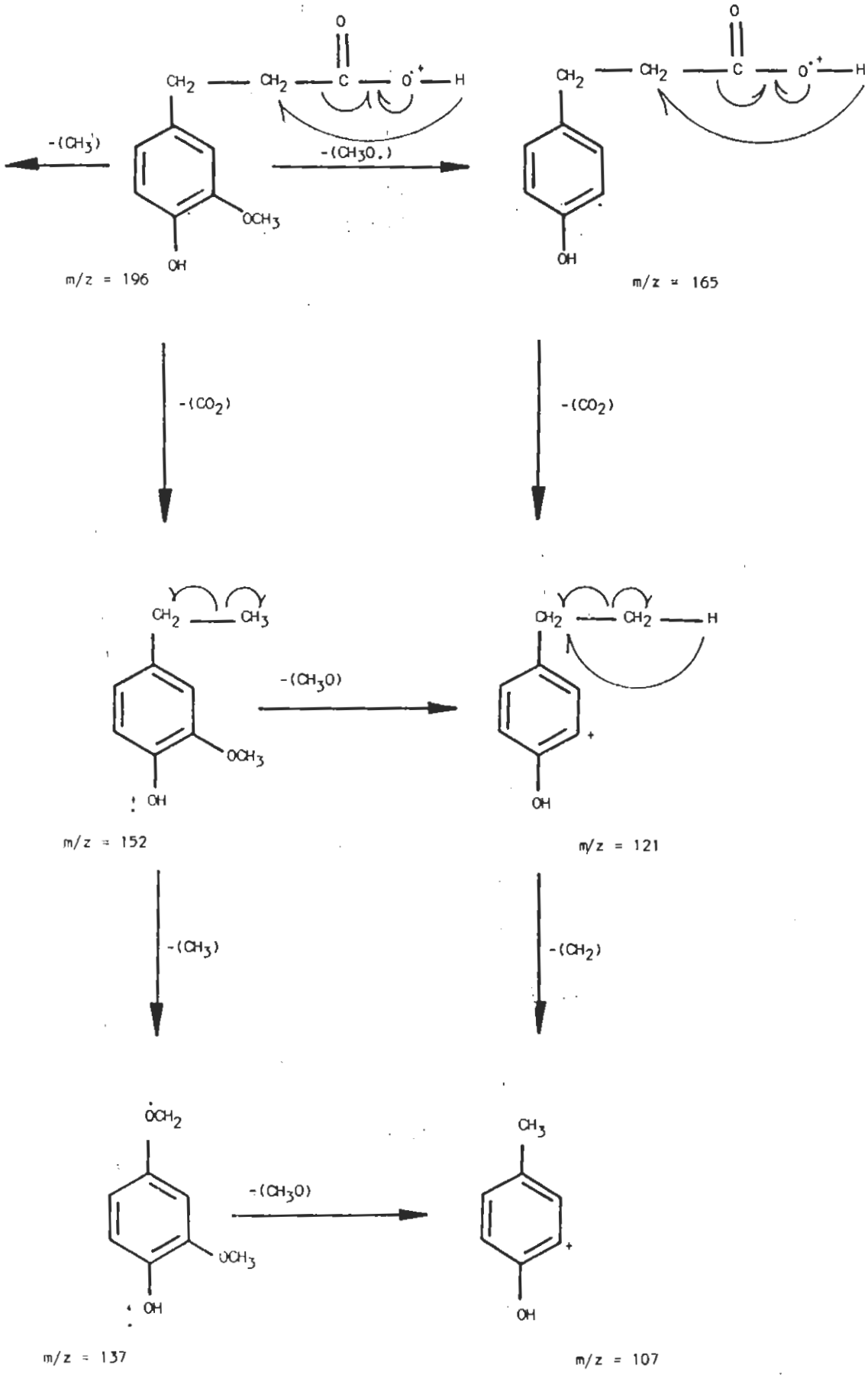
The mass spectrum fragmentation pattern (Scheme-7) for compound also proved the structure 40. A peak at m/z 181 shows the loss of methyl group and a peak at m/z 165 (Base peak) indicates the loss of methoxy unit. Two peaks at m/z 152 and 121 show the loss of CO_2 from m/z 196 and 165 respectively. The relative intensity of each peak is indicated in Fig.-8.



	cleavage	m/z	Formula for ion	(rel.int.%)
$^{+}$	-	196	$C_{10}H_{12}O_4$	46
$^{+}$-CH_3	(a-b)	181	$C_9H_9O_4$	12
$^{+}$-CH_3O	(c-d)	165	$C_9H_9O_3$	100
$^{+}$-CO_2	(e-f)	152	$C_9H_{12}O_2$	8
$^{+}$-CH_2COOH	(g-h)	137	$C_8H_9O_2$	30
$^{+}$-CO_2-CH_3O	(a-b and e-f)	121	C_8H_9O	22
$^{+}$-CH_2COOH-CH_3O	(a-b and g-h)	107	C_7H_7O	16

Fig.-8

81


-(CH3·)
-(CH3O·)
m/z = 196
m/z = 165
-(CO2)
-(CO2)
-(CH3O)
m/z = 152
m/z = 121
-(CH3)
-(CH2)
-(CH3O)
m/z = 137
m/z = 107

(Scheme-7)

4 EXPERIMENTAL

4.1 General Notes

4.1.1 Instrumentation and chromatographic materials

The ultra violet spectra were scanned on Shimadzu UV 240 spectrophotometer and the Infra red spectra were recorded on JASCO A-302 spectrophotometer. The ^{1}H and ^{13}C-NMR were scanned on Bruker AM-300 and Bruker AM-400 Nuclear Magnetic Resonance Spectrometer, using TMS as an internal standard.

The mass spectra were measured on Varian MAT-112 and MAT-312 spectrometer connected to MAT-188 data system and PDP 11/34 computer system. All the melting points were determined on a Gallenkamp melting point apparatus and are uncorrected.

4.1.2 X-Ray analysis of cadabicine

All crystallographic calculations were done on a PRIME 850 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were: REDUCE and UNIQUE, data reduction programs by M.E. Leonowicz, Cornell University 1978; MULTAN 78 and MULTAN 80, systems of computer programs for the automatic solution of crystal structures from X-ray diffraction data written by P. Main, S.E. Hull, L. Lessinger, G. Germain, J.P.De-clearcq and M.M. Wollfson, University of York England, 1978 and 1980; BLAS78A, and anisotropic block diagonal least squares refinement written by K. Hirotsu and

E. Arnold, Cornell University 1980; PLUTO 78, and an isotropic block diagonal least squares refinement written by K. Hirotsu and E. Arnold, Cornell University 1980; PLUTO 78, a crystallographic illustration program by W.D.S. Motherwell, Cambridge Crystallographic Data Centre 1978; and BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu, Cornell University, 1978. Crystallographic parameters have been deposited with the Cambridge Crystallographic Data File, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, England and are available from them.

Flash column chromatography was performed on Eyala Flash Chromatography CF-10 model, using silica gel 60, 230-400 mesh size (E. Merck).

Thin layer chromatography (TLC) was performed on silica gel F_{254} precoated aluminium cards (E. Merck), while for column chromatography silica gel 60 (70-230 mesh, E. Merck) was used.

For confirming the purity of the samples high performance thin layer chromatography (HPTLC) silica gel 60, F_{254} precoated, glass plates, (nano tlc, E. Merck) were used. Solvents used for different chromatographic purposes were mostly supplied by E. Merck.

The alkaloids were detected on UV lamp (254 nm) and by spraying with Dragondorff's reagent.

The sesquiterpenes were detected on UV lamp (254 nm), and by spraying with

Ehrlich reagent.

4.1.3 Plant Material

The stem bark of *Cadaba farinosa* was collected from the Karachi University Campus and identified by a taxonomist of the Botany Department of the University.

The stem bark was removed, washed with water, chapped into small pieces and dipped into ethyl alcohol. After 24 hours the material was allowed to disperse in solvent with the help of ultra-disperser, and kept for 15 days. The solvent after filtration was evaporated which yielded a thick gummy material.

4.2 Isolation of cadabicine (1)

The residue obtained on evaporation of the alcoholic extraction was partitioned between ethyl-acetate and water at pH-6. The aqueous layer was then basified with ammonia (pH-10) and extracted repeatedly with $CHCl_3$. The alkaloid containing $CHCl_3$ layers were combined, washed, dried and concentrated. This solution on storing at 0° deposited crystals of cadabicine. The crystals were washed with ether and chloroform. After recrystallization from hot methanol pure cadabicine (300 mg from 31 kg barks) was obtained. M.P. 270-272° decomp.).

A solution of cadabicine in methanol was allowed to react with a solution of neutral ferric chloride solution as a result of which, dark brown precipitates of ferric cadabicinate were obtained, which showed the presence of phenolic group. Due to the absence of any chiral centre, the cadabicine (1) is optically inactive.

Spectral data of cadabicine (1):

UV (MeOH): λ_{max} 217 ($\log \varepsilon$ =3.55), 284 ($\log \varepsilon$ =3.76) and 310 nm (shoulder).

IR (KBr): ν_{max} 3400-3200 (br., OH and NH), 1655 (α,β-unsaturated amide) and 1600 cm^{-1} (aromatic ring).

^{1}H-NMR (DMSO-d_6, 400 MHz, Inter.stand.TMS): δ 1.22-1.84 (m, 6H, for 2xH-12,

dH-16 and 2xH-17), 2.70-3.12 (m, 8H, for 2xH-11, 2xH-13, 2xH-15 and 2xH-18), 84, 6.63, 7.12 and 7.44 (each d, 1H, J=15.5 Hz, olefinic protons of trans nnamic acid residues), 6.36 (d, 1H, J=1.8 Hz, for H-27), 6.95 (dd, 1H, J=8.0 and 1.8 Hz, for H-24), 7.18, 7.64 (each d, 2H, J=7.8 Hz, for para substituted benzene ring), 7.21 (d, 1H, J=8.5 Hz, for H-25).

^{13}C-NMR (DMSO-d_6, 100 MHz): δ C-1) 151.80 3) 155.15 4) 122.33[a] 5) 129.49 133.51[b] 7) 137.62/137.70[*c] 8) 124.90/124.44[*d] 9) 164.00 11) 45.09/64.21[*]) 27.01/27.70[*] 13) 38.21/38.40[*] 15) 35.31/35.91[*] 16) 26.40/26.51[*] 17) 25.89) 42.43/42.72[*] 20) 164.78/164.88[*] 21) 124.66/124.71[*d] 22) 136.90/137.05[*c]) 133.50[b] 24) 123.89 25) 110.70 26) 141.10 27) 122.21[a] 28) 122.30[a] 29) 9.51.

b,c,d = Assignments may be interchanged.

= Doubling of signals due to confermers with regard to amide bond.

HRMS: m/z 435.2145 (calcd. for $C_{25}H_{29}N_3O_4$, 435.2157), 407.2002 (calcd. for $_1H_{27}N_2O_4$, 407.1970), 349.1327 (calcd. for $C_{21}H_{19}NO_4$, 349.1313), 307.0798 alcd. for $C_{18}H_{13}NO_4$, 307.0844), 291.0661 (calcd. for $C_{18}H_{11}O_4$, 291.0657), 1.0812 (calcd. for $C_{17}H_{12}O_2$, 264.0786), 250.1309 (calcd. for $C_{13}H_{18}N_2O_3$,).1317), 235.0772 (calcd. for $C_{16}H_{11}O_2$, 235.0758), 234.1371 (calcd. for $_3H_{18}N_2O_2$, 234.1368), 234.1138 (calcd. for $C_{13}H_{16}NO_3$, 234.1130), 218.1184 alcd. for $C_{13}H_{16}NO_2$, 218.1181), 205.1161 (calcd. for $C_{12}H_{15}NO_2$, 205.1102), .1161 ($C_{12}H_{15}NO$, 189.1153), 146.0359 (calcd. for $C_9H_6O_2$, 146.0367), 131.0512 alcd. for C_9H_7O, 131.0496).

.3 Acetylation of cadabicine

Cadabicine 1 (20 mg) was allowed to react with acetic anhydride (2 ml) in e presence of pyridine (0.5 ml). The mixture was warmed slighly and kept ernight. Ice was added to the reacted mixture and immediately a colourless ecipitate were formed. The precipitate were filtered and dired. It was aringly soluble in cold methanol, but soluble in hot methanol. The hot lution of methanol deposited shining rods of cadabicine diacetate 2 (M.P. 5-268°).

ectral data of cadabicine diacetate (2):

UV (MeOH): λ_{max} 210 (log ε=4.12), 273 (log ε=4.02) and 305 nm (soulder).

IR (KBr): ν_{max} 3400 (br., NH), 1760 (phenolic acetate), 1745 (N-acetate), 5 (α,β-unsaturated amide) and 1590 cm^{-1} (aromatic ring).

^{1}H-NMR (DMSO-d_6, 400 MHz): δ 1.95 and 1.97 (each s, togather integration, for N-acetate and δ 2.34 (s, 3H) for phenolic acetate. Other signals were ost similar to compound 1.

^{13}C-NMR (DMSO-d_6, 100 MHz): δ C-1) 151.81 3) 155.16 4) 122.31[a] 5) 129.54 133.54[b] 7) 137.62/137.72*[c] 8) 124.44/124.54*[d] 9) 164.09[e] 11) 45.00/47.42* 27.04/27.73* 13) 38.26/38.44* 15) 35.36/35.94* 16) 26.48/26.58* 17) 25.89 42.43/43.72* 20) 164.78/164.88*[e] 21) 124.66/124.70*[d] 22) 136.98/137.06*[c]

) 133.52[b] 24) 124.01 25) 110.78/110.81* 26) 140.33 27) 122.22[a] 28) 122.31[a]
) 129.54 $O\underline{C}OCH_3$ 168.54, $OCO\underline{C}H_3$ 20.41, $N\underline{C}OCH_3$ 169.01/169.04*, $NCO\underline{C}H_3$
.08/21.20*

b,c,d,e = These assignments are interchangeable.

The doubling of peaks is due to Z and E confermers, with regards to amide nd.

Mass: m/z 519 (Mol. ion peak for $C_{29}H_{33}N_3O_6$), 447 (M^+-CH_2CO) and 434 $^+-CH_2CO-CH_3CO$). Other peaks were same as in case of compound 1.

.4 Isolation of cadabicine methyl ether (7)

After the separation of crystals of cadabicine (1), the crude alkaloidal ıterial from the stem bark was partitioned between H_2O and CH_2Cl_2 at pH-11. ıe alkaloid containing CH_2Cl_2 layers were combined, washed with water, dried ıd finally converted into a dark brown gummy material. This mixture was ıromatographed by flash column chromatography and eluted with $CHCl_3$ with ıcreasing polarity of methanol. The mixture was partially separated into two ·rtions, the less polar alkaloidal mixture and relatively more polar kaloidal mixture.

The separated more polar alkaloidal mixture was subjected to silica gel lumn chromatography, using CH_2Cl_2, CH_3OH and NH_3 (90:9:1) as mobile phase, ich furnished yellowish crystals of cadabicine methyl ether (7). :rystallization from methanol yielded pale yellow shining crystals of pure labicine methyl ether (7) M.P. 190-192°C.

This compound was readily soluble in MeOH, and gave negative test for nol with ferric chloride reagent. It was also optically inactive.

Spectral data of cadabicine methyl ether (7):

UV (MeOH): λ_{max} 220 (log ε=2.84), 282 (log ε=2.78) and 310 nm (shoulder).

IR (KBr): ν_{max} 3400-3200 (br. NH), 1660 (α,β-unsaturated amide) and 1600

n^{-1} (aromatic ring).

^{1}H-NMR (DMSO-d_6, 400 MHz): δ 1.22-1.71 (m, 6H, 2xH-12, 2xH-16 and 2xH-17), .15-3.39 (m, 8H, 2xH-11, 2xH-13, 2xH-15 and 2xH-18), 3.56 (sharp s, 3H, OCH_3), .83, 6.59, 7.14 and 7.48 (each d, 1H, J=15.5 Hz, for olefinic protons of trans innamic acid residues), 6.42 (d, 1H, J=2.2 Hz, H-27), 6.97 (d, 1H, J=7.7 Hz, -25), 7.02 (dd, 1H, J=7.8 Hz and 2.0 Hz, H-24), 7.19 and 7.84 (each d, 2H, :7.6 Hz, para disubstituted benzene ring).

^{13}C-NMR (DMSO-d_6, 100 Hz): δ C-1) 148.71 3) 155.41 4) 123.34 5) 129.26 6) 3.72 7) 137.54/137.62*a 8) 125.82/125.97*b 9) 164.61c 11) 46.05/46.16*d 12) .96/26.13*e 13) 38.63/39.40 15) 35.28/35.95* 16) 22.29/22.66* 17) 25.63 18) .93/44.24*d 20) 164.94/165.08*c 21) 125.43/125.77*b 22) 137.79/138.14*a 23) 8.8 24) 121.76 25) 112.08 26) 140.12 27) 118.94 28) 120.26 29) 9.33/129.45*, OCH_3 56.22.

b,c,d,e = Assignments may be interchanged.

= Doubling of signals due to Z and E confermers.

HRMS: m/z 449.2282 (calcd. for $C_{26}H_{31}N_3O_4$, 449.2314), 421.2132 (calcd. for $_3H_{29}N_2O_4$, 421.2127), 419.2206 (calcd. for $C_{25}H_{29}N_3O_3$, 419.2208), 407.2003 alcd. for $C_{24}H_{27}N_2O_4$, 407.1970), 363.1466 (calcd. for $C_{22}H_{21}NO_4$, 363.1470),).1326 (calcd. for $C_{21}H_{19}NO_4$, 349.1313), 321.1011 (calcd. for $C_{19}H_{15}NO_4$, .1001), 307.0911 (calcd. for $C_{18}H_{13}NO_4$, 307.0844), 306.0876 (calcd. for $_3H_{14}O_4$, 306.0892), 292.0698 (calcd. for $C_{18}H_{12}O_4$, 292.0735), 278.1001 (calcd.

or $C_{18}H_{14}O_3$, 278.0942), 264.0813 (calcd. for $C_{17}H_{12}O_3$, 264.0786), 264.1502 calcd. for $C_{14}H_{20}N_2O_3$, 264.1473), 250.1013 (calcd. for $C_{17}H_{14}O_2$, 250.0993), 18.1291 (calcd. for $C_{14}H_{18}NO_3$, 248.1286), 248.1552 (calcd. for $C_{14}H_{20}N_2O_2$, 18.1524), 236.0875 (calcd. for $C_{16}H_{12}O_2$, 236.0837), 232.1341 (calcd. for $_{14}H_{18}NO_2$, 232.1337), 204.1071 (calcd. for $C_{12}H_{14}NO_2$, 204.1024), 189.1160 calcd. for $C_{12}H_{15}NO$, 189.1153), 146.0361 (calcd. for $C_9H_6O_2$, 146.0367), 31.0514 (calcd. for C_9H_7O, 131.0496).

4.5 Formation of N-acetyl cadabicine methyl ether (12)

20 mg of cadabicine methyl ether (7) were dissolved in about 0.5 ml of pyridine and allowed to react with acetic anhydride for overnight at normal temperature. On addition of ice to the reacted mixture, the solution become turbid. The acetyl derivative thus formed was extracted with chloroform in the presence of ammonia (pH=11). The chloroform layer was separated and evaporated which yielded an amorphous solid. It was recrystallized from hot methanol. The colourless micro crystals of N-acetyl cadabicine methyl ether (12) melted at 180-182°C.

Spectral data of N-acetyl cadabicine methyl ether (12):

UV (MeOH); λ_{max} 220 (log ε=2.84), 283 (log ε=2.72) and 310 nm (shoulder).

IR (KBr); ν_{max} 3400 (br.-NH), 1740 (N-acetate), 1660 (α,β-unsaturated amide) and 1590 cm^{-1} (aromatic ring).

^{1}H-NMR (DMSO-d_6, 400 MHz); δ 1.93 and 1.95 (each s, totally for 3H, N-acetate), 1.19-1.52 (m, 6H, 2xH-12, 2xH-16 and 2xH-17), 3.19-3.42 (m, 8H, H-11, 2xH-13, 2xH-15 and 2xH-18), 3.57 (s, 3H, OCH_3), 5.86, 6.62, 7.32 and 7.58 (each d, 1H, J=15.5 Hz, olefinic protons of trans cinnamic acid residues), 7.48 (d, 1H, J=2.5 Hz, H-27), 6.73 (dd, 1H, J=2.6 and 7.6 Hz, H-24), 7.05 (d, 1H, J=7.7 Hz, H-25), 7.18 and 7.49 (each d, 2H, J=7.8 Hz, para disubstituted benzene ring).

^{13}C-NMR (DMSO-d_6, 100 MHz); δ C-1) 149.16 3) 153.28 4) 122.24 5) 122.23 6) 130.14 7) 140.38/140.49*a 8) 125.76/125.89*b 9) 166.62c 11) 46.08/46.22*d 12) 27.74/27.88*c 13) 37.18/38.16* 15) 35.62/35.74*16) 23.45/23.50* 17) 26.85e 18) 45.07/45.62*d 20) 168.24/169.13*c 21) 126.24/126.44*b 22) 140.13/140.32*a 23) 128.34 24) 122.84 25) 114.08 26) 147.34 27) 118.70 28) 125.85 29) 129.28/129.46*, O$\underline{C}H_3$ 52.82, NO$\underline{C}CH_3$ 170.84, NOC$\underline{C}H_3$ 21.82.

a,b,c,d,e = Assignments may be interchanged.

* = Doubling of peaks due to Z and E conformers.

HRMS; m/z 491.2418 (calcd. for $C_{28}H_{33}N_3O_5$, 491.2420), 461.2327 (calcd. for $C_{27}H_{31}N_3O_4$, 461.2314), 449.2288 (calcd. for $C_{26}H_{31}N_3O_4$, 449.2314), 419.2194 calcd. for $C_{25}H_{29}N_3O_3$, 419.2208), 306.1574 (calcd. for $C_{16}H_{22}N_2O_4$, 306.1579), 90.1626 (calcd. for $C_{16}H_{22}N_2O_3$, 290.1630), 264.1501 (calcd. for $C_{14}H_{20}N_2O_3$, 64.1473), 248.1551 (calcd. for $C_{14}H_{20}N_2O_2$, 248.1524).

ll other peaks were similar to compound (7).

4.6 Synthesis of cadabicine methyl ether

4.6.1 Formation of 3-(4-formyl phenoxy) -4-methoxy Benzaldehyde (17)

p-Bromobenzaldehyde (15) and Isovanilline (m-hydroxy-p-methoxy benzaldehyde) (16) in 1:1 molar ratio were allowed to react in boiling N,N-dimethyl acetamide, in the presence of Cu_2O (1 m mole of bromobenzaldehyde, 1 m mole of Isovanilline and 0.5 m mole of Cu_2O in 20 ml of solvent). The mixture was heated continuously for 72 hours under nitrogen atmosphere with stirring. During the reaction, the mixture changes its colour from red to green and finally blackish brown. It was then cool down to room temperature and filtered of through a small layer of silica gel present on a filter paper in a funnel. The residue with silica gel was washed with dry ether, several times. The filtrate was diluted with water and partitioned between ether and H_2O. The ether layers were separated, combined and evaporated, it yielded a thick dark brown semi solid material.

The material thus obtained was chromatographed on a column packed with silica gel, by using ether with increasing amounts of chloroform, as mobile phase. It furnished colourless crystals of pure diphenyl ether (17).

Spectral data of Compound (17):

IR ($CHCl_3$); λ_{max} 1700-1710 (two overlapped bands for two "CHO" groups) and 590 cm^{-1} (aromatic ring).

^{1}H-NMR ($CDCl_3$, 400 MHz); δ 3.49 (s, 3H, $OC\underline{H}_3$), 8.90 (s, 1H, $C\underline{H}O$) and 9.10 (s, 1H, $C\underline{H}O$). Some other peaks also observed for aromatic protons.

HRMS; m/z 256.0756 (calcd. for $C_{15}H_{12}O_4$, 256.0735), 227 (M^+-CHO) and 198 (M^+-2xCHO). Two other fragments at m/z 185 and 152 were also observed, which were due to 15 and 16 formed as a result of the cleavage of ether bond and hydrogen transfer.

4.6.2 Formation of Methyl cinnamate, methyl ferulate ether (18)

A phosphonium yild [$(C_6H_5)_3$-P=CH-CO-OCH_3] was prepared by the action of triphenylphosphine [$(C_6H_5)_3$-P] on α-bromomethyl acetate (Br-CH_2-CO-OCH_3). The triphenyl phosphine was dissolved in dry benzene and then α-bromomethyl acetate was added dropwise with contineous stirring. The pale yellow precipitates thus formed were filtered and dried. They were treated with a solution of K_2CO_3 in a mixture of water and MeOH, which furnished the shining colourless crystals of ylid.

Compound 17 was allowed to react with ylid in benzene (Wittig reaction) at 70°C. The residue thus obtained was partitioned between water and ethyl acetate. The ethyl acetate layers were separated and evaporated which yielded a crystalline material containing compound 18 with small amount of triphenyl phosphorous oxide. The mixture was dissolved in ethyl acetate and ether was added dropwise, as the result of which a turbid solution was formed which was

kept for some time. The triphenyl phosphine oxide compound crystallized out and compound 18 remained in solution. After evaporation the material was subjected to short-column chromatography (silica gel) and eluted with a mixture of ethyl acetate and ether, which furnished colourless crystals of compound 18.

Spectral data of Compound (18):

IR ($CHCl_3$); ν_{max} 1720, 1725 (for two ester groups) and 1600 cm^{-1} (aromatic ring).

^{1}H-NMR ($CDCl_3$, 300 MHz); δ 3.75, 3.77 (each s, 3H, $COOCH_3$), 3.82 (s, 3H, XCH_3), 6.24, 6.32, 7.52, 7.63 (each d, 1H, J=15.6 Hz, for olefinic protons of two trans cinnamic acid residues), 6.91 (dd, 1H, J=3.1 and 9.0 Hz, meta and ortho coupling), 7.00 (d, 1H, J=9.0 Hz, ortho coupling), 7.21 (d, 1H, J=3.1 Hz, meta coupling), 7.31, 7.63 (d, 2H, J=9.0 Hz, ortho coupling).

HRMS; m/z 368.1262 (calcd. for $C_{21}H_{20}O_6$, 368.1259), 337 (M^+-OCH_3), 310 (M^+-$COOCH_2$), 279 (M^+-OCH_3-$COOCH_2$), 252 (M^+-2x$COOCH_2$), 221 (M^+-OCH_3-2x$COOCH_2$), 177, 61, 147, 131 and 103.

5.3 Formation of Ether of -p-cinnamic acid-m-ferulic acid (19)

The compound 18 was hydrolysed with alkali in a mixture of 70% methanol and 30% water on strong heating. After cooling the reacted mixture was acidified, as a result of which white precipitates of compound 19 were obtained. On

recrystallization from a mixture of benzene and methanol (8:2) yielded colourless crystals of compound 19. It gave effervesence with $NaHCO_3$ solution.

Spectral data of Compound (19):

IR ; ν_{max} 1700 and 1695 (for two carboxylic groups) and 1605 cm^{-1} (aromatic ring).

^{1}H-NMR ($CDCl_3$, 300 MHz); δ 9.97 (2xCOOH).

MASS; m/z M^+ (not observed), 296 (M^+-CO_2), 266 ($M^+-CO_2-CH_2O$), 252 (M^+-2CO_2), 222 ($M^+-2CO_2-CH_2O$).

.6.4 Formation of Thiazolidine compound (20)

'reparation of thallium salt of thiazoline-2-thione:

A solution of thiazolidine-2-thione (4.56 g) in methanol (120 ml) was added ropwise to a solution of thallium (I) acetate (10.52 g) in methanol (400 ml) uring 15 min. with continuous stirring, during which time a yellow precipitate ppeared. Stirring was continued on magnetic stirrer for further four hours at oom temperature. The precipitate was filtered off and washed with large amount f methanol and ether to give a yellowish shining powder of thallium salt $(C_3H_4NS_2)^- Tl^+$].

Preparation of Compound (20):

The diacid 19 was refluxed with thionyl chloride under dry conditions using a calcium chloride tube in benzene solvent for one hour. The solvent was evaporated at high vacuume. The thick oily acid chloride thus obtained was allowed to react with thallium (I), salt of thiazolidine-2-thione with continuous stirring in THF at room temperature under nitrogen atmosphere for 20 hours. A dirty white solid (TlCl) settled down. The solution was filtered off. The residue washed with CH_2Cl_2. The filtrate alongwith washing was partitioned between CH_2Cl_2 and water. The CH_2Cl_2 layer was separated again washed with water, and evaporated. The gummy material thus obtained was recrystallized from $CHCl_3$, which yielded compound 20 as micro crystals.

Spectral data of Compound (20):

IR ($CHCl_3$); ν_{max} 1680 (CO-N), 1600 cm^{-1} (aromatic ring).

^{1}H-NMR ($CDCl_3$, 400 MHz); δ 3.65 (s, 3H, OCH_3), 3.37, 3.56, 3.68, 3.98 (each t, 2H, four methylene of two thiazolidine moieties. The peaks at aromatic region were similar to compound 18.

4.6.5 Formation of Cadabicine methyl ether from thiazolidine compound (20)

A solution of thiazolidine compound (20) in CH_2Cl_2 (1 m mole in 100 ml solvent) was added dropwise using a micro droping funnel into a solution of

permidine (1.5 m mole) in 100 ml of CH_2Cl_2 during 3 hours, under nitrogen with onteneous stirring at room temperature. The mixture was stirred for further hours under the same conditions. After evaporation of the solvent under acuume, the residue was washed with CH_2Cl_2. The gummy material thun obtained is separated on RP_{18} plates (HPTLC), using $CHCl_3$, MeOH, H_2O and NH_3 7:2:1.5:0.5) system. Few mg of cadabicine methyl ether (7) were obtained ongwith minor amount of its regio isomer.

The synthetic cadabicine methyl ether when treated with acetic anhydride the presence of pyridine at room temperature afforded N-acetyl cadabicine thyl ether (12), M.P. 180-182°C.

The spectral data of synthetic cadabicine methyl ether were identical with tural cabicine methyl ether (7). Similarly M.P. and spectral data of N-acetyl rivatives were also similar.

4.7 Isolation of Cadabicine diacetate (21)

The ethanolic extract of stem bark was partitioned between hexane and water at slightly acidic pH. The aqueous layer was basified with ammonia to pH-9 and extracted with chloroform. The alkaloid-containing chloroform layers were combined, washed with water, dried and concentrated. The thick material thus obtained deposited crystals of cadabicine (1). After removal of crystals of cadabicine, the material was subjected to column chromatography (silica gel), using chloroform as mobile phase, with gradually increasing polarity of methanol. The fractions thus obtained yielded a mixture of cadabicine and cadabicine diacetate (21).

The short column chromatography (Silica gel) of the mixture, with $CHCl_3$, furnished cadabicine diacetate (21) as yellowish brown amorphous solid, which on recrystallization from hot methanol produced colourless shining, needle like crystals, M.P. 265-267°. The crystals were readily soluble in hot methanol and also in a mixture of MeOH-C_6H_6 (1:1) at room temperature.

Cadabicine diacetate (21) gave positive test for alkaloid with Dragondorff's reagent, but negative test for phenol with neutral ferric chloride solution. It was optically inactive.

Spectral data of cadabicine diacetate:

UV (MeOH); λ_{max} 210 (log ε=4.12), 273 (log ε=4.08) and a shoulder at 305 nm.

IR (KBr); ν_{max} 3400 (br., NH), 1760 (phenolic acetate), 1745 (N-acetate), 1655 (α,β-unsaturated amide) and 1590 cm^{-1} (aromatic ring).

^{1}H-NMR (DMSO-d_6, 400 MHz, Inter. stand. TMS); δ 1.95-1.97 (two s, total integration 3H, N-$COCH_3$), 2.32 (s, 1H, $OCOCH_3$), 1.23-1.60 (m, 6H, for 2xH-12, 2xH-16 and 2xH-17), 3.00-3.35 (m, 8H, for 2xH-11, 2xH-13, 2xH-15 and 2xH-18), 5.98, 6.52, 7.19, 7.46 (each d, 1H, 15.5 Hz, olefinic protons of two trans cinnamic acid residues), 6.43 (d, 1H, J=1.7 Hz, for H-27), 7.15 (dd, 1H, J=8.1 and 1.7 Hz, for H-24), 7.13, 7.71 (each d, 2H, J=7.8 Hz, for para disubstituted benzene ring), 7.21 (d, 1H, J=8.1 Hz, for H-25).

^{13}C-NMR (DMSO-d_6, 100 MHz); δ C-1) 151.80 3) 155.17 4) 122.33[a] 5) 129.54 6) 133.54[b] 7) 137.62/137.70*[c] 8) 124.43/124.54*[d] 9) 164.10[e] 11) 45.00/47.42* 12) 27.04/27.71* 13) 38.27/38.43* 15) 35.36/35.94* 16) 26.48/26.59* 17) 25.90 18) 42.44/43.71* 20) 164.79/164.87*[e] 21) 124.63/124.69*[d] 22) 136.97/137.06*[c] 23) 133.50[b] 24) 124.01 25) 110.78/110.84* 26) 140.33 27) 122.23[a] 28) 122.30 29) 129.54, $OCOCH_3$ 168.54, $OCOCH_3$ 20.43, $NCOCH_3$ 169.00/169.02*, $NCOCH_3$ 21.10/21.20*.

a,b,c,d,e = Assignments may be interchanged.

* = Doubling of peaks due to Z and E confermers.

HRMS; m/z 519.2684 (calcd. for $C_{29}H_{33}N_3O_6$, 519.2732), 477.2258 (calcd. for $C_{27}H_{31}N_3O_5$, 477.2263), 435.2146 (calcd. for $C_{25}H_{29}N_3O_4$, 435.2157), 407.1963 calcd. for $C_{24}H_{27}N_2O_4$, 407.1970), 349.1321 (calcd. for $C_{21}H_{19}O_4$, 349.1313),

334.1532 (calcd. for $C_{17}H_{22}N_2O_5$, 334.1528), 307.0852 (calcd. for $C_{18}H_{13}NO_4$, calcd. for 307.0844), 292.1409 (calcd. for $C_{15}H_{20}N_2O_4$, 292.1423), 291.0660 (calcd. for $C_{18}H_{11}O_4$, 291.0657), 276.1481 (calcd. for $C_{15}H_{20}N_2O_3$, 276.1473), 276.1241 (calcd. for $C_{15}H_{18}NO_4$, 276.1235), 264.0791 (calcd. for $C_{17}H_{12}O_2$, 264.0786), 260.1293 (calcd. for $C_{15}H_{18}NO_3$, 260.1286), 250.1300 (calcd. for $C_{13}H_{18}N_2O_3$, 250.1317), 235.0771 (calcd. for $C_{16}H_{11}O_2$, 235.0758), 234.1136 (calcd. for $C_{13}H_{16}NO_3$, 234.1130), 234.1370 (calcd. for $C_{13}H_{18}N_2O_2$, 234.1368), 218.1183 (calcd. for $C_{13}H_{16}NO_2$, 218.1181), 205.1131 (calcd. for $C_{12}H_{15}NO_2$, 205.1102), 189.1149 (calcd. for $C_{12}H_{15}NO$, 189.1153), 146.0361 (calcd. for $C_9H_6O_2$, 146.0367), 131.0501 (calcd. for C_9H_7O, 131.0496).

4.8 Isolation of Capparisine (32)

The crude alkaloidal material after separation of crystals of cadabicine was subjected to column chromatography using $CHCl_3$, MeOH and Ammonia (8:1.5:0.5) as mobile phase. The material partially separated into two portions. A polar (slower moving) alkaloidal mixture and non-polar (faster moving) alkaloidal mixture. The polar fraction showed four spots on TLC plates for alkaloids in MeOH-NH_3 (10:0.1) system. These compounds were separated through preparative TLC, using $CHCl_3$, methanol and ammonia (7:2.5:0.5) as mobile solvent system. The upper most band removed from the plates and extracted with $CHCl_3$, which on evaporation afforded light yellow crystals of capparisine (32). M.P. 160-162°C.

Spectral data of Capparisine (32):

UV (MeOH); λ_{max} 218 (log ε=2.71), 282 (log ε=2.91) and a shoulder at 308 nm.

IR (KBr); ν_{max} 3300-3400 (br., NH and OH), 1600 (α,β-unsaturated amide) and 1600 cm^{-1} (aromatic ring).

^{1}H-NMR (DMSO-d_6, 300 MHz); δ 1.17-1.68 (m, 6H, 2xH-12, 2xH-13 and 2xH-17), 3.16-3.41 (m, 8H, 2xH-11, 2xH-14, 2xH-16 and 2xH-18), 5.90, 6.65, 7.15 (each d, 1H, J=15.4 Hz, olefinic protons of two trans cinnamic acid residues), 6.33 (d, 1H, J=2.1 Hz, H-27), 6.55 (dd, 1H, J=9.2 and 2.1 Hz, H-28), 6.86 (d, 1H,

J=9.1 Hz, H-29), 6.91 (d, 1H, J=2.2 Hz, H-4), 7.18 (d, 1H, J=9.0 Hz, H-25), 7.20 (dd, 1H, J=9.0 and 2.1 Hz, H-24).

^{13}C-NMR (DMSO-d_6, 75.43 MHz); δ C-1) 152.10 3) 155.71 4) 138.54 5) 148.11 6) 134.59 7) 137.65 8) 125.49/125.67* 9) 166.01[a] 11) 47.20 12) 25.61[b] 13) 39.10 14) 37.34/37.82* 16) 28.01/28.51* 17) 25.49/25.58*[b] 18) 42.30 20) 165.79[a] 21) 124.11 22) 137.81/138.22* 23) 133.22 24) 123.53 25) 110.23 26) 143.61 27) 142.90 28) 122.05 29) 120.37, $\underline{OCH}_3$ 56.10.

a,b = Assignments may be reversed.

* = Doubling of signals due to Z and E conformers.

HRMS ; m/z 465.2258 (calcd. for $C_{26}H_{31}N_3O_5$, 465.2263), 435.2142 (calcd. for $C_{25}H_{29}N_3O_4$, 435.2157), 366.1697 (calcd. for $C_{22}H_{24}NO_4$, 366.1705), 264.0789 (calcd. for $C_{17}H_{12}O_3$, 264.0783), 263.1391 (calcd. for $C_{14}H_{19}N_2O_3$, 263.1395), 248.1291 (calcd. for $C_{14}H_{18}NO_3$, 248.1286), 247.1442 (calcd. for $C_{14}H_{19}N_2O_2$, 247.1446), 235.1078 (calcd. for $C_{12}H_{15}N_2O_3$, 235.1082), 232.1341 (calcd. for $C_{14}H_{18}NO_2$, 232.1337), 219.1129 (calcd. for $C_{12}H_{15}N_2O_2$, 219.1133), 204.1019 (calcd. for $C_{12}H_{14}NO_2$, 204.1024), 189.0794 (calcd. for $C_{11}H_{11}NO_2$, 189.0789), 161.0597 (calcd. for $C_{10}H_9O_2$, 161.0602), 133.0691 (calcd. for C_9H_9O, 133.0653), 118.0422 (calcd. for C_8H_6O, 118.0418).

4.9 Isolation of Cadabicilone (39)

The residue obtained on evaporation of alcoholic extract of stem bark of the plant was partitioned between $CHCl_3$ and water at pH-6. The chloroform layer, containing neutral portion of the alcoholic extract, was separated and washed with water. The $CHCl_3$ layers were combined and evaporated. The residue thus obtained was a yellowish powder. It was chromatographed on a column of silica gel.

Elution with chloroform:hexane (1:1) yielded an amorphous solid, which on fractional crystallization with a mixture of chloroform and ether (1:1) furnished colourless crystals of cadabicilone (39), M.P. 118-120°C.

Spectral data of Cadabicilone (39):

IR (KBr): ν_{max} 1760 (lactone) and 1700 cm^{-1} (ketone).

^{1}H-NMR ($CDCl_3$, 400 MHz); δ 1.18 (s, 3xH-14), 1.20 (d, J=7.0 Hz, 3xH-15), 1.22 (d, J=7.0 Hz, 3xH-13), 1.55 (m, 1xH-7), 1.58 (dd, J=11 Hz and 5 Hz, 1xH-5), 1.62 (ddd, J=11.5 Hz, 5.5 Hz and 3.1 Hz, 1xH-9β), 1.68 (ddd, J=11.5 Hz, 5.5 Hz and 3.1 Hz, 1xH-9α), 1.72 (m, 2xH-1), 1.85 (m, 2xH-8), 2.25 (m, 1xH-4), 2.40 (ddd, J=12 Hz, 5.5 Hz and 3.0 Hz, 1xH-2β), 2.49 (ddd, J=12 Hz, 5.5 Hz and 3.0 Hz, 1xH-2α), 2.50 (m, 1xH-11), 3.90 (dd, J=11 Hz and 12 Hz, 1xH-6).

^{13}C-NMR ($CDCl_3$, 100 MHz); δ C-1) 40.53, 2) 37.20 3) 211.39 4) 40.40 5) 52.79 6) 83.02 7) 53.43 8) 23.12 9) 40.10 10) 34.21 11) 44.86 12) 178.94 13) 13.81 14) 18.29 15) 12.35.

These assignments also confirmed by DEPT experiment.

^{13}C-^{1}H Hetero nuclear coupling ; δ ^{13}C-NMR (δ ^{1}H-NMR, coupled with) 1) 40.53 (1.72) 2) 37.20 (2.40 and 2.49) 3) 211.39 (no coupling) 4) 40.40 (2.25) 5) 52.79 (1.58) 6) 83.02 (3.90) 7) 53.43 (1.55) 8) 23.12 (1.85) 9) 40.10 (1.62 and 1.68) 10) 34.21 (no coupling) 11) 44.86 (2.50) 12) 178.94 (no coupling) 13) 13.81 (1.22) 14) 18.29 (1.18) 15) 12.35 (1.20).

HRMS; m/z 250.1554 (calcd. for $C_{15}H_{22}O_3$, 250.1568), 235.1321 (calcd. for $C_{14}H_{19}O_3$, 235.1334), 206.1684 (calcd. for $C_{14}H_{22}O$, 206.1670), 194.1290 (calcd. for $C_{12}H_{18}O_2$, 194.1306), 177.1258 (calcd. for $C_{12}H_{17}O$, 177.1279), 161.0941 (calcd. for $C_{11}H_{13}O$, 161.0961), 153.1271 (calcd. for $C_{10}H_{17}O$, 153.1279), 149.0942 (calcd. for $C_{10}H_{13}O$, 149.0966), 135.0791 (calcd. for $C_9H_{11}O$, 135.0809), 123.1166 (calcd. for C_9H_{15}, 123.1173), 121.0648 (calcd. for C_8H_9O, 121.0653), 109.1014 (calcd. for C_8H_{13}, 109.1017), 93.0704 (calcd. for C_7H_9, 93.0704), 81.0702 (calcd. for C_6H_9, 81.0704).

(rel.int. %); 250 (38), 235 (26), 206 (13), 194 (21), 177 (24), 161 (35), 153 (32), 149 (30), 135 (27), 123 (32), 121 (59), 109 (65), 93 (100), 81 (72).

.10 Isolation of α,β-Dihydro ferulic acid (40)

The ethanolic extract of stem bark of the plant was evaporated and the esidue thus obtained was partitioned between ethyl acetate and water at pH-6. he ethyl acetate layers were combined, washed with water and concentrated. he gummy material thus obtained was allowed to solvent separation.

After separation of methanol, hot methanol, methanol-acetate and acetone oluble fractions, the brown gummy material was allowed to dissolve in benzene t normal temperature.

The TLC of benzene soluble fraction showed a single spot under UV-light. ne extraction of material with hot benzene afforded a pale yellow solid, which ı recrystallization from a mixture of benzene and methanol (1:1) produced olourless needle like crystals of α,β-dihydro ferulic acid (40), a low melting olid, M.P. 32-34°C. When a solution of compound 40 in benzene-methanol mixture is allowed to react with a neutral solution of ferric chloride, raddish brown ecipitate was produced which showed the presence of phenolic group in mpound.

Similarly a solution of compound 40, on treatment with a solution of $NaHCO_3$ oduced brisk effervescences, which indicated the presence of a carboxylic oup in compound.

Spectral data of α,β-dihydro ferulic acid (40):

UV (MeOH); λ_{max} 210 and 291 nm.

IR ($CHCl_3$); ν_{max} 3450-3300 (br.-OH), 1640 (carboxylic group) and 1610 cm^{-1} (aromatic ring).

^{1}H-NMR ($CDCl_3$, 300 MHz); δ 1.87 (t, 2xH at β to COOH group), 1.91 (t, 2xH at α to COOH group), 3.63 (s, 3H, $OC\underline{H}_3$), 6.52 (d, 1xH-5), 6.73 (dd, 1xH-9), 7.08 (d, 1xH-8), 10.04 (s, 1H, $COO\underline{H}$).

^{13}C-NMR ($CDCl_3$, 75.43 MHz); δ C-1) 205.52 2) 27.29 3) 23.60 4) 118.27 5) 120.46 6) 146.72 7) 148.72 8) 116.31 9) 122.13, $OC\underline{H}_3$ 59.69.

HRMS; m/z (rel.int. %) 196.0746 (46) (calcd. for $C_{10}H_{12}O_4$, 196.0735), 181.0511 (12) (calcd. for $C_9H_9O_4$, 181.0500), 165.0621 (100) (calcd. for $C_9H_9O_3$, 165.0551), 152.0832 (8) (calcd. for $C_9H_{12}O_2$, 152.0837), 149.0661 (14) (calcd. for $C_9H_9O_2$, 149.0602), 137.0614 (30) (calcd. for $C_8H_9O_2$, 137.0602), 123.0461 (25) (calcd. for $C_7H_7O_2$, 123.0446), 121.0687 (22) (calcd. for C_8H_9O, 121.0653), 107.0514 (16) (calcd. for C_7H_7O, 107.0496), 93.0344 (16) (calcd. for C_6H_5O, 93.0340), 77.0411 (22) (calcd. for C_6H_5, 77.0391).

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6 PUBLICATIONS

LIST OF PUBLICATIONS

1. Viqar Uddin Ahmad, Aziz-ur-Rehman Amber, Shoib Arif, Marie H.M. Chen and Jon Clardy - The structure of cadabicine, A new alkaloid from *Cadaba farinosa*, **Phytochemistry, 24**(11), 2709 (1985).

2. Viqar Uddin Ahmad, Shoib Arif, Aziz-ur-Rehman Amber, Khan Usmang hani and Ghulam Abbas Miana - A new spermidine alkaloid from *Capparis decidua*, **Heterocycles, 23**(12), 3015 (1985).

3. Viqar Uddin Ahmad, Shoib Arif, Aziz-ur-Rehman Amber, M. Ajmal Nasir and K. Usmanghani - A new alkaloid from root bark of *Capparis decidua*, **Z. Naturforsch., 41b**, 1033 (1986).

4. Viqar Uddin Ahmad, Shoib Arif, Aziz-ur-Rehman Amber and Kaniz Fizza - Capparisinine, A new alkaloid from *Capparis decidua*, **Liebigs Ann. Chem.**, 161 (1987).

5. Viqar Uddin Ahmad, Kaniz Fizza, Aziz-ur-Rehman Amber and Shoib Arif - Cadabicine and cadabicine diacetate from *Crataeva nuruvala* and *Cadaba farinosa*, **J. Nat. Prod.**, 50(6), 1186 (1987).

6. Viqar Uddin Ahmad, Aziz-ur-Rehman Amber et.al. - Application of spectroscopic methods in the structure elucidation of some natural products, in Atta-ur-Rahman (Editor), **Recent Advances in Natural Product Chemistry**, Shamim Printing Press, Karachi, p-19 (1987).

7. Viqar Uddin Ahmad, Nargis Ismail and Aziz-ur-Rehman Amber - Isocodonocarpine from *Capparis decidua*, **Phytochemistry, 28**(9), 2493 (1989).

8. Viqar Uddin Ahmad, Kaniz Fizza and Aziz-ur-Rehman Amber - Odontin and Odonticin, two new eudesmane sesquiterpenes from *Pluchea arguta* Boiss., **J. Nat. Prod.**, 52(4), 861 (1989).

9. Viqar Uddin Ahmad, Aziz-ur-Rehman Amber, Kaniz Fizza and Arshad Kamal - Cadabicilone, A sesquiterpene lactone from *Cadaba farinosa*, **Z. Naturforsch., 45b** (in press, 1990).

10. Viqar Uddin Ahmad, Aziz-ur-Rehman Amber, Kaniz Fizza and Shoib Arif - The Isolation and total synthesis of cadabicine methyl ether (Manuscript under preparation).

11. Viqar Uddin Ahmad, Nargis Ismail, Aziz-ur-Rehman Amber and Shoib Arif - Two new spermidine alkaloids, N-acetyl Isocodonocarpine and N-acetyl capparisine from *Capparis decidua* (Manuscript under preparation).

12. Viqar Uddin Ahmad and Aziz-ur-Rehman Amber - Isolation of an aromatic acid, α,β-dihydro ferulic acid from *Cadaba farinosa* (Manuscript under preparation).