ISOLATION, IDENTIFICATION AND PHARMACOLOGICAL SCREENING OF THE ALKALOIDS.OF THE LEAVES OF SYNCLISIA SCABRIDA MEIRS

BY

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A Thesis Submitted to the Postgraduate School, Ahmadu Bello University, in Partial Fulfilment of the Requirements for the Degree of

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OCTOBER 1986

DECLARATION

I solemnly declare that the work reported in this thesis has been carried out by me under the supervision of Dr. B.K. Chowdhury, Dr. A. Mustapha, and Dr. S.L. Bodhankar. It has not been accepted in any previous application for a higher degree elsewhere. The works of other investigators are acknowledged and referred to accordingly.

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DEDICATION

To my Parents, Brothers and Sisters

and

To all those who strive for knowledge

for the service of humanity.

CERTIFICATION

This thesis entitled ISOLATION, IDENTIFICATION AND PHARMACOLOGICAL SCREENING OF THE ALKALOIDS OF THE LEAVES OF SYNCLISIA SCABRIDA MEIRS by UMARU FAROUQ BAKO meets the regulations governing the award of the adegree of MASTER OF SCIENCE of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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U. F. BAKO (MR.)

ABSTRACT

The leaves of Synclisia scabrida (Menispermaceae), a plant used in the treatment of gynaeco-obstetrical and mental problems in traditional medicine was investigated for its alkaloidal content.

The alkaloidal fractions of the ethanolic extract of the leaves of Synclisia scabrida showed the presence of five alkaloids by TLC. Three of the alkaloids were isolated and designated as alkaloids D, E and F.

Alkaloid D m.p. 276-278°C was identified as isochondodendrine from colour reactions, spectral data and methylation experiments. This is the first report of occurence of isochondodendrine in Synclisia scabrida.

Alkaloid F m.p. 268-270°C was identified as cycleanine from colour reactions, spectral data and comparison with authentic sample. This is the first report of the isolation of cycleanine from the leaves of Synclisia scabrida.

The colour reactions and spectral properties of alkaloid E m.p. 230-232°C have been studied.

In order to obtain further information about structure, alkaloid E was methylated to the O-methyl derivative (alkaloid E-M). The spectral properties of alkaloid E-M were studied. From the available information some suggestions have beer; made regarding the structure of alkaloid E, but no definite structure could be predicted for this alkaloid.

Pharmacological screening showed that alkaloid D antagonised acetylcholine induced contractions of the frog rectus abdominis tissue. It showed weak antagonism to oxytocin induced contractions of the rat uterus. Anti-psychotic tests showed that apomorphine induced stereotyped behaviour in chicks was suppressed by alkaloid D.

Alkaloid E showed no effect on acetylcholine induced contractions of the frog rectus abdominis tissue but it produced a weak antagonism to ocytocin induced contractions of the rat uterus.

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ABBREVIATIONS

NaOH	=	Sodium hydroxide
P.m.r.	=	Proton magnetic reasonance
e.g.	=	for example
U.V.	=	Ultraviolet
I.R.	=	Infrared
i.p.	=	intra peritoneal
mg	=	milligramme
ug	=	micrgramme
ng	=	nanogramme
TLC	=	Thinlayer chromotography
NMR	=	Nuclear magnetic reasonance
H_2SO_4	=	Sulphuric acid
HNo_3	=	Nitric acid
MS	=	Mass spectrum
m.p.	=	melting point
m.m.p.	=	mixed melting point
Rf	=	retardation factor
Ach	=	acetylcholine
P^H	=	hydrogen ion concentration
Na_2SO_4	=	Sodium sulphate
HCl	=	hydrochloric acid

CHAPTER ONE

LITERATURE REVIEW

1.1 General Introduction

Menispermaceous plants, though widely distributed in the tropics, have been studied World wide. Their application in traditional medicine is responsible for their World wide study. They are closely related to Berberidaceae, Papaveraceae, Annonaceae, Rutaceae and Ranunculaceae both in the range and type of alkaloids found. They are however unique in producing the hasubanan skeleton.

Alkaloids found in the Menispermaceous plants include I-benzylisoquinolines, proaporphines, aporphines, protoberberines, morphines, hasubanan and bisbenzylisoquimoline alkaloids. The bisbenzyl-isoquinoline alkaloids are however predominant (Thornber, 1970).

1.2 Traditional Medicinal Uses of Menispermaceous Plants

Tiliacora dinklagei, a woody climber indigenous to Ghana and other parts of West Africa has been used natively as a tie for securing house parts and as a medicinal in the treatment of various fevers and menstrual irregularities. Tiliacora

funifera has been used similarly and both species were found to contain some bisbenzylisoquinoline alkaloids (Tackie et al, 1979). Various species of Triclisia especially I. patens and T. subcordata, woody climbers of the forest and coastal areas of Ghana have been used in the treatment of malaria, diarrhoa, pyorrhoe, swelling in the extremities, anaemia, joint pains and as arrow poisons. They have also been found to contain alkaloids of the bisbenzylisoquinoline types (Irvine, 1961). Cissampelos pareira linn, a climbing shrub distributed throughout warm parts of Asia, East Africa and America is extensively used as medicine in Africa and some parts of the world. In Tanganyika the roots are used as sexual stimulants and for the relief of abdominal pains, rheumatic pains and headache. In some places it is used in the treatment of blennorrhagia, haematuria, colic and as a diuretic (John et al, 1962). In India the leaves are used as local application for itch (Oliver-Bever, 1968). The roots of Cocculus pendulus (Forst) Diels are used in Nigeria as antipyretic and in Senegal as cholagogue and diuretic (Dalziel, 1937). Sciadotenia toxifera growing in the upper Amazon Andean valleys of Peru was reportedly used for the treatment of sterility and malaria.

Synclisia scabrida found mainly in some parts of Sierra Leone, Nigeria and Ghana has been used by native doctors for the treatment of a number of ailments (Irvine, 1961; Ogbeide *et al*, 1982).

In Nigeria the roots are used for the treatment of a number of gynaeco-obstetrical problems (e.g. threatened abortion) or mental disorder (e.g. phychosis). The roots or chips of the roots may be soaked in locally brewed alcohol or boiled in water placed in earthenware pots. The concoction is then ingested orally at regular intervals (Wambebe *et al*, 1982).

These examples though not exhaustive show how extensive memispermaceous plants are used in traditional medicine.

1.3 Phytochemistry of Menispermaceous Plants

Apart from alkaloids there have been very few reported cases of isolation of glycosides, saponins tannins and mineral elements from menispermaceous plants (Oliver-Bever, 1983). Bisbenzylisoquinoline alkaloids being more predominant are discussed in details.

1.3.1a Bisbenzylisoquinoline Alkaloids

The bisbenzylisoquinoline alkaloids are built up of two benzylisoquinoline units linked by ether

bridges. In addition to ether bridging, methylenoxy bridging or direct carbon-carbon bonding is also found between the two benzylisoquinoline units. These alkaloids can be divided into groups and sub-groups based on the structural differences that may arise due to differences in the following: (Guha et al, 1979).

(i) The number of aromatic oxygen substituents.

(ii) The number of ether linkages.

(iii) The nature of the bridge(s) i.e. diphenyl ether, or benzylphenyl ether or direct carbon-carbon bonding.

(iv) The sites on the two benzylisoquinoline units at which the ether or the carbon bond originates.

Individual members in each group can be differentiated by simply considering the following:

(i) The stereochemistry of the assymmetric centres.

(ii) The nature of the substitution of the two nitrogen atoms (-NH, NCH_2, $N^+(CH_3)_2$)

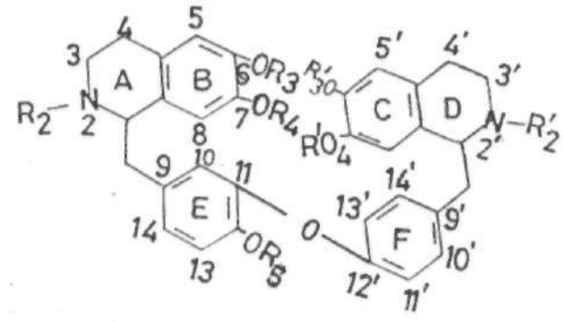
(iii) The nature of oxygenated substituents (OH, OCH_3, OCH_2O).

(iv) The degree of unsaturation of the heterorings.

Examples:

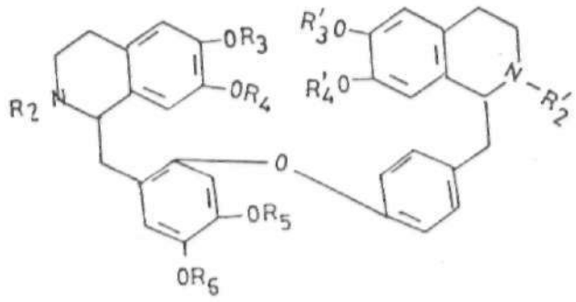
A: Alkaloids containing one diphenyl ether linkage.

Tail to tail



Dauricine (I) $R_2=R_3=R_4=R'_2=R'_3=R'_4=CH_3$, $R_5 = H$

Magnoline (II) $R_2=R_3=R'_2=R'_3=CH_3$, $R_4=R_5=R'_4= H$

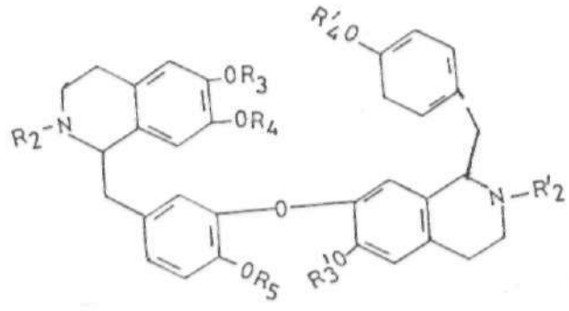


Magnolamine (III) $R_2=R_3=R_5=R'_2=R'_3=CH_3, R_4=R_6=R'_4 = H$

Tetramethylmagnolamine (IV) $R_2=R_3=R_4=R_5=R_6$

$R_2=R_3=R_4 = H$

Head to tail

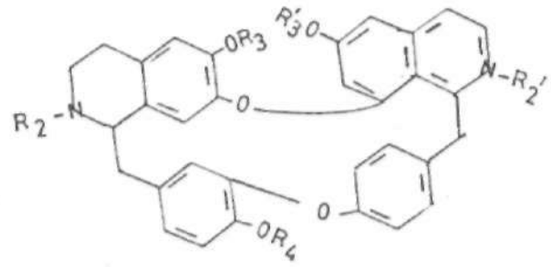


Isoliensimine (V) $R_2=R_3=R'_2=R'_3=R'_4 = CH_3, R_4=R_5= H$

Liensinine (VI) $R_2=R_3=R_4=R'_2=R'_3=CH_3, R_5=R'_4 = H$

B: Alkaloids containing two diphenyl ether linkages.

Head to head and tail to tail

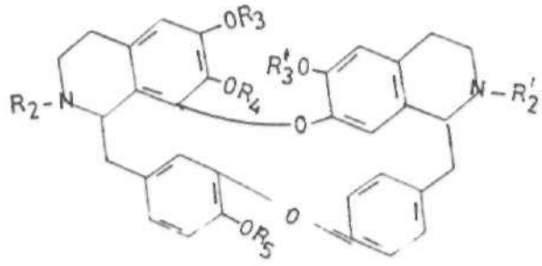


Oxyacanthine (VII); $R_2=R_3=R'_2=R'_3=R'_4 = CH_3, R_4 = H$

Daphnandrine (VIII); $R_3=R_4=R'_2=R'_3 = CH_3, R_2=R'_4 = H$

Cepharanthine (IX); $R_2=R_3=R_4=R'_2 = CH_3, R'_3-R'_4 = -CH_2$

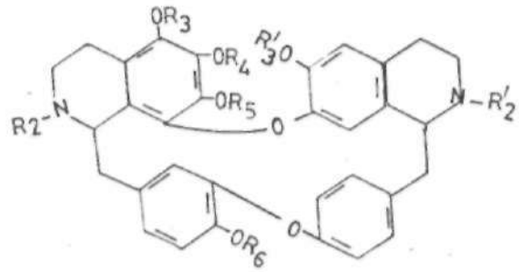
Daphnoline (x); $R_2=R_4=R'_4=H, R_3=R'_2=R'_3=CH_3$



Berbamine (XI); $R_2=R_3=R_4=R'_2=R'_3=CH_3, R_5 = H$

Isotetrandrine (XII); $R_2=R_3=R_4=R_5=R'_2=R'_3= CH3$

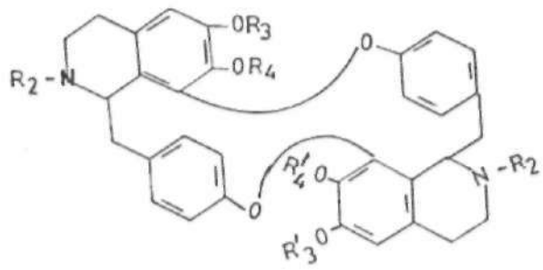
Tetrandrine (XIII); $R_2=R_3=R_4=R_5=R'_2=R'_3=CH_3$



Hernandezine (XIV); $R_2=R_3=R_4=R_5=R_6=R'_2=R'_3 = CH_3$

Thalidezine (XV); $R_2=R_4=R_5=R_6=R'_2=R'_3=CH_3, R_3 = H$

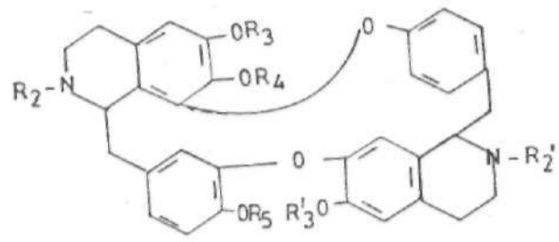
Head to tail



Cycleanine (XVI); $R_2=R_3=R_4=R'_2=R'_3=R'_4 = CH_3$

Norcycleanine (XVII); $R_3=R_3=R_4=R'_2=R'_3=CH_3, R'_4=H$

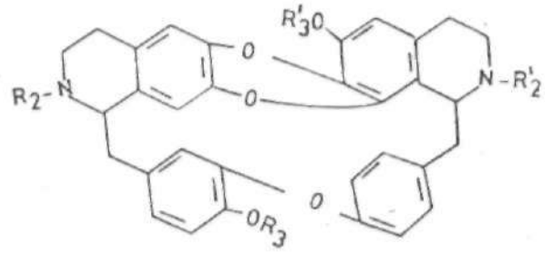
Isochondodendrine (XVIII); $R_2=R_3=R'_2=R'_3=CH_3, R_4=R'_4=H$



Curine (XIX); $R_2=R_3=R'_2=R'_3=CH_3, R_4=R_5=H$

Cycleacurine (XX); $R_2=R_3=R'_2=CH_3, R_4=R_5=R'_3 = H$

C: Alkaloids containing three diphenyl ether linkages.



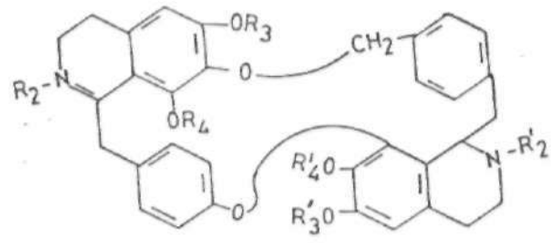
Cocsoline (XXI); $R'_2=R'_3=R_3, CH_3, R_2=R_3 = H$

Cocsuline (XXII); $R_2=R'_2=R'_3=CH_3, R_3=H$

Isotrilobine (XXIII) $R_2=R_3=R_2=CH_3$

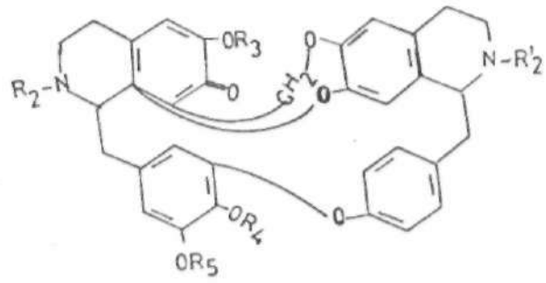
Trilobine (XXIV); $R_2=R_3=R'_3=CH_3, R'_2 = H$

D: Alkaloids containing one diphenyl ether and one benzylphenyl ether linkages.



Cissampareine (XXV) $R_3=R'_2=R'_3=R'_4=CH_3, R_4=OH$

E: Alkaloids containing two diphenyl ether and one phenylbenzyl ether linkages.

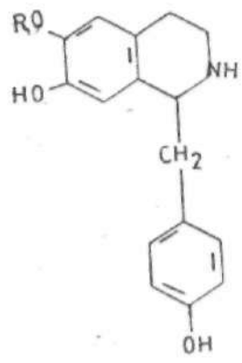


Repanduline (XXVI); $R_2=R_3=R'_2=CH_3$, $R_4-R_5=CH_2$

1.3.1b Biogenesis

The theory proposed by Faltis that all bisbenzylisoquinoline alkaloids are derived biogenetically from two molecules of coclaurine (or nor-coclaurine) through carbon-oxygen-carbon phenolic oxidative coupling has now been accepted with slight modifications (Faltis 1932). This was further supported by instances where coclaurine type bases occur with bisbenzylisoquinoline alkaloids in the same plant. Such instances include coclaurine and trilobine in Coccus laurifolius

(Tomita and Kusuda, 1953); Magnocurarine (the quaternized form of N-methyl coclaurine) and phaenthine in the bark of Gyrocarpus americanus (McKenzie and Price, 1956).



Coclaurine; R = CH_3

Norcoclaurine; R = H

The theory was able to explain the structure of most of the bisbenzylisoquinoline alkaloids except a few like magnolamine (III) and tenuipine, that contain one more oxygen function per 1-benzylinoquinoline unit than coclaurine. These are considered to arise by hydroxylation possibly after the phenolic coupling has occured (Dyke, 1978).

The occurrence of fully and partially methylated bisbenzylisoquinoline alkaloids in the same plant suggests that union of the two benzylisoquinoline units occurs before N-methylation. Thus trilobine (XXIV) and isotrilobine (N-methyltrilobine) (XXIII) occur together in Cocculus trilobus; daphnoline (x)

and N-methyladaphnoline (aromoline) occur together in Daphnandra aromatica (Brick et al, 1963).

Biosynthetic pathways of various bisbenzylinoquinoline alkaloids have been studied utilizing ^{3}H and ^{14}C labelled coclaurine, norcoclaurine and N-methyl coclaurine. The study supported the following biosynthetic pathways:

Oxyacanthine (VII)

Tyrosine ⟶ norcoclaurine ⟶ coclaurine ⟶ (+) - (s) - methylcoclaurine + (- (R) - N-methyl coclaurine ⟶ oxidative dimerization ⟶ oxyacanthine (Bhakurni et al, 1978).

Isotetrandrine (XII)

Caclaurine ⟶ (+) - (S) - N-methylcoclaurine + (-) (R) N-methylcoclaurine ⟶ inter and intromolecular oxidative coupling ⟶ isotetrandrine (Bhakuni et al, 1980).

Tetrandrine (XIII)

Tyrosine ⟶ norcoclaurine ⟶ coclaurine ⟶ (+) (S) - N-methylcoclaurine ⟶ oxidative dimerization ⟶ tetrandrine (Bhakuni et al, 1980).

1.3.1c Isolation

The isolation of compounds from natural sources has always been a challenge to natural products chemist. Thus, though there are general methods for the isolation of alkaloids from plants, individual alkaloids or group of alkaloids are isolated with reference to their chemical and physical properties.

In the case of bisbenzylisoquinoline alkaloids ecology was found to play a role in the nature and amounts of these alkaloids in plants. Indian Mahonia plants have been found to contain in many instances both oxyacanthine and berbamine against isotetrandrine and berbamine found in Japanese Mahonia. Only Mahonia fortunei (Hort) Fedde was found to contain oxyacanthine among Japenese Mahonia (Tomita and Abe, 1952(a); Tomita and Abe, 1952(b)). Leaves of Magnolia fuscata collected from Japan gave only magnolamine while those collected from Russia gave magnoline and magnolamine (Proskurnina and Orekhov, 1938). Stephania rotunda collected from India does not contain cycleanine, while that collected from the Caucasus contained cycleanine (Shchelchkova et al 1965). Cissampelos pareira alkaloids were also

found to vary from one source to another. The plant collected from Kashmir yielded hayatine and hayatinine, whereas the one collected from Pilibhit yielded hayatine and curine but not hayatinine (Bhattacharji, et al, 1956). In addition to hayatine and curine the plant collected from Madras and Madagasca contained isochondodendrine, (Boissier et al, 1965).

The alkaloids were also found to vary in nature and relative proportion in different parts of the plant. A typical case is that of Mahonia fortunei where the trunk was found to contain berbamine and oxyacanthine whereas the leaves do not contain any bisbenzyl isoquinoline alkaloid (Tomita and Sugamoto, 1961). A similar case is that of Menispermanum canadense Linn where the stem, roots and rhizome contain alkaloids of the magnolamine type but the leaves contain no alkaloid (Manske, 1943).

The general method of isolation involves extraction by percolation at room temperature with methanol or ethanol or 1-5% acidic alcohol. The acid is normally acetic acid. The use of methanolic tartaric acid solution has been reported (Barton et al, 1966, Guha, et al, 1979). Hot alcohol

extractions have also been reported (Boissier et al, 1965; Wambebe et al, 1982). In rare cases the plant material is initially defatted (Padilla and Herran, 1962; Wambebe et al 1982).

The solvent is then removed in vacuum leaving a gummy mass which is treated with 1-5% aqueous solution of acetic citric, tartaric, hydrochloric or sulphuric acid. The use of phosphoric acid and boiling 1% hydrochloric acid has been reported (Guha et al, 1979). Aqueous acid solutions have also been used for the extraction of the alkaloids directly from plants (Barltrop and Jeffreys, 1954, King, 1948). Removal of a non-alkaloidal matters from the aqueous acid extracts is generally achieved by treatment with light petroleum ether or ether. There are however, reported cases of the use of lead acetate to precipitate non-alkaloidal matters; the excess lead being removed as lead sulfide (King, 1948; Tomita and Abe, 1952(a)). The aqueous acid extracts are then basified with alkali solution to precipitate the total alkaloidal fractions (Tomita and Abe, 1952(b); Wabambe et al, 1982; Iriah, 1984).

Paper and thinlayer chromatography (TLC) are generally used to assess the number of alkaloids in the crude alkaloidal mixtures. In certain cases the papers or plates are impregnated with certain chemicals. Tomita and co-workers (1956) used paper pretreated with buffer PH 3.5 and the solvent system: n-butanol: acetic acid: water (67:10:23). Chan and co-workers (1967) on the other hand used Whatman paper Nos. 1 and 4 impregnated with 0.2M potassium hydrogen orthophosphate prior to descending paper chromatography with the following solvent systems: (1) n-butanol: glacial acetic acid: water (63:10:27) and (2) benzene: glacial acetic acid water (6:7:3). Recently Iriah (1984) reported the use of Whatmann No. 1 with Butanol: acetic acid: water (10:1:3) as the solvent system.

Thinlayer chromotography is now more widely used than paper chromotography to detect and separate crude alkaloidal mixtures. This may be due to its versatility and convenience. Silica gel plates are most commonly used and in some cases the plates may be pretreated. Boissier and co-workers (1963) for instance used silica

gel plates prepared with 0.1N NaOH for the detection of phaenthine in Triclisia patens. This method has also been used for the separation of isotetrandrine, pycnamine, berbamine and oxyacanthine due to their greater basicity (Guha et al, 1979).

Dragendorff's reagent or a mixture of aqueous solution of chloroplatinic acid and aqueous potassium iodide is generally used for visualization. Dragendoff's reagent is poor for the detection of phenolic alkaloids because the red spots obtained in almost all cases are tootransient. Folin-Denis reagent followed by alcoholic ammonia was however found to be more satisfactory (Guha et al, 1979).

Having determined the number of alkaloids, the individual alkaloids are them isolated using various methods. One of such methods is to separate the alkaloids into phenolic and cryptophenolic bases using 5% NaOH and Claisen reagent (25% methanolic potassium hydroxide solution) (Bick et al, 1972). Acid constants have also been used as a basis for separating individual bisbenzylisoquinoline bases by countercurrent distribution of the alkaloids between chloroform and buffers

of different P^H. Gallefi and co-workers (1975) successfully isolated the alkaloids of Guatteria megaphylla Diels and those of Sciadotenia toxifera by this method. Countercurrent distribution was also reported for the isolation of the alkaloids of Abuta splendida (Saa et al, 1975) and Thalictrum rochebrunianum (Fong et al, 1966).

In most cases the various alkaloids are then purified by column chromotography and recrystallization. For this purpose silica gel and alumina (neutral or basic) columns are most common. The use of partition chromatography (Knapp et al, 1967) as well as preparative thin layer chromatography has been reported (Roychoudhury, 1972; Kupchan et al, 1973).

Quaternary bisbenzylisoquinoline alkaloids are generally precipitated as reineckates in weakly acidic medium after the removal of tertiary bases (King, 1948). The use of ion-exchange columns have also been reported for the isolation of the quaternary alkaloids (Kupchan et al, 1973).

1.3.2 Structure Elucidation

Structure elucidation of natural products is as challenging as their isolation in pure forms.

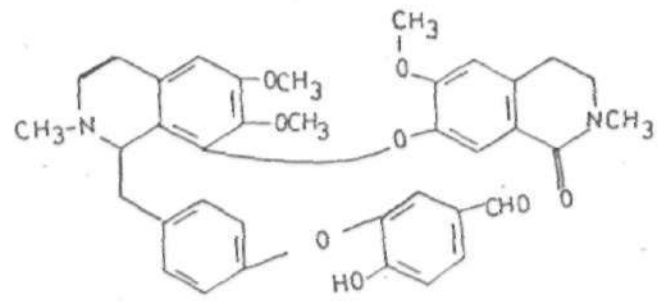
Chemical properties of bisbenzylisoquinoline alkaloids especially degradation reactions have been used to elucidate their structures. However, only after the application of mass spectrometry could chemists obtain accurate molecular weights and composition of these alkaloids.

Presently spectroscopy plays a leading role in predicting the structure of bisbenzylisoquinoline alkaloids. Degradation reactions are still indispensable most especially because spectroscopic data alone cannot elucidate completely the structure of a new bisbenzylisoquinoline alkaloid.

1.3.2a Degradation Studies

Oxidation of bisbenzylisoquinoline alkaloids with aqueous solution of potassium permanganate leads to falile cleavage of the benzylic methylene groups B to nitrogen atom. Identification of the fragments leads to the structure of the parent compound. In some cases identification of these fragments cannot lead to a single structure for the parent compound. A typical case is that of obamegine and isotetrandrine where aqueous permanganate oxidation products of the two compounds are the same (Guha et al, 1979). A controlled oxidative

method with potassium permanganate in acetone has been developed and successfuly applied to a number of bisbenzylisoquinoline alkaloids (Shamma and Foy, 1975). In this case cleavage occurs at the benzylic bond of the isoquinoline moiety which is unsubstituted at C8(or C8') producing a tertiary lactam and an aromatic aldelyde. This is irrespective of the configurations of the chiral centers. This reaction when applied to oxyacanthine (VII) gave baluchistanamine (XXVII) which coincidentally is a natural product obtained from Berberis baluchistanica.

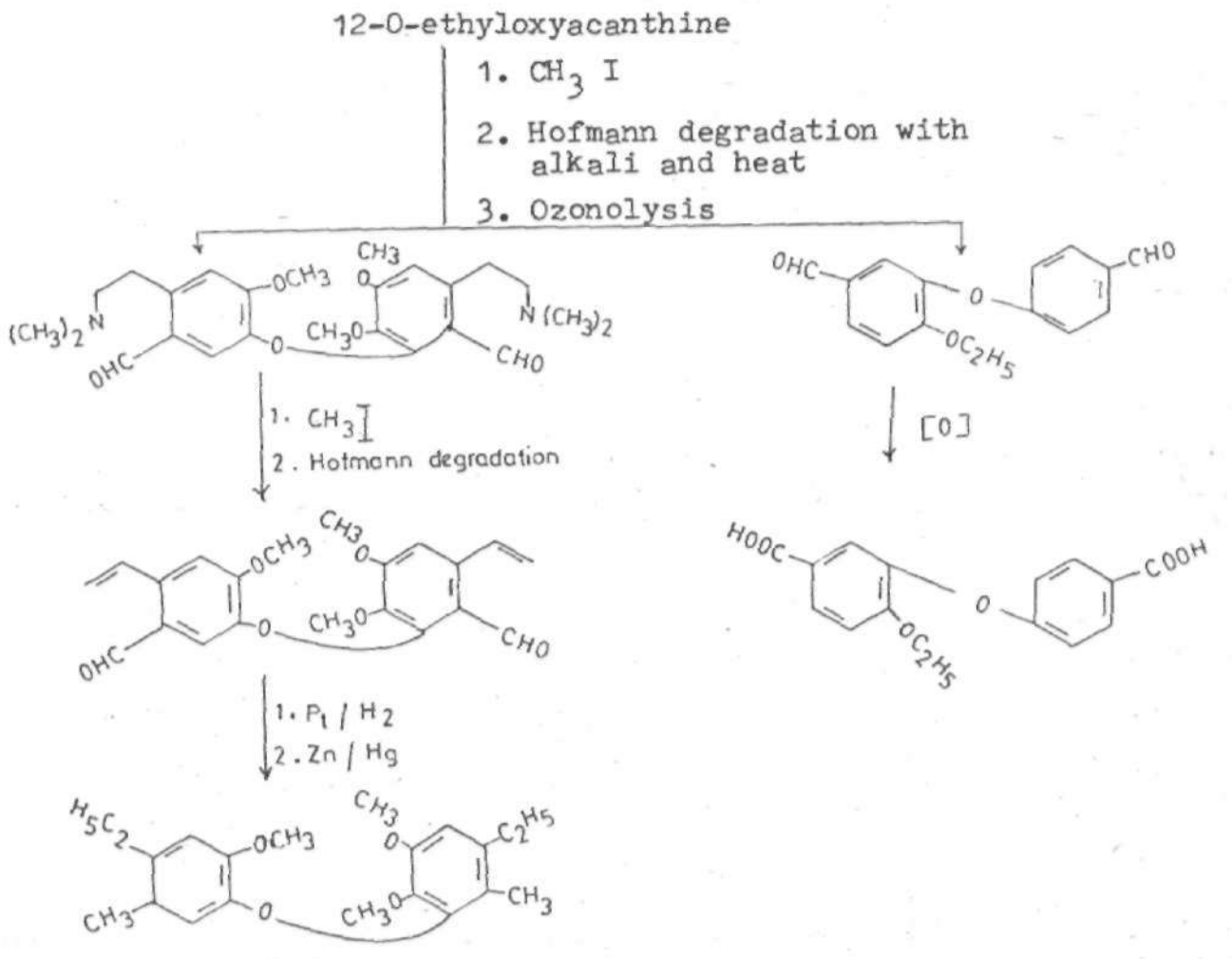


Baluchistanamine (XXVII)

The aromatic p.m.r. signals of the oxidized products unlike those of the parent compounds are

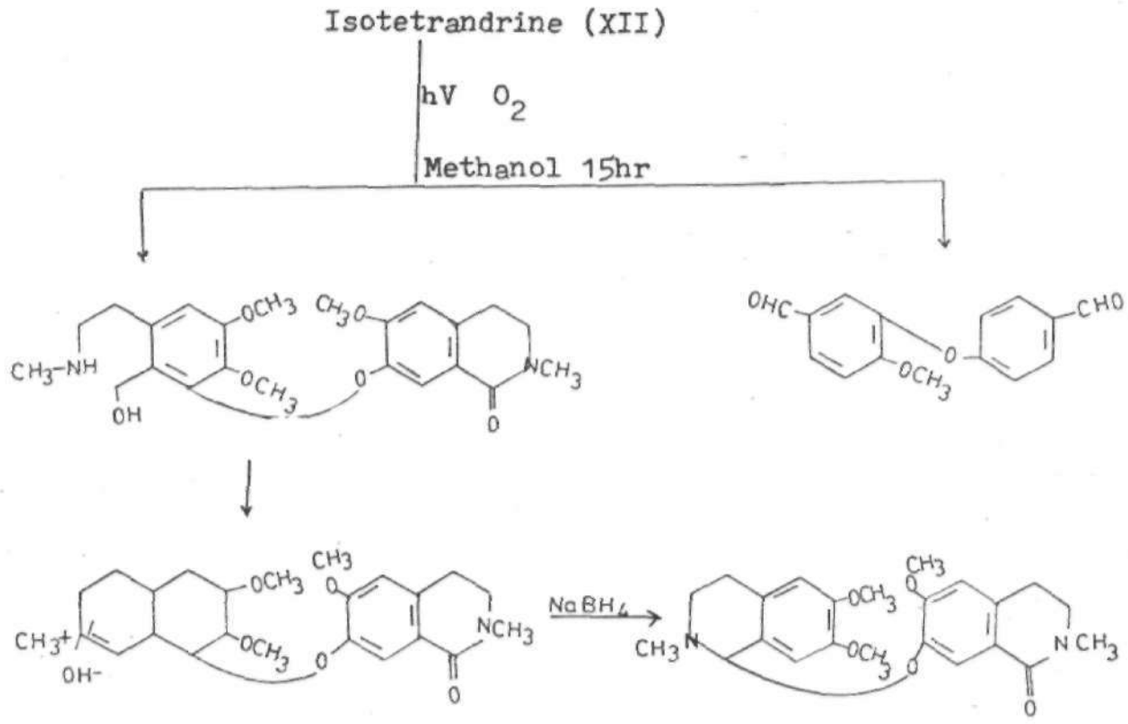
spread over a large range and are generally easier to identify. This controlled oxidative method is however limited by its low yield.

Hofmann degradation has been widely used in structural elucidation of bisbenzylisoquinoline alkaloids. Its main limitation is that a single structure cannot be predicted. For instance 12-O-ethylderivatives of oxycanthine (VII) and berbamine (Xi) gave the same degradation products. Hoffmann degradation with 12-O-ethyloxycanthine is illustrated below (Guha et al, 1979)

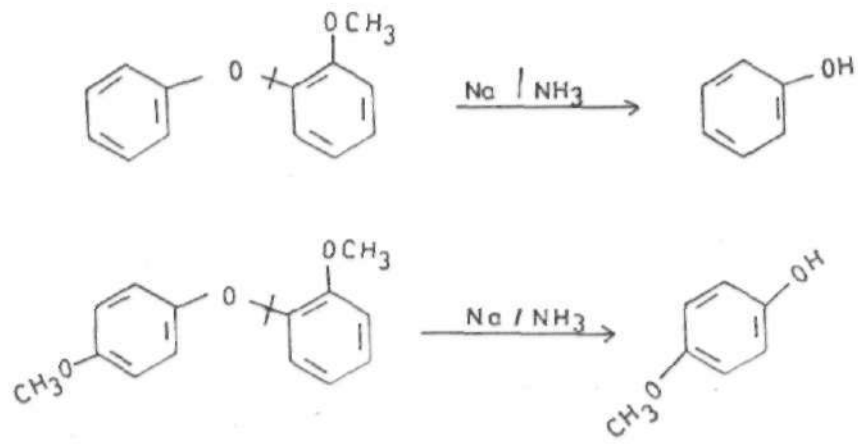


Despite its limitations it helped in elucidating the structure of a number of bisbenzylisoquinoline alkaloids including oxyacanthine (VII) and trilobine (XXIV), (Dyke, 1978).

A photo-oxidative cleavage reaction of some bisbenzylisoquinoline alkaloids has been studied. In their study Brick and co-workers (1971) observed that bisbenzylisoquinoline alkaloids on irradiation with U.V. light in the presence of oxygen undergo cleavage at the C-1 and C-1' benzylic centres, the reaction has been applied in structure elucidation of a number of these alkaloids including isotetradrine, tenuipine and miranthine. The mechanism of this photo oxidation is still uncertain. With isotetrandrine (XII) the reaction goes as follows:



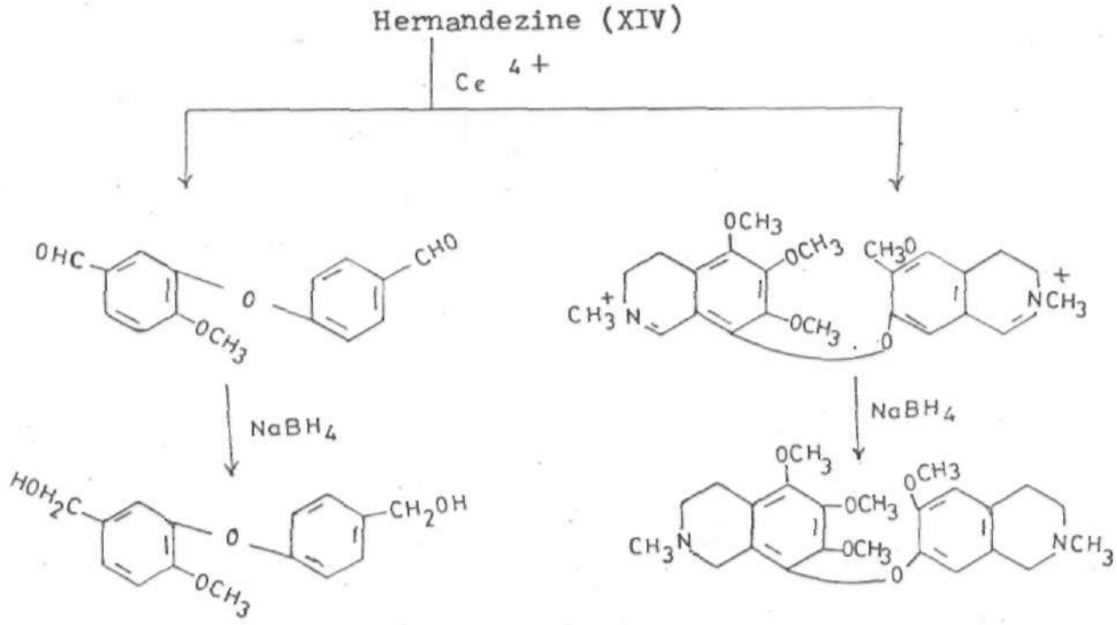
Sodium in liquid ammonia cleavage is another important and widely used degradation reaction applied to bisbenzylisoquinoline alkaloids in their structure determination. This reagent is employed due to its versatility in cleaving diphenyl ether linkages. Compounds having a methoxyl group ortho to the ether linkage under goes preferential cleavage of the C-O bond ortho to this group. (Dyke, 1978).



Simple Na/NH_3 (⟨) cleavage cannot give definite conclusion about the sites of attachment of the ether linkages. To solve this problem an alternative method has been developed whereby ND_3 was used instead of NH_3. Deuterium marks the terminals of the ether linkages (Bick et al, 1971). Another method involves O-methylation dimer followed by treatment with 3% DCl in D_2O at 120°C in sealed tubes. Under these conditions only protons ortho to methoxyl groups are exchanged. Subsequently sodium in liquid ammonia cleavage is applied. An arromatic hydrogen ortho to methoxyl group indicates the site of the original diphenylether linkage (Inubushi, et al, 1972). It is important to point out here that this reaction

is ineffective in bisbenzylisoquinoline alkaloids containing a biphenyl unit. In this case Hofmann degradation is the ultimate alternative despite its limitations (Guha, et al, 1979).

The use of ceric ammonium nitrate for oxidative degradation of bisbenzy isoguinoline alkaloids has been reported (Bick et al, 1978; Wu et al, 1980). The reaction has been applied successfully to tetrandrine, hernandezine and O-methylmiranthine. When applied to berbamine, thalibrunine and tenuipine it gave good yields of nitrogen containing fragments only. The products after reduction gave a diamine and a diol. With hernandezine the reaction goes as follows:



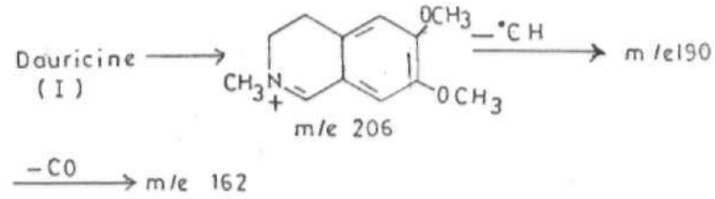
1.3.2b Spectroscopic methods

Spectroscopy is now used extensively for the structure elucidation of bisbenzylisoquinoline alkaloids. It is helpful in reducing to the barest minimum the structural possibilities of a new bisbenzylisoquinoline alkaloid. Various reviews have shown that correlations exist between spectral data and structure of these alkaloids.

The U.V. spectra of most of these alkaloids show a characteristic absorption at max $\sim$283 (Sangster and Staurt, 1965). Bathochromic shifts as expected have been observed in the U.V. spectra of phenolic bisbenzylisoquinoline alkaloids on addition of alkali (Bhakuni et al, 1970). The I.R. spectra show characteristic absorption indicating the presence of ether and substituted aromatic functions. Where the alkaloids are phenolic absorptions due to hydronyl groups have been observed (Bhakuni et al, 1970). The I.R. spectra apart from being useful in identifying functional groups could be used to establish identity or otherwise of a new alkaloid with that of a reference compound.

Mass spectrometry has been applied widely at one stage or another in structural elucidation of

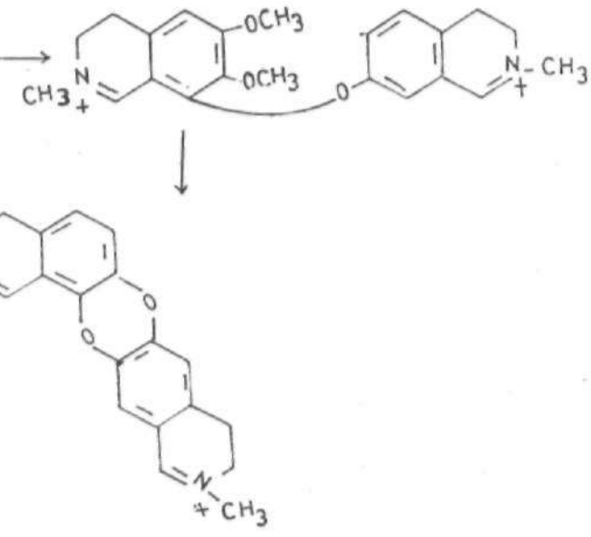
bisbenzylisoquinoline alkaloids. It is a very easy way of obtaining correct molecular weights of new compounds. Various reviews have shown that alkaloids of similar skeletal types follow characteristic fragmentation patterns (Baldas et al, 1966; Baldas, et al, 1972; Baldas et al, 1972; Baldas et al, 1972). In most cases the most stable peak is as a result of cleavage of the bonds β - to nitrogen and to two aromatic systems. Dauricine (1) for instance has a base peak at m/e 206.



Similar patterns were observed with tetramethylmagnolamine (IV) (Tomita et al, 1966).

The oxyacanthine-berbamine type of alkaloids have as a base peak a doubly charged ion resulting from double benzylic fission of the doubly charged molecular ion. Isoetetrandrine (XII) for example has a base peak at m/e 198 followed by elimination of the elements of dimethyl ether to give ions having dibenzo-1,4; dioxin structure.

Isotetrandrine (XII) ⟶



m/e 179

Cepharanthine (IX) is an exception in this type of transition because it has a methylene dioxy group in place of vicinal dimethoxy grouping of isotetrandrine (XII). In addition to doubly charged ions, singly charged ions are also produced by a double benzylic fission of the singly charged molecular ion. In case of isotetrandrine it can be represented as:

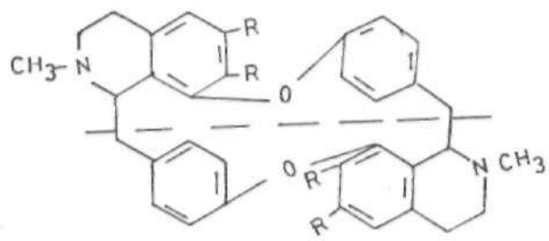
Isotetrandrine (XII) —— m/e 396 $\xrightarrow{-CH_3}$ m/e 381

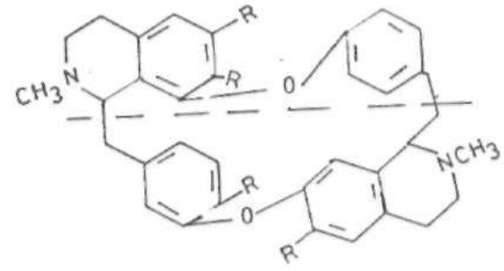
m/e 622

With the exception of cepharanthine (IX) all alkaloids of oxyacanthine - berbamine group show strong

ions at M-191 and M-192. This is as a result of loss of rings C and D.

In case of curine-isochondodendrine group of alkaloids the base peak is as a result of double benzylic fission (across the dotted lines) with hydrogen transfer.



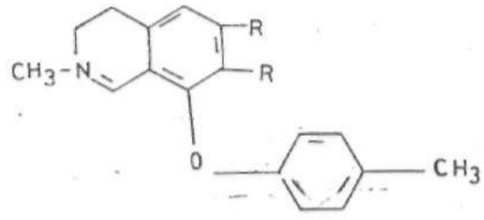


Isochondodendrine type Curine type

(R = OH or OCH_3)

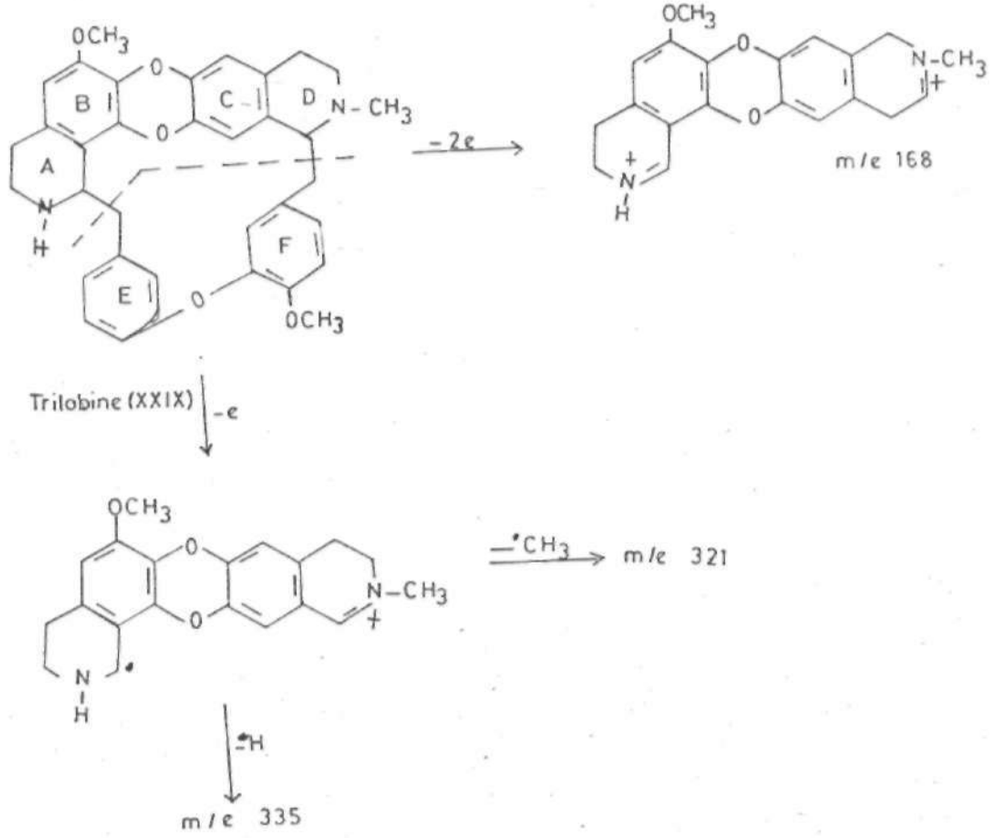
In both cases the base peak has this structure:

Cycleanine (XVI) m/e 312
Isochondodendrine (XVIII) m/e 298
Curine m/e (XIX) 298



A fragment at $\frac{1}{2}$ $(M-91)^{++}$ occurs in the spectra of all curine-Isochondodendrine type alkaloids. This may be due to loss of the unsubstituted benzylic group (C_7H_7) from the doubly charged parent ion. Complementary to the neutral fragment an ion at m/e 91 is seen in all curine-isochondodendrine type alkaloids.

The trilobine type of alkaloids are characterized by the presence of a dibenzo-P-dioxin moiety in their structure. In this case fussion occurs (across the dotted line) giving doubly charged ions as the most abundant fragments (base peak).



The absence of ions at M-191 and M-192 is of diagnostic value in these alkaloids because the formation would involve the rapture of the two C-O bonds. Ions resulting from loss of ring E has not been observed.

Nuclear magnetic reasonance has proven to be quite useful in solving many structural problems such as location of bsubstituents and configuration of chiral centres. Structural correlations in the NMR spectra of bisbenzylisoquinoline alkaloids has been reported (Bick, 1961). The anisotropic nature of benzene and the macrocylic nature of bisbenzyl-isoquinoline alkaloids affect the chemical shift of some groups of protons in a characteristic manner. For instance, the hydrogen nuclei of a methoxyl group lying in the plane of an aromatic ring will have a lower chemical shift than those lying below or above the plane of the ring. This helps in explaining the marked differences between chemical shifts among methoxyl groups and methyl-mino group in these types of alkaloids. In oxyacanthine berbamine series methoxyl groups at position 4' have a chemical shift δ3.87-3.95, whereas the same at position 7 has a very higher value of δ3.02-3.2. This has been attributed to the fact that in many of its orientations the 7 methoxyl groups passes closely over the top of

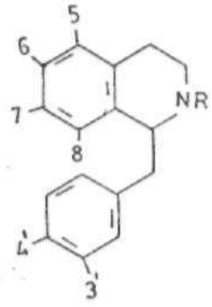
adjacent ring B. It was regularly observed that when assymetric carbon centres are paired (+-) or (-.+) the 6 methoxyl reasonance has a chemical shift near δ 3.6 whereas it is near δ3.35 when the pairing is (+,+) or (-,-). In the curine-isochondodendrine series the two halves of the molecule are joined head to tail giving the molecule more extended structures whereby the 6' methoxyl group is not cin close proximity to a benzene ring. This is why relatively normal chemical shifts δ 3.75 - 3.93 are observed for these compounds compared to that of berbamine-oxyacanthine series. Hydroxyl resonance are generally observed near δ 5 (Shiff, 1983). Hydrogen bonded phenolic hydroxyl groups resonate considerably down field (Jackman and Steruhell, 1969). Recently carbon (^{13}C) magnetic resonance is also being used in the structure elucidation of bisbenzy-lisoquinoline alkaloids. In addition efforts are being made to correlate luminescence spectra, circular dichroism and specific rotation to chemical structure, (Schiff, 1983).

1.3.2c Colour Reactions

Colour reactions have also proved to be of assistance in classifying certain bisbenzylisoquinoline alkaloids. A blue colouration with a mixture of concentrated HNO_3 and concentrated H_2SO_4 is quite characteristic of bisbenzylisoquinoline alkaloids with diphenylene dioxide function e.g. cocsolin (XXI); trilobine (XXIV) and isotrlobine (XXIII); (Bhakumi and Joshi, 1975). Ferric chloride in perchloric acid as a spray reagent has been used to differentiate among various berbamine type alkaloids (Schiff, 1983). The colour reaction was noted immediately after spraying and after heating for five and ten minutes with a hair dryer.

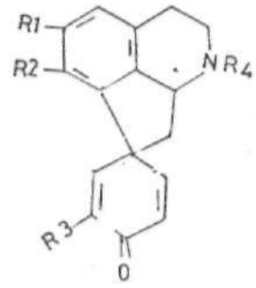
1.3.3 Other Alkaloids of Menispermaceous Plants

1 - Benzylisoquinolines



	R	3'	4'	6	7
Coclaurine (XXVIII)	H		OH	OCH_3	OH
Isococlaurine (XXIX)	H		OH	OH	OCH_3
Laudanine (XXX)	CH_3	OH	OCH_3	OCH_3	OCH_3

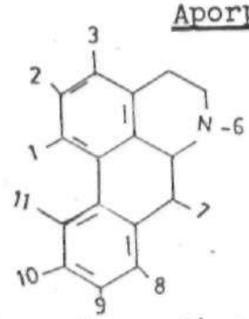
Proaporphines



Stepharine (XXXI);$R_1 = R_2 = OCH_3$, $R_3 = R_4 = H$

Pronuciferine (XXXII);$R_1 = R_2 = OCH_3$, $R_3 = CH_3$, $R_4 = H$

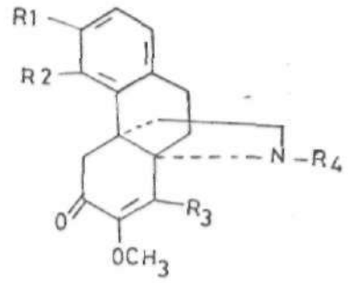
Aporphines



Stephanine (XXXIII);1 OCH_20^2, 6=CH_3, 8=OCH_3

Tuduranine (XXXIV);1 = OCH_3, 2=OCH_3, 6=H, 10=OH

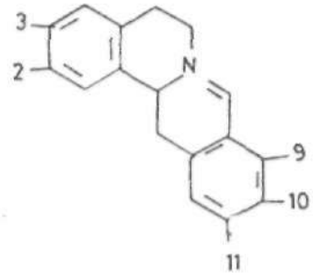
Hasubanan alkaloids



Homostephanoline (XXXV); R_1=OH, R_2=R_3=OCH_3, R_4=CH_3

Hasubanonine (XXXVI); R_1=R_2=R_3=OCH_3, R_4=CH_3

Protoberberines



	2	3	9	10	11
Palmatine (XXXVII);	OCH_3	OCH_3	OCH_3	OCH_3	
Columbamine (XXXVIII);	OH	OCH_3	OCH_3	OCH_3	

1.3.4 Pharmacology of the alkaloids of Menispermaceous Plants

Menispermaceous plants have been shown to possess a variety of pharmacological actions. In general they are associated with curare-like action. The tertiary bases have little or no curariform activity. When converted to quarternary salts however, they exhibit potent curaizing actions, (Craig, 1968). The rather very potent action of bisbenzylisoquinoline alkaloids compared to quaternized coclaurine derivatives suggests that the presence of two quarternary nitrogen atoms in a single molecule is responsible for the high activity. In general alkaloids with the head to tail arrangement (curine-isochondodendrine type) exhibit greater activity than those with head to head arrangement, (Bick, 1974). This may be due to the fact that those with head to tail arrangement are more flexible and more planar, which would presumably result in much effective contact with the receptor. Methylation of phenolic groups in all curine types alkaloids leads to increase in curarizing action. In case of oxyacanthine methylation of the free phenolic group leads to decrease in activity (Craig, 1968).

A number of bisbenzylisoquinoline alkaloids have been shown to possess antimicrobial action

against Mycobacterium smegmatis (e.g. thalfine, thalibrine and hernandezine) while few in addition are active against Staphyllococcus aureus. Antimicrobial activity against Candide albicans has been shown by O-methylthalibrine (Wu, et al, 1980, Schiff, 1983).

A number of alkaloids show significancant and reproducible anti-tumor activity (e.g. cissampareine, curine, d-isochondodendrine and fangchinoline) (Kupchan et al, 1965). Tetrandrine, Oxyacanthine and Cepharanthine have been shown to be tuberculostatic. Cepharanthine in addition is anti-leprotic (Buchi, 1945, 1946, Tolkachev et al, 1978).

A number of alkaloids mainly isolated from various species of thalictrum have been shown to be hypothensive. Examples include N-desmethylthalistyline from Thalictrum longistylum and O-methylthalicberine from Thalictrum revolutum (Wu et al, 1980; Schiff, 1983).

1.4 Synclisia Scabrida

1.4.1 Phytochemistry

West African plants of the Menispermoceaehave been studied extensively and have been shown to

contain various bisbenzylisoquinoline alkaloids (Dwuma-Badu et al, 1975).

Although Synclisia scabrida has been used by the Binis for a very long time, the first report on the extractives of this plant appeared only in 1980. That was the isolation of cycleanine and another aklaoid designated as alkaloid B (Scheiman et al, 1980). Both cycleanine and alkaloid B were isolated from the alcoholic extract of the roots after defatting with petroleum ether. Further work led to the isolation of cycleanine (XVI), nor-cycleanine (XVII), cocsolin (XXI), cocsulin (XXII) and a new alkaloid cycleanine N-oxide from the stems of this plant (Ohiri et al, 1983). One important observation is that all the alkaloids so far isolated and identified from this plant belong to either isochondodendrine or trilobine group. Recently preliminary work on the leaves of Synclisia scabrida led to the isolation of another alkaloid designated alkaloid C. Alkaloid C was isolated from cold ethanol extract and purified by passing through alumina column. The U.V. and I.R. data suggest that alkaloid C could be of the bisbenzylisoquinoline type (Iriah, 1984).

1.4.2 Pharmacology

Cycleanine has been known for quite some time before its isolation from Synclisia scabrida. It has been isolated from other sources, e,g. the roots and leaves of Epinetrum cordifolium (Debray et al, 1967) and the Bulbs of Stephania glabra (Rabinovich et al, 1965).

A report by Nikitina and Trutneva (1967) showed that cycleanine exhibited antiphlogistic, antipyretic, analgesic and adrenolytic properties, when administered i.p to rats. It also reduces the extent of inflammatory oedema in rats with formalin induced athritis. Cycleanine has also been associated with decreased serum hyaluronidase activity when given i.p. to rats (Nikitina and Trutneva, 1971). The pharmacological effects of cycleanine on behaviour and electrocortical activity of young chicks has been studied, (Ogbeide et al, 1982). It has been found that cycleanine (0.5-2mg/kg i.p) sedated young chicks dose dependently In addition cycleanine was observed to potentiate nitrazepam induced sleep while cycleanine induced sleep was potentiated by primozide. Apomorphine on the other hand antagonized cycleanine induced sedation (Wambebe et al, 1981). In a related study on rat uterus and guinea pig ileum

it was found that cycleanine decreased both the magnitude and frequency of oxytocin induced contractions of rat uterus. Similarly it antagonised acetylcholine induced contractions of the uterus. Both effects were concentration dependent and reversible. When compared to atropine cycleanine was less potent on weight to weight basis. However, it has no effect on acetylcholine and histamine induced contractions of the guinea pig ileum (Wambebe, and Ogbeide, 1982). The combination of uterolytic effect with sedative hypnotic property of cycleanine may be responsible for the use of the roots of Synclisia scabrida in the management of threatened abortion and severe dysmenorrhoe.

Pharmacological effects of alkaloid B on some isolated muscle preparations has been studied (Wambebe, et al, 1982). It was found that electrically induced stimulation of the rat diaghragm via the phrenic nerve was reversibly blocked by alkaloid B. The alkaloid had no effect on the responses of the diaphragm elicited by direct electrical stimulation. The responses of the frog rectus abdominis muscle to acetycholine were inhibited by the alkaloid. On the rat uterus

specific inhibition of the contractile effect of oxytocin was observed.

The crude extracts obtained by extracting the leaves with cold water, hot water, cold ethanol and hot ethanol were found to have some pharmacological activity (Iriah, 1984). All the four fractions blocked acetylcholine induced contractions of the frog rectus abdominis muscle but only the cold ethanol and hot ethanol extracts have blocking effect on the nerve induced contractions of the rat phrenic nerve diaphragm preparation. Reversible antagonism was observed on acetylcholine induced contractions of the rabbit duodenun but no effect on histamine induced contractions of the ileum. Oxytocin induced contracts of the rat uterus was antagonized by all the extracts in a concentration dependent pattern similar to that observed with cycleanine and alkaloid B. In vivo studies showed that all the extracts antagonized apomorphine induced behaviour in chicks.

Antimicrobial tests showed that only the hot ethanol extract has antimicrobial activity which was demonstrated against Pseudomonas aeruginosa (gram-ve) and Staphylococcus auereus (gram+ve). The minimum

inhibitory concentration being 7.5± 0.7 ug/ml for the Staphylococcus strain and 3.75± 0.8 ug/ml for *Pseudomonas* strain compared to cycleanine with 0.25 mg/ml as the minimum inhibitory concentration against *Pseudomonas aeruginosa*.

CHAPTER TWO

PHYTOCHEMICAL STUDIES

2.1 Aims and Objectives

Traditional medicine though as old as antiquity is still practised in many parts of the world especially in Africa and Asia.

Synclisia scabrida has been used for the treatment of a number of gynaeco-obstetrical and mental problems by native doctors in southern parts of Nigeria.

Phytochemical investigation of the roots led to the isolation of cycleanine and one other alkaloid designated as alkaloid B. Cycleanine and alkaloid B both exhibited chrane-like and anti-oxytocic actions. Cycleanine in addition was shown to have sedative hypnotic property (Wambebe et al, 1982, Wambebe and Ogbeide, 1982). These actions were found to be compatible with the use of the plant in traditional medicine.

The investigation of the stem lead to the isolation of five alkaloids: cocsolin, cocsulin, cycleanine, Norcycleanine and cycleanine-N-oxide (Ohiri et al, 1983)

Preliminary work on the leaves showed that the crude alkaloidal fractions of the leaves are biologically active. The biological actions include, curare-like action, anti-oxytocic action, anti-psychotic action, hypnotic and sedative property and antimicrobial actions (Iriah, 1984). These actions were found to be compatible with the use of the plant in gynaeco-obstetrical and mental problems.

Although the pharmacological properties of the crude alkaloidal fractions were determined, little effort was made to isolate and identify the active principles present in the alkaloidal extracts. It is therefore of interest to isolate the active principles of the alkaloidal fractions of the leaf extract of Synclisia scabrida elucidate their structures and evaluate the biological activities of the pure compounds. With this view in mind the phytochemical screening of the alkaloids of the leaves of Synclisia scabrida was undertaken.

2.2 Phytochemical Screening of Synclisia Scabrida: Isolation of Alkaloids D, E and F

The dried leaves of Synclisia scabrida were extracted with ethanol at room temperature in a

percolator. The extract after evaporation of the solvent was digested with 5 percent aqueous hydrochloric acid at 45°C.

The aqueous acid layer was extracted with chloroform. Further purification of this chloroform extract furnished needle like crystals (Dragendoff positive) m.p. 268-270°C which has been designated alkaloid F.

The aqueous acid layer on basification with ammonia furnished a solid which was filtered. The solid on crystallization gave white crystals (Dragendorf positive) m.p. 278-280, which has been designated as alkaloid D.

The aqueous basic filtrate on extraction with chloroform gave light brownish solid which on recrystallization furnished light brown crystals (Dragendorf positive) m.p. 230-232°C, which has been designated as alkaloid E.

2.3 Studies on Alkaloid D

2.3.1 Homogeneity of alkaloid D

Alkaloid D was found to be homogeneous by TLC. A single spot was obtained when chromatographed in different solvent systems. The TLC result is as shown in table 2.1.

Table 2.1 Rf Values of Alkaloid D on TLC

Solvent System	Rf
Chloroform-Methanol-Acetic Acid (90:10:0.5)	0.15
Butanone-Xylene-dicthylamine Methanol 20:10:1.5	0.4

2.3.2 Colour Tests

Colour reactions of alkaloid D with certain reagents have been studied. The results are shown in table 2.2.

Table 2.2 Colour Reactions of Alkaloid D

Reagent	Colour
Sulphuric acid (conc.)	No colour
Nitric acid (conc)	Reddish brown
Sulphuric acid + Nitric acid	Dark brown
Ferric chloride	Orange-yellow changing to green

The formation of green colour when the methanolic solution of the compound was treated with ferric chloride solution indicated the presence of phenolic hydroxyl group in alkaloid D.

Table 2.3 Test for Alkaloids

Reagent	Colour/ Precipitate	Inference
Dragendorff's reagent	Orange colour	Alkaloid present
Dragendorff's spray reagent	Orange ppt.	Alkaloid present
Mayer's reagent	Cream colour	alkaloid present

2.3.3 U.V. Absorption Spectrum of Alkaloid D

The U.V. absorption spectrum (fig. 2.1) of alkaloid D was taken in absolute ethanol. The spectrol data are shown in table 2.4.

Table 2.4 U.V. Absorption Spectrum of Alkaloid D in Ethanol

Max (nm)	Log E
216	4.8
232(sh)	4.54
278	3.89
285	3.83

The U.V. absorption spectrum of alkaloid D has similarity with the bisbenzylisoquinoline alkaloids. The bisbenzylisoquinoline

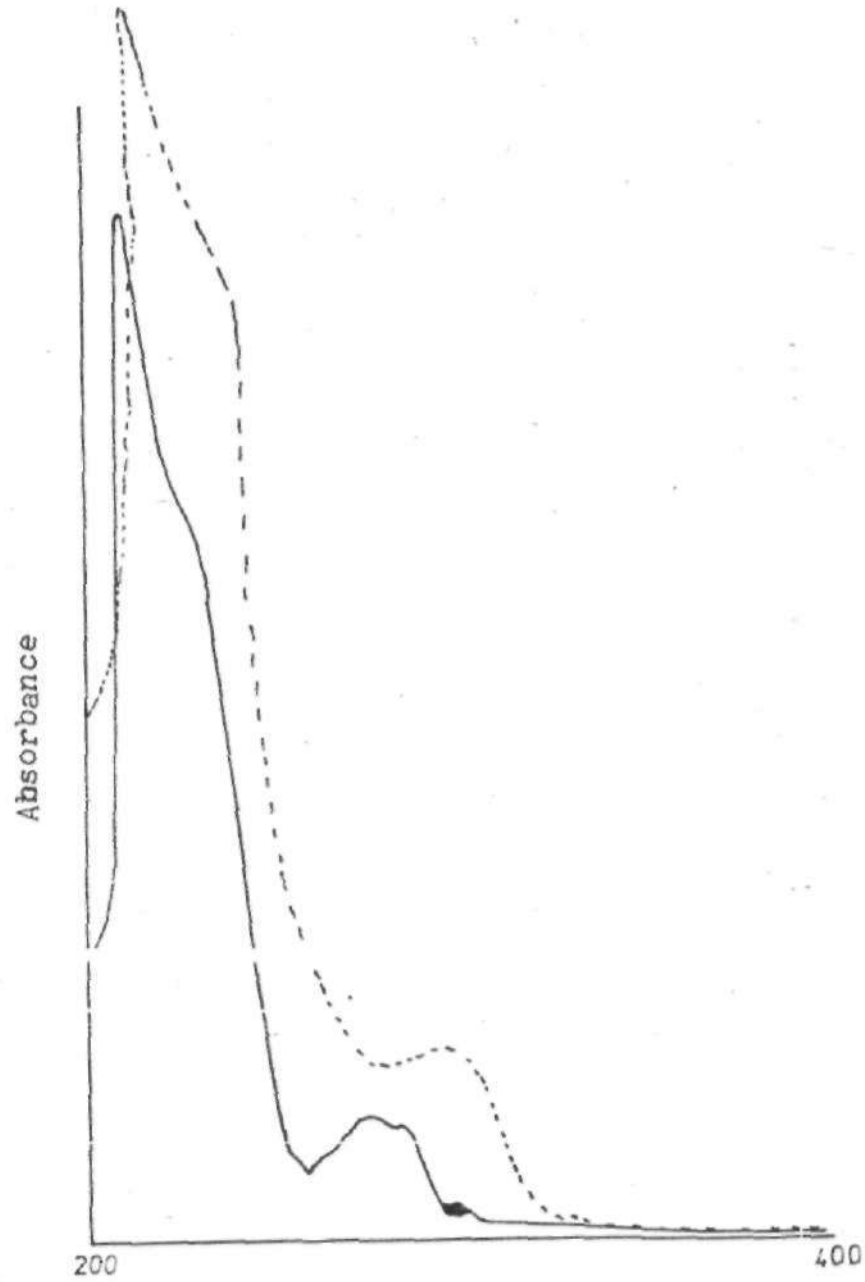


Fig. 2.1 U.V. absorption spectrum of alkaloid D (-) in ethanol (----) in ethanolic sodium hydroxide

alkaloids have been reported to show characteristic maxima at ~283nm (log E 3.8) Sangster and Stuart, 1965). The maximum at 285nm could be due to the presence of bisbenzylisoquine chromophore.

2.3.4 U.V. Absorption Spectrum of Alkaloid D in Ethanolic Sodium Hydroxide

The U.V. absorption spectrum (fig. 2.1) of alkaloid D was taken in ethanolic sodium hydroxide. It was found that the band at 285nm was shifted bathochromically to 300nm. U.V. shifts to higher wavelengths are generally observed in compounds with phenolic hydroxyl groups. This bathochromic shift is due to the formation of the phenolate ion with sodium hydroxide.

Bisbenzylisoquinoline alkaloids with phenolic hydroxyl groups have been reported to show the shift of the 283mm band with alkali (Bhakuni and Joshi, 1975). The above shift in alkaloid D therefore suggested the presence of phenolic hydroxyl groups in it.

2.3.5 I.R. Spectrum of Alkaloid D

The I.R. spectrum (Fig. 2.2) of alkaloid D was taken in liquid paraffin. The diagnostic peaks are shown in Table 2.5.

Table 2.5 I.R. Spectral Data of Alkaloid D

Absorption Band (Cm^{-1})	Inference
1620, 1510, 1590, 1570	Aromatic system
1300, 1220	Aromatic ether
840	Aromatic Substitution

The aromatic carbon-oxygen vibrations have been reported to appear as strong bands near 8 ($12500cm^{-1}$) Silverstein et al, 1974). The appearance of strong bands at 1300 and 1220 cm^{-1} could be attributed to the presence of aromatic ether functions in alkaloid D. The I.R. spectrum also showed the presence of aromatic system and aromatic substitution.

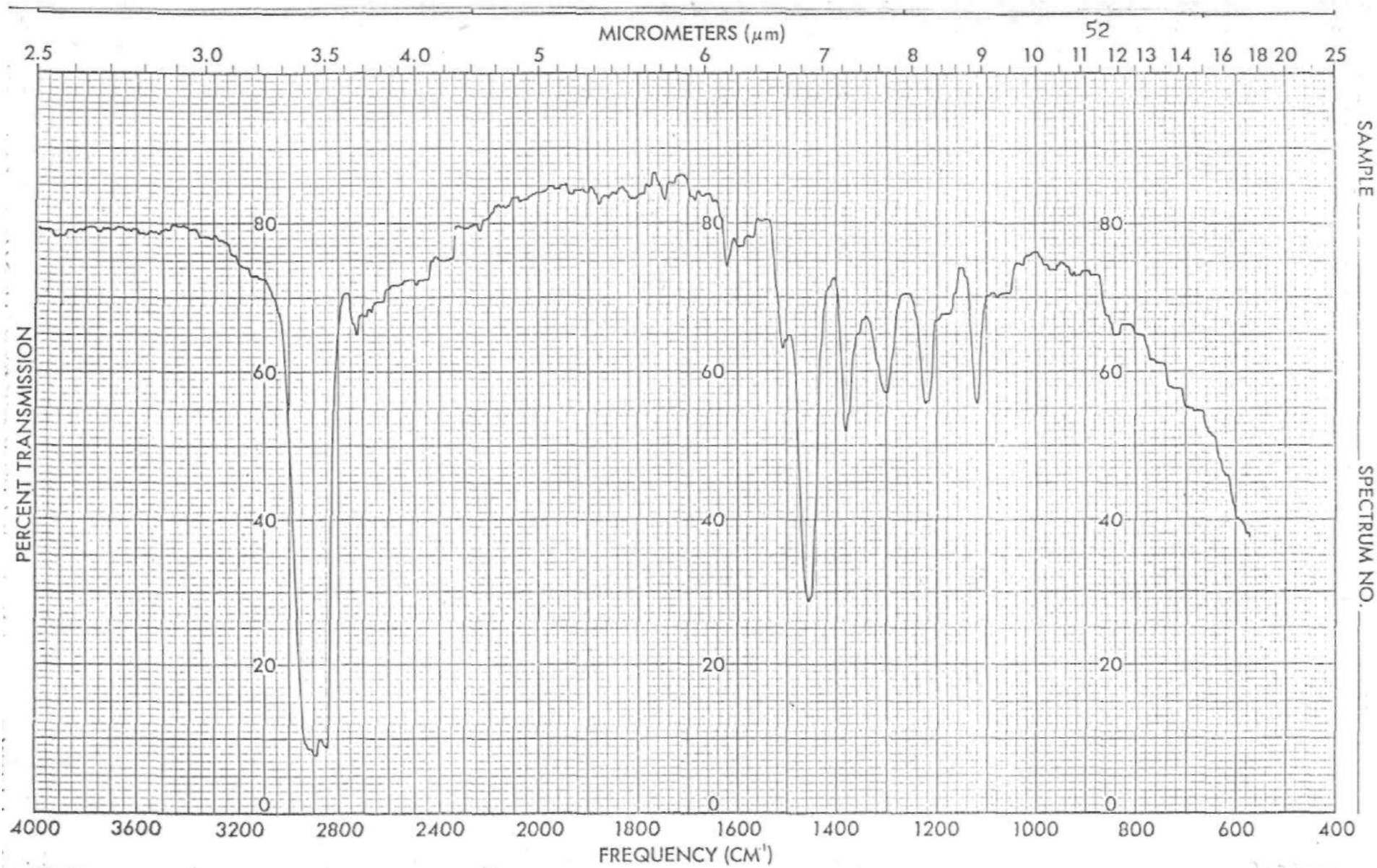


Fig. 2.2 I.R. spectrum of alkaloid D

2.3.6 Mass Spectrum of Alkaloid D

The mass spectrum (Fig. 2.3) of alkaloid D showed peaks at m/e 594 (M+), 593, 595, 487, 299, 298) base peak), 297, 251.5, 191, 190, 162 and 91.

The mass spectral data and C,H,N analysis showed that alkaloid D has a molecular weight of 594 and a molecular formula, $C_{36}H_{38}O_6N_2$.

The ion at m/e 251.5 suggested that alkaloid D may be a bisbenzylisoquinoline alkaloid with a head to tail arrangement, because ions at $(M-91)^{++}$ are quite characteristic of these types of alkaloids (Tomita et al, 1966; Baldas et al, 1966). This involves the loss of the unsubstituted benzyl group (C_7H_7) from the doubly charged parent ion. The head to tail arrangement is further supported by the base peak at m/e 298. Most bisbenzylisoquinoline alkaloids with a head to tail arrangement have been reported to have a base peak near m/e 300 (Milne and Plimmer, 1966). The head to tail arrangement suggested that alkaloid D either belongs to the isochondodendrine or curine series.

The ion at m/e 191 is characteristic of isoquinoline moeity having structure (xxxix) (Brick et al, 1972).

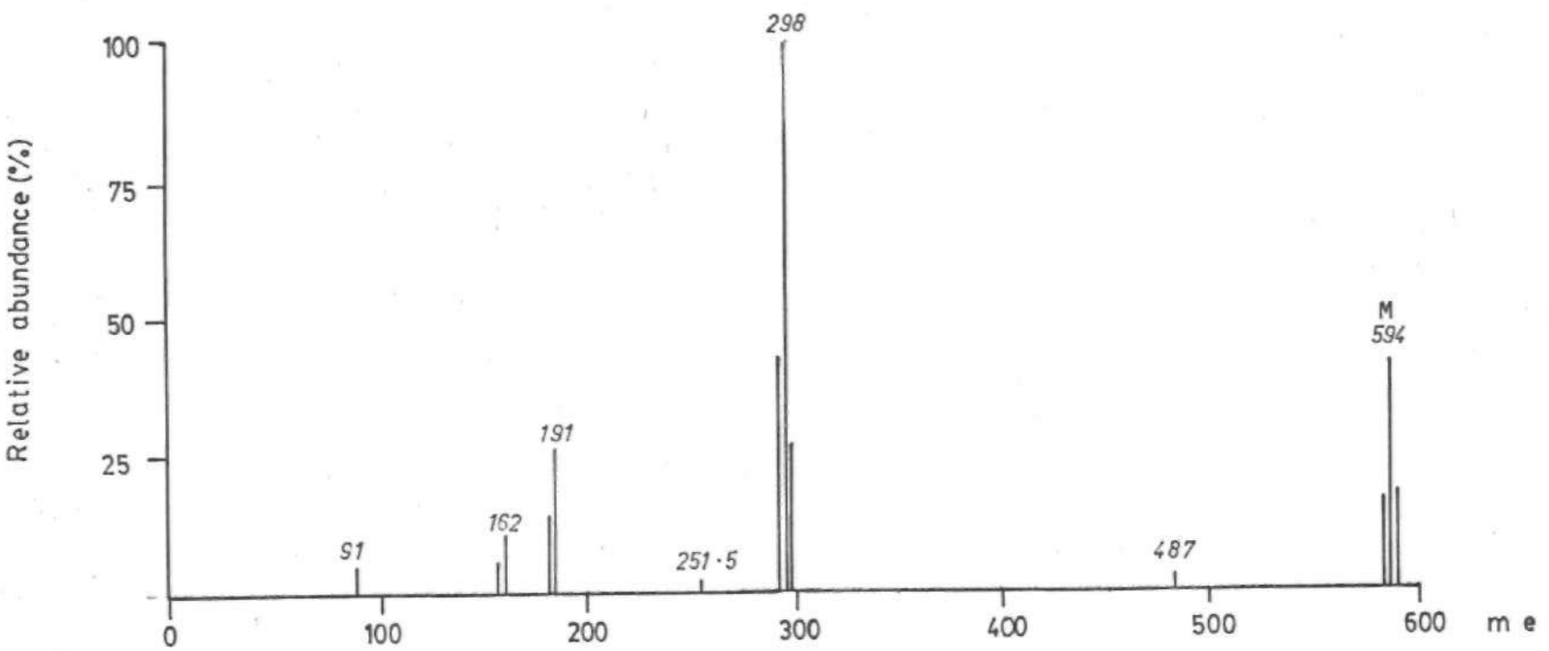
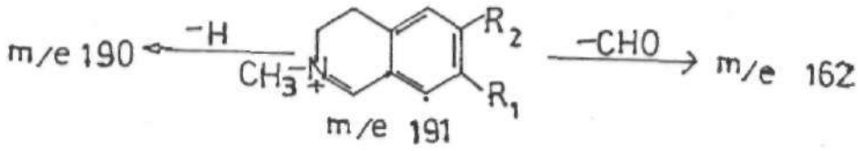
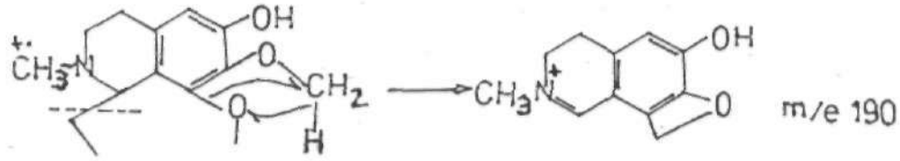


Fig 2·3 Mass spectrum of alkaloid D



(xxxix) $R_1 = OCH_3$, $R_2 = OH$ or vice-versa.

The bisbenzylisoquinoline alkaloids with a head to tail arrangement having 7 (or 7') hydroxyl group have been reported to show a strong peak at m/e 191. The compounds having 7 (or 7') methoxyl group on the other hand have been reported to show a prominent peak at m/c 190 due to the following rearrangement (Brick et al, 1972).



Alkaloids of the bisbenzylisoquinoline group with a head to tail arrangement having two vicinal methoxyl groups give strong peaks at m/e 204 due to similar rearrangement shown above

(Bick et al, 1972). The absence of peaks at m/e 204 indicated that the methoxyl groups in alkaloid D are not vicinal. Since methoxyl groups in positions 5 (and 5') are rarely encountered, it is also suggested that the two methoxyl groups are situated in different isoquinoline nuclei.

The absence of a prominent peak at m/e 190 suggested that the methoxyl groups in alkaloid D are not in the 7 (or 7') positions. However, a weak peak at m/e 190 appeared due to elimination of a hydrogen atom from m/e 191. The ion at m/e 191 also eliminated a CHO group to give a peak at m/e 162 (Bick et al, 1972).

2.3.7 'H NMR Spectrum of Alkaloid D

The 'H NMR spectrum (Fig. 2.4) of alkaloids D was taken in dimethylsulfoxide. The spectral data are shown in table 2.6.

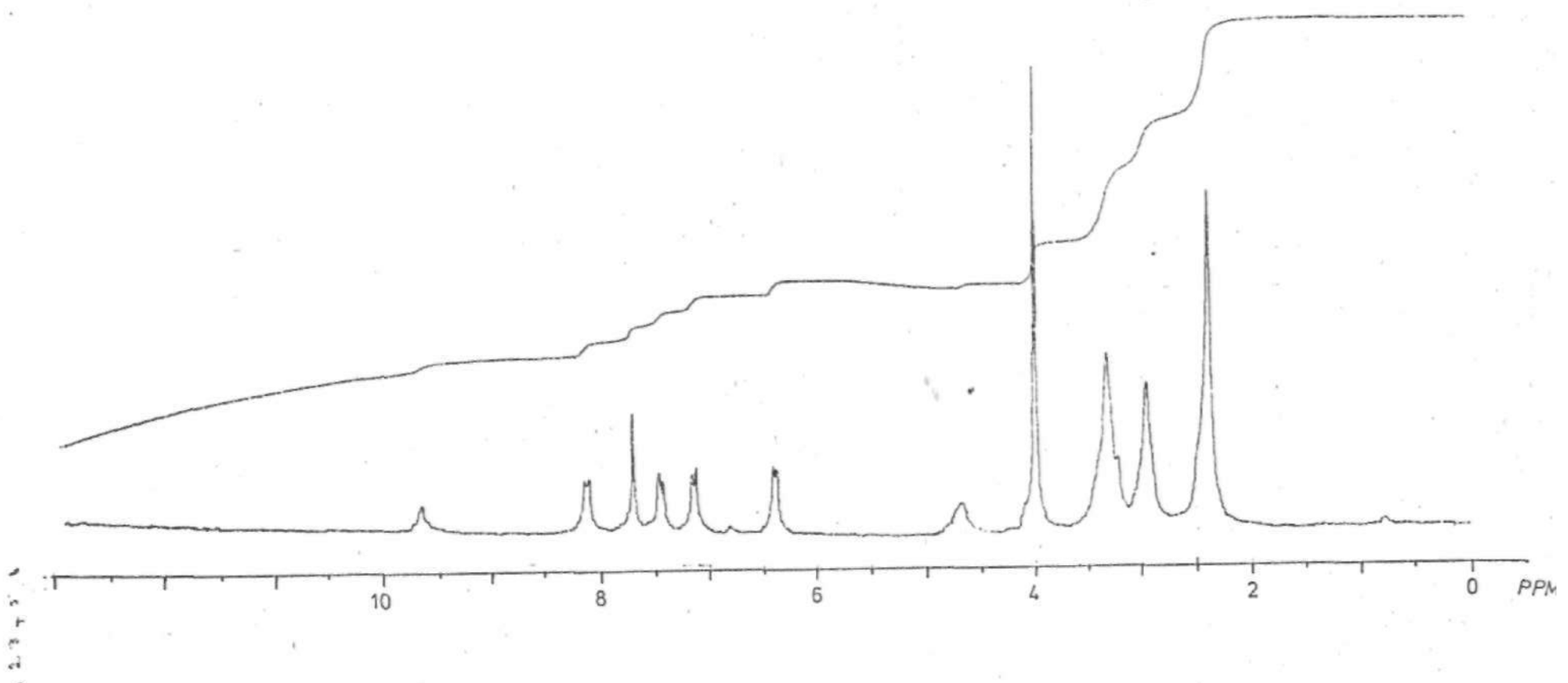


Fig. 2.4 'HNMR spectrum of alkaloid D

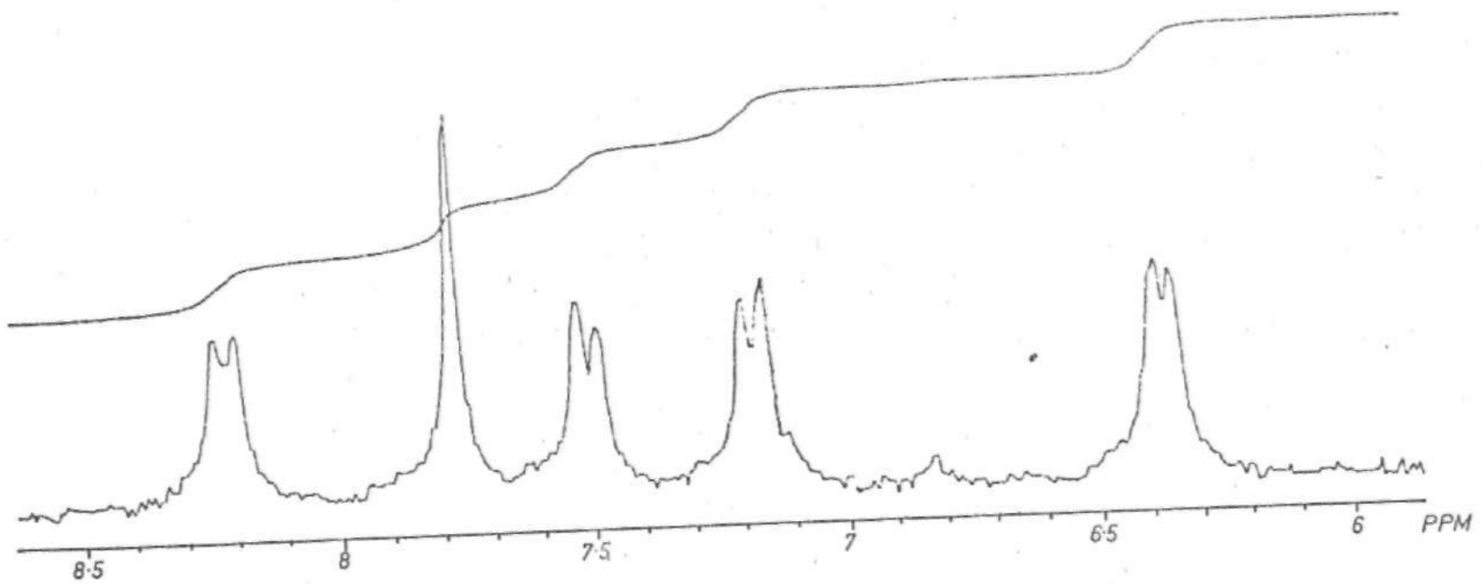


Fig. 2.4 'HNMR Spectrum of alkaloid D (low field)

Table 2.6 'H NMR spectral data of Alkaloid D

Chemical Shifts (δ)	Multiplicity	No. of Protons	Assignment of Peaks
2.50	Singlet	6	2 N-CH_3
3.00	Singlet	8	4 ring CH_2
3.30	Singlet	4	2 benzylic-CH_2
3.90	Singlet	6	2 aromatic OCH_3
6.40	Doublet	10	Aromatic protons
7.20	Doublet		
7.50	Doublet		
7.80	Singlet		
8.25	Doublet		

The 'H NMR spectral data showed that alkaloid D is a bisbenzylisoquinoline derivative having two N-CH_3 groups and two aromotic methoxyl functions. It has been observed that the 6 and 6' methoxyl groups of the alkaloids of isochondodendrine type show relatively normal chemical shifts (δ3.75-3.93). The 7 and 7' methoxyl groups on the other hand have somewhat higher values (δ 3.35). The other groups of bisbenzylisoquine alkaloids, for example

repandine-berbamine series show somewhat high values for the 6 and 6' methoxyl groups (Bick et al, 1966). The appearance of the methoxyl groups at δ 3.90 therefore suggested that the two methoxyl groups in alkaloid D are situated at 6 and 6' positions.

It has been reported that in most cases bis-benzylisoquinoline alkaloids with isochondodendrine-curine structures show signals for the two methoxyl groups (6 and 6') at the same field. However, in the repandine-oxyacanthine-berbamine series the two methoxyl groups appear at different fields. (Bick et al, 1966). The appearance of the two methoxyl groups of alkaloid D at the same field (δ 3.90) further supported the presence of curine-isochondodendrine skeleton in it.

The 'HNMR spectrum also revealed the presence of two benzylic groups. The signal at δ 3.0 for eight protons is due to the four methylene groups of the two isoquinoline nuclei.

Among the aromatic protons eight appeared as doublets whereas two protons appeared as a singlet. The appearance of the eight aromatic protons as doublets is due to O- and m- coupling, which

suggested the absence of substituents at ortho or meta positions. The singlet at δ 7.80 for two protons indicated the presence of substituents ortho and meta to them.

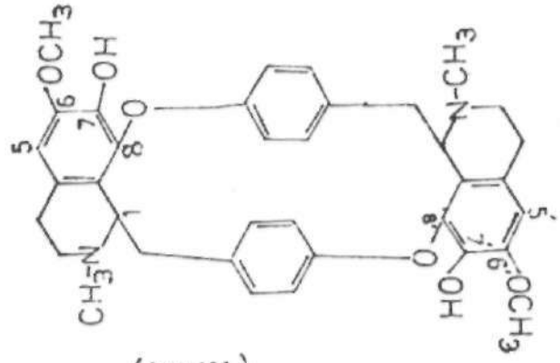
It has been suggested earlier that the two methoxyl groups and the two hydroxyl groups of alkaloid D are in 7, 7' and 6, 6' positions respectively. Therefore the singlet at 7.80 is due to aromatic protons in positions 5 and 5'. This is in conformity with the placement of the two hydroxyl and two methoxyl groups at 7, 7' and 6,6' positions respectively.

2.3.8 Assignment of Structure of Alkaloid D

From C,H,N analysis and mass spectral data, the molecular formula of alkaloid D has been established as $C_{36}H_{38}O_6N_2$. The spectral data (U.V., I.R., NMR and MS) showed that alkaloid D is a bisbenzylisoquinoline alkaloid of the isochondodendrine series having two methoxyl and two N-methyl functions. The absence of blue colour with conc. H_2SO_4 and conc. HNo_3 indicated that alkaloid D does not possess a dibenzo-dioxin function (Bhakuni and Joshi, 1975). The colour tests and U.V. spectral shifts in alkali indicated the presence of phenolic hydroxyl group(s).

The mass spectrum suggested the presence of two phenolic hydroxyl groups which are in 7,7' positions. The spectral data (MS and NMR) showed that the two methoxyl groups of Alkaloid D are in 6 and 6' (positions.

Based on these informations, the following structure was assigned to alkaloid D.



(XVIII)

The above structure (XVIII) is the same as isochondodendrine (Dutcher, 1946). The spectral data therefore identified alkaloid D as isochondodendrine.

With a view of obtaining further proof regarding the identify of alkaloid D with isochondodendrine it was converted to its O-methylderivative.

2.4 Methylation of Alkaloid D

Alkaloid D was methylated with excess ethereal diazomethane generated from nitrosomethylurea. The purified methylated product appeared as white crystals M.p. 267-269°C. It has been designated as alkaloid D-M.

The methylated product was found to be homogeneous by TLC. The TLC data are shown in Table 2.7

Table 2.7 Rf Values of Alkaloid D-M

Solvent system	Rf
Chloroform-Methanol-Acetic Acid (90:10:0.5)	0.7
Butanone-Xylene-Methanol-diethylamine 20:10:1.5	0.61

2.4.1 U.V. Spectrum of alkaloid D-M

The U.V. spectrum (Fig. 2.5) of alkaloid D-M was taken in absolute ethanol. The spectral data are shown in Table 2.8

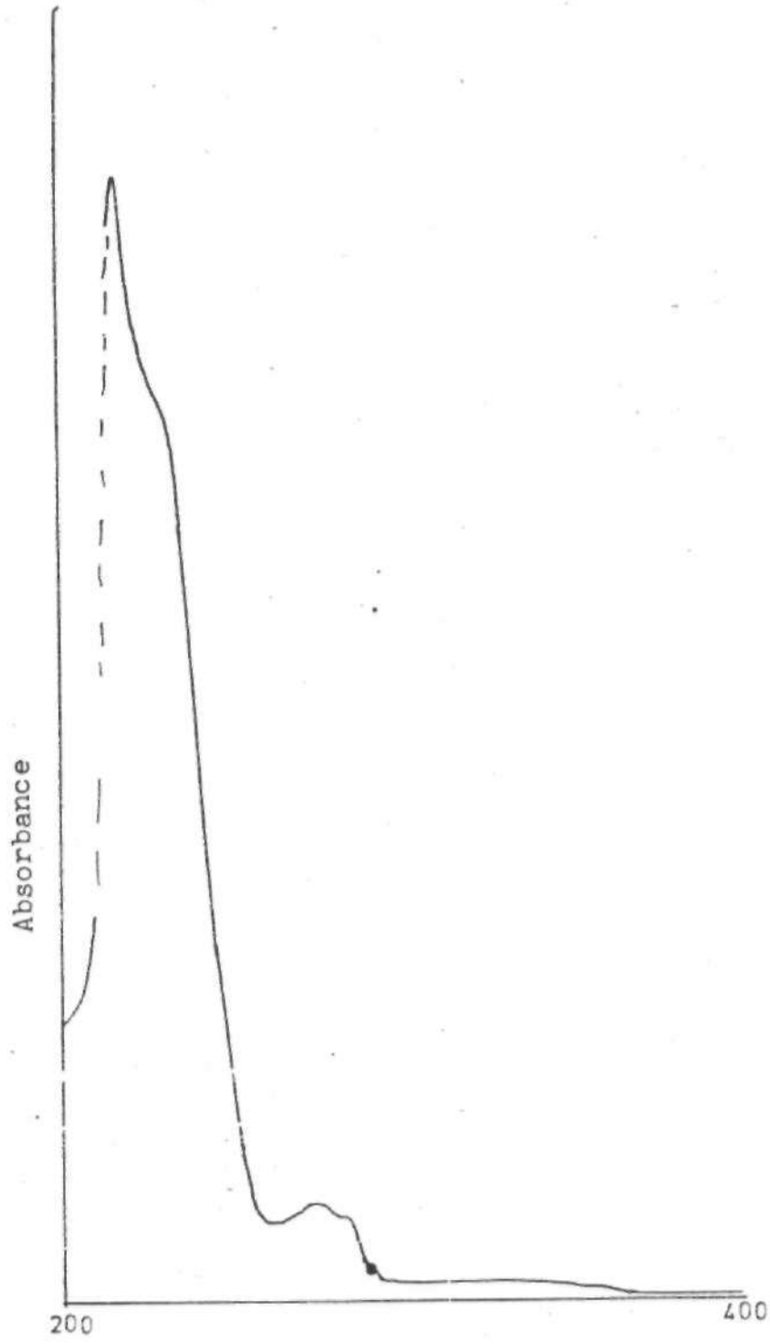


Fig. 2.5 U.V. absorption spectrum of alkaloid D-M

Table 2.8 U.V. Absorption Spectrum of Alkaloid D-M in Ethanol

max (nm)	Log E
216	4.72
232 (Sh)	4.57
276	3.74
284 (Sh)	3.72

The U.V. spectral data are quite similar to that of alkaloid D. T is is mainly because O-methylation does not change the chromophonic system of alkaloid D appreciably.

2.4.2 U.V. Spectrum of Alkaloid D-M in Ethanolic Sodium Hydroxide

The U.V. spectrum did not show any bathochromic shift with alkali, which indicated that both the phenolic hydroxyl groups of alkaloid D had been methylated.

From spectral properties alkaloid D has been identified as isochondodendrine (XVIII). Isochondodendrine on methylation should furnish cycleanine (XVI). Therefore the methylated product (alkaloid D-M) was compared with a reference sample of cycleamine (TLC, I.R. and m.m.p.)

Comparison of Alkaloid D-M
With Cycleanine by TLC

Alkaloid D-M was compared with cycleanine by TLC. The TLC data are as shown in Table 2.9.

Table 2.9 Rf Values of Alkaloid D-M and Cycleanine on TLC

Solvent system	Rf D-M	Rf mix	Rf cy
Chloroform-Methanol-Acetic acid (90:10:0.5)	0.61	0.61	0.61
Butanone-Xylene-Diethylamine (20:10:5.1)	0.7	0.7	0.7

D-M = alkaloid D-M, Cy = Cycleanine, Mix. = mixture of cycleanine and alkaloid D-M.

The TLC data therefore showed that alkaloid D-M is identical with cycleanine.

2.4.3 Mixed Melting of Alkaloid D-M with Cycleanine

Alkaloid D-M, m.p. 267-269°C did not show any depression of melting point when mixed with cycleanine, 268-270°C.

2.4.4 Comparison of Alkaloid D-M with Cycleanine by I. R. Spectroscopy

The I.R. spectra (fig. 2.6) of alkaloid D-M and cycleanine were taken in liquid paraffin and

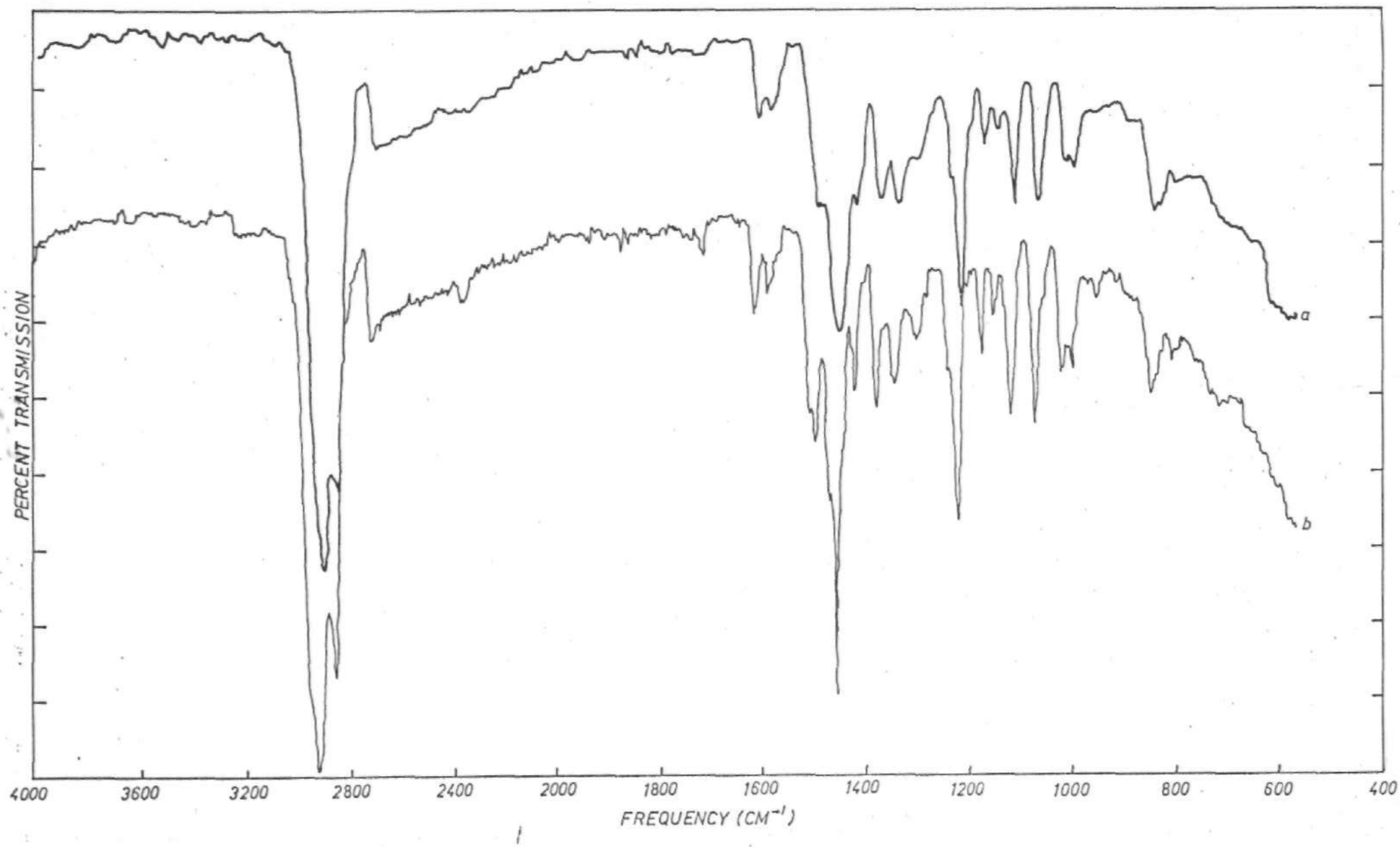


Fig. 2.6 I.R. spectrum cycleanine (a) and (b) alkaloid D-M

the spectra were found to be superimposable.

Alkaloid D-M was therefore identical with cycleanine (m.p., m.m.p., TLC and I.R.)

The above findings and the result of the spectral analysis established the identity of alkaloid D as isochondodendrine (XVIII). This is the first report of the isolation of isochondodendrine from _ynclisia scabrida_.

2.5 Studies on Alkaloid E

2.5.1 Homogeneity

Alkaloid E was found to be homogeneous by TLC. A single spot was obtained when chromatographed in different solvent systems. The TLC data are as shown in Table 2.10.

Table 2.10 Rf Values of Alkaloid E on TLC

Solvent System	Rf
Chloroform-Methanol-Acetic Acid (90:10:0.5)	0.62
Butanone-Xylene-Diethylamine-methanol (20:10:1.5)	0.33

2.5.2 Colour Tests

The colour reactions of alkaloid E with certain reagents have been studied. The results are as shown in Table 2.11.

Table 2.11 Colour Reactions of Alkaloid E

Reagent	Colour
Nitric acid (conc)	Yellow
Sulphuric acid (conc)	No colour
Nitric acid+ Sulphuric acid	Orange
Ferric chloride	Green

The green colour with ferric chloride indicated the presence of phenolic hydroxyl group.

Table 2.12 Test for Alkaloids

Reagent	Colour/ Precipitate	Inference
Dragendorff's reagent	Orange ppt.	Alkaloid present
Daagendorff's spray reagent	Orange colour	Alkaloid present
Mayer's reagent	Cream ppt.	Alkaloid present

2.5.3 U.V. Absorption Spectrum of Alkaloid E

The U.V. absorption spectrum of alkaloid E (Fig. 2.7a and 2.7b) was taken in methanol. The spectral data x are shown in Table 2.13.

Table 2.13 U.V. Absorption Spectrum of Alkaloid E in Methanol

max (nm)	Log E
242	4.5
276	4.2
281.6 (Sh)	3.6
322	3.4
332.6	3.2

The U.V. spectrum of alkaloid E has similarity with aporphines and other tetrahydroisoquinoline group of alkaloids (Sangster and Stuart, 1965).

2.5.4 U.V. Absorption Spectrum of Alkaloid E in Methanolic Sodium Hydroxide

The U.V. absorption spectrum (Fig. 2.7a) of alkaloid E was taken in methanolic sodium hydroxide. The spectral data are shown in table 2.14.

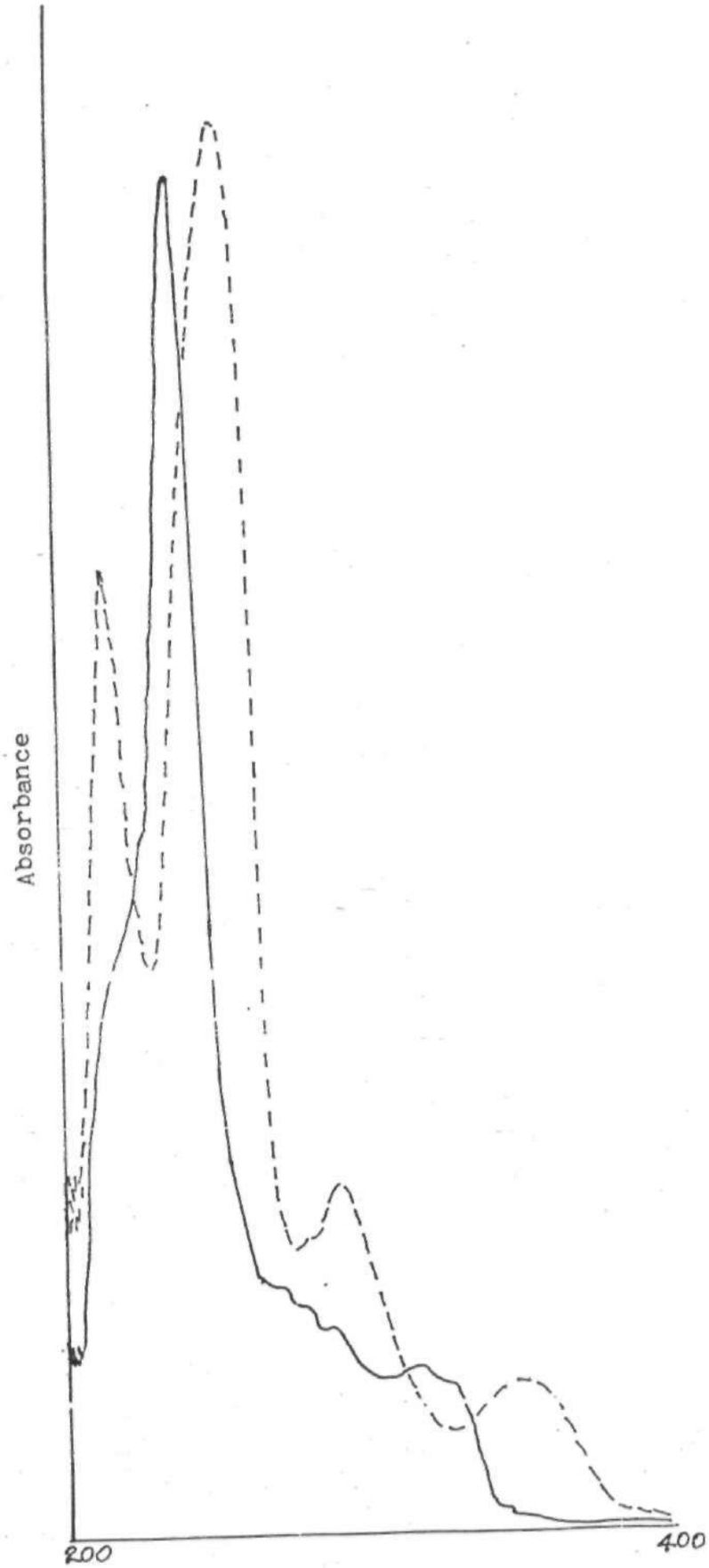


Fig. 2.7(a) U.V. absorption spectrum of alkaloid
(——) in methanol (---) in methanolic NaOH

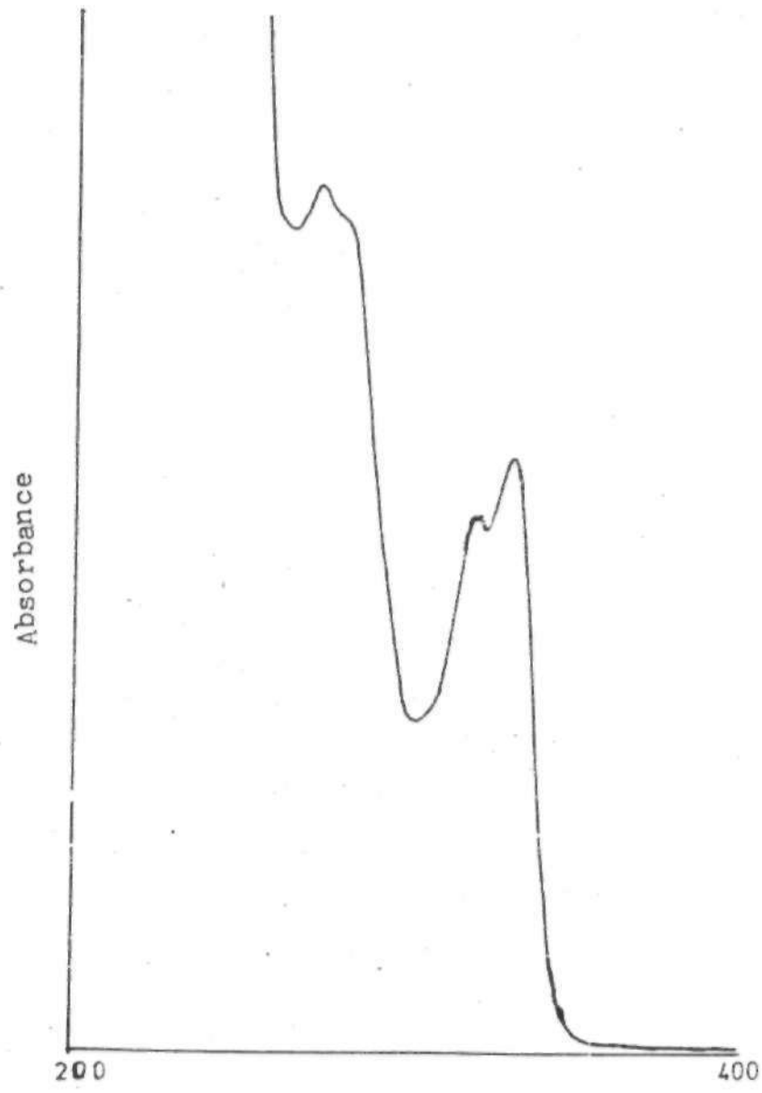


Fig. 2.7(b) U.V. absorption spectrum of alkaloid E in methanol

Table 2.14 U.V. Absorption Spectrum of Alkaloid E in Methanolic Sodium Hydroxide

max (nm)	Log E
250	4.7
284 (Sh)	4.1
296	3.8
354	3.5

The bathochromic shifts of the maxima in alkali confirmed the presence of phenolic hydroxyl group which was suggested from the colour reactions.

2.5.6 I.R. Spectrum of Alkaloid E.

The I.R. spectrum of alkaloid E (Fig. 2.8) was taken in liquid paraffin. The diagnostic peaks are shown in Table 2.15.

Table 2.15 I.R. Spectral Data of Alkaloid E

Absorption band (cm^{-1})	Inference
3500	hydroxyl group
1510 1600 1610	Aromatic system
1240 1270	Aromatic ether
840 860	Aromatic sub-stitution

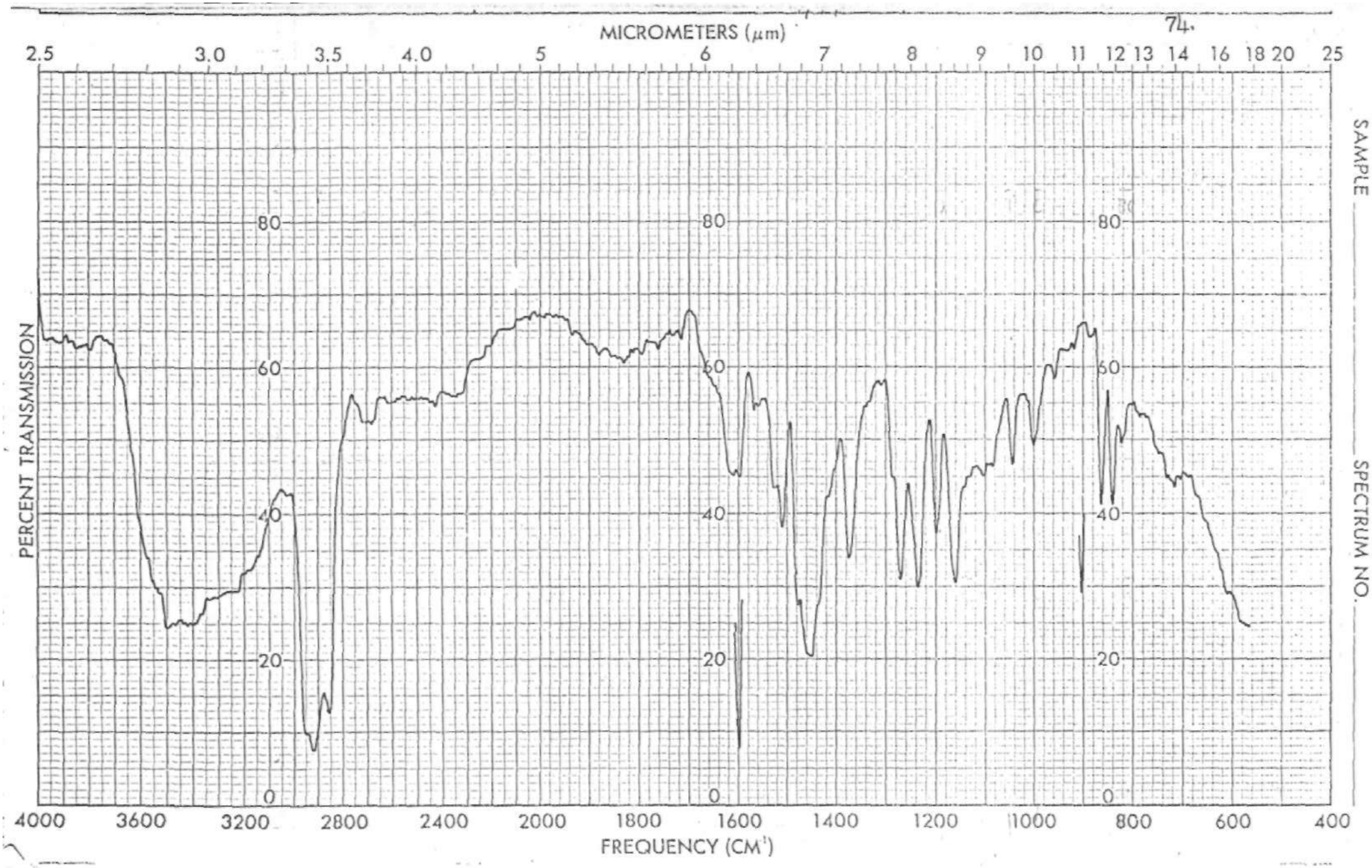


Fig. 2.8 I.R. spectrum of alkaloid E

The I.R. spectrum showed the presence of aromatic system and aromatic ether function as well as the presence of a hydroxyl group.

2.5.7 Mass Spectrum of Alkaloid E

The mass spectrum (fig. 2.9) showed peaks at m/e 281 (M^+), 280 (base peak,) 264, 265, 248, 249, 220, 140.5, 107, 77, 236, 237.

The striking features about the mass spectrum of alkaloid E is that the $(M-1)^+$ peak is the base peak and a doubly charged molecular ion appeared at m/e 140.5.

It was observed that in the mass spectrum of most aporphine alkaloids the base peak is the $(M-1)^+$ peak especially those oxygenated at 1,2,9 and 10 positions. A doubly charged ion has also been observed in the mass spectra of aporphine alkaloids (Jackson and Martins, 1966).

Aporphine alkaloids are known to occur in Menispermaceous plants (Tornber, 1970). The appearance of the base peak of alkaloid E at m/e 280 (M-1)+ as well as the presence of the doubly charged molecular ion at m/e 140.5 was therefore taken as an indication that alkaloid E could belong to the aporphine group. However, aprophine have

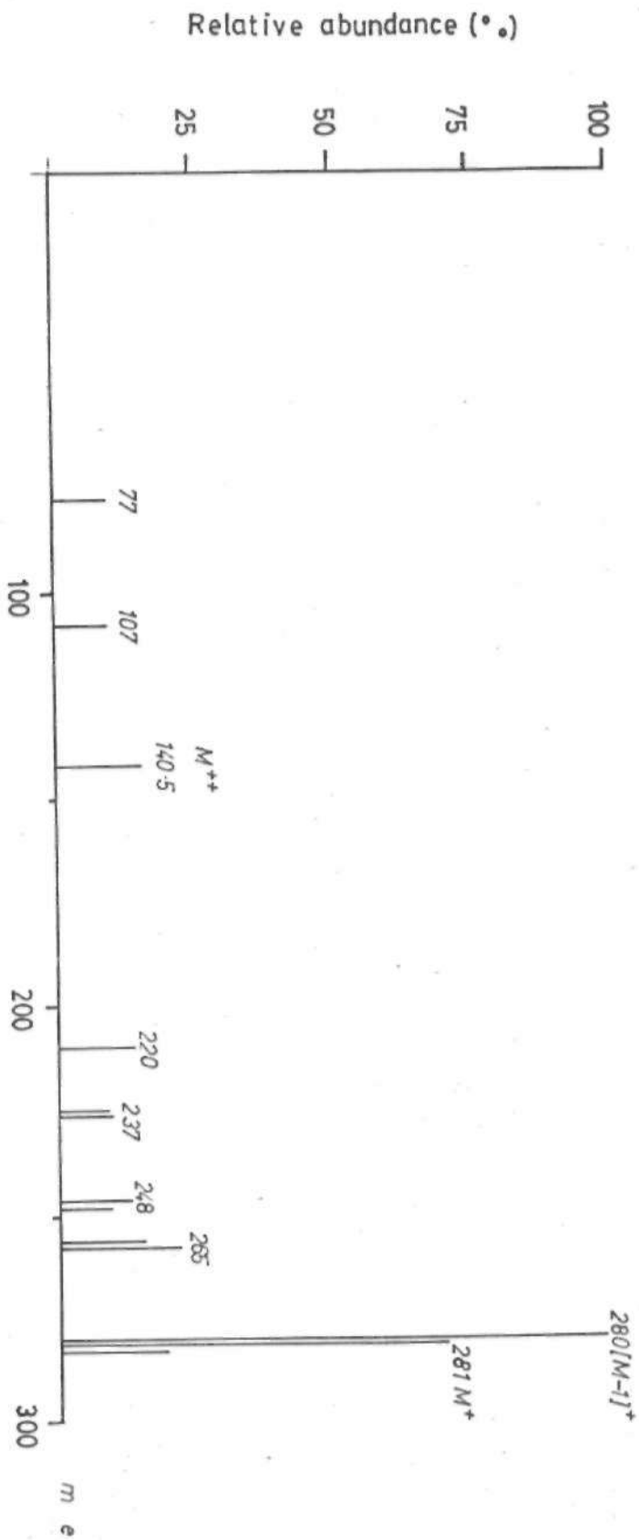
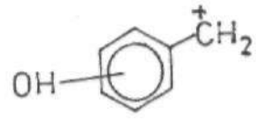


Fig 2·9 Mass spectrum of alkaloid E

been reported to show characteristic peaks at $(M-43)^+$ for the N-methyl derivatives while the secondary amines show intense peaks at $(M-29)^+$ (Dyke, 1978). Neither of these ions were observed in the mass spectrum of alkaloid E.

The peak at m/c 77 could be attributed to a phenyl group, $C_6H_5^+$ while that at m/c 105 could be attributed to a hydroxybenzylic group (XL)



m/e 105

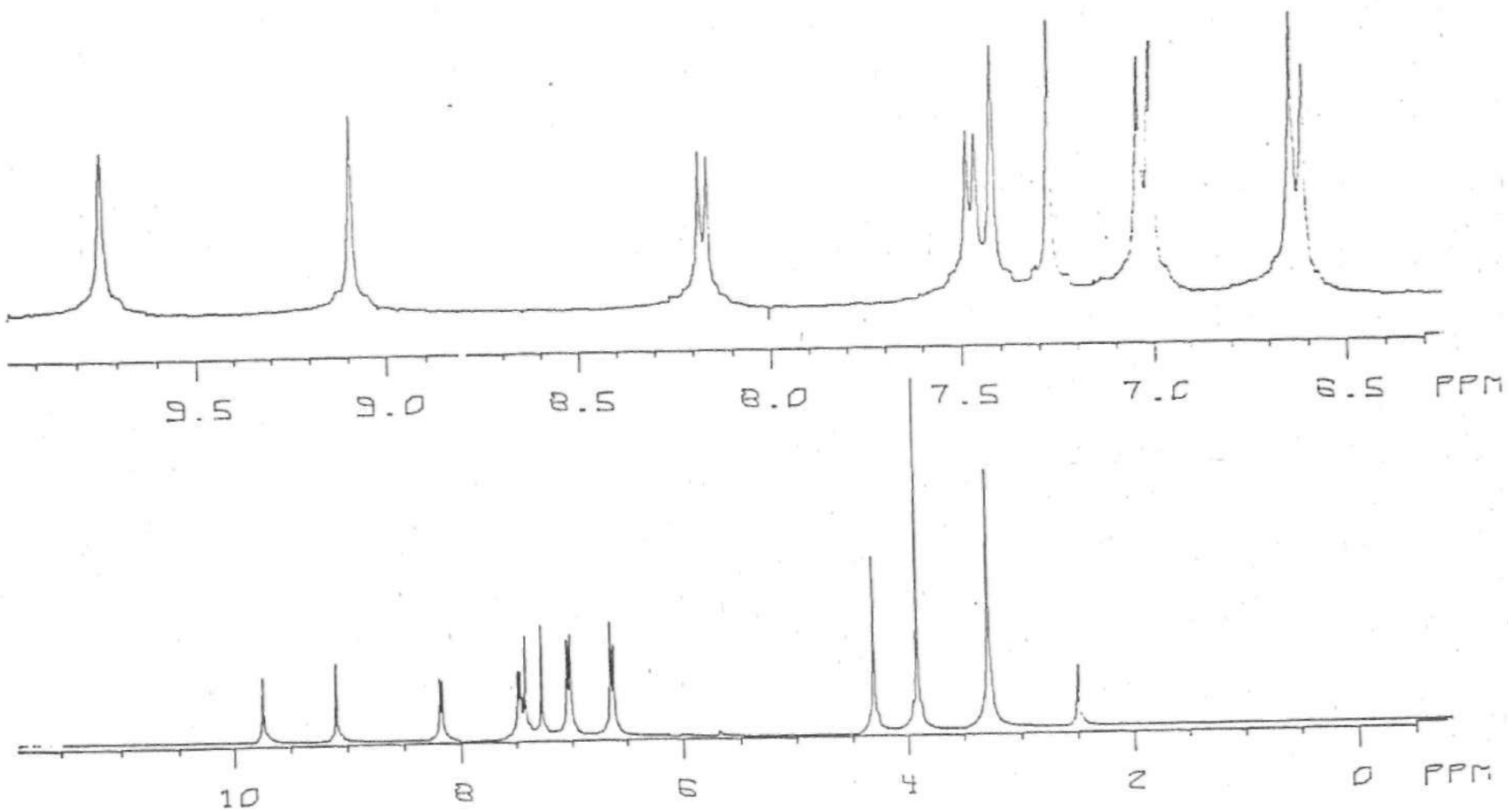
(XL)

The presence of the hydroxybenzylic ion m/c 105 (strong peak) and the absence of either $(M-43)^+$ or $M-29)^+$ peaks are not in conformity with the aporphine skeleton suggested for alkaloid E.

The peak at m/c 264 might have been formed by the elimination of a hydroxyl group $(M-OH)^+$.

2.5.8 'H NMR spectrum of Alkaloid E

'H NMR spectrum (Fig. 2.10) of alkaloid E was taken in dimethylsulfoxide. The spectral data are shown in table 2.16.



2.10: 'H NMR spectrum of alkaloid E

Table 2.16 'H NMR Spectrum of Alkaloid E

Chemical shift(s)(δ)	Multiplicity	No. of protons	Assignment of peaks
2.5	Singlet	1	Could not be assigned
3.29	Singlet	3	Aromatic OCH_3
3.91	Singlet	3	Aromatic OCH_3
4.31	Singlet	2	Benzylic CH_2
6.65	Doublet	2	Aromatic protons
7.05	Doublet	2	
7.29	Singlet	1	
7.45	Singlet	1	
7.50	Doublet	1	
8.20	Doublet	1	
9.10	Singlet	1	Hydrogen Bonded hydroxyl groups
9.75	Singlet	1	

The 'H NMR spectrum of alkaloid E showed 19 protons of which 8 are aromatic protons. The two protons at δ 4.31 could be due to benzylic methlene protons. The spectrum also showed the presence of two methoxyl groups at δ 3.29 and δ 3.91. The appearance of the two methoxyl groups at different fields suggested that the two groups are located in different structural environments.

Six of the eight aromatic protons appeared as doublets indicating that they are ortho or meta coupled. This suggested the absence of substituents at positions ortho and meta to them. Two aromatic protons appeared as singlets suggesting the presence of substituents at positions ortho and meta to them.

The presence of eight aromatic protons does not conform with the aporphine structure earlier suggested by the mass spectrum.

Hydrogen bonded phenolic hydroxyl groups have been reported to resonate considerably downfield (Jackman and Sternhell, 1969). The presence of hydroxyl groups in alkaloid E was indicated by the results of I.R., U.V. and colour reactions. The appearance of two protons at δ 9.10 and δ 9.75 therefore suggested the presence of two hydrogen bonded phenolic hydroxyl functions.

2.6.1 O-Methylation of Alkaloid E

The colour reactions, U.V. spectral shifts in alkali, mass and ^{1}H NMR results indicated the presence of two phenolic hydroxyl groups in alkaloid E. In order to confirm this and to obtain further information regarding the structure, alkaloid E was converted to its O-methyl derivative.

A known amount of alkaloid E was treated with excess ethereal diazomethane. The methylated product was isolated and purified by passing through silica gel column. It appeared as white crystals which has been designated as alkaloid E-M.

2.6.1 Homogenity of Alkaloid E-M

Alkaloid E-M was chromotographed together with alkaloid E on TLC. The TLC data are as shown on Table 2.17.

Table 2.17 TLC Data of Alkaloids E and E-M

Solvent System	Rf E	Rf E-M
Butanone-xylene-Methanol-diethylamine (20:10:5:1)	0.33	0.88

The results showed that alkaloid E-M is homogeneous. It also showed that alkaloid E has been methylated.

2.6.2 Ferric Chloride Test for Alkaloid E-M

No colour change was observed when methanolic solution of alkaloid E-M was treated with aqueous solution of ferric chloride. The absence of characteristic colour for phenolic compounds with ferric chloride indicated that the phenolic hydroxyl

groups in alkaloid E have been methylated.

2.6.3 U.V. Absorption Spectrum of Alkaloid E-M in Methanol

The U.V. absorption spectrum (Fig. 2.11 and 2.12) of alkaloid E-M was taken in methanol. The spectral data are as shown in table 2.18.

Table 2.18 U.V. Absorption Spectral Data of Alkaloid E-M in Methanol

max (nm)	236, 276, 282 (Sh), 318, 328)

The U.V. absorption spectrum of alkaloid E-M is quite similar to that of alkaloid E. This is mainly because the chromophoric system in alkaloid E is not appreciably changed by O-methylation.

2.6.4 U.V. Absorption Spectrum of Alkaloid E-M in Methanolic Sodium Hydroxide

The U.V. absorption spectrum of alkaloid D-M was taken in methanolic sodium hydroxide. The U.V. shifts in alkali observed in the U.V. spectrum of alkaloid E was not observed in alkaloid E-M. This further indicated that the phenolic hydroxyl groups in alkaloid E have been methylated.

2.6.5 Mass Spectrum of Alkaloid E-M

The mass spectrum of alkaloid E-M (Fig. 2.13) showed peaks at m/e 309 (M^+), 308 (base peak), 295, 261, 262, 263, 278, 279, 250, 236, 220, 204, 250

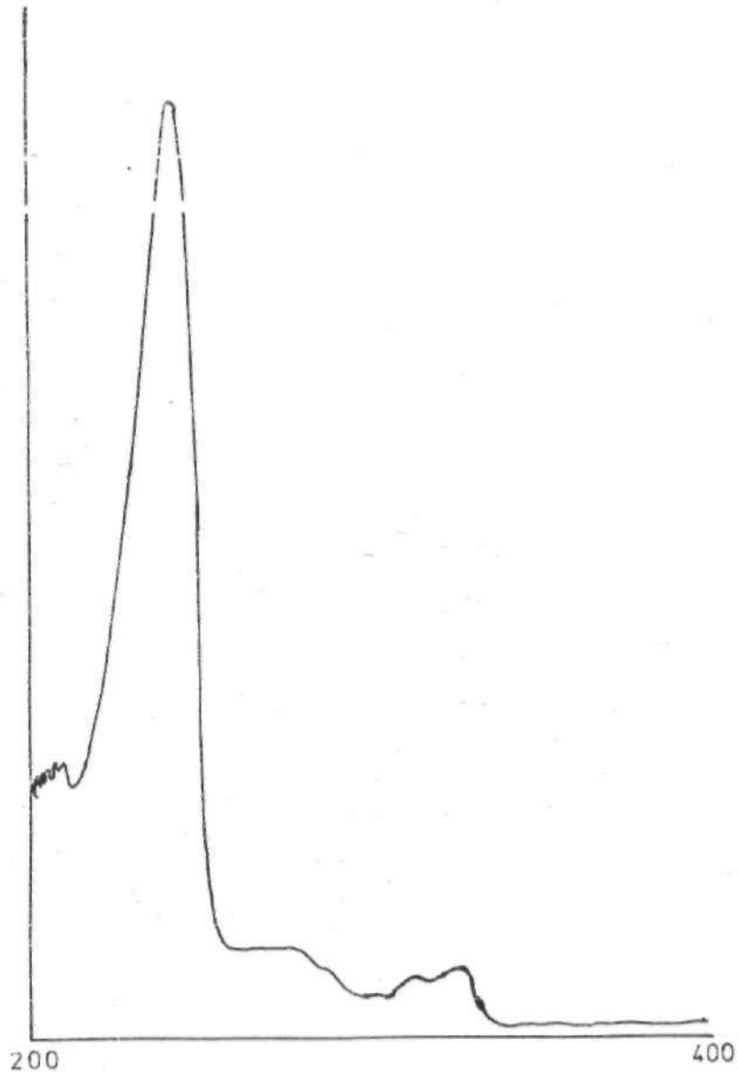


Fig. 2.11 U.V. absorption spectrum of alkaloid E-M in methanol

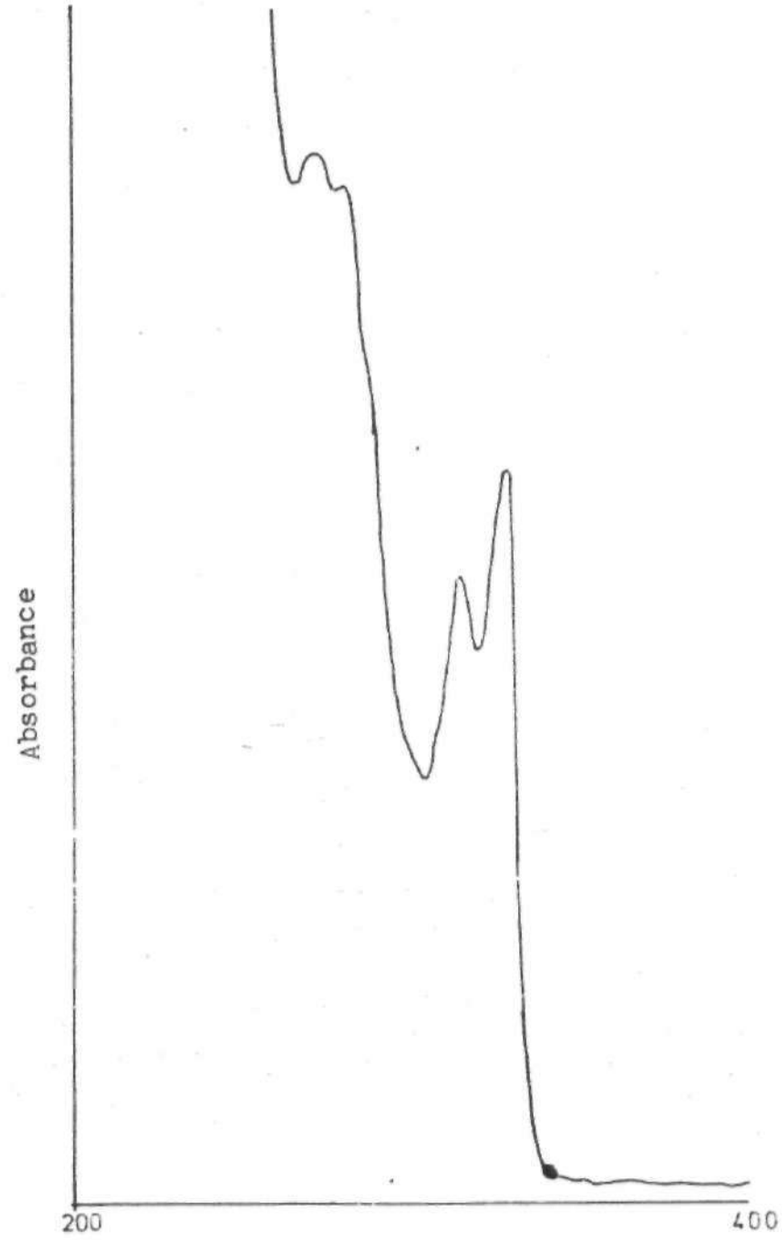
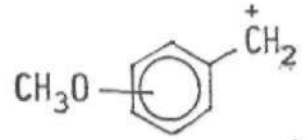


Fig. 2.12 U.V. absorption spectrum of alkaloid E-M in methanol

191, 192, 154.5, 121, 77, 78.

The M^+ of alkaloid E-M appeared at m/e 309, indicating that the M^+ of alkaloid E has increased by twenty eight mass units after methylation, thus confirming the presence of two hydroxyl groups in it. Similar to alkaloid E a doubly charged ion appeared at m/e 154.5 in alkaloid E-M, representing the doubly charged molecular ion. The peak at m/e 107 in alkaloid E representing the hydroxybenzylic ion shifted to m/e 121 in alkaloid E-M (a shift of 14 mass units) representing a methoxybenzylic ion (XLI).



(XLI)

This also confirmed the presence of phenolic hydroxyl group in alkaloid E.

The peak at m/e 77 due to $C_6H_5^+$ which appeared in the mass spectra of alkaloid E also appeared in alkaloid E-M. The peak at m/e 220 is also common to the two alkaloids.

The peak at m/e 294 is due to $(M-CH_3)^+$ while that at m/e 278 could be due to $(M-OCH_3)^+$.

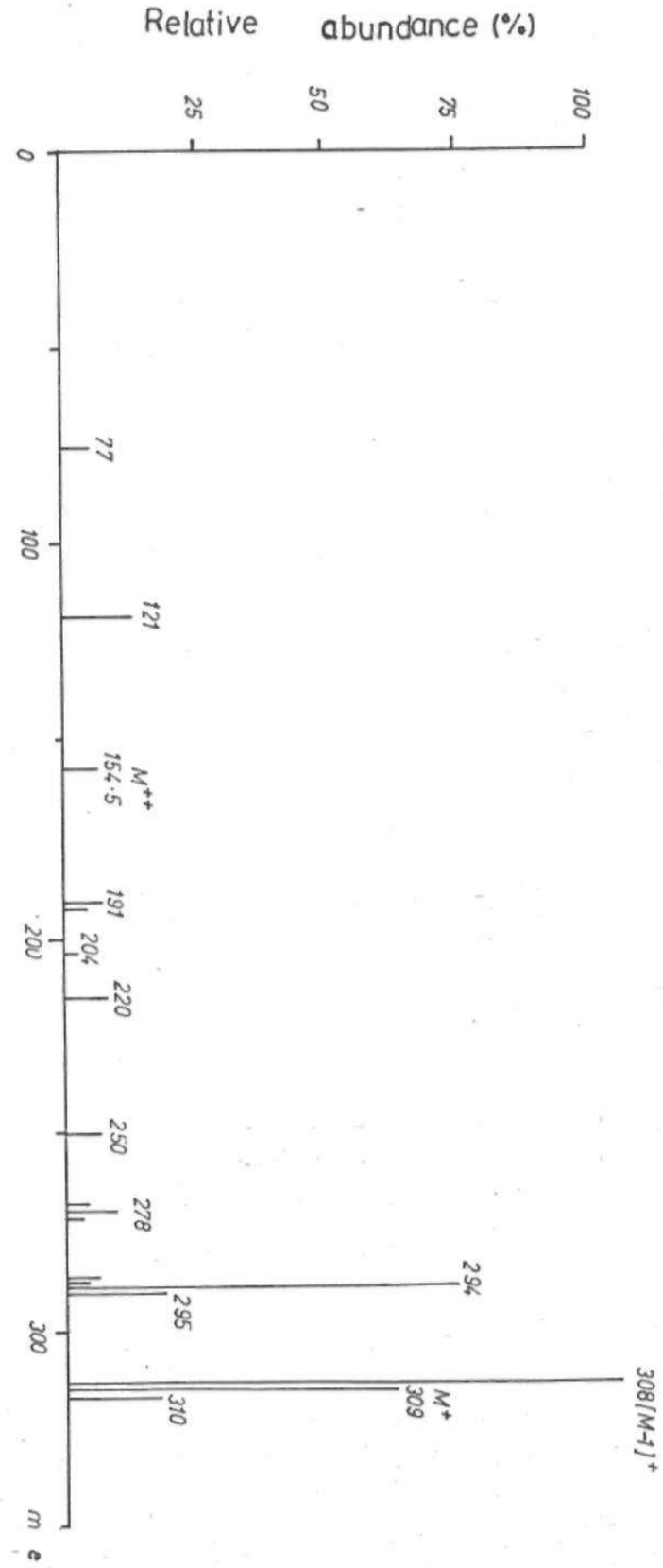


Fig 2·13 Mass spectrum of alkaloid E-M

In addition to bisbenzislisoquinoline group of alkaloids, menispermaceous plants have been reported to elaborate other groups of alkaloids, such as benzylisoquinolines, aporphines, pro-aporphines, morphines, hasubanan alkaloids and protoberberines (Thornber, 1970). From the results of spectral data, colour reactions and methylation experiment alkaloid E could not be assigned to any of these groups of alkaloids.

Based on the available information no definite structure could be assigned to alkaloid E. More work (spectral and degradative studies) has to be done in order to elucidate the structure of alkaloid E.

2.7 Studies on Alkaloid F

2.7.1 Homogenity

Alkaloid F was found to be homogeneous by TLC. A single spot was obtained when chromatographed in different solvent systems. The data are as shown in Table 2.19.

Table 2:19 Rf Values of Alkaloid F

Solvent System	Rf
Chloroform methanol-acetic acid 90: 10: 0.5	0.6
Butanone-xylene-methanol-diethylamine 20:10:5:1	0.69

2.7.2 Colour tests

Colour reactions of alkaloid F with certain reagents have been studied. The result is as shown in table 2.20.

Table 2.20 Colour reactions of Alkaloid F

Reagent	Colour
Sulphuric acid (conc)	Yellowish brown
Nitric acid (conc)	Dark brown
Sulphuric acid + Nitric acid	Yellowish brown turning to reddish brown
Ferric chloride	No colour change

The absence of characteristic colour for phenolic compounds with ferric chloride indicated that alkaloid F may lack phenolic hydroxyl group.

Table 2:21 Test for Alkaloids

Reagent	Colour/ Precipitate	Inference
Dragendorff's reagent	Orange ppt.	Alkaloid present
Dragendorff's spray reagent	Orange colour	Alkaloid present
Mayer's reagent	Cream ppt.	Alkaloid present

2.7.3 U.V. spectrum of Alkaloid F

The U.V. spectrum (Fig. 2.14) of alkaloid F was taken in absolute ethanol. The spectral data are shown in table 2.22.

Table 2.22 U.V. Absorption Spectrum of Alkaloid F

max (nm)	Log E
216	4.72
232 (sh)	4.57
276	3.74
284 (sh)	3.72

The peak at 284nm suggested that alkaloid F could be of the bisbenzylisoquinoline group (Sangster and Stuart, 1965).



Fig. 2.14 U.V. spectrum of alkaloid F

2.7.4 U.V. Absorption Spectrum of Alkaloid F in Ethanolic sodium Hydroxide

The U.V. absorption spectrum of alkaloid F did not show any bathochromic shift with alkali, which indicated the absence of phenolic hydroxyl groups in alkaloid F.

2.7.5 I.R. spectrum of alkaloid F

The I.R. spectrum (fig. 2.15) of alkaloid F was taken in liquid paraffin, the diagnostic peaks are shown in table 2.23 below.

Table 2.23 I.R. Spectral Data of Alkaloid F

Absorption band (Cm^{-1})	Inference
1490	
1580	Aromatic system
1610	
1220	Aromatic ether
840	Aromatic substitution

The I.R. spectrum of alkaloid F showed the presence of aromatic ether function, aromatic system and aromatic substitution.

The above data (m.p. colour test, U.V. and I.R.) and results of C,H,N, analysis for alkaloid F suggested that it could be identical with cycleanine (XVI), a non-phenolic bisbenzylisoquinoline alkaloid already isolated from the roots and stem of Synclisia scabrida

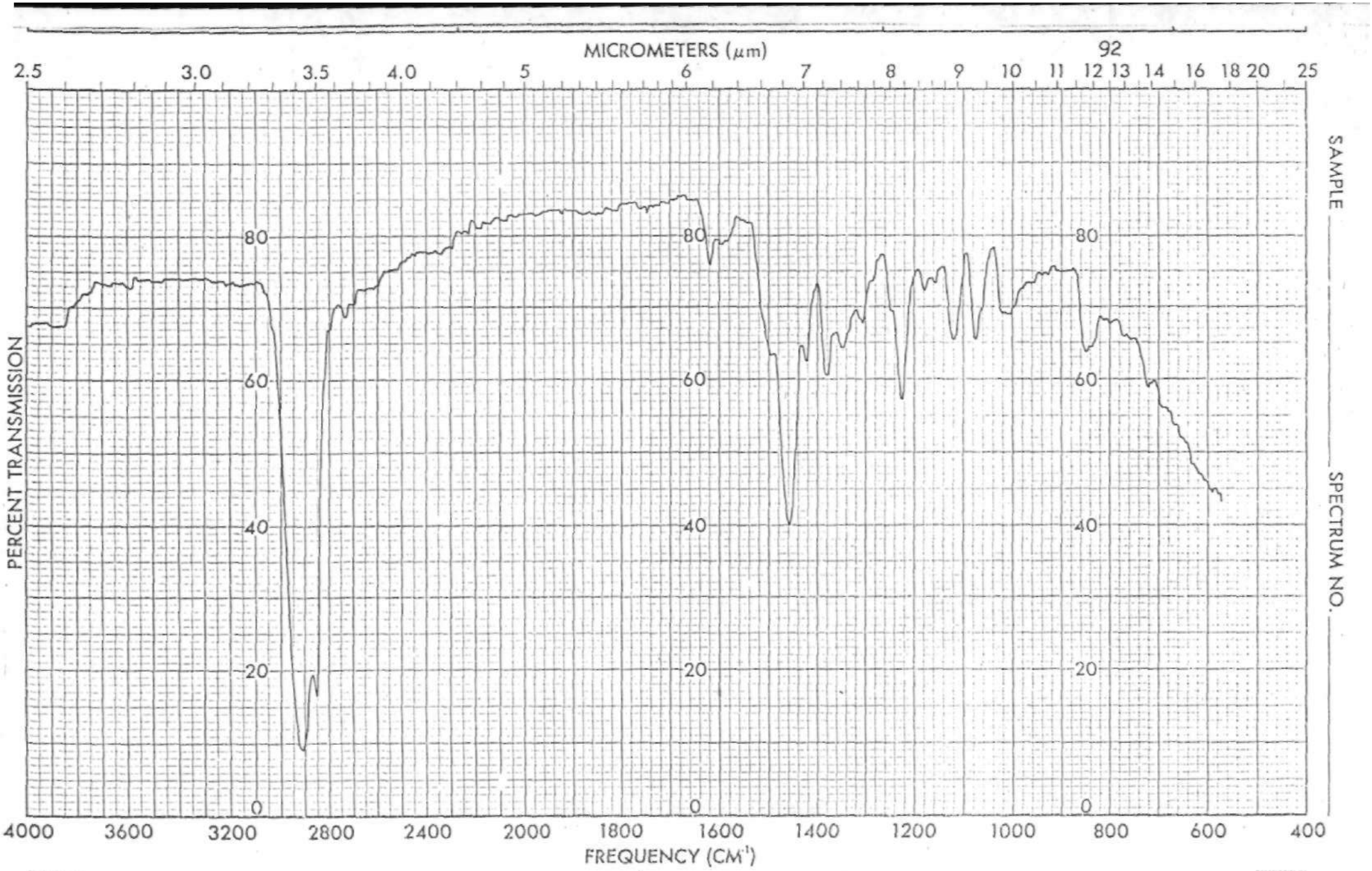


Fig. 2.15 I.R. spectrum of alkaloid F

Alkaloid F was therefore compared with an authentic specimen of cycleanine.

2.7.6 Comparison of alkaloid F with Cycleanine by TLC

Alkaloid F was compared with cycleanine by TLC. The TLC data are as shown in table 2.24.

Table 2.24: Rf values of Alkaloid F and Cycleanine on TLC

Solvent system	Rf F	Rf mix	Rf cy
Butanone-xylene-Methanol-diethylamine 20:10:5:1	0.69	0.69	0.69
Chlorform-Methanol-Acetic acid 90:10:0.5	0.6	0.6	0.6

F = alkaloid F, Cy = Cycleanine, Mix = mixture of alkaloid F and cycleanine.

The TLC data therefore showed that alkaloid F is identical with cycleanine.

2.7.7 Mixed Melting Point of Alkaloid F With Cycleanine

Alkaloid F, M.p. 268-270^{o}C did not show any depression of melting point when mixed with a reference sample of cycleanine, 269-271^{o}C.

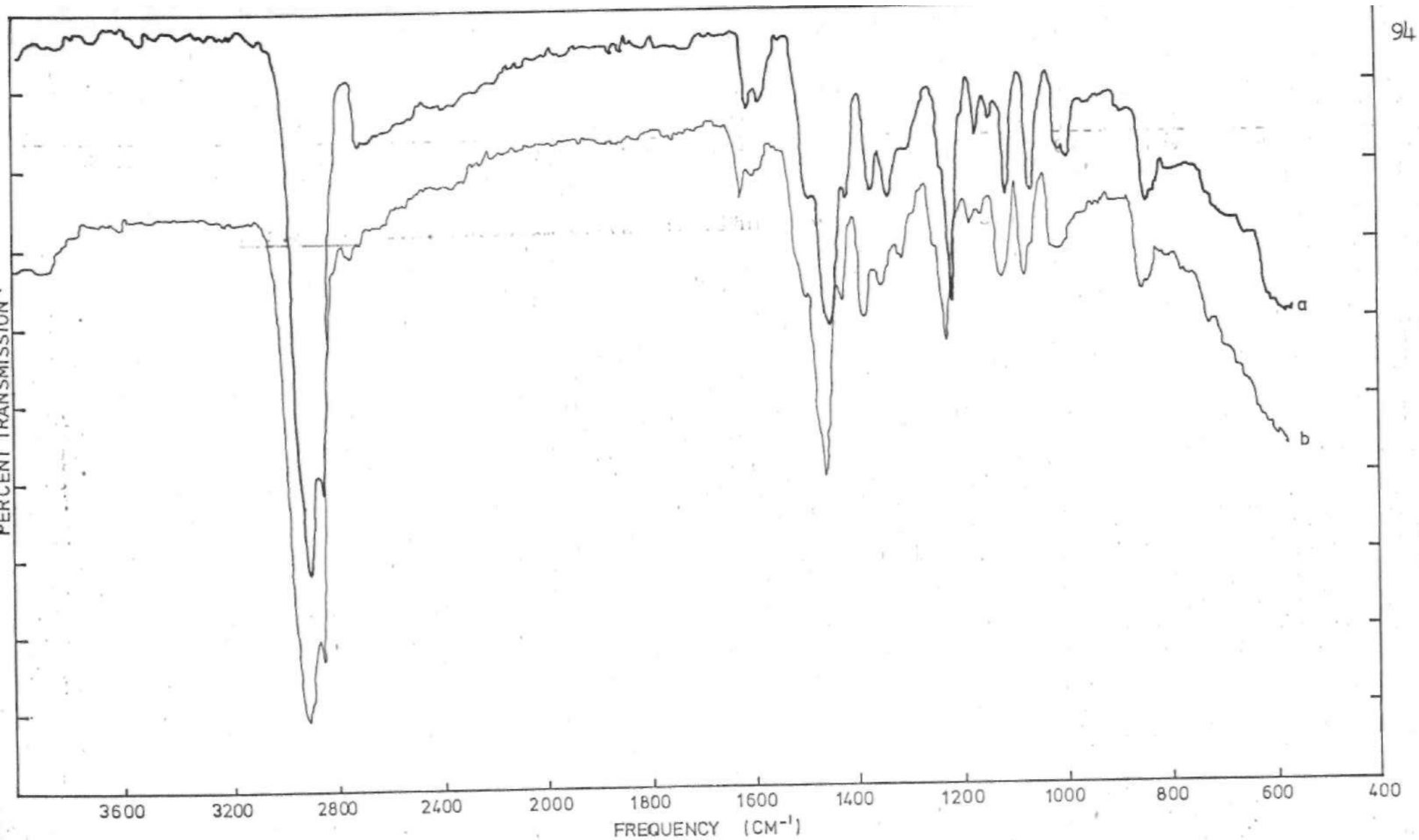


Fig. 2.16 I.R. spectrum of (a) cycleanine and (b) alkaloid F

2.7.8 Comparison of Alkaloid F with Cycleanine by I.R. Spectroscopy

The I.R. spectra (fig. 2.16) of alkaloids F and cycleanine were taken in liquid paraffin and the spectra were found to be identical.

Alkaloid F was therefore identical with cycleanine (m.p., m.m.p., TLC and I.R.). This is the first report of the isolation of cycleanine from the leaf of Synclisia scabrida.

EXPERIMENTAL

2.8 Plant Material

The leaves of Synclisia scabrida were obtained from rubber plantation in Benin, Bendel State, Nigeria. The plant was identified by Mr. A.O. Ohaeri of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

2.8.1 Extraction and Separation

The leaves were oven dried at 40°C for 24 hours and then milled. The powdered and dried leaves of the plant (750g), were percolated to exhaustion with 95 percent ethanol. This was achieved by soaking the leaves overnight in 2L of ethanol. The extract was collected by the following day and fresh ethanol was added to the leaves and left for another day. This process was repeated until the ethanol extract obtained appeared colourless.

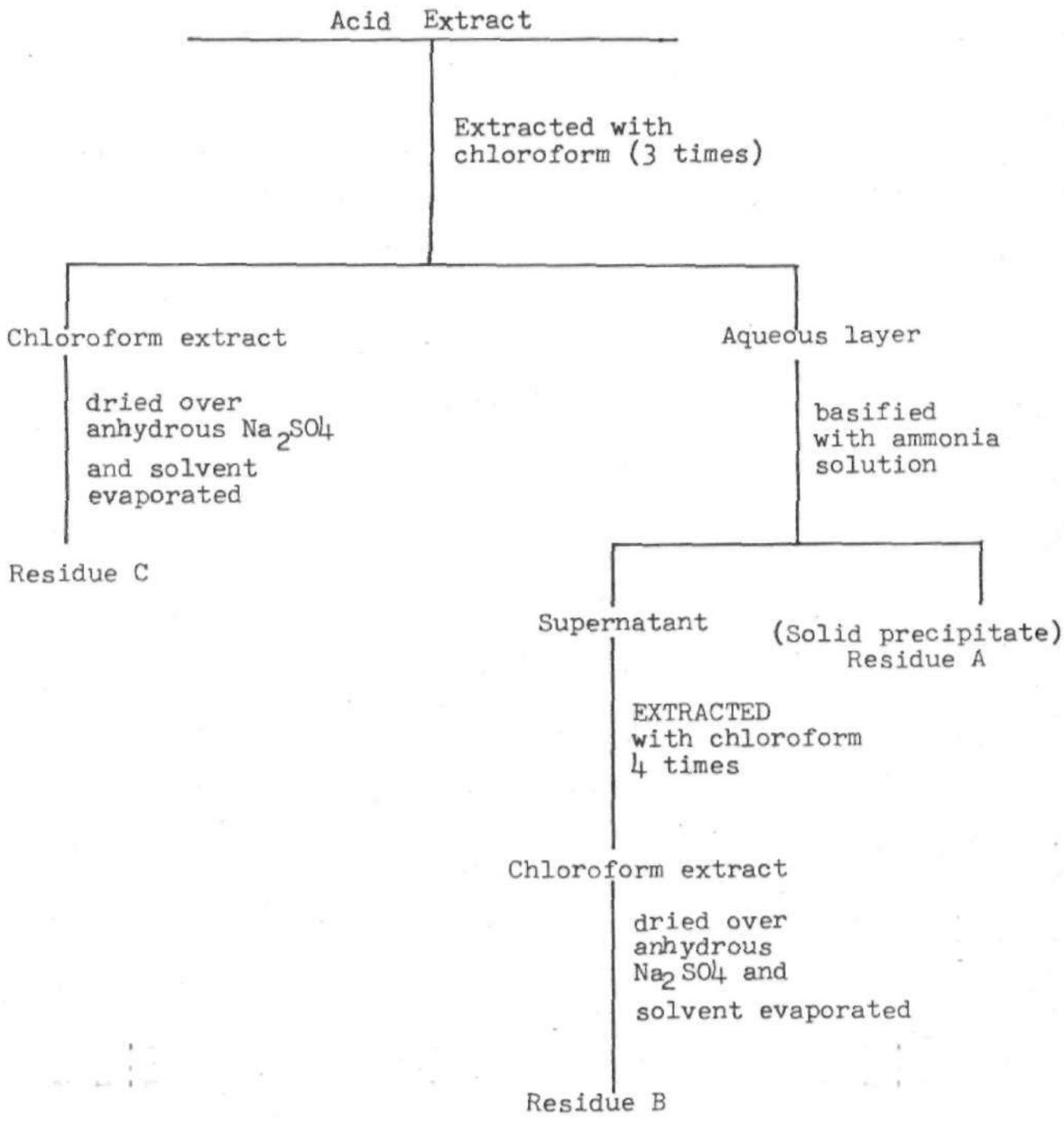


Fig. 2.17 Flow Sheet for Separation of alkaloids D, E and F

The residue after evaporation of the solvent under reduced pressure was acidified with 5 percent HCl (w/v) to $P^H 1$ in order to convert the alkaloids to their hydrochloride salts which are water soluble. The extract was processed for alkaloid content by the procedure summerized in figure 2.17

2.8.2 Isolation for Alkaloid D

TLC examination of residue A showed the presence of one alkaloid Rf 0.4 Butanone-xylene-methanol-diethylami (20:10:5:1). The alkaloidal extract was percolated several times with chloroform in a conical flask (500ml) until the chloroform extract appeared colourless. The dried (Na_2 SO4) chloroform extract gave (120mg) of crude alkaloid on evaporation of solvent.

The crude alkaloid was then chromotographed on silica gel (E. merk) column with petroleum ether as the initial solvent followed by benzene and then chloroform with increasing ammounts of methanol. The 5 percent methanolic chloroform effluent gave a residue (95mg) on evaporation that crystallized from ethanol as white crystals. This has been designated as alkaloid D, m.p. 268-280°C. (Anal. found c_1 72.3; H, 6.5; N. 4.6, calc. for C_{36} $H_{38}O_6$ N_2: C, 72.7; H. 6.5; N. 4.7%).

2.8.3 Isolation of Alkaloid E

TLC examination of residue B showed five spots 0.2, 0.33, 0.4, 0.5 and 0.69. Butanone-xylene-methanol-diethylamine (20:10:5.1). The residue dissolved in hot methanol and on cooling a light brownish compound (60mg) m.p. 230-232°C crystallized out. On recrystallization with methanol (twice) it app ared as light brownish needle like crystals. It has been designated as alkaloid E.

2.8.4 Isolation of alkaloid F

TLC examination of residue C showed the presence of five spots. Rf 0.2, 0.33, 0.4, 0.5, 0.69 Butanone-xylene-diethylamine-methanol (20:10:1.5). The fraction was taken in chloroform and extracted three times with 5 percent Hcl. The acid layer was extracted with chloroform. The dried Na_2 SO4) chloroform residue on crystallization gave 12mg of white crystals m.p. 268-270°C. It has been designated as alkaloid F (Anal. found: C, 73.5; H, 6.8; N, 4.7. Calc. for ($C_{38}H_{42}O_6N_2$: C, 73.3, H. 6.7 N. 4.5%).

2.8.5 Preparation of Nitrosomethyl urea

Nitrosomethylurea was prepared by the method described by Vogel (1972).

2.8.6 Preparation of Diazomethane

A mixture of 35ml of 45 percent KOH solution and 250ml of ether was placed in a flask provided with a stirrer. The mixture was cooled to $5^{o}C$. 17g of finely powdered nitrosomethylurea was added with vigorous stirring in small portions as rapidly as the crystals dissolved. The diazomethane formed dissolved in the ether layer making it deep yellow. The deep etheneal diazomethane layer was seperated from the auqeous layer by decantation. The solution was then dried over pellets of pure Kolt. The dried etheneal diazomethane was used for O-methylation of alkaloids D and E (Vogel, 1972).

2.8.7 O-methylation of alkaloid D

Alkaloid D (30mg) in methanol (20mls) was treated with excess ethereal diazomethane and the solution was allowed to stand at room temperature for 48 hours. The solution was concentrated partially, then treated with a second portion of diazomethane in ether. The solvent was evaporated to yield a syrupy residue shown to be homogeneous on TLC (Rf 0.88, Butanone-xylene-methanol-diethylamine 20:10:5.1). The residue was chromatographed on silica gel and eluted with 5% methanolic chloroform. The chloroform residue

recrystallized from acetone as white crystals m.p. 268-270°C, (Vogel, 1972). The methylated product has been designated as alkaloid D-M.

2.8.8 O-methylation of alkaloid E

Alkaloid E (10mg) was methylated using the same procedure as alkaloid D above. In this case the methylated product was eluted with chloroform and recrystallized from methanol. The methylated product has been designated as alkaloid E-M.

2.8.9 Analytical methods

1. Melting Point (M.P.)

Melting point determinations were carried out using Gallenkhamp capillary melting point apparatus. The melting points are uncorrected.

2. Ultraviolet Spectroscopy (U.V.)

U.V. spectra were recorded with Sp8-100 Pye Unicam spectrophotometer.

3. Infrared Spectroscopy (I.R).

I.R. Spectra were taken in liquid paraffin (mule technique) using Perkin-Elmer 710B Norwalk Connecticut (USA) spectrophotometer.

4. Mass Spectrometry

Mass spectra were obtained using AE1-MS spectrophotometer.

5. Nuclear Magnetic Reasonance Spectroscopy (NMR)

Nuclear magnetic reasonance spectra were taken in dimethylsulfoxide (DMSO).

6. Chromatography

(i) Thin Layer Chromatography TLC

Technique: Ascending, development distance about 12cm.

Adsorbents: Silica gel G. Type 60 (Merk, Damstadt, W. Germany). Thin layer plates were activated at 110°C for one hour and kept in a dessicator before use.

Development: Spots were mannually applied using fine capillary tubes and a template. The plates were developed in Shandon chromoto-tanks (22x24x9cm) (Shandon, London.

Visualization: The following methods of visualization were used.

(a) U.V. lamp at wavelength 254.

(b) Iodine. A chamber saturated with iodine was used.

(c) Dragendorff's spray reagent

(ii) Column Chromatography

Technique: Gradient elution.

Column: Glass column

Adsorbent: Silica gel (60-120 mash) for chromatographic adsorption analysis (B.D.H. chemicals Ltd., Poole, England).

CHAPTER THREE

PHARMACOLOGICAL STUDIES

3.1 Aims and Objectives

The crude alkaloidal extracts of the leaves of Synclisia scabrida have been shown to possess a number of pharmacological actions. Such actions include muscle relaxant (curare-like) activity, anti-oxytocic action, antipsychotic action and antimicrobial actions, (Iriah, 1984).

The aim of this study is to isolate the alkaloids present in the leaves of Synclisia scabrida and evaluate the biological activity of the pure alkaloids.

The objective is to ascertain whether the biological activity of the pure compounds could explain the scientific basis for the use of this plant in the treatment of gynaeco-obstestrical and mental problems by traditional medical practitioners.

3.2 Materials and Methodology

3.2.1 Test Animals

Frogs were obtained locally. Adult female, wistar albino rats were inbred in the animal house, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. Chicks were obtained from Arewa Agricultural Enterprises Limited, Zaria, Nigeria.

3.2.2 Drug Solutions

The stock solutions of alkaloids D and E were made by dissolving a weighed ammount in 0.1ml of 0.1N HCl. Appropriate dilutions were made with physiological solutions. A control vehicle was prepared by appropriately diluting 0.1ml of 0.1N Hcl. In all cases the alkaloid being tested was added to the tissue bath 15 minutes prior to the addition of any agonist.

3.2.3 Standard Drugs Used

The following drugs were purchased from Sigma Chemical Company, d-Tubocurarine hydrochloride, Acetylcholine chloride, apomorphine. Oxytocin was purchased from Ritcher International Generic Ltd., London. Apo morphine solution was stabilized by adding an equivalent weight of sodium metabisulphite as an antioxidant before dissolving in normal saline.

3.3 Isolated Tissues

The isolated Tissues were removed from freshly killed animals and quickly placed in a petri dish prior to setting up in the organ bath. In all cases a period of 30 minutes was allowed for the tissue to stabilize before studying the ffects of

drugs on it. Responses were recorded using Ugo Basil recording microdynamometer (model number 7050) with appropriate transducer.

3.3.1 Physiological solutions (See Appendix)

Dejalon solution was used for the rat uterus while Ringers solution was used for the frog rectus abdominis tissue.

3.3.2 Frog Rectus Abdominis

The frog rectus abdominis tissue was prepared using Burn's (1952) technique.

The spinal cord of the frog was destroyed by a probe after the frog was decapitated. It was then placed in a supine position and the skin of the abdomen was cut out to expose the whole abdomen. The two rectus abdominis muscles running along each side of the midline from the base of the sternum to the fork were removed and each was used for a set up. The muscles were stretched for 30 minutes with 1g weight before the experiment was begun.

The bath temperature was maintained at room temperature, about 27^{o}C and aerated continuously with air.

Rat Uterus Preparation

The rat uterus was prepared following the procedure contained in the British Pharmacopoiea (1968).

Adult female rats were pretreated with stilboesterol 0.1mg/kg 24 hours before the experiment was carried out in order to initiate oestrus phase.

The animals were killed by a blow on the head and the abdomen cut open to the reveal the uterine horns. The two horns were removed and each used for a different set up. The tissues were aerated with air and maintained at a temperature of 37°C.

3.4 Antipsychotic Test

Apomorphine (0.15 mg/kg) was administered subcutaneously to a pair of chicks of known weights, kept in a cage containing food and water.

Doses of alkaloid D (5mg/kg and 10mg/kg) were administered subcutaneously to a group of other chicks (two in a group) 10 minutes prior to the administration of apomorphine. Activities were recorded using the following parameters:

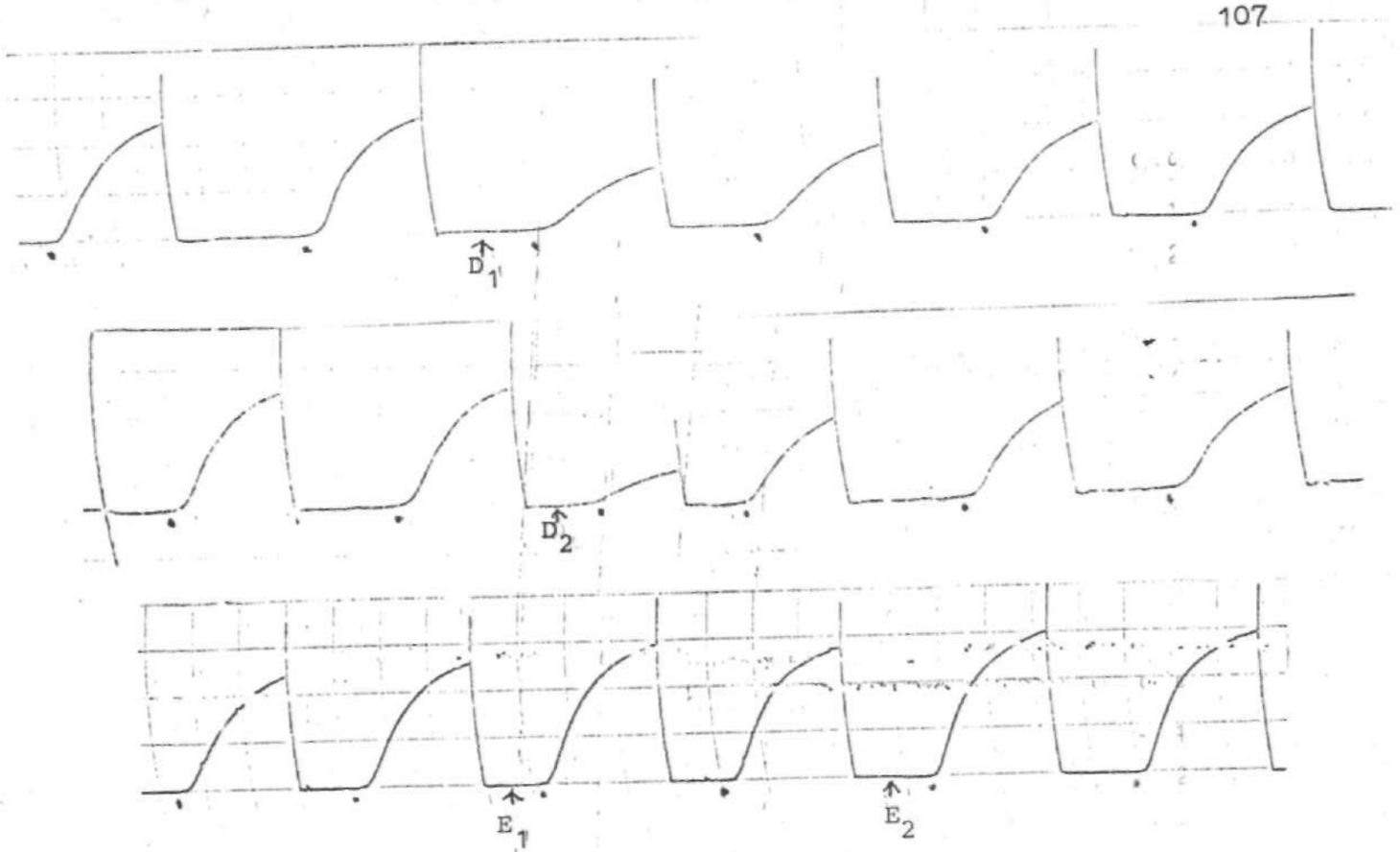


Fig. 3.1 Effects of alkaloids D and E on acetylcholine induced contractions of the frog rectus adabdominis muscle (. = Ach. 1.6 ug/ml, D_1 = 64 ug/ml Alk. D, D_2 = 128 ug/ml alk. D, E_1 = 32 ug/ml alk. E and E_2 = 64 ug/ml alk. E bath concentrations).

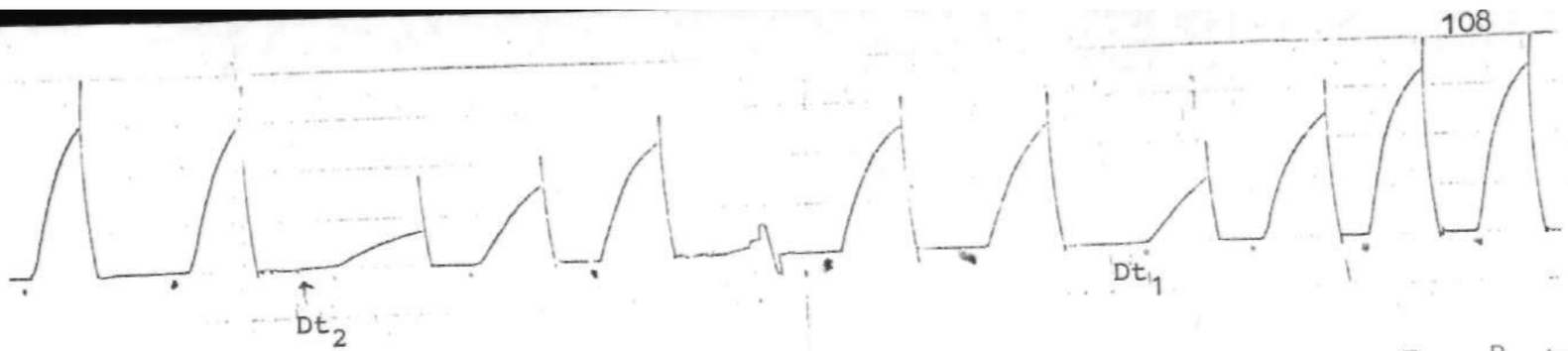


Fig. 3.2 Effects of D-tubocurarine on acetylcholine induced contractions of the Frog Rectus abdominis muscle (.= Acl 1.6 ug/ml, Dt_1 = D-tubocurarine 0.8 ug/ml and Dt_2 = 1.6 ug/ml D. tubocurarine, bath concentrations.

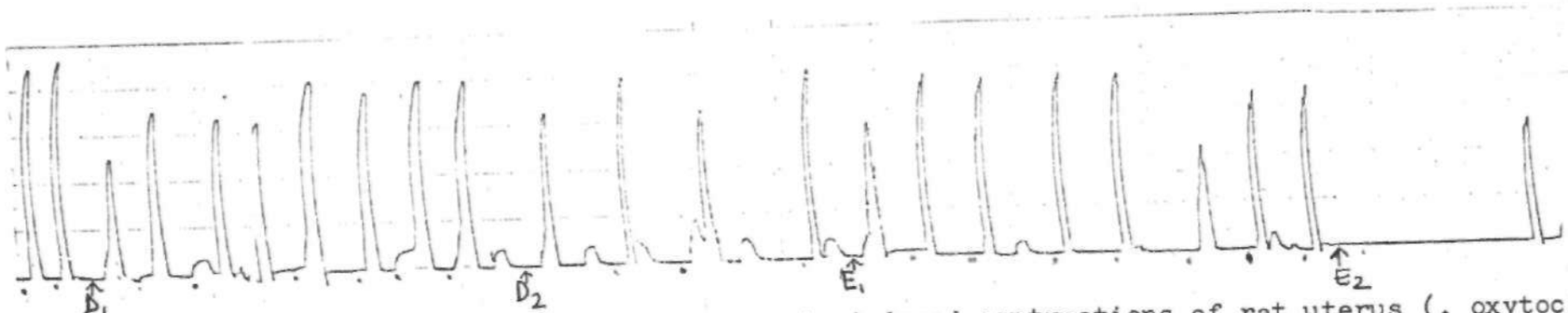


Fig. 3.3 Effects of alkaloids D and E on oxytocin induced contractions of rat uterus (. oxytocin 0.8 ng/ml, D_1 = 32 ug/ml alkaloid D, D_2 = 64 ug/ml alk. D, E_1 = 16 ug/ml alk. E and E_2 = 32 ug/ml alk. E, bath concentrations)

Pecking of each other

Pecking of non-food objects

Pecking of self and

fighting episodes

These parameters were observed for a period of one hour.

3.5 Results

3.5.1 Frog Rectus Abdominis Muscle

Alkaloid D (64-128 ug/ml) antagonised acetylcholine (1.6ug/ml) induced contractions of the frog rectus abdominis muscle in concentration dependent manner (Fig. 3.1) when compared to d-tubocurare however, alkaloid D appeared to be about 80 timelless potent than D-tubocurarine (0.8-1.6 ug/ml) (Fig. 3.2).

Alkaloid E (32-64 ug/ml) had no effect on acetyl choline (1.6 ug/ml) induced contractions of the frog rectus abdominis muscle (Fig. 3.1).

3.5.2 Rat Uterus

Alkaloid D (32-64 ug/ml) showed a weak antagonism to oxytocin (0.8 ug/ml) induced contractions of the rat uterus. This antagonism is not concentration dependent (Fig. 3.3).

Alkaloid E (16-32 ug/ml) similarly showed weak antagonism to oxytocin (0.8ng/ml) induced contractions of the rat uterus (Fig. 3.4).

3.5.3 Antipsychotic Test

The result of the antipsychotic test using alkaloid D on chicks is shown in table 3.1.

Table 3:1 Effects of Alkaloid D (5mg/kg and 10mg/kg) on Apormorphine (0.15mg/kg) induced Stereotyped Behaviour on Chicks

Stereotyped behaviour	Control	5mg/kg	10mg/kg
Pecking of self	195	29	137
Pecking of each other	184	161	278
Pecking of non-food objects	604	391	437
Fighting episodes	41	44	85
Total	1024	625	937

The central nervous system of the chicks was excited by apo morphine leading to the abnormal behaviour as observed in the increase in fighting episodes and pecking.

Alkaloid D (5mg and 10mg/kg) reduced the pecking of self from 195 to 29 and 137 respectively. Pecking of each other was however reduced to 161 and then increased to 278 from 184 by the two doses of alkaloid D.

Pecking of non-food objects was reduced from 604 to 391 and 437 while fighting episodes were increased from 41 to 44 and 85.

The overall parameters were reduced from 1024 to 625 and 975. This indicated that the effect of alkaloid D is not concentration dependent. The experiment could not be repeated due to lack of sample.

3.6 Discussion

The blocking effect of alkaloid D on acetylcholine induced contractions of the frog rectus abdominis muscle suggested antagonistic activity at the nicotinic receptor sites located on the end plates of skeletal muscles. The reversible and concentration dependent manner of this blocking effect indicated that alkaloid D competes with acetylcholine at the motor end plates. Similar actions were observed with cycleanine and alkaloid B (Wambebe et al, 1982).

The effects of alkaloids D and E on oxytocin induced contractions of the rat uterus suggested that the two alkaloids possessed a weak relaxant effect on smooth muscles. Cycleanine and alkaloid B were however, reported to have strong anti-oxytocic action on the rat uterus, (Wambebe and Ogbeide, 1982; Wambebe et al, 1982).

The result of the antipsychotic test showed that the abnormal behaviour induced by apormorphine on chicks has been weakly suppressed by alkaloid D in a concentration independent manner.

3.7 Conclusion

The skeletal muscle relaxant effect of alkaloid D the weak anti-oxytocic action of alkaloids D and E and the weak anti-psychotic action of alkaloid D could be beneficial in gynaeco-obstetrical and mental problems.

Thus these actions of alkaloids D and E and the cycleanine content of the leaves justifies the use of this plant by traditional medical practitioners.

In view of the preliminary nature of this study further studies on the pharmacological effects of alkaloids D and E is worthwhile.

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APPENDICES

I: Dragendorff's Reagent

Solution I:

Bismuth oxynitrate	0.85g
Glacial acetic acid	10ml
Water	40ml

Solution II:

Potassium Iodide	8.0g
Distilled water	20ml .

Solutions I and II were mixed to make the stock solution. 10ml of stock solution was diluted with 20ml of glacial acetic acid and the volume adjusted to 100mls with water to prepare the spray reagent.

II: Preparation of Physiological Self Solutions

	QUANTITIES FOR TEN LITRES OF	
	RINGER	DE JALON
$NaH_2PO_4 \cdot 2H_2O$	1ml	-
NaCl	65	90g
Kcl 10%	14ml	42ml
D-Glucose	20g	5g
$NaHCO_3$	4g	5g
$CaCl_2$ (Molar)	10.8ml	2.7ml
$CaCl_2$ 10%	-	6ml