MACROCYCLIC PYRROLIZIDENE ALKALOIDS
FROM CROTALARIA ROSENII

A thesis
Presented to
The School of Graduate Studies
Addis Ababa University

In Partial Fulfillment of
the Requirements for the Degree of
Master of Science in Chemistry

by

Getachew Atnafu

June 1986

TO MY FATHER, ATO ATNAFU ALEMAYEHU
AND MY MOTHER W/o ASELEFECH BEKELE
FOR MAKING MY EDUCATION POSSIBLE

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Macrocyclic Pyrrolizidine Alkaloids from *Crotalaria rosenii*

by

Getachew Atnafu

Chemistry Department

Science Faculty

pproved by:

r. Berhanu Abegaz Advisor	Berhanu Abegaz
rof. Edwin G.E. Jahngen Examiner	Edwin G. E. Jahngen
r. Tarekegne G/Yesus Examiner	
r. Ermias Dagne Examiner	Ermias Dagne

Table of Contents

ACKNOWLEDGEMENTS

I would like to express my appreciation for the constant guidance and supervision I received from my advisor Dr. Berhanu Abegaz and his consistent and stimulating advice in the research conducted and in the preparation of this dissertation. I also gratefully acknowledge Prof. Helmut Duddeck of Bochum University, FRG for determining all spectra. I am in debt to Dr. Mesfin Tadesse, Ato Zemedie Asffaw and Tamirat Bekele who help me incollecting and identifying the plant sample. I would like to thank Dr. Ermias Dagne and Ato Gizachew Alemayehu for helping me receive the spectra. My special thank goes to W/t Ejegayehu Tessema and Ato Snshaw Alamrew for their consistent encouragement.

I would like to express my thanks to Ato Esayas Birhanu, Abiy Yenesew, Alemayehu Abebaw, Bekele Dinku, Amha Bekele, Aberra Fura, Tadele Tenaw, Yilma Tamiru, Adnew Geletu, Abdu De Tango, Tekle Habte, W/t Senait Dagne, Yordanos Woldu and all others who contributed some to this work in one or another way. Finally first but not last I want to thank the Chemistry Department for providing the necessary facilities to conduct the research work.

List of Tables

List of Figures

Page

ABSTRACT

Macrocyclic Pyrrolizidine Alkaloids from *Crotalaria rosenii*

by

Getachew Atnafu

Research Advisor: Dr. Berhanu Abegaz

Crotolaria rosenii, family Leguminosae, is a poisonous endemic shrub or small slender tree found in mountain scrub and forest clearings between 2000 and 3000 m. The powdered leaves and flowers of *C. rosenii* were defatted with petroleum ether (60-80°C), extracted with methanol (soxhlet) and subjected to acid-base extraction to give a raw basic extract containing nine alkaloids. Column chromatographic separation of the raw extract on silica gel with chloroform-methanol mixture resulted in the isolation of three alkaloids. The structures of these alkaloids were determined by 2-dimensional nuclear magnetic resonance techniques as well as mass and infrared spectra. The three alkaloids were identified as madurensine, 7-acetyl madurensine and Crotaflorine.

Application of homo and hetero nuclear shift correlations have enabled unequivocal assignments of the carbon chemical shifts of these compounds and pointed out erraneous conclusion made by other workers.

1. INTRODUCTION

Crotalaria species belong to the family Leguminose sub-family Papilionoideae. Many of them are small herbs or shrubs found besides roads, on eroded soils and as weeds between 600 and 3000 m (1). Some 600 species are found throughout the tropics and sub-tropics particularly of the Southern hemisphere. About 500 Crotalaria species are recorded in tropical Africa (2). There are 85 species of Crotalaria listed for Ethiopia out of which 15 are endemic. Crotalaria rosenii which is also known by the synonyms C. bieberi, C. raffillii and Erythrina rosenii (2) is a poisonous endemic shrub or small, slender tree up to 4 m tall. It is found in mountain scrub and forest clearings from 2000 to 3000 m. Its flowers are yellow with black and brown tips and markings.

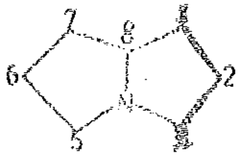
Plants from the genus Crotalaria are known to contain pyrrolizidine alkaloids. Up to the end of 1985 about 51 pyrrolizidine alkaloids have been isolated from over 59 species. The structures of these alkaloids, classified according to the type of necine base they contain are given in Table 1. The economic importance of pyrrolizidine alkaloids is now more widely appreciated because of the increasing number of deaths to livestock attributable to consumption of plants containing pyrrolizidine alkaloids. Many cases of human liver disease are now also known to be due to ingestion of pyrrolizidine alkaloids (3).

No phytochemical investigations are reported on Crotalaria rosenii prior to the initiation of this project. The presence of nine alkaloids has been established and the structures of three macrocyclic pyrrolizidine alkaloids were determined on the basis of IR, Mass, ^{1}H NMR and ^{13}C NMR spectroscopy.

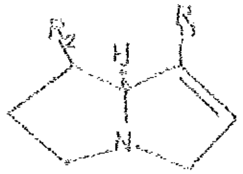
2. THEORETICAL BACKGROUND

2.1 Pyrrolizidine Alkaloids

Pyrrolizidine alkaloids contain one nitrogen atom in the molecule and this is present in a pyrrolizidine nucleus(I), a system also known as 1-azabicyclo [0,3,3]-octane. Quite a number of pyrrolizidine alkaloids contain eighteen carbon atoms. Most of the alkaloids are esters which on hydrolysis give alkanolamines (known as necines) and carboxylic acids (known as necic acids). They fall into three main categories: monoesters of the necine with a monocarboxylic necic acid(II), diesters of the necine with two different monocarboxylic necic acids(III) and macrocyclic diesters of the necine with a dicarboxylic necic acid(IV). Most of them are reported to have a 1,2-double bond in the necine and are known to be allylic esters (4). The most common ring size in the macrocyclic pyrrolizidine alkaloids is 11 or 12 membered. However, few examples of 13 membered macrocyclic diesters are known for platynecine (5-10) and retronecine (11-15). Recently a new 14-membered derivative of retronecine has been reported (11-13).



I



II. Heleurine

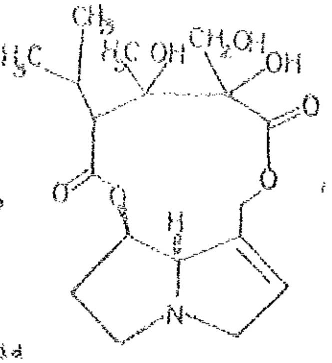
R_1 = lasiocarpinic acid

R_2 = H

III. Lasiocarpine

R_1 = lasiocarpinic acid

R_2 = angelic acid



IV. Junceine

13 plant families are known to contain pyrrolizidine alkaloids of which Compositae, Boraginaceae, Leguminosae and Orchidaceae contain the largest number of species investigated (16). The majority of pyrrolizidine alkaloids are isolated from the genus Senecio of Compositae. It contains monoesters (17) and macrocyclic diesters (18,19) pyrrolizidine alkaloids with hydroxy group on saturated necine base not isolated from other plant families. The genus Crotalaria consists of pyrrolizidine alkaloids with six different type of necine bases (Table 1) of which croalbinecine (20) and crotanecine (21,22) type alkaloids are only isolated from this species. Crotanecine is a tri-hydroxyl amino alcohol in which one hydroxyl group is present as the 1-hydroxymethyl grouping and the other two hydroxyls are attached to C-6 and C-7 (21). It contains

12 and 13 membered macrocyclic rings. The 12 membered ring have the ester attachement at C-7 and C-9 while the 13-membered ring have the ester attachement at C-6 and C-9. The conformation of the saturated 12-membered ring is exo-buckled and is endo for 13-membered ring (21).

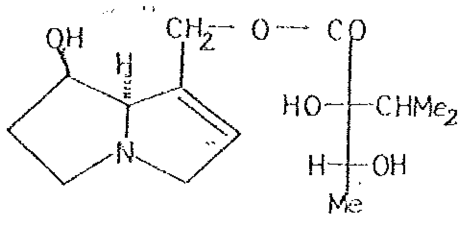
Most macrocyclic pyrrolizidine alkaloids have methyl substituents on the α-position of the ester groups but in six Crotalaria and three Senecio species pyrrolizidine alkaloids having hydroxy methyl substituent on the α-position of ester group have been isolated (23,24,25,26,22). Macrocyclic diesters with epoxide ring in the necic acid portion were reported to occur in species of Senecio (18) and Crotalaria (27).

2.1.1 Pharmacology

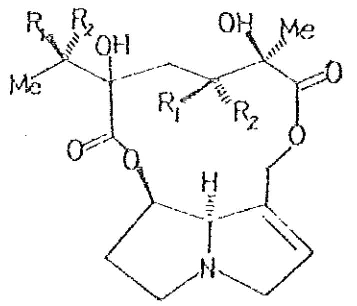
Plants containing pyrrolozidine alkaloids have been of medicinal interest since the fourth century B.C. and there has been reports of their use for treatment of tumors (30). A recent report confirmed the antitumor effect of monocrotaline (Table 1 section c (3)) and this pyrrolizidine alkaloid was reported to be more destructive to malignant cells than to normal cells (34). The N-oxide of indicine(VI) does display antitumor activity with less toxic effect than related pyrrolizidne alkaloids. There has been a data on its test on humans (35)

Many pyrrolizidine alkaloids are found to be toxic acting specifically on the liver (31). According to studies made on 15 pyrrolizidine alkaloids by Culvenor (32) and Robertson (33) hepatotoxic pyrrolizidine alkaloids have a 1,2-double bond in the necine, and are esterified at C-9. Substitution at the C-7 hydroxy-group is associated with increasing toxicity of the alkaloid. The toxic metabolites of pyrrolizidine alkaloids are pyrrole derivatives produced by hepatic mixed function oxidase. In order to form these toxic pyrrolic metabolites, the alkaloids must posses 1,2-unsaturation in the necine, and be esterified at C-9 (16).

Pyrrolizidine alkaloids were reported in Cow milk samples (36). The hepatotoxic alkaloids senecionine, seneciphylline (Table 1 section c (b)), jacoline(VII) and jaconine(VIII) from Senecio jacobaea L, were found in honey produced from nectars of these species (37). The transmission of such hepatotoxic alkaloids or their metabolites to consumers through meat and dairy products from exposed animals thus, poses possible health hazards.



VI



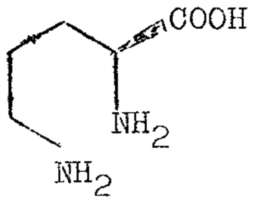
VII. R_1=OH, R_2=H

VIII. R_1=Cl, R_2=OH

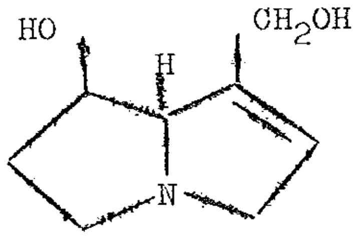
2.1.2 Biosynthesis

Pyrrolizidine alkaloids occur largely as esters of amino alcohols (necines) and carboxylic acids (necic acids.) Biosynthetic studies have been made on the common necine bases and necic acids.

Ornithine(IX) is known to be incorporated into a variety of plan bases. These include the pyrrolizidine alkaloids typified by retronecine. Retronecine(X) is the most common necine and most of the biosynthetic studies reported have been carried out on this base. Results from the study of feeding labelled ornithine, show that ornithine derived fragments are incorporated.



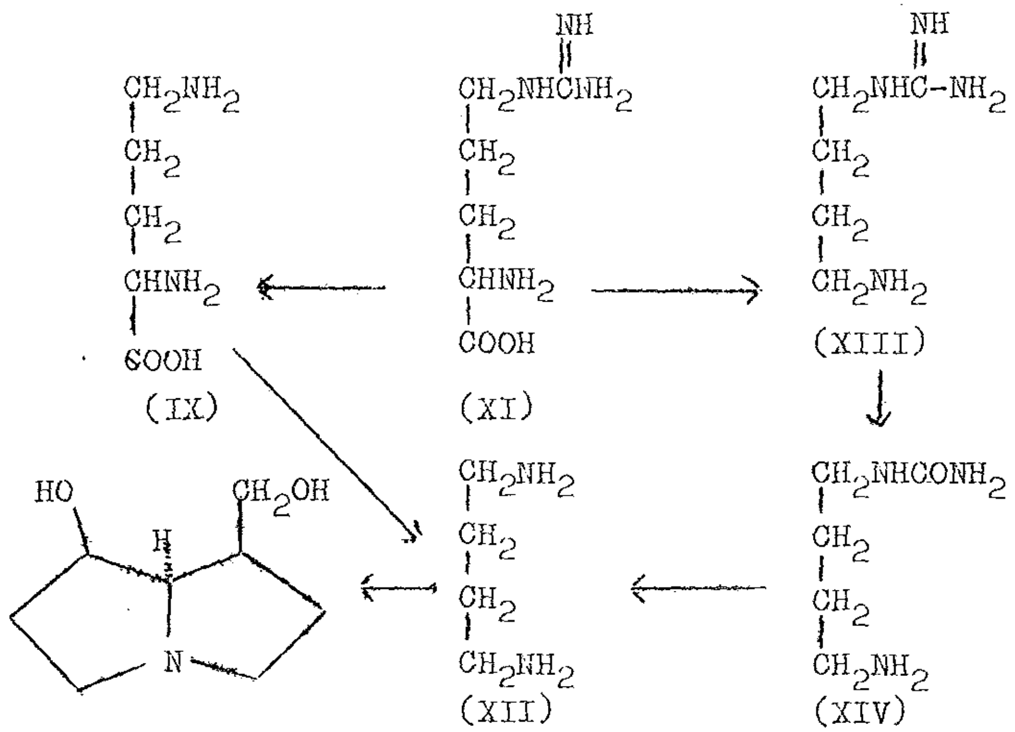
IX



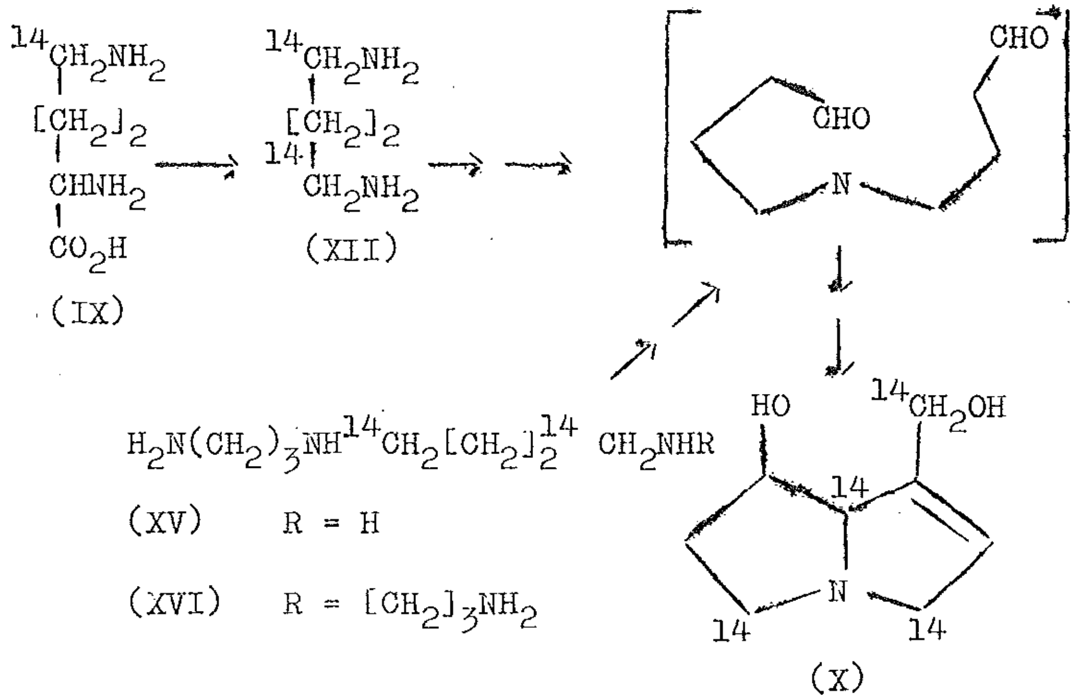
X

Nowacki and Byerrum (58) fed [2-^{14}C] - ornithine to Crotalaria spectabilis plant and obtained monocrotaline, which was shown to be radioactive only in the retronecine portion (necine part). Hughes et al (39) fed [2-^{14}C] ornithine to Senecio isatideus plant. They found necine actively at C-9, but no actively at C-5 and C-3. Instead, the rest of the activity was at C-(7 + 8). These workers concluded that two molecules of ornithine are incorporated into retronecine.

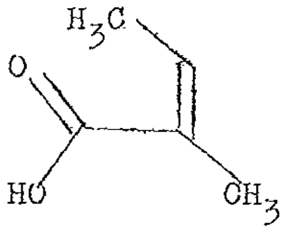
Bale and Crout (40) have carried out feeding experiments with arginine(XI) and ornithine labelled with different isotopes (^{3}H and ^{14}C) and found out that ornithine is a slightly more efficient precursor than arginine for retronecine biosynthesis. It is probable that ornithine is incorporated into retronecine(X) after decarboxylation to putrescine(XII) since labelled putrescine has also been shown to be specifically incorporated into retronecine. Putrescine can be also formed from arginine via agmatine(XIII) and N-carbamylputrecine(XIV) as shown below.



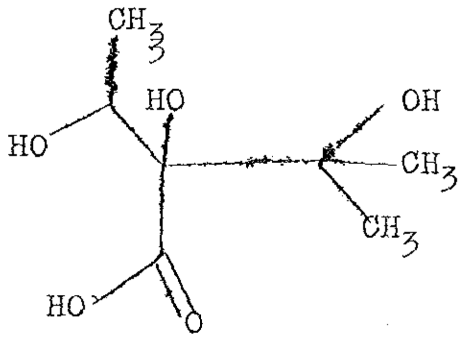
Robins and Sweeny (41) showed that putrescine(XII) sepermidine(XV) and spermine(XVI) are efficient precursors of retronecine(X) biosynthesis. Degradation of labelled alkaloids demonstrated a symmetrical intermediate of the type C_4-N-C_4 in the biosynthesis. They have found putrenscine to be a much more efficient precursor than ornithine which supports the theory that putrescine follows ornithin in the biosynthetic pathway.



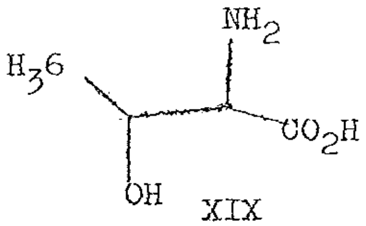
The acids with which the necines are esterified are generally not found elsewhere in alkaloids. For sometime, the acids which include the C_5 monocarboxylic acid, angelic acid(XVII), the C_7 monocarboxylic acid, echimidinic acid(XVIII), and a number of C_{10} dicarboxylic acids such as senecic acid(XXII), were thought to be acetate (mevalonate) derived (42). Because many of the necic acids contain ten carbon atoms, they were originally believed to be of terpenoid origin eventhough the mode of coupling of the C_5 units was quite different from those of terpenoid compounds. However, experiments with labelled precursors have established that a number of necic acids are formed from one or more of the common branched chain amine acids threonine(XIX), isoleucine(XX) and valine(XXI) (43). Thus, Crout (44) has shown that isoleucine is a precursor of angelic acid(XVII) and that valine is a specific precursor of echimidinic acid(XVIII).



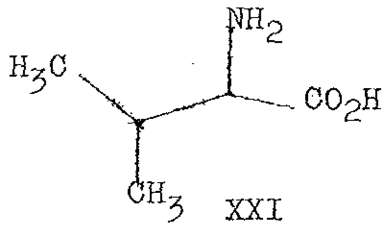
XVII



XVIII



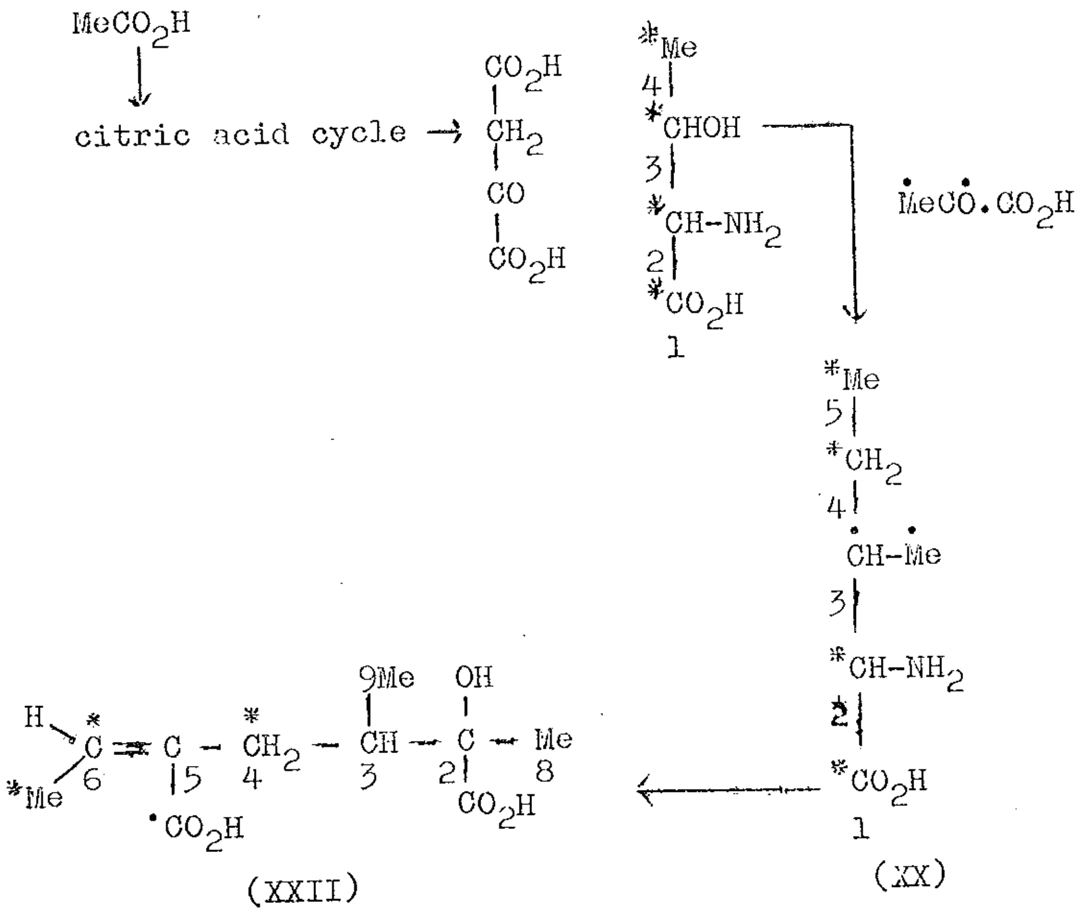
XIX



XXI

Senecic acid(XXII) has been the most widely studied of C_{10} acids and belongs to the main structural type found within this group of acids. It was shown that isoleucine(XX) is incroporated into senecic acid by the loss of both carboxyl carbons(44). The mechanism by which the tso isoleucine units are coupled in the senecic acid biosynthesis is not obvious.

Crout et al. (45) showed by feeding experiment incorporation of two five - carbon units derived from isoleucine(XX) and its biological precursor threonine(XIX) into senecic acid(XXII) in Senecio magnificus. With uniformly labelled [^{14}C] - L - isoleucine as precursor; the five - carbon component comprising (C-4, -5, -6, -7 and -10) of senecic acid was most heavily labelled, and it was suggested that isoleucine was incorporated according to the schem below.



Results obtained from experiments done with a wide range of labelled compounds (46-50) show that the necic acids of most pyrrolizidine alkaloids arise by a biosynthetic pathway involving the coupling of metabolites derived from isoleucine(XX).

2.1.3 Isolation and Characterization

In the most common isolation procedure, the crude extract obtained after preliminary processing is purified by dissolution in dilute acid and filteration from insoluble material. The alkaloids are then reprecipitated with alkali or extracted with an immiscible solvent from an alkali solution. Individual alkaloids are separated from each other through differences in solubilities of the bases and their salts. Many isolation procedures have been described for pyrrolizidine alkaloids in the screening of various plant materials. The most commonly used technique is column chromatography with silica gel (51), alumina (52), kieselguhr (53) or cellulose (54) and preparative thin-layer chromatography (55).

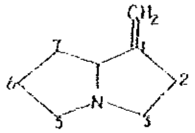
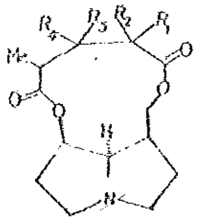
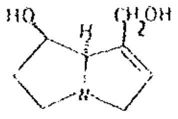
A considerable number of improvements to existing procedures for the detection, isolation and separation of pyrrolizidine alkaloid mixtures has been reported. The alkaloids often occur naturally in the form of their N-oxides, and these are usually reduced with zinc in acidic solution to liberate the free bases. Due to the low yield obtained with zinc acid reduction and the possibility of decomposition another method, which employs a redox polymer on anion exchange resin was developed (56). Thin layer chromatography provides a rapid separation of pyrrolizidine alkaloids. Reproducible R_f values for pyrrolizidine alkaloids were obtained by using silica gel plates impregnated with methanol saturated with potassium chloride (57). A sensitive reagent for detection of pyrrolizidine alkaloids was developed by direct treatment of o-chloranil (tetrachloro-o-benzoquinone) followed by spraying with the Ehrlich's reagent (p-dimethylamino benzaldehyde in absolute ethanol containing boron trifuloride etherate) (58). Improved separation of mixtures of pyrrolizidine alkaloids have been obtained by high performance liquid chromatography (59-61) and gas chromatography (62).

Alkaline hydrolysis of the alkaloids has been employed as a useful degradative tool in the study of most of the alkaloids of the pyrrolizidine type, since hydrolysis gives the necine and necic acid (or modified necic acid) portions which constitute the parent alkaloid. The other general degradative procedure which has been exceedingly useful is that of hydrogenolysis of the alkaloid to give a modified necine and necic acid. This practice of hydrolysis or hydrogenation of the alkaloid to its constituent basic (necine) and acidic (necic acid) moieties, and identifying both portions is not now carried out by all workers, sometimes due to the small amounts of material available.

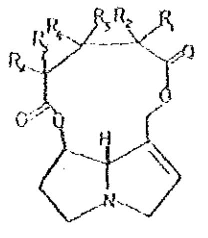
There has been an increasing reliance on spectral data chiefly ^{1}H ^{13}C nuclear magnetic resonance (n.m.r) and mass-spectrometry. Useful information on stereochemical details and conformations have been obtained from X-ray diffraction analysis (23,63-66). The introduction of new techniques such as NOE and two dimensional (2-D) NMR spectroscopy have opened possibilities for the analysis of spectra and is able to provide information previously not accessible. Various techniques of two-dimensional NMR spectroscopy made possible correlation of spectral parameters (chemical shifts, spin-spin coupling constants) of different nuclei. Measurments of homonuclear $^{1}H-^{1}H$ shift correlated spectra and hetronuclear $^{13}C-^{1}H$ shift correlated spectra (COSY) greatly assisted the assignment of signals and structural determinations (67).

The mass spectra of pyrrolizidine alkaloids are very distinctive and often permit structural assignments to be made by comparison with mass spectra of alkaloids of known structure. These alkaloids display characteristic fragment ions of their necine base. For example, those alkaloids containing otonecine as base (Table 1 section D) display characteristic fragment ions at m/z 168, 151, 150, 122, 110 and 94 (68,69). The N-oxides of pyrrolizidine alkaloids usually show the presence of ions at M-16, M-17, and M-18 (70).

Table 1. The Structures of Pyrrolizidine Alkaloids for Genus *Crotalaria*

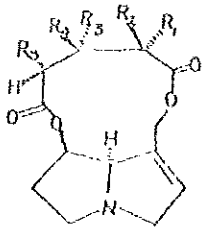
	Alkaloid	Plant species	References
A. Simple pyrrolizidine base			
	1-methylenepyrrolizidine	*C. anagyroides* *C. damarensis* *C. grandistipulata* *C. lachnophora* *C. natalitia* *C. rhodesiae* *C. stolzii* *C. verrucosa*	18,27
B. Turneforcidine group			
	$R_1=R_3=Me, R_2=OH, R_4=H$ Retusine	*C. retusa* *C. spectabilis* (*C. sericea*)	18
	$R_1=R_4=Me, R_2=H, R_3=OH$		
	Crocandine	*C. candicans*	62
	Isocrocandine	*C. [illegible]*	62
C. Retronecine group			
1. Acyclic derivative			
 1	Retronecine	*C. aridicola* *C. retusa*	18

II. 11-membered cyclic diesters



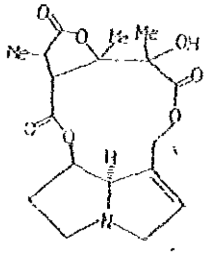
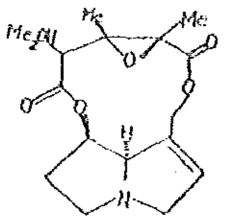
2

Alkaloid	Plant species	References
$R_1=R_2=R_6=H, R_4=Me, R_3=OH, R_5=F$ Merotaline	*C. globifera* *C. dura*	19
$R_1=R_4=R_6=H, R_2=CHOMe, R_3=Me=R_5$ Croaburhine	*C. nana*	72
$R_1=R_4=R_5=Me, R_2=OH, R_3=OAc, R_6=H$ Spectabiline	*C. spectabilis* (*C. sericea*)	18
$R_1=R_4=Me, R_2=OH, R_3=R_5=R_6=H$ Crobarbatine	*C. barbata*	73
$R_1=R_4=R_5=Me, R_2=OH, R_3=OH, R_6=Et$ Crotolarine (Croburhine)	*C. burhia*	74
$R_1=R_3=OH, R_2=CH_2OH, R_4=R_6=H, R_5=Me_2CH$ Junceine	*C. rubiginosa* *C. juncea*	75
$R_1=R_3=OH, R_2=Et, R_4=R_6=H, R_5=Me_2CH$ Axillaridine	*C. axillaris*	67
$R_1=CHOHMe, R_2=R_4=OH, R_3=H, R_5=Me_2CH$ Axillarine		67
$R_1=CHOHMe, R_2=OH, R_3=R_4=H, R_5=Me_2CH$ Deoxyaxillarine		67

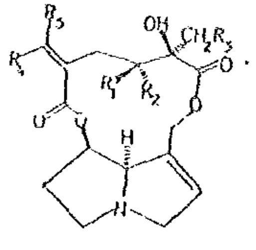


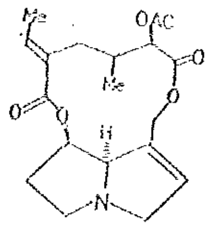
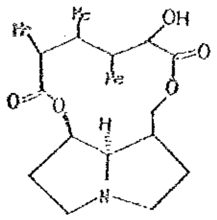
3

$R_1=R_3=R_5=Me, R_2=H, R_4=OH$ Fulvine	*C. madurensis* *C. paniculata* *C. crispata* *C. fulva*	21 18
$R_1=R_4=R_5=Me, R_2=H, R_3=OH$ Crispatine	*C. crispata* *C. madurensis* *C. leschenaultii*	76,77,18
Diastereomers of Crispatine:-		
Cromadurine	*C. madurensis*	78
Isocromadurine	*C. madurensis*	79
$R_1=R_3=R_5=Me, R=OH, R_4=OH$ Monocrotaline	*C. aegyptiaca* *C. leiloba* *C. burhia* *C. crispata* *C. grahamiana* *C. leschenaultii* *C. mysorensis* *C. novaehollandiae* *C. quinquefolia* *C. retusa* *C. sagittalis* *C. spectabilis* *C. stipularia*	80,76,27, 82,83,18 81
$R_1=R_3=R_5=Me, R_2=R_4=O_2CMe$ Monocrotalinine	*C. grahamiana*	83,80
$R_1=R_3=R_5=Me, R_2=OH, R_4=OCOCHMeEt$ Grahamine	*C. grahamiana*	83,80
$R_1=R_3=Me, R_2=R_4=OH, R_5=Me_2CH$ Trichodesmine	*C. junceine* *C. rubiginosa* *C. tetragona*	82,18,24

	Alkaloid	Plant species	References
 4	Grantianine	*C. grantiana*	27,19
 5	Grantaline	*C. grantiana*	27

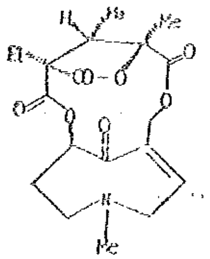
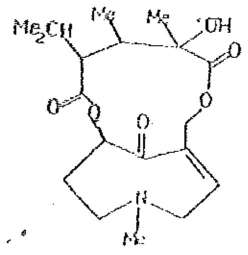
iii. 12-membered cyclic diesters

	Alkaloid	Plant species	References
 6	$R_1=R_3=R_5=H, R_2=R_4=Me$ Senecionine	*C. anagyroides* *C. juncea* *C. usaramoensis*	21,84,18
	$R_1,R_2=CH_2, R_3=R_5=H, R_4=Me$ Seneciphylline	*C. juncea*	18
	$R_1=R_5=H, R_2=R_4=Me, R_3=OH$ Retrorsine	*C. spartioides* *C. usaramoensis*	86
	$R_1,R_2=CH_2, R_3=OH, R_4=Me,$ $R_5=H$ Riddelline	*C. juncea*	18
	$R_1=R_3=R_4=H, R_2=R_5=Me$ Integerrimine	*C. intermedia* *C. tetragona* *C. usaramoensis* *C. brevifolia* *C. incana* *C. mucronata*	76,84,25,82 19,86
	$R_1=R_4=H, R_2=R_5=Me, R_3=OH$ Usaramine (Mucronatine)	*C. brevifolia* *C. incana* *C. intermedia* *C. mucronata* *C. usaramoensis*	76,25,27 84,86

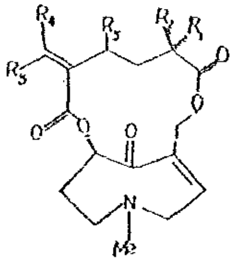
	Alkaloid	Plant species	References
 7	Crotastriatine (pilgrine acetate)	*C. mucronata* (*C. striata*)	67
 8	Crotananine	*C. nana*	88

D. Otonecine group

1. 11-membered cyclic diesters

	Alkaloid	Plant species	References
 1	Retusamine	*C. crassipes* *C. mitchelli* *C. novae-hollandiae* *C. retusa*	18,81
 2	Crosemperine	*C. aegyptiaca* *C. semperflorens*	89,90

ii. 12-membered cyclic diesters

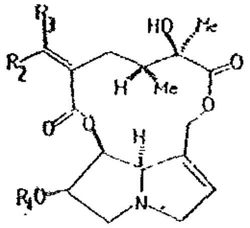


3

	Alkaloid	Plant species	References
	$R_3=R_4=H, R_1=Me, R_2=OH, R_5=Me$ Senkirkine	*C. laburnifolia*	26
	Isosenkirkine	*C. walkeri*	76
	$R_1=R_5=Me, R_2=OAC, R_3=R_4=H$ O-Acetylsenkirkine	*C. walkeri*	76,91
	$R_1=CH_2OH, R_2=OH, R_3=R_4=H, R_5=Me$ Hydroxysenkirkine	*C. laburnifolia*	26
	$R_1=OH, R_2=R_3=R_4=H, R5=Me$ Crotafoline	*C. laburnifolia*	26
	$R_1=OH, R_2=R_4=Me, R_3=R_5=H$ Crotaverine	*C. walkeri* *C. verrucosa*	91,92
	$R_1=OAC, R_2=R_4=Me, R_3=R_5=H$ O-Acetylcrotaverine	*C. verrucosa* *C. walkeri*	92

E. Crotanecine group

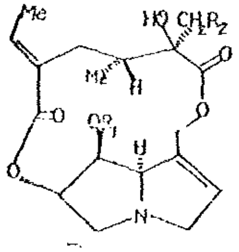
i. 12-membered cyclic diesters



1

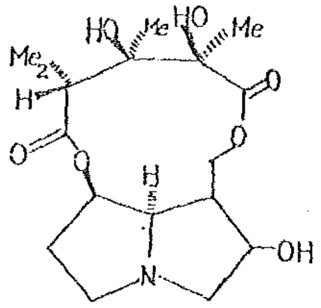
Alkaloid	Plant species	References
$R_1=R_3=H, R_2=Me$ Anacrotine (Crotalaburine)	*C. laburnifolia* *C. agatiflora* *C. anagyroides* *C. incana*	22,21,27 93,26
$R_1=Ac, R_2=Me, R_3=H$ 6-Acetylanacrotine	*C. agatiflora*	22
$R_1=Ac, R_2=H, R_3=Me$ 6-Acetyl-trans-anacrotine	*C. agatiflora*	22
$R_1=$angelyl$, R_2=H, R_3=Me$ 6-Angelyl-trans-anacrotine	*C. agatiflora*	22

ii. 13-membered cyclic diesters



2

Alkaloid	Plant species	References
$R_1=R_2=H$: Madurensine	*C. madurensis* *C. agatiflora* *C. laburnifolia*	21,26,93
$R_1=Ac, R_2=H$: 7-Acetylmadurensine	*C. agatiflora*	22
$R_1=Ac, R_2=H$: 7-Acetyl-cis-madurensine	*C. agatiflora*	22
$R_1=H, R_2=OH$: Crotaflorine	*C. agatiflora*	22

Alkaloid	Plant species	Reference
F. Croalbinecine group		
 Croalbidine	*C. albida* (*C. montana*)	20
G. Miscellaneous group		
1. 7β-hydroxy-1-methyl pyrrolizidine	*C. podocarfa* *C. goreensis* *C. cylinddro-carfa* *C. aegyptica*	72 [illegible] 72 83
2. 1-hydroxymethyl-1,2-eppxy pyrrolizidine	*C. grantiana*	63
3. Methyl esthers of supinidine and retronecine	*C. medicaginea* *C. trifotiastrum*	18

3. RESULTS AND DISCUSSION

3.1 Extraction and Fractionation of Crotaloria rosenii

A schematic flow chart for extraction and fractionation of the leaves and the flowers of Crotalaria rosenii is given in Figure 1. Defatting the powdered leaves with petroleum ether (60-80°C) and extraction with methanol and subsequent purification by acid base extraction gave a raw basic extract which showed three alkaloids on t.l.c. These alkaloids were labelled CR-1 to CR-3 in the order of decreasing R_f values. Alkaloid CR-1 was easily isolated from this mixture by column chromatography on silica gel as detailed in the experimental section. Extraction and purification of the flowers of C. rosenii gave a raw basic extract which showed nine alkaloids on t.l.c. labelled from CR-4 to C-12 in the order of decreasing R_f values. Three alkaloids CR-5, CR-7 and CR-9 were isolated by column chromatography (see experimental section). Alkaloid CR-1 isolated from the leaves was found to be identical with CR-5 isolated from the flowers (t.l.c, mixed melting point, MS and ^1H NMR). Since CR-5 and CR-9 were found to be derivatives of CR-7, the structure of CR-7 will be discussed first.

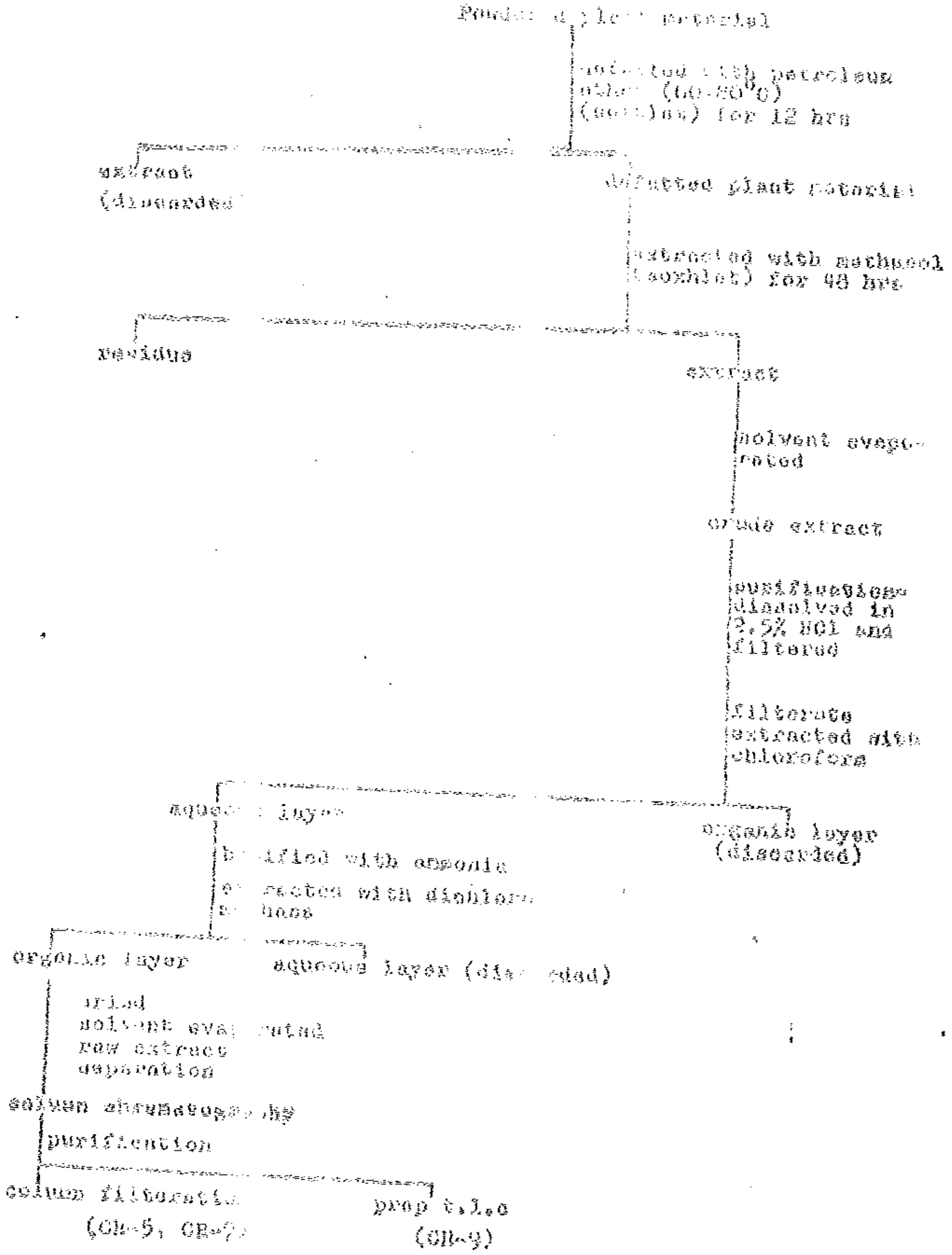


Figure 1. Flow chart for extraction and fractionation of C. rosenii

3.2 Structure of CR-7

CR-7 is a colorless crystalline (needles) alkaloid m.p. of 174-175°C (dec.). It is soluble in acetone, chloroform and methanol. The infrared spectrum of CR-7 (Figure 2) showed strong ester carbonyl absorption at 1720 cm^{-1} and a band at 3420 cm^{-1} due to hydroxy-group. The structure of CR-7 was identified as madurensine(XXV) based on information obtained from mass, ^{1}H NMR and ^{13}C NMR spectra as discussed below.

3.2.1 Mass Spectrum of CR-7

The high resolution mass spectrum of CR-7 showed a prominent molecular ion of 351.1687 corresponding to the molecular formula $C_{18}H_{25}NO_6$. The most important structural information obtained from the mass spectrum is the existence of the trihydroxy amine as the necine base of the alkaloid CR-7. It is already established that the ion fragmentation of macrocyclic pyrrolizidine alkaloids proceeds by initial cleavage of the ester linkages (94). This is then followed by subsequent loss of fragments until the necine base remains (21).

The region of the mass spectrum corresponding to the necine base consists of intense ions and provides useful information on the type of substitution present on the pyrrolizidine ring. For example, non-substituted necine base retronecine ester alkaloids (Table 1 section c) show fragments at m/z 138, 136, 120, 119 (97) while crotanecine ester alkaloids (Table 1 section e) show fragments at m/z 154, 152, 136, 135 which are 16 units greater than that retronecine indicating hydroxyl substituted necine base of crotanecine ester alkaloids (21).

In the mass spectrum of CR-7 ions at m/z 307.1788 (M^{+} -44), 264.1843 (M^{+} -87) and 236* (M^{+} -115) are attributable to the loss of CO_2, $H_2C{=}C(OH){-}COO$, and $H_2C{=}CH{-}C(CH_3)(OH){-}COO$ respectively. This sequence is the major fragmentation path way for the fission of ester linkages in pyrrolizidine alkaloids (94). Anacrotine(XXIII) is a macrocyclic diester pyrrolizidine alkaloid of a trihydroxy pyrrolizidine base crotanecine (Table 1 section e). It undergoes characteristic fragmentation pattern of the crotanecine pyrrolizidine base as shown in figure 3 (21). Thus, in the low mass region *CR-7 gave fragments at m/z 154, 152, 137, 136, 135, 95, 94 and 93 (Figure 4) a fragmentation pattern characteristic of the pyrrolizidine base of the crotanecine group. Eight crotanecine pyrrolizidine alkaloids have been isolated from _Crotalaria agatiflora_ (95). The structures of these alkaloids appear in Table 1 section e.

---

*Accurate mass not determined

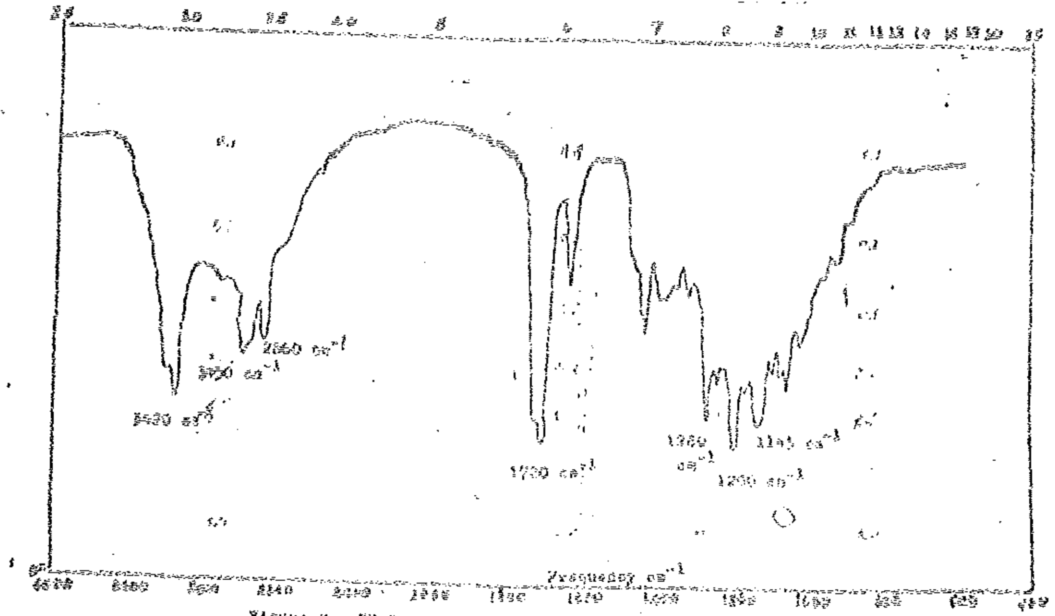


Figure 2. IR Spectrum of OHA-7

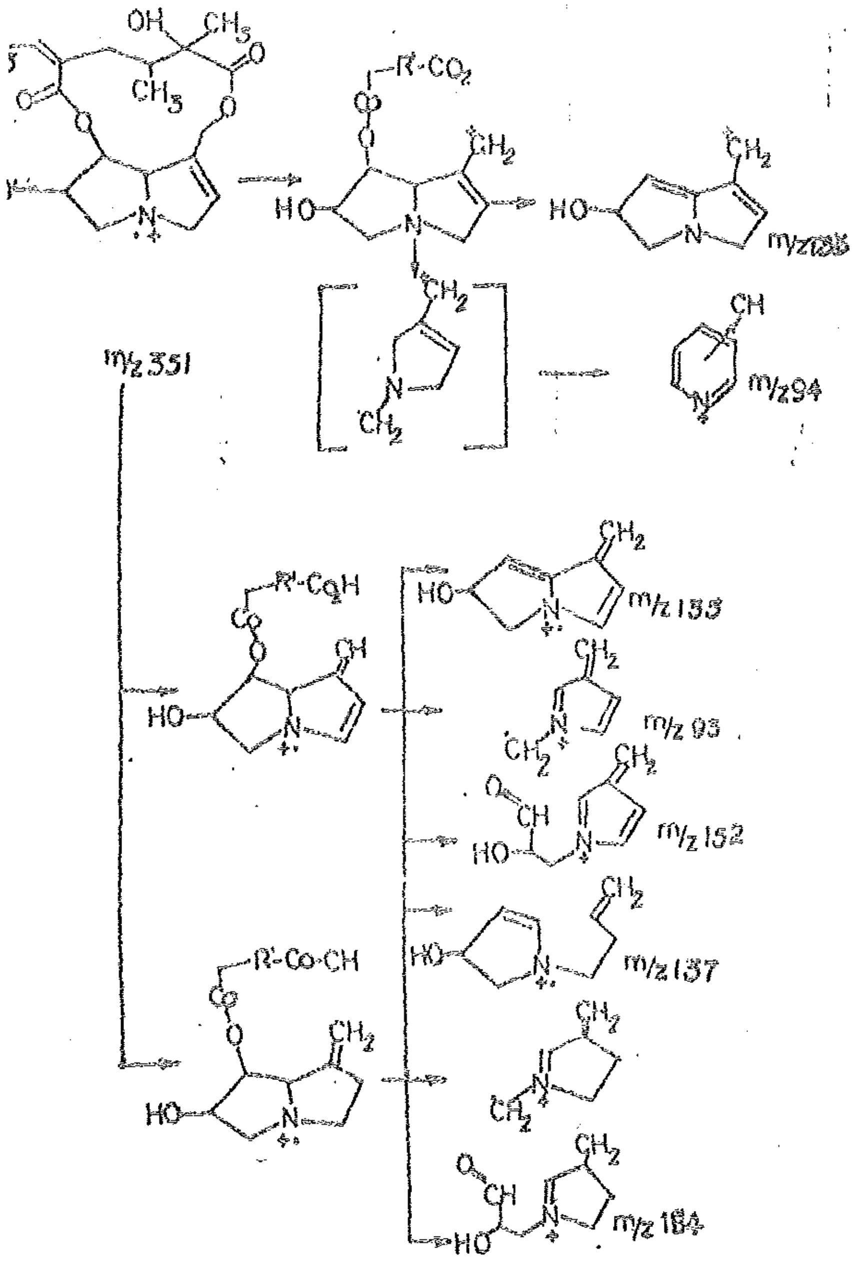
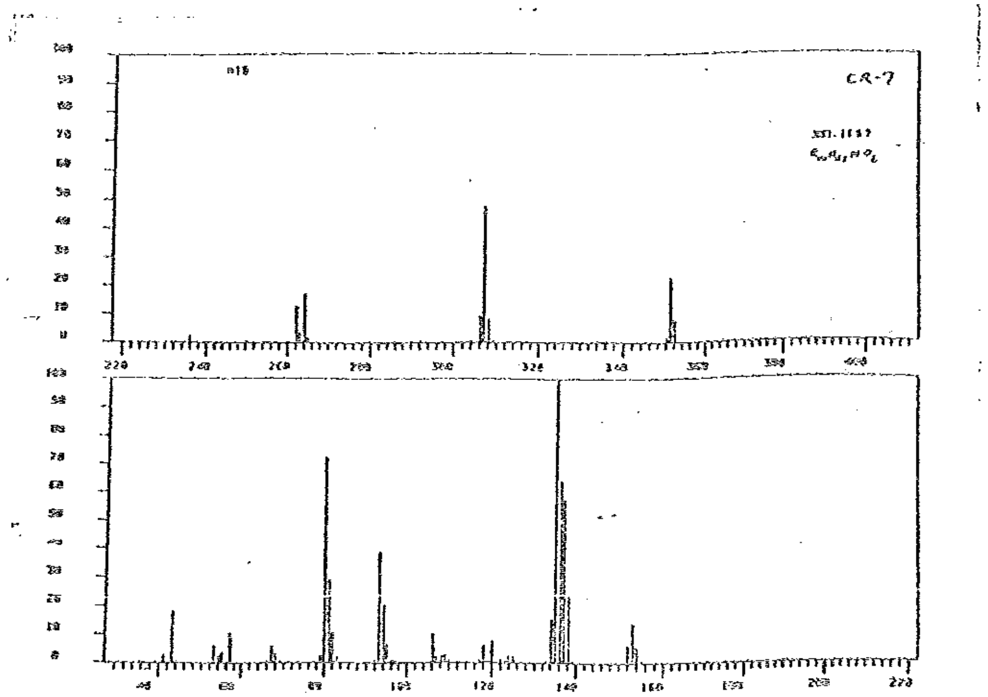
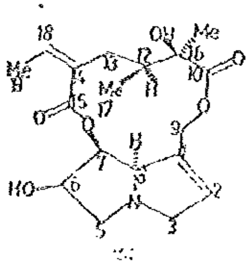


Figure 3. Fragmentation sequence for anacrotine

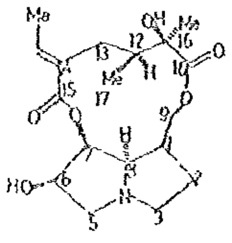


Mass Spectrum of CR-7

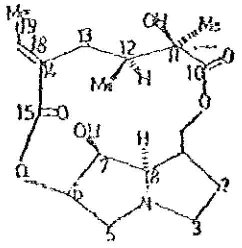
The first crotanecine based alkaloids, anacrotine (XXIII) and madurensine(XXIV) were reported in 1966 by Atal et al (21). These workers wrongly assigned the structure of madurensine as the integrrinecic acid ester of crotanecine with the free - OH group at C-6. Later in 1970 Culvenor and Smith (29) revised the structure of madurensine(XXV) based on ^{1}H NMR measurments and suggested the secondary ester attachement at C-6 and not at C-7 as suggested earlier.



XXIII



XXIV



XXV

3.2.2 Proton Nuclear Magnetic Resonance of CR-7

The structures of madurensine(XXV) and anacrotine(XXIII) were determined by Atal et al (21) by hydrolysis and based on ^{1}H NMR spectra to be the diesters of integrrinecic acid and senecic acid with crotanecine base (21,29). These workers have also reported the partial 60 MH$_{z}$ ^{1}H NMR spectra of madurensine and anacrotine (Table 2). The presence of an additional OH group on the necine base of madurensine (at C-7) and anacrotine (at C-6) simplifies the spectrum by reducing the coupling to adjecent hydrogens. In compounds of th same structure without the additional OH group more complex multiplets are observed. These authors report multiplets for the magnetically nonequivalent CH_2-O-CO protons, a multiplet for $C\underline{H}$-OCOR proton and signals for groups, $C\underline{H}_3$-CH (doublet), $C\underline{H}_3$-C-OH (singlet) and CH_3-$C\underline{H}$=C(CO)- (quartet) of the esterifying acids.

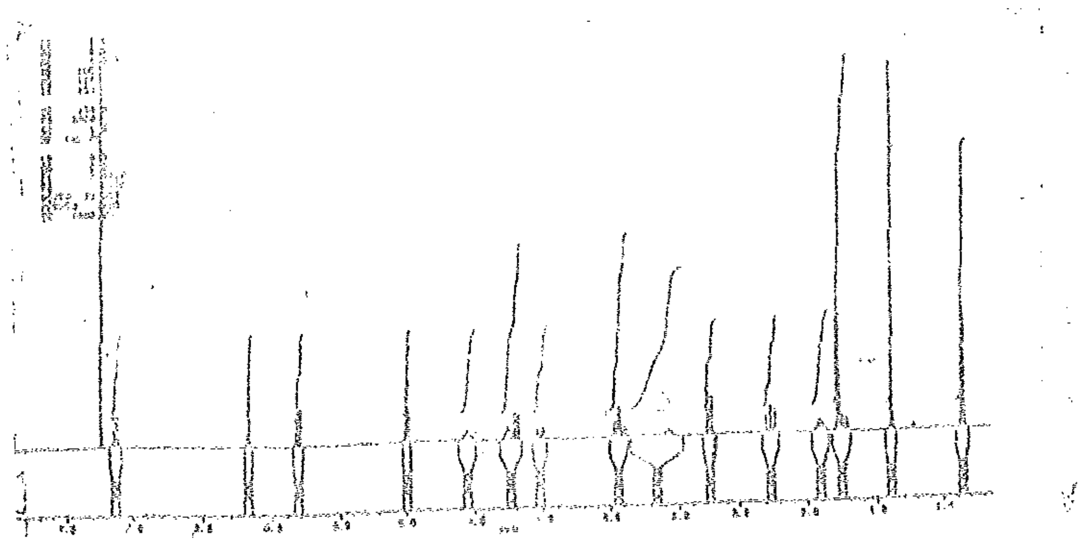
It is generally observed that the signal of a $C\underline{H}$-OH proton moves to lower field when the alcohol is acetylated. The location of the $C\underline{H}$-OCOR proton of madurensine(XXV), which appears at lower field than the $C\underline{H}$-OH proton was determined by Culvenor et al (29) to be at C-6 and not at C-7 by decoupling experiments which showed this proton to be vicijal to the C-5 proton. In anacrotine(XXIII) the $C\underline{H}$OCOR proton was found to be located at C-7 by similar decoupling experiment which showed it to be located vicinal to the C-6 hydrogen. There is significant difference in chemical shifts of madurensine and anacrotine in the ethylidene group of the necic acid due to its configuration which is <u>trans</u> with respect to the carbonyl group in madurensine(XXV) due to the appearance of the olefinic proton at 7.14 ppm. The down field shift of the olefinic proton is because of the diamagnetic anisotropic effect of the ester carbonyl. In anacrotine(XXIII) the olefinic proton appears at 5.74 ppm due to the <u>cis</u> configuration.

The 60 MH_z spectrum of Madurensine is not sufficiently well resolved to allow the assignments of all the protons in the molecule. However, the 400 MH_z spectrum provides adequate resolution to permit the complete assignment of the protons. Further experiments involving $^1H-^1H$ spectral correlations permit the unequivocal assignment of protons. The complete assignments of chemical shifts for CR-7 is given in Table 2.

In the spectrum of CR-7 (Figure 5 a) the C-3-α proton appear at 4.02 ppm (14.4H_z) as double of multiplets and C-3-Hβ proton at 3.42 ppm (J3H_z,2H_z) as a double of doublets. Similarly the C-5-α proton appear at 3.45 ppm (dddd, J14.4H_z, 6H_z, 2H_z) and C-5β proton does not couple with C-6-α proton and as a result appear at 2.77 ppm (J14.4 H_z) as a doublet (Figure 5 c). The mageneti-cally non-equivalent protons of C-9 appear at 5.81 ppm (J10.3 H_z) and 4.19 ppm (J10.3 H_z) as doublets (Figure 5 b, 5 d). The appearance of the C-5-β proton at 2.77 ppm (J14.4 H_z) as a doublet and the olefinic proton at 7.12 ppm (J6.7 H_z) as a quartet (Figure 5 e) indicates C-6 ester linkage and *trans* configuration of the ethylidene group in CR-7. Of the esterifying acid protons, signals at 0.86 ppm (d,J6.7 H_z), 1.38 ppm (s) and 1.78 ppm (dd, J6.7 H_z, 1.8 H_z) (Figure 5 f and 5 g) are due to methyl protons on C-12, C-11 and C-18 respectively.

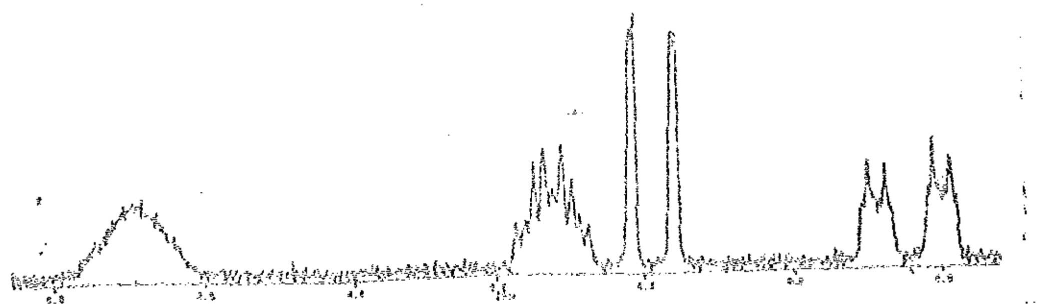
Table 2. ^1H NMR Chemical Shifts of Madurensine(XXV) Anacrotine(XXIII) and CR-7.

	δ (ppm)		
Proton	CR-7	Madurensine(21)	Anacrotine(21)
C-2-H	6.17,S,H	6.19,s,H	
C-3-Hα	4.02,dm,H(J14.4H_z)		
C-3-Hβ	3.42,dd,H(J14.4H_z)		
C-5-Hα	3.45,dddd,H(J14.4,6, 2H_z)		
C-5-Hβ	2.77,d,H(J14.4H_z)	2.77,d,H	2.6,m,H
C-6-Hα	4.98,t,H(J2.36H_z)	5.02,H	4.56,m,H
C-7-Hα	4.55,m,H		
C-8-Hα	4.26,m,H		
C-9-H_A	5.81,d,H(J10.3H_z)	5.83,m,H	4.05,m,H
C-9-H_B	4.19,d,H(J10.3H_z)	4.2,m,H	5.46,m,H
C-12-H	1.91,m,H		
C-13-H_A	2.26,t,H(J11.7H_z)		
C-13-H_B	1.73,d,H(J11.7H_z)		
C-16-H_3	1.38,s,3H	1.42,s,3H	1.34,5,3H
C-17-H_3	0.86,d,3H(J6.7H_z)	0.89,d,3H	0.95,d,3H
C-18-H	7.12,q,H(J6.7H_z)	7.14,q,H	5.74,q,H
C-19-H_3	1.78,dd,3H(J6.7,1.8 H_z)	1.82,H	1.83,3H
-OH	3.05-3.1,2H		



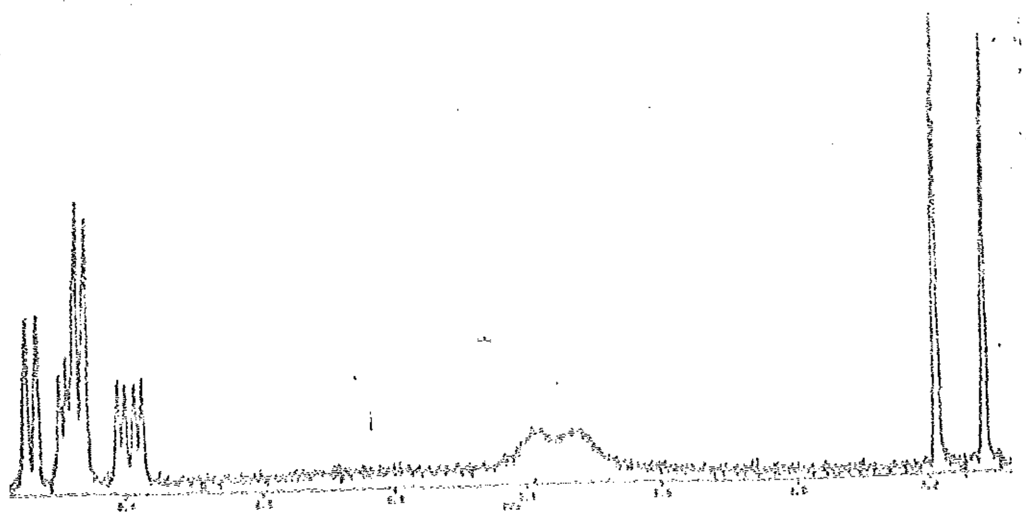
^{1}HNMR spectra of CH-7

Fig. 5a



^{1}HNMR Spectrum of CH-7 (δ 4.0 – δ4.6 ppm)

Fig. 5b



^{1}HNMR Spectrum of CH-7 (δ 2.5 – δ 3.3 ppm)

Fig. 5c

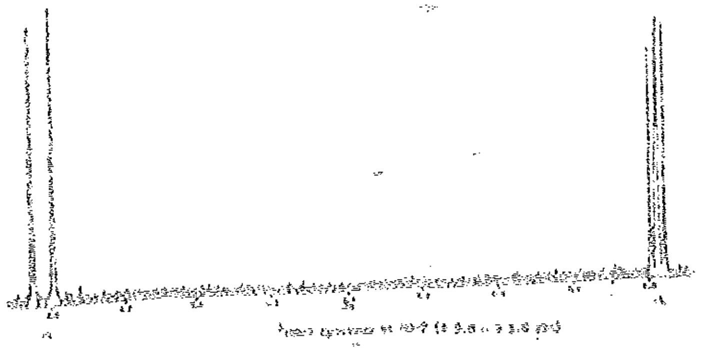


Fig. 5d

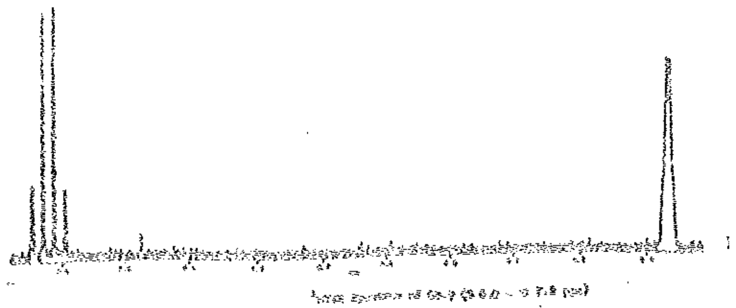


Fig. 5e

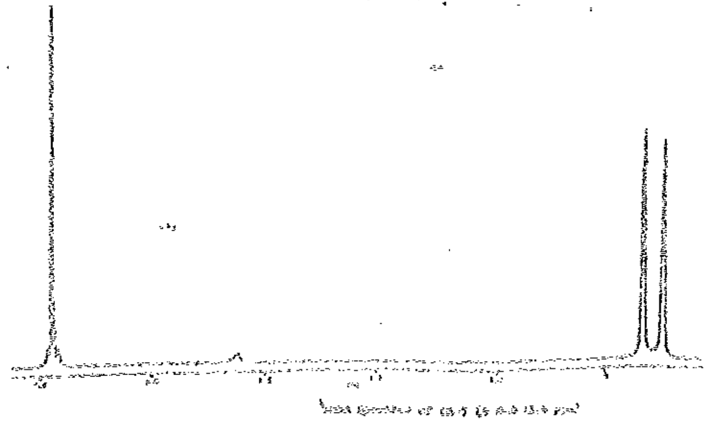


Fig. 5f

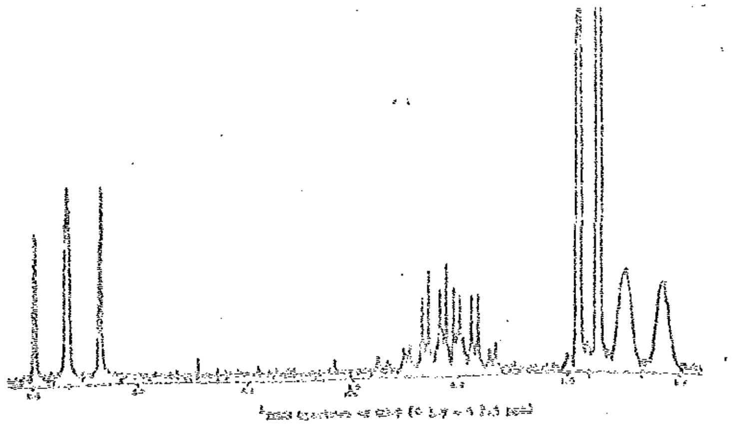
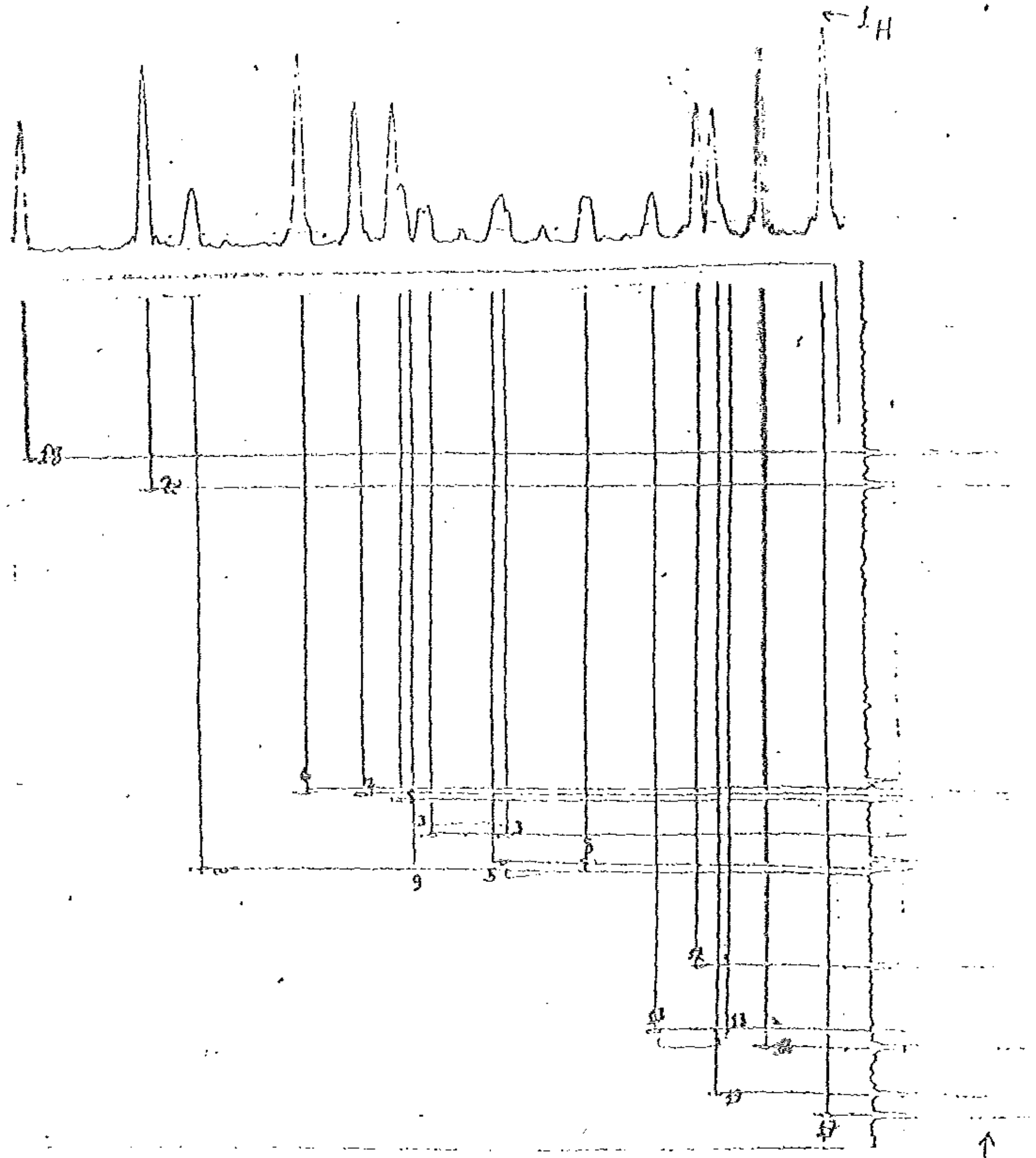


Fig. 5g

3.2.3 ^{13}C NMR Spectrum of Cr-7

The chemical shifts of fourteen out of eighteen carbon resonance signals were unequivocally determined by 2-dimensional ^{13}C-^{1}H shifts correlated spectrum (Figure 6). Carbon resonance signals for the remaining four quaternary carbon atoms were assigned by comparing chemical shift values with those of similar compounds (96,97). Recently Mody et al. have reported the ^{13}C spectrum of Madurensine (98). They assigned chemical shifts of carbon resonance signals by comparing acquired values of chemicals shifts with those of similar compounds (98). Comparision of values obtained for CR-7 by 2-dimensional techniques with those of Mody et al. reveal that their data contain misassignments for C-3, C-5, C-6, C-7, C-8 and C-9 carbons.



$^{13}C-^{1}H$ Shift Correlated Spectrum of CR-7

Fig. 6

Table 3. ^{13}C NMR Chemical Shifts for CR-7 and Madurensine(XXV)

	δ, ppm	
Carbon number	CR-7	Madurensine (98)
1	135.5	129.9
2	136.2	136.1
3	66.4	59.3
5	61.5	66.4
6	75.1	74.7
7	74.7	73.7
8	73.7	75.2
9	59.5	61.5
10	177.1	176.9
11	76.3	76.3
12	40.5	40.5
13	27.5	27.6
14	129.7	135.6
15	167.0	167.0
16	24.5	24.6
17	10.8	10.8
18	142.6	142.5
19	15.0	15.0

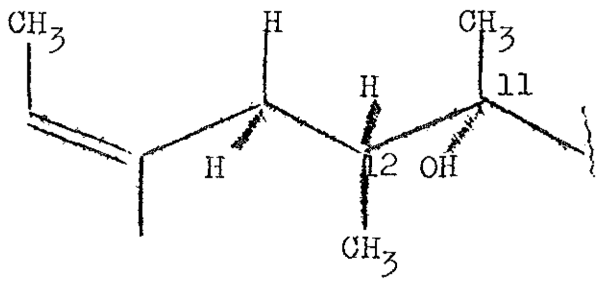
3.2.4 NOE Difference Spectral of CR-7

NOE experiments were done on CR-7 to establish the relative stereochemistry of the necine base at C-6, C-7, and C-8 as well as the configuration of the necic acid at C-11 and C-12. These experiments are also used to confirm the configuration of the ethylidene group of the esterifying acid. NOE difference spectral data for C-2-H, C-7-H, C-16-H_3, C-17-H_3, C-18-H_3 and C-19-H_3 protons are given in Table 4.

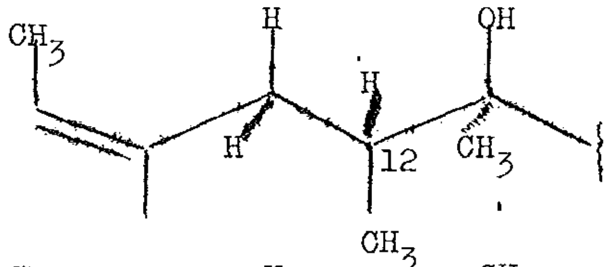
Irradiation of C-7-Hα (δ 4.54 ppm) (Figure 7 a) showed NOE on C-5-Hα, C-6-Hα and C-8-Hα which requires that these protons be on the same side of C-7-Hα confirming the stereochemistry of the necine base at C-5, C-6 and C-8.

The trans configuration of the ethylidene group is also supported from the NOE experimental data. Irradiation of C-18-H (δ 7.12 ppm) (Figure 7 b) showed NOE on C-19-H_3 and no enhancement was observed for the C-13 protons due to the fact that C-18 and C-13 protons are not in close proximity with one another. This is apparently true if the C-18 proton is away from C-13 protons as would be expected in the trans configuration.

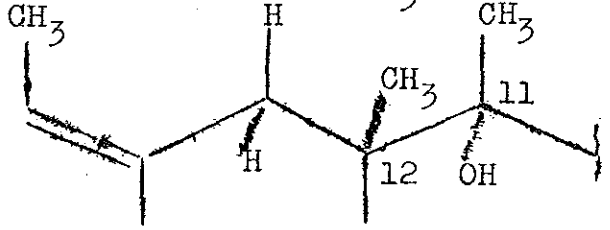
There are two chiral center at C-11 and C-12 in the necic part of CR-7 which give rise to four possible configurations. These are: 11S and 12S (XXVI) 11R and 12S, (XXVII) 11S and 12R(XXVIII) 11R and 12R(XXIX).



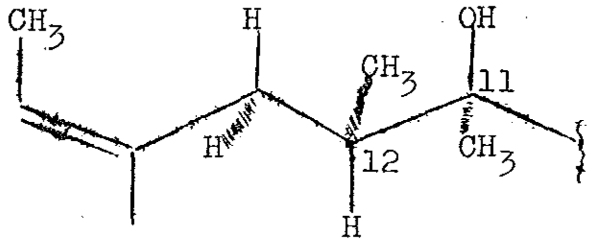
XXVI



XXVII



XXVIII



XXIX

Table 4. NOE Difference Spectral Data for CR-7

Irradiated proton	NOE%
C-2H(δ 6.16 ppm)	C-3Hα (2.4%) C-3Hβ (2.0%)
C-7-H(δ 4.54 ppm)	C-5Hα (2.2%), C-6Hα (6.2%), C-8Hα (7.3%)
C-16-H_3(δ 1.38 ppm)	C-17-H_3 (22.7%), C-12-H (24.4%)
C-17-H_3(δ 0.86 ppm)	C-16-H_3 (5.3%), C-12-H (8.7%), C-13-H (4.7%), C-19-H_3 (4.2%)
C-18-H(δ 7.12 ppm)	C-19-H_3 (9.6%)
C-19-H_3(δ 1.78 ppm)	C-17-H_3 (6.2%), C-13-H_A(10%)

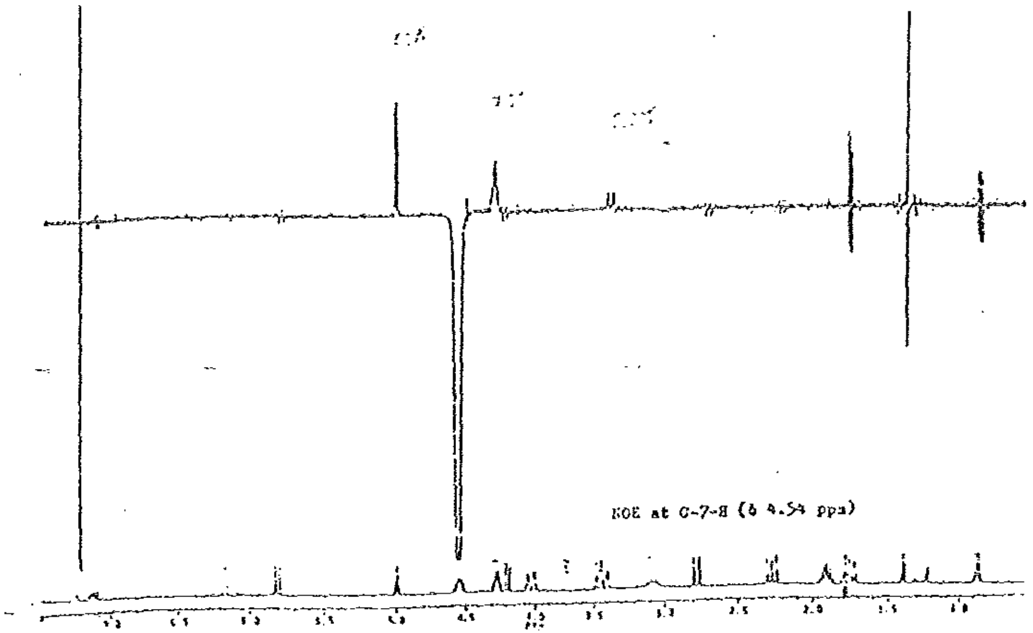


Fig. 7a

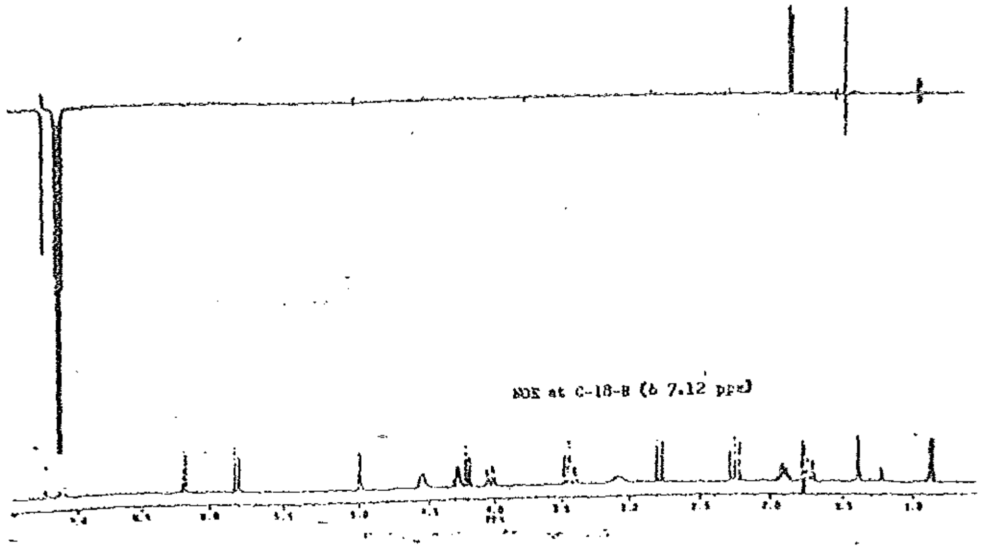


Fig. 7b

Irradiation of C-16-H_3 (Figure 7 c) showed significant NOE on C-17-H_3 (22.7%) which suggests special proximity of C-16-H_3 and C-17-H_3. Irradiation of C-17-H_3 (Figure 7 d) enhanced C-16-H_3 and C-19-H_3 which requires the close proximity of C-17-H_3 and C-19-H_3. In the first configuration(XXVI) C-16-H_3 and C-17-H_3 are on the same side away from C-19-H_3 and therefore cannot account for the observed NOE effects of C-19-H_3 on C-17-H_3. In the second configuration(XXVII) C-17-H_3 and C-19-H_3 are not in close proximity and C-17-H_3 as well as C-16-H_3 are also on opposite side. Therefore, this configuration is not likely. In the third configuration(XXVIII) even if C-19-H_3 and C-17-H_3 are in close proximity C-17-H_3 is far away from C-16-H_3 and cannot explain the 22% NOE effect. The fourth configuration(XXIX) is the most likely, because C-19-H_3, C-17-H_3 and C-16-H_3 are in close proximity and can account for the NOE effect observed. Therefore, configuration of CR-7 at the chiral centers is possibly 11R and 12R.

Hrbeck et al (99) reported circular dichroism measurments for madurensine. For madurensine since the ester ring attachement is at C-6 but not at C-7, the conformation of the diester ring system changes drastically. They indeed observed this change from the CD (ethanolic solution) of madurensine which is of enanthiomorphic type to that of the pyrrolizidine alkaloids in which the attachement is at C-7 with exo-buckled ring. Circular

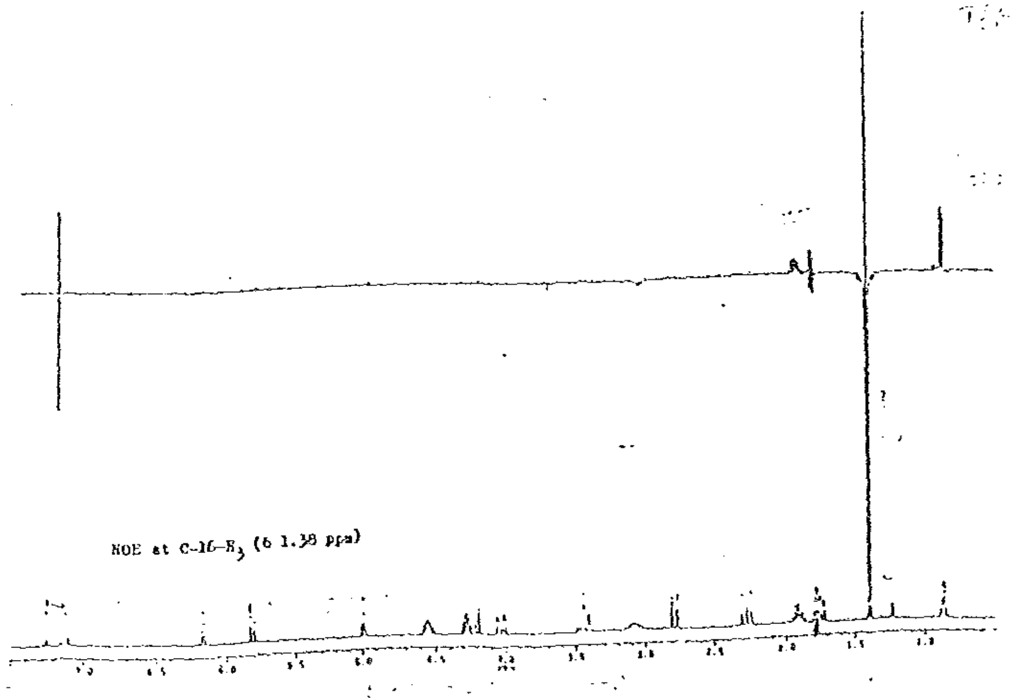

NOE at C-16-H$_3$ (δ 1.38 ppm)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm
3.5
3.0
2.5
2.0
1.5
1.0

Fig. 7c

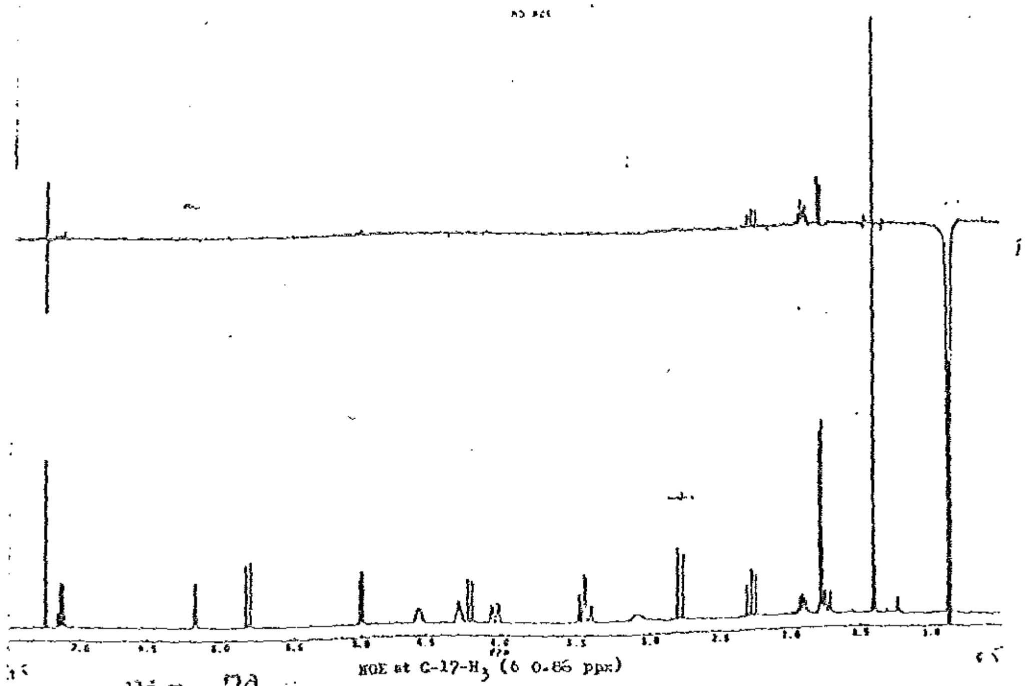

7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm
3.5
3.0
2.5
2.0
1.5
1.0
NOE at C-17-H$_3$ (δ 0.86 ppm)

Fig. 7d

dichroism measurments for CR-7 (Figure 8) were identical with values reported for madurensine except the appearance of a fine band at 285 nm for CR-7 which may be due to the use of modern instrument. We do not know whether this band is significant or not.

The ultimate proof for the structure is the direct comparison with authentic sample and X-ray. But based on mass spectrum, ^{1}H NMR spectrum and ^{13}C NMR spectrum data CR-7 is identified to be madurensine(XXV). CR-7 is identified to be diester of integrrenecic acid with crotanecine base (madurensine). There is a possibility for CR-7 to have an alternate structure. The linkage of the diester bond may be as shown(XXV) or the oxygen on C-9 may be joined to the C-15 carbonyl. COLOC experiment was done on CR-7 to establish the mode of linkage of the ester group. COLOC experiment ignores all couplings greater than $6H_z$ and only long range coupling is observed. In COLOC spectrum of CR-7 (Figure 7e) coupling was observed between proton on C-9 and C-10 carbonyl carbon. No coupling was observed between proton on C-9 and C-15 carbonyl carbon. Therefore, this is a proof for the structure of CR-7 to be the same as that of madurensine (XXV) in which the oxygen on C-9 is attached to the C-10 carbonyl not to the C-15 carbonyl.

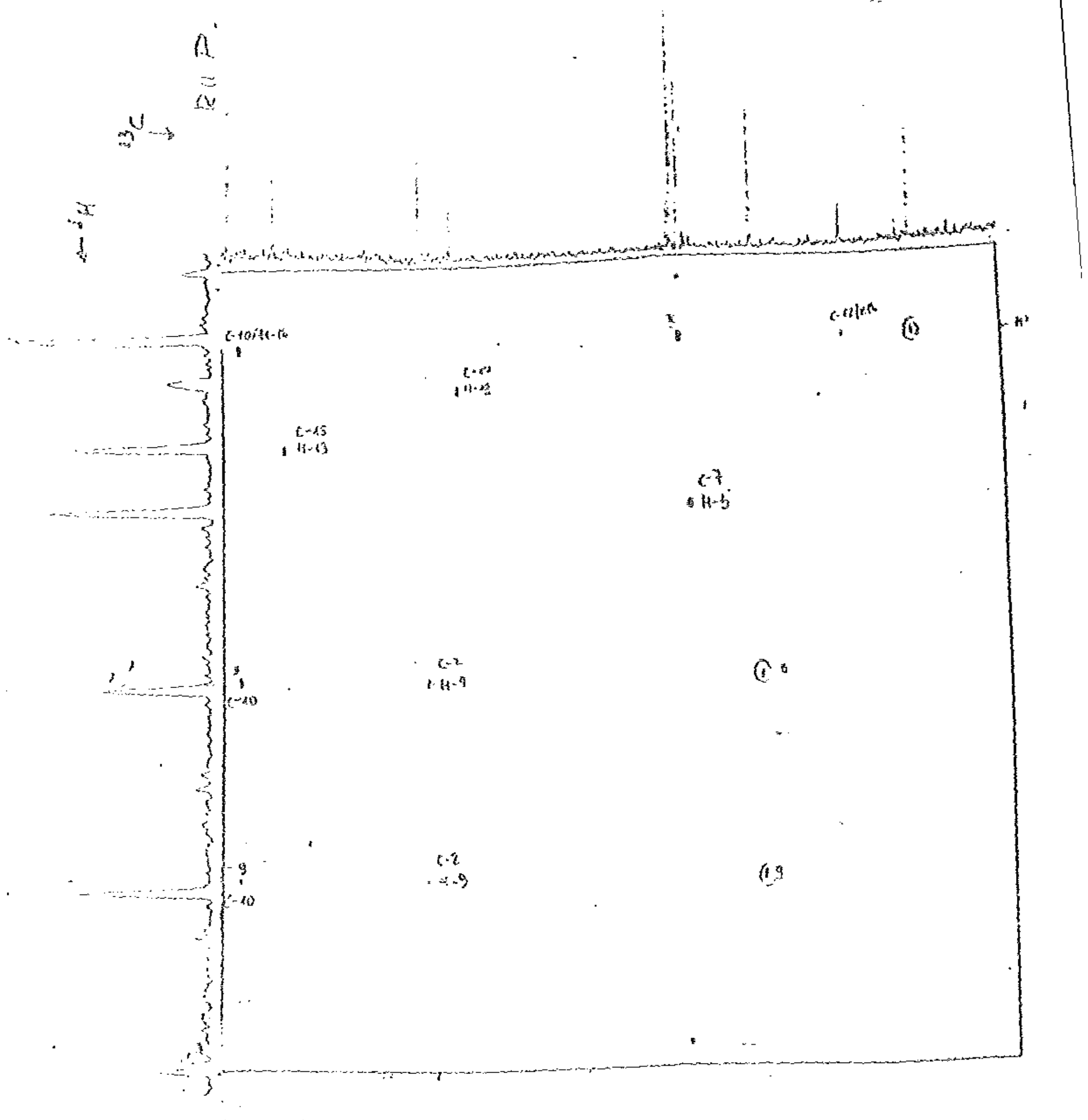


Figure 7e. COLOC Spectrum of CR-7

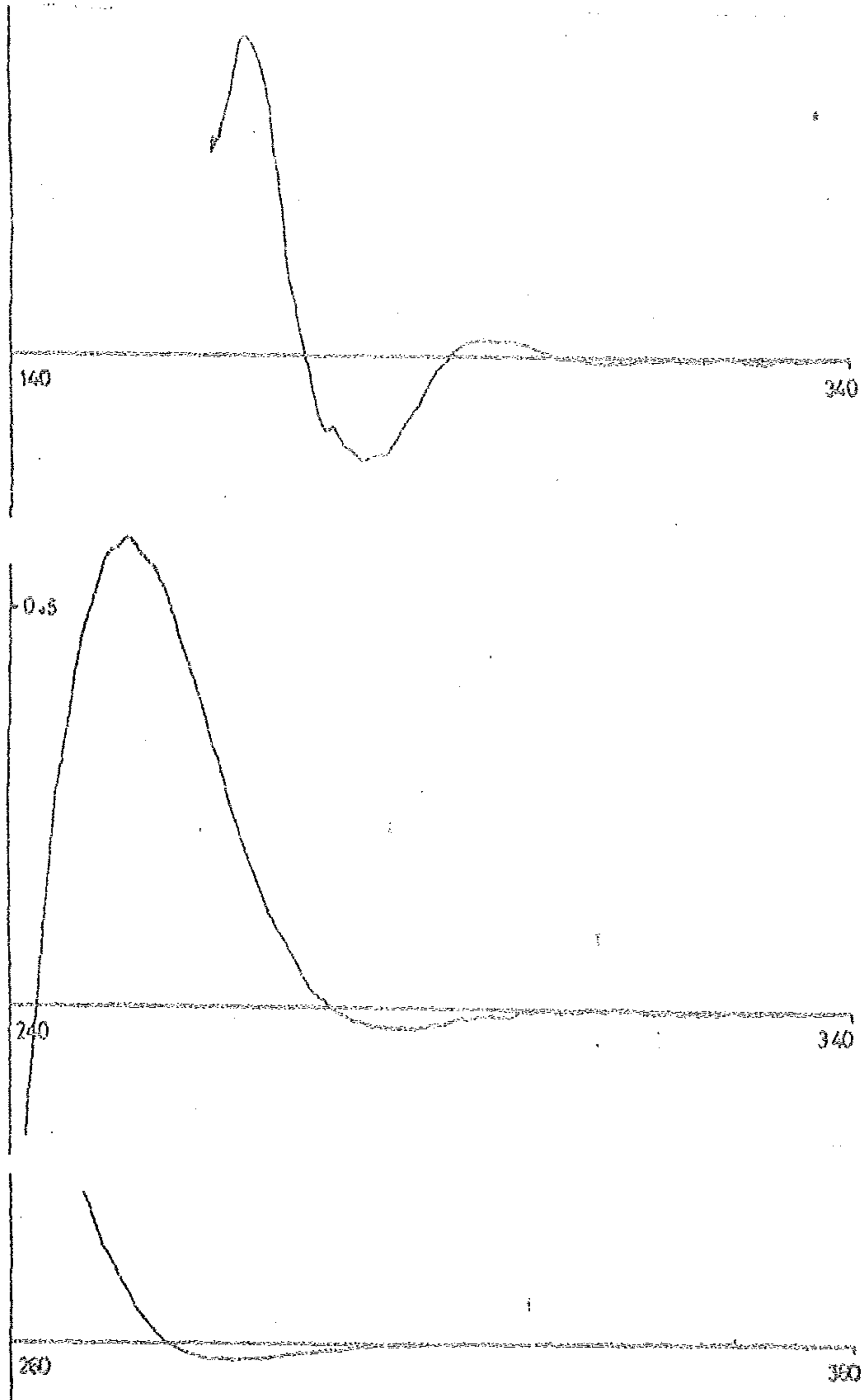
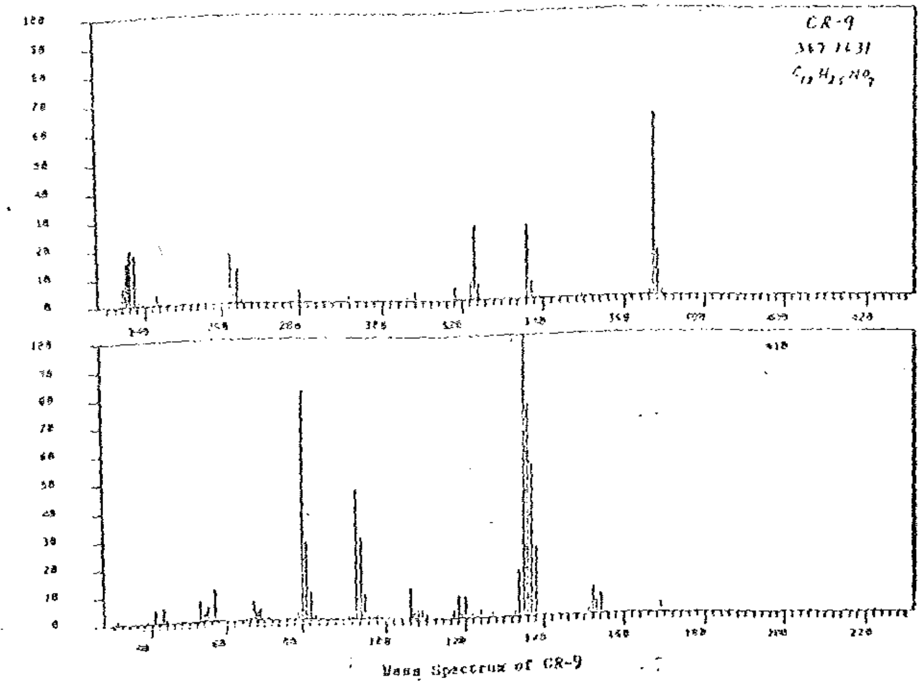


Fig. 8

3.3 Structure of CR-9

CR-9 is a colorless crystalline alkaloid m.p. 178-181°C (dec.). It is soluble in acetone, chloroform and methanol. Its high resolution mass spectrum gave molecular formula $C_{18}H_{25}NO_7$ (367.1631). In the mass spectrum ions at m/z 323.1644 (M^+ -44) due to loss of CO_2 and 280.1580 (M^+ -87) which is due to loss of $H_2C=C(OH)-COO$ indicates the major fragmentation path way of ester linkages in pyrrolizidine ester alkaloids (94). In the mass spectrum of CR-9 (Figure 9) there are fragments at m/z 137*, 136, 135, 95, 94, and 93 which are characteristic of crotanecine base (21).

The ^1H NMR chemical shifts assignments for CR-9 were made from ^1H-^1H shift correlated spectrum (COSY) (Figure 10). It closely resemble that of CR-7 (Table 5, Figure 11 a) in having chemical shift for trans $CH_3-CH=C-CO$ at 7.14 ppm (J8.6 H_z) (Figure 11 b), non-equivalent C-9 protons, a triplet at 4.99 ppm (J2.7 H_z) (Figure 11 c) and a doublet at 2.78 ppm (J14.7 H_z) for C-5-Hβ (Figure 11 d). The spectrum differs from that of CR-7 in that the singlet for CH_3-C-OH at 1.38 ppm is missing and is replaced by a double of doublets of two protons at 3.77 ppm with geminal coupling of 21.6 H_z (Figure 11 e). These data are consistent with the presence of a hydroxymethyl (CH_2OH) grouping at C-11 and



Mass Spectrum of CR-9

Fig. 9

^1H-^1H - Shift correlated spectrum of CR-9

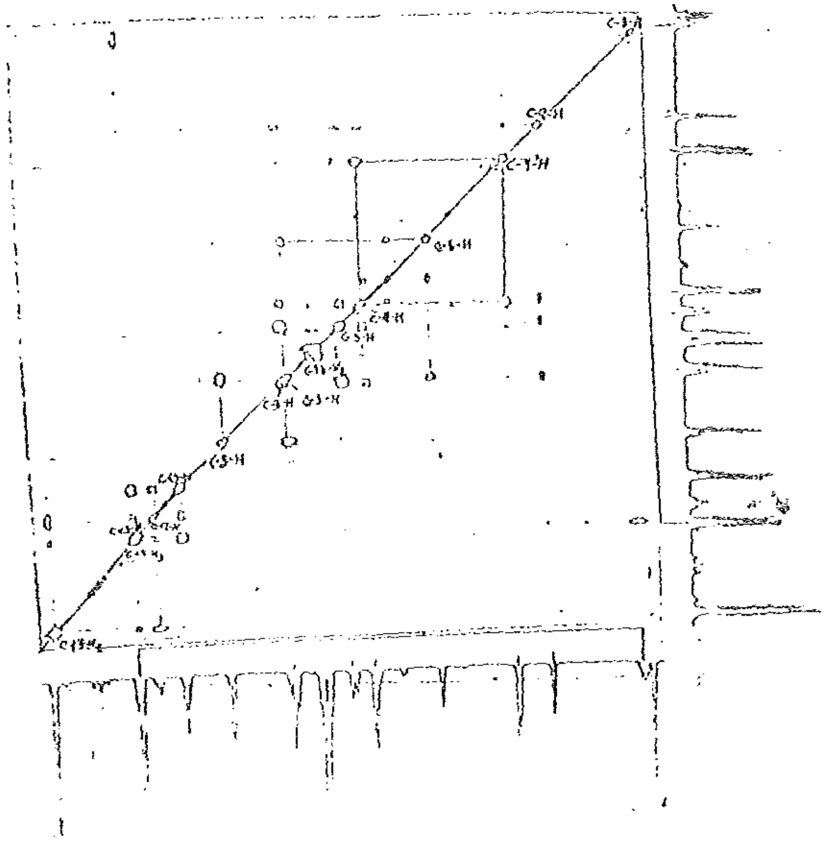
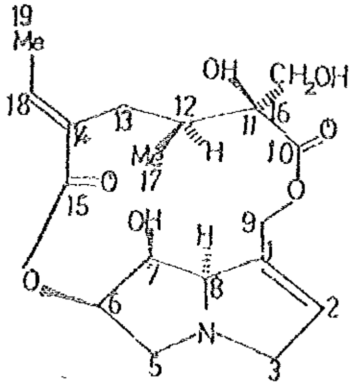
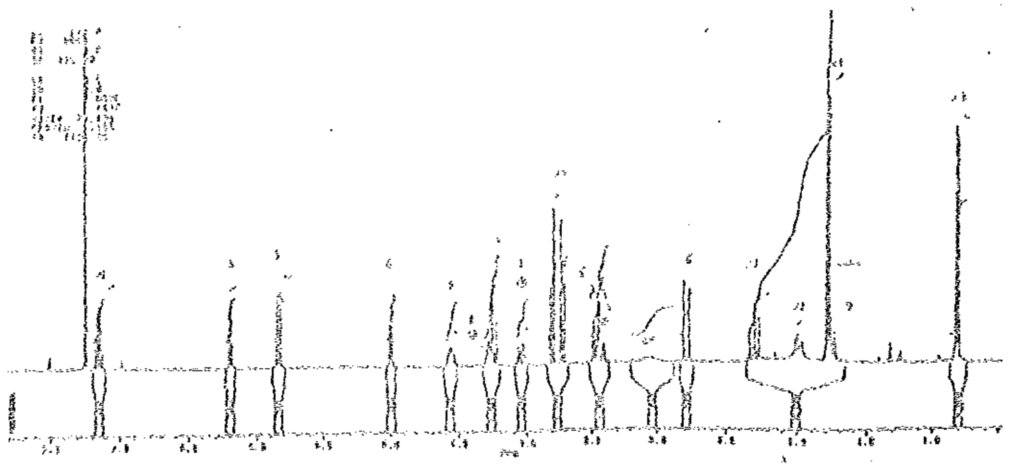


Fig. 10

suggests the esterfying dicarboxylic acid to be retronecic acid (100, 101). Since the chemical shift for C-5-Hβ in CR-9 is a doublet at 2.78 ppm (J14.7 H_z), the macrocyclic ring is formed through C-6. The IR spectrum of CR-9 (Figure 12) has extended OH absorption at 3570 cm^{-1} due to the additional -OH group. Further, the presence of additional -OH group is indicated from the difference in molecular weight between CR-9 and CR-7 by 16 mass units. Therefore, CR-9 is a hydroxy methyl of CR-7 at C-11 and the structure can written as (XXX). The assignment of ^{13}C NMR data for CR-9 (Table 6) was made by comparing with chemical shift values assigned for CR-7.

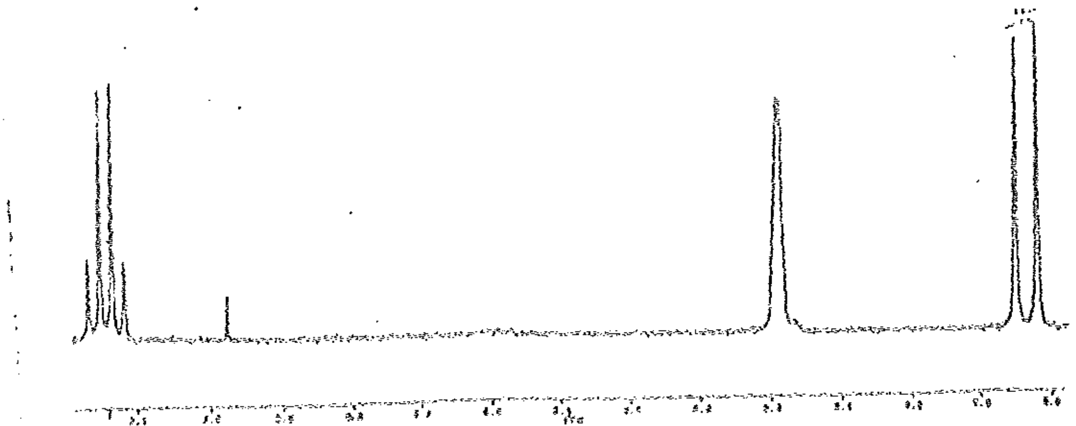


XXX



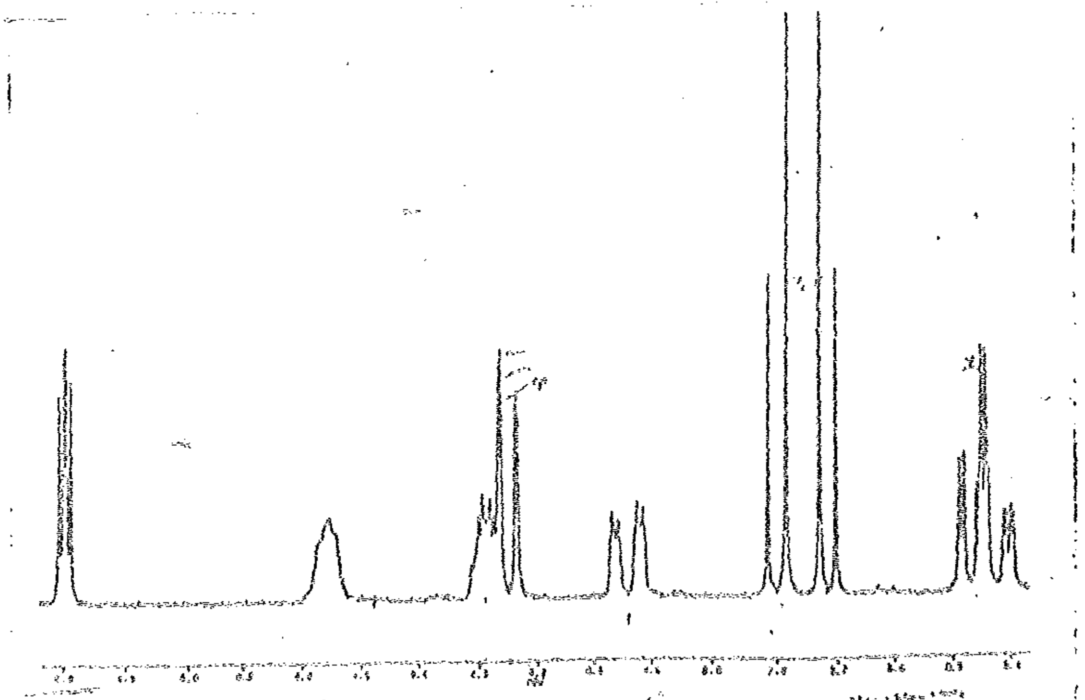
¹H NMR Spectrum of CS-9

Fig. 11a



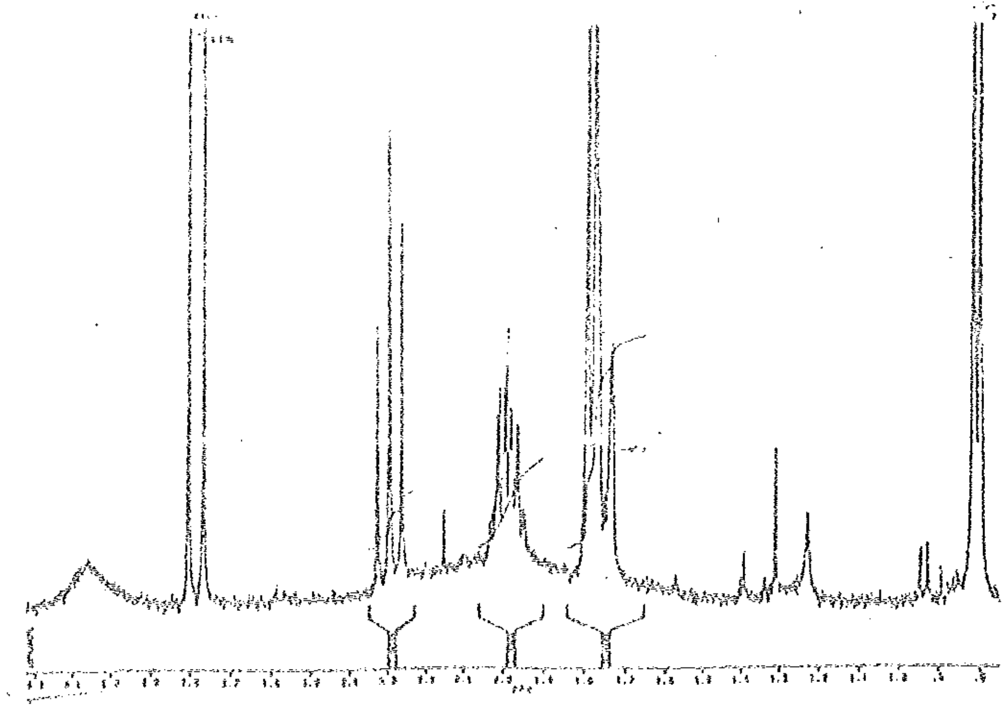
¹H NMR Spectrum of CS-9 (δ5.6 – 7.2)

Fig. 11b



¹H NMR Spectrum of CS-5 (δ5.6 – 3.0)

Fig. 11c



^{1}HNMR Spectrum of CS-9 (δ0.7 - 9.2)

Fig. 11d

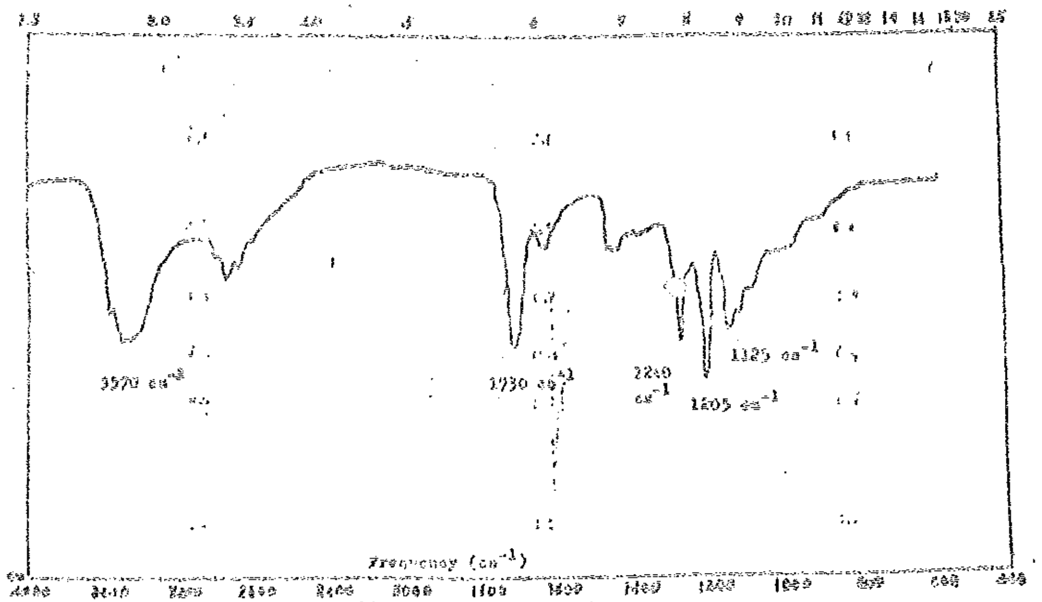


Figure 12. IR Spectrum of CS-9

Fig. 12

Table 5. ^{1}H NMR Chemical Shifts of CR-7(XXV), CR-9(XXX) and Crotaflorine

Proton	δ (ppm)		
	CR-7	CR-9	Crotaflorine (95)
C-2-H	6.17,s,H	6.19,s,H	6.19H
C-3-Hα	4.02,dm,H(J14.4H_z)	4.04,dm,H (J16.8H_z)	
C-3-Hβ	3.42,dd,H(J14.4,3H_z)	3.44,dd,H (J14.7,3.3 H_z)	
C-5-Hα	3.45,dddd,H(J14.4, 6,2H_z)	3.46,ddd,H (J14.7,2H_z)	
C-5-Hβ	2.77,d,H(J14.4H_z)	2.78,d,H (J14.7H_z)	2.79,H(J15H_z)
C-6-Hα	4.98,t,H(J2.36H_z)	4.99,t,H (J2.7H_z)	5.01,H
C-7-Hα	4.55,m,H	4.56,m,H	
C-8-Hα	4.26,mH	4.28,m,H	
C-9-H_A	5.81,d,H(J10.3H_z)	5.83,d,H (J11.5H_z)	
C-9-H_B	4.19,d,H(J10.3H_z)	4.25,d,H (J11.4H_z)	
C-12-H	1.91,m,H	1.98,m,H	
C-13-Hα	2.26,tH(J11.7H_z)	2.29,tH (J12.3H_z)	
C-13-Hβ	1.73,d,H(J11,7H_z)	1.73,dH (J12.3H_z)	
C-16-H_3/H_2	1.38,s,3H	3.75,dd,2H (J21.6,10H_z)	3.71,2H
C-17-H_3	0.86,d,3H(J6.7H_z)	0.81,d,3H (J8.6H_z)	0.82,3H

Table 5. Cont.

	δ (ppm)		
Proton	CR-7	CR-9	Crotaflorine (96)
C-18-H	7.12,q,H(J6.7H$_z$)	7.14,q,H (J8.6H$_z$)	7.15,H
C-19-H$_3$	1.78,dd,H(J6.7), 1.8H$_z$)	1.78,d,H (J7.88H$_z$)	1.79.3H
-OH	3.05 - 3.1,2H	3.0 - 3.1, 3H	

Table 6. ^{13}C NMR Chemical Shifts for CR-9

Carbon number	δ, ppm
1	135.4
2	136.3
3	66.4
5	61.4
6	75.2
7	74.6
8	73.7
9	59.8
10	174.9
11	81.2
12	37.2
13	27.3
14	129.1
15	166.9
16	66.5
17	11.5
18	143.0
19	15.0

Crotaflorine (Table 1 section e) is a crotanecine ester alkaloid with molecular formula $C_{18}H_{25}NO_7$ (367) isolated from Crotalaria agatiflora (95). This compound has a hydroxy methyl group at the α-position of the ester and macrocyclic ring attachement at C-6. The probable structure of crotaflorine was assigned by Culvenor et al (95) based only on the partial ^1H NMR data given in Table 5. With high resolution ^1H NMR, ^{13}C NMR, and MS data obtained the structure for CR-9 is correctly established to be(XXX) which is identical with structure reported for crotaflorine (95).

3.4 Structure of CR-5

CR-5 is an alkaloid with molecular formula of $C_{20}H_{27}NO_7$ (393.1787) as indicated in a high resolution mass spectrum. It has typical fragment ions at m/z 349.1888 (M^+-44), 306.1714 (M^+-87) and 278 (M^+-115) which are also prominent in the spectrum of CR-7. The ion series 196., 194, 178, 153, 137, 136, 135 (Figure 13) are 42 units greater than the ion series in CR-7 which is characteristic ion series of crotanecine pyrrolizidine base (21). This is an indication of a madurensine type nucleus with acetate group for CR-5.

The ^{1}H NMR chemical shift of CR-5 (Table 7, Figure 14a) shows signals for CH_3-CH=CO, CH_3-C-OH and CH_3-CH which are appropriate to integerrinecic acid ester as in madurensine (95). The ester linkage of CR-5 is at C-6 like in CR-7 (madurensine) due to the presence of a doublet at 2.85 ppm (J14.4 H_z) for C-5Hβ (Figure 14 b). ^{1}H NMR chemical shift of CR-7 is identical with CR-5 (Table 7) except the additional methyl signal at 2.15 ppm (s,H) (Figure 14 c) due to the acetyl group. There are also down field chemical shifts of protons on C-6, C-7 and C-8 to 5.25, 5.35 and 4.55 ppm respectively because of the acetate group at C-7. CR-7 (madurensine) was acetylated with acetic anhydride in pyridine. Under this reaction condition only the secondary alcohol is acetylated to give the mono-acetate.

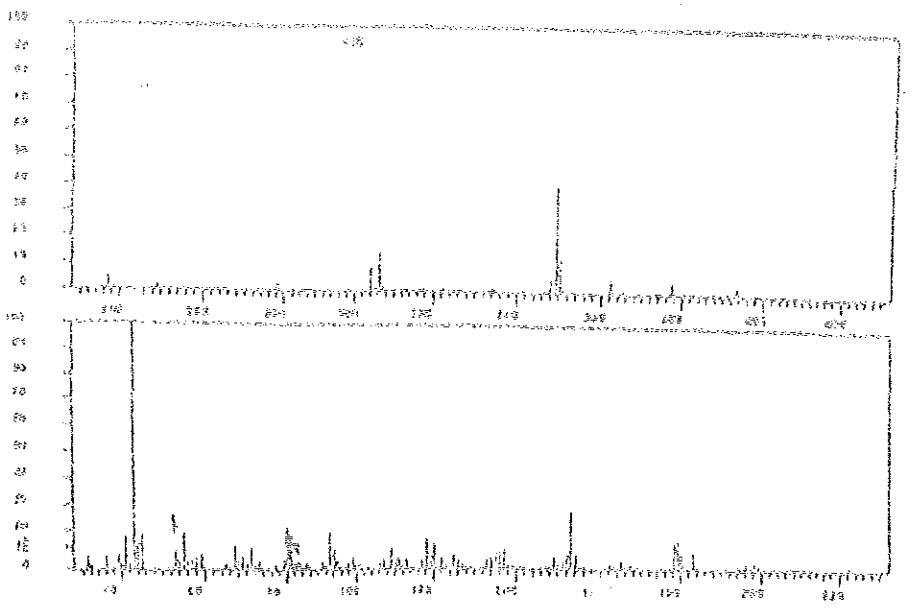
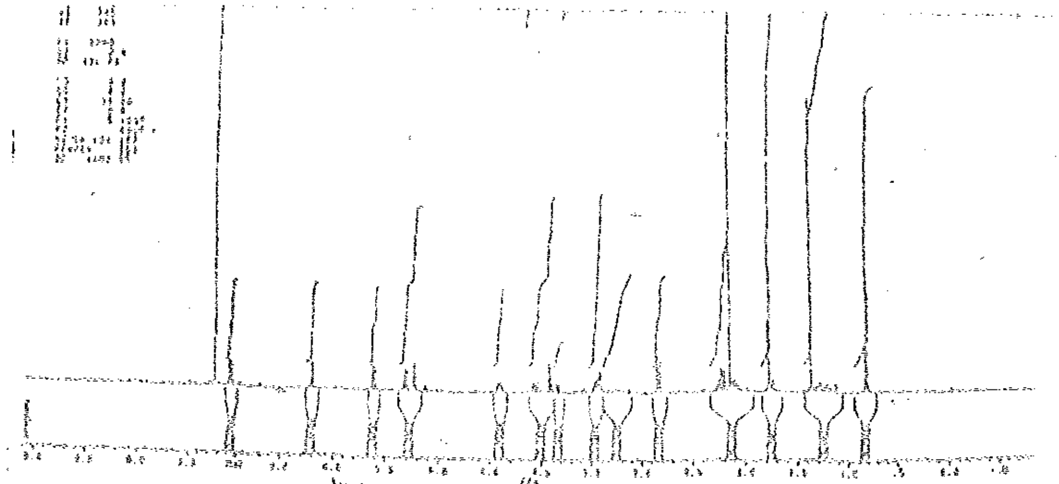
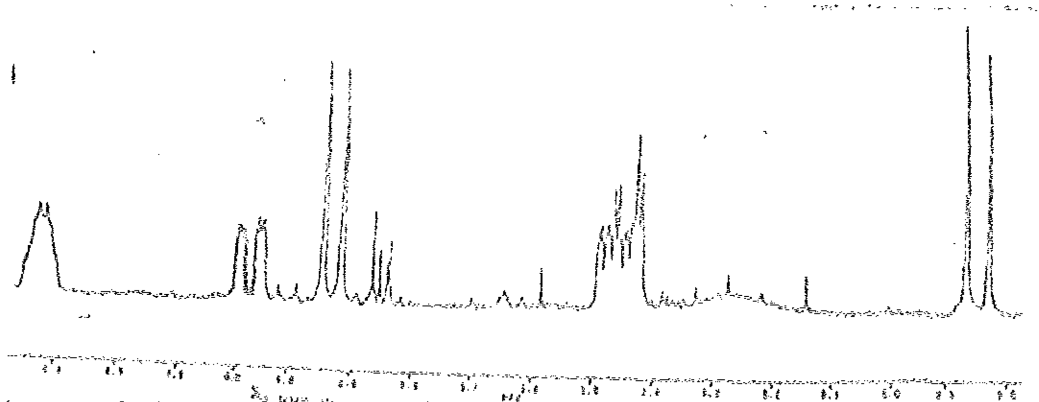


Figure 13. Mass Spectrum of GB-5



^{13}C NMR Spectrum of GB-5

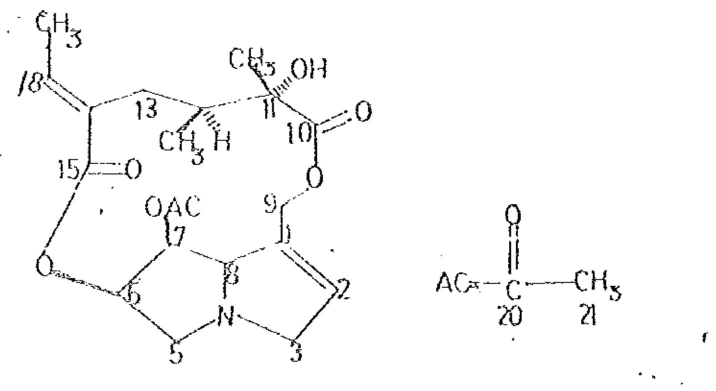
Figure 14a



^{1}H NMR Spectrum of GB-5 ([illegible])

Figure 14b

The acetylation product gave molecular formula of $C_{20}H_{27}NO_7$ (393) in the mass spectrum with the same fragment ions as CR-5. T.l.c comparision of CR-5 and acetylated CR-7 gave identical spot with the same R_f value of 0.72 and 0.85 in solvent systems chloroform: methanol (80:20) and chloroform: methanol: ammonia (85:14:1) respectively. ^1H NMR chemical shifts observed in the spectrum of the natural product (CR-5) (Figure 11 a) were also observed in the spectrum of acetylated CR-7 (Figure 15). Based on these results the structure of CR-5 (CR-7 monoacetate) can be written as (XXXI). ^{13}C NMR chemical shift assignments for CR-5 (Table 8) was made by comparing with chemical shift of CR-7.



XXXI

7.Acetyl madurensine (Table 1 section e) a compound isolated from C. agatiflora has molecular formula of $C_{20}H_{27}NO_7$ (393). The partial ^1H NMR chemical shift of this compound reported by Culvenor et al (96) shows signals appropriated for $CH_3CH{=}C{-}CO$, $CH_3{-}C{-}OH$ and $CH_3{-}CH$ groups as in madurensine. The structure of this compound is the same as the structure assigned for CR-5.

Table 7. ^{1}H NMR Chemical Shifts of CR-7 and CR-5

	δ (ppm)	
Proton	CR-7	CR-5
C_2-H	6.17,s,H	6.2,s,H
C_3-Hα	4.02,dm,H(J14.4H_z)	3.92,dH(J11.7H_z)
C_3-Hβ	3.42,dd,H(H14.4,3H_z)	3.85,m,H
C_5-Hα	3.45,dddd,H(J14.4,6,2H_z)	3.49,dd,H(J16.2,2H_z)
C_5-Hβ	2.77,d,H(J14.4H_z)	2.85,d,H(J14.4H_z)
C_6-H	4.98,t,H(J2.36H_z)	5.23,t,H(J4.2H_z)
C_7-H	4.55,m,H	5.35,m,H
C_8-H	4.26,m,H	4.45,m,H
C_9-Hα	5.81,d,H(J10.3H_z)	5.6,d,H(J10.8H_z)
C_9-Hβ	4.19,d,H(J10.3H_z)	3.95,d,H(J10.8H_z)
C_{12}-H	1.91,m,H	2.1m,H
C_{13}-Hα	2.26,t,H(J11.7H_z)	2.25,t,H(12H_z)
C_{13}-Hβ	1.73,d,H(J11.7H_z)	1.73,H
C_{16}-H_3	1.38,3.3H	1.39,s,BH
C_{17}-H_3	0.86,d,3H(J6.7H_z)	0.83,d.3H(J6.8H_z)
C_{18}-H	7.12,q,H(J6.7H_z)	7.06,qH(J7.4H_z)
C_{19}-H_3	1.78,dd,H(J6.7,1.8H_z)	1.78,d,3H(6.8H_z)
-OH	3.05 - 3.1, 2H	3.2 - 3.3, H
C_{21}-H_3	-	2.15,s,3H

Table 8. ^{1}H NMR Chemical Shifts for CR-5, Acetylated CR-7 and 7-Acetylmadurensine

	δ (ppm)		
Proton	CR-5	Acetylated CR-7	7-Acetyl-modurensine (95)
C_2-H	6.2,s,H	6.2,s,H	6.2,H
C_3-Hα	3.92,d,H(J11.7H_z)	4.09,d,H (J12.6H_z)	
-Hβ	3.85,m,H	3.92,m,H	
C_5-Hα	3.49,dd,H(J16.2, 2H_z)	3.45,dd,H (J2H_z)	
-Hβ	2.85,d,H(J14.4H_z)	2.85,d,H (J12.8H_z)	2.84,H(J14.6H_z)
C_6-H	5.23,t,H(J4.2H_z)	5.23,t,H (J2H_z)	5.3,H
C_7-H	5.35,m,H	5.35,m,H	
C_8-H	4.45,m,H	4.45,m,H	
C_9-Hα	5.6,d,H(J10,8H_z)	5.6d,H(J8H_z)	
-Hβ	3.95,d,H(J10,8H_z)	3.95,d,H (J8H_z)	
C_{12}-H	2.1m,H	2.1m,H	
C_{13}-Hα	2.25,t,H(J12H_z)	2.25,t,H (J9H_z)	
-Hβ	1.73,H	1.72,m,H	
C_{16}-H_3	1.39,s,3H	1.39,s,3H	1.4,3H
C_{17}-H_3	0.83,d,3H(J6.8H_z)	0.82,d,3H (J5.4H_z)	0.83,3H
C_{18}-H	7.06,q,H(J7.4H_z)	7.06,q,H (J5H_z)	7.08,H
C_{19}-H_3	1.78,d,3H(6.8H_z)	1.78,d,3H (J5H_z)	1.79,3H
C_{21}-H_3	2.15,s,3H	2.15,s,3H	
OH	3.2 - 3.3	3.6 - 3.7	

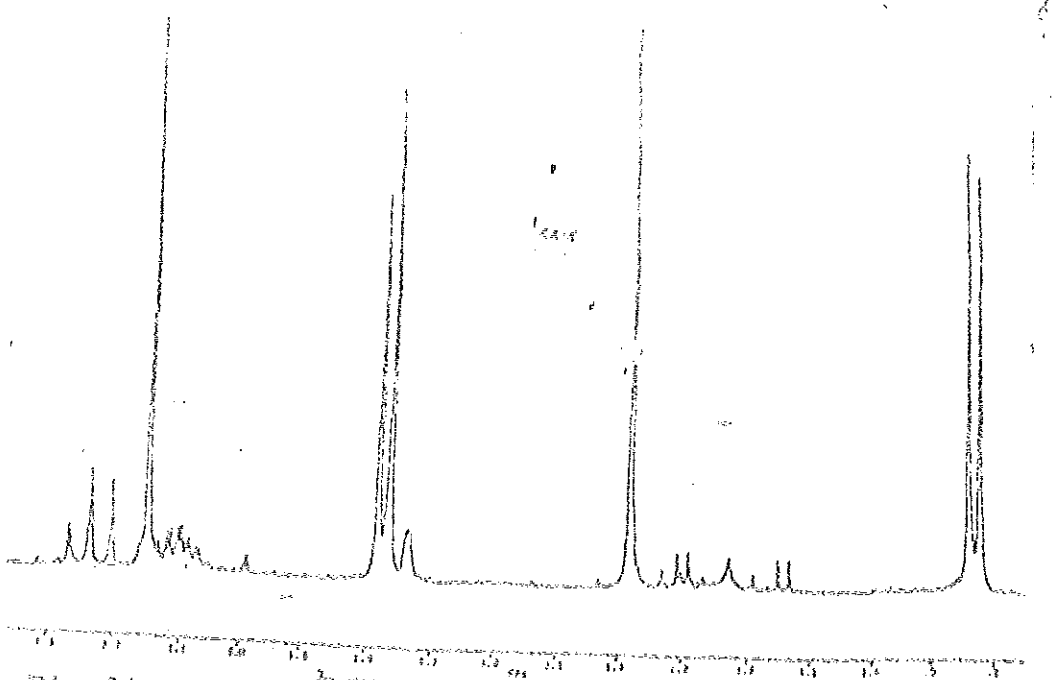


Fig. 14c ^{1}H NMR Spectrum of GR-9 (0.8 - 8.5)

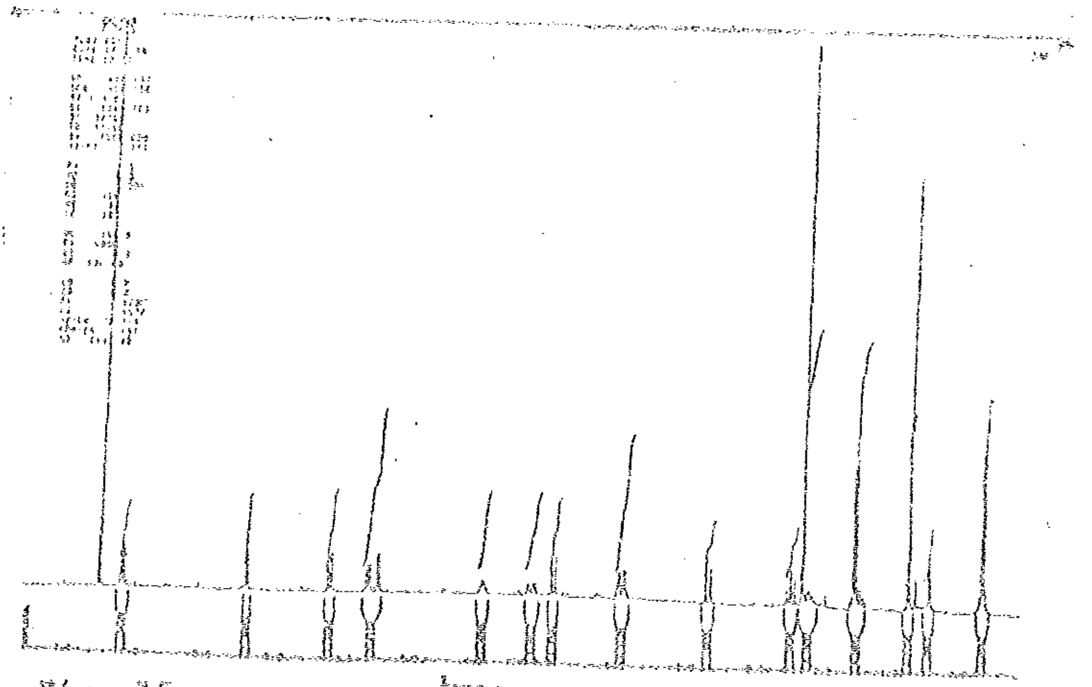


Fig. 15 ^{13}CNMR Spectrum of Acetylated GR-7

Table 9. ^{13}C NMR Chemical Shift of CR-5

Carbon number	δ, ppm
1	135.37
2	136.06
3	66.51
5	61.08
6	74.45
7	76.6
8	71.51
9	58.92
10	178.02
11	75.9
12	39.62
13	27.18
14	131.79
15	167.28
16	24.28
17	10.63
18	142.09
19	14.97
20	170.33
21	20.78

4. CONCLUSION

There has been a tremendous growth in interest in pyrrolizidine alkaloids due to their increasing economic importance. Crotalaria species are one possible source of pyrrolizidine alkaloids. Out of 85 species of Crotalaria found in Ethiopia only 10 of them were studied. This is the first project to study plants of these species in the country. This project which is based on the study of the chemical composition of Crotalaria rosenii will enable to develop methods for the study of chemical composition of these species. Hence it opens the way for further investigations.

The alkaloidal composition of the flowers, leaves and seeds of C. rosenii have been investigated. It has been found that nine alkaloids were found in the flowers and out of these nine two were detected in the seeds and three in the leaves in different amounts. There was also seasonal variation in the number and type of alkaloids found in the leaves. This is evident from the detection of the fourth alkaloid in the sample collected in November which is absent from that collected in Feburary.

The structures of the compounds isolated were determined chemically and spectroscopically. The use of new spectroscopic techniques such as 2-dimensional NMR homo and heteronuclear shift correlated spectra and access to high field instruments have enabled to assign the chemical shifts of the crotanecine ester alkaloids and to point out some errors in the litrature.

The three alkaloids isolated from Crotalaria rosenii have previously been isolated from Crotalaria agatiflora. These two species are morphologically very similar differing from one another by the size of the petiole and shape of the beak of the keel. They are also chemically similar in containing three identical alkaloidal which were isolated and characterized from both plants. Four of the eight alkaloids isolated from C. agatiflora are isomers but the nine alkaloids detected from C. rosenii are not isomers as observed on t.l.c. so the isolation and characterization of these alkaloids may possibly show the difference in chemical compositons of these two plants.

5. EXPERIMENTAL

Melting points were determined on hot stage Bockmonoscope 'M' melting point apparatus. Infrared spectra were determined in KBr pellets using a Perkin Elmer model 727 B grating spectrometer. Optical rotations were measured in chloroform on Perkin Elmer 241 polarimeter. The nuclear magnetic resonance spectra measurements were performed for 1.5 M $CDCl_3$ solutions in 5 mm NMR tubes on a Bruker AM-400 spectrometer. The chemical shifts were referenced to the deuterochloroform signal (δ=7.24 for ^1H and δ=77.0 for ^{13}C). Mass spectra were recorded on a Varian CH-5 and CH-7 spectrometer.

Analytical thin layer chromatography (t.l.c) were run on a 0.25 mm thick layer silica gel GF 254 (Merck) and the products were detected by their ultraviolet fluorescence and Dragendorff's spray reagent Munier modification. Preparative thin layer chromatography were run on 1.0 mm thick layer of silica gel G(type 60) and the products were detected by their ultraviolet fluorescence. Column chromatography were performed on silica gel 60 (70-230 Mesh) (Merck).

5.1 Extraction of the Leaves of Crotalaria rosenii (102, 103)

340 grams of powdered leaves of Crotalaria rosenii was defatted with petroleium ether (60-80°C)

in a soxhlet apparatus for 12 hrs. Defatted plant material was extracted with methanol in a soxhlet apparatus for 48 hrs. The methanolic extract was distilled at atmospheric pressure giving 39.95 gms (11.6%) crude extract (GA-1).

5.2 Purification of GA-1

GA-1 was dissolved in 250 ml of 2.5% HCl and filtered. The filterate was extracted with 600 ml of chloroform. The aqueous layer was made alkaline (ammonia) and extracted with 500 ml of dichloromethane. The organic layer was dried with anhydrous sodium sulfate. Evaporation of the organic layer gave 1.487 grams (0.43%) of raw extract (GA-2).

5.3 Column Chromatography of GA-2

1.45 gms of GA-2 dissolved in 5 ml of chloroform was applied to a 2.5 cm diameter column packed with 85 grams of silica gel. The column was eluted with 300 ml of methanol-dichloromethane (4:1) (50 ml fractions were collected). Evaporation of the solvent under reduced pressure gave 100 mgs of brown crystals. Dissolving the crystals in acetone and repeated filteration through a micro-pipette gave 20 mgs (0.006%) of colourless alkaloid crystals (CR-1). It gave a single spot (R_f 0.72, chloroform: Methanol (85:15) on t.l.c, m. pt 157-160 (decomp.) The mass spectrum showed a molecular ion peak at m/z 393.

5.4 Extraction of Flowers of *Crotalaria rosenii* (102, 103)

400 gms of powdered flowers of *Crotalaria rosenii* were defatted with petroleum ether (60-80°C) in a soxhlet apparatus for 12 hrs. Defatted plant material was extracted with methanol in a soxhlet apparatus for 48 hrs. The methanolic extract was concentrated by distilling under reduced pressure giving 155 gms (38.7%) of crude extract (GA-3).

5.5 Purification of GA-3

GA-3 was dissolved in 900 ml of 2.5% HCl and filtered. The filtrate was extracted with 1000 ml chloroform. The aqueous layer was basified with ammonia and extracted with 800 ml of dichloromethane. The organic layer was dried with anhydrous sodium sulfate. Evaporation of the organic layer gave 3.01 grams (0.75%) raw extract (GA-4).

5.6 Column Chromatography of GA-4

3 gms of GA-4 dissolved in 8 ml of chloroform was applied to a 2.5 cm diameter column packed with 100 grams of silica gel. The column was first eluted with chloroform and then with chloroform-methanol mixtures. Chloroform containing 3% methanol eluted 35 mg of brown (mass) (GA-5). Chloroform containing

5% methanol eluted 250 mgs of light brown crystals (GA-6). Chloroform containing 30% methanol eluted 250 mg of crystal (GA-7).

5.7 Purification and Characterization of GA-5

35 mgs of GA-5 was applied to a micro-column packed with silica gel. The column was eluted with chloroform containing 20% methanol and gave 15 mg (0.004%) of colourless alkaloid crystals (CR-5). It gave single spot (R_f = 0.73, chloroform methanol (85:15) R_f = 0.85, chloroform: Methanol: Ammonia (85:14:1) on t.l.c, m. pt 158-160° (decomposition) which is the same as that of CR-1.

5.7.1 Mass Spectrum

High resolution mass spectrum gave molecular formula of $C_{20}H_{27}NO_7$ (393.1787) with major fragment ions at m/z 350.1843, 349.1888, 306.1714, 196, 194, 179, 178, 153, 137, 136, 135, 94, 93, and 80.

5.7.2 Nuclear Magnetic Resonance Spectrum

The ^1H nmr spectrum showed signals (δ,ppm):- 0.83 (d,3H), 1.39 (S,3H), 1.78 (d,3H), 2.1 (m,H), 2.25 (t,H), 2.85 (d,H), 3.49 (dd,H), 3.85 (m,H), 4.06 (dd,H), 5.23 (t,H), 5.6 (d,H), 6.2 (S,H), 7,06 (q,H).

^{13}CNMR assignments were made from the sequences in the DEPT spectrum (Table 9).

5.7.3 Infrared Spectrum

The IR spectrum showed absorptions at 1740 cm^{-1} and 3550 cm^{-1}.

5.7.4 Optical Rotation (C=0.2, Chloroform)

λ	589	578	546	436	365
$[\alpha]^{22}$	+66.5	+68.7	+79°	+138.8	+206.7

5.7.5 Circular Dichroism (λ(nm),$\Delta\varepsilon$)

Acetonitrile:- +195.5 (+5.78), +213 (-2.65), +217 (-2.9), +219.6 (-2.98), 221.6 (-2.96), +224.2 (-2.94), +245 (+0.48), +247.9 (+0.540).

5.8 Purification and Characterization of GA-6

Preparative thin layer chromatography of GA-6 was run with chloroform containing 15% methanol giving 110 mgs (0.027%) of alkaloid component (CR-7). It gave a single spot (R_f = 0.52, chloroform.methanol (85:15) R_f = 0.76, chloroform; Methanol: Ammonia (85:14:1) on t.l.c., m. pt 174-175°C (decomposition).

5.8.1 Mass Spectrum

High resolution mass spectrum gave molecular formula of C_{18} C_{25} NO_6 (m/z 351.087 with major fragment ions at m/e 307.1788, 264.1843, 262.1715, 236, 154, 152, 137, 136, 135, 95, 94, 93, and 80.

5.8.2 Nuclear Magnetic Resonance Spectrum

The ^{1}HNMR spectrum showed signals (δ, ppm):- 0.86 (d,3H), 1.38 (s,3H), 1.73 (d,H), 1.78 (dd,3H), 1.91 (m,H), 2.26 (t,H), 2.73 (d,H), 3.45 (dddd,H), 4.02 (dm,H), 4.26 (m,H), 4.98 (t,H), 5.81 (d,H), 6.2 (s,H), 7.12 (q,H).

^{13}C NMR assignments were made from the sequences in the DEPT spectrum and hetero nuclear spin correlation. (Table 3).

5.8.3 Infrared Spectrum

The IR spectrum showed strong absorptions at 1720 cm^{-1} and 3420 cm^{-1}.

5.8.4 Optical Rotation (C = 0.2, Chloroform)

λ	589	578	546	436	365
$[\alpha]^{22^\circ}$	+190°	+17°	+21°	+40°	+74°

5.8.5 Circular Dichroism ((nm),)

Acetonitrile:- +195.4 (+8.04), +22.32 (-2.72), +253 (0.42), +283.9 (-0.019), +285.9 (-0.022).

5.9 Purification and Characterization of GA-7

Solid crystals of GA-7 were dissolved in chloroform and filtered through a micro-pipette to give 150 mg (0.038/) of colourless crystaline alkaloid (CR-9). It gave a single spot (R_f = 0.29, chloroform: methanol (85:15) R_f = 0.5, chloroform: Methanol: Ammonia (85:14:1) on t.l.c, m. pt 178-181°C (decomp.).

4.9.1 Mass Spectrum

High resolution mass spectrum gave molecular formula of $C_{18}H_{25}NO_7$ (367.1631) with major fragment ions at m/z 154, 152, 137, 136, 135, 95, 94, 93, and 80.

5.9.2 Nuclear Magnetic Spectrum

The ^{1}HNMR spectrum showed signals (δ, ppm):- 0.81 (d,3H), 1.78 (d,3H), 1.86 (m,H), 1.98 (m,H). 2.29 (t,H), 2.78 (d,H), 3.46 (ddd,H), 3.75 (dd,2H), 4.04 (dm,H), 4.28 (m,H), 4.56 (m,H), 4.99 (t,H), 5.83 (d,H), 3.19 (s,H) 7.14 (q,H).

^{13}C NMR assignments were made from the sequences in the DEPT spectrum and hetero nuclear spin correlation (Table 6).

5.9.3 Infrared Spectrum

The IR spectrum showed absorptions at 1730 cm^{-1} and 3570 cm^{-1}.

5.9.4 Optical Rotation (C = 0.2, Chloroform)

λ	589	578	546	436	365
$[\alpha]^{22}$	+32	+34.5	+39	+73.5	+128.5

5.9.5 Circular Dichroism (λ(nm), $\Delta\varepsilon$)

Acetonitrile:- +195.6 (+5.02), +199.8 (+4.767), +226.2 (-2.023), +253.2 (+0.5), +284.7 (-0.016), +286.8 (-0.016), +289.6 (-0.015), +299.4 (-0.005).

5.10 Acetylation of CR-7

A mixture of 10 mg of CR-7, 1 ml of acetic anhydride and 2 drops of Pyridine was stirred with a magnetic stirrer at room temperature for 3 days. 10 ml of ice water was added and the solution stirred for 2 hrs. The solution was neutralized with 25 ml of saturated $NaHCO_3$ solution and extracted with 100 ml of dichloromethane. The organic layer was dried with anhydrous sodium-sulfate. The solvent was evaporated to give 8 mg (80%) of acetylated product (AC-CR-7). It gave a single spot (R_f = 0.72, chloroform: methanol (85:15)) which is the same as the R_f value for CR-5 on t.l.c.

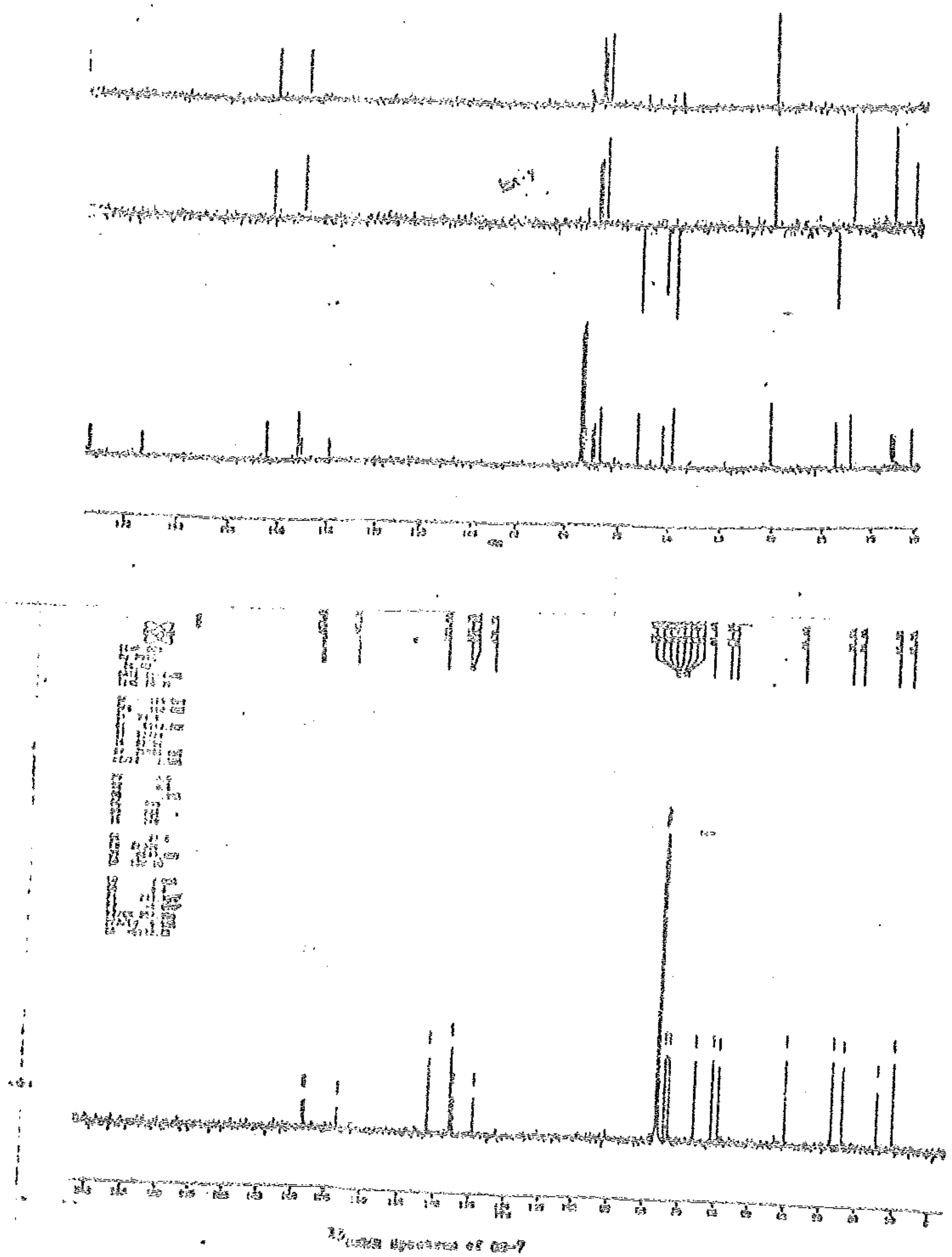
5.10.1 Mass Spectrum

Mass spectrum gave molecular formula of $C_{20}H_{27}NO_7$(393) with major fragment ions at m/e 350, 349, 306, 196, 179, 178, 153, 137, 136, 135, 94, 93, and 80.

5.10.2 Nuclear Magnetic Resonance

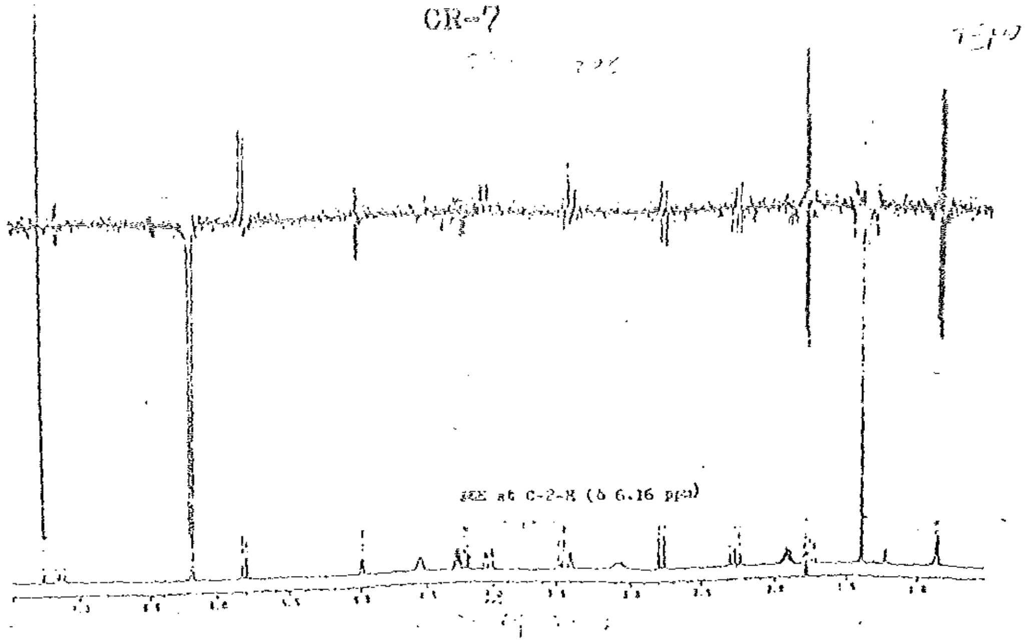
The ^{1}HNMR spectrum showed signals (δ, ppm):- 0.83 (d,3H), 139 (s,3H), 1.78 (d,3H), 2.15 (m,H), 2.2 (t,H), 2.85 (d,H), 3.45 (dd,H), 3.95 (d,H), 4.09 (dd,H), 5.6 (d,H), 6.2 (s,H), 7.05 (q,H).

Appendix



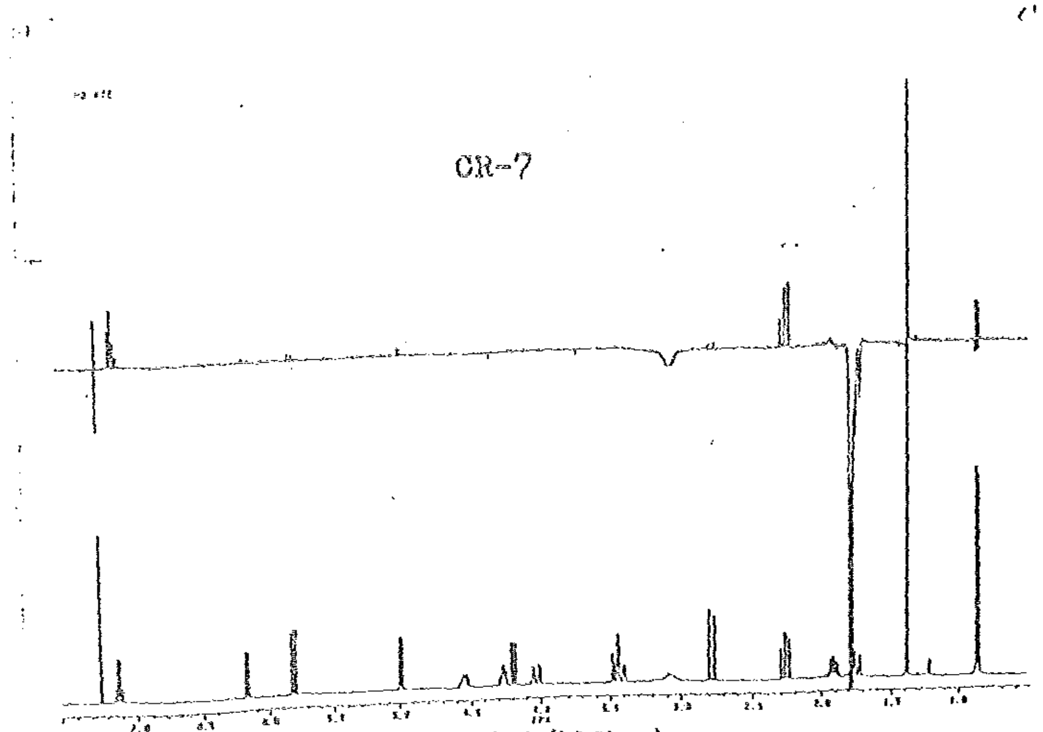
^{13}C-NMR Spectrum of GB-7

CR-7

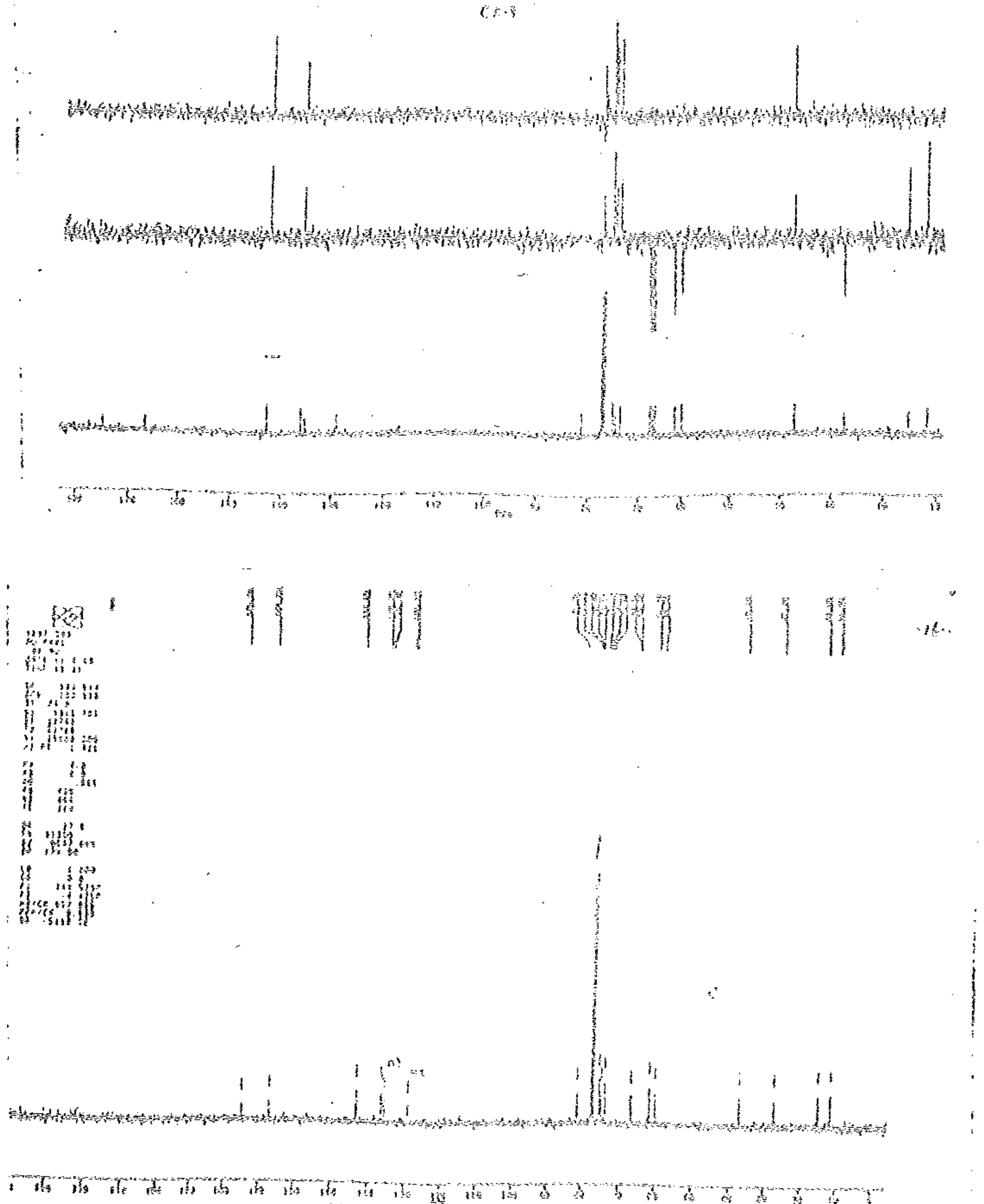


NOE at C-2-H (δ 6.16 ppm)

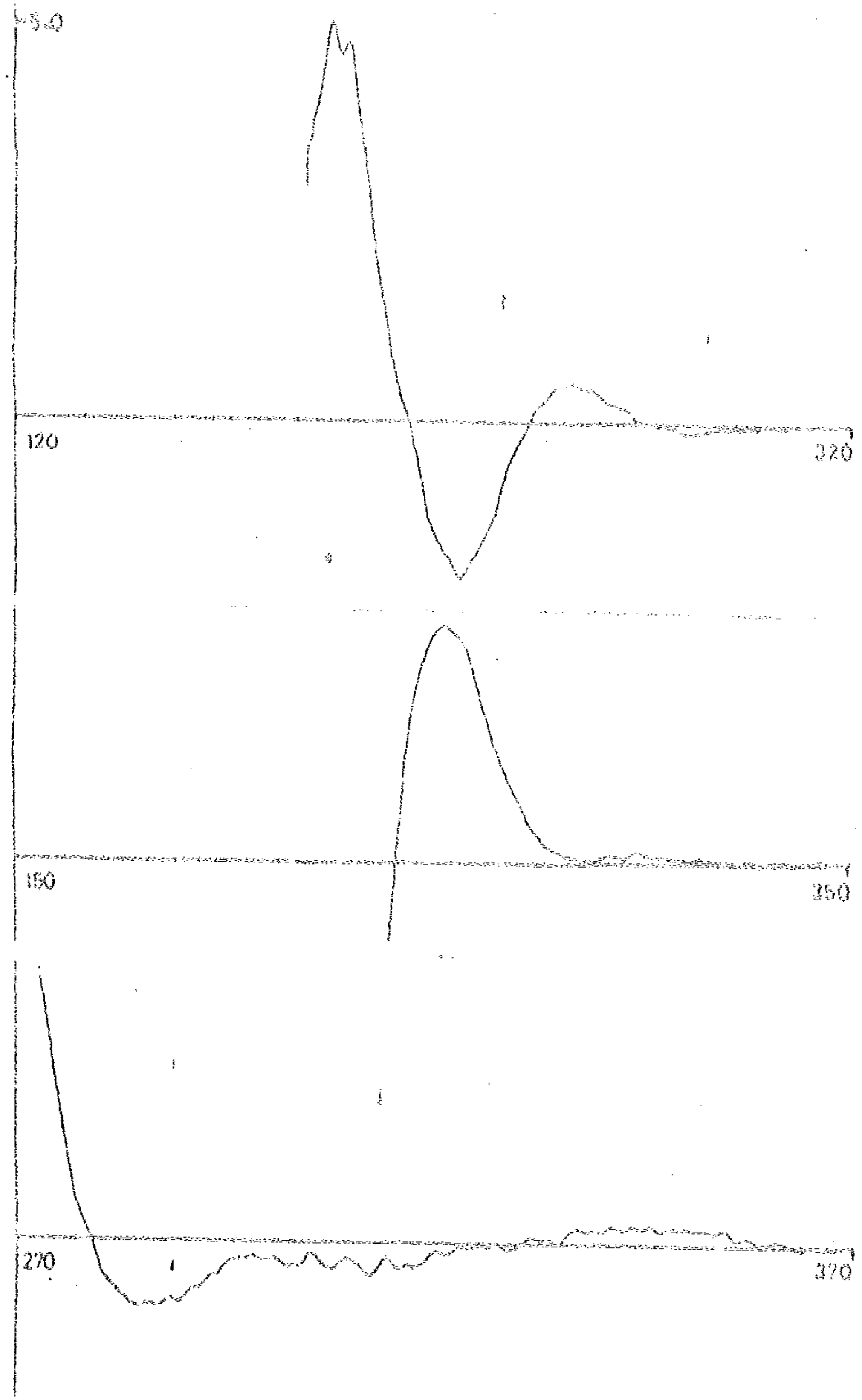
CR-7

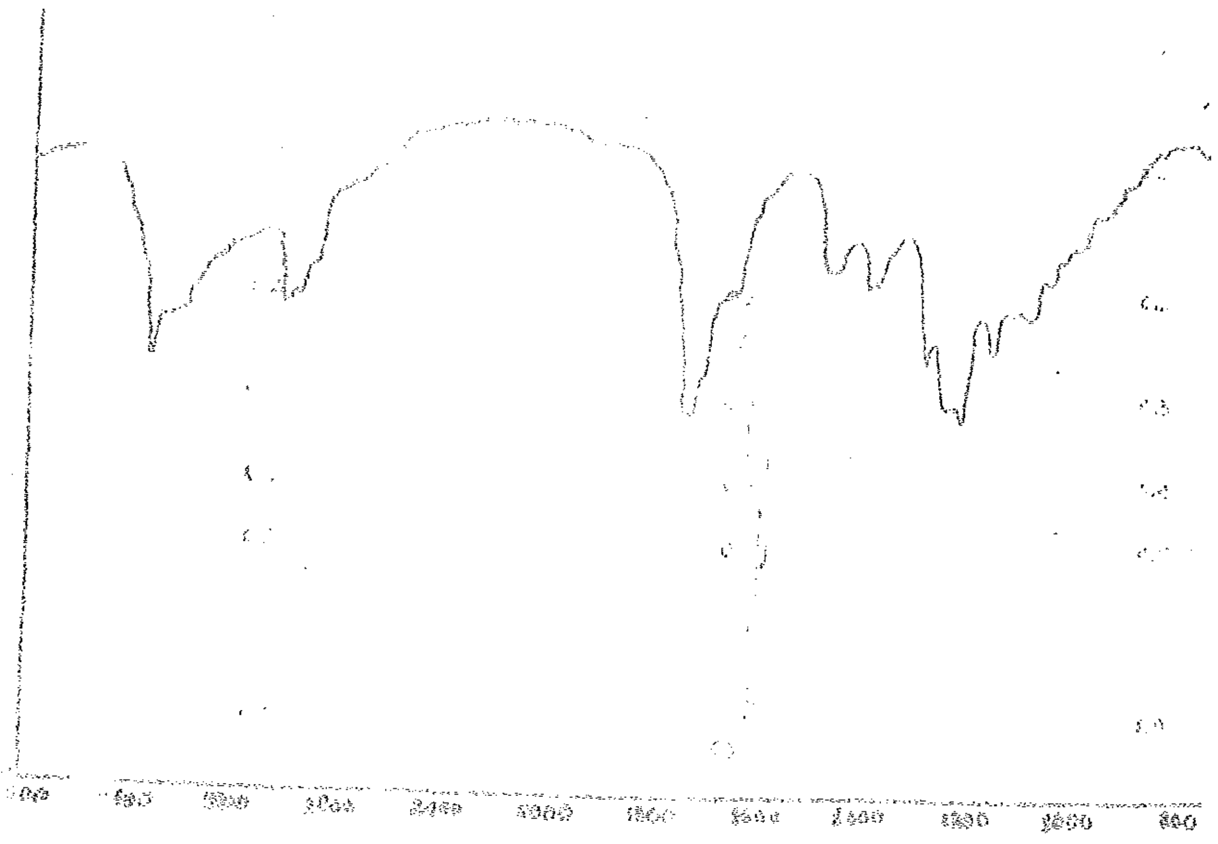


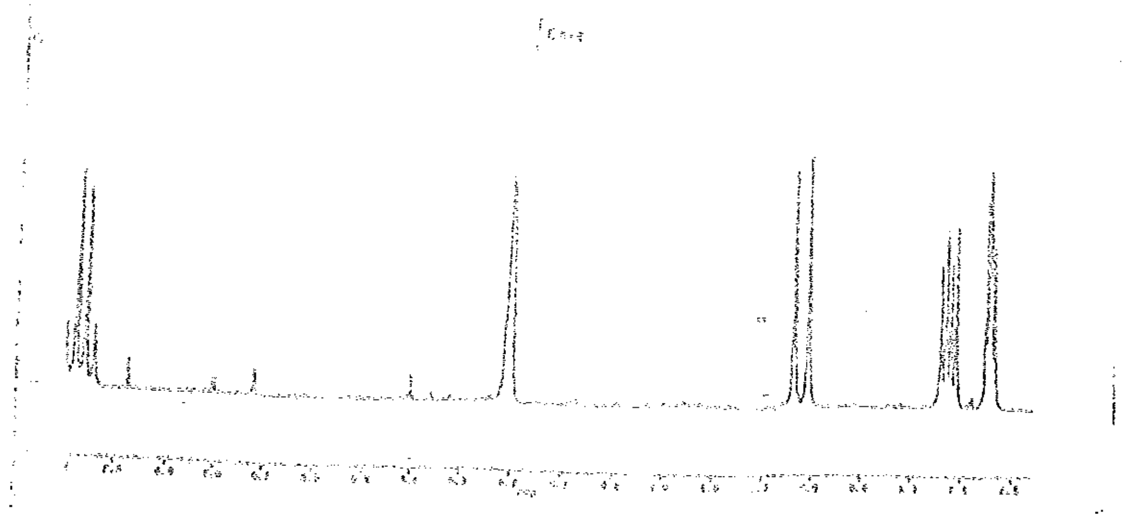
NOE at $C-19-H_3$ (δ 1.78 ppm)

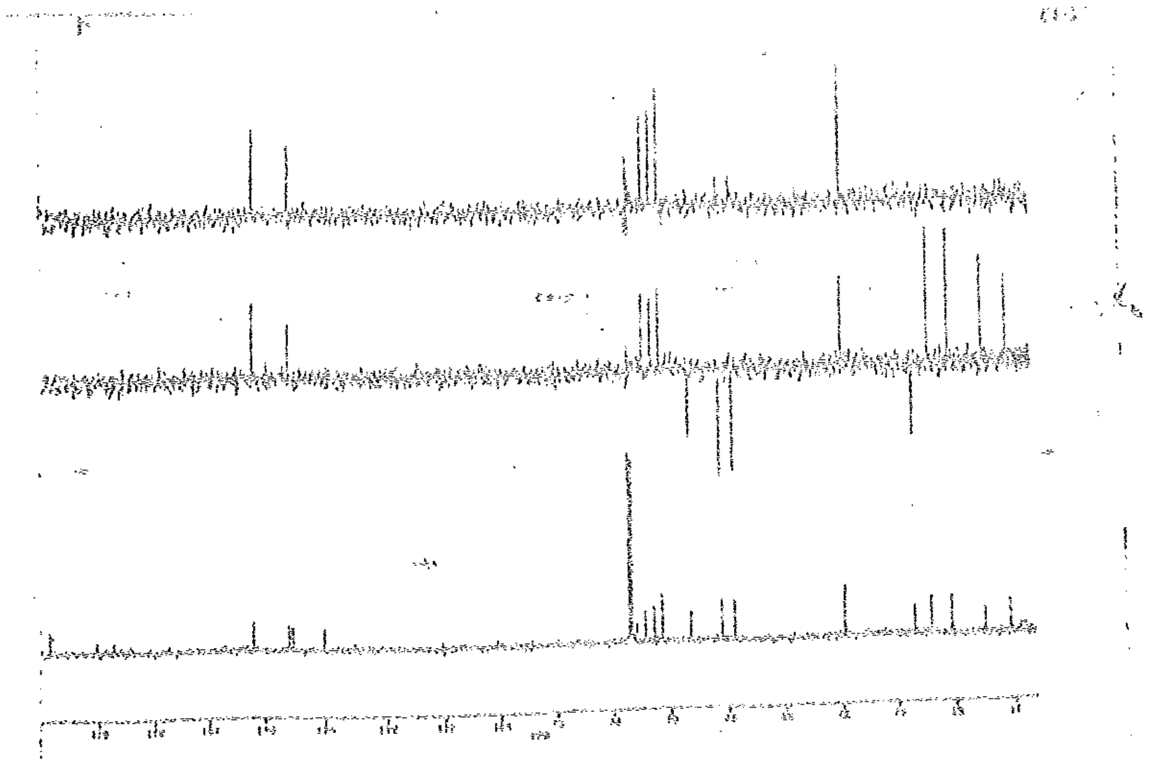


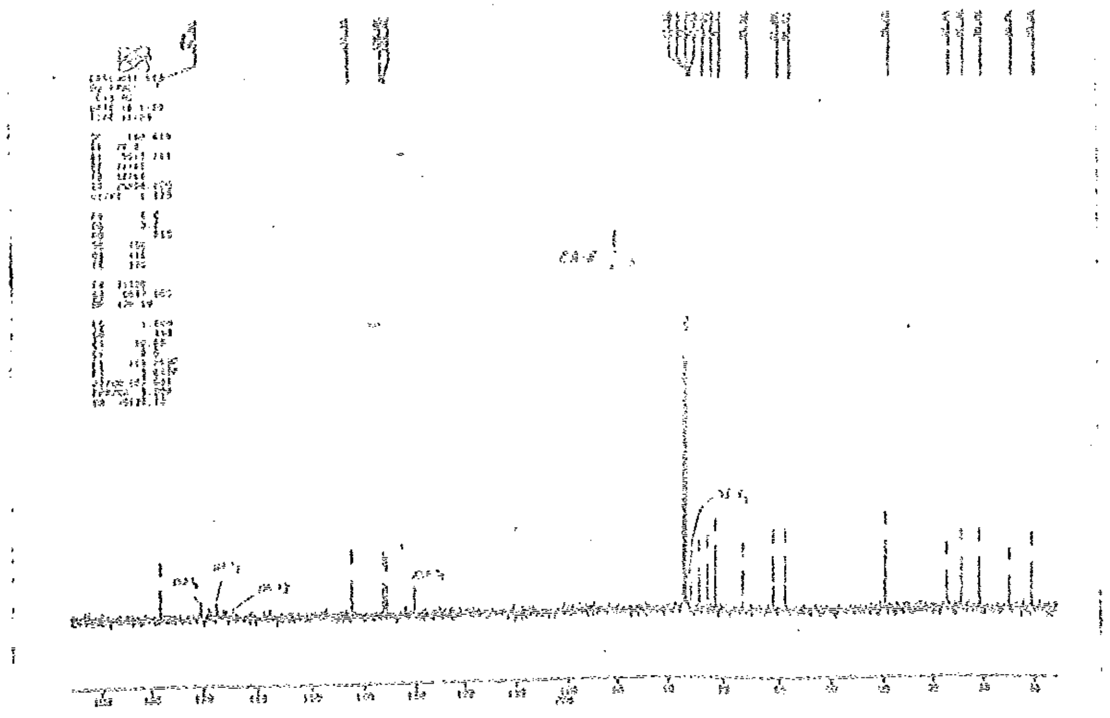
^{13}CNMR Spectrum of CE-3

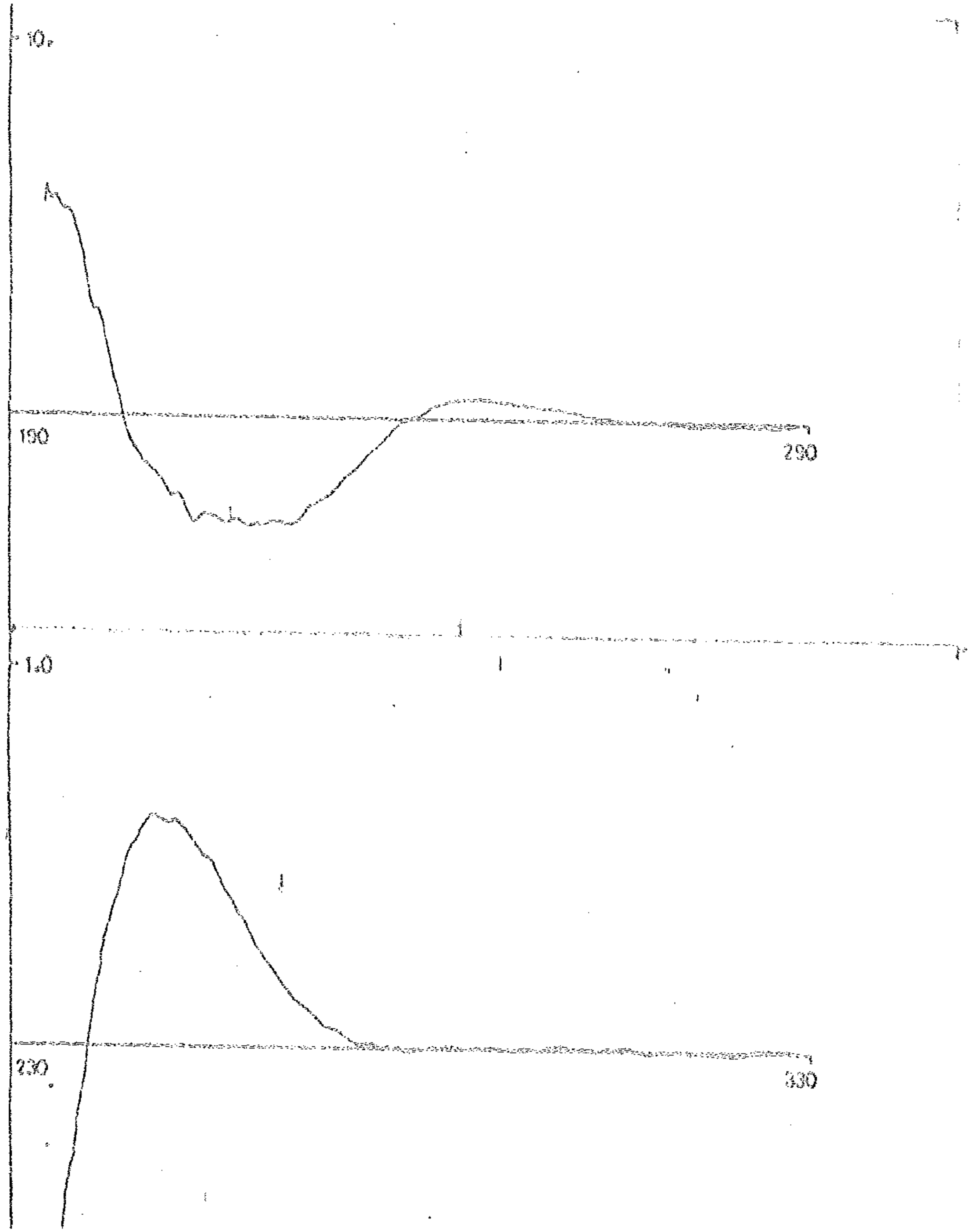

+5.0
120
320
150
350
270
370

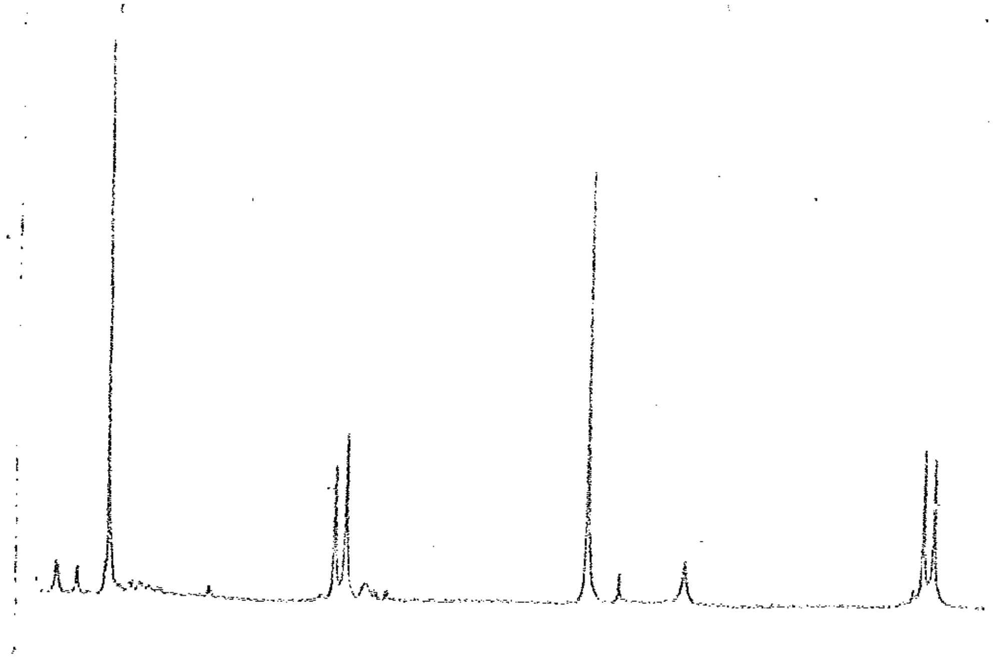

2400
2000
1800
1600
1400
1200
1000
800



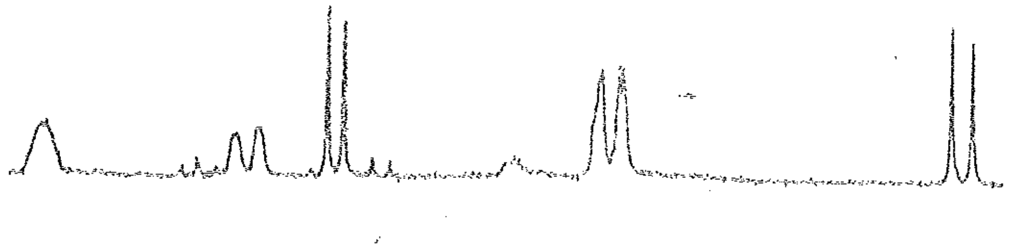




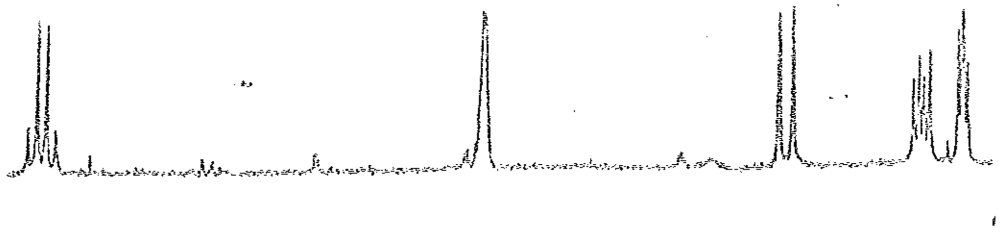

10
190
290
1.0
230
330



^{1}HNMR Spectrum of Acetylated CS-7 (δ0.8 - 2.3)



^{1}HNMR Spectrum of Acetylated CS-7 (δ 2.8 - 4.4)



^{1}HNMR Spectrum of Acetylated CS-7 (δ5.3 - 7.1)

REFERENCES

1. Sue Edwards, "Some wild Flowering plants of Ethiopia," Addis Ababa University Press 1976, p. 24.
2. M. Thulin, "Leguminosea of Ethiopia" Copenhagen, 1983, pp. 154-159.
3. H.D., B.N. Tandon, and N.C. Nayak; Indian J. Med. Res. 65. 679, (1977).
4. G.A. Swan, "An introduction to the Alkaloids" Black well scientific publication, 1967, p. 58.
5. P. Klasek, A.P. Sedmera, A. Boeva, and F. Santauy; Collect. Czech. Chem. Commum. 38, 2504 (1973).
6. P. Klasek, A.P. Sedmera, J. Vokun, A. Boeva, S. Dvorackova, and F. Santauy; Collect. Czech. Chem. Commun. 45, 548 (1980).
7. N.I. Nghia, P. Sedmera, A. Klasek, A. Boeva, L. Djanovska, L. Doleijs, and F. Santauy; Collect. Czech. Chem. Commun. 41, 2952 (1976).
8. H.S. Toleckli-Evans: Acta Crystallogr. Sect. B 36, 3150 (1980).
9. F.L. Warren: Fortshr. Chem. Org. Naturst. 12, 198 (1955).
10. F.L. Warren: Fortshr. Chem. Org. Naturst. 24, 329 (1966).
11. J.A. Edgar, N.J. Eggers, A.J.J. Ones, and G.B. Rusell: Tetrahedron Lett. 2657 (1980).

12. N.J. Egger, and G.J. Gainsfor: *Cryst. Struct. Commun.* *8*, 597 (1979).

13. G.J. Gainafor: *Cryst. Struct. Commun.* *9*, 173 (1980).

14. A. Kirfel, G. Will, H. Wilddonfeld, and E. Roder: *Cryst. Struct. Commun.* *9*, 353 (1980).

15. E. Roder, H. Wiedenfeld, and M. Frisse: *Phytochemistry*, *19*, 1275 (1980).

16. D.J. Robins: The Pyrrolizidine Alkaloids, *I. Fortschr. Chem. Org. Naturst*, *41*, 114 (1981).

17. A.J. Aosen, C.C.J. Culvenor, and L.W. Smith: *J. Org. Chem.* *34*, 4137 (1969).

18. F.L. Warren: The Pyrrolizidine Alkaloids, *I. Fortschr. Chem. Org. Naturst.* *12*, 198 (1955).

19. F.L. Warren: The Pyrrolizidine Alkaloids, *I. Fortschr. Chem. Org. Naturst.* *12*, 320 (1955).

20. R.S. Sawhney and C.K. Atal: *Indian J. Chem.* *11*, 88 (1973).

21. C.K. Atal, C.C.J. Culvenor, and L.W. Smith: *Tetrahedron Lett.* 537 (1966).

22. N.M. Davies, and D.H.G. Crout: *J. Chem. Soc., Perkin Trans I*, 2079 (1974).

23. H. Stockii-Evans, and D.H.G. Crout. *Helv. Chem. Acta* *59*, 2168 (1976).

24. C.K. Atal. R.K. Sharma, C.C.J. Culvenor, and L.W Smith: *Aust. J. Chem.* *19*, 2189 (1966).

25. N.S. Bhacca, and R.K. Sharma: Tetrahedron 24, 6319 (1968).

26. C.C.J. Culvenor and L.W. Smith: Tetrahedron Lett. 3603 (1969).

27. L.B. Bull and C.C.J. Culvenor: Aust. J. Chem. 21, 275, (1968).

28. C.C.J. Culvenor, M.L. Hefferman and W.G. Woods, Aust. J. Chem. 18, 1605 (1965).

29. C.C.J. Culvenor, L.W. Smith, and R.I. Willing: Chem. Commun. 65 (1970).

30. L.H. Zalkow, S. Bonetti, L. Gelbaum, M.M. Gordon: J. Natural Product, 42, 603 (1979).

31. R.J. Huxtable: Gen. Pharmacol. 10, 159 (1979).

32. C.C.J. Culvenor, A.T. Dann and A.T. Dick: Nature, 195, 570 (1962).

33. K.A., J.L. Seymour, and J.R. Allen: Cancer Res. 37, 3141 (1977).

34. G.A. Cordell and N.R. Fornsworth: Lioydia 40, 1 (1977).

35. J.S. Kovach, J.S., M.M. Ames, G. Powis, C.G. Molrtel, R.G. Hahan and E.T. Crolgon: Cancer Res. 39, 4540 (1979).

36. J.O. Dickinson, M.P. Cooke, R,R. King, and P.A. Mohamed: J. Am. Vet. Assoc. 169, 1192 (1976).

37. M.L. Deinzer and P.A. Thomson: Science, 195, 497 (1977).

38. E. Nowacki, and R.V. Byerrum: Life Science 1, 157 (1962).

39. C.A. Hughes, R.Letcher, and F.L. Warren: J. Chem. Soc. 4974 (1964).

40. N.M. Bale and D.H.G. Crout: Phytochemistry 14, 2617 (1975).

41. D.J. Robins and J.R. Sweenly: J. Chem. Soc. Chem. Commun. 120 (1979).

42. D.R. Dalton, "The Alkaloids A Biogenetic Approach" New York, 1979, pp 56-61.

43. J.A. Devlin and D.J. Robins: J. Chem. Soc., Perkin Trans. I 1329 (1984).

44. D.H.G. Crout, M.H, Benor, H. Imaaseki, and T.A. Geissman: Phytochemistry 5, 1 (1966).

45. D.H.G. Crout, N.M. Davies, E.H. Smith, and D. Whitehouse: J. Chem. Soc., Perkin Trans. I 671 (1972).

46. D.H.G. Crout, N.M. Davies, E.H. Smith, and D. Whitehouse: Chem. Commun 635 (1970).

47. D.H.G. Crout: J. Chem. Soc. (C) 1968 (1966).

48. D.H.G. Crout: J. Chem. Soc. (C) 1233 (1967).

49. B.A. MC. Gaw and J.G. Wolly: Phytochemistry 18, 1647 (1979).

50. D.J. Robins, N.M. Bale, and O,H.G. Crout: J. Chem. Soc. Perkin Trans. I 2082 (1974).

51. T.R. Rajagapolam and V. Botra: *Indian J. Chem. 15B.* 494 (1977).

52. L.H. Zalkow, L. Gebaum and E. Klinan: *Phytochemistry* *17*, 172 (1978)

53. C.C.J. Culuenor: *Aust. J. Chem.* *7*, 287 (1954).

54. R.B. Bradbury and C.C.J. Culvenor: *Aust. J. Chem.* *7*, 378 (1954).

55. A. Ulubelen and F. Ocal: *Phytochemistry* *16*, 499 (1977).

56. H.J. Huizing, and T.M. Malingre: *J. Chromatogr.* *173*, 187 (1979).

57. H.J. Huizing, and T.M. Malingre: *J. Chromatogr.* *205*, 218 (1981).

58. R.J. Molyneux, and J.N. Roctman: *J. Chromatogr.* *195*, 412 (1980).

59. G.P. Dimenna, T.P. Krick, and H.J. Segall: *J. Chromotogr.* *192*, 474 (1980).

60. C.W. Oualls, and H.J. Segall: *J. Chromatogr.* *150*, 202 (1978).

61. G.H. Tittel, and H. Wagner: *Planta Med.* *37*, 1 (1979).

62. A.H. Chalmers and C.C.J. Culvenor: *J. Chromatogr.* *20*, 270 (1965).

63. M.T. Hikichi, and Y. Iitaka: *Tetrahedron Lett.* 767 (1978).

64. G.I. Birnbaum: *J. Am. Chem. Soc.* *96*, 6165 (1974).

15, 455 (1977).

79. T.R. Rajagopolan, and V. Batra: Indian J. Chem. 13, 835 (1975).

80. C.K. Atal, C.C.J. Culvenor and L.W. Smith: Aust. J. Chem. 22, 1773 (1969.

81. C.C.J. Culvenor and L.W. Smith: Aust. J. Chem. 19, 2127 (1966).

82. S.C. Puri, R.S. Sawhey and C.K. Atal J. Indian Chem. Soc. 51, 628 (1974).

83. T.R. Rajagopolan and V. Batra: Indian J. Chem. Sec. B 15, 455 (1977).

84. C.C.J. Culvenor, and L.W. Smith: Aust. J. Chem. 21, 2027 (1968).

85. A.K. Lasek, O. Sedmera, Collect. Czech. Chem. Commun. 36, 956 (1970).

86. R.S. Sawhrey, R.N. Girotra, C.K. Atal, C.C.J. Culvenor, and L.W. Smith: Indian J. Chem. 5, 655 (1967).

87. R.N. Gandhi and T.R. Seshadri: Curr. Sci. 37, 285 (1968).

88. O.P. Suri and C.K. Atal Indian J. Chem. Sec. B 16, 1132 (1978).

89. C.C.J. Culvenor and L.N. Smith: Tetrahedron Lett. 3603 (1969).

90. A.V. Daniloua, and L.M. Utkin: Zh. Obshch. Khim 30, 345 (1960).

91. K.A. Suri and R.S. Sawhney: Indian J. Pharm. 37, 69 (1975).

92. O.P. Suri, M.S. Bhatia, and C.K. Atal, Phytochemistry 15, 1061 (1976).

93. R.S. Sawhney and C.K. Atal, J. Indian Chem. Soc. 47, 741 (1970).

94. D.H.G. Crout: J. Chem. Soc., Perkin I. 13, 1602 (1972).

95. C.C.J. Culvenor and L.W. Smith: Anales De Quim. 68. 883 (1972).

96. S.E. Drewes, I. Antonwitz, and P. T. Kaye: J. Chem. Soc., Perkin Trans. I 287, (1981).

97. H. Wiedenfeld: Phytochemistry 21, 2767 (1982).

98. N.V. Mody, R.S. Sawhney and S.W. Pettetier: J. Natural Products 42, (4), 417 (1979).

99. J. Hrbek, L. Hruban, A. Klasek, N.K. Kochetkou, A.M. Likhoshestou and G. Snatzke: Coll. Czech. Chem. Commun. 37, 3918 (1972).

100. C.K. Atal, R.S. Sawhney, C.C.J. Culvenor and L.W. Smith: Tetrahedron Lett 54, 5605 (1968).

101. M.J. Pestchanker, M.S. Ascheri and O.S. Geordano, Planta Med. 166 (1985).

102. H. Wiedenfeld, E. Roeder: Phytochemistry 18, 1083 (1979).

103. H. Wiedenfeld, E. Roeders and E. Anders: Phytochemistry, 24, 376 (1983).

DECLARATION

I, the undersigned, declare that this thesis is my work and that all sources of material used for the thesis have been duly acknowledged.

Signature ____________________

Place and Date of Submission: Chemistry Department,
Addis Ababa University
June 1986