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MACROCYCLIC PYRROLIZIDINE ALKALOIDS OF *CROTALARIA ROSENII*

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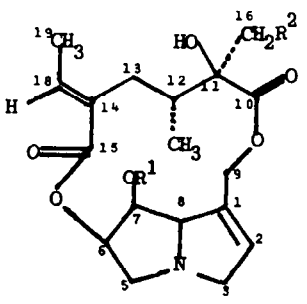
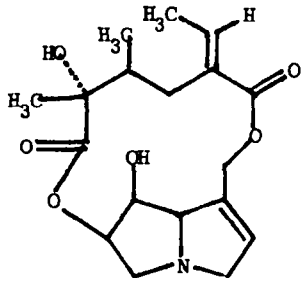
Abstract - Three macrocyclic pyrrolizidine alkaloids have been isolated from the flowers of *Crotalaria rosenii*. Two of them , madurensine 1 and 7-acetylmadurensine 2 are rare but known alkaloids for which full spectroscopic data have been generated and some erroneous ^{13}CNMR data in the literature corrected. Crotaflorine 3, an alkaloid previously given a "probable" structure has been subjected to detailed spectroscopic analysis and shown to have structure 3. The recently introduced "COLOC" NMR technique has been used to distinguish between the two alternative structures 1 and 4.

The genus *Crotalaria* represents some 600 species throughout the tropics and subtropics of which 500 species occur in Africa[1]. In Ethiopia the genus is represented by 85 species[1] of which only 10 have been subjected to varying degrees of chemical investigation. *Crotalaria rosenii* is one of the 15 endemic species and is reported to be one of the toxic plants[2] causing livestock poisoning. We have recently examined the various parts of the plant and identified the macrocyclic pyrrolizidine alkaloids madurensine 1, 7-acetylmadurensine 2 and crotaflorine 3. The first two alkaloids are rather rare and have been found only in *C. madurensis* and *C. agatiflora*[3,4]. The structures of these two alkaloids were determined on the basis of IR, MS and ^{1}HNMR data only. We have now conducted detailed NMR studies including ^{1}H-^{1}H and ^{13}C-^{1}H shift correlated as well as NOE spectra which have enabled us to make unequivocal assignments of the proton and carbon chem ical shifts and also to revise some erroneous assignements made recently by other workers[5].

The third alkaloid, crotaflorine 3, has been isolated from *C. agatiflora* by Culvenor and Smith[6] as one of eight alkaloids and a "probable structure" assigned to it on the basis of partial NMR and MS data. We have also subjected this compound to rigorous NMR spectroscopic studies and confirmed the structure to be 3.





4

1 R^1 = R^2 = H

2 R^1 = Ac, R^2 = H ; 3 R^1=H, R^2=OH

The application of the recently introduced "COLOC" NMR technique (correlation spectroscopy via long range couplings), which is used to investigate small-range heteronuclear couplings, has also been used to establish the mode of attachment of the esterifying diacid to the crotanecine base as shown in structure 1 and not as in 4.

RESULTS AND DISCUSSION

Alkaloid 1 showed m.p. 174–175°C and a molecular formula of $C_{18}H_{25}NO_6$ (HRMS 351.1687). The proton NMR spectra at 60 and 90 MHz were not sufficiently resolved to allow complete assignments of the various protons. The 400 MHz spectrum, however showed clearly all the protons (Fig.1) and the suggested assignments in Table 1 were confirmed by the ^1H–^1H shift correlated studies (Fig.2).

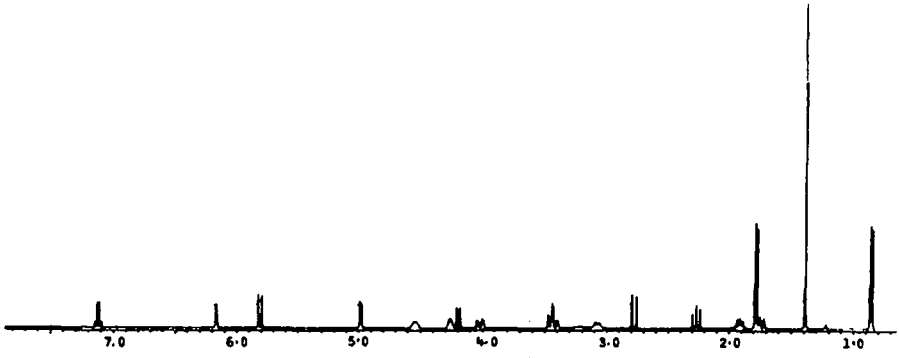


Fig.1 ^{1}HNMR spectrum of madurensine 1 at 400 MHz in $CDCl_3$

Table 1 ^{1}HNMR (400 MHz, $CDCl_3$, δ TMS = 0 ppm) chemical shifts of 1,2 and 3

Proton	1	2	3
C2-H	6.17 b	6.20 b	6.19 b
C3-H_α	4.02 dm, (J=14.4)	3.92 d(J=11.7)	4.04 dm (J=16.8)
-H_β	3.42 dd, (J=14.4)	3.85 m	3.44 dd(J=14.7,3.3)
C5-H_α	3.45 dddd(J=14.4, 6.2)	3.49 dd (J=16.2,2)	3.46 dddd(J=14.4,2)
-H_β	2.77 d(J=14.4)	2.85 d(J=14.4)	2.78 d(J=14.7)
C6-H_α	4.98 t(J=2.36)	5.23 t (J=4.2)	4.99 t (J=2.7)
C7-H_α	4.55 m	5.35 m	4.56 m
C8-H_α	4.26 m	4.45 m	4.28 m
C9-H	5.81 d(J=10.3)	5.60 d (J=10.8)	5.83 d(J=11.5)
-H	4.19 d(J=10.3)	3.95 d(J=10.8)	4.25 d(J=11.4)
C12-H	1.91 m	2.10 m	1.98 m
C13-H_a	1.73 d(J=11.7)	1.73 d(J=11.3)	1.73 d(J=12.3)
-H_b	2.26 t(J=11.7)	2.25 t (J=12.0)	2.29 t (J=12.3)
C16-Me	1.38 s	1.39 s	—
C16-CH_2	—	—	3.75 dd(J=21.6,10)
C17-Me	0.86 d(J=6.7)	0.83 d(J=6.8)	0.81 d(J=8.6)
C18-H	7.12 q(J=6.7)	7.06 q(J=6.8)	7.14 q(J=8.6)
C19-Me	1.28 dd(J=6.7, 1.8)	1.78 d(J=7.4)	1.78 d(J=7.9)
-OH	3.05-3.1	3.2-3.3	3.0-3.1
C21-Me	—	2.15 s	—

NOE experiments were also done on 1 to establish the stereochemistry of the necine base at C-6, C-7 and C-8. Irradiation of C7-H_a (4.54 ppm) showed NOE enhancement of C5-H_a, C6-H and C8-H protons, which requires that these protons be on the same side of C7-H_a. NOE experiments also confirm the configuration of the ethylidene group of the esterifying acid. Irradiation of C18-H

(7.12 ppm) showed NOE enhancement of C19-Me but not of the C13-protons. This is consistent with the location of the C18-proton away from the C13-hydrogens as would be expected in the trans configuration. The position of the quartet observed at lower field than expected for the C18-H is due to the anisotropic effect of the C15-carbonyl group. Further NOE rxperiments with 1 proved the configuration at C11 and C12 as well as the conformation of the diester part in the macrocyclic ring:

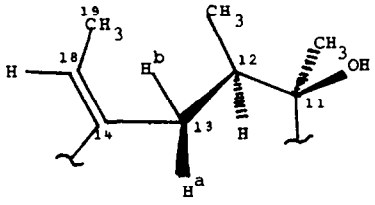


Fig. 2

The C19-hydrogens are close to C13-H_b which is antiperiplanar to C12-H, since its triplet-type signal indicates a three-bond coupling constant of 11-12 Hz in contrast to C13-H_a signal which is a doublet. Moreover, the C17-hydrogens are near neighbours to the C16- as well as the C19-hydrogens and, finally, irradiating the C16-protons gave a significant NOE at C12-H. Dreiding models clearly show that all these NOE findings are consistent only with the one configuration depicted in Fig.2. For all others "impossible" conformations would have to be assumed. Thus, not only the configuration of 1 is determined to be exactly as reported earlier[7]; we even could arrive at a good estimate of the preferred conformation of the macrocyclic ring. It was further noted that the CD spectrum of 1 is in excellent agreement with literature data[7].

The chemical shifts of the fourteen out of the eighteen carbon resonance signals in the ^{13}CNMR spectrum of 1 were unequivocally assigned by two-dimensional ^{13}C-^{1}H shift correlated and DEPT spectra. The carbon resonance signals of the remaining four quaternary carbons were assigned by comparing chemical shift values with those of similar compounds in the literature[8,9]. Recently Mody et al have reported the ^{13}CNMR spectrum of madurensine[5]. Comparison of the values we obtained by 2D-techniques with those of Mody et al reveals that the report of these workers contains incorrect assignments for C-3, C-5, C-6, C-7, C-8 and C-9 carbons.

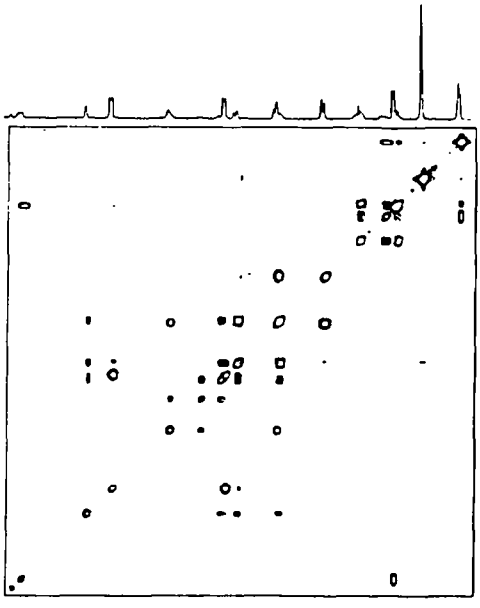


Fig.3 ^{1}H-^{1}H Chemical shift correlated 2-D (Cosy 45) NMR spectrum of madurensine 1 in $CDCl_3$ at 400 MHz.

Alkaloid 2 showed m.p. 157-160°C and a molecular formula of $C_{20}H_{27}NO_7$ (HRMS 393.1787). Treatment of 1 with acetic anhydride and pyridine led to 2, 7-acetylm adurensine, identical in all

respects with the natural product isolated from the plant. The NMR spectral data for 2 are given in Tables 1 and 2.

Alkaloid 3, crotaflorine, showed m.p. 178-181°C and a molecular formula of $C_{18}H_{25}NC_7$ (HRMS 367.1631). The ^{1}HNMR spectrum closely resembled that of madurensine 1. The spectrum however differed by the absence of the HO-C-Me signal at 1.38 ppm and the appearance of a doublet of doublet of two protons at 3.75 ppm, with a geminal coupling constant of 21.6 Hz, appropriate to a HO-C-CH_2-OH group of retronecic acid (Fig.4). Since the chemical shift of C5-H_β hydrogen in crotaflorine is a doublet at 2.78 ppm (J=14.7Hz), the macrocyclic ring is formed through C-6[4]. The ^{13}C-NMR data for crotaflorine was assigned by the application of 2D-techniques and by comparing with chemical shift values for madurensine 1. This compound has so far been reported from *C. agatiflora* only.

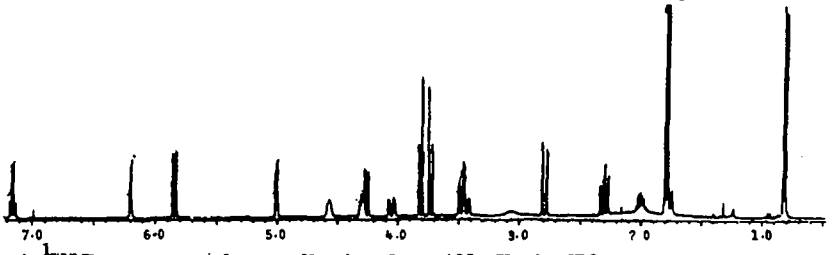


Fig.4 ^{1}HNMR spectrum of crotaflorine 3 at 400 MHz in $CDCl_3$

Table 2 ^{13}CNMR (100.61 MHz , $CDCl_3$, δ TMS = Oppm) chemical shifts of 1,2 and 3

Carbon No.	1	2	3
1	135.5	135.4	135.4
2	136.2	136.1	136.3
3	66.4	66.5	66.4
5	61.5	61.1	61.4
6	75.1	74.5	75.2
7	74.7	76.6	74.6
8	73.7	71.5	73.7
9	59.5	58.9	59.8
10	177.1	178.0	174.9
11	76.3	75.9	81.2
12	40.5	39.6	37.2
13	27.5	27.2	27.3
14	129.7	1 31.8	129.1
15	167.0	167.3	166.9
16	24.5	24.3	66.5
17	10.8	10.6	11.5
18	142.6	142.1	143.0
19	15.0	15.0	15.0
20	—	170.3	—
21	--	20.8	--

One of the most intriguing aspects of the structure of macrocyclic diesters is the difficulty to distinguish between a structure like that of madurensine 1 and an alternative structure represented by structure 4. These alternative structures result from the consideration that the same esterifying diacid may be linked to the C-9 and C-6 alcohols of the necine base in two different ways. The usual spectroscopic techniques do not avail themselves to enable unequivocal distinction between these two structures. A lot of spectroscopic data presented for macrocyclic pyrrolizidine alkaloids in the literature, except those backed by x-ray studies do not infact rule out such alternative structures. The current knowledge in biosynthesis of these alkaloids does not also enable us to make such distinctions.

We have found that the "COLOC" NMR technique, recently proposed by Kessler and coworkers[10], to be specially suitable to the solution of such problems. The COLOC technique is a heteronuclear 2D-NMR experiment which is performed by optimizing the pulse sequences in such a way that the large coupling constants are ignored and couplings of relatively small values, say, 4-6 Hz are observed. In contrast to other heteronuclear correlation spectra, cross peaks can be observed in the COLOC spectrum even for signals of quaternary carbons. ^{13}C-^{1}H correlated spectra of this type may enable one to observe long-range couplings which may exist even when there are oxygen or other hetero atoms in between the coupling carbon and hydrogen atoms.

The COLOC spectrum of madurensine 1 is given in Fig.5. Thus to distinguish between the two structures 1 and 4, one would need to establish with which of the two carbonyl groups of the esterifying diacid the C9-protons couple. The chemical shifts of the two carbonyls (177.1 and 167.0 ppm) are easily assigned since only one of the two is a conjugated carbonyl. In the COLOC spectrum a cross peak is observed for the C9-protons and the non-conjugated carbonyl (177.1 ppm). This enables us to conclude that madurensine has the structure represented by 1 and not 4.

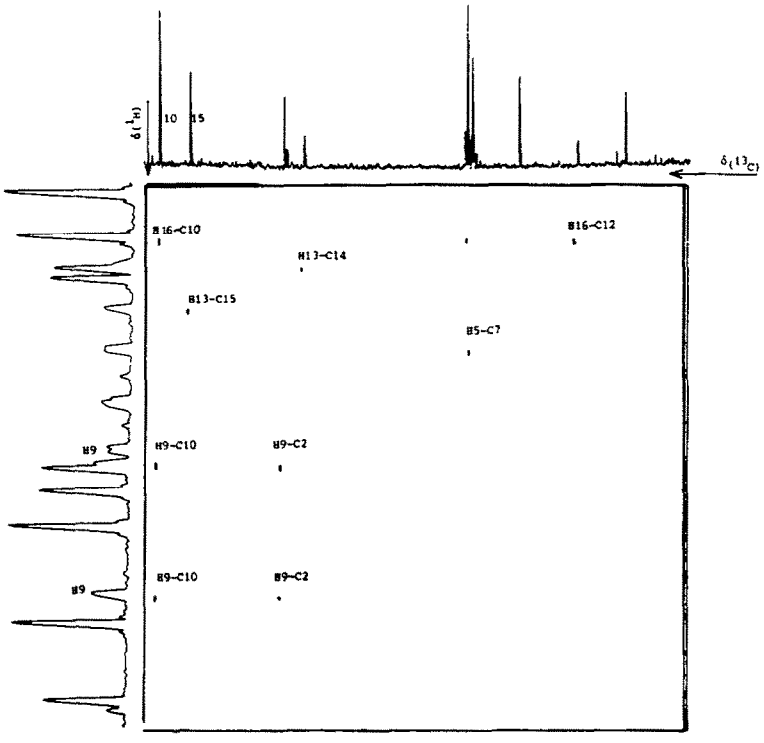


Fig.5 "COLOC" spectrum of madurensine 1, couplings optimized at 6 Hz.

EXPERIMENTAL

General Experimental Procedures

Melting points were determined on hot stage Bock Monoscope apparatus and are uncorrected. IR spectra were determined on a Perkin Elmer model 727 instrument. Optical rotations were measured on a Perkin Elmer model 241 polarimeter. NMR spectra were obtained using a Bruker AM400 spectrometer. Mass spectra were recorded on a Varian CH-5 and CH-7 spectrometer.

Plant Material: *C. rosenii* was collected in Lepis forest, ca 250km South of Addis Ababa, in April 1985.

Extraction: 400 g of dry and powdered flowers, after defatting with hexane, were extracted with methanol (soxhlet) to give 155 g of extract. This was subjected to the usual acid (2.5% HCl)-base (ammonia) extraction to give 3.01 g (0.75%) of alkaloidal extract.

Chromatography: 3.0 g of the alkaloidal extract was put on 100 g silica gel (Merck 60) and subjected to methanol-chloroform gradient elution. 3%, 5% and 30% methanol in chloroform eluted 35, 250 and 250 mg of impure but crystalline alkaloids respectively. These were purified by several micro-column chromatography. The first fraction (35 mg) gave, after such purification, 15 mg of 7-acetylmadurensine 2 (0.004%). The second fraction (250 mg) gave 110 mg of madurensine 1 (0.027%). The third fraction (250 mg) gave 150 mg (0.038%) of crotaflorine 3.

Acetylation of Madurensine 1: A mixture of 10 mg of madurensine, 1 ml of acetic anhydride and 2 drops of pyridine was stirred at room temperature for 3 days. 10 g of ice was added and the solution stirred for 2 hours, neutralized with 25 ml of saturated sodium bicarbonate and extracted with 100 ml of dichloromethane, dried and the solvent evaporated to give 8 mg of 7-acetylmadurensine 2.

Physical and Spectroscopic Data of Alkaloids:

Madurensine 1, m.p. 174-175°C (Lit. 175-176°[3]); IR KBr cm^{-1}: 1720, 3420; MS, M/z (Rel.Int.): 351.1687 (M^+, 23), 307(49), 264(17), 137(56), 136(64), 135(100), 93(40), 80(73). For NMR data see Tables 1 and 2.

7-Acetylmadurensine 2: m.p. 158-160°C; IR KBr cm^{-1}: 1740, 3550; MS, M/z (Rel.Int.) 393.1787 (M^+,3), 349(37), 307(13), 153(43), 93(10), 81(17), 80(14), 43(100). For NMR data see Tables 1 and 2.

Crotaflorine 3: m.p. 178-181°C (Lit. 179-180°[6]); IR KBr cm^{-1}: 1730, 3570; MS, M/z (Rel.Int.) 367.1631 (M^+, 67), 336(29), 323(26), 137(56), 136(76), 135(100), 93(49), 80(86). For NMR data see Tables 1 and 2.

Optical Rotations: solvent: chloroform; concentration c=2

Alkaloid	Wavelength in nm				
	589	578	546	436	365
Madurensine 1	+190	+17	+21	+40	+74
7-Acetylmadurensine 2	+ 66	+68	+79	+138	+206
Crotaflorine 3	+ 32	+35	+39	+74	+129

ACKNOWLEDGEMENT

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