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NOVEL SECOPYRROLIZIDINE ALKALOIDS FROM *CROTALARIA VERRUCOSA**

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Key Word Index—*Crotalaria verrucosa*; Leguminosae; secopyrrolizidines; crotaverrine; *O*-acetyl crotaverrine; otonecine esters; diastereoisomer integerrinecic acid.

Abstract—Crotaverrine and *O*-acetylcrotaverrine, isolated from the seeds of *C. verrucosa* Linn., have been shown by spectroscopy and chemical evidence to be the macrocyclic diesters of otonecine and diastereoisomeric integerrinecic acid. Hitherto, diastereoisomeric integerrinecic acid esters were not known to occur in nature.

INTRODUCTION

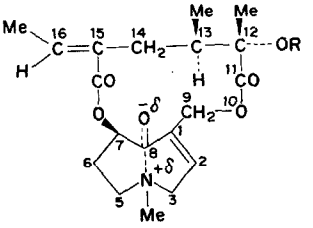
Crotalaria verrucosa Linn. is a shrub with blue flowers which grows in South India and is used in medicine [1]. Earlier workers [2] have reported the isolation of sitosterol, isovitexin, vitexin and crotalaburnine (anacrotine [3]). However, our investigations on the alkaloids from the seeds of this species have shown the presence of two new otonecine macrocyclic esters and the absence of anacrotine.

RESULTS AND DISCUSSION

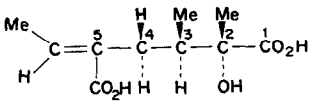
Structure of crotaverrine (**1**). The NMR spectrum of the alkaloid exhibits signals at δ 0·92 (*d*, *J* 6·5Hz, >CH–C$\underline{H}_3$), 1·22 (*s*, >C(OH)C$\underline{H}_3$), 2·10 (*d*, *J* 7Hz, C$\underline{H}_3$–CH=), 2·30 (*s*, N–C$\underline{H}_3$), 2·82 (*m*, OH and C-5), 3·25 (*m*, C-3), 4·73 (*ABq*, *J* 11Hz, C-9), 4·98 (*t*, *J* 5Hz, C-7), 6·10 (*q*, *J* 7Hz, Me–C$\underline{H}$=) and 6·17 (*m*, C-2). The quartet centred at δ 6·10 does not indicate whether the C-16 proton is *cis* or *trans* in respect of the secondary ester carbonyl. However, eventually it was found to be *cis* from the NMR of the acid, obtained by hydrolysis of crotaverrine with either sodium hydroxide or barium hydroxide (see below).

The presence of significant ions at *m/e* 94, 96, 110, 122, 123, 149, 150, 151 and 168 in the MS of crotaverrine clearly shows it to be an otonecine (**3**) type ester [4]. The cracking pattern of the base is the same as that of senkirkine (**4**). This gives definite evidence that the sequence of the various groups in the acid part of crotaverrine is same as that found in senkirkine. Crotaverrine exhibits an M^+-17 (3%) fragment attributable to the loss of a hydroxyl group which must be at C-12 because those alkaloids having a hydroxyl group at positions other than C-12 e.g. floricaline and floridanine, do not show the M^+-17 peak [4].

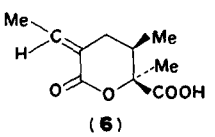
* Part 24 in the series "*Genus Crotalaria*." For part 23 Rao, P. G., Sawhney, R. S. and Atal, C. K. *Indian J. Chem.* (In Press).



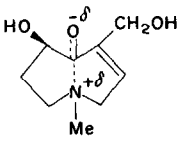
(**1**) R = H
(**2**) R = Ac



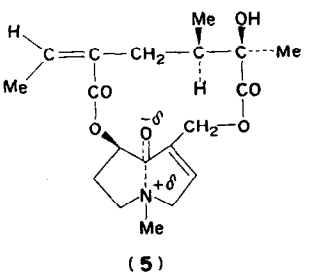
(**4**) 2S, 3R



(**6**)



(**3**)

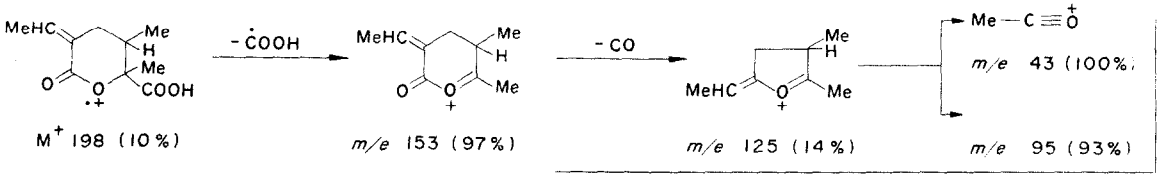


(**5**)

Table 1. Comparison of crotaverrine acid with the diastereoisomeric integerrinecic acid

	(−) Diastereoisomeric integerrinecic acid lactone (synthetic) (2*R*, 3*S* or 2*S*, 3*R*)	Crotaverrine acid lactone	(−) Diastereoisomeric integerrinecic acid (synthetic)	Crotaverrine acid
Mp of acid	134–136°	134–135°	132–133°	132–133°
Mp of Brucine salt	187–189°	184–185°	—	—
$[\alpha]_D$	−10·0°	−12·4°	−24·0°	−22·3°

Crotaverrine on acid hydrolysis afforded otonecine hydrochloride and an acid lactone (**5**). The NMR spectrum (D_2O) of the lactone showed signals at δ 1·17 (*d*, *J* 5·5Hz, $C\underline{H}_3$–CH–), 1·62 (*s*, $C\underline{H}_3$–C–CO_2H), 1·78 (*d*, *J* 7Hz, $C\underline{H}_3$–CH=), *ca* 2·28 (*m*, $C\underline{H}_2$–$C\underline{H}$–), 6·52 (COO$\underline{H}$) and 7·23 (*q*, *J* 7Hz, Mc–$C\underline{H}$=). The quartet at δ 7·23 due to a proton of an ethylidene group clearly shows it to be *cis* to the lactone carbonyl and this represents its position in the parent alkaloid. The MS (M^+ at *m/e* 198) of the acid lactone was also in good agreement with the structure (Scheme 1). The lactone, mp 134–135°, was found to be different from natural integerrinecic acid lactone (2*R*, 3*R*).



It is known [5] that when senecic acid is lactonised with HCl, integerrinecic acid lactone is obtained. To establish that the lactone obtained by acid hydrolysis of crotaverrine is not a geometrical isomer of that actually present in crotaverrine, samples of the base were hydrolysed with either sodium hydroxide or barium hydroxide at room temp. The NMR spectrum [$(CD_3)_2CO$] of the acid obtained showed a quartet at δ 7·04 due to the ethylidene proton, thus confirming its configuration. The MS of the acid showed the parent peak at *m/e* 198 (M^+-H_2O). The base peak at *m/e* 43 supported the view that acetylium ion is very easily formed from lactones having a methyl group on the carbon atom which bears a CO_2H group and oxygen [6].

Since the lactone and the dicarboxylic acid obtained from crotaverrine were found to be different from their natural counterparts, they were compared with the synthetic diastereoisomers [7]. The pair of synthetic diastereoisomeric integerrinecic acids have mp 132–133°; the optical rotation of one isomer is −24° while that of the other is +26°. The (−) isomer can be compared (Table 1) with the crotaverrine acid. Direct comparison could not be made due to non availability of the synthetic compound.

Lead tetraacetate oxidation [8] of crotaverrine acid afforded a keto acid (**6**), which was converted into its 2:4 DNP, mp 145–146°. Similar lead-tetraacetate oxidation of natural integerrinecic acid also yielded a keto acid, the 2:4 DNP of which was found to be identical with that of (**6**) by mmp (144–145°) and superimposable IR spectra. This suggests that the crotaverrine acid and the natural integerrinecic acid may only differ in their configuration at C-2 or C-3. Since natural integerrinecic acid is 2*R*, 3*R*, the crotaverrine acid may be *cis*-(2*S*, 3*R*) or *cis*-(2*R*, 3*S*)-5-ethylidene-2-hydroxy-2,3-dimethyl adipic acid. The structure of crotaverrine may therefore be represented as **1** (*cis*-15-ethylidene-12-hydroxy-4, 12ξ,13ξ-trimethyl-8-oxo-4,8-secosenec-1-enine).

Structure of O-acetyl crotaverrine (**2**). The MS (M^+ at *m/e* 407) of the base showed peak at *m/e* 364 (M^+-COMe) with the rest of the fragmentation pattern being similar to that of crotaverrine. In addition to protons due to crotaverrine, the NMR spectrum ($CDCl_3$) of the base exhibited a 3 proton singlet at δ 2·05 which is assigned to the acetyl group. The alkaloid on acid hydrolysis afforded compound **5** as confirmed by mmp; co-TLC; UV; IR and NMR. The amino alcohol was confirmed to be otonecine hydrochloride. Crotaverrine on treatment with acetyl chloride yields *O*-acetyl crotaverrine (**2**) (*cis*- 15-ethylidene- 12-acetoxy-4, 12ξ,13ξ-trimethyl-8-oxo-4, 8-secosenec-1-enine).

EXPERIMENTAL

Mps are uncorrected. NMR spectra were recorded using TMS as internal reference in $CDCl_3$ or $(CD_3)_2CO$.

Extraction of alkaloids from the seeds of C. verrucosa. Powdered seeds (1·4 kg) containing 0·16% tertiary bases and 0·13% N-oxides were defatted with *n*-hexane and subsequently extracted with EtOH. The EtOH extract on further processing [9] yielded a mixture of alkaloids.

Separation of crotaverrine and O-acetylcrotaverrine. The mixture was resolved by partition chromatography [10]. In one of the typical experiments, crude bases (578 mg) were applied to a column of celite 525 (120 g) moistened with 1N NaH_2PO_4 (120 ml) and the alkaloids were eluted with CCl_4, CCl_4-$CHCl_3$ mixtures and subsequently with $CHCl_3$. Acetylcrotaverrine (**2**, 250 mg) was eluted with 10–40% $CHCl_3$ in CCl_4 whereas crotaverrine (**1**, 192 mg) was eluted with $CHCl_3$. Crotaverrine partially crystallised from EtOAc to give hygroscopic crystals, mp 142–144°; $C_{19}H_{27}O_6N$ (M^+ 365); $[\alpha]_D^{25}$ +32·7° (*C* 1·04 MeOH); λ_{max}^{EtOH} 220 nm; ν_{max}^{film} cm^{-1}: 3333 (OH), 1750 (ester CO), 1725 (α,β unsaturated CO), 1650 (C=C). Acetylcrotaverrine, $C_{21}H_{29}O_7N$, $[\alpha]_D^{25}$ + 45·54° (*C* 1·01 MeOH); λ_{max}^{EtOH} 220 nm; could not be induced to crystallise.

Acid hydrolysis of crotaverrine. The base (300 mg) was heated with 12% HCl (7 ml) on a steam bath for 16 hr. Working up of the reaction product afforded necic acid (125 mg) and necine hydrochloride (117 mg). The necic acid on TLC (C_6H_6–MeOH–$MeCO_2H$, 20:4:3) showed two spots having R_f values 0·45 (major) and 0·35 (minor) which corresponded

to integerrinecic acid lactone and integerrinecic acid respectively. To the mixture were added a few drops of HCl followed by evaporation to dryness to convert the free dicarboxylic acid to its lactone (**5**), $C_{10}H_{14}O_4$, mp 134–135° (Et_2O-petrol); $[\alpha]_D^{25}$ − 12·4° (EtOH). The acid lactone did not respond to the ferric-chloride test for α-hydroxy acids. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1735 (COOH), 1710 (−lactone) and 1635 (C=C). The necine hydrochloride was charcoaled and separated from other impurities by preparative PC. On trituration with Me_2CO, it crystallised, mp 147–148°; $[\alpha]_D^{22}$ − 9·6° (*C* 1·45 EtOH) (lit. mp 145–147° [11]; $[\alpha]_D^{23}$ − 13 ± 1°). The NMR (D_2O) was identical with that of otonecine hydrochloride [12]. Its MS (M^+ at *m/e* 185) exhibited prominent peaks at *m/e* 168, 128, 126, 110, 98 and 82.

Alkaline hydrolysis of crotaverrine. The base (350 mg) was treated with 2 N methanolic NaOH (10 ml) at room temp, the reaction was complete after 3 hr. MeOH was removed and the residue taken in dil HCl and filtered. The filtrate, on extraction with Et_2O afforded a dicarboxylic acid, mp 132–133° (Et_2O–petrol), $[\alpha]_D^{25}$ − 22·3 (*C* 0·818 EtOH). (Found: C, 55·2; H, 7·4; $C_{10}H_{16}O_5$ requires C, 55·6; H 7·4%).

Hydrolysis of crotaverrine with Ba(OH)₂. The base (250 mg) was treated with 6% $Ba(OH)_2$. The reaction mixture was allowed to stand overnight at room temp. Excess of baryta was precipitated as $BaCO_3$ and the soln filtered. The filtrate was acidified with dil HCl and extracted with Et_2O to give necic acid, mp 132–133°. The acid was found to be identical (TLC mmp, IR) with the acid obtained by NaOH hydrolysis of the alkaloid.

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