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PYRROLIZIDINE ALKALOIDS FROM *CROTALARIA LACHNOSEMA* AND *C. NARAGUTENSIS*

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Key Word Index—*Crotalaria lachnosema*; *C. naragutensis*; pyrrolizidine alkaloid; dicrotaline; acetyl dicrotaline; integerrimine; acetyl integerrimine; nilgirine; usaramine.

Abstract—*Crotalaria lachnosema*, from Nigeria, was found to contain two pyrrolizidine alkaloids, dicrotaline and its acetyl derivative, the natural occurrence of which is reported for the first time. The major alkaloid in *C. naragutensis* was nilgirine, together with integerrimine, usaramine and a new alkaloid, acetyl integerrimine.

INTRODUCTION

Pyrrolizidine alkaloids, many of which are toxic and hazardous to livestock, have been found in a large number of *Crotalaria* species [1–3]. *Crotalaria lachnosema* Stapf. and *C. naragutensis* Hutch. are both woody undershrubs 0.9–1.8 m high, which occur in northern Nigeria. The former is tawny, covered with hairs and has orange streaked flowers, while the latter has soft scanty hairs, with yellow flowers in stalked racemes up to 30 cm or more long.

The present study was undertaken to determine whether these plants contain pyrrolizidine alkaloids. Okopido and Ogunbiyi [4] did not detect any alkaloids in the leaves of *C. lachnosema* while Pilbeam *et al.* [2] reported detecting, in the seeds, an alkaloid that was not monocrotaline. However, the tests used in both studies were not specific for pyrrolizidine alkaloids. No report has been found on any chemical investigations of *C. naragutensis*.

RESULTS

Specific tests for unsaturated pyrrolizidine alkaloids [5] showed that these alkaloids and their *N*-oxides were present in the leaves of *C. lachnosema* (seeds not available), and in the seeds and seed pods, but not the leaves, of *C. naragutensis*. Subsequently all extracts were reduced to convert *N*-oxides to tert-bases before isolating the alkaloids.

The crude basic extract (0.14%) from *C.lachnosema* contained two alkaloids as shown by TLC and capillary GC. The major component, R_f 0.55, R_t 9.4 min comprising 86% of the total alkaloids, was shown to be dicrotaline (1). High resolution GC-MS indicated the formula $C_{14}H_{19}NO_5$. Fragments, *m/z* 80, 93, 106, 119 and 136 were characteristic of the amino alcohol moiety, retronecine [6], and fragments at *m/z* 236–238, 222–223 and 178 – 179 represented sequential losses of $CO_2(H)$, CH_2 and MeCOH from the acid chain. Treatment of the crude alkaloids with hydrochloric acid gave a hydrochloride, mp 196 – 198° (decomp) after several recrystallizations. Marais [7] prepared dicrotaline HCl in two forms, mp 200° (decomp) and 258–260° (decomp), and suggested that the latter was the more stable. Brown *et al.* [8] reported its mp as 210–211° (decomp) but the mp was very variable (D. J. Robins, personal communication). The IR spectrum (KBr) of our purified HCl salt and the ^{1}H NMR spectrum ($CDCl_3$) of the base recovered from it were identical with authentic dicrotaline spectra supplied by Dr D. J. Robins. The alternative structure, 13-*epi*-dicrotaline [8] was ruled out by the lower mp (158–161°) of its HCl salt. Alkaline hydrolysis of the alkaloid afforded a crystalline acid, identified as 3-hydroxy-3-methylglutaric acid (dicrotalic acid) by the mass spectrum of its tri-TMSi derivative. EIMS failed to show a molecular ion, but CIMS gave a peak at 379 (MH^+) and prominent fragments, *m/z* 363, 289, 273 and 247 representing $(M-Me)^+$, $(M-Me-TMSiH)^+$, $(M-Me-TMSiOH)^+$ and $(M-CH_2CO_2TMSi)^+$ respectively.

The minor alkaloid from *C. lachnosema*, R_f 0.64, R_t 10.5 min, was recovered from the mother liquors after crystallization of the major alkaloid hydrochloride. The high resolution mass spectrum indicated the formula $C_{16}H_{21}NO_6$, M_r 323. Fragments, *m/z* 263 and 220 showed loss of acetate and ($MeCO_2H+CO_2$) respectively. The ^{1}H NMR spectrum also showed the presence of an acetyl group (δ 2.04 ppm) and the structure was confirmed as acetyl dicrotaline (**2**) by comparing it (MS, NMR) with an authentic sample prepared by acetylation of dicrotaline (cf. [8]).

Crotalaria naragutensis seeds yielded a mixture of crude bases (0.15%) containing four alkaloids (A–D), characterized by capillary GC-MS and TLC (Table 1). Seed pods of the same plant contained only alkaloids A and D (total, 0.07%). The MS of all the alkaloids showed fragments characteristic of the base moiety retronecine [6]. The major alkaloid (A) was identified as nilgirine, $C_{17}H_{23}NO_5$ (3). Its EIMS showed a strong M^+, 321, and fragments, *m/z* 276, 246, 220 and 206 indicating loss of $CO_2(H)$ followed by progressive cleavage of the acid chain. The ^{1}H NMR spectrum (60 MHz, $CDCl_3$) showed

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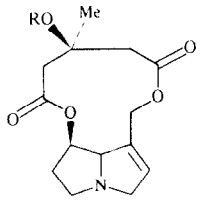
Table 1. Alkaloids from *Crotalaria naragutensis*

Alkaloid	TLC R_f	Capillary GC R_t (min)	Capillary GC Relative amount (% of total bases)	EIMS $[M]^+$
A	0.53	9.9	69	321
B	0·60	10.0	9.5	335
C		11·2	1.5	377
D	0.36	11.9	20	351



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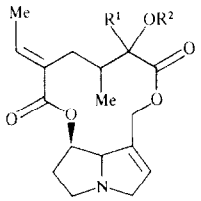
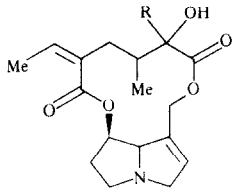
2 R = Ac



	R^1	R^2
3	H	H
4	Me	H
5	CH_2OH	H
6	Me	Ac



7 R = Me
8 R = CH_2OH

signals characteristic of nilgirine [9], with δ (ppm) 1.05 (3H, *d*, MeCH, collapsed to a singlet by irradiation at δ 2.1); 1.75 (3H, *d*, Me=CH); 2.18 (2H, *m*, CH_2); 2.75 *br* (1H, OH); 4.04 (1H, *s*, CHOH); 4.17 and 5.41 (2H, *dd*, J = 12 Hz, H-9); 6.18 (1H, *m*, H2: 12-membered macrocyclic diester) and 6.56 (1H, *q*, J = 7.5 Hz, *trans*-ethylidene [10]).

Alkaloids (B) and (D) were identified as integerrimine (**4**) and usaramine (**5**) respectively, by comparing their GC characteristics and mass spectra with those of authentic standards (Table 2). The mass spectrum of integerrimine is almost identical with that of its geometric isomer senecionine (**7**) [11,12] and our spectra of alkaloid B were contaminated with nilgirine. However their retention times distinguished alkaloids B and D as the *trans* isomers (**4**) and (**5**) respectively. The EIMS of alkaloid D corresponded with that of usaramine (**5**) rather than that of its *cis* isomer, retrorsine (**8**). The mass spectra of these two alkaloids are very similar, but retrorsine gives rise to a small fragment, *m/z* 306 whereas usaramine gives *m/z* 307. Also, peaks at *m/z* 246 and 248 from usaramine are of similar size, whereas retrorsine gives a much weaker peak at 248 than that at 246. TLC of usaramine and integerrimine (Table 2) gave spots corresponding with the weaker spots shown by the crude alkaloid mixture.

The minor alkaloid, C, $[M]^+$ 377, did not match any known pyrrolizidine alkaloid. However a major fragment, *m/z* 290 representing the loss of carboxyl and acetyl groups, was consistent with the structure of either acetyl integerrimine (**6**) or acetyl senecionine. The structure was confirmed as the former by comparison of its GC and MS with authentic (**6**) prepared by acetylation of integerrimine.

DISCUSSION

Dicrotaline (**1**) was first identified in 1944 by Marais [7] in *Crotalaria dura* and *C. globifera* (although more recently Brown *et al.* [13] were unable to find it in the latter plant). It was only the third pyrrolizidine alkaloid to be isolated from *Crotalaria* species (the first two being monocrotaline [14] and grantianine [15]). It is thus surprising that until now dicrotaline has never again been encountered in studies of over 100 species of this genus (or in any other plant) although a great many other pyrrolizidine alkaloids have been discovered.

Nilgirine (**3**) has previously been found only in *Crotalaria mucronata* collected in India; African species of *C. mucronata* contained usaramine (**5**) as the major alkaloid [9]. Integerrimine (**4**) and acetyl nilgirine (crotastriatine) have also been found in *C. mucronata* [1]. It is therefore interesting to find nilgirine again associated with usaramine and integerrimine in *C. naragutensis*. These alkaloids all possess a *trans* ethylidene group; the *cis* isomers retrorsine (**8**) and senecionine (**7**) occur less often in *Crotalaria* species. Acetyl nilgirine (crotastriatine) was not detected in *C. naragutensis*, but a small amount of the new alkaloid acetyl integerrimine (**6**) was found. This and acetyl discrotaline from *C. lachnosema*, are thus new additions to the growing number of acetylated pyrrolizidine alkaloids to be found in plants.

Table 2. Characteristics of alkaloid standards

Alkaloid	TLC R_f	Capillary GC R_t (min)	EIMS $[M]^+$	EIMS Fragments (*m/z*)
Integerrimine (**4**)	0·60	10·0	335	320, 291, 248, 246, 220
Usaramine (**5**)	0·36	11·9	351	320, 307, 248, 246, 220
Acetyl integerrimine (**6**)	0·65	11·2	377	290, 246, 207, 153
Senecionine (**7**)	0·61	9·6	335	291, 248, 246, 220, 153
Retrorsine (**8**)	0·36	11·4	351	320, 306, 248, 246, 220

EXPERIMENTAL

Plant material. Both species were collected from Zaria, Nigeria and identified by Mr A. O. Ohaeri of the Herbarium Section, Department of Biological Sciences, Ahmadu Bello University, Zaria, where voucher specimens have been deposited. *C. lachnosema* was confirmed by Dr R. M. Polhill at the Royal Botanic Gardens, Kew, U.K.

Extraction. Ground, dried plant material (*C. lachnosema* leaves, 64 g: *C. naragutensis* seeds, 4.1 g; seed pods, 16 g) were extracted (Soxhlet) for 12 hr with industrial methylated spirit (EtOH, 95% + MeOH, 4%). The evapd extracts were dissolved in 0.1 M HCl and the solns defatted with Et_2O, reduced (1 hr) with Zn dust, basified (NH_3) and the bases extracted with $CHCl_3$ (x 3). The dried (Na_2SO_4) extracts were conc. under red. pres. Yields: *C. lachnosema*, 88.4 mg (0.14%); *C.naragutensis* seeds 6.3 mg (0.15%); seed pods 11 mg (0.07%).

Chromatography. TLC was on silica gel-coated Al sheet, developed with EtOAc–Me_2CO–EtOH–aq. NH_3 (5:3:1:1); alkaloids were visualized by spraying with *O*-chloranil followed by Ehrlich reagent [16]. Capillary GC (FID) employed 20 m × 0.3 mm silica WCOT columns (OV-1), with He at 2 ml/min; injection vol. 0.5 μl; injector temp. 280°, detector 300°; programme 100° for 1 min;then 20°/min up to 300°.

C. lachnosema *alkaloids.* The crude basic extract was a gum which failed to crystallize. GC-MS showed two components: A, R_t 9.4 min, $[M]^+$ 281.1263 (calc. for dicrotaline, $C_{14}H_{19}NO_5$: 281.1263); B, R_t 10.5 min, $[M]^+$ 323.1410 (calc. for $C_{16}H_{21}NO_6$: 323.1369; calc. for $C_{17}H_{25}NO_5$: 323.1733). The crude bases (25 mg) with HCl yielded a hydrochloride (22.5 mg) which formed needles, mp 179–180° (slight decomp) after 2 recrystallizations from EtOAc–Et_2O, rising to 198° after drying (15 min, 100° *in vacuo*). Base recovered from this salt was pure dicrotaline (NMR; GC-MS). It was hydrolysed [$Ba(OH)_2$, 100°, 45 min] to give a crystalline acid, mp (crude) 101–103°, identified (MS) as dicrotalic acid (lit. [7] mp 109°).

Base (1.05 mg) recovered from the mother liquors after crystallization of dicrotaline HCl was almost pure alkaloid B (TLC; GC-MS). Refluxing dicrotaline HCl (2.85 mg) with Ac_2O + AcCl for 2 hr gave acetyl dicrotaline, $C_{16}H_{21}NO_6$ (2) (2.53 mg). identical (TLC; GC-MS; NMR) with alkaloid B. The ^{1}H NMR spectrum ($CDCl_3$; 250 MHz) corresponded with the data of ref. [8].

C. naragutensis alkaloids. The bases extracted from seeds formed a hard gum containing 4 components, A, B, C, D (Table 1). The ^{1}H NMR spectrum (60 MHz, $CDCl_3$) showed signals characteristic of the major component (A). A, B and D were identified (GC-MS) as nilgirine, integerrimine and usaramine respectively.

Alkaloid C was characterized as acetyl integerrimine by comparison (GC-MS) with an authentic sample prepared by heating integerrimine (100 mg) under reflux with Ac_2O (0.5 ml), AcCl (0.25 ml) and 4-dimethylaminopyridine (2 mg) for 5 hr. The product was isolated as a gum, R_f 0.65, R_t 11.2 min; ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.93 (3H, *d*, $J = 7$ Hz, MeCH); 1.70 (3H, *s*, MeC–O); 1.78 (3H, *d*, $J = 7$ Hz, ethylidene Me); 2.10 (3H, *s*, acetyl Me); 4.1 and 5.4 (2H, *dd*, $J = 12$ Hz, H-9); 4.96 (1H, *m*, H-7); 6.25 (1H, *m*, H-2); 6.55 (1H, *q*, $J = 8$ Hz, *trans*-ethylidene).

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