Phytochemistry, Vol. 00, No. 0, pp. 0000 0000, 1991
Printed in Great Britain.

Phyto 2193-4456 H SR

0031 9422/91 $3.00 + 0.00

PYRROLIZIDINE ALKALOIDS FROM SEEDS OF *CROTALARIA CAPENSIS*

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(*Received* 5 *June* 1991)

Key Word Index—*Crotalaria capensis*; Fabaceae seeds; pyrrolizidine alkaloids; *trans*-anacrotine; madurensine.

Abstract—Madurensine and a new pyrrolizidine alkaloid, *trans*-anacrotine, were found as the only alkaloids in seeds of *Crotalaria capensis*. Structural elucidation of the new alkaloid proved that it is the *trans* isomer of anacrotine.

INTRODUCTION

Crotalaria, the nominate genus of the tribe Crotalarieae, has been the only reported source of macrocyclic pyrrolizidine alkaloids in the Fabaceae until 1987 [1]. Pyrrolizidine alkaloids were only recently isolated from the genera *Lotononis* and *Buchenroedera* [2, 3] and were thus far the only other sources of these natural products in the Fabaceae. In our continued survey of alkaloids as characters in the chemosystematics of the Fabaceae, we have extracted and isolated two pyrrolizidine alkaloids from the seeds of *Crotalaria capensis*, a species from which alkaloids have not been isolated previously. Alkaloids have, however, been detected in earlier studies [4, 5]. Madurensine (1) and a new product, *trans*-anacrotine (2), were the only alkaloids isolated from the extract. The structure of this newly isolated product was in fact the structure first reported for madurensine (1) by Atal *et al.* [6]. The corrected structure for 1, in which the macrocyclic ester is attached to C-6 of the pyrrolizidine base, rather than to C-7 as suggested earlier, was subsequently reported by Culvenor *et al.* [7]. In spite of the isolation of the acetyl ester of *trans*-anacrotine (3) and the C-6 angeloyl ester of *trans*-anacrotine *N*-oxide (4) from *C. agatiflora* [8], the free alcohol (2), however, has never been recorded before.

RESULTS AND DISCUSSION

Seeds of *C. capensis* were collected from trees in Grahamstown in the Cape Province, South Africa. The seeds were ground to a fine powder and extracted with acid. Basification of the acidic solution with ammonia and extraction with methylene chloride gave a crude mixture of alkaloids. TLC of the crude extract and GC analysis indicated the presence of only two compounds. The crude extract was then subjected to column chromatography to afford 1 as the major constituent and 2 as the minor component. Madurensine (1) was identified by comparison of its physical and spectral data with those reported in the literature [6, 9–11].

In order to verify the structure of 2, its ^1H and ^{13}C NMR spectra were recorded and compared with those of structurally related compounds such as integerrimine (6-deoxy-*trans*-anacrotine) (5), anacrotine (6), senecionine (7), platyphylline (8), neoplatyphylline (9) and madurensine (1). In the ^1H NMR spectrum of 2, the H-21 quartet at δ6.57 (compared to δ6.48 for 5 and δ6.50 for 9) clearly indicated a *trans* configuration for the carboxy-

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Table 1. ^{1}H NMR spectral data for compound (2) (200 Mhz)

H	
2	6.14 *dddd* ($J_{2,3\alpha} \simeq J_{2,3\beta} \simeq 2$, $J_{2,8} \simeq 2$, $J_{2,9} \simeq 1$)
3α	3.93 *ddd* ($J_{2,3\alpha} \simeq J_{3\alpha,8} \simeq 2$, $J_{3\alpha,3\beta} = 16.0$)
3β	3.43 *ddd* ($J_{2,3\beta} \simeq 2$, $J_{3\alpha,3\beta} = 16.0$, $J_{3\beta,8} \simeq 4$)
5α	3.40 *dd* ($J_{5\alpha,5\beta} = 10.03$, $J_{5\alpha,6} = 6.20$)
5β	2.53 *dd* ($J_{5\alpha,5\beta} = 10.03$, $J_{5\beta,6} = 7.62$)
6	4.43 *ddd* ($J_{5\alpha,6} = 6.20$, $J_{5\beta,6} = 7.62$, $J_{6,7} = 3.71$)
7	5.21 *dd* ($J_{6,7} = 3.71$, $J_{7,8} = 4.83$)
8	4.31 *dddd* ($J_{2,8}$) = 6.14, $J_{3\alpha,8} \simeq 2$, $J_{3\beta,8} \simeq 4$, $J_{7,8} = 4.83$)
9a	4.24 *d* ($J_{9a,9b} = 12.20$)
9b	5.22 *dd* ($J_{2,9b} \simeq 1$, $J_{9a,9b} = 12{,}20$)
13	1.88 *ddq* ($J_{13,14a} = 8.70$, $J_{13,14b} = 4.05$, $J_{13,19} = 6.78$)
14a	2.22 *dd* ($J_{13,14a} = 8.70$, $J_{14a,14b} = 13.66$)
14b	2.08 *dd* ($J_{13,14b} = 4.05$, $J_{14a,14b} = 13.66$)
18	1.29 *s* —
19	0.92 *d* ($J_{13,19} = 6.78$)
21	6.57 *q* ($J_{21,22} = 7.08$)
22	1.74 *d* ($J_{21,22} = 7.08$)
OH	3.25 *br s* —

Table 2. ^{13}C NMR spectral data of compound 2 and related pyrrolizidine alkaloids

	cis-Ethylidene				*trans*-Ethylidene		
C	8*	7*	6†	2	5*	9*	1
1	37.3	131.5	131.5	132.7	131.7	36.9	135.4
2	30.8	136.3	136.5	137.5	136.7	30.2	136.1
3	52.0	62.8	63.6	63.5	62.6	51.6	66.3
5	53.9	53.1	58.5	59.8	53.1	52.9	61.4
6	35.3	34.8	74.8	73.7	33.8	35.0	75.1
7	69.4	74.9	75.5	76.4	75.5	68.8	74.5
8	74.2	77.7	74.4	73.6	77.2	73.4	73.6
9	65.4	60.6	60.7	61.9	60.9	67.1	59.4
11	177.7	178.2	178.3	178.4	178.2	178.7	176.9
12	76.2	76.8	76.8	76.9	76.6	75.4	76.2
13	39.9	38.5	38.3	39.1	39.5	39.5	40.4
14	39.2	38.4	38.4	29.8	29.5	32.8	27.5
15	131.7	133.1	132.8	132.9	133.9	131.8	129.7
16	167.4	167.5	169.6	170.5	169.1	167.5	167.0
18	26.1	25.0	25.2	25.5	25.1	26.4	24.4
19	13.0	11.1	11.3	12.4	11.7	14.3	10.7
20	136.0	134.3	134.3	135.5	135.3	137.7	142.5
21	15.5	15.0	15.2	14.4	14.2	14.4	14.9

*^{13}C NMR data recorded for alkaloids isolated from species of *Lotononis* and *Buchenroedera* in an earlier study [3].

†Data from ref. [9].

ethylidene moiety. A significant difference in the δ-values for H-21 in **2** and **1** (δ7.12) is probably due to conformational [6, 12] and size differences in the macrocyclic rings of the two compounds. The presence of a hydroxyl group in the molecule was evident from the downfield shift of H-7 (from δ4.97 in **5** to δ5.21 in **2**), the appearance of a multiplet at δ4.43 (H-6) and a broad singlet δ3.25 representing the alcohol proton. D_2O exchange of the hydroxyl signal resulted in the coalescence of the multiplet which, upon decoupling experiments, showed spin–spin interaction with the two C-5 protons as well as with H-7. These results were consistent with a C-6 position for the hydroxyl group. The coupling constants for this carbinol proton were in accordance with a β-orientation for the hydroxyl group, similar to anacrotine (**6**), with H-6 coupling constants closely matching those of H-6 in **2** [6, 8]. A weak spin–spin interaction ($J = 3.71$ Hz) between H-6 and H-7 indicated a dihedral angle of *ca* 45° and, therefore, a *cis*-orientation for these two protons. From the crystal structure of the isomer of **2**, anacrotine (**6**), it was evident that the H-6/H-7 dihedral angle is in fact *ca* 41° for the *exo*-buckled pyrrolizidine nucleus. The vicinal coupling constants with the two C-5 protons ($J_{5\alpha,6} = 6.20$ and $J_{5\beta,6} = 7.62$ Hz) were much larger than their counterparts in uspallatine (**10**) with an α-oriented hydroxyl group at C-6 [13]. All these data supported a β-orientation for the C-6 hydroxyl group. The ^{1}H NMR data are presented in Table 1.

The only significant difference in the ^{13}C NMR spectra of **2** and anacrotine (**6**) was in the chemical shift of C-14. It had a value of δ29.8 for **2** compared to δ38.4 for **6**, indicative of a rather strong shielding of the C-14 nucleus by the methyl group of the ethylidene functionality. These values correspond well with those of other alkaloids with *cis* and *trans* orientations of the ethylidene group, such as integerrimine (**5**), senecionine (**7**), platyphylline (**8**) and neoplatyphylline (**9**). Table 2 compares the ^{13}C NMR data of **2** and structurally related pyrrolizidine alkaloids.

EXPERIMENTAL

Mps: uncorr. IR spectra were recorded for thin films of $CHCl_3$ solns. Optical rotations were measured for a path length of 1 cm in the solvents stated for each sample. ^{1}H and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively, in $CDCl_3$ using the $CHCl_3$ signal (δ7.24) as ref. TLC was performed on Merck 60 F_{254} type E alumina plates using MeOH–$CHCl_3$ (3:97). Chromatograms were visualized after drying by spraying with iodoplatinate soln. CC was performed using Fluka neutral alumina type 507C using the eluent as specified for TLC.

Ripe seeds (50 g) of *C. capensis* were collected in January 1990 from plants of a natural population on a hill slope below Settler's Monument in Grahamstown (voucher specimen: B.-E. van Wyk 2993a in JRAU). The finely ground material was suspended in 0.05 M H_2SO_4 for 30 min and the solids filtered off. Conc NH_3 soln was used to basify the soln and extraction with CH_2Cl_2 gave 790 mg of crude alkaloidal mixt. CC afforded madurensine (**1**) (519 mg) and *trans*-anacrotine (**2**) (29 mg).

Madurensine (**1**). Mp 175–176° (lit. 175–176° [6]). $[\alpha]_D^{22} + 48°$ ($CHCl_3$; *c* 2). IR $\nu_{max}^{CHCl_3}$ 3550 *br* (OH), 1720 (ester C=O). EIMS (probe) 70 eV (rel. int.): *m/z* $[M]^+$ (4), 307 (5), 264 (3), 153 (13), 137 (49), 136 (53), 135 (100), 93 (35), 80 (67).

Trans-*Anacrotine* (**2**). $[\alpha]_D^{22} + 11°$ ($CHCl_3$; *c* 1.7). IR $\nu_{max}^{CHCl_3}$ 3520 *br* (OH), 1725 (ester C=O). EIMS (probe) 70 eV (rel. int.): *m/z* $[M]^+$ (5), 307 (7), 264 (9), 236 (8), 154 (15), 153 (23), 152 (29), 137 (32), 136 (52), 135 (87), 94 (87), 93 (100), 83 (31), 67 (35), 43 (85).

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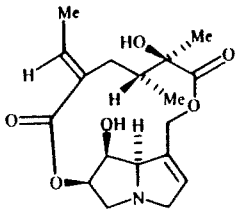

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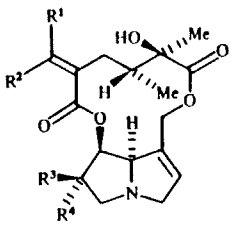
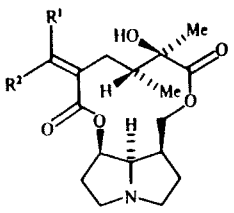


	R^1	R^2	R^3	R^4	
2	Me	H	OH	H	
3	Me	H	OAc	H	
4	Me	H	OAng	H	N-oxide
5	Me	H	H	H	
6	H	Me	OH	H	
7	H	Me	H	H	
10	H	Me	H	OH	



	R^1	R^2
8	H	Me
9	Me	H