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GLOBIFERINE, A PYRROLIZIDINE ALKALOID FROM *CROTALARIA GLOBIFERA*

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Key Word Index—*Crotalaria globifera*; Leguminosae; globiferine; grantaline; grantianine; trichodesmine; pyrrolizidine alkaloids.

Abstract—Seeds of *Crotalaria globifera* from two separate locations in South Africa yielded different pyrrolizidine alkaloids. One batch gave trichodesmine and grantaline, while the other afforded grantianine and a new pyrrolizidine alkaloid, globiferine.

INTRODUCTION

Pyrrolizidine alkaloids have been isolated from about 60 *Crotalaria* species (Leguminosae) [1]. *Crotalaria globifera* E. Mey. is a yellow-flowered, multi-stemmed, perennial, up to 50 cm high. This species has been reported to contain the pyrrolizidine alkaloid dicrotaline [2]. We required a sample of dicrotaline for comparison with our synthetic sample [3, 4].

RESULTS AND DISCUSSION

The initial batch of *C. globifera* seeds was collected from a coastal population in Natal. The seeds were extracted and the two alkaloids present were separated by TLC. Dicrotaline was not detected. Instead the major component was readily identified as the pyrrolizidine alkaloid trichodesmine (**1**) by comparison of its spectra with those of authentic material [5]. The less polar, minor component of the alkaloidal mixture was shown to be the much rarer pyrrolizidine alkaloid grantaline (**3**) from spectroscopic data, including the close similarity with the published ^{13}C NMR spectrum [6]. The identification was confirmed by direct comparison with an authentic sample of grantaline supplied by Dr. C. C. J. Culvenor. Grantaline (**3**) has been isolated once before [7] from an impure sample of grantianine obtained from *Crotalaria grantiana* Harvey [8, 9], and was believed to contain an epoxide group in the acid portion of the alkaloid. Recently, the structure of grantaline has been revised to **3**, containing the rare oxetane ring system, on the basis of an X-ray crystallographic study [10].

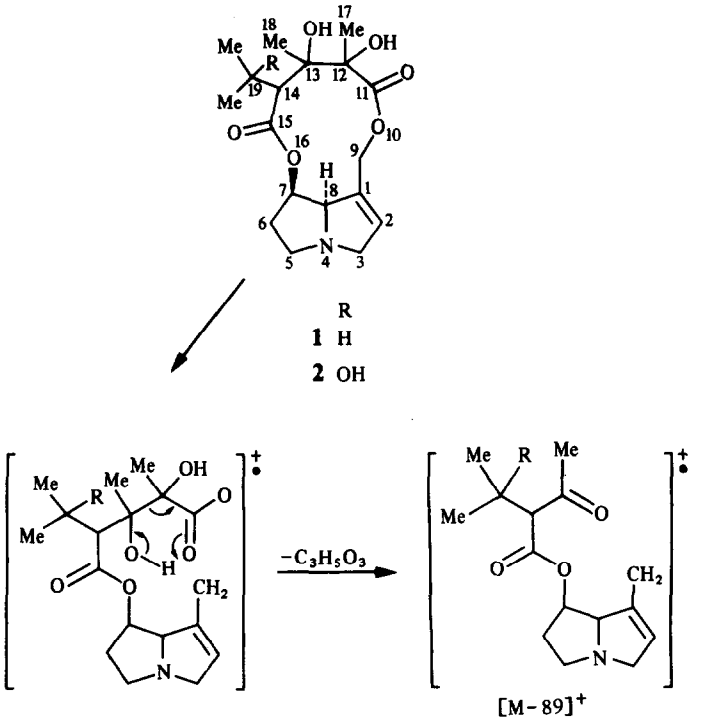
Extraction of a second batch of *C. globifera* seeds, obtained from inland Natal at *ca* 1050 m above sea level, also failed to yield any dicrotaline. The alkaloidal mixture was separated into three fractions by TLC. The major component of the mixture was the least polar band, and was identified as grantianine (**4**) by comparison of IR and ^1H NMR spectra with those of grantianine supplied by Dr. Culvenor. Grantianine was originally isolated from *C. grantiana* by Adams and co-workers [8, 9]. Mass spectra and ^1H NMR data showed that the central band from the TLC separation contained a mixture of two pyrrolizidine alkaloids. High resolution mass spectra indicated that these had the molecular formulae $C_{18}H_{25}NO_7$ and $C_{18}H_{27}NO_6$. Close inspection of the ^1H NMR spectrum of the mixture suggested that the latter alkaloid is trichodesmine. Lack of material prevented further attempts to separate this mixture.

The most polar band from the TLC separation contained a new pyrrolizidine alkaloid, named globiferine. High resolution mass spectral data indicated a molecular formula of $C_{18}H_{27}NO_7$. The mass spectra of globiferine and trichodesmine (**1**) were similar. In particular, the presence of ions at m/z 138, 137, 136 and 120 in the mass spectra of both alkaloids is characteristic of a 1,2-didehydropyrrolizidine base [7]. Furthermore, strong $[M-89]^+$ ions are evident in the mass spectra of both alkaloids, indicating similarity in the acid part of the alkaloids as shown (Scheme 1). The difference in structure between trichodesmine and globiferine therefore lies in the acid portion, and is due to an extra hydroxyl group in globiferine. The location of this hydroxyl group is evident from the ^1H NMR spectrum of globiferine. The presence of four methyl singlets deshielded by adjacent oxygen atoms leads immediately to the formulation of **2** for globiferine. The rest of the ^1H NMR spectrum contained the signals of a 1H singlet for H-14, three exchangeable hydroxyl protons, and the expected signals for the retronecine portion. The ^{13}C NMR spectrum of globiferine showed the presence of four methyl carbons, three quaternary carbons, a CH doublet, and two carbonyl carbons in addition to the signals for retronecine. These data clearly rule out possible structures with 12-membered rings, and confirm the structure **2** for globiferine.

Although the two batches of *C. globifera* seeds collected from different locations yielded two different sets of pyrrolizidine alkaloids, it is evident that these structures are all closely related. Experiments are in hand to demonstrate this biogenetic relationship.

EXPERIMENTAL

Seeds of *Crotalaria globifera* were collected at (a) Port Edward, Natal and (b) Umzinto, Natal, and were identified by Mr. B. D. Schrire (Vouchers B. Schrire 232 and 869, Natal Herbarium). TLC was carried out on silica gel (Merck).



Scheme 1.

Isolation of the alkaloids: *batch* (*a*). The seeds (5 g) were finely ground and continuously extracted with EtOH for 48 hr. The extract was concd *in vacuo* and the residual dark syrup was dissolved in 2% citric acid (15 ml). The acid layer was washed with CH_2Cl_2 (5 × 5 ml), basified with conc NH_3 (2 ml), and extracted with CH_2Cl_2 (5 × 5 ml). The CH_2Cl_2 extracts were dried and concd *in vacuo* to an oil, 38 mg. Analytical TLC in $CHCl_3$–MeOH–conc NH_3 (85:14:1) showed two alkaloids, R_f 0.50 and 0.55, which were separated by TLC.

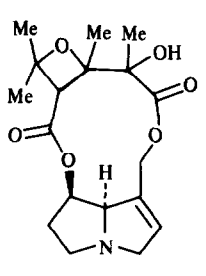
Trichodesmine (**1**). R_f 0.50, 22 mg, mp 159–161° (lit. [5] mp 158–160°). Identity was confirmed by comparison with authentic material (mmp of alkaloid and picrate, IR and co-TLC).

Grantaline (**3**). R_f 0.55, 10 mg, mp 218–220° (from aq. MeOH); $[\alpha]_D^{18}$ +33° ($CHCl_3$; *c* 0.065); $[M]^+$ 351.1679 ($C_{18}H_{25}NO_6$ requires $[M]^+$ 351.1682), $[M-89]^+$ 262.1440 ($C_{15}H_{20}NO_3$ requires 262.1443); IR ν_{max}^{KBr} cm^{-1}: 3440, 1734, 1729, 1232 and 1145. ^1H NMR (360 MHz, $CDCl_3$): δ1.37, 1.44, 1.62 and 1.68 (all 3H, *s*, 4 × Me), 2.14 (2H, *m*, H-6), 2.59 (1H, *m*, H-5), 3.18 (1H, *m*, H-5), 3.39 (1H, *br s*, OH), 3.45 (1H, *dd*, *J* = 4 and 16 Hz, H-3), 3.71 (1H, *s*, H-14), 3.87 (1H, *m*, H-3), 4.14 (1H, *dd*, *J* = 1 and 12 Hz, H-9), 4.29 (1H, *m*, H-8), 4.95 (1H, *m*, H-7), 5.28 (1H, *d*, *J* = 12 Hz, H-9), 6.15 (1H, *d*, *J* = 2 Hz, H-2). ^{13}C NMR: see ref. [6]. MS (probe) 70 eV, *m/z* (rel. int.): 351 $[M]^+$ (12), 262 (50), 220 (90), 138 (80), 137 (75), 136 (85), 121 (75), 120 (100), 93 (85) and 83 (95). Identity was confirmed by comparison with authentic material (mmp, IR, NMR and co-TLC).

Batch (*b*). The seeds (15 g) were extracted as above to yield an oil, 176 mg. Analytical TLC indicated three components, R_f 0.61, 0.50 and 0.39, which were separated by TLC.

Grantianine (**4**). R_f 0.61; 104 mg; mp 223–224° (from Me_2CO) (lit. [7] mp 205°) $[\alpha]_D^{18}$ +44.4° ($CHCl_3$; *c* 1.78); $[M]^+$ 365.1461 ($C_{18}H_{23}NO_7$ requires $[M]^+$ 365.1474). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3510, 1775, 1730, 1460 and 1455. ^1H NMR (360 MHz, $CDCl_3$): δ1.21 (3H, *d*, *J* = 7 Hz, H-20), 1.50 and 1.56 (both 3H, *s*, H-17 and H-18), 2.17 (2H, *m*, H-6), 2.62 (1H, *dt*, *J* = 6 and 10 Hz, H-5), 3.16 (1H, *m*, H-5), 3.17 (1H, *d*, *J* = 11 Hz, H-14), 3.25 (1H, *m*, H-19), 3.51 (1H, *dd*, *J* = 4 and 17 Hz, H-3), 3.67 (1H, *br s*, OH), 3.85 (1H, *dd*, *J* = 3 and 17 Hz, H-3), 4.33 (1H, *m*, H-8), 4.38 (1H, *d*, *J* = 17 Hz, H-9), 5.0 (1H, *m*, H-7), 5.22 (1H, *d*, *J* = 17 Hz, H-9), 6.18 (1H, *s*, H-2). ^{13}C NMR (25 MHz, $CDCl_3$): δ14.0 (*q*), 19.8 (*q*), 23.0 (*q*), 33.7 (*t*), 38.0 (*d*), 51.2 (*d*), 53.0 (*t*), 60.8 (*t*), 63.1 (*t*), 76.2 (*d*), 77.4 (*s*), 77.5 (*d*), 85.3 (*s*), 131.7 (*s*), 137.1 (*d*), 170.3 (*s*), 174.1 (*s*), 175.8 (*s*). MS (probe) 70 eV, *m/z* (rel. int.): 365 $[M]^+$ (10), 294 (41), 276 (12), 220 (7), 138 (15), 137 (10), 136 (39), 120 (100), 119 (32), 94 (47), 93 (77). The identity of grantianine was confirmed by comparison of the IR and ^1H NMR spectra with those of authentic grantianine.

Globiferine (**2**). R_f 0.39; 23 mg; mp 126–129° (from petrol); $[\alpha]_D^{18}$ −8.6° ($CHCl_3$; *c* 0.232); $[M]^+$ 369.1807 ($C_{18}H_{27}NO_7$ requires $[M]^+$ 369.1788), $[M-89]^+$ 280.1544 ($C_{15}H_{22}NO_4$ requires 280.1548). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3510, 1740, 1460, 1110 and 1080. ^1H NMR (360 MHz, $CDCl_3$): δ1.24, 1.43, 1.53 and 1.59 (all 3H, *s*, 4 × Me), 2.20 (2H, *m*, H-6), 2.70 (1H, *m*, H-5), 2.99 (1H, *s*, H-



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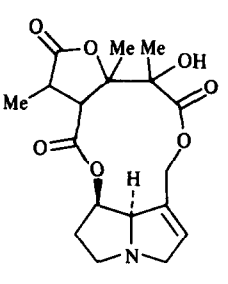

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14), 3.62 (2H, *m*, H-3 and H-5), *ca* 4.0 (3H, *br*, 3 × OH), 4.20 (1H, *d*, J = 18 Hz, H-3), 4.72 (1H, *d*, J = 12 Hz, H-9), 4.79 (1H, *br s*, H-8), 4.87 (1H, *d*, J = 12 Hz, H-9), 5.12 (1H, *t*, J = 4 Hz, H-7), 6.12 (1H, *s*, H-2). ^{13}C NMR (25 MHz, CD_3CN): δ22.7 (*q*), 22.8 (*q*), 25.8 (*q*), 30.9 (*q*), 34.1 (*t*), 54.2 (*t*), 56.9 (*d*), 60.1 (*t*), 61.4 (*t*), 74.7 (*s*), 75.7 (*d*), 77.4 (*d*), 78.7 (*s*), 80.7 (*s*), 134.2 (*s*), 135.6 (*d*), 172.2 (*s*), 175.3 (*s*). MS (probe) 70 eV, *m/z* (rel. int.): 369 $[M]^+$ (2), 280 (37), 227 (5), 220 (39), 192 (17), 139 (26), 138 (49), 137 (51), 136 (84), 121 (44), 120 (100), 119 (80), 118 (24), 108 (17), 95 (26), 94 (55), 93 (78).

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