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GRANTIANINE AND GRANTALINE, ALKALOIDS OF *CROTALARIA VIRGULATA* SUBSP. *GRANTIANA*

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Key Word Index—*Crotalaria virgulata* subsp. *grantiana*; Leguminosae; alkaloids; grantianine; grantaline; 1-hydroxy-1β,2β-epoxy-8αH-pyrrolizidine.

Abstract—Characterization data are presented for grantianine and grantaline, alkaloids of *Crotalaria virgulata* subsp. *grantiana*. The plant growing in Australia also contains 1-hydroxymethyl-1β,2β-epoxy-8αH-pyrrolizidine.

The alkaloids of *Crotalaria virgulata* subsp. *grantiana* [1] were first studied by Adams and Gianturco who described the isolation of grantianine, $C_{18}H_{23}NO_7$, of probable structure **1** [2, 3]. Some years ago, we separated a second alkaloid, grantaline, $C_{18}H_{25}NO_6$, from a sample of crude grantianine supplied by Professor Adams. The NMR and mass spectral data were partially described and possible structures for grantaline proposed in a review [4]. The details of the separation and other characterization of the alkaloids have not been previously reported.

Grantaline has recently been shown to have the four-membered oxide ring structure **2** by an X-ray crystallographic study [5] and its ^{13}C NMR spectrum has been described as part of a general study of pyrrolizidine alkaloids [6]. The isolation of grantaline and grantianine from *C. virgulata* subsp. *grantiana* of Australian origin, along with 1-hydroxymethyl-1β,2β-epoxy-8αH-pyrrolizidine (**3**) is also described. The latter alkaloid was previously isolated from *Crotalaria medicaginea* Lam. (*C. trifoliastrum* Willd.) [7]. Alkaline hydrolysis of grantaline gives retronecine and an acid, grantalinic acid, which was not fully characterized. The IR spectrum of the acid has a peak, 1723 cm^{-1}, appropriate for a carboxylic acid and/or a δ-lactone with hydrogen bonding. The ^1H NMR spectrum shows the expected four methyl peaks and a single-proton singlet. The mass spectrum of the methyl ester has an ion of highest mass, *m/z* 229, corresponding with $[M+1]^+$ for a lactone-methyl ester, $C_{11}H_{16}O_5$, derived from the dicarboxylic acid of **2** or a rearrangement product. In the absence of further data, the structure of grantalinic acid remains uncertain.

EXPERIMENTAL

R_f values refer to PC in a solvent which is the upper phase resulting from shaking *n*-BuOH with an equal vol. of 5% HOAc.

Separation of grantianine and grantaline. The crystalline alkaloid, mp 203–205°, from *C. virgulata* subsp. *grantiana* (syn. *C. grantiana* Harvey), supplied by Professor Adams, showed two spots on PC, R_f 0.34 and 0.40. The alkaloid (1.04 g) was applied to a column (3 cm diameter) packed with powdered Pyrex glass (1320 g) moistened with pH 8.1 KPi buffer (224 ml). The eluants used were petrol–CCl_4 (1:1) (500 ml), CCl_4 (700 ml) and CCl_4–$CHCl_3$ (4:1) (2 l.). Alkaloid emerged only with the last solvent so that the first solvent, at least, probably had no effect on the separation. The fractions obtained were grantaline (325 mg), R_f 0.38, then a mixture of grantaline and grantianine (300 mg), and finally grantianine (420 mg), R_f 0.33.

Grantaline (**2**) was crystallized from EtOH to give colourless prisms, mp 219.5–220°, $[\alpha]_D^{20}$ +100.9° (*c* 0.8 in EtOH) (Found: C, 61.7; H, 7.1; N, 4.0. Calc. for $C_{18}H_{25}NO_6$: C, 61.5; H, 7.2; N, 4.0%). IR ($CHCl_3$) cm^{-1}: 3510, 1735; ^1H NMR (60 MHz, $CDCl_3$): δ 1.36, 1.44, 1.60, 1.68 (12H, 4*s*, H-17, H-18, H-20, H-21), 4.14, 5.26 (2H, AB*q*, H-9), *ca* 5.0 (1H, *m*, H-7) 6.15 (1H, *m*, H-2). EIMS *m/z* (rel. int.): $[M]^+$ 351 (2), 280 (2), 262 (18), 220 (69), 192 (2), 178 (9), 170 (3), 149 (2), 141 (18), 138 (27), 137 (16), 136 (60), 121 (22), 120 (96), 119 (42), 118 (34), 117 (81), 104 (34), 101 (23), 94 (48), 90 (41), 89 (34), 83 (100), 80 (27).

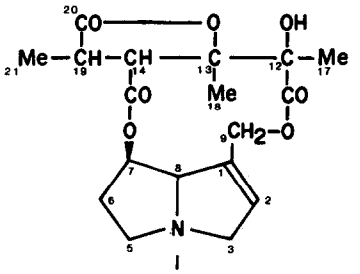
Grantianine (**1**) was recrystallized from EtOH to give colourless prisms, mp 209–209.5°, $[\alpha]_D^{20}$ +63.8° (*c* 1.32 in EtOH), $[\alpha]_D^{20}$ +43.8° (*c* 0.5 in $CHCl_3$) (previously described as mp 205°, (dec.), $[\alpha]_D^{27}$ +50.6° ($CHCl_3$) [2]. ^1H NMR (60 MHz, $CDCl_3$): δ 1.23 (3H, *d*, H-21), 1.53 (3H, *s*, H-18), 1.57 (3H, *s*, H-17), 4.28, 5.28 (2H, AB*q*, H-9), 5.10 (1H, *m* H-7), 6.25 (1H, *m*, H-2).

Isolation from plant material of Australian origin. Dried aerial parts of *C. virgulata* subsp. *grantiana* (Harvey) Polhill (2.2 kg) collected in Queensland were extracted with hot MeOH. After removal of solvent, the residue was extracted with 0.5 N H_2SO_4 made 2 N with respect to H_2SO_4 and reduced with Zn for 3 hr. Excess Zn was removed by filtration and the soln made alkaline with aq. NH_3 and extracted with $CHCl_3$ to give crude base (7.1 g) as a brown gum. The aq. mother liquor was made more alkaline with aq. NaOH and extracted with $CHCl_3$ to give crude base (1.5 g), R_f 0.20.

The alkaloid extracted from aq. NH_3 was taken up in $CHCl_3$ and the soln extracted with 0.5 N H_2SO_4. The acid extract was washed once with $CHCl_3$, made alkaline with $NaHCO_3$ and extracted with $CHCl_3$ to give a brown gum (2.5 g) which slowly crystallized on standing. Shaking with cold EtOH gave crystals (1.1 g), R_f 0.33, 0.38. CC as described above gave grantianine, mp 210.5–211.5°, undepressed on admixture with authentic grantianine, and grantaline, mp 220–221°, undepressed on admixture with authentic grantaline.

The aq. $NaHCO_3$ mother liquor, further basified with aq. NaOH and extracted with $CHCl_3$, gave a brown gum (2.4 g), R_f 0.20, which was not investigated further.

1-Hydroxymethyl-1β,2β-epoxypyrrolizidine (**3**). The alkaloid extracted from aq. NaOH (see above) formed a picrate, mp



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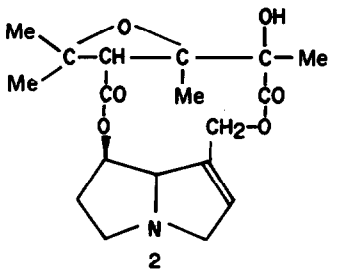

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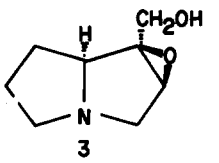

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170–171°, undepressed on admixture with authentic 1-hydroxymethyl-1β,2β-epoxypyrrolizidine picrate [7] (Found: C, 43.7; H, 4.4; N, 14.1. Calc. for $C_8H_{13}NO_2$ $C_6H_3N_3O_7$: C, 43.8; H, 4.2; N, 14.6%).

Hydrolysis of grantaline. Grantaline (88 mg) was refluxed for 2 hr with a soln of hydrated $Ba(OH)_2$ (150 mg) in H_2O (3 ml). After cooling, the soln was saturated with CO_2 and the precipitated $BaCO_3$ removed by filtration. The filtrate was acidified with dilute H_2SO_4 and extracted with Et_2O to give grantalinic acid (45 mg). Recrystallization from EtOAc–petrol gave colourless needles, mp 147–148°. IR (Nujol) cm^{-1}: 1723. ^1H NMR (60 MHz, D_2O): δ 1.43, 1.47, 1.56, 1.59 (12H, 4*s*, H-1, H-6, H-8, H-10), 4.12 (1H, *s*, H-4). EIMS (dimethyl ester) *m/z* (rel. int.): 229 (2), 201 (2), 185 (2), 171 (7), 157 (16), 143 (19), 116 (13), 115 (100), 111 (12), 104 (31), 83 (8), 59 (8), 55 (11), 43 (62).

The mother liquor remaining after extraction of the acid was run through a column of Deacidite FF ion-exchange resin. The alkaline eluate was evapd and the residue crystallized from Me_2CO to give colourless prisms, mp 117–118°, undepressed on admixture with authentic retronecine.

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