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ALKALOIDS OF *ROTHIA TRIFOLIATA* AND *ROTHIA HIRSUTA*[1]

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Pursuant to our interest in the distribution of alkaloids in the legume subfamily Papilionoideae (1), we have studied extracts of specimens of the two species that constitute the genus *Rothia*, namely, *Rothia trifoliata* Pers. (syn. *Rothia indica* L.) and *Rothia hirsuta* (Gwill. & Perr.) Bak. The genus *Rothia* is classified in the tribe Crotalarieae and is, therefore, closely related taxonomically to the genus *Crotalaria*, whose species commonly accumulate hepatotoxic pyrrolizidine alkaloids (2,3). In a preliminary investigation, however, *R. trifoliata* was found to produce the quinolizidine alkaloid lupanine rather than bases of the pyrrolizidine type (4).

In the present communication, detailed quinolizidine alkaloid profiles are reported for vegetative and herbarium specimens of *R. trifoliata* and *R. hirsuta* and were established using capillary gc-ms. Esters of 13-hydroxylupanine, which were conveniently identified in this investigation by cims (5) using NH_3 as the reactant gas, were found to be characteristic of both species. ^{13}C-nmr chemical shifts have been determined for four *R. trifoliata* constituents, 13α-angeloyloxylupanine, cinevanine, 13α-(2-methylbutyryl)oxylupanine, and 13α-tigloyloxylupanine. 13α-(2-Methylbutyryl)oxylupanine has previously been identified only tentatively as a natural product, when present in a cell suspension culture of *Lupinus polyphyllus* (6). Its structure and stereochemistry were confirmed in the present investigation by catalytic hydrogenation of 13α-angeloyloxylupanine. The presence of quinolizidine alkaloids in the two *Rothia* species studied, as well as the concomitant absence of pyrrolizidine bases, is of chemotaxonomic importance. Prior to the present study, esters of 13-hydroxylupanine have only been reported in legume species in the tribes Sophoreae and Genisteae (7). Given the known toxic potential of quinolizidine alkaloids (7), the practice of using *R. trifoliata* as a vegetable in India in times of scarcity (8) is not to be recommended.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Specific rotations were measured on a Perkin-Elmer 241 polarimeter. The uv and ir spectra were obtained on a Beckman DU-7 spectrometer and a Nicolet MX-1 FT-IR spectrometer, respectively. ^{1}H-nmr and ^{13}C-nmr spectra were recorded in $CDCl_3$ on a Nicolet NMC-360 instrument, using TMS as internal standard and operating at 360 MHz and 90.8 MHz, respectively. Low-resolution electron impact mass spectra were obtained on a Varian MAT 112S instrument, operating at 70 eV. Gc-ms analysis was performed on a Finnigan 4510 mass spectrometer, with retention times established for each compound using two fused silica capillary columns (J&W Scientific Co., Folsom, California). Samples were initally injected onto a DB-5 column using the ei mode, with all parameters the same as previously described (1). With the ci mode, samples were injected onto a DB-1 column, 30 m × 0.25 mm i.d. × 0.25 μm film thickness, and NH_3 was employed as the reactant gas at 0.35 torr. The electron voltage was adjusted to 90 eV, the scan-to-scan ratio was 2 s, and the mass range scanned was 100–550 au. All other conditions were the same as those used for

[1]Part 4 in the series "Alkaloids of Papilionoideae." For part 3 see Kinghorn *et al.* (1).

eims above, except that the ionizer temperature was at 90° instead of 110°.

PLANT MATERIAL AND EXTRACTION FOR GC-MS.—The aerial parts of *R. trifoliata* (3.6 g) were collected near Warangal, India, in August 1987, and a voucher specimen representing this collection has been deposited in the herbarium of the Field Museum of Natural History, Chicago. In addition, herbarium samples of the pods of *R. trifoliata* collected near Madras, India (Wight 571, 0.08 g) and near Mysore, India (Edgeworth 2010, 0.02 g) and of the pods of *R. hirsuta* collected at Msembi, Tanzania (Greenway and Kanuri 14,269, 0.04 g) were kindly supplied from the Herbarium of the Royal Botanic Gardens, Kew, Surrey, UK. The entire amounts of these specimens available to us were ground and extracted as previously described (1).

REFERENCE ALKALOIDS.—Authentic samples of the following alkaloids, either in the form of free bases or salts, were available to us as described before (1,9): ammodendrine, 13α-hydroxylupanine, α-isolupanine, α-isosparteine, lupanine, and nuttalline (4β-hydroxylupanine). Cinevanine (13α-vanillyloxylupanine) and 13α-tigloyloxylupanine were kindly donated by other workers in this area. 13α-Angeloyloxylupanine was present as a major constituent of a C_6H_6-soluble extract of the aerial parts of *R. trifoliata* that was made available to us and was purified by preparative tlc on Si gel G plates (Analtech, Newark, Delaware, 0.25 mm, R_f 0.60), using $CHCl_3$-MeOH (9:1) as developing solvent. Catalytic hydrogenation of 13α-angeloyloxylupanine (6 mg), using Pt/C (5%, 10 mg) under an atmosphere of H_2 for 8 h, led to the production of 13α-(2-methylbutyryl)oxylupanine (5 mg). Hydrolysis of 0.5-mg aliquots of 13α-angeloyloxylupanine and 13α-(2-methylbutyryl)oxylupanine in 10% methanolic KOH (0.5 ml) by reflux under N_2 for 2 h led in both cases to the generation of 13α-hydroxylupanine, which was identified by gc-ms.

IDENTIFICATION OF ALKALOIDS.—The following alkaloids were identified by direct comparison to authentic compounds, using a combination of two capillary gc columns. For every compound identified in each *Rothia* specimen, the following data refer to relative retention time (RR_t) values to lupanine with DB-5 and DB-1 stationary phases, respectively, the cims characteristics, and the percent of each identified compound in the alkaloid extract, as determined by internal normalization. *R. trifoliata*, aerial parts: 13α-Angeloyloxylupanine, RR_t 1.99, 1.85; ms *m/z* $[M+H]^+$ 347 (100), 23.4%. Ammodendrine, RR_t 0.57, 0.47; ms *m/z* $[M+H]^+$ 209 (100), 11.8%. Cinevanine, RR_t 2.67, 2.53; ms *m/z* $[M+H]^+$ 415 (55%), 249 (76), 246 (28); 0.1%. 13α-Hydroxylupanine, RR_t 1.41, 1.53; ms *m/z* $[M+H]^+$ 265 (100%); 2.1%. α-Isolupanine, RR_t 0.91, 0.91; ms *m/z* $[M+H]^+$ 249 (100%); 1.2%. α-Isosparteine, RR_t 0.35, 0.34; ms *m/z* $[M+H]^+$ 235 (100%); 0.3%. Lupanine, RR_t 1.00, 1.00; ms *m/z* $[M+H]^+$ 249 (100%); 29.3%. 13α-(2-Methylbutyryl)oxylupanine, RR_t 1.95, 1.97; ms *m/z* $[M+H]^+$ 349 (100%), 249 (41); 0.04%. Nuttalline, RR_t 1.17, 1.14; ms *m/z* $[M+H]^+$ 265 (100%); 1.2%. 13α-Tigloyloxylupanine, RR_t 2.05, 1.89; ms *m/z* $[M+H]^+$ 347 (100); 0.1%. Also found were two unknown compounds, representing 30.5% of the total alkaloids. *R. trifoliata*, pods (Madras): ammodendrine, 35.7%, cinevanine, 7.5%, lupanine, 11.0%, and nuttalline, 43.2%. *R. trifoliata*, pods (Mysore): ammodendrine, 51.7%, cinevanine, 25.9%, and lupanine, 5.2%. *R. hirsuta*, pods: cinevanine, 72.9%, lupanine, 0.8%, and nuttalline, 2.2%. One unknown compound (4.9% of total alkaloids) was also detected in this specimen.

TENTATIVE ALKALOIDAL IDENTIFICATION.—A constituent of several of the samples studied was tentatively identified as 13α-cinnamoyloxylupanine (either *cis*- or *trans*-) (10), on the basis of its ms characteristics (6). This compound exhibited RR_t to lupanine on DB-5 and DB-1 columns, respectively, 2.39, 2.22; eims *m/z* $[M]^+$ 394 (1%), 247 (7), 246 (18); cims, *m/z* $[M+H]^+$ 395 (83%), 247 (28), 246 (13), and occurred to the extent of 2.6% and 17.2% in *R. trifoliata* pods from Mysore and Madras, respectively, and 19.2% in *R. hirsuta* pods. This compound was present at concentration levels too low to permit its isolation and proof of structure by analysis of its ^1H- and ^{13}C-nmr parameters.

CHARACTERIZATION OF 13α-HYDROXYLUPANINE ESTERS.—*13α-Angeloyloxylupanine.*—Mp 220–223°; $[\alpha]D$ 36.3° (*c* = 0.2, MeOH); uv (MeOH) λ max 256 nm (log ε 2.52); ir (KBr) ν max 3010, 1743, 1709, 1653, 1305, 1275, 1160 cm^{-1}; ^1H nmr δ 6.07 (1H, m, 3′-H), 5.42 (1H, m, 13-H_{eq}), 4.35 (1H, m, 10-H_{eq}), 3.96 (1H, m, 4-H_{eq}), 3.35 (1H, m, 6-H), 1.93 (3H, m, 5′-Me), 1.84 (3H, m, 4′-Me); ^{13}C nmr δ 14.74 (q, C-4′), 18.96 (q, C-5′), 19.83 (t, C-4), 26.31 (t, C-5), 27.56 (t, C-8), 28.33 (t, C-14), 32.68 (d, C-9), 32.90 (t, C-3), 33.85 (d, C-7), 35.09 (t, C-12), 46.81 (t, C-10), 49.86 (t, C-15), 51.49 (t, C-17), 58.87 (d, C-11), 60.50 (d, C-6), 67.84 (d, C-13), 126.45 (s, C-2′), 139.61 (d, C-3′), 166.25 (s, C-1′), 171.33 (s, C-2); eims see Wink *et al.* (6).

Cinevanine.—Mp, $[\alpha]D$, uv, and ir see Faugeras and Paris (11); ^1H nmr, δ 7.70 (1H, dd, *J* = 8, 2 Hz, 7′-H), 7.56 (1H, d, *J* = 2 Hz, 3′-H), 6.93 (1H, d, *J* = 8 Hz, 6′-H), 5.30 (1H, m, 13-H_{eq}), 4.54 (1H, m, 10-H_{eq}), 3.94 (3H, s, 4′-OMe), 3.86 (1H, m, 4-H_{eq}), 3.42 (1H, m, 6-H); ^{13}C nmr, δ 19.73 (t, C-4), 26.54 (t, C-5), 27.50

(t, C-8), 28.71 (t, C-14), 32.44 (d, C-9), 33.04 (t, C-3), 34.12 (d, C-7), 36.43 (t, C-12), 46.78 (t, C-10), 49.85 (t, C-15), 51.86 (t, C-17), 56.04 (q, OMe), 58.17 (d, C-11), 60.66 (d, C-6), 68.43 (d, C-13), 110.37 and 111.85 (2d, C-6′ and C-7′), 123.03 (s, C-2′), 123.63 (d, C-3′), 148.55 (s, C-5′), 152.86 (s, C-4′), 170.98 (s, C-1′), 171.26 (s, C-2); eims see Faugeras and Paris (11).

13α-(2-Methylbutyryl)oxylupanine.—Mp 192–195°; $[\alpha]_D$ 24.2° ($c = 0.4$, MeOH); uv (MeOH) λ max 217 nm (log ε 2.13); ir (KBr) ν max 3040, 2910, 1712, 1645, 1562, 1461 cm^{-1}; ^1H nmr, δ 5.20 (1H, m, 13-H_{eq}), 4.58 (1H, m, 10-H_{eq}), 4.22 (1H, m, 4-H_{eq}), 3.41 (1H, m, 6-H), 2.55 (1H, m, 1′-H), 1.25 (3H, m, 5′-Me), 1.20 (2H, m, 3′-H_2), 0.92 (3H, t, 4-Me); ^{13}C nmr δ 11.30 (q, C-4′), 16.74 (q, C-5′), 19.46 (t, C-4), 26.55 (t, C-3′), 26.81 (t, C-5), 27.36 (t, C-8), 27.52 (t, C-14), 31.94 (d, C-9), 33.01 (t, C-3), 33.66 (d, C-7), 35.75 (t, C-12), 41.07 (d, C-2′), 46.41 (t, C-10), 50.42 (t, C-15), 51.51 (t, C-17), 58.91 (d, C-11), 60.62 (d, C-6), 67.54 (d, C-13), 167.19 (s, C-1′), 170.93 (s, C-2); eims *m/z* $[M]^+$ 348 (13%), 347 (11), 275 (9), 247 (23), 246 (48), 134 (16), 97 (24), 85 (55), 83 (89), 71 (56), 57 (100).

13α-Tigloyloxylupanine.—Mp 213–216°; $[\alpha]_D$ and ir see Bratek-Wiewiórowska *et al.* (10); uv (MeOH) λ max 259 nm (log ε 2.37); ^1H nmr δ 6.72 (1H, m, 3′-H), 5.16 (1H, m, 13-H_{eq}), 4.50 (1H, m, 10-H_{eq}), 4.12 (1H, 4-H_{eq}), 3.36 (1H, m, 6-H), 1.82 (3H, m, 5′-Me), 1.74 (3H, m, 4′-Me); ^{13}C nmr δ 12.16 (q, C-5′), 14.47 (q, C-4′), 19.66 (t, C-4), 26.37 (t, C-5), 27.50 (t, C-8), 28.30 (t, C-14), 32.81 (d, C-9), 33.01 (t, C-3), 33.80 (d, C-7), 35.89 (t, C-12), 46.69 (t, C-10), 49.93 (t, C-15), 51.49 (t, C-17), 58.25 (d, C-11), 60.63 (d, C-6), 67.73 (d, C-13), 128.76 (s, C-2′), 137.63 (d, C-3′), 167.10 (s, C-1′), 171.14 (s, C-2); eims see Wink *et al.* (6).

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