IDENTIFICATION OF 11-HYDROXYVITTATINE IN *STERNBERGIA LUTEA*

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Earlier investigations (1) on *Sternbergia lutea* Ker Gawl resulted in the isolation of six alkaloids: lycorine, tazettine, hyppeastrine, haemanthidine, galanthamine, and galanthine. Recently, in the course of our studies on the chemistry and biological properties of lycorine, we identified hippamine (2) and the new alkaloid sternbergine (3), structurally related to lycorine and to pseudolycorine, respectively.

Extraction of this species and fractionation of the constituents by chromatography has now resulted in the isolation of 11-hydroxyvittatine, an alkaloid already isolated from two Amaryllidaceae species: *Pancratium maritimum* and *Rhodophiala bifida* (4).

To my knowledge, this represents the first isolation of 11-hydroxyvittatine from *S. lutea*; it is present at a low level (1.9 mg in 100 g of dried bulbs) in this plant.

Spectral data indicated a crinine-type skeleton for this alkaloid. In particular, the configuration of the two hydroxylated asymmetric carbons (C-3 and C-11) was established by ^{1}H-nmr and ms data. The vicinal proton coupling constants for the AMX and the ABX systems [$H_2C(4)$-CH(3) and $H_2C(12)$-CH(11), respectively] in its ^{1}H-nmr spectrum suggest a β configuration for the hydroxyl group on C-3 and a haemanthamine-like stereochemistry for the group at C-11. As expected, this result is in agreement with the values of the coupling constants observed for the same systems in the ^{1}H-nmr spectrum of haemanthamine. The prominent peaks at *m/z* 269, 268, 258, 227, 225, and 181 in its mass spectrum, in comparison with the fragmentation pathway described for haemanthamine-type alkaloids (5), confirmed the stereostructure assigned to 11-hydroxyvittatine.

The alkaloid formed an *O,O'*-diacetylderivative by reaction with pyridine and Ac_2O and was converted in good yield into apohaemanthamine by treatment with mineral acid (6).

EXPERIMENTAL

PLANT MATERIAL.—A voucher specimen of *S. lutea* used in this study is deposited at the Istituto di Botanica, Università di Bari, Bari, Italy.

EXTRACTION AND FRACTIONATION OF ALKALOIDS.—The dried and powdered bulbs (1 kg) of *S. lutea* were extracted with 1% H_2SO_4. The neutralized aqueous solution was partitioned with EtOAc according to our reported procedure (3) and afforded a crude oily residue. The oil was chromatographed on a SiO_2 column; elution with $CHCl_3$-EtOAc-MeOH (1:1:1) furnished fractions containing 11-hydroxyvittatine together with an unknown alkaloid. Purification of the residue obtained from this eluate, performed on SiO_2 plates (same solvent), yielded pure 11-hydroxyvittatine (18.6 mg, 0.0019%): mp 244-247° ($CHCl_3$); $[\alpha]^{25}D$ +11.3° (c=0.88 MeOH) [lit. (4) mp 248-250°; $[\alpha]^{25}D$ +12.0° in MeOH]; uv λ max (EtOH) nm (log ε) 293 (3.57), 236 (3.42); ir ($CHCl_3$) ν max 3685, 3600, 1600, 1505, 1485 cm^{-1}; ^{1}H nmr (270 MHz, CD_3OD) δ 6.94 (s, H-10), 6.56 (s, H-7), 6.41 (d, *J*=9.9 Hz, H-1), 6.19 (dd, *J*=9.9, 4.8 Hz, H-2), 5.89 (s, 2H, H-13), 4.35 (d, *J*=16.9 Hz, H-6X), 4.28 (ddd, *J*=4.8, 4.8, 1.5 Hz, H-3), 3.98 (dd, *J*=7.0, 3.7 Hz, H-11), 3.84 (d, *J*=16.9 Hz, H-6A), 3.50 (dd, *J*=13.6, 7.0 Hz, H-12A), 3.47 (dd, *J*=13.6, 4.8 Hz, H-4A), 3.20 (dd, *J*=13.6, 3.7 Hz, H-12B), 2.28 (ddd, *J*=13.6, 4.8, 13.6 Hz, H-4M), 1.85 (ddd, *J*=1.5, 4.8, 13.6 Hz, H-5); ^{13}C nmr (67.88 MHz, CD_3OD) δ 148.4 (C-8), 147.9 (C-9), 136.9 (C-6a), 133.2 (C-2), 127.6 (C-1), 126.3 (C-10a), 107.9 (C-7), 104.4 (C-10), 102.4 (C-13), 80.8 (C-3), 64.6 (C-11), 64.0 (C-5), 63.7 (C-12), 61.5 (C-6), 51.4 (C-10b), 32.8 (C-4); ms *m/z* (rel. int.) 287 (M^+) (35), 269 (37), 268 (37), 258 (73), 243 (77), 227 (86), 225 (57), 211 (40), 181 (100).

Acetylation of 11-hydroxyvittatine, carried out with pyridine and Ac_2O under the usual conditions, afforded the corresponding *O,O'*-diacetylderivative: mp 170-174° [lit. (4): 178-180°]; $[\alpha]^{25}D$ −63.1° (c=1.72 $CHCl_3$); uv λ max (EtOH) nm (log ε) 290 (3.62), 235 (3.50); ir ($CHCl_3$) ν max 1735, 1730, 1600, 1505, 1485 cm^{-1}; ^{1}H nmr (270 MHz, $CDCl_3$) δ 5.36 (ddd, *J*=4.5, 4.5, 1.5 Hz, H-3), 4.97 (dd, *J*=7.0, 4.0 Hz, H-11), 1.99 and 1.96 (s, 3H each, two MeCO), the other ^{1}H resonances being very similar to those reported for the 11-hydroxyvittatine; ms *m/z* (rel. int.) 371 (M^+) (49), 312 (22), 311 (16), 269 (41), 268 (37), 251 (37), 224 (100), 181 (74), 43 (64). Treatment of the alkaloid with 6N HCl, according to the described procedure (6), gave apohaemanthamine: $[\alpha]^{25}D$ +182° (c=0.21 EtOH); uv λ max (EtOH) nm (log ε) 293 (3.35); ir (CCl_4) ν max 2960, 1505, 1485, 1020 cm^{-1}; [lit. (6): mp 146-148°; $[\alpha]^{25}D$ +204° (c=1.03 EtOH); uv λ max (EtOH) nm (log ε) 296 (3.73); ir (CCl_4) ν max 3067 cm^{-1}]; ^{1}H nmr (500 MHz, $CDCl_3$) δ 6.91 (dd, *J*=8.5, 5.5 Hz, H-2), 2.51 (br d, *J*=15.1, 1.5 Hz, H-5), the other ^{1}H resonances being very close to those reported for the 11-hydroxyvittatine; ms *m/z* (rel. int.) 269 (M^+) (100), 240 (38), 225 (20), 211 (20), 181 (48). Apohaemanthamine and the product obtained from haemanthamine (6), applying the same procedure, showed the same Rf value in three tlc systems [SiO_2,

$CHCl_3$-EtOAc-MeOH, 2:2:1, and $CHCl_3$–iPrOH, 9:1, and reverse phase (Stratocrom C-18, Whatman), H_2O-EtOH, 6:4].

Full details on the isolation and identification of the compounds are available on request to the author.

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LITERATURE CITED

1. G.K. Phokas, *Farmakeftikon Deltion*, **1**, 9 (1971).
2. A. Evidente, I. Iasiello, and G. Randazzo, *J. Nat. Prod.*, **47**, 1061 (1984).
3. A. Evidente, I. Iasiello, and G. Randazzo, *J. Nat. Prod.*, **47**, 1003 (1984).
4. W.C. Wildman, in: *The Alkaloids*. Ed. by R.H.F. Manske, Vol. XI, Academic Press, New York, 1968, p. 355.
5. A.M. Duffield, R.T. Aplin, H. Budzikiewicz, C. Djerassi, C.F. Murphy, and W.C. Wildman, *J. Am. Chem. Soc.*, **87**, 4902 (1965).
6. H.M. Fales and W.C. Wildman, *J. Am. Chem. Soc.*, **82**, 197 (1960).

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THE FLAVONOIDS OF *AUREOLARIA VIRGINICA*

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Aureolaria virginica L. (Scrophulariaceae) is a wild, annual herb distributed over most of the southern United States (1). The plant is usually found in dry, open woods where it is parasitic upon the roots of oaks (2). No chemical work has previously been reported on this plant. During our search for the pharmacologically active compounds, the flavonoids apigenin, apigenin-7-*O*-glucoside, kaempferol-3-*O*-rhamnoside, quercitrin, and quercetin-3-*O*-arabinopyranoside were isolated from the leaves.

EXPERIMENTAL

PLANT MATERIAL.—Leaves of *A. virginica* were collected in August 1984, from the Oconee Forest, School of Forest Resources, University of Georgia, Athens, Georgia. The plant was identified by Nancy C. Coile, Herbarium, Department of Botany, University of Georgia. A voucher specimen is deposited in the Medicinal Chemistry and Pharmacognosy Department, College of Pharmacy, University of Georgia.

EXTRACTION, SEPARATION AND IDENTIFICATION.—The leaves (700 g) were cold extracted with 85% MeOH followed by 50% MeOH. The combined methanolic extracts were concentrated under reduced pressure until only the H_2O remained. The aqueous layer was then extracted with *n*-hexane followed by $CHCl_3$ and finally EtOAc. Paper chromatography indicated that only the EtOAc and $CHCl_3$ fractions contained flavonoids. The material from the $CHCl_3$ fraction (10.2 g) was chromatographed over a silica gel column (5×70 cm) using $CHCl_3$-MeOH mixtures. Apigenin (31 mg) was obtained from the $CHCl_3$-MeOH (96:4) fraction. THe EtOAc fraction (8.2 g) was chromatographed over a polyamide column (5×80 cm) eluted with MeOH-H_2O (3:13) with increasing amounts of MeOH to 100%. The flavonoids isolated from this column were apigenin-7-*O*-glucoside (95 mg), kaempferol-3-*O*-rhamnoside (25 mg), and a mixture of quercitrin and quercetin-3-*O*-arabinopyranoside (105 mg). This mixture was further separated by droplet counter current chromatography (dccc) using the upper layer of $CHCl_3$-MeOH-H_2O (7:13:8) as the mobile phase. All the flavonoids were purified before spectral analysis on columns of Sephadex LH20 (100×2 cm) eluted with MeOH. The flavonoid structures were determined by standard methods of uv, ^1H nmr, ^{13}C nmr, and mass spectroscopy (3-5). Sugars were identified from their gas liquid chromatograms after hydrolysis and trimethylsilylation (6). Further confirmation of the structures was made by comparison of the aglycones with reference compounds.

LITERATURE CITED

1. W.H. Duncan and L.E. Foot, "Wild Flowers of the Southeastern United States," The University of Georgia Press, Athens, 1975, p. 176.