Journal of Natural Products
Vol. 49, No. 2, pp. 351-353, Mar-Apr 1986

SESQUITERPENE LACTONES AND OTHER CONSTITUENTS OF *VERNONIA MOLLISSIMA* AND *VERNONIA SQUAMULOSA*

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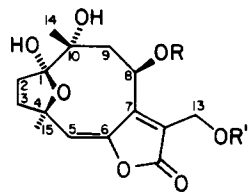
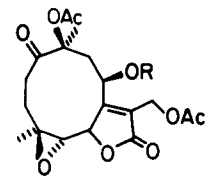
In an earlier article (1), two of us described isolation of several glaucolides from *Vernonia fulta* as part of our continuing study of Argentine Compositae. We now report isolation of the piptocarphol esters **1b-1d** from *Vernonia mollissima* Don. and piptocarphin A (**1e**) and glaucolide A (**2a**) from *Vernonia squamulosa* Hook *et* Arn. Glaucolide G (**2b**) accompanied **2a** in *V. squamulosa* but was not isolated in pure form. Lactone **1d**, the acetate of piptocarphin F (**1f**) is new. Various triterpenes, triterpene acetates, and sterols were also found in both species; in addition, *V. mollissima* contained scopoletin (**3**) and loliolide (**4**).

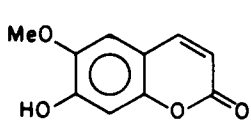
Compounds **1b-e**, which are derivatives of the unknown piptocarphol (**1a**), were identified by nmr spectrometry and comparison with data in the literature (2-4). Lactone **1b** was originally found in *Vernonia scorpioides* and *Vernonia saltensis*, although the C-10 stereochemistry was misrepresented (3,4). Piptocarphin D (**1c**) and piptocarphin A (**1e**) have been isolated from *Piptocarpha chontalensis* together with **1f** and related compounds (3). In the ^{1}H-nmr spectrum of **1d**, the signal of H-8 is a broadened doublet at δ 6.32 characteristic of a β-oriented ester function (2-4) while the chemical shift of the AB system of H-13a,b, centered at 4.38 is evidence for attachment of the ethoxy group (methyl triplet at δ 1.20, methylene quartet at δ



1a R, R′=H
1b R, R′=Ac
1c R=H, R′=Ac
1d R=Ac, R′=Et
1e R=MeAcr, R′=Ac
1f R=H, R′=Et



2a R=MeAcr
2b R=Ang



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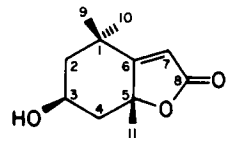

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3.55) to C-13. It is possible that **1d** is an artifact of the procedure used for the isolation of the constituents. Glaucolide A is a common lactone constituent of Western hemisphere *Vernonia* species (5).

EXPERIMENTAL

PLANT MATERIAL.—Aerial parts of *V. mollissima* were collected by Dr. J.C. Oberti and associates of the Universidad Nacional de Córdoba on December 11, 1981, in Potrero de Garay, Lago Los Molinos, Dpto. Santa Maria, Cordoba, Argentina, and were identified by Dr. Luis Ariza Espinar of the Museo Botanico, Universidad Nacional de Cordoba. Aerial parts of *V. squamulosa* were collected by CANC and associates in Horco Molle, Tucuman, Argentina, on September 5, 1982. (voucher no. CANC 21 deposited in Instituto Miguel Lillo, S.M. de Tucuman).

EXTRACTION OF *V. MOLLISSIMA*.—Dried and ground plant material (2.00 kg) was extracted with 2×11 liters of $CHCl_3$ at room temperature for 6 days to yield 51 g of extract which was suspended in 600 ml of EtOH at 50-60°, diluted with 400 ml of H_2O and extracted successively with hexane (3×250 ml) and $CHCl_3$ (4×200 ml). Evaporation of the hexane extract gave a syrup (32 g) which was dissolved in hexane/EtOAc, decolorized with charcoal, filtered, and chromatographed over Si gel using hexane and increasing amounts of Et_2O, all fractions being monitored by tlc. This gave 901 mg of triterpene acetates, 3.873 g of pentacyclic triterpenes, and 660 mg of sterols. Reversed-phase hplc of the pentacyclic triterpene fraction using a semipreparative Altex Ultrasphere ODS column (5 μm, 10 mm id×25 cm) and MeOH as eluting solvent at a flow rate of 2.0 ml/min gave three cleanly separated peaks in the ratio 5:21:74 which were collected separately and identified as lupeol [rt 42.5 min, mp 214-215°, ^{1}H nmr (100 MHz) and ms identical with that of authentic material], β-amyrin [rt 51 min, mp 199-200°, ^{1}H nmr (300 MHz) and ms identical with that of authentic material) and α-amyrin, [rt 56 min, mp 184-186°, ^{1}H nmr (300 MHz) and ms identical with that of authentic material]. Saponification of the triterpene acetates and separation of the tree alcohols by reversed phase hplc yielded lupeol, β-amyrin, and α-amyrin in the ratio 11:29:60. Separation of the sterol fraction by hplc with MeOH at a flow rate of 1.8 ml/min gave four peaks in the ratio of 7:109:82:2 which were identified as cholesterol (rt 49.5 min), stigmasterol (rt 54 min), sitosterol (rt 61 min) and sitostanol (rt 68 min). All compounds were characterized by ms, ^{1}H nmr, and comparison with authentic material.

Evaporation of the $CHCl_3$ extract gave a residue (18 g) which was worked up in the usual fashion (6). The crude gum (12.1 g) was purified by flash chromatography over Si gel (350 g) using $CHCl_3$ and increasing amounts of Et_2O (0-20%). The first fractions consisted of 9.8 g of oily lipids, while the last fractions (750 mg) contained lactones (ir absorption at 1730-1770 cm^{-1}). Repeated preparative tlc of the latter (Si gel, hexane-Me_2CO-EtOAc, 5:2:2) gave 81 mg of pure **1b**; the remaining lactone fractions were further purified by reversed phase hplc (Altex column, MeOH-H_2O, 4:3, flow rate 2.0 ml/min), thus affording **1d**.

Diacetylpiptocarphol (**1b**), (2) colorless gum, ir (KBr) 3450 (br), 1770-1730 (very br), 1370, 1225, 1015, 935 cm^{-1}; uv (MeOH) λ max 286 nm (ε 13800); ^{1}H nmr (60 MHz, $CDCl_3$) δ 6.28 (brd, J=8 Hz, H-8), 5.93 (H-5), 5.10 and 4.95 (AB quartet, J=13 Hz, H-13), 4.2 and 2.4 (br, disappear on D_2O exchange, OH), 2.56 (dd, J=15,8 Hz, H-9), 2.09 and 2.07 (two Ac), 1.57 and 1.53 (2 Me); ms *m/z* (%) 396 (M^+, 2), 336 (1), 321 (1.5), 294 (3), 276 (8), 234 (19), 218 (15), 216 (16), 191 (10), 188 (16), 163 (13), 148 (19), 43 (100).

8-Acetyl-13-ethoxypiptocarphol (**1d**, 35 mg) colorless gum, rt 23 min; ir (KBr) 3470 (br) and 1760-1730 (very br); uv (MeOH) λ max 285 nm (ε 17000); ^{1}H nmr (60 MHz, $CDCl_3$) δ 6.32 (dbr, J=9 Hz, H-8), 5.84 (H-5), 4.46 and 4.30 (AB quartet, J=12 Hz, H-13a,b), 3.60 (br, 2H, exchangeable with D_2O), 3.55 (2H, q, J=6.5 Hz, OCH_2-CH_3), 2.50 (dd, J=15.5, 9 Hz, collapses, to d on irradiation at frequency of H-8, H-9β), 2.10 (Ac), 1.57 and 1.23 (2 Me), 1.20 (t, J=6.5 Hz, collapses to singlet on irradiation at δ 3.55, -OCH_2CH_3). Due to decomposition in transit, the ms was not determined.

Piptocarphin D (**1c**, 3 mg) (3), colorless gum, rt 13.5 min; ^{1}H nmr (270 MHz, $CDCl_3$) 6.10 (d, J=12 Hz, 8-OH, disappeared after addition of D_2O), 5.87 (H-5), 5.45 (td, J=12,2 Hz, collapsed to dd on addition of D_2O, H-8), 4.96 and 4.83 (AB system of H-13a,b, J=13 Hz), 4.79 (br) and 4.50 (d, J=2 Hz, two -OH, disappeared on addition of D_2O), 2.53 (dd, J=15, 12 Hz) and 1.91 (dd, J=15, 2 Hz, H-9a,b, 2.42 (ddd, H-2a or H-3a partially superimposed on H-9a) 2.08 (Ac, superimposed on complex multiplet 1.65 (H-15 partially superimposed on complex multiplet), 1.22 (br, H-14).

Scopoletin (**3**, 8 mg), mp 203-205°, rt 10 min, was identical with an authentic sample (ir, uv, ^{1}H nmr). Loliolide (**4**, 10 mg), mp 149-150°, rt 12 min, had properties which corresponded with those in the literature; the previously unreported ^{13}C-nmr spectrum (67.89 MHz, $CDCl_3$) exhibited signals at δ 35.85s (C-1), 47.42t and 45.76t (C-2 and C-4), 66.91d (C-3), 86.50s (C-5), 113.13s (C-6), 113.03d (C-7),

171.66s (C-8), 30.67q (probably C-9, deshielded by OH), 27.12q and 26.59q (C-10 and C-11).

EXTRACTION OF *V. SQUAMULOSA*.—Dried and ground plant material (342 g) was extracted successively with hexane and $CHCl_3$. Column chromatography (Si gel) of the residue (11.6 g) from the hexane extract as described in the previous section furnished 1.22 g of pentacyclic triterpenes, which were separated by hplc to give lupeol, β-amyrin, and α-amyrin in the ratio 27:23:50, and 180 mg of an equimolecular mixture of sitosterol and stigmasterol which were also separated by hplc. Workup of the residue from the $CHCl_3$ extract in the usual fashion and column chromatography (Si gel, 250 g) of the crude gum (7.5 g), initially with $CHCl_3$ (500 ml) and then with mixtures of $CHCl_3$ containing increasing proportions of Et_2O, afforded 3.62 g (1.06%) of glaucolide A (**2a**) mp 154°, whose spectral properties coincided with those reported in the literature (8). A contaminant (approx 15%) of slightly higher Rf in the first two fractions containing glaucolide A was the angelate analog glaucolide G (**2b**) (9) since the ^{1}H-nmr spectrum of the mixture exhibited an extra multiplet at δ 6.40 characteristic of H-3′ of an angelate and since the ms of the mixture, if compared with the ms of pure glaucolide A, exhibited peaks at *m/z* 478 (M^+ of **2b**), 418 (M^+-AcOH), 378 (M^+-$C_5H_8O_2$), and an intense peak at *m/z* 83 ($C_5H_7O^+$).

The last fractions from the $CHCl_3$ extract yielded 0.931 g 0.27%) of piptocarphin A (**1e**), ir (KBr) 3350-3450 (broad) and 1760-1720 cm^{-1} (broad); uv (MeOH) λ max 286 nm (ε 15000); ^{1}H nmr (300 MHz, $CDCl_3$) δ 6.59 (brd, J=9 Hz, H-8), 6.28 (br) and 5.68 (br, H-3′a,b), 5.90 (H-5), 5.33, and 4.90 (AB quartet, J=13 Hz, H-13a,b), 4.1 (br) and 3.85 (br, -OH), disappeared on exchange with D_2O), 2.62 (dd, J=16, 11 Hz, H-9β), 2.43 (td, J=7, 12 Hz, one of H-2a,b or H-3a,b), 2.08 (Ac partially superimposed on a 1p multiplet), 1.95 (H-4′ partially superimposed on a 1p multiplet) 1.58 (H-15 partially superimposed on 2p multiplet) and 1.24 (br, H-14); ms *m/z* (%) 422 (M^+, 2), 362 (0.7), 344 (0.7), 336 (0.5), 320 (1), 300 (0.4), 276 (7), 234 (18), 148 (35), 69 (100).

ACKNOWLEDGMENTS

Thanks are due to Dr. J.C. Oberti for one of the collections. Work in Tucumán was suppored by a grant from SECYT; we also thank CONICET and the Rectorado de la Universidad Nacional de Tucumán for financial support.

LITERATURE CITED

1. C.A.N. Catalán, P.R. Legname, B.V. Crist, and D.I.A. de Iglesias, *Phytochemistry*, **24**, 2113 (1985).
2. F. Bohlmann, P.K. Mahanta, and L.N. Dutta, *Phytochemistry*, **18**, 289 (1979).
3. P.L. Cowall, J.M. Cassady, C.J. Chang, and J.F. Kozlowski, *J. Org. Chem.*, **46**, 1108 (1981).
4. W. Herz and P. Kulanthaivel, *Phytochemistry*, **22**, 1286 (1983).
5. F.C. Seaman, *Bot. Rev.*, **48**, 121 (1982).
6. W. Herz and G. Högenauer, *J. Org. Chem.*, **27**, 905 (1962).
7. G.R. Pettit, C.L. Herald, R.H. Ode, P. Brown, D.J. Gust, and C. Michael, *J. Nat. Prod.*, **43**, 752 (1980).
8. W.G. Padolina, H. Yoshioka, N. Nakatani, T.J. Mabry, S.A. Monti, R.E. Davis, P.J. Cox, G.A. Sim, W.H. Watson, and I. Beth Wu, *Tetrahedron*, **30**, 1161 (1974).
9. M. Betkouski, T.J. Mabry, T.W. Adams, W.H. Watson, and S.B. Jones, *Rev. Latinoamer. Quim.*, **7**, 111 (1976).

Received 11 October 1985