Pergamon

PII: S0031-9422(97)00703-6

Phytochemistry, Vol. 47, No. 3, pp. 425–428, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

GLAUCOLIDES AND HIRSUTINOLIDES FROM *VERNONANTHURA SQUAMULOSA*

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(*Received in revised form* 19 *May* 1997)

Key Word Index—*Vernonanthura squamulosa*; Asteraceae; Vernonieae; Vernoniinae; sesquiterpene lactones; glaucolides; hirsutinolides.

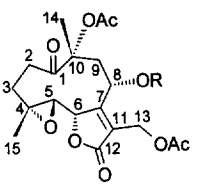
Abstract—Reinvestigation of the aerial parts of *Vernonanthura squamulosa* afforded a known and a new glaucolide, and eleven hirsutinolides of which five are new.

INTRODUCTION

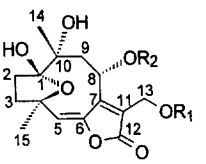
In continuation with our work on Argentine species of Vernoniinae, we have carried out an exhaustive examination of the minor constituents of *Vernonanthura squamulosa* (Hook. et Arn.) H. Robinson, formerly *Vernonia squamulosa* Hook. *et* Arn. [1]. In a previous article [2] we reported glaucolide A (**1b**) [3, 4], glaucolide G (**1c**) [5] and piptocarphin A (**3d**) [6]. In addition, this work describes the isolation of the new natural product glaucolide J (**1a**) and the new hirsutinolides **2a**, **2b**, **3a**, **3b** and **3c**, together with the previously described hirsutinolides **3d**–**3h**, known as piptocarphins due to their apparent natural origin in *Piptocarpha chontalensis* [6]. These results reinforce the fact that glaucolides are characteristic constituents of *Vernonia* and *Vernonanthura* [7, 8]

RESULTS AND DISCUSSION

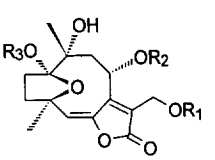
The structure of lactone **1a** was determined when its ^{1}H NMR spectrum was compared with that of glaucolide A (**1b**) [3, 4]. As is characteristic for glaucolide derivatives, the ^{1}H NMR spectrum of **1a** in $CDCl_3$ at room temperature exhibited broad signals due to a conformational equilibrium. Therefore, ^{1}H NMR measurements were carried out in C_6D_6 at 45°C (Table 1). The signals corresponding to the isobutyrate residue at δ 2.22, 0.91 and 0.89 together with ^{1}H-^{1}H decoupling experiments, the ^{13}C NMR data (Table 2) and the EI mass spectrum were in agreement with the structure and the relative stereochemistry of **1a**, now named glaucolide J.



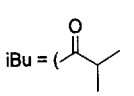
	R
1a	iBu
1b	Meacr
1c	Ang

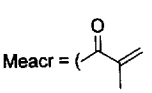


	R_1	R_2
2a	Ac	Tigl
2b	Et	Meacr



	R_1	R_2	R_3
3a	Et	H	H
3b	Ac	Meacr	Me
3c	Ac	iBu	H
3d	Ac	Meacr	H
3e	Ac	Tigl	H
3f	H	Meacr	H
3g	Ac	H	H
3h	Ac	Meacr	Et
3i	Et	Meacr	H





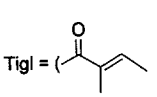


Table 1. ^{1}H NMR spectra of compounds **1a**, **2a**, **2b** and **3a**–**3c*** (measured at 300 MHz)

H	1a†	1a‡	2a‡	2b‡	3a‡	3b‡	3c‡
H-2a	1.50–1.90	2.50–2.80	1.70–2.70	1.70–2.70	2.41 *m*	2.30 *m*	2.43 *m*
H-2b	2.28 *ddd* (16, 11, 5)	2.50–2.80	1.70–2.70	1.70–2.70	2.15 *m*	1.80–2.10	1.80–2.20
H-3a	1.90–2.50	2.50–2.80	1.70–2.70	1.70–2.70	1.80–2.10	1.80–2.10	1.80–2.20
H-3b	1.24 *br m*	1.60 *br m*	1.70–2.70	1.70–2.70	1.80–2.10	1.80–2.10	1.80–2.20
H-5	2.09 *br d* (10)	2.50–2.80	6.08 *s*	6.00 *s*	5.81 *s*	5.93 *br s*	5.91 *br s*
H-6	4.68 *br d* (10)	4.88 *br d* (10)	—	—	—	—	—
H-8	4.82 *dd* (8, 1)	4.78 *br s*	5.52 *t* (3)	5.49 *t* (3)	5.54 *ddd* (12, 11, 2)	6.49 *br m*	6.47 *br m*
H-9a	2.38 *dd* (16, 8)	2.90 *br m*	2.65 *dd* (15, 3)	2.60 *m*	2.51 *dd* (16, 11)	2.54 *dd* (16, 9)	2.60 *m*
H-9b	1.50–1.90	2.50–2.80	2.31 *dd* (15, 3)	1.50–2.30	1.96 *dd* (16, 2)	2.10 *m*	1.80–2.20
H-13a	4.99 *br d* (13)	4.93 *br d* (13)	4.89 *s*	4.32 *d* (13)	4.30 *s*	5.21 *br d* (13)	5.29 *br d* (13)
H-13b	4.80 *br d* (13)	4.80 *br d* (13)	4.89 *s*	4.19 *d* (13)	4.30 *s*	4.92 *d* (13)	4.91 *br d* (13)
Me-14¶	1.47 *s*	1.56 *br s*	1.25 *s*	1.35 *s*	1.23 *s*	1.25 *s*	1.24 *s*
Me-15¶	1.47 *s*	1.65 *s*	1.59 *s*	1.58 *s*	1.67 *s*	1.62 *s*	1.56 *s*

*Coupling constants (Hz) in parentheses. Substituent signals for **1a**†: 1.72 *s* and 1.60 *s* [acetates]; 2.22 *hept* (7), 0.91 *d* (7) and 0.89 *d* (7) [isobutyrate]. **1a**‡: 2.13 *s* and 2.07 *br s* [acetates]; 2.57 *hept* (7), 1.19 *d* (7) and 1.15 *d* (7) [isobutyrate]. **2a**: 2.05 *s* [acetate]; 6.95 *dq* (7, 1), 1.83 *br d* (7) and 1.82 *br s* [tiglate]; 3.85 *br s* and 3.75 *br s* [2 OH]. **2b**: 6.23 *br s*, 5.71 *br s* and 1.98 *br s* [methacrylate]; 3.65 *br s* [OH]; 3.50 *q* (7) and 1.21 *t* (7) [OEt]. **3a**: 6.20 *d* (12), 4.82 *br s* and 4.50 *br s* (3 OH); 3.55 *dq* (7, 2) and 1.21 *t* (7) [OEt]. **3b**: 2.09 *s* [acetate]; 6.31 *br s*, 5.69 *m* and 1.97 *br s* [methacrylate]; 3.54 *s* [OMe]; 3.43 *br s* [OH]. **3c**: 4.08 *br s* and 3.93 *br s* [2 OH]; 2.08 *s* [acetate]; 2.57 *hept* (7), 1.19 *d* (7), 1.17 *d* (7) [isobutyrate].

†In C_6D_6.

‡In $CDCl_3$.

¶Intensity three protons.

Table 2. ^{13}C NMR data of compounds **1a** and **3c** (measured at 75.4 MHz in $CDCl_3$)

C	1a*	3c
C-1	—†	108.7
C-2	32.9	31.8
C-3	32.1	37.5‡
C-4	61.5	82.1
C-5	59.1	126.8
C-6	80.8	150.0
C-7	162.8	144.0
C-8	63.9	66.5
C-9	49.7	38.1‡
C-10	—†	78.1
C-11	—†	131.2
C-12	169.6	166.5
C-13	55.2	55.8
C-14	18.1‡	25.4
C-15	18.9‡	29.1
C-1′	166.3	175.4
C-2′	33.5	34.1
C-3′	18.8	18.9
C-4′	18.4	18.4
C-1″	170.7	170.3
C-2″	20.9	20.7
C-1‴	170.2	—
C-2‴	21.3	—

*Assigned by comparison with data described in ref. [13].

†Not observed.

‡Assignments may be interchanged.

Inspection of the ^{1}H NMR spectra of lactones **2a** and **2b** revealed that **2a** is the C-1 epimer of piptocarphin B (**3e**), while **2b** corresponds to the C-1 epimer of piptocarphin F (**3i**) [6]. We already pointed out [9, 10] that the ^{1}H NMR spectra of such hemiketals permit assignment of such compounds to two groups, according to the stereochemistry at C-1. The members of each group can be readily identified according to the chemical shift and coupling constants observed in the H-8 signal. Group A includes those compounds having the α-hydroxyl group at C-1. In this group, the coupling constants $J_{8,9a}$ and $J_{8,9b}$ differ greatly in magnitude ($J_{8,9a}$ = 1–3 Hz and $J_{8,9b}$ = 8–11 Hz) and the H-8 resonance is unusually downfield for a proton geminal to an ester residue (δ 6.30–6.50 in $CDCl_3$). Group B, with the β-hydroxyl group at C-1, is characterized by equal and relatively small (*ca.* 3 Hz) $J_{8,9a}$ and $J_{8,9b}$ coupling constants and the H-8 signal is found usually near δ 5.50. Since in compounds **2a** and **2b** the H-8 signal appears as a triplet ($J_{8,9a}$ and $J_{8,9b} \approx 3$ Hz) at 5.52 and 5.49, respectively, they clearly belong to group B.

The ^{1}H NMR spectra of lactones **3a**, **3b** and **3c** suggested that they have a piptocarphin framework with various substituents at C-1, C-8 and C-13. In the spectrum of lactone **3a**, the chemical shift of the C-13 methylene hydrogens at δ 4.30 and the methyl triplet at δ 1.21 and methylene quarter at δ 3.55 evidenced the presence of an ethoxyl group at C-13, as in piptocarphin F (**3i**) [6]. The position of the H-8 resonance at δ 5.54 (*ddd*) and its coupling to the proton at δ 6.20 (exchangeable with D_2O) demonstrates the presence of a free hydroxyl group at C-8. Lactone **3b** is probably an artifact derived from piptocarphin A (**3d**) with a methyl ether at C-1 as shown by the chemical shift

of a singlet at δ 3.54. Finally, lactone **3c** is a piptocarphin A derivative in which the methacrylate residue at C-8 has been replaced by an isobutyrate residue, as deduced by the presence of a septet at δ 2.57 and of two secondary methyl groups at δ 1.19 and 1.17 in the ^{1}H NMR spectrum. Since the transformation of sesquiterpene lactones like glaucolide A (**1b**) into hirsutinolides has been shown to occur during the isolation procedures [11], lactone **3c** also could be an artifact derived from glaucolide J (**1a**). Several related hirsutinolides are known [12].

EXPERIMENTAL

General. HPLC with differential refractometer was used for the sepn of mixtures. The columns employed were: (A) a Beckman Ultrasphere C-8 (5 μ, 10 × 250 mm) and (B) a Phenomenex C-18 (5 μ, 10 × 250 mm). R_fs were measured from the solvent peak.

Plant material. Aerial parts of *Vernonanthura squamulosa* (Hook. *et* Arn.) H. Robinson were collected at the flowering stage in Horco Molle, Tucumán, Argentina, in August 1993. A voucher specimen (CANC No. 21) is on deposit in the herbarium of the Fundación Miguel Lillo, Tucumán, Argentina).

Extraction and isolation. Plant material was divided in two portions and extracted and processed in two different ways.

Procedure 1: flowers and leaves (342 g) were extracted successively with hexane and $CHCl_3$ at room temp. for 7 days. Evapn under vacuum gave 7.5 g of residue (2.2%) which was subjected to CC over silica gel using $CHCl_3$ with increasing amounts of Et_2O as the eluent to give 80 fractions. Frs 15–19 (210 mg) were processed by HPLC (column A, MeOH—H_2O 4:3, 2 ml min^{-1}) to give **3e** (1.1 mg, R_t 24 min) and **2b** (2.4 mg, R_t 26 min). Frs 20–34 (113 mg) were processed by HPLC (column B, MeOH—H_2O 4:3; 2 ml min^{-1}) to give **3a** (1.5 mg; R_t 10 min), **3d** (30.6 mg, R_t 29 min) and **2a** (4.5 mg, R_t 41 min). From frs 35–45, glaucolide A (927 mg) was isolated as the main constituent [2, 5]. HPLC (column B, MeOH—H_2O 4:3, 2 ml min^{-1}) of frs 46–62 (151 mg) gave **3g** (1.1 mg, R_t 14 min).

Procedure 2: flowers and leaves (642 g) were extracted with $CHCl_3$ (2 × 4 l.) at room temp. for 4 days to give 58.5 g (9.1 %) of a residue which was suspended in 503 ml of EtOH at 50°, diluted with 377 ml of H_2O, and extracted successively with hexane (2 × 200 ml), C_6H_6 (2 × 200 ml), $CHCl_3$ (2 × 150 ml) and EtOAc (free of AcOH, 2 × 100 ml). Evapn of the C_6H_6 extract under vacuum, gave 16.4 g of a residue which was flash chromatographed over silica gel using C_6H_6-EtOAc 5:1, to give 69 fractions. Frs containing sesquiterpene lactones, as indicated by IR absorption at *ca.* 1765 cm^{-1}, were further purified by HPLC. Frs 21–28 (400 mg) were chromatographed by HPLC (column B, MeOH—H_2O 3:2, 2.5 ml min^{-1}) to give **3d** (17.8 mg, R_t 14 min), **1b** (29.4 mg, R_t 7 min), **1a** (13.3 mg, R_t 19 min), **3b** (6.4 mg, R_t 21 min) and **3h** (15.4 mg, R_t 35 min). Frs 29–41 (738 mg) contained glaucolide A (723.2 mg) as the major constituent together with small amounts of **3d** (10.1 mg). Frs 42-52 (150 mg) were processed by HPLC (column B, MeOH-H_2O 3:2, 2 ml min^{-1}) to give **3d** (31.6 mg, R_t 20 min) and glaucolide A (40.8 mg, R_t 27 min). HPLC (column B, MeOH-H_2O 4:3, 2 ml min^{-1}) of frs 53–69 (340 mg) afforded **3f** (5.9 mg, R_t 11 min), **3d** (113.6 mg, R_t 25 min) and **3c** (5.1 mg, R_t 31 min).

(4R*,5R*,6S*,8S*,10R*)-10,13-*diacetyloxy*-4,5-*epoxy*-8-*isobutyryloxy*-1-*oxogermacr*-7(11)-*en*-6,12-*olide* (**1a**) *Glaucolide J.* Gum; UV λ_{max}^{MeOH} nm (log ε): 211 (4.51), 286 (3.37); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3028, 1770, 1742, 1458. $[\alpha]_{589}$ − 50°, $[\alpha]_{578}$ − 50°, $[\alpha]_{546}$ − 60°, $[\alpha]_{436}$ − 95°, $[\alpha]_{365}$ − 130° (*c* 0.2, EtOH). DCIMS (NH_3) *m/z* (rel. int.): 467 $[MH]^+$ (57.9), 407 $[MH—AcOH]^+$ (15.2), 347 $[MH—2AcOH]^+$ (2.5), 319 $[MH—iBuOH—AcOH]^+$ (8.9), 71 $[C_3H_7C=O]^+$ (59.9).

(1R*,4R*,8S*,10R*)-13-*acetyloxy*-1,4-*epoxy*-1,10-*dihydroxy*-8-*tigloyloxygermacra*-5E, 7(11)-*dien*-6,12-*olide* (**2a**). Gum; UV $\lambda_{max}^{MeOH/H_2O}$ nm: 287 (measured with the HPLC equipment). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1775, 1760, 1730, 1715, 1220.

(1R*,4R*,8S*,10R*)-1,4-*epoxy*-13-*ethoxy*-1,10-*dihydroxy*-8-*methacryloxygermacra*-5E, 7(11)-*dien*-6, 12-*olide* (**2b**). Gum; UV $\lambda_{max}^{MeOH/H_2O}$ nm: 283 (measured with the HPLC equipment).

(1S*,4R*,8S*,10R*)-1,4-*epoxy*-13-*ethoxy*-1,8, 10-*trihydroxygermacra*-5E,7(11)-*dien*-6, 12-*olide* (**3a**). Gum; UV $\lambda_{max}^{MeOH/H_2O}$ nm: 287 (measured with the HPLC equipment).

(1S*,4R*,8S*,10R*)-13-*acetyloxy*-1,4-*epoxy*-10-*hydroxy*-8-*methacryloxy*-1-*methoxygermacra*-5E,7(11)-*dien*-6,12-*olide* (**3b**). Gum; UV λ_{max}^{MeOH} nm (log ε): 210 (4.50), 286 (4.49), 343 (3.56). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3026, 2930, 1762, 1380. $[\alpha]_{589}$ + 35°, $[\alpha]_{578}$ + 38°, $[\alpha]_{546}$ + 45°, $[\alpha]_{436}$ + 115° (*c* 0.4, EtOH). DCIMS (NH_3) *m/z* (rel. int.): 405 $[MH—MeOH]^+$ (14.1), 387 $[MH—MeOH—H_2O]^+$ (2.5), 345 $[MH—AcOH—MeOH]^+$ (16.5), 319 $[MH—Meacr—MeOH]^+$ (11.5), 301 $[MH—Meacr—MeOH—H_2O]^+$ (9.6), 69 $[C_3H_5C=O]^+$ (59.5).

(1S*,4R*,8S*,10R*)-13-*acetyloxy*-1,4-*epoxy*-1,10-*dihydroxy*-8-*isobutyryloxygermacra*-5E,7(11)-*dien*-6,12-*olide* (**3c**). Gum; UV λ_{max}^{MeOH} nm (log ε): 214 (3.82), 286 (4.39). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540, 3016, 2852, 1760, 1462. $[\alpha]_{589}$ + 41°, $[\alpha]_{578}$ + 45°, $[\alpha]_{546}$ + 53°, $[\alpha]_{436}$ + 137° (*c* 0.7, EtOH). FABMS (MeOH/NBA/NaCl) *m/z* (rel. int.): 425 (4.5) $[MH]^+$, 407 (7.2) $[MH—H_2O]^+$, 347 (2.5) $[MH—AcOH—H_2O]^+$, 337 (3.3) $[MH—iBuOH]^+$, 319 (3.9) $[MH—iBuOH—H_2O]^+$, 277 (4.8) $[MH—iBuOH—AcOH]^+$.

Acknowledgments—The work in Tucumán was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina) and Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT). Partial financial support from CoNaCyT, México, and stimulating support from CYTED, Spain, is also acknowledged.

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