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GUAIANOLIDES AND OTHER CONSTITUENTS FROM *VERNONIA NITIDULA*

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IN MEMORY OF TONY SWAIN, 1922–1987

Key Word Index—*Vernonia nitidula*; Compositae; Vernonieae; guaianolides; sesquiterpene lactones.

Abstract—Chemical investigation of *Vernonia nitidula* resulted in isolation of 11 guaianolides, eight of them new, as well as other common plant constituents.

INTRODUCTION

In continuation of our work on Argentine *Vernonia* species [1–4] we have studied the aerial parts of *Vernonia nitidula* Less. Isolated were 11 closely related guaianolides **1a–f**, **2a–d** and **4** as well as apigenin, luteolin, isorhamnetin, hydroquinone and various commonly encountered triterpenes and sterols.

RESULTS AND DISCUSSION

Guaianolide **1c** was the major sesquiterpene lactone constituent; the other lactones were obtained in very small quantities only. Compounds **1a** (desacylcyanopicrin) [5], **1b** (aguerin B) [6] and **2a** (8α-acetoxyzaluzanin D) [7] are known, while **1c–f**, **2b–d** and **4** have not been described previously. Structures of **1c–f** and **2b–d** were obvious when their ^{1}H NMR spectra (Table 1, all connections and coupling constants established by spin decoupling) were compared with those of analogues such as cynaropicrin (**1h**) in our files and in the literature, e.g. [8, 9]. Additional chemical evidence was provided by acetylation of **1c** to naturally occurring **2c** and its oxidation with pyridinium chlorochromate to an α,β-unsaturated ketone **3**. ^{13}C NMR spectra of **1c** and **2c** are listed in Table 2 and confirm the tentative assignments reported recently [10] for **1i**. The absolute stereochemistry of these compounds is that represented in the formulas [11].

Structure and stereochemistry of the C-14 oxygenated guaianolide **4** were deduced from the MS and the ^{1}H NMR spectrum listed in Table 1. An alternative C-15 oxygenated structure was excluded by sequential decoupling involving H-5 through H-9.

Guaianolides of type **1** or derivatives thereof are typically found in Cardueae and to a lesser degree in Lactuceae, while glaucolides and related sesquiterpene lactones containing a 7,(11)-double bond and an acyl group on C-13 are characteristic constituents of the large genus *Vernonia* and its relatives. In a limited number of instances, however, guaianolides similar to or derived from compounds of type **1** have been isolated from *Vernonia* species, but generally only from the roots [12–21]. Whether this possesses any significance will require further study.

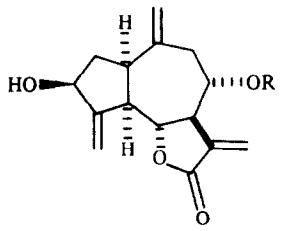
EXPERIMENTAL

General. For separation of mixtures Waters HPLC equipment (M45 pump, U6K injector with 2 ml loop and R-401 differential refractometer) was used. The column employed was an ALTEX Ultrasphere ODS column (5 μm, 10 mm inner diameter × 25 cm). R_fs were measured from the injection point.

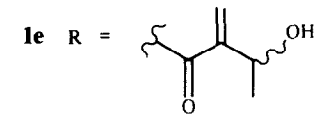
Plant material. Aerial parts of *Vernonia nitidula* Less. were collected at the flowering stage in October 1986 in Paraná, Entre Rios Province, Argentina by Mr J. M. Retamal (IPNAYS, Facultad de Ingeneria Química, Universidad Nacional del Litoral) (voucher CANC No. 25, Instituto Miguel Lillo, Tucumán.

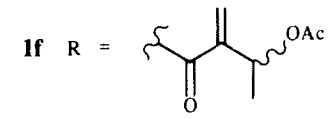
Extraction of V. nitidula. Flowers and leaves (450 g) were extracted with $CHCl_3$ (2 × 6 l) at room temp. for 7 days to give 27 g of crude extract (yield 6.0%) which was suspended in 230 ml of EtOH at 50–55°C, diluted with 172 ml of H_2O and extracted successively with *n*-hexane (3 × 500 ml) and $CHCl_3$ (3 × 500 ml). Evapn of the hexane extract gave 14.4 g of residue, a portion of which (6.0 g) was chromatographed over silica gel with *n*-hexane–Et_2O mixtures (20–50% Et_2O) as eluent. This afforded 900 mg of pentacyclic triterpenes, 50 mg of sterols and 600 mg of a fraction with R_f lower than the sterols. Reversed-phase HPLC of the triterpene mixture (MeOH, flow rate 2.7 ml/min) gave three peaks which were identified as lupeol, β-amyrin and a mixture of α-amyrin and Ψ-taraxasterol (GC, OV-17, 3%, 270°) by coinjection with authentic material in HPLC and GC-MS. The ratio lupeol: β-amyrin; α-amyrin: Ψ-taraxasterol was 4:3:5:2. The sterol fraction was saponified with dil. KOH in MeOH; the unsaponifiable material was separated by HPLC (MeOH, flow rate 2.4 ml/min) to give an equimolecular mixture of stigmasterol and sitosterol. The last fraction from the chromatogram (600 mg) furnished 490 mg of **1c**.

Evaporation of the $CHCl_3$ extract gave a residue (7.0 g) which showed a major spot on TLC. A 5 g portion of the residue was chromatographed (silica gel $CHCl_3$–Et_2O with 0–50% Et_2O, 51 fractions). Rechromatography of frs 5 and 6, which showed a

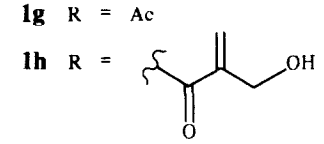


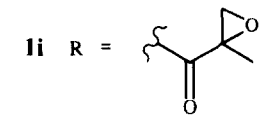
1a R = H
1b R = MeAcr
1c R = Ang
1d R = Tig

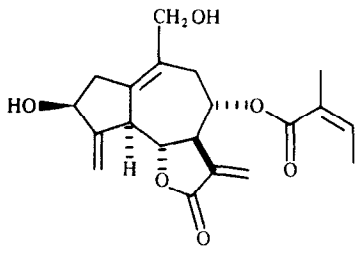




1g R = Ac







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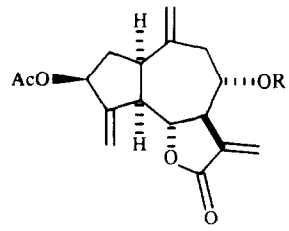
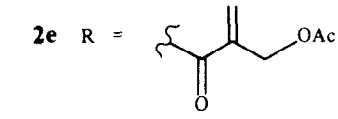
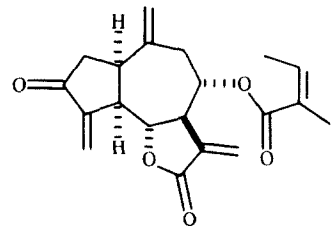


2a R = Ac
2b R = MeAcr
2c R = Ang
2d R = Tig





3

major spot on TLC, combined wt. 42 mg, over Florisil yielded 31 mg of **2c** as a gum. Frs 7–10 (20 mg) afforded after separation by RP-HPLC (MeOH–H_2O, 2.5:1, flow rate 1.5 ml/min) the following compounds: 8α-acetoxyzaluzanin D (**2a**, 1.7 mg, gum identified by IR, MS, PCI and ^1H NMR in the literature [7], **2b** (R_t 25 min, gum, 1.7 mg), **2d** (R_t 35 min, gum, 1.3 mg) and an additional 6.2 mg of **2c**.

Frs 11–22 (1.93 g) which showed a major spot on TLC were combined. Rechromatography (silica gel, $CHCl_3$–Et_2O 2:1) yielded 1.58 g of pure **1c**. Frs 23–31 (combined wt 0.54 g) after CC (silica gel, C_6H_6–Et_2O 1:1) gave 100 mg of hydroquinone identified by comparison with an authentic sample and then 306 mg of **1c**. Frs 32–34 (30 mg) on RP-HPLC (MeOH–H_2O, 1:1, flow rate 1.5 ml/min) gave 1 mg of aguerin B(**1b**, R_t 25 min) which was identified by direct comparison of IR, MS, MS PCI and ^1HNMR spectra with authentic material [22], 0.8 mg of desacylcynaropicrin (**1a**, R_t 28 min) identified by comparison (IR, MS, MS PCI, ^1HNMR with the literature [5], 1 mg of **1d** (R_t 33 min) and an additional 22 mg of **1c** (R_t 36 min).

Fr 35–41 which showed a major spot on TLC were combined to give 4 mg of apigenin, mp 346–348° (MeOH) identified by mmp, UV and ^1H NMR. Frs 42 and 43 were combined; purification by HPLC (MeOH–H_2O 2:1, flow rate 1.5 ml/min) gave 0.6 mg of **1f** (R_t 22 min), 1 mg of aguerin B (**1b**), R_t 26 min), 0.6 mg of **1a** (R_t 30 min), 0.5 g of **1d** (R_t 38 min) and 6.6 mg of **1c** (R_t 42 min). Intermediate fractions were mixtures. Frs 44 and 45 were combined and purified by HPLC to give 3 mg of a 2:1 mixture of luteolin and isorhamnetin (R_t 10 min) identified by paper chromatography and comparison of the eluted material with authentic material, 1 mg of **1f** (R_t 17 min) and 5 mg of **1c** (R_t min).

Purification of frs 46–48 by HPLC (MeOH–H_2O 4:3, flow rate 1.5 ml/min yielded 4 mg of luteolin, mp 327° (R_t 13 min). Fractions 49–51 were combined; separation by RP-HPLC (MeOH–H_2O 4:3) gave 8.5 mg of **1e** (R_t 9-10 min) and 10 mg of **4**.

(1S, 3S, 5R, 6R, 7R, 8S)-8-*Angelyloxy*-3-*hydroxyguai*-3 (15), 10 (14), 11(13)-*trien*-6,12-*olide* (**1c**). Mp 98.5–99° (Et_2O), IR ν^{KBr}

Table 1. ^{1}H NMR spectra of compounds **1c–f**, **2b–d** and **4** ($CDCl_3$, 270 MHz)

H	1c	1d	1e	1f	2b	2c	2d	4*	4
1	2.98*ddd*	2.97	3.00	2.99	3.03	3.04	3.01	—	—
2a	2.24*ddd*	2.25	2.25	2.23	2.32	2.38	2.38	2.39*dd*	2.17*dd*
2b	1.74*ddd*	1.75	1.75	1.76	1.80	1.82	1.80	2.24*dd*	2.07*dd*
3	4.57*brdd*	4.58	4.58	4.56	5.58	5.57	5.57	5.08*brt*	4.76*brt*
5	2.85*brdd*	2.85	2.87	2.85	2.85	2.86	2.85	2.95*brd*	2.78*d*
6	4.25*dd*	4.25	4.25	4.25	4.19	4.19	4.19	4.96*dd*	4.12*dd*
7	3.17*dddd*	3.19	3.21	3.18	3.20	3.19	3.19	3.39*dddd*	3.35*dddd*
8	5.10*ddd*	5.11	5.14	5.12	5.07	5.09	5.11	5.02*ddd*	5.07*ddd*
9a	2.72*dd*	2.70	2.74	2.70	2.70	2.70	2.69	2.87*dd*	2.71*dd*
9b	2.41*dd*	2.39	2.41	2.40	2.40	2.42	2.38	2.53*dd*	2.57*dd*
13a	6.23*d*	6.21	6.24	6.23	6.23	6.23	6.22	6.12*d*	6.18*d*
13b	5.62*d*	5.61	5.62	5.61	5.62	5.64	5.62	5.49*d*	5.61*d*
14a	5.13*br*	5.14	5.15	5.15	5.10	5.11	5.14	5.71‡	5.96‡
14b	4.96*br*	4.95	4.96	4.96	4.93	4.97	4.97		
15a	5.51*brs*	5.51	5.51	5.50	5.55	5.54	5.55	5.61*brd*	5.44*s*
15b	5.37*brs*	5.38	5.39	5.36	5.37	5.36	5.37	5.03*brd*	5.09*s*
-OAC†	—	—		2.03	2.10	2.10	2.10	4.71*s*(OH)	4.30*s*(OH)
3′	6.19*qq* (7.5, 1.5)	6.97*qq*	4.70*brq* (6)	5.70*brq*	6.19*d*(1) 5.69*dq*(2)	6.20*qq*	6.96*qq*	5.80*qq*	6.20*qq*
4′†	2.04*dq* (7, 1.5)	1.86*dq*	1.44*d* (6)	1.43*d*	2.00*br*	2.04*dq*	1.86*dq*	1.96*qd*	2.02*dq*
5′	1.94*t*† (1.5)	1.89*q*† (1)	6.30*br* 5.96*br*	6.32*br* 5.90*br*		1.94*t*†	1.89*q*†	1.81*t*†	1.93*t*†

*In C_6D_6.

†Intensity three protons.

‡Centre of AB system.

J(Hz): Compounds **1c–f**, **2b–d**: 1, 2a = 7.5; 1, 2b = 10.5; 1, 5 = 6, 7 = 7, 8 = 9; 2a, 2b = 13; 2a, 3 = 2b, 3 = 7; 3, 15 = 3, 5 = 1.5; 5, 6 = 10; 7, 13a = 3.5; 7, 13b = 3; 8, 9a = 5; 8, 9b = 4; 9a, 9b = 14; Compound **4**: 2a, 2b = 9a, 9b = 14; 2a, 3 = 2b, 3 = 8; 5, 6 = 11; 6, 7 = 9; 7, 8 = 9.5; 7, 13a = 3.5; 7, 13b = 3; 8, 9a = 8, 9b = 5.

cm^{-1} 3444, 1768, 1710, 1643, 1456, 1270, 1133, 1042 and 988; ^{1}H and ^{13}C NMR spectra in Tables 1 and 2; MS *m/z* (rel. int. %) 344 (M^+, 0.35), 244 (5), 226 (4.2), 148 (6.7), 120 (5), 83 (100), 55 (18.6); MS PCI *m/z* (rel. int. %) 345 ($M^+ + 1$, 100), 331 (5.9), 245 (46), 227 (28.2), 83 (15.2).

Acetylation of 0.2 g of **1c** (Ac_2O–pyridine overnight at room temp.) and purification of the crude product by chromatography over silica gel gave 177 mg of a gum identical in all respects with naturally occurring **2c** (*vide infra*). Oxidation by addition of 0.35 g of **1c** in 2 ml $CHCl_3$ to 1.5 mmol pyridinium chlorochromate in 2 ml $CHCl_3$ followed by stirring for 2 hr and the usual work-up [23] furnished 110 mg of (1*S*, 5*R*, 6*R*, 7*R*, 8*S*)-8-angelyloxy-3-oxoguai-3 (15), 10 (14), 11 (13)-trien-6,12-olide (**3**), mp 104–106°, UV λ_{max} 224 nm (ε 20 000); IR ν^{KBr} cm^{-1} 1770, 1723, 1642, 1269, 1234 and 1146. The material decomposed before the ^{1}H NMR spectrum could be determined.

(1S, 3S, 5R, 6R, 7R, 8S)-3-*Hydroxy*-8-*tiglyloxyguai*-3(15), 10(14),11(13)-*triene*-6,12-*olide* (**1d**). Gum; ^{1}H spectrum in Table 1; MS *m/z* (rel. int. %) 344 (M^+, 0.39), 326 (01), 244 (11.9), 226 (7.7), 148 (5.9), 120 (4.7), 97 (11.3), 83 (100), 71 (15), 55 (44.5). MS PCI *m/z* (rel. int. %) 345 ($M^+ + 1$, 85.0), 245 (100), 228 (15.4), 227 (92.2), 217 (20.0), 199 (8.4), 131 (24.2), 101 (16.5), 83 (48.1).

(1S, 3S, 5R, 6R, 7R, 8S)-3-*Hydroxy*-8-[2-(1-*hydroxyethyl*) *propenoyloxylguai*-3 (15),10(14, 11 (13)-*triene*-6, 12-*olide* (**1e**). Gum; ^{1}H NMR spectrum in Table 1; MS *m/z* (rel. int. %) 360 (M^+, 5.6), 342 (1.1). 262 (41), 244 (100), 226 (63), 216 (37.4), 197 (38.8), 148 (49.2), 99 (41.5), 71 (10.2); MS PCI *m/z* (rel. int. %) 361 ($M^+ + 1$, 72.7), 343 (39.7), 261 (21.3), 245 (100), 227 (90), 117 (39.9) 99 (22.8).

(1S, 3S, 5R, 6R, 7R, 8S)-8-[2-(1-*Acetoxyethyl*)-*propenoyloxyl*-3-*hydroxyguai*-3(15),10(14),11(13)-*trien*-6,12-*olide* (**1f**). Gum; ^{1}H NMR spectrum in Table 1; MS *m/z* (rel. int. %) 402 (M^+, 0.7), 343 (0.4), 270 (13.3), 244 (34.2), 226 (21.8), 141 (78.2), 99 (40), 81 (100); MS PCI *m/z* (rel. int. %) 403 ($M^+ + 1$, 21.6), 385 (3.8), 345 (20.1), 343 (6.5), 285 (12), 271 (12.8), 257 (25.4), 245 (63.3), 227 (81.8), 201 (23), 159 (69.7), 141 (30), 99 (100).

(1S, 3S, 5R, 6R, 7R, 8S)-3-*Acetoxy*-8-*methacrylyloxyguai*-4-(15),10(14),11(13)-*trien*-6,12-*olide* (**2b**). Gum; ^{1}H NMR spectrum in Table 1; MS PCI *m/z* (rel. int. %) 373 (M^+1, 69), 313 (37.6), 287 (2.5), 279 (10.7), 227 (100), 201 (7.6), 85 (11.8).

(1S, 3S, 5R, 6R, 7R, 8S)-3-*Acetoxy*-8-*angelyloxyguai*-4 (15),10 (14),11(13)-*trien*-6,12-*olide* (**2c**). Gum; ^{1}H and ^{13}C NMR spectra in Tables 1 and 2; MS *m/z* (rel.int. %) 386 (M^+, 0.02), 326 (0.03), 286 (0.17), 226 (4.2), 83 (100) 55 (16.2); MS PCI *m/z* (rel. int. %) 387 ($M^+ + 1$, 93.3), 327 (34), 227 (100), 83 (21.1).

(1S, 3S, 5R, 6R, 7R, 8S)-3-*Acetoxy*-8-*tiglyloxyguai*-4 (15),10 (14),11(13)-*trien*-6,12-*olide* (**2d**). Gum; ^{1}H NMR spectrum in Table 1; MS *m/z* (rel. int. %) 386 (M^+, 0.14), 326(0.15), 226 (8.8), 83 (100), 55 (46.7); MS PCI *m/z* (rel. int. %) 387 ($M^+ + 1$, 53), 327 (34.3), 227 (100).

(3S, 5R, 6R, 7R, 8S)-8-*Angelyloxy*-3,14-*dihydroxyguai*-1(10), 4 (15),11(13)-*trien*-6,12-*olide* (**4**). Gum; IR ν^{CHCl_3} cm^{-1} 3400, 1765, 1715, 1640, 1430, 1225, 1065, 1040, 970; ^{1}H NMR spectrum in Table 1; MS *m/z* (rel. int. %) 360 (M^+, 0.2), 342 (0.4), 277 (0.5),

Table 2. ^{13}C NMR spectra of **1c** and **2c** ($CDCl_3$, 67.89 MHz)

C	1c	2c	1h†
1	45.14*d*	45.67*d*	45.3
2	36.96*t*	36.36*t**	38.3
3	73.30*d*	74.68*d**	73.2
4	152.12*s*	147.23*s*	151.8
5	51.15*d*	51.73*d**	51.5
6	78.52*d*	78.07*d**	78.1
7	47.50*d*	47.75*d**	46.9
8	73.47*d*	73.37*d**	75.2
9	38.90*t*	37.37*t**	35.6
10	141.80*s*	141.53*s*	141.6
11	137.50*s*	137.57*s*	136.8
12	169.00*s*	170.69*s*	169.5
13	122.16*t*	122.41*t*	123.6
14	117.70*t*	118.28*t**	118.1
15	113.19*t*	116.05*t**	113.8
1′	166.70*s*	166.83*s*	174.8
2′	126.99*s*	127.17*s*	76.0
3′	139.67*d*	139.93*d*	68.1
4′	15.70*q*	15.84*q*	21.6
5′	20.30*q*	20.45*q*	
Ac		170.69*s*	
		21.20*q*	

*Assignments by single frequency decoupling.

†Taken from ref. [10] with assignments confirmed by comparison with **1c** and **2c**.

260. (0.5), 242 (6), 83 (100), 55 (55.8), MS PCI (rel. int. %) 361 ($M^+ + 1$, 80.2), 343 (55.1) 260 (36.4), 243 (58.6), 225 (20), 85 (100).

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