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GLAUCOLIDES FROM *VERNONIA FULTA*

CÉSAR A. N. CATALÁN, PABLO R. LEGNAME*, B. VINCENT CRIST† and DORA I. A. DE IGLESIAS

Instituto de Química Orgánica, Facultad de Bioquímica Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 491, (4000) S. M. de Tucumán, Argentina; *Fundación Miguel Lillo, Miguel Lillo 205, (4000) Tucumán, Argentina; †Surface Science Laboratories, Inc., 1206 Charleston Road, Mountain View, CA 94043, U.S.A.

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Key Word Index—*Vernonia fulta*; Compositae; sesquiterpene lactones; germacranolides; glaucolides; vernofultanin; isovernofultanin; glaucolide-B.

Abstract—Four sesquiterpene lactones were isolated from the aerial parts of *Vernonia fulta*. Spectral characterization showed that three were new and one was known (i.e. glaucolide-B). The biogenetic relationship among these sesquiterpene lactones is discussed briefly.

INTRODUCTION

In our current survey of Argentinian plants, we have started a study of several species of the genus *Vernonia*, which is known to produce a variety of highly oxygenated sesquiterpene lactones [1–4].

RESULTS AND DISCUSSION

From the aerial parts of *Vernonia fulta* Schreb., we isolated a mixture of lactones that could only be separated by reversed-phase HPLC. Two of the lactones (**1**, **2**) accounted for about 85% of the mixture. The major component (45%) was determined to have the molecular formula $C_{21}H_{26}O_{10}$ and was identified as glaucolide-B (**1**) in accordance with its physical and spectroscopic properties [5].

A second major component (40%) was found to have the molecular formula $C_{19}H_{24}O_8$ and was identified as a new glaucolide which was given a trivial name, vernofultanin (**2**). Its IR spectrum displayed carbonyl absorptions at 1780, 1745–1725 and 1705 cm^{-1} and its UV spectrum showed a weak absorption at λ_{max} 289 nm ($\varepsilon = 65$). Its ^1H NMR spectrum (Table 1) indicated the presence of two acetate groups (note the two singlets at δ2.04 and 2.11), an isolated methyl group (indicated by the singlet at δ1.47) and a methyl group α to a carbonyl group (inferred from the doublet at δ1.16). Irradiation of the doublet at δ1.16 simplified the multiplet at 2.72. Subsequent irradiation of the multiplet caused changes in signals at δ1.16, 2.09 and 2.54. Additional decoupling experiments showed that the latter two signals were coupled to each other and to the signal at δ4.83. Irradiation of the doublet at δ2.74 (assigned as H-5) simplified the doublet at 4.90 into a singlet. This change, when correlated with the chemical shift of the 15-Me group (which was attributed to the signal at δ1.47), indicated that a 4,5-epoxide group must be present. This being the case the doublets at δ4.90 and 4.83 were assigned to H-6 and H-8, respectively. The coupling pattern of the signals at δ4.82 and 4.99 was interpreted as the usual AB coupling of the C-13 protons which is typical of many glaucolides [2–6]. (These protons are adjacent to acetoxy and olefinic groups.) Taken collectively, these data indicated that **2** must be a 10-desacetoxy derivative of **1**. In addition, by assuming a β configuration for the C-6 lactonic proton (see [6, 7]) a Dreiding stereo-model showed that a β orientation for H-10 would fit best with the observed proton–proton couplings. Consequently, the 14-Me group of **2** was assigned to the α or 10*R* configuration as shown. (N.B.

Table 1. ^1H NMR spectral data of lactone **2** (300 MHz, $CHCl_3$ as internal standard)

H	$CDCl_3$	C_6D_6
2	2.75–2.9* *m*	1.6–1.85*
2′	2.3–2.5* *m*	
3	2.3–2.5* *m*	
3′	1.65–1.8* *m*	1.0–1.15*
5α	2.74 *d*	1.99
6β	4.90 *d*	4.81
8β	4.83 *dd*	4.85
9β	2.54 *ddd*	2.33
9α	2.09 *ddd*	1.70
10β	2.72 *dqd*	1.95
13	4.99 *d*	4.96
13′	4.82 *d*	4.76
14	1.16 *d*	0.60
15	1.47 *s*	1.18
OAc_1	2.04 *s*	1.40
OAc_2	2.11 *s*	1.61

*Not first order.

J (Hz): 5α, 6β = 9.3; 8β, 9β = 3.7; 8β, 9α = 11.7; 9α, 9β = 13; 9β, 10β = 12.8; 9α, 10β = 2.4; 10β, 14 = 6.9; 13, 13′ = 12.7.

Table 2. ^{1}H NMR spectral data of lactone **3** (300 MHz, $CHCl_3$ as internal standard)

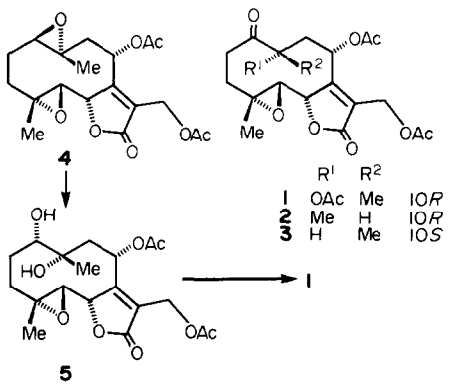
H	$CDCl_3$	C_6D_6
2α	2.65 *ddd*	—
2β	2.69 *ddd*	—
3α	1.71 *ddd*	—
3β	2.28 *ddd*	—
5α	2.54 *d*	2.25
6β	4.83 *d*	4.67
8β	4.82 *dd*	4.67
9β	2.15 *ddd*	—
9α	2.49 *ddd*	—
10α	2.94 *dqd*	2.34
13	4.96 *d*	4.90
13′	4.88 *d*	4.82
14	1.13 *d*	0.57
15	1.58 *s*	1.27
OAc_1	2.06 *s*	1.54
OAc_2	2.13 *s*	1.63

J (Hz): 2α, 2β = 14.5; 2α, 3α = 5.8; 2α, 3β = 5.5; 2β, 3α = 9.6; 2β, 3β = 5.0; 3α, 3β = 13.8; 5α, 6β = 9.2; 8β, 9β = 4.0; 8β, 9α = 4.5; 9α, 9β = 15; 9β, 10α = 2.5; 9α, 10α = 11.5; 10α, 14 = 6.9; 13, 13′ = 12.8.

Table 3. ^{1}H NMR spectral data of lactone **4** (300 MHz, $CHCl_3$ as internal standard)

H	$CDCl_3$	C_6D_6
1α	2.68 *d*	2.05
2β	1.56 *dddd*	0.88
2α	2.13 *ddd*	1.60
3β	2.30 *ddd*	1.52
3α	1.34 *ddd*	0.77
5α	2.57 *d*	1.90
6β	4.93 *d* (*br*)	4.63
8β	5.19 *d*	4.97
9α	1.98 *d*	1.70
9β	2.69 *d*	2.32
13	5.03 *dd*	5.05
13′	4.79 *d* (*br*)	4.78
14	1.51 *s*	1.07
15	1.48 *s*	0.92
OAc_1	2.08 *s*	1.50
OAc_2	2.11 *s*	1.63

J (Hz): 1α, 2β = 10.5; 1α, 2α = 0; 2α, 2β = 13.8; 2β, 3β = 5.2; 2β, 3α = 13.4; 2α, 3α = 6; 2α, 3β = 1.8; 3α, 3β = 13.5; 5α, 6β = 8.8; 6β, 13 = 0.8; 8β, 9α = 9.1; 8β, 9β = 0; 9α, 9β = 14; 13, 13′ = 13.4.



Bohlmann *et al.* [8] have isolated two glaucolides, which are quite similar to structures **2** and **3**. The 400 MHz ^{1}H NMR data ($CDCl_3$) gathered from those glaucolides were found to correlate well with those measured from glaucolides **1–3**.)

A third lactone (**3**) was isolated as a minor constituent (5% of the total mixture). The virtual overlap of its spectroscopic properties with those of vernofultanin (**2**) indicated that it was a C_{10} epimer of vernofultanin. The 0.22 difference in chemical shifts for the respective C-10 protons (i.e. δ2.74 for **2** vs 2.94 for **3**) served as further evidence for the epimeric relationship of **2** and **3** (note the similarity of the splitting pattern and constants of these two signals). It was quite surprising to find that both lactones exhibited the same aromatic solvent induced shifts for their respective 14-Me groups (i.e. 0.56). This suggested that the dihedral angle between the carbonyl double bond and the adjacent methyl–methine single bond was quite similar for compounds **2** and **3** [9]. If this is true, then the conformation of the 10-membered ring of **2** must be different from that of **3**. (N.B. Lactone **3** is most likely an artifact of the isolation procedure. A two day old solution of pure **2** and lightly acidified chloroform contained a small amount (4%) of **3**.)

A fourth and final lactone was isolated as a crystalline solid in 10% overall yield. Elemental analysis indicated that it was an isomer of lactones **2** and **3**. Its ^{1}H NMR spectrum was consistent with the presence of two acetate groups (denoted by the 3H singlets at δ2.08 and 2.11) and two tertiary methyl groups (denoted by 3H singlets at δ1.48 and 1.51). The latter two groups were apparently attached to oxygen bearing carbons. Double resonance experiments simplified the complete assignment of all signals and clearly revealed the presence of two epoxide groups at positions 1, 10 and 4, 5. Our interpretation of the data led us to propose structure **4**. Inspection of a Dreiding stereo-model of structure **4** showed that the proposed stereochemistry would agree reasonably well with the observed coupling constants. (Just recently a C-8 substituted methacrylate analogue of **4** was isolated from a South African *Vernonia* by Bohlmann and Zdero [10].)

Compound **4**, a diepoxy-lactone, is the pre-eminent biogenetic precursor to structures **2** and **3**. Isomerization of oxiranes such as those found in structure **4** [11] has been effected on similar systems by a mild reaction with mineral or Lewis acids [12–15]. The formation of glaucolide-B (**1**) probably occurred by the hydration of **4**, which would produce glycol **5**. Subsequent transformation by biological oxidation and acetylation would yield structure **1**.

EXPERIMENTAL

General. ^{1}H NMR spectra were measured in $CDCl_3$ and C_6D_6 at 100 and 300 MHz. Mass spectra were obtained from a single focussing system at 70 eV.

Plant material. The aerial parts of *Vernonia fulta* were collected in Horco Molle in the Tucuman province. A voucher specimen was deposited in the Lillo Institute in Tucuman (voucher CC 21).

Extraction and isolation. The aerial parts (355 g, mainly flowers) of *V. fulta* were extracted and worked up in the usual fashion [5, 16] to afford, after treatment with lead (II) acetate, 4.13 g (1.16%) of crude extract. TLC gave partial purification. The mixture was separated by using reversed-phase HPLC (Whatman Partisil M9 10/50 ODS-2) with MeOH–H_2O (1:1) at a flow rate of 3.0 ml/min. Four cleanly separated fractions were collected; the R_ts were 16, 21, 28 and 38 min. The respective fractions gave compounds **4**, **3**, **2** and **1** in the ratio 2:1:8:9.

10-*Desacetoxy*-10-*isoglaucolide-B* (*vernofultanin*) (**2**) was isolated as colourless crystals with mp 156–157° (hexane–EtOAc); IR ν_{max}^{KBr} cm^{-1}: 1780 (γ-lactone), 1745–1725 and 1230 (two acetates), 1705 (C=O); UV λ_{max}^{EtOH} at 289 nm (ε65); MS (solid probe) *m/z* (rel. int.): 278 (3), 260 [M − 2AcOH]$^+$ (9), 111 (64), 109 (74), 99 (32), 95 (30), 91 (32), 60 [AcOH]$^+$ (100), 55 (94). (Found: C, 59.95; H, 6.40. $C_{19}H_{24}O_8$ requires: C, 59.97; H, 6.36.)

10-*Desacetoxy-glaucolide-B* (*isovernofultanin*) (**3**) was isolated as a colourless gum with: IR ν_{max}^{neat} cm^{-1}: 1780–1745 (γ-lactone and two acetates), 1710 (C=O); UV λ_{max}^{EtOH} at 288 nm (ε68); MS (solid probe) *m/z* (rel. int.): 278 (1), 260 [M − AcOH]$^+$ (3), 111 (33), 109 (37), 99 (33), 95 (21), 91 (21), 60 [AcOH]$^+$ (100), 55 (74). (Found: C, 60.03; H, 6.43. $C_{19}H_{24}O_8$ requires C, 59.97; H, 6.36.)

8*S*,13-*Diacetoxy*-1R,(10R)-4R,5R-*diepoxygermacra*-7(11)-*en*-6S,12-*olide* (**4**) was isolated as white crystals with mp 164–166° (hexane–EtOAc); IR ν_{max}^{KBr} cm^{-1}: 1770 (γ-lactone), 1740 (acetate); MS (solid probe) *m/z* (rel. int.): 320 [M − AcOH]$^+$ (1), 278 (8), 260 [M − 2AcOH]$^+$ (2), 250 (7), 161 (45), 97 (70), 85 (62), 69 (100), 60 (63), 55 (99). (Found C, 59.91; H, 6.40. $C_{19}H_{24}O_8$ requires C, 59.97; H, 6.36.)

Isomerization of **2**. Lactone **2** (10 mg) was dissolved in 10 ml $CHCl_3$ containing 5 μl trifluoroacetic acid and the soln kept at room temp. for 2 days. The soln was washed with 5% $NaHCO_3$, water, dried and the solvent evaporated. Analysis of the residue by HPLC showed two peaks with R_t 20 and 26 min (abundance ratio was 1:24), corresponding to structures **3** and **2**, respectively.

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