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DITERPENES FROM *VERNONANTHURA AMPLEXICAULIS*

SUSANA BORKOSKY, ALICIA BARDÓN, CÉSAR A. N. CATALÁN, JESÚS G. DÍAZ* and WERNER HERZ*

Instituto de Química Organica, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 491, 4000 S.M. de Tucumán, Argentina; *Department of Chemistry, The Florida State University, Tallahassee, Florida 32306, U.S.A.

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Key Word Index—*Vernonanthura amplexicaulis*; Vernonieae; Compositae; diterpenes; kaurane, pimarane.

Abstract—Aerial parts of *Vernonanthura amplexicaulis* furnished a new kaurane and a new pimarane.

INTRODUCTION

Vernonanthura amplexicaulis (R. E. Fries) H. Robinson (old binomial *Vernonia amplexicaulis* R. E. Fries) [1, 2] is a species limited to the extreme northwest of Argentina and adjacent areas of Bolivia [1, 3]. We now report isolation of the new diterpenes **1** and **2** from a small Bolivian collection of *V. amplexicaulis*. Apigenin and 1-acetylglycerol were also found.

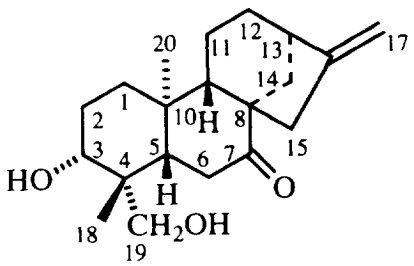
Structure determination of **1** and **2**, of which only 1.7 and 2.7 mg, respectively, were available, is best discussed in terms of the final structures. The mass spectrum of **1** suggested formula $C_{20}H_{30}O_3$ which, together with the ^{1}H NMR spectrum (Table 1), indicated a tetracyclic diterpene containing two quaternary methyls at δ1.25 and 1.17 (H-8 and H-20), a $-CH_2OH$ residue attached to a quaternary carbon (H-19a, b at δ4.33 and 3.46, chemical shifts characteristic of axial orientation on C-4), an equatorial secondary hydroxyl (H-3ax at δ3.52, J values = 12, 4, 1 Hz), an exocyclic methylene (H-17a, b, slightly broadened triplets, $J \sim 3$ Hz, at δ4.90 and 4.86) whose protons were allylically coupled to a methinyl hydrogen responsible for the broadening (H-13 at δ2.73) and to the protons of a methylene group (H-15a,b at δ3.08 and 2.05). The appearance of H-17a, b as triplets rather than as quartets indicated that the substance was a 16-kaurene rather than a 16-atisene [4, 5].

The third oxygen atom was that of a carbonyl group sited at C-7 because of the presence in the ^{1}H NMR spectrum of a three proton system comprised of H-5 at δ1.50 coupled to H-6ax at δ2.39 at H-6eq at δ2.52. This also explains the paramagnetic shift of H-15a which lies in the plane of the C = O bond (model).

The location of the equatorial hydroxyl on C-3 was established by NOE difference spectrometry (Table 2) which showed a significant NOE between H-3ax and the equatorial methyl (H-18) on C-4. These experiments also indicted the existence of a hydrogen bond between the hydroxyl groups on C-3 and C-19; thus only one of the protons on C-19, i.e. H-19a, exhibited an NOE with H-20 but not with H-18, whereas the other, H-19b, interacted with H-6ax and H-18, but not with H-20. This orientation of the C-19 substituents apparently also accounts for a small W-coupling (1 Hz) between H-3ax and H-19b.

The empirical formula $C_{20}H_{32}O_3$ of **2** and its ^{1}H NMR spectrum (Table 1), which exhibited the signals of a vinyl group (*dd* of H-15 at δ5.78, J values = 17.5, 11 Hz, *d* values of H-16a, b at δ4.94, J = 17.5, and δ4.93, J = 11 Hz), a vinylic proton (*brs* of H-14 at δ5.55), an axial secondary hydroxyl (H-7 triplet at δ4.21, J = 3 Hz), an equatorial secondary hydroxyl (*ddd* of H-3 at δ3.58, J values = 12, 4, 1 Hz), an axial $-CH_2OH$ similar to that of **1** and three methyl singlets at 1.04 (H-17), 1.21 (H-18) and 0.80 (H-20) suggested the presence of a pimara- or sandaracopimara-7,15-diene. H-14 was allylically coupled to H-9 which was in turn coupled to two partially submerged signals (H-11a, b) near δ1.65 and 1.48, while H-7, the proton under the axial hydroxyl, was coupled to H-6eq and δ1.87 and H-6ax at δ1.48, both of which were, in turn, coupled to H-5 at δ1.83. The location of the axial hydroxyl on C-3 and the preferred orientations of the protons on C-19 followed from NOE spectrometry in C_6D_6 as in the case of **1** (Table 2). This also allowed assignment of the δ0.99, 1.11 and 0.42 singlets of H-17, H-18 and H-20, respectively.

In view of the paucity of material ^{13}C NMR spectrometry which, because of the preferred stereochemistry adopted by ring C, readily distinguishes members of the pimaradiene (C-17 quasi-equatorial) from members of the sandaracopimaradiene series (C-17 quasiaxial) [6–8, see also 9] could not be used to establish the stereochemistry of **2** at C-13. However, the NOE between H-14 and H-15 (Table 2) demonstrated that the C-13 methyl was almost in the plane of the 8(14)-double bond and hence α and pseudoequatorial, if **2** belongs to the normal, or β and pseudoequatorial if **2** belongs to the enantiomeric series. Although the absolute configurations of **1** and **2** were not established we provisionally enrol them in the *ent*-series as shown in the formulas.



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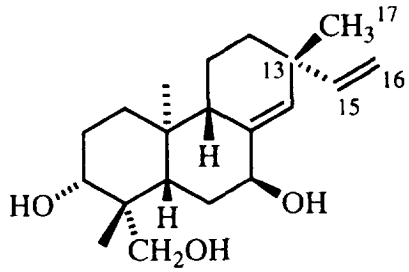

2

Table 1. ^{1}H NMR spectra of compounds **1** and **2** (500 MHz)

	1 ($CDCl_3$)	**1** (C_6D_6)	**2** ($CDCl_3$)	**2** (C_6D_6)
1ax	in 1.55	obsc.	1.65 *m*	0.76 *m*
1eq	– 1.75 *c*	obsc.	1.48 *m*	obsc.
2ax	1.65 *m*	1.24	1.56 *qd* (12, 3)	1.26
2eq	1.96 *m*	1.63	1.94 *dq* (13, 4)	1.75
3ax	3.52 *ddd* (12, 4, 1)	3.16	3.58 *ddd* (12, 4, 1)	3.39
5	1.50 *dd* (14.5, 3)	1.33	1.83 *dd* (13, 2.5)	1.62
6ax	2.39 *t* (14.5)	1.81	1.48 *td* (14, 3)	1.01
6eq	2.52 *dd* (15, 3)	2.20	1.87 *dt* (13, 3)	1.55
7eq	—	—	4.21 *dd* (3, 3)	3.82
9	1.38 *dd* (7.5, 2.5)	0.87 *m*	2.11 *atd* (7, 1.5)	1.82
11a	1.78 *m*	obsc.	1.65 *m*	obsc.
11b	in 1.55	obsc.	1.48 *m*	obsc.
12a, b	– 1.75 *c*	obsc.	obsc.	1.32‡
13	2.73 *brq* (~ 3.5)	2.51	—	—
14α	1.90 *dd* (12.5, 2.5)	1.51	5.55 *brs*	5.33
14β	1.46 *dd* (12, 4)	1.3	—	—
15α	3.08 *ddd* (17, 3, 3)	3.39	5.77 *dd* (17, 10.5)	5.75
15β	2.05 *brd* (17)	1.92 *dq* (17, 2)	—	—
16*cis*	—	—	4.93 *brd* (10.5)	4.97 *dd* (11, 1)
16*trans*	—	—	4.94 *brd* (17)	4.96 *dd* (17, 1)
17a	4.90 *brt* (3)	4.94 *m*	1.04 *s**	0.99*
17b	4.86 *brt* (3)	4.93 *m*	—	—
18*	1.25 *s*†	0.87	1.21 *s*	1.11
19a	4.33 *d* (11)	3.95	4.35 *d* (11)	4.12
19b	3.46 *dd* (11, 1)	3.11	3.45 *dd* (12, 11)	3.27
20†	1.17 *s*†	0.56	0.80 *s*	0.42

*Intensity three protons.
†Assignments may be interchanged.
‡H-12eq.

If the genus *Corymbium* is excluded from the tribe Vernonieae as proposed on the basis of pollen morphology and chemistry [10] *V. amplexicaulis* is the only member of the tribe so far shown to produce diterpenoids, albeit in very small amounts.

EXPERIMENTAL

General. For sepn of mixts HPLC with a differential refractometer was used. The columns employed were (A) a Phenomenex Maxsil 10 C8 (10 μm, 10 × 500 mm) and (B) a Phenomenex Ultremex C18 (5 μm, 10 × 250 mm). Retention times were measured from the solvent peak.

Plant material. Aerial parts of *V. amplexicaulis* were collected at the flowering stage in October 1992 near Santa Cruz de la Sierra, Bolivia. A voucher specimen LIL # 595774 is on deposit in the herbarium of the Fundación Miguel Lillo, Tucumán.

Extraction and isolation. Flowers and leaves (250 g) were extracted with EtOAc freed of HOAc (2 × 2.5 l) at room temp. for 7 days to give 8 g of crude extract which was suspended in 69 ml of EtOH at 55°, diluted with 51 ml of H_2O and extracted successively with hexane

Table 2. NOE difference spectra of compounds **1** and **2** (C_6D_6)

1		**2**	
Irradiated	Observed (% enhancement)	Irradiated	Observed
H-2eq	H-2ax (8.2), H-3ax (2.3)	H-2eq	H-2ax (9.1), H-3ax (2.1)
H-3ax	H-2eq (7.2), H-18 (8.4)	H-3ax	H-2eq (1.1), H-5 (1.0), H-18 (2.1), H-1ax (1.5)
H-6eq	H-6ax (8.2), H-18 (3.9)	H-5	H-3ax (2.0), H-9 (8.0), H-18 (3.4)
H-6ax	H-6eq (9.1), H-19b (4.9), H-20 (1.0)	H-6eq	H-6ax (4.7), H-7eq (0.9), H-18 (3.4)
H-9 + H-18	H-3ax + H-19b (3.5), H-6eq (3.9) H-15β (3.3), H-5 (3.9)		
H-13	H-14β (14.0), H-17a (6.6)	H-6ax + H-17	H-6eq (4.8), H-7eq (0.9), H-14 (0.8)
H-14α	H-13 (2.4), H-14β (4.0), H-20 (1.4)	H-7eq	H-6ax (3.1), H-14 (11.5)
		H-9	H-1ax (1.0), H-5 (4.4)
H-14β	H-13 (2.0), H-14α (2.1)	H-14	H-7eq (9.6), H-15 (3.0), H-16$_{cis}$ (0.8), H-17 (1.7)
H-15α	H-15β (16.2)		
H-15β	H-15α (10.9), H-9 (6.0)	H-15	H-12a (2.0), H-16$_{cis}$ (2.8)
H-17a	H-13 (1.8)	H-16$_{cis}$	H-15 (2.0)
H-19a	H-19b (17.1), H-20 (7.6)	H-18	H-3ax (1.0), H-5 + H-6eq (9.2), H-19b (1.0)
H-19b	H-6ax (5.6), H-18 (3.9), H-19a (13.1)	H-19a	H-19b (10.2), H-20 (7.0)
H-20	H-2ax (1.4), H-6ax (1.5), H-14ax (1.4), H-19a (1.0)	H-19b	H-6ax (3.2), H-18 (1.2), H-19a (7.6)
		H-20	H-6ax (1.5), H-19a (1.9)

(3 × 100 ml) and EtOAc (3 × 100 ml). Evaporation of the EtOAc extract at red. press. furnished 1.2 g of residue which was chromatographed on Florisil using hexane and increasing amounts of EtOAc (0–100%) to give 26 frs. Frs 6–9 (27 mg) were processed by HPLC using column A (MeOH–H_2O, 3:2, 2 ml min^{-1}) to give 2.0 mg of unidentified material, R_t 55 min. Frs 10–17 (127 mg) on HPLC by column A (MeOH–H_2O, 4:3, 2 ml min^{-1}) gave 1.6 mg of unidentified material, R_t 57 min, 1.6 mg of **1**, R_t 80 min, and 2.7 mg of **2**, R_t 99 min. Rechromatogrpahy of the subsequent fractions by HPLC gave small amounts of mixtures related to **1** and **2**. Frs 18–20 (59 mg) on HPLC, column B (MeOH–H_2O, 8:5, 2 ml min^{-1}) furnished 3.5 mg of apigenin, R_t 28 min, identified by MS, UV and ^{1}H NMR, and unidentified mixtures while frs 21–22 (50 mg) on HPLC by column A (MeOH–H_2O, 4:3, 2 ml min^{-1}) gave a peak which on prep TLC gave 2.4 mg of 1-acetylglycerol identified by MS and ^{1}H NMR.

ent-3β,19-*Dihydroxy*-7-*oxokaur*-16-*ene* (**1**). Gum; MS PCI (NH_3) *m/z* (rel. int.) 336 $[M + NH_4]^+$ (100), 318 (39.9); ^{1}H NMR spectrum in Table 1.

ent-3β,7α,19-*Trihydroxypimara*-8(14),15-*diene* (**2**). Gum; MS PCI (NH_3) *m/z* (rel. int.) 338 $[M + NH_4]^+$ (36.7), 320 (100); ^{1}H NMR spectrum in Table 1.

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