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GUAIANE SESQUITERPENOIDS FROM *HAPLOPAPPUS FOLIOSUS*

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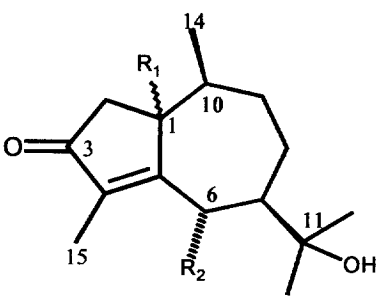
Key Word Index—*Haplopappus foliosus*; Compositae; Asteraceae; sesquiterpenes; guaianes.

Abstract—RP-18 chromatography of the methanolic extract of the leaves of *Haplopappus foliosus* D.C. led to the isolation of (1α,7β,10β)-11-hydroxy-4-guaien-3-one and the two new isomeric guaianes: (1β,7β,10β)-1,11-dihydroxy-4-guaien-3-one and (1α,6α,7β,10β)-6,11-dihydroxy-4-guaien-3-one. The structure and relative configurations of the new compounds are proposed on the basis of spectroscopic evidence.

INTRODUCTION

As part of a quest for new sources of natural biopesticides [1, 2], we analysed the methanolic extracts of over a hundred Chilean plants, using RP-18-HPLC for detection of the characteristic UV absorption (ca 243 nm), arising from the γ-hydroxy-α,β-unsaturated ketone chromophore present in the structure of β-ecdysone and related phytoecdysteroids [3]. During the screening we found that the chromatogram of the extract of the aerial parts of *H. foliosus* showed three intense peaks that could be attributed to the presence of this type of compound. As a previous chemical study on this species had only reported the isolation of a neoclerodane diterpenoid and some aromatic compounds of common occurrence in the genus [4], we decided to study the polar extract of *H. foliosus* in order to isolate and identify the compounds detected by HPLC.



	R_1	R_2
1	αH	H
2	βOH	H
3	αH	OH

RESULTS AND DISCUSSION

Repeated column chromatography of the extract led to the isolation of a main compound (**1**) and a more polar mixture of two compounds (**2**, **3**) that could only be resolved by preparative RP-18-TLC.

An analysis of NMR (Tables 1 and 2), IR and mass spectra (see Experimental) showed that the three compounds were closely related bicyclic sesquiterpenoids. The main structural features in common included an α,β-unsaturated cyclopentanone (1690, 1640 cm^{-1}) with a fully substituted double bond; a tertiary alcohol and four methyl groups, one of them secondary. Compounds **2** and **3** differed from **1** only by the presence of an extra hydroxyl group.

A study of 2D-NMR spectra (HETCOR and COSY) and DEPT multiplicities of the major compound ($C_{15}H_{24}O_2$), led to a structural sequence which was consistent with an 11-hydroxy-4-guaien-3-one structure. It is interesting to note on the misleading low field shift of the C-5 signal (δ 176) in the ^{13}C spectra (Table 2) of this type of guaiane. The relative stereochemistry of H-10 (α), H-1 (α) and H-7 (α) depicted in **1** was deduced from observed NOE effects and the consideration that the isopropyl group attached to C-7 is usually β for guaianes isolated from species belonging to the Compositae [5]. Thus, irradiation of the H-1 signal produced a 2.5% enhancement of H-10, while a NOE (3.6%) was observed at H-1 upon irradiation of H-7. Examination

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Table 1. ^{1}H NMR spectral data of compounds **1**–**3** [$CDCl_3$, δ values, J (Hz) at 20°]

	1 (360 MHz)	**2** (300 MHz)*	**3** (300 MHz)
H-1	3.08 *br*		3.28 *br*
H-2α	2.53 *ddd*	2.60 *d*	2.51 *dd*
	(18.7; 6.4; 1.2)	(18.1)	(18.3; 6.5)
H-2β	2.01 *dt*	2.47 *d*	2.10 *dd*
	(18.7;1.5)	(18.1)	(18.2; 1.6)
H-6α	3.05 *brd*	2.77 *dd*	4.83 *d*
	(12.3)	(12.4, 3.7)	(8.8)
H-6β	2.18 *dm*	2.47 *t*	
	(12.1)	(11.9)	
H-7	1.65 *m*		1.91 *dd*
			(9.3, 9.6)
H-8	1.90 *m*		1.31 *m*
H-8′	1.22 *m*		0.91 *m*
H-9	1.67 *m*		1.19 *m*
H-9′	1.84 *m*		1.65 *m*
H-10	2.10 *m*		2.28 *m*
Me-12	1.18 *s*	1.28 *s*	1.37 *s*
Me-13	1.22 *s*	1.28 *s*	1.21 *s*
Me-14	0.60 *d*	1.11 *d*	0.63 *d*
	(7.1)		(6.9)
Me-15	1.65 *m*	1.76 *s*	1.82 *d*
			(1.5)

* Resonances for H-7 to H-10 were overlapping and could not be identified.

Table 2. ^{13}C NMR spectral data of compounds 1–3 (75.4 MHz, in $CDCl_3$).

	1	**2**	**3** (55°C)
1	45.7	79.4	42.9
2	41.3	50.4	40.4
3	208.8	205.9	210.5
4	137.7	136.5	139.2
5	176.4	174.5	173.9
6	33.7	24.3	70.0
7	47.9	45.7	53.1
8	27.0	27.3	21.9
9	36.7	27.7	34.2*
10	35.3	44.4	33.6*
11	73.1	73.8	74.0
12	27.2	27.5	28.7
13	25.9	26.2	23.3
14	12.1	17.7	13.7
15	7.9	7.6	7.2

* Interchangeable assignments.

of models suggests that the secondary methyl group at C-10 should be in a *pseudo*-axial disposition in order to account for these effects. In this conformation it becomes shielded by the carbonyl group at C-3, in agreement with the observed high field shifts [4] of the corresponding resonances in the NMR spectra (Tables 1 and 2).

A compound with the same structural features discussed above, was isolated from *Euryopis pedunculatus* [5] and the reported data (^{1}H-NMR, IR, MS) coincide with ours, thus identifying **1** as (1α,7β,10β)-11-hydroxy-4-guaien-3-one.

The H-NMR spectrum of **2** ($C_{15}H_{24}O_3$) did not have a resonance signal for H-1, which suggested that the extra hydroxyl group was situated at C-1. This conclusion is supported by the observed β-shifts in the resonances for C-2 and C-10 in the ^{13}C NMR spectrum (Table 2) in comparison to those of **1**. An axial disposition of this hydroxyl substituent would account for the high field shifts (γ-effect) in the resonances assigned to C-6 and C-9. Additionally, the observed low field shifts of the carbon and proton resonances associated with C-14 (Tables 1 and 2) are consistent with a change in conformation in which the secondary methyl group becomes equatorial. This change is better accounted for with a β-hydroxyl group, which would push back the secondary methyl and also produce the low field shift observed in the resonance of H-2β, as compared with **1** (Table 1).

In related 1β,10β-guaianes, the secondary methyl group resonances have been reported [6, 7] to have similar values (ca δ_C20 and δ_H0.9) to those found for **2**. On the basis of this evidence we propose structure **2** for the new compound which corresponds to (1β,7β,10β)-1,11-dihydroxy-4-guaien-3-one.

The ^{13}C NMR spectrum of third compound ($C_{15}H_{24}O_3$) had only 10 signals at room temperature and the missing resonances could only be detected at 55°, which suggested a slow equilibrium between conformers. A study of HETCOR and COSY spectra, as well as a comparison with the NMR data recorded for **1** and **2** (Tables 1 and 2), allowed the assignment of signals in the spectra and showed that C-2, C-4, C-9, C-10 and C-14 were the centres affected by conformational changes that must be associated with the presence of a secondary hydroxyl group at C-6 in the structure of **3**. Inspection of models showed that in order to account for the observed $J_{6,7}$ coupling (8.8 Hz) the hydroxyl group must be α because in a β configuration, H-6 and H-7 become almost eclipsed, in the most stable conformation, giving rise to a small coupling constant, as reported [4, 8] for a related compound isolated from *Pleocarphus revolutus* (Asteraceae). The existence of conformers could then be explained by the formation of hydrogen bonding between the OH groups at C-6 and C-11. Lack of material prevented us from performing other experiments in order to give further support to this assumption. Structure **3**, proposed for this new compound, corresponds to (1α,6α,7β,10β)-6,11-dihydroxy-4-guaien-3-one.

Absolute configurations were not established and have been proposed in structures **1**–**3** relative to a 7β-configuration of the isopropyl group, as found in most of the guaianes isolated from the Asteraceae.

To our best knowledge this is the first report on the presence of guaiane sesquiterpenes in *Haplopappus*

species. This type of compound was not detected in extracts of *H. velutinus*, *H. illinitus* and *H. chrysanthemifolius* when they were analysed by RP-HPLC under the same experimental conditions used for *H. foliosus* in the present study.

EXPERIMENTAL

^{1}H and ^{13}C NMR were recorded at 300 and 75.4 MHz respectively. EIMS (probe) at 75 eV.

Plant material

Haplopappus foliosus was collected near Los Vilos (V Region, Chile) in September 1995 and was identified by Dr Sebastian Teillier. A voucher specimen (M-11-95) is kept in the Chemistry Department, Universidad de Chile, Santiago.

Extraction and isolation

Ground air-dried leaves (200 g) were macerated in MeOH (2 l, room temp., 8 h). Evapn of solvent gave 9.6 g of crude extract which was then submitted to repeated low pressure CC using silica gel (Merck 60 H) and different solvent systems (CH_2Cl_2–EtOAc; petrol–EtOAc) in fractions of increasing polarity. Prep. TLC (petrol–hexane, 1:1) gave the major compound **1** (12 mg). The more polar fractions were digested in H_2O (10 ml), filtered through a RP-18 Sep-Pack cartridge and eluted with H_2O–*iso*–PrOH mixtures of decreasing polarity. Further purification of the intermediate fractions was achieved by RP-18 prep. TLC (50% H_2O–*iso*–PrOH) to give: **2** (14 mg) and **3** (5 mg).

HPLC analyses

HPLC was carried out at 55° on an RP-18 column (LiChroCART 15.5 × 0.4 cm., Merck) using a flow rate of 1.0 ml min^{-1} and 12% *iso*-PrOH in H_2O as the mobile phase and employed an UV diode-array detector. Fractions were monitored at 242 nm for the detection of **1** (R_t 28.1 min), **2** (R_t 7.1 min) and **3** (R_t 12.2 min).

(1α,7β,10β)-11-*hydroxy*-4-*guaien*-3-*one* (**1**). Oil. IR ν_{max}^{film} cm^{-1}: 3595 (OH), 1687, 1628. MS *m/z* rel. int.): 218 $[M-H_2O]^+$ (35), 203 $[M-H_2O-Me]^+$(6), 178 $[M-Me_2CO]^+$ (7), 163 $[178-Me]^+$ (20), 149 (32), 110 (38), 59 $[Me_2COH]^+$ (100). ^{1}H NMR in Table 1. ^{13}C NMR in Table 2.

(1β,7β,10β)-1,11-*dihydroxy*-4-*guaien*-3-*one* (**2**). Oil. IR ν_{max}^{film} cm^{-1}: 3415 (OH), 1691, 1643. MS *m/z* (rel. int.): 234 $[M-H_2O]^+$ (35), 216 $[234-H_2O]^+$ (18), 201 $[216-Me]^+$ (17), 176 $[234-Me_2CO]^+$ (8), 59 (100). ^{1}H NMR in Table 1. ^{13}C NMR in Table 2.

(1α,6α,7β,10β)-6,11-*dihydroxy*-4-*guaien*-3-*one* (**3**). Oil. IR ν_{max}^{film} cm^{-1}: 3362 (OH), 1689, 1635, MS *m/z* (rel. int.): 234 $[M-H_2O]^+$ (35), 219 $[234-Me]^+$ (5), 176 $[234-Me_2CO]^+$ (8), 161 $[176-Me]^+$ (20), 59 (100). ^{1}H NMR in Table 1. ^{13}C NMR in Table 2.

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