PERGAMON

Phytochemistry 52 (1999) 1547–1550

PHYTOCHEMISTRY

Diterpenes from *Haplopappus chrysanthemifolius*

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Received 4 November 1998; received in revised form 26 March 1999; accepted 29 April 1999

Abstract

Three new diterpenes were isolated from the aerial part of *Haplopappus chrysanthemifolius* and assigned the structures 6α-hydroxy-*ent*-labd-8(17)-en-15-oic acid, 3β-acetoxy-*ent*-labd-8(17)-en-15-oic acid and 18α-acetoxylabd-8(17)-en-15-oic acid. The structures were elucidated by high field NMR spectroscopy.

Keywords: *Haplopappus chrysanthemifolius*; Asteraceae; Astereae; Diterpenes

1. Introduction

The genus *Haplopappus* (Asteraceae, Astereae), largely present in South America, is represented in Chile by 63 species (Marticorena & Quezada, 1985). Diterpenes of the labdane (Urzúa & Mendoza, 1989; Maldonado, Honeisen & Silva, 1993; Marambio & Silva, 1989) or clerodane types (Silva & Sammes, 1973; Bittner, Zabel, Smith & Watson, 1978), triterpenes (Silva & Sammes, 1973), flavonoids (Maldonado et al., 1993; Marambio & Silva, 1989; Nuñez-Alarcón, Dolz, Quiñones & Carmona, 1993) and coumarins (Chiang, Bittner, Silva, Mondaca, Zemelman & Sammes, 1982) have been reported as constituents of these species. As a part of our phytochemical research on the resinous Asteraceae, we have studied the dichloromethane extract of *Haplopappus chrysanthemifolius* (Less) D.C., an herbaceous plant that inhabits coast zones of Central Chile from Coquimbo to Maule. The wide variety of ecological conditions under which this species grows has favoured the development of a large number of forms and consequently of synonyms (Hall, 1928). Antimicrobial activity against *Bacillus cereus* has been reported as the result of a previous biological investigation of the ethanolic extract of the whole plant (Zuñiga, Wilkens, Labbé & Faini, 1995).

This paper deals with the structure determination of three new diterpenes (**1**–**3**) with a labdane skeleton, and of two known flavonoids, 5,3′-dihydroxy-3,7,4′-trimethoxyflavone (ayanin) (**4**) and 5,3′,5′-trihydroxy-3,7,4′-trimethoxyflavone (5′-hydroxyayanin) (**5**).

2. Results and discussion

The ^{1}H- and ^{13}C-NMR spectral data (Tables 1 and 2), including APT experiment, established the presence in compound **1** of three quaternary methyl groups, one methyl group linked to a methine, an exocyclic methylene, a carboxyl and a secondary alcohol in a structure of 20 carbon atoms. Some of the above groups were confirmed by IR absortions at 3477 (OH), 1702 (CO), 1647 and 892 (double bond) cm^{-1}. In the mass spectrum, the molecular peak at *m*/*z* 322, relatable to a molecular formula $C_{20}H_{34}O_3$, corresponded to a bicyclic diterpene containing an hydroxyl group in the decaline system, as suggested by the loss of H_2O (*m*/*z* 304) prior to the loss of the $C_6H_{11}O_2$ side chain (*m*/*z* 189).

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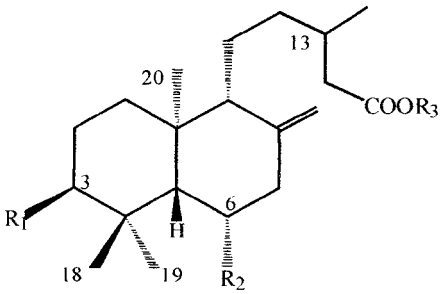
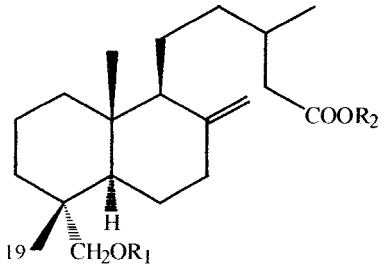
0031-9422/99/$ - see front matter
PII: S0031-9422(99)00395-7





1 $R_1 = R_3 = H$, $R_2 = OH$
1a $R_1 = H$, $R_2 = OH$, $R_3 = Me$
2 $R_1 = OOCMe$, $R_2 = R_3 = H$
2a $R_1 = OOCMe$, $R_2 = H$, $R_3 = Me$

3 $R_1 = COMe$, $R_2 = H$
3a $R_1 = COMe$, $R_2 = Me$
3b $R_1 = R_2 = H$

The location of the hydroxyl group at C-6 was substantiated by an INEPTL experiment, which related the H-6 irradiated signal to the resonances of C-4, C-8 and C-10 carbons ($^3J_{H,C}$). Moreover, NOE experiments revealed the proximity of 19-Me with 20-Me and of H-6 with 18-Me. These findings addressed to 6β-hydroxylabd-8(17)-en-15-oic acid, previously isolated as methyl ester ($\alpha_D = +2.4$) from *Cistus psilosepalus* (Sweet) (de Pascual Teresa, Urones & Montes Sánchez, 1978). Accordingly, the methyl ester **1a** showed spectral data comparable with those reported in the literature, mainly the presence in the mass spectrum of significant fragments at *m/z* 207, 189, 153, and 109 (de Pascual Teresa et al., 1978). However, the negative α_D values of **1** (−2) and **1a** (−8.8) suggested that they rather belong to the *ent*-labdane series.

The C-13 configuration was not determined but the comparison is valid since, according to the Carman report (Carman, 1966), the contribution of the asymmetric centre at C-13 to the rotatory power of labdanes is largely smaller than that one of the decaline system. Therefore, compound **1** was assigned the structure 6α-hydroxy-*ent*-labd-8(17)-en-15-oic acid.

^{1}H- and ^{13}C-NMR spectral data (Tables 1 and 2) indicated that compound **2** (M^+ at *m/z* 364) had also a labdane skeleton, containing an acetylated hydroxyl function in the bicyclic system, as suggested by the finding of fragments at *m/z* 304 and 189 (de Pascual Teresa et al., 1978) in the mass spectrum. INEPTL experiments revealed a $^3J_{H,C}$ connectivity between the proton signal at 4.53 ppm (double doublet) and the

Table 1
Selected ^{1}H-NMR spectral data for compounds **1**–**3**[a]

H	1	2	3
H-3		4.53 *dd* (12, 5)	
H-6	4.37 *bq* (4.5)		
H-7	2.38 *dd* (15, 5.5)		
H-7′	2.14 *dd* (15, 8.5)		
H-9	2.33 *t* (2.5)		
H-16	0.98 *d* (6.9)	0.97 *d* (6.6)	0.98 *d* (6.6)
H-17	5.00 *s*	4.83 *s*	4.83 *s*
H-17′	4.77 *s*	4.49 *s*	4.49 *s*
H-18	0.99 *s*	0.87 *s*	3.85 *d* (10.5)
H-18′			3.64 *d* (10.5)
H-19	1.20 *s*	0.85 *s*	0.82 *s*
H-20	0.97 *s*	0.70 *s*	0.71 *s*
MeAc		2.06 *s*	2.08 *s*

[a] 300 MHz, $CDCl_3$, TMS as internal standard.; *J* (Hz).

Table 2
^{13}C-NMR spectral data for compounds **1**–**3a**[a]

C	1	1a	2	2a	3	3a
1	43.9	43.9	38.0	38.0	38.5	38.5
2	19.5	19.5	24.3[b]	24.3	18.6	18.6
3	42.0	42.0	80.8	80.8	35.9[b]	36.0
4	34.4	34.4	39.3	39.3	38.0	38.1
5	57.4	57.4	54.7	54.7	49.4	49.5
6	69.4	69.3	23.8[b]	23.8	20.9	20.0
7	47.7	47.7	38.0	38.0	36.9	36.9
8	144.2	144.3	147.8	147.9	148.1	148.1
9	57.7	57.8	56.7	56.8	57.2	57.2
10	40.9	40.9	36.7	36.7	39.6	39.6
11	21.0	21.0	21.1	21.2	21.0	21.0
12	35.5	35.6	35.7	35.8	35.8[b]	35.8
13	30.8	31.0	30.9	31.1	31.0	31.1
14	41.2	41.4	41.1	41.4	41.0	41.4
15	178.8	173.7	178.7	173.9	177.9	173.8
16	19.9	20.0	19.9	20.0	19.9	19.9
17	110.3	110.2	106.8	106.8	106.7	106.6
18	23.6	23.6	28.2	28.2	73.0	73.0
19	33.6	33.7	16.5	16.5	17.5	17.5
20	17.1	17.1	14.5	14.6	14.9	14.9
COAc			171.0	171.0	171.4	171.3
MeAc			21.3	21.3	21.1	20.9
OMe		51.3		51.4		51.3

[a] 75.4 MHz, $CDCl_3$, TMS as internal standard.
[b] In the same column may be interchanged.

carbon resonated at δ 28.2 and 16.5 ppm (18-Me and 19-Me, respectively) and vice versa. The location at C-3 was thus established for the OAc group, which moreover is in β-position because of the coupling constant and the mutual NOE effect between H-3 and 19-Me. The negative value of the optical rotation opposite to those of 3β-acetoxylabdanes (de Pascual Teresa, Urones, Basabe, Carrillo, Muñoz & Marcos, 1985) required compound **2** to be 3β-acetoxy-*ent*-labd-8(17)-en-15-oic acid.

Compound **3** was isomeric with **2** (M^+ at *m/z* 364) and for the same considerations must feature an acetoxy substituent in the decaline moiety. ^{1}H- and ^{13}C-NMR spectral data (Tables 1 and 2) showed that an acetylated hydroxymethyl group (AB system centred at 3.75 ppm) was present, instead of the 18-methyl group. The chemical shifts of the C-18 protons were in close agreement with values for analogous diterpenes with equatorial C-4 substituents (Henrick & Jefferies, 1965). The corresponding alcohol **3b** was reported by two of the authors (Delle Monache, d' Albuquerque, Delle Monache & Marini Bettolo, 1970) as the hydrogenation (at Δ 13, 14) product of copaiferolic acid, isolated from *Copaifera multijuga*. Acetylation (pyridine/Ac_2O) of **3b** gave a product identical (TLC, NMR and MS) with **3**. Compound **3** was thus assigned the structure 18α-acetoxylabd-8(17)-en-15-oic acid.

Labdanes and *ent*-labdanes have been previously reported in the same plant (de Pascual Teresa et al., 1985) as well as in the genus *Haplopappus* (Zdero, Bohlmann & Niemeyer, 1990).

UV (with additives) and ^{1}H-NMR spectral data of compounds **4** and **5** were consistent with those reported in the literature for ayanin (Wang, Hamburger, Gueho & Hostettmann, 1989) and 5′-hydroxyayanin (Yu, Fang & Mabry, 1987), respectively. Since the first one has been reported as fungicide against *Cladosporium cucumerinum* (Wang, Hamburger, Gueho & Hostettmann, 1989), the antimicrobial activity of the extract (Zuñiga, Wilkens, Labbé & Faini, 1995) can be partially explained.

3. Experimental

3.1. Plant material

Haplopappus chrysanthemifolius was collected at Cuesta Cavilolén (IV Region, Chile) in March 1994 and was identified by Dr Sebastián Teillier. A voucher specimen is deposited in the Chemistry Department, Facultad de Ciencias, Universidad de Chile, Santiago.

3.2. Extraction and isolation

Ground air-dried leaves (400 g) were macerated in CH_2Cl_2 (room temperature, 6 h). Evaporation of solvent gave a crude extract (40.2 g), which was fractionated by extended CC (silica gel) with different solvent systems (petrol–EtOAc, CH_2Cl_2–EtOAc or CCl_4–EtOAc). The fractions were further purified by Chromatotron and prepared TLC to give **1** (50 mg), **2** (80 mg), **3** (45 mg), **4** (50 mg) and **5** (60 mg).

3.3. 6α-Hydroxy-ent-labd-8(17)-en-15-oic acid (*1*)

Oil; $[\alpha]_D$ −2 ($CHCl_3$, *c* 1.1); IR (film) ν_{max} cm^{-1}: 3477, 3088, 2926, 2835, 1702, 1647, 1261, 1032, 892, 869. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. MS 70 eV (direct inlet) *m/z* (rel. int.): 322 $[M]^+$ (2), 321 (4), 320 (16), 304 $[M\text{-}H_2O]^+$ (45), 289 (25), 261 (7), 235 (7), 193 (13), 189 [304-chain]$^+$ (19), 153 (65), 123 (44), 109 (91), 69 (100). Methyl ester (with CH_2N_2) (**1a**): oil, $[\alpha]_D^{25}$ −8.8 ($CHCl_3$; *c* 0.4); ^{13}C-NMR: see Table 2.

3.4. 3β-Acetoxy-ent-labd-8(17)-en-15-oic acid (*2*)

Oil; $[\alpha]_D^{25}$ −22.35 ($CHCl_3$, *c* 0.85). IR (film) ν_{max} cm^{-1}: 3340, 2974, 2938, 2854, 1746, 1708, 1642, 1268, 896, 776. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. MS 70 eV (direct inlet), *m/z* (rel. int.): 364 $[M]^+$ (2), 304 $[M\text{-}AcOH]^+$ (58), 289 (21), 261 (33), 235 (6), 203 (6), 189 [304-chain]$^+$ (21), 175 (39), 135 (100), 121 (30), 119 (24), 107 (33). Methyl ester (with CH_2N_2) (**2a**): oil, $[\alpha]_D$25 −17 ($CHCl_3$, *c* 0.14); ^{13}C-NMR: see Table 2.

3.5. 18α-Acetoxylabd-8(17)-en-15-oic acid (*3*)

Oil; $[\alpha]_D^{25}$ +20 ($CHCl_3$, *c* 0.2). IR (film) ν_{max} cm^{-1}: 3340, 3080, 2970, 2940, 2855, 1748, 1708, 1638, 1271, 896, 775; ^{1}H- and ^{13}C-NMR: see Tables 1 and 2; MS 70 eV (direct inlet), *m/z* (rel. int.): 364 $[M]^+$ (6), 304 $[M\text{-}AcOH]^+$ (100), 291 (32), 289 (21), 261 (6), 209 (16), 203 (10), 189 [304-chain]$^+$ (16), 175 (32), 135 (63), 119 (21), 109 (42), 107 (58). Methyl ester (with CH_2N_2) (**3a**): oil, $[\alpha]_D^{25}$ +18 ($CHCl_3$, *c* 0.1); ^{13}C-NMR: see Table 2.

Acknowledgements

This work was supported by DID (Universidad de Chile) Projects Q3546 and E-059/97, DICYT (USACH) and the Project for academic exchange CONICYT-Fundación Andes CNR (Italia).

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