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Diterpenoids from *Haplopappus rigidus*

Glauco Morales *, Patricia Sierra, Luis A. Loyola, Jorge Borquez

Laboratorio de Productos Naturales, Departamento de Química, Facultad de Ciencias Básicas, Universidad de Antofagasta, Casilla 170, Antofagasta, Chile

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Abstract

Fractionation of the aerial parts of *Haplopappus rigidus* Phil., directed by the brine shrimp lethality test (BST), has led to the isolation of two new diterpenoids, rigidusol and deacetylrigidusol. Their structures were established as 13-hydroxy-18-acetoxy-*cis*-cleroda-3,14-diene (8βH, 10βH, 19β, 20α form) and 13,18-dihydroxy-*cis*-cleroda-3,14-diene (8βH, 10βH, 19β, 20α form), respectively. Rigidusol exhibit moderate cytotoxic activity against human breast adenocarcinoma cell line MCF-7. Their structures were established by spectral data, in particular using 2D NMR spectroscopy (DEPT, DQF-COSY, HMQC and HMBC). © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Haplopappus rigidus* Phil.; Asteraceae; Diterpenoids; *cis*-clerodanes; 13-Hydroxy-18-acetoxy-*cis*-cleroda-3,14-diene (8βH; 10βH; 19β; 20α form); 13; 18-Dihydroxy-*cis*-cleroda-3,14-diene (8βH; 10βH; 19β; 20α form)

1. Introduction

The phytochemical study of the Asteraceae species from northern Chile has been the object of our attention over the last 20 years. The genus *Haplopappus* is well represented in Chile by 61 species, about 40% of the total (Marticorena, 1985). *Haplopappus rigidus* Phil., popular name ''baylahuén'', is an endemic species generally found at a height of over 3000 m in the Andes mountains. In this region, the infusion of *H. rigidus* has been used to cure or prevent liver diseases, gastrointestinal disorders, cough and has also been employed as a sexual stimulant (Montes and Wilkomirsky, 1985; Wickens, 1993; Monterrey, 1996). In this paper, we report on the isolation and structural elucidation of two diterpenoids designated rigidusol **1** (13-hydroxy-18-acetoxy-*cis*-cleroda-3,14-diene) (8βH, 10βH, 19β, 20α form) and deacetylrigidusol **2** (13,18-dihydroxy-*cis*-cleroda-3,14-diene (8βH, 10βH, 19β, 20α form), from *H. rigidus* Phil.

2. Results and discussion

The leaves and aerial parts of *H. rigidus* were extracted with PEM (petrol–Et_2O–MeOH 1:1:1). The PEM residue was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 extract was partitioned between aq MeOH (1:9) and hexane. The most strongly bioactive extract, as tested by the brine shrimp lethality test BST (Meyer et al., 1982; McLaughlin, 1991), was the hexane extract (LC_{50} 140.6) and it was submitted to successive CC Si gel, directed by BST at each step, to yield rigidusol **1** and desacetylrigidusol **2**. Rigidusol **1** was isolated as a colourless gum, $[\alpha]_D^{24}-31.8^\circ$ ($CHCl_3$; *c* 0. 11). The molecular formula $C_{22}H_{36}O_3$ was assigned by ^{13}C NMR, DEPT^{13}C NMR and CIMS. No molecular ion peak in the EIMS was observed, but one appeared at *m*/*z* 348 (16%) in CIMS (ammonia). The highest peak in the EIMS of **1** appeared at *m*/*z* 288 (0.4%) and the exact mass in the HREIMS (288.2452) indicated a formula $C_{20}H_{32}O$ which was interpreted as $[M\text{-HOAc}]^+$. The IR spectrum showed absorption bands assigned to hydroxyl groups (3460 cm^{-1} *br*), to an acetate group (1740, 1380, 1230 cm^{-1}) and to an olefinic system (1660, 1640, 1415, 920 cm^{-1}). Deacetylrigidusol **2** was also isolated as a colourless gum, $[\alpha]_D^{24}$ -21.1° ($CHCl_3$; *c* 1.21). The molecular formula $C_{20}H_{34}O_2$ was assigned by

* Corresponding author.

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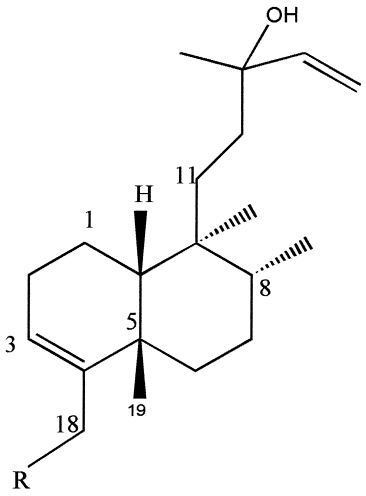

1 R = OAc
2 R = OH

Table 1
^{13}C data for compounds **1**, **2** and sagittariol monoacetate **1***(75 MHz, TMS as int. standard, $CDCl_3$)[a]

C	DEPT	1	2	1*	C	DEPT	1	2	1*
1	CH_2	17.55	17.45	17.0	12	CH_2	35.39	35.15	34.8
2	CH_2	24.30	23.76	23.7	13	C	73.77	73.38	73.1
3	CH	129.57	124.49	128.9	14	CH	145.43	145.22	144.8
4	C	138.38	143.20	137.9	15	CH_2	112.21	111.72	111.5
5	C	36.59	36.25	36.0	16	CH_3	27.98	27.63	27.5
6	CH_2	37.70	36.87	37.1	17	CH_3	16.22	15.81	15.7
7	CH_2	28.78	28.63	28.5	18	CH_2	67.00	64.73	66.4
8	CH	37.46	37.38	37.1	19	CH_3	34.98	34.96	34.4
9	C	40.13	39.84	39.5	20	CH_3	17.77	17.45	17.2
10	CH	45.36	45.22	44.7	OAc		171.31		170.5
11	CH_2	31.90	31.60	31.3			21.54		21.0

[a] Data for sagittariol monoacetate (Sharma et al., 1984).

^1H, ^{13}C NMR, DEPT ^{13}C NMR and EIMS. The IR spectrum showed absorption bands assigned to hydroxyl groups (3450–3300 cm^{-1} *br*), and to an olefinic system (1660, 1640, 1415, 920 cm^{-1}). The ^1H and ^{13}C NMR spectra of **1** and **2** were very similar, suggesting that both compounds had closely related molecular structures. Moreover, analysis of the spectra suggested a diterpenoid nature, and the easy chemical interconversion of **1** in **2** and vice-versa, caused by hydrolysis and acetylation respectively, indicated that rigidusol **1** was the monoacetyl derivative of diol **2**. The ^1H NMR spectra of **1** and **2** showed very similar signals except for the change of a primary acetate to a hydroxymethyl group. The ^1H NMR spectra of **1** and **2** (see Section 3) exhibited signals for: four CH_3 groups, one secondary, two tertiary, and a tertiary carrying an oxygen function; a signal attributable to a geminal methylene adjacent to an acetate group (δ 4.57, *br s*) in **1** or a hydroxymethyl group in **2** (δ 4.04 *dd* and 4.15 *dd*); a proton olefinic signal adjoining a methylene group (δ 5.66 *t*, $J=3.3$ Hz) and, finally, a 12 line system characteristic of an ABC system. Additionally, a methyl group of an acetate group (δ 2.05) in **1** was observed. The ^{13}C NMR spectrum of **1** (Table 1) also showed well-resolved resonances for all 22 carbon atoms. The multiplicity of each carbon atom was determined using DEPT 90° and 135° experiments which revealed the presence of five methyl groups, eight methylene groups, four methine groups and five quaternary carbons, indicating the presence of 35 hydrogen atoms connected to carbon atoms. The ^{13}C NMR spectrum of **1** displayed signals characteristic of two double bonds: a trisubstituted double bond (δ 129.57 *d* and 138.68 *s*), a monosubstituted double bond (δ 112.21 *t* and 145.43 *d*), and a primary acetate group (δ 171.31, 21.54 *q* and 67.00 *t*). The ^{13}C NMR spectrum of **2** (Table 1) showed resonances for 20 carbon atoms. DEPT experiments revealed the presence of four methyl groups, eight methylene groups, four methine groups and five quaternary carbons indicating the presence of 32 hydrogen atoms connected to carbon atoms. The ^{13}C NMR spectrum of **2** displayed signals characteristic of two double bonds: a trisubstituted double bond (δ 124.49 *d* and 143.20 *s*), a monosubstituted double bond (δ 111.71 *t* and 145.22 *d*), and a hydroxymethyl group (δ 64.73 *t*). The signals at δ 73.77 in **1** and 73.38 in **2** were assigned to a quarternary carbon bonded to an OH group. All spectral data for **1** and **2** permitted deduction that both compounds are bicyclic and strongly suggests that each contains a 3,14-clerodadiene-13-ol skeleton.

Compound **1** showed a mass spectral peak at *m*/*z* 189.1665 $[C_{14}H_{21}]^+$ and 71.0560 $[C_4H_7O]^+$ (base peak) in its HRMS spectrum,which were in accordance with to the presence of a bicyclic system and a methyl vinyl carbinol group on the side chain. The presence of an OH group in **2**, and an acetate group in **1**, connected to C-18 is deduced from the change in chemical shifts of carbon atoms of a mono-substituted double bond observed due to the acetylation of **2** in order to produce **1**. The magnitude of these effects observed in C-18 ($\Delta\delta+2.27$), C-4 ($\Delta\delta-4.82$) and C-3 ($\Delta\delta+5.08$) respectively, are similar to values found for crotonic acid, a *cis*-clerodane isolated from *Croton chilensis*, whose structure has been confirmed by X-ray crystallographic studies (C-18 ($\Delta\delta$ 2.11), C-4 ($\Delta\delta$ −4.62) and C-3 ($\Delta\delta$ 4.34) (Borquez et al., 1995).

The ^1H NMR and ^{13}C NMR spectral chemical shifts of **1** and **2** were assigned through direct and long-range C–H correlations using HMBC, HMQC techniques and DFQ–COSY. Among the most relevant couplings observed in the HMQC spectrum of **1** are: methyl protons signal at δ 0.74 (3H, *d*, $J=6.7$ Hz) with carbon atom at δ 16.22 assigned to C-17; 0.76 (3H, *s*) with a carbon atom at δ 17.77 assigned to C-20; 1.09 (3H, *s*)

with a carbon atom at δ 34.98 assigned to C-19; 1.29 (3H, *s*) with a carbon atom at δ 27.98 assigned to C-16; and 2.05 (3H, *s*) with a carbon atom at δ 21.54 assigned to the methyl functionality, an acetate group; a methylene proton signal at δ 4.57 (2H, *br s*) with a carbon atom at δ 67.00 assigned to C-18; 5.06 (1H, *dd*, $J=10.7$, 1.2 Hz) and 5.21 (1H, *dd*, $J=17.4$, 1.2 Hz) with a carbon atom at δ 112.21 assigned to C-15; methine proton signals at δ 1.42 with the carbon atom at δ 37.46 assigned to C-8; 1.35 with carbon atom at δ 45.36 assigned to C-10; 5.86 (*dd* $J=17.4$, 10.7 Hz) with carbon atom at δ 145.43 assigned to C-14; 5.66 (t, $J=3.3$ Hz) with carbon atom at δ 129.57 assigned to C-3.

The connectivities observed in the HMBC spectrum are shown in Fig. 1.

The chemical shifts of carbon C-19 are considered as a direct evidence whereby the mode of A–B ring fusion can be distinguished. (Sharma et al., 1884; Manabe and Nishino 1986; Lopes, Bolzani and Trevisan, 1987). The A–B *cis*-fused structures were proposed on the basis of low field ^{13}C NMR signals of the bridge-head methyl carbons (δ 34.98 in **1** and 34.96 in **2**). The relative stereochemistry of all the stereogenic centres, except for at C-13, in **1** was derived from its ROESY spectrum. In the ROESY of **1** there were significant NOEs effects between Me-20 and H-7α; Me-20 and H-2α; H-10 and H-8; H-1β and Me-19; H-14 and H-15a,b and finally between H-3 and OAc. Therefore, all data above suggests that rigidusol **1** (colourless gum, $[\alpha]_D^{24}$ -31.8° ($CHCl_3$; c 0. 11) and deacetylrigidusol **2** (colourless gum, $[\alpha]_D^{24}$ -21.1° ($CHCl_3$; c 1.21) have the stereochemistry of an homobicyclic system defined as 8βH, 10βH, 19β, 20α form and which correspond to diastereomers of sagittariol monoacetate and sagittariol (white needles, mp 109°, $[\alpha]_D$ $+41^\circ$), respectively (Sharma, Tandon and Dhar, 1975; Sharma et al., 1984). The two pairs of diastereomers are epimeric at C-13, or, having the same configuration at C-13, but differ in the absolute configuration of the centres of the bicyclic system. We are currently attempting to prepare a crystal suitable to establish the absolute sterochemistry for rigidusol **1** and deacetylrigidusol **2**.

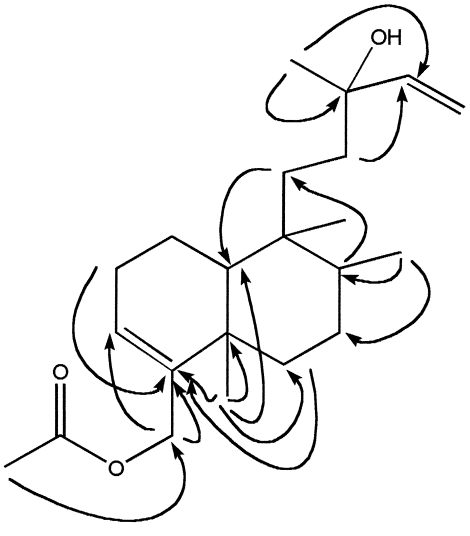

Fig. 1. HMBC correlations for rigidusol **1**.

Rigidusol **1** has moderate cytotoxicity to the human breast adenocarcinoma cell line MCF-7 showing ED_{50} 3.8 μg/ ml compared with adriamicin with ED_{50} 0.5 μg/ ml (Soule et al., 1973) while the compound was found to be inactive against human lung (A-549) and colon (HT-29) tumor cell lines (ED_{50} > 10 μg/ml).

3. Experimental

3.1. Plant material

Leaves and aerial parts of *H. rigidus* were collected in Socaire in northern Chile (23°36′40 s S; 67°50′33 s W, 3230 m) The material was identified by Professor Clodomiro Marticorena, Facultad de Ciencias Biológicas y de Recursos Naturales de la Universidad de Concepción, and voucher specimens are kept at the Herbarium of Universidad de Concepción, Chile.

3.2. Isolation procedure

Dried and ground plant material (1.9 kg) was extracted at r.t. with PEM (petrol, 60–80 °C, Et_2O and MeOH 1:1:1). The PEM ext. (189 g) (F001, BST LC_{50} 350 ppm) was partitioned between CH_2Cl_2 and H_2O to yield a H_2O soluble residue (63 g, F002, BST LC_{50} > 1000 ppm) and a CH_2Cl_2 soluble residue (98 g, F003, BST LC_{50} 298 ppm). F003 was further partitioned between 90% aq. MeOH and hexane resulting in a MeOH aq soluble residue (68 g, F005, BST LC_{50} 680 ppm) and a hexane soluble residue (22 g, F006, BST LC_{50} 140 ppm). A sample of F006 (15 g) was separated by column chromatography over Si gel using hexane and EtOAc gradient with increasing amounts of EtOAc; 250 fractions were collected, and fractions 120–175 (6.3 g) were combined on the basis of TLC and further resolved on another Si gel column eluted with a mixture of hexane and EtOAc. 60 fractions were collected and pooled according to their similar TLC patterns. The active pool (Pool 5, 1.75 g, BST LC_{50} 98 ppm) was subject to another Si gel column eluted with a mixture of hexane and EtOAc, to yield rigidusol **1** (0.58 g, BST LC_{50} 33.3 ppm, MCF-7 ED_{50} 3.8 ppm, A-549 ED_{50} > 10 ppm, HT-29 ED_{50} > 10 ppm). The successive CC Si gel of the fractions 185–230 (3.2 g) using mixture of hexane and EtOAc, yielded deacetylrigidusol **2** (0.25 g, BST LC_{50} (300 ppm, MCF-7 ED_{50} > 10 ppm, A-549 ED_{50} (10 ppm, HT-29 ED_{50} > 10 ppm).

3.3. Biological testing

The extracts, column fractions and pure compounds were evaluated for lethality to brine shrimp larvae (BST). In vitro cytotoxicities against human solid tumour cells were measured in 7-day MTT assays at Purdue Cancer Center, Cell Culture Laboratory, for the A-549 (lung carcinoma), MCF-7 (human breast carcinoma) and HT-29 (human colon adenocarcinoma). Adriamycin was used as a positive cytotoxic control.

3.4. Rigidusol **1**

Colourless gum, $[\alpha]_D^{24} = -31.8^\circ$ ($CHCl_3$; *c* 0.11). IR ν_{max} (film) cm^{-1}: 3460, 3090, 2940–2880, 1740, 1660, 1640, 1465, 1450, 1415, 1380, 1370, 1230, 1040, 1020, 1000, 1075, 980, 920, 895.

^1H NMR (300 Mz, $CDCl_3$) δ 0.74 (3H, *d*, $J = 6.7$ Hz, Me-17), 0.76 (3H, *s*, Me-20), 1.09 (3H, *s*, Me-19), 1.20 (1H, *m*, H-7a), 1.25 (1H, *m*, H-11a), 1.28 (1H, *m*, H-6a), 1.29 (3H, *s*, Me-16), 1.32 (2H, *m*, H-12a; H-7b), 1.35 (1H, *m*, H-10), 1.37 (1H, *m*, H-6b), 1.42 (2H, *m*, H-12b; H-8), 1.78 (1H, *m*, H-1a), 1.92 (1H, *m*, H-2a), 1.96 (1H, *m*, H-1b), 2.05 (3H, *s*, CH_3COO), 2.13 (1H, *m*, H-2b), 3.46 (1H, *s*, OH), 4.57 (2H, *s*, CH_2OAc), 5.06 (1H, *dd*, $J = 10.7$, 1.2 Hz, H-15 *cis*), 5.21 (1H, *dd*, $J = 17.4$, 1.2 Hz, H-15 *trans*), 5.66 (1H, *t br*, $J = 3.3$, H-3), 5.86 (1H, *dd* $J = 17.4$, 10.7 Hz, H-14). ^{13}C NMR (75 Mz, $CDCl_3$) (Table 1).

CIMS (ammonia) *m*/*z*: 348(16%), 330(6), 306(20), 288(18), 271(100), 240(5). EIMS *m*/*z* 288(0.4%), 277(0.4), 270(1), 255(2), 241(1.2), 227(3), 213(1), 199(1), 189(20), 175(5), 159(8), 145(12), 133(14), 121(12), 119(22), 109(13), 107(33), 105(32), 95(24),93(30), 91(36), 81(31), 79(25), 77(13)71(100), 67(20). HREIMS *m*/*z* 288.2452 for $C_{20}H_{32}O$ (calcd 288.2453), 189.1665 for $C_{14}H_{21}$, (calcd 189.1624), 71.0560 for C_4H_7O (calcd 71.0498).

3.4.1. Hydrolysis of **1**

1 (108 mg) was treated with anhydrous K_2CO_3 in MeOH (2.5 ml) at r.t. After 2 h the mixture was acidified with 5% HCl, extracted with Et_2O, concd and purified by cc (silica gel) to give a product whose spectral and physical properties are in agreement with desacetylrigidusol **2**.

3.5. Deacetylrigidusol **2**

Colourless gum, $[\alpha]_D^{24} = -21.1^\circ$ ($CHCl_3$; *c* 1.21). IR ν_{max} (film) cm^{-1}: 3450–3300, 3090, 2950–2830, 1660, 1640, 1465, 1450, 1415, 1370, 1040, 1020, 1000, 1075, 980, 920, 895, 780.

^1H NMR (300 Mz, $CDCl_3$) δ 0.67 (3H, *d*, $J = 6.4$ Hz, Me-17), 0.70 (3H, *s*, Me-19), 1.04 (3H, *s*, Me-20), 1.23 (3H, *s*, Me-16), 4.04 (1H, *dd*, $J = 13$, 1.3 Hz, H-18a), 4.15 (1H, *dd*, $J = 13$, 1.3 Hz, H-18b), 5.02 (1H, *dd*, $J = 10.6$, 1.2 Hz, H-15 *cis*), 5.17 (1H, *dd*, $J = 17.3$, 1.3 Hz, H-15 *trans*), 5.54 (1H, *t br*, $J = 3.3$, H-3), 5.83 (1H, *dd* $J = 17.3$, 10.4 Hz, H-14). ^{13}C NMR (75 Mz, $CDCl_3$) (Table 1).

3.5.1. Acetylation of **2**

A soln of **2** (50 mg) in Ac_2O/C_5H_5N (1:1, 25 ml) was kept at r.t. overnight. The reaction mixture was poured into ice water and the resulting precipitate was extracted with Et_2O. Evapn of the neutral Et_2O layer yielded a gum, which was purified by silica gel CC to afford **1** (38 mg).

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