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SESQUITERPENES FROM *TESSARIA ABSINTHIOIDES*

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Key Word Index—*Tessaria absinthioides*; Compositae; sesquiterpenes; eudesmanes; eremophilanes.

Abstract—The sesquiterpenes tessaric acid, 2-deoxytessaric acid, ilicic acid, 3-oxo-4,11(13)-eudesmadien-12-oic acid and 3β,5α-dihydroxycostic acid have been isolated from the aerial parts of *Tessaria absinthioides*. The structure of a new eudesmane, 3β,5β-dihydroxicostic acid, was established by spectroscopic data. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Tessaria absinthioides (Hook. et Arn.) DC is a very frequent species inhabiting sandy and wet soils in Bolivia, Chile, Uruguay and a great part of Argentina, where it covers thousands of square miles in the river basins.

Tessaria absinthioides infusions have been employed as an antihypercholestrolaemic in folk medicine. The potential therapeutic usefulness of the extract has stimulated research on the isolation of the pharmacologically active components from this plant. We have previously described the isolation of two eremophiladien-12-oic acids, tessaric acid (**1**) and 2-deoxytessaric acid (**2**) [1, 2]. In our continuing research on this species, we have isolated a new eudesmadien-12-oic acid (**3**) together with three known compounds, ilicic acid (**4**) [3], 3-oxo-4,11(13)-eudesmadien-12-oic acid (**5**) [4] and 3β,5α-dihydroxycostic acid (**6**) [5]. This paper describes the isolation and structural elucidation of **3**.

RESULTS AND DISCUSSION

The ethanol extract of *T. absinthioides* was fractioned into acid and neutral fractions. The crude mixture of acids was chromatographed, as described in the Experimental section, to yield the compounds **1**–**6**. However, sesquiterpenes were not found in the neutral fraction.

The acidic compound **3** was obtained as an oil that gave no molecular ion peak in its EI-mass spectrum. The HR EI-mass spectrum revealed the presence of two hydroxyl groups showing two dehydrated ion peaks at *m/z* 248.1413 $[M-H_2O]^+$ $[C_{15}H_{20}O_3]$ and *m/z* 230.1316 $[C_{15}H_{18}O_2]$, which suggested a molecular formula $C_{15}H_{22}O_4$. A peak at *m/z* 145.1009 $[C_{11}H_{13}]$ displayed in the mass spectrum was in agreement with the loss of the hydroxyl groups, the angular methyl group and the acrylic moiety at C-6 from the $[M]^+$. The ^{13}C NMR spectrum of **3** (Table 1) showed the resonances attributed to one methyl, five methylenes, one methine, one quaternary carbon, four olefinic carbons, one oximethine, one oxygenated quaternary carbon and one carboxylic carbon. The presence of only

Table 1. ^{13}C NMR spectral data of compounds **3** (125.7 MHz, $CDCl_3$), **8** and **10** (50.23 MHz, $CDCl_3$)

C	3	8	9 [6]	10
1	30.2	30.7	—	32.8
2	28.2	29.0	—	20.9
3	74.9	70.5	—	29.3
4	146.9	144.4	—	34.8
5	76.9	85.8	87.1	87.9
6	36.6	33.2	32.7	34.2
7	36.0	34.2	34.6	34.4
8	25.6	28.0	—	24.8
9	33.0	31.2	—	31.3
10	38.6	38.6	39.1	38.6
11	144.4	138.4	138.9	139.1
12	171.3	164.9	165.6	166.5
13	124.2	127.9	127.3	127.2
14	22.3	21.0	22.4	21.9
15	116.4	106.6	—	15.1
16	—	169.9	—	—
17	—	22.3	—	—

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Table 2. ^{1}H NMR spectral data of compounds **3** (500 MHz, $CDCl_3$), **8** and **10** (200 MHz, $CDCl_3$)

H	3	8	10
2α	1.85 *m*		—
2β	1.77 *m*	—	—
3	4.34 *t*	5.36 *q*	—
4	—	—	2.22 *ddq*
6α	2.13 *dd*	2.42 *dd*	2.05 *dd*
6β	1.53 *t*	1.75 *m*	1.91 *dd*
7	2.79 *m*	2.93 *t*	2.92 *t*
13	6.23 *s*	6.51 *s*	6.47 *s*
13′	5.62 *s*	5.58 *s*	5.53 *s*
14	1.09 *s*	0.99 *s*	1.07 *s*
15	5.52 *s*	5.41 *s*	0.89 *s*
15′	5.27 *s*	5.05 *s*	—
OAc		2.15 *s*	—

J[Hz]: Compound **3**: 2α,2β = 13; 2α,3α = 2β, 3α = 4,5; 6α,6β = 6β, 7α = 14; 6α,7α = 4,5. Compound **8**: 6α,6β = 14; 6α,7 = 6β, 7 = 4,5. Compound **10**: 4,3α = 13;4,3β = 8;4, 15 = 6;6α,6β = 14; 6α,7 = 6β,7 = 7.8α = 7,8β = 3.

one tertiary methyl group at δ 1.09 and two pairs of vinyl protons (δ 5.52 and 5.27, δ 6.23 and 5.62) in the ^{1}H NMR spectrum (Table 2) suggested that **3** must be a dihydoxyderivative of costic acid. The positions of the two oxygenated carbons were assigned at C-3 and C-5. The presence of a hydroxyl group on C-3 was deduced on the basis that the geminal proton appeared as a triplet at δ 4.34, indicating an allylic position. The stereochemistry of the hydroxyl group was presumed to be axial from the coupling constant (*dd* 3.6; 5.1 Hz) of H-3. On the other hand the position of the second hydroxyl group was supported by the downfield shift of H-14 (δ 1.09) and H-6α (δ 2.13). The HMBC spectrum (Table 3) showed three-bond connectivities between the two H-15 protons and two carbons each bearing a hydroxyl group at δ 74.98 and 76.99. In addition the C-5 signal showed three-bond correlations with H-3 and the methyl protons. This spectrum resembled those of **6** [5], presented signals for the same proton systems, but with some chemical shift differences. Based on the above data compound **3** was presumed to be 3β,5β-dihydroxy costic acid. The *cis*-fused ring system, as well as the stereochemistry of the functional groups in **3**, were confirmed by 2D NOESY experiments (Fig. 1). The chemical transformation of **3** (Scheme 1) into **8** by acetylation with acetic anhydride and pyridine reflected the β-orientation of the tertiary hydroxyl group at C-5. The ^{1}H NMR spectra of **8** (Table 2) was in part close to those of eudesmane 4(15),11(13)-dien-12,5β-olide (**9**) [6], but an additional acetyl group at C-3 was the difference. The $W_{1/2}$ = 14.1 Hz observed for H-3 was explainable in terms of a conformational inversion. The acetylation of the C-3 hydroxyl group was therefore accompanied by the conversion of the *cis*-decalin system from a steroid-like to non-steroid conformation.

Table 3. Correlations observed in the HMBC spectrum of **3**

	^{13}C	
^{1}H	^2J	^3J
1H-3	—	30.2 (C-1), 76.9 (C-5), 116.4 (C-15)
2H-6	76.9 (C-5)	38.6 (C-10), 146.9 (C-4)
2H-13	144.4 (C-11)	36.0 (C-7), 171.3 (C-12)
2H-14	38.6 (C-10)	30.2 (C-1), 33.0 (C-9), 76.9 (C-5)
2H-15	—	74.9 (C-3), 76.9 (C-5)

The structure of the known compounds **1**, **2**, **4**, **5** and **6** were established by direct comparison with authentic samples. The co-occurrence of all these compounds in one species is consistent with previous reports on eremophilanes biosynthesis models (Scheme 2). In order to identify the presence of the metabolite **7** [6] in the plant material this compound was prepared by dehydratation of **4** with *p*-toluenesulphonic acid in dry benzene which yielded **7** and the lactone **10** (Scheme 1). The ^{13}C NMR spectral data of **10** (Table 1) suggested for this eudesmanolide the same relative configuration that was observed for the compound **9** [6] and the derivative **8** here reported.

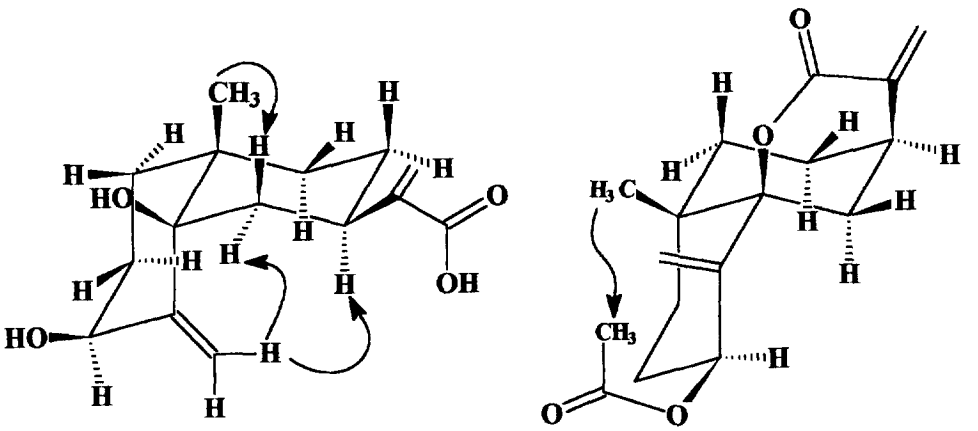
The metabolite **7**, which is most likely the biogenetic precursor of **3**, **5** and **6**, was not identified in the acidic fraction. In order to confirm our hypothesis about the biogenetic pathway we have analysed the bioconversion of **7** with a cell-free extract from fresh plant material. The preliminary results showed that the presence of **3** increased considerably in the assays inoculated with **7**.

EXPERIMENTAL

General experimental procedure. The ^{1}H NMR were recorded in $CDCl_3$ at 200.13 and 500.13 MHz, the ^{13}C NMR were obtained at 50.23 and 125.7 MHz. COSY, HMQC, HMBC, NOESY, XH-CORR and COLOC experiments were obtained using standard software. EIMS were collected at 70 eV. Mps were taken on a hot plate microscope. CC were performed on silica gel G 70-230 mesh and Kieselgel 60 H; TLC were carried out on silica gel 60 F_{254} 0.2mm thick plates using C_6H_6–dioxano–AcOH, 30:5:1 as solvent.

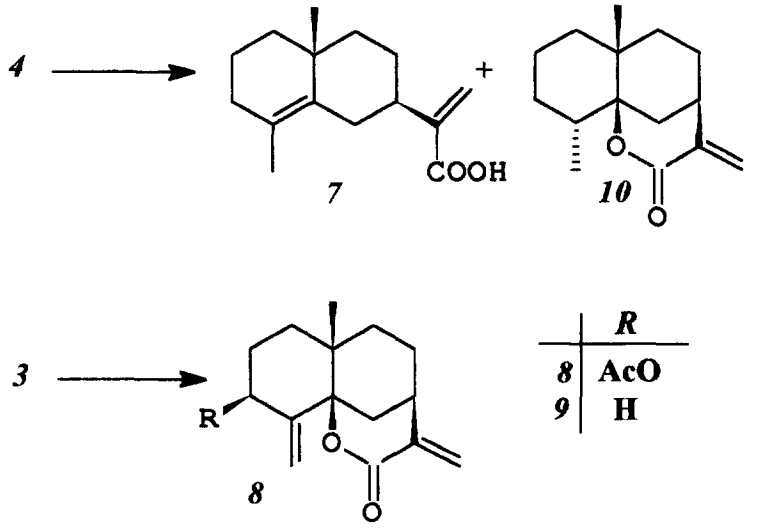
Plant material. Tessaria absinthioides was collected in Mendoza, Argentina, in December 1994, and identified by Luis Del Vitto. A voucher specimen Number 7922 (UNSL) is deposited in the Herbarium of the San Luis University.

Extraction and isolation. Dried and ground aerial part (5 kg), were extracted with EtOH (3 × 48 hr) at room temp. The EtOH extracts were combined and conc *in vacuo*. The residual syrup was suspended in 10% $NaCO_3H$ (5 l) and extract with CH_2Cl_2 (2 l × 4). The aq. layer was acidified with 10% HCl and partitioned against CH_2Cl_2 (2 l × 4). The CH_2Cl_2 extracts were combined and subjected repeatedly to CC on silica gel using *n*-hexane–EtAcO mixtures of increasing



3 8

Fig. 1.



	R
8	AcO
9	H

Scheme 1.

polarity. The yields of the known acids **1**, **2**, **4**, **5** and **6** were 25.0, 0.760, 0.450, 0.150 and 0.095 g, respectively.

3β,5β-Dihydroxycostic acid. (**3**, 105 mg) oil. $[\alpha]_D^{22}$ +122° ($CHCl_3$; *c* 0.05). IR ν_{max}^{KBr} cm^{-1}: 3300, 2950, 1680, 1100, 900. HR EIMS *m/z* (rel. int.) 248.1414 [M-H_2O] (14), 230 (62), 215 (49), 206 (29), 191 (37), 178 (78), 164 (33), 145 (42), 133 (40), 119 (59). NMR: Tables 1, 2 and 3.

Acetylation of **3**. Compound **3** (30 mg) in pyridine-Ac_2O (1:1) was left at room temp. for 24 hr. Usual work-up and CC gave **8** (28 mg). ν_{max}^{KBR} cm^{-1}: 2990, 2940, 1738, 1712, 1625, 1300; NMR spectra see Tables 1 and 2.

Dehydratation of **4**. To a soln of **4** (100 mg, 0.375 mmol) in dry C_6H_6 (40 ml), TsOH (100 mg, 0.581 mmol) was added and the mixture heated at 100°C under a N_2 atmosphere for 5 min. Usual work-up afforded **7** (98 mg). When the reaction was carried out at 100° for 6 hr, it gave, after purification, **10** (38 mg); HR EIMS *m/z* (rel. int.) 234.1617 $[M]^+$ $[C_{15}H_{22}O_2]$. EIMS 234 $[M]^+$ (28), 219 (5), 205 (2), 193 (5), 175 (6), 163 (5), 149 (7), 136 (17), 122 (8), 109 (7), 95 (11); NMR. Tables 1 and 2. Further elution afforded **7** (52 mg).

Bioconversion with cell-free extracts. The biosynthetic capabilities of the cell-free system were investigated by incubation of aliquots of the cell-free extracts prepared from fresh plant material according to ref. [7] using the compound **7** (4 mg) with incubation times of (0, 1.5, 3.0, 6.0, 9.0, 18.0 and 30.0 hr at 28±1°). The incubations were terminated by the addition of 10 ml $CHCl_3$–HCl (9.9:0.1). The $CHCl_3$ soluble fraction was extracted from the reaction mixture. The dried extracts were taken-up in 500 μl Et_2O and analysed by HPTLC. Each incubation was performed in duplicate.

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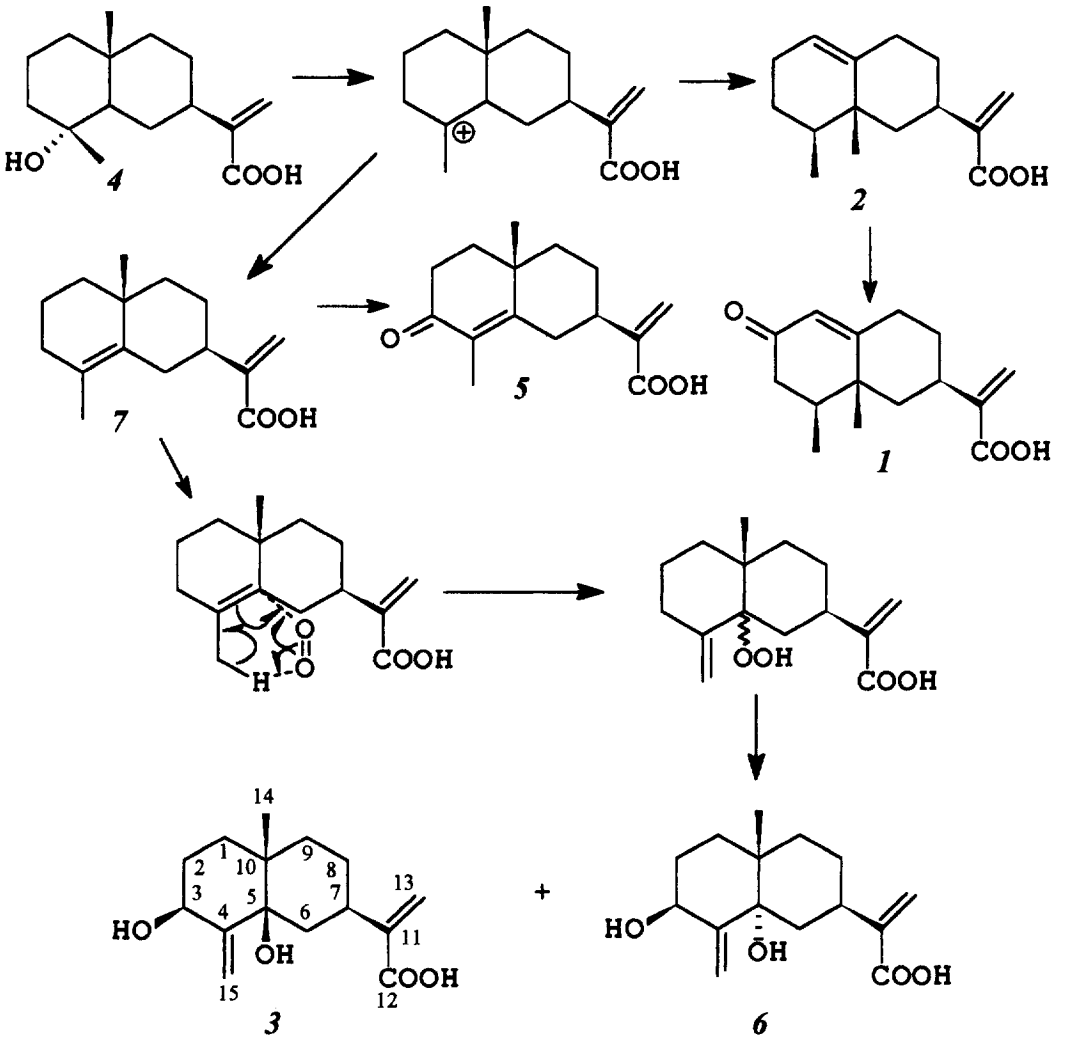

Scheme 2.

L. A. Del Vitto for identification of plant material, to LANAIS-EMAR-CONICET-U.B.A. for MS and HRMS, to CONICET and U.N.S.L. for the financial support.

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