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SESQUITERPENE LACTONES FROM *GOCHNATIA PALOSANTO* AND COUMARINS FROM *G. ARGENTINA*

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Key Word Index—*Gochnatia palosanto* and *G. argentina*; Compositae; aerial parts; sesquiterpene lactones; coumarins; flavonoids.

Abstract—Two sesquiterpene lactones were isolated from the aerial parts of *Gochnatia palosanto*. Their structures were established as desacyldeoxyelephantopin 2-methylbutyrate and desacylisodeoxyelephantopin 2-methylbutyrate. Three coumarins and two flavonoids were isolated from the aerial parts of *Gochnatia argentina*. Their structures were established as capensin, fraxidin, fraxetin and luteolin 7-methyl ether and hispidulin, respectively.

INTRODUCTION

In a recent study of Argentinian *Gochnatia*, we isolated and characterized two *ent*-pimaradine diterpenes [1], acacetin, quercetin 3,3'-dimethyl ether, quercetin 3,4'-dimethyl ether and eriodictyol, from *G. glutinosa*. In the present paper we report the results on the study of *G. palosanto* Cabr. and *G. argentina* (Cabr.) Cabr, which differs from all the other species studied [1–9] in containing coumarins. *Gochnatia palosanto* afforded two sesquiterpene lactones of the deoxyelephantopin type which were previously found in other species of this genus [6], and it differed only in their ester side chains.

RESULTS AND DISCUSSION

Systematic fractionation of a methanolic extract of the aerial parts of *G. palosanto* led to the isolation of two crystalline sesquiterpene lactones (**1** and **2**). Lactones **1** and **2**, $C_{20}H_{24}O_6$, exhibited ^{1}H NMR (Table 1) spectra similar to those of **3** and **4** from *Elephantopus carolinianus* [10], except for the signals due to the ester side chain at C-8 which were characteristic of a 2-methylbutyrate residue. This was confirmed by the mass spectrum, *m/z* 258 $[M-C_5H_{10}O_2]^+$, 85 $[C_5H_9O]^+$ and 57 $[C_4H_9]^+$ (100%) and by ^{1}H NMR signals at δ 2.31 (*m*, 1H), 1.41 (*m*, 1H), 1.68 (*m*, 1H), 0.88 (*t*, $J = 7$ Hz, 3H) and 1.19 (*d*, $J = 7$ Hz, 3H) due to H-17, H-18$_a$, H-18$_b$, H-19 and H-20, respectively.

The aerial parts of *G. argentina* afforded a complex mixture of flavonoids (**6** and **7**) and coumarins (**5**, **8** and **9**) which could be separated first by column chromatography (silica gel), and then on Sephadex LH-20. Compounds **6** and **7** were identified as luteolin 7-methyl ether and hispidulin, respectively, from their UV, ^{1}H NMR and mass spectral data. The coumarins were identified as capensin (**5**) [11], fraxidin (**8**) and fraxetin (**9**) by comparison of our spectral and physical data with those published.

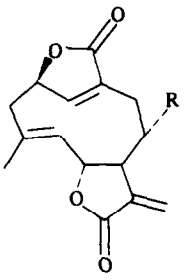
EXPERIMENTAL

Mps: uncorr; ^{1}H NMR: 60 MHz, $CDCl_3$ and C_6D_6 (coumarins and flavonoids), 300 MHz, $CDCl_3$ (lactones), TMS as int. standard; ^{13}C NMR: 75.429 MHz, $CDCl_3$; MS 70 eV, direct

Table 1. ^{1}H NMR spectral data of **1** and **2** (300 MHz, $CDCl_3$, δ-values)

H	1	2
1	7.08 *s*	7.15 *s*
2	5.45 *dd*	5.37 *br d*
3a	2.68 *dd*	2.38 *dd*
3b	2.83 *dd*	2.94 *br d*
5	4.77 *d*	5.10 *d*
6	5.10 *dd*	5.15 *dd*
7	2.96 *m*	3.12 *dddd*
8	4.55 *ddd*	4.47 *ddd*
9a	2.72 *dd*	2.67 *dd*
9b	2.96 *dd*	2.96 *dd*
13a	5.69 *d*	5.72 *d*
13b	6.29 *d*	6.28 *d*
14	1.83 *s*	1.77 *s*
17	2.31 *m*	2.33 *m*
18a	1.41 *m*	1.41 *m*
18b	1.68 *m*	1.68 *m*
19	0.88 *t*	0.88 *t*
20	1.19 *d*	1.19 *d*

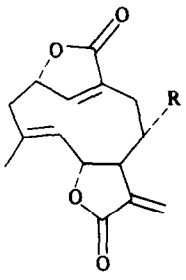
Proton coupling constants in Hz: Compound **1**: 2, 3a = 2; 2, 3b = 4; 3a, 3b = 14; 5, 6 = 10; 6, 7 = 7.5; 7, 8 = 4; 7, 13a = 3.5; 7, 13b = 4; 8, 9a = 11; 8, 9b = 2; 9a, 9b = 12. Compound **2**: 2, 3a = 5; 3a, 3b = 14; 5, 6 = 10.5; 6, 7 = 7.5; 7, 8 = 4; 7, 13a = 3.5; 7, 13b = 4; 8, 9a = 4.5; 8, 9b = 12; 9a, 9b = 13.



R

1 2-MeBu

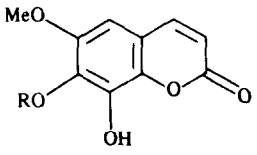
3 MeAcr



R

2 2-MeBu

4 MeAcr



R

5 —$CH_2CH{=}C(Me)_2$

8 Me

9 H

Table 2. ^{13}C NMR spectral data of **1** and **2** (75.429 MHz, $CDCl_3$, δ-values)

C	1	2
1	153.1	149.2
2	81.3	79.0
3	33.6	30.2
4	128.6*	131.5*
5	133.7	125.4
6	77.9	78.8
7	52.1	49.8
8	71.0	73.3
9	41.3	40.1
10	128.8*	133.8*
11	135.9	135.3
12	169.3	169.3
13	123.9	123.6
14	172.2	174.2
15	20.1	21.5
16	175.6	175.9
17	41.0	41.0
18	25.9	26.0
19	11.6	11.7
20	16.7	16.8

*Assignments may be interchanged.

inlet; TLC: silica gel UV-254, solvent systems C_6H_6–dioxane–HOAc (45:5:1 and 90:25:4).

Plant material. Gochnatia palosanto and *G. argentina* were collected in Tucuman and Entre Rios (Argentina), respectively, and identified by Luis del Vitto.

Extraction and isolation. The aerial parts (1.3 and 1.5 kg) were air-dried, finely ground and extracted at room temp. with MeOH (3 times × 24 hr). The crude extract obtained by evapd red. pres. was dissolved in MeOH containing H_2O (10, 20 and 30%) then partitioned between *n*-hexane, CCl_4 and $CHCl_3$, respectively. The $CHCl_3$ extract was adsorbed on silica gel packed in C_6H_6 and eluted with C_6H_6–EtOAc mixture of increasing polarity.

Gochnatia palosanto. Fractions 7–9 (C_6H_6–EtOAc, 9:1) yielded crystalline residues contained two substances, **1** and **2**, which were separated by successive chromatography over silica gel, 0.020 g of **1** and 0.11 g of **2**.

Desacyldeoxyelephantopin 2-methylbutyrate (**1**). Colourless crystals, mp 163–164°; IR ν_{max}^{KBr} cm^{-1} 3100, 1760, 1750, 1745, 1640; MS *m/z* (rel. int.): 360[M]$^+$ (1), 276 [M−84]$^+$ (3), 258 [M−102]$^+$ (8), 230 [M−102−28]$^+$ (2), 172 (7), 162 (5), 134 (4), 85 (54), 83 (64), 57 (100). Accurate mass peak *m/z* 276 ($C_{15}H_{16}O_5$): 276.0973 $C_{15}H_{16}O_5$ requires 276.0998.

Desacylisodeoxyelephantopin 2-methylbutyrate (**2**). Colourless crystals, mp 196–198°; $[\alpha]_D$ +206.9° ($CHCl_3$; *c*0.095); IR ν_{max}^{KBr} cm^{-1} 3100, 1760, 1750, 1745, 1635; MS *m/z* (rel. int.): 360 [M]$^+$ (0.5), 276 [M−84]$^+$ (1), 258 [M−102]$^+$ (9), 230 [M−102−28]$^+$ (2) 172 (5), 162 (8), 134 (4), 85 (61), 83 (69), 57 (100). Accurate mass of peak *m/z* 276 ($C_{15}H_{16}O_5$): 276.0959 $C_{15}H_{16}O_5$ requires 276.0998.

Gochnatia argentina. Fractions 12–15 (C_6H_6–EtOAc, 85:15) contained two substances **5** and **6**, which were separated by successive chromatography over Shephadex LH-20, afforded 0.35 g of **5** and 0.06 g of luteolin 7-methyl ether (**6**). 8-Hydroxy-6-methoxy-7-[(3-methyl-2-butenyl)oxy]-2H-1-benzopyran-2-one, Capensin (**5**). Colourless crystals from C_6H_6–petrol (60–80°), mp 132–134°; IR ν_{max}^{KBr} cm^{-1} 3500–3400, 1700, 1620, 1540; UV λ_{max}^{MeOH} 312, 253 nm; λ_{max}^{NaOMe} 335, 272 nm; ^1H NMR [$CDCl_3$ (C_6D_6)]: δ7.59 (6.57) (1H, *d*, *J* = 9.5 Hz, H−4), 6,48 (5.78) (1H, *s*, H-5), 6.31 (5.84) (1H, *d*, *J* = 9.5 Hz, H-3), 5.52 (5.42) (1H, *t*, *J* = 7 Hz, >CH=CH_2O–), 4.63 (4.58) (2H, *d*, *J* = 7 Hz, >CH=CH_2O–), 3.88 (3.26) (3H, *s*, OMe), 1.75 (1.73) (3H, *s*, Me–C(I)=), 1.68 (1.65) (3H, *s*, Me–C(I)=), MS *m/z* (rel. int.): 276 [M]$^+$ (1.5), 261 [M−15]$^+$ (0.5), 208 [M−68]$^+$ (100), 193 [M−68−15]$^+$ (18), 180 [M−68−28]$^+$ (10), 165 (6.5), 161 (3), 137 (8), 123 (6), 109 (7), 95 (7), 69 (53). Accurate mass of M$^+$ ($C_{15}H_{16}O_5$): 276.0990 $C_{15}H_{16}O_5$ requires 276.0998.

Fractions 20–24 (C_6H_6–EtOAc, 70:30) contained two substances, **7** and **8**, which were separated and purified by successive chromatography over Shephadex LH-20. 0.085 g of Hispidulin (**7**) and 0.01 g of fraxidin (**8**).

Fractions 34–36 (C_6H_6–EtOAc, 50:50) contained 0.06 g. fraxetin (**9**), purified by successive chromatography over Shephadex over LH-20.

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EPI-DANSHENSPIROKETALLACTONE FROM *SALVIA MILTIORRHIZA*

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Key Word Index—*Salvia miltiorrhiza*; Labiatae; abietanoid; *epi*-Danshenspiroketallactone

Abstract—The structure of a new abietanoid pigment from the Chinese traditional medicine Dan-shen, *Salvia miltiorrhiza*, was isolated and its structure determined by NMR spectroscopy.

INTRODUCTION

The dried roots of the Chinese sage, *Salvia miltiorrhiza* Bunge, are used in the traditional medicine, Dan-shen, to treat heart disease, [1] hepatitis, [2] and more recently, in the treatment of tuberculosis [3, 4] and leprosy [5]. Numerous abietanoid pigments have been isolated from this drug and identified as physiologically active natural products [6–13]. Several synthetic investigations have also been devoted to these compounds [14–21]. The activities of these Dan-shen constituents in the pure state, however, has not matched the activity of the crude drug itself [10]. For this reason, considerable effort is still being devoted to identifying the minor constituents of this traditional medicine, as well as determining the most effective mixture of components for eliciting its therapeutic value.

Recently, the structure of a new spirolactone, named danshenspiroketallactone (**1**) was determined by X-ray analysis [12]. In our work on the constituents of Dan Shen, we have also isolated **1**, but prior to recrystallization, had been unable to separate **1** from a minor compound by any chromatographic method. We have now identified this minor component as *epi*-danshenspiroketallactone (**2**). For reasons discussed below, we demonstrate that **2** is not an artifact of the isolation process (via epimerization of **1**), but more likely that **1** results from the epimerization of **2**.

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RESULTS AND DISCUSSION

The 95% ethanolic extract of Dan-shen was fractionated as previously described [11]. The residue from evaporation of the mother liquor from the tanshinone IIA recrystallization yielded 50 mg of ether soluble material. Chromatography on silica gel with cyclohexane and cyclohexane–CH_2Cl_2 afforded **1** and **2** as a 7:1 mixture (**1**:**2**), and tanshinlactone (**3**) [13]. An exhaustive effort to resolve **1** and **2** with normal and reverse phase HPLC using numerous solvent systems was not successful. Recrystallization from ethanol, however, gave pure **1** and a mother liquor enriched in **2**, thus accounting for the ability of the Shanghai group to obtained **1** in pure form for X-ray analysis [12]. Two additional recrystallizations ultimately afforded a 4:6 mixture of **1**:**2** (3 mg total wt). Due to the limited amount of material, no further attempts to purify **2** via additional recrystallizations were made.

From both the ^1H and ^{13}C NMR spectra it was apparent that **1** and **2** were epimers (Table 1) with only the ^1H- and ^{13}C-nuclei of the isoprene moieties differing in their chemical shift values. The mass spectrum also indicated only a single molecular ion (EIMS: M^+ at m/z 268). The ^1H-homonuclear coupling connectivities of the isoprene moieties of **1** and **2** could be independently mapped in the ^1H-homonuclear COSY spectrum (Fig. 1) of the initially obtained mixture (prior to recrystallization). The ^1H-aromatic signals of **1** and **2** coincided in benzene-d_6.

Additional NMR evidence revealed **2** to be the C-15