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GUAIANOLIDES AND EUDESMANOLIDES FROM *CENTAUREA ORNATA*

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ABSTRACT.—Four sesquiterpene lactones and three other compounds have been isolated from the EtOH-H_2O-soluble fraction of a $CHCl_3$ extract of *Centaurea ornata*. A new grosshemin derivative **3** is described, and the stereochemistry of natural 11-*epi*-dihydroreynosin [**2**] is revised.

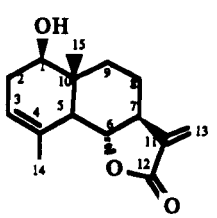
As a part of our study of sesquiterpene lactones from species of the family Compositae (1,2), we have investigated *Centaurea ornata* Will., for which no phytochemical analysis has been reported. The aerial parts of this plant have hypoglycemic effects at high doses in rats, and the root extracts show antispasmodic and analgesic effects.[1]

From that portion of the $CHCl_3$ extract of the aerial parts of the plant soluble in EtOH/H_2O, we have isolated two eudesmanolides, santamarin [**1**] and 11-*epi*-dihydroreynosin [**2**], and two guaianolides, **3** and **5**. Compound **3** is a new natural product related to grosshemin. Vanillin, 5-methyl-8-hydroxycoumarin (3), and the flavonoid salvigenin (4) were also isolated.

RESULTS AND DISCUSSION

Compound **2** was obtained as colorless needles, mp 142–145°, and displayed a molecular ion peak at *m/z* 250 ($C_{15}H_{22}O_3$). The ir absorptions (hydroxyl, α-methylene-γ-butyrolactone, and methylidene groups) and the ^{1}H-nmr data were coincident with those previously reported (5) for synthetic 11-*epi*-dihydroreynosin [**2**]. This substance has also been isolated from *Artemisia herba-alba* (6), but the configuration assigned at C-11 must be reversed. The aromatic-induced solvent shift for the C-11 methyl group, $\Delta\delta CDCl_3$-$C_6D_6 = 0.41$ ppm, is characteristic of a reported pseudoaxial methyl group (7). Moreover, the ^{13}C-nmr data of **2** confirm the β configuration for the C-11 methyl group, which is shielded at δ 9.7 in agreement with the observations of Pregosin *et al.* (8), while the chemical shift reported for the C-11 α-methyl group in dihydroreynosin is δ 12.5 (9). The ^{13}C-nmr spectrum also differed at C-7, C-8, C-11, and C-12 from that of dihydroreynosin.

Compound **3** was obtained as an amorphous solid and showed a molecular ion peak at *m/z* 364 ($C_{19}H_{24}O_7$). The ir spectrum exhibited absorption bands typical of an α,β-unsaturated-γ-lactone group (1760 cm^{-1}), hydroxyl groups (3400 cm^{-1}), cyclopentanone, and acyl carbonyl groups (1740, 1730 cm^{-1}). In the ^{1}H-nmr spectrum exomethylene protons typical of a conjugated γ-lactone were observed at δ 6.25 and δ



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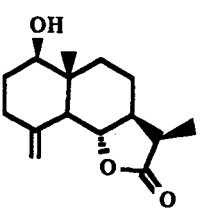

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[1]Personal communication from Professor San Román, Faculty of Pharmacy, Salamanca University.

TABLE 1. ^{1}H-nmr Spectral Data of Compounds **3**–**7**.[a]

Proton	Compound					
	3	**3**[b]	**4**	**5**	**6**	**7**
H-1	3.20 ddd (8.5, 1.5)	3.39 ddd	3.18 ddd	2.96 ddd (12.2, 8.5, 7.6)	2.93 ddd (12.2, 8.0, 7.5)	2.92 ddd
H-2α, -2β	2.20–2.50 m	2.83 dd (18.4, 8.5)	2.18–2.48 m	1.74 ddd (13.6, 12.2, 7.3)	1.70 ddd (14, 12.2, 8.0)	1.69 ddd
		2.94 dd		2.24 ddd (13.6, 7.6, 7.3)	2.24 dt (14, 8.0)	2.21 d
H-3	—	—	—	4.54 tt (7.3, 1.9)	5.43 dt (8.0, 1.8)	5.51 dt (8.0, 1.8)
H-4	2.41 dq (9.5, 7.1)	3.00 dq	2.18–2.48 m	—	—	—
H-5	2.20–2.50 m	2.41 ddd (10.4, 9.5, 8.5)	2.18–2.48 m	2.78 dd (10.5, 8.5)	2.75 dd (10.7, 7.5)	2.73 dd (10.7, 7.5)
H-6	4.06 dd (10.4, 9.3)	4.03 dd	4.04 dd	4.30 dd (10.5, 9.0)	4.11 dd (10.7, 9.1)	4.11 dd (10.7, 9.1)
H-7	3.28 dddd (9.7, 9.3, 3.3, 3.1)	3.48 dddd	3.30 dddd	3.13 dddd (9.3, 9.0, 3.3, 3.1)	3.08 dddd (9.3, 9.1, 3.4, 3.1)	3.07 dddd (9.3, 9.1, 3.4, 3.1)
H-8	5.06 ddd (9.7, 6.3, 5.7)	5.68 ddd	5.08 ddd	5.15 ddd (9.3, 5.0, 2.8)	5.10 ddd (9.3, 5.0, 3.1)	5.08 ddd
H-9	2.84 dd (13.6, 6.3)	2.72 dd	2.86 dd	2.67 dd (15, 5.0)	2.60 dd (15, 5.0)	2.35 dd
	2.20–2.50 m	2.10 dd (13.6, 5.7)	2.18–2.48 m	2.38 dd (15, 2.8)	2.35 dd (15, 3.1)	2.39 dd
H-13a, -13b	6.25 d (3.3)	7.32 d	6.28 d	6.28 d (3.3)	6.10 d (3.4)	6.16 d
	6.07 d (3.1)	7.03 d	5.90 d	5.90 d (3.1)	5.60 d (3.1)	5.75 d
H-14a, -14b	5.05 br s	5.55 br s	5.10 s	5.13 br s	5.06 d (1.1)	5.08 d
	4.92 br s	5.41 br s	4.93 s	4.86 br s	4.85 d (1.8)	4.98 d

TABLE 1. Continued.

Proton	Compound					
	3	3[b]	4	5	6	7
H-15	1.21 d (7.1)	2.04 d	1.20 d	5.44 br s 5.36 br s	5.40 br s	5.44 br s
H-3′a, -3′b	3.82 d (11.3)	4.60 d	4.54 d	3.86 d (10.7)	4.26 d (11.6)	4.51 d
	3.61 d (11.3)	4.38 d	4.32 d	3.61 d (10.7)	4.08 d (11.6)	4.25 d
H-4′	1.34 s	2.14 s	1.57 s	1.34 s	1.35 s	1.54 s
OAc			2.05 s 2.05 s		2.02 s 2.02 s	2.02 s 2.02 s 2.00 s

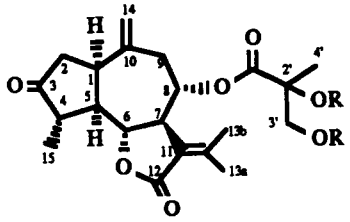
[a]Chemical shifts are in ppm from TMS, coupling constants are in parentheses in Hz.
[b]In C_6D_6.

6.07 in addition to two broad singlets at δ 5.05 and δ 4.92 which are attributed to another exomethylene group. The trans configuration of the γ-lactone ring was deduced from the coupling constants (Table 1) for the β-methyne proton of the γ-lactone ring at δ 3.28. This is coupled to the exomethylene protons with $J > 3$ Hz in agreement with Samek's rule (10) for a *trans*-lactone, and showed *trans*-coupling ($J = 9.3$ and 9.7 Hz in Table 1), with the signals at δ 4.06 (H-6) and δ 5.06 (H-8). The presence of a cyclopentanone ring was deduced from the ^{13}C-nmr (δ 218.2) and the ir (1740 cm^{-1}) data, which suggested a guaianolide structure for the lactone **3**. Also observed were the two double doublets at δ 2.94 and δ 2.83 (C_6D_6, Table 1) of the C-2 methylene group and a double quartet at δ 2.41 coupled with a methyl group at δ 1.21. The cis bicyclic junction was assigned from the coupling constants $J_{1,5} = 8.5$ Hz and $J_{5,6} = 10.4$ Hz (11).

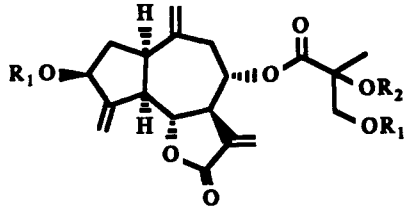
All the above data are very similar to those of grosshemin (12–14). Hence both substances have the same ring structure, but **3** also possesses an ester group. The presence of a -CH_2O- group [two doublets at δ 3.82 and δ 3.61 ($J = 11$ Hz)] (15) and a quaternary methyl group (δ 1.34, s) suggests that the acyl group is an α,β-dihydroxyisobutyryl moiety in agreement with the peak at *m/z* 244 in the mass spectrum, due to loss of an acid ($C_4H_8O_4$). The structure **3** was reinforced by a 2D ^{1}H-^{1}H homonuclear correlation experiment (COSY); this compound therefore is grosshemin α,β-dihydroxyisobutyrate.

There are small differences in chemical shifts of some signals of **3** compared with other lactones of the same type (16). The observed differences for H-13b (δ 6.07) and H-9β (δ 2.38) could be interpreted as a change of stereochemistry at C-2′, which would have the greatest steric and anisotropic effects at both protons as was proposed by Merrill and Stevens (17) to explain the differences in the epimeric epoxides. These differences are reduced in the diacetate **4** (Table 1).

Compound **5** showed ir and mass spectra coincident with those described for a lactone isolated from *Centaurea kotschyi* (18). Their ^{1}H-nmr spectra were identical except for a small difference in the chemical shift for H-13b, and we propose that both substances differ in configuration at C-2′ and are diastereomers. Acetylation of **5** gave the corresponding triacetate **7**, the structure of which was confirmed by a 2D ^{1}H-^{1}H homonuclear correlation experiment. The ^{13}C-nmr spectral data of the acetates **6** and **7** are presented in Table 2.



3 R=H
4 R=Ac



5 $R_1=R_2=H$
6 $R_1=Ac$, $R_2=H$
7 $R_1=R_2=Ac$

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ on a Perkin-Elmer 241 polarimeter. Spectra were recorded with the following instruments: ir, Beckman AcuLab VIII spectrophotometer; nmr, Bruker WP 200 SY (^{1}H, 200 MHz; ^{13}C, 50.3 MHz) recorded in $CDCl_3$ or C_6D_6 with TMS as internal standard; mass spectra, Hewlett-Packard 5930A with direct inlet probe at 70 eV.

TABLE 2. ^{13}C-nmr Data of Compounds **3–7**.

Carbon	Compound				
	3	**4**	**5**	**6**	**7**
C-1	40.7	41.1	45.6	45.5	45.4
C-2	42.3[a]	42.4[a]	36.1	36.5[a]	35.8[a]
C-3	218.2	217.7	73.6	75.3	74.8
C-4	46.7[b]	46.7[b]	152.1	147.1	146.7
C-5	51.5	52.0	51.8	51.7	51.6
C-6	80.6	80.6	78.2	77.5	80.6
C-7	46.5[b]	47.2[b]	47.4	47.4	47.2
C-8	75.9	75.8	75.6	74.8	74.1
C-9	40.7[a]	40.0[a]	39.2	36.2[a]	34.9[a]
C-10	141.5	141.6	141.8	141.1	140.7
C-11	135.6	136.4	137.0	137.5	137.0
C-12	174.7	170.0	175.0	173.4	170.0
C-13	125.2	124.3	123.5	121.9	122.0
C-14	117.3	117.7	118.2	118.5	118.2
C-15	15.1	15.4	113.9	116.0	115.9
C-1′	168.9	168.7	169.2	170.4	169.4
C-2′	75.5	78.6	77.2	73.6	77.2
C-3′	67.9	65.4	68.1	68.7	64.9
C-4′	21.8	20.0	21.9	22.3	19.4
OAc	—	169.3	—	169.9	169.2
	—	169.8	—	168.4	168.8
					168.1
		20.6	—	20.9	20.0
		20.8	—	20.4	20.3
					20.6

[a,b]Values in the same column with the same superscript may be interchanged.

PLANT MATERIAL, EXTRACTION, AND ISOLATION.—The plant was collected at Aldealengua, Salamanca, Spain, and a voucher specimen was deposited in the Botany Department (SALA 7315), University of Salamanca. The air-dried aerial part of *C. ornata* (4 kg) was extracted with $CHCl_3$ in a Soxhlet apparatus for 12 h to give 90 g of crude extract. The fraction of this extract soluble in EtOH-H_2O (2:3) was extracted with $CHCl_3$ (20 g) and fractionated on a column of Si gel (Merck 7733, 500 g), eluting with hexane/Et_2O mixtures of increasing polarity. The compounds vanillin (65 mg), 5-methyl-8-hydroxycoumarin (90 mg), salvigenin (75 mg), santamarin [**1**] (125 mg), 11-*epi*-dihydroreynosin [**2**] (140 mg), **3** (3.1 g), and **5** (4 g) were isolated by repeated chromatographic separations (cc and tlc on Si gel) and crystallization.

Santamarin [**1**].—Eluted from a Si gel column with $CHCl_3$-MeOH (98:2); colorless crystals, mp 133–135° (Et_2O/hexane); $[\alpha]^{24}$ (λ nm) +46.8° (589), +63.1° (578), +66.4° (546), +67.6° (436) ($c = 0.7$); ir γ max (KBr) 3380, 3030, 1775, 1680, 860, 820 cm^{-1}; ^{1}H nmr δ 0.89 (3H, s, H-15), 1.85 (3H, br s, H-14), 3.68 (1H, dd, $J = 9.7$ and 6.5 Hz, H-1), 3.95 (1H, dd, $J = 11.1$ and 10.8 Hz, H-6), 5.36 (1H, br s, OH), 5.41 (1H, d, $J = 3.0$ Hz, H-3), 6.02 (1H, d, $J = 3.3$ Hz, H-13) ppm.

11-epi-*Dihydroreynosin* [**2**].—Eluted from Si gel with $CHCl_3$-MeOH (9:1); colorless needles mp 142–145° (hexane/Et_2O); $[\alpha]^{24}$ (λ nm) + 1.8° (589), +1.8° (578), +2.1° (546), +3.6° (436) ($c = 0.9$); ir γ max 3450, 3070, 1775, 1660, 1210, 1180, 1040, 1000, 910 cm^{-1}; ^{1}H nmr δ 0.82 (3H, s, H-15), 1.20 (3H, d, $J = 7$ Hz, H-13), 2.65 (1H, q, $J = 7.6$ Hz, H-11), 3.51 (1H, dd, $J = 11.4$ and 4.6 Hz, H-1), 4.27 (1H, dd, $J = 10.8$ Hz, H-6), 4.84 (1H, d, $J = 1.4$ Hz, H-14b), 4.98 (1H, d, $J = 1.4$ Hz, H-14a) ppm; ^{13}C nmr δ 78.5 (C-1), 31.5 (C-2), 36.2 (C-3), 143.0 (C-4), 53.2 (C-5), 78.3 (C-6), 48.3 (C-7), 20.4 (C-8), 33.7 (C-9), 42.8 (C-10), 38.8 (C-11), 179.9 (C-12), 9.7 (C-13), 110.5 (C-14), 11.7 (C-15); ms *m/z* (%) 250 (26), 232 (100), 192 (78), 178 (40), 165 (80), 149 (51), 121 (83), 107 (78), 91 (93).

Grosshemin α,β-*dihydroxyisobutyrate* [**3**].—Pale yellow oil eluted with $CHCl_3$-MeOH (9:1); $[\alpha]^{24}$ (λ nm) + 1.9° (546) ($c = 1.8$); ir γ max 3400, 3080, 1770, 1745, 1740, 1645, 1270, 1050, 980, 895 cm^{-1};

^{1}H nmr see Table 1; ^{13}C nmr see Table 2; ms *m/z* (%) 364 (2), 346 (1), 333 (9), 262 (12), 244 (36), 176 (6), 75 (72), 43 (100).

Acetate **4**.—Compound **3** (500 mg) in pyridine (3 ml) and Ac_2O (3 ml) was left overnight at room temperature. After the usual workup acetate **4** (480 mg) was isolated: ir γ max 3080, 1775, 1750, 1740, 1645, 1250, 1170, 1040, 980, 895 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

3α-*Dihydro*-4(15)-*dehydrogrosshemin* α,β-*dihydroxyisobutyrate* [**5**].—Eluted with $CHCl_3$-MeOH (8:2); white solid, mp 128°, $[\alpha]^{24}$ (λ nm) +1.3° (578), +1.5° (546), +2.6° (436) (*c* = 1.2); ir γ max 3400, 3080, 1750, 1730, 1645, 1270, 1135, 1060, 1020, 940, 850 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; ms *m/z* (%) 364 (5), 346 (1), 333 (8), 261 (14), 244 (27), 119 (38), 91 (68), 75 (100).

Acetates **6** *and* **7**.—Compound **5** (2 g) and excess Ac_2O/pyridine was left overnight at room temperature. After the usual workup the crude product was chromatographed on Si gel yielding acetate **6** (1.4 g) and acetate **7** (0.5 g).

Compound **6** formed white crystals, mp 132.5° ($CHCl_3$); $[\alpha]^{24}$ (λ nm) +0.8° (589), +0.8° (576), +0.9° (546), +1.6° (436) (*c* = 1.3); ir γ max (KBr) 3080, 1780, 1750, 1650, 1250, 1150, 1115, 1060, 1035, 1025, 960, 910, 880, 810 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

Compound **7** was a pale yellow oil, $[\alpha]^{24}$ (λ nm) +0.5° (579), +0.6° (546), +1.4° (436) (*c* = 1.0); ir γ max 3450, 3080, 1775–1740, 1645, 1250, 1115, 1070, 980, 930, 835 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

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