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SESQUITERPENE LACTONES FROM *CENTAUREA UNIFLORA* SUBSP. *NERVOSA*

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Key Word Index—*Centaurea uniflora* subsp. *nervosa*; Compositae; sesquiterpene lactones.

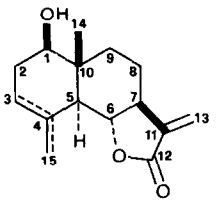
Abstract—Besides known compounds, the leaves of *Centaurea uniflora* subsp. *nervosa* afforded a new highly oxygenated guaianolide, whose structure was established by spectral data and chemical reactions.

INTRODUCTION

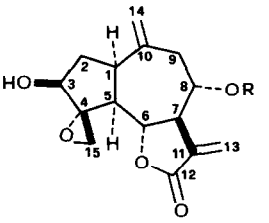
As a part of an investigation of alpine plants [1–4], we have studied the constituents of *Centaurea uniflora* Turra subsp. *nervosa* (Willd.) Bonnier et Layens. We report here the isolation of five sesquiterpene lactones from the leaves of this plant.

RESULTS AND DISCUSSION

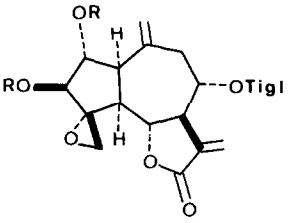
Column chromatography of a chloroform extract gave the eudesmanolides santamarin (**1**) and reynosin (**2**), and a mixture of three guaianolides (**3**–**5**) which could be only partially separated by column chromatography. Pure products were obtained by preparative HPLC (see Experimental). Compounds **3** and **4**, both colourless gums, were dihydroxyguaianolides esterified at C-8 by hydroxymethacrylic and 4-hydroxytiglic acids respectively. Compound **5**, a crystalline compound, was a trihydroxyguaianolide bearing a tigloyl group at C-8. Compound **3** was identified as janerin [5], and **4** as a guaianolide recently isolated from the South African plant *Berkheya pauciflora* Roessl. [6]. Compound **5** is new. Comparison of the spectra of **5** with those of **3** and **4** showed that in the former a hydroxyl group had been introduced at C-2. The coupling pattern of the protons in the segment C-1/C-5/C-9 was virtually identical in all



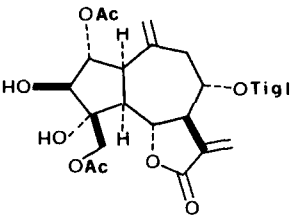
1 $\Delta^{3,4}$
2 $\Delta^{4,15}$



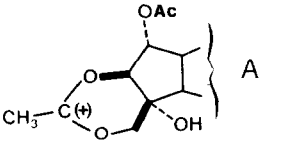
R
3 4-OH Mac
4 4-OH Tigl



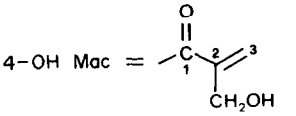
R
5 H
6 Ac

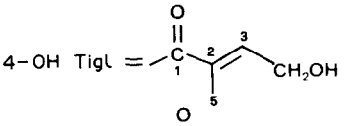


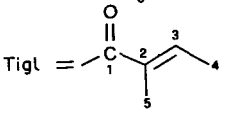
7



A







these compounds, suggesting that the relative stereochemistry at these centres was also the same. In compound **5**, the interpretation of ^3J values over the cyclopentane moiety was complex, due to the presence of several substituents and to the heavy concentration of donors and acceptors of H-bonding, which can cause conformational changes.

The relative orientation of the oxygenated functions at C-2, C-3 and C-4 was thus established by chemical means, by the same reactions used for epoxyrepdiolide, the methacrylate analogue of **5** [7]. Treatment of **5** with anhydrous cupric sulphate in dry acetone or 2,2-dimethoxypropane and *p*-toluenesulphonic acid gave no isopropylidene derivative, showing that the hydroxyls at C-2 and C-3 are *trans*. Acetylation with acetic anhydride–pyridine afforded diacetate **6**, which when treated with acids, underwent 1,4-acyl rearrangement to diol **7**. Reactions of this type are believed to proceed through a dioxolanylium ion intermediate (A) and to comply with the Furst–Plattner rule of diaxial opening of epoxides [8]. Inspection of models shows that this requires a *trans*-relationship between the epoxide at C-4, C-15) and the acetyl at C-3.

The presence of W-coupling (1.1 Hz) between H-5 and H-3 showed that these protons are *cis*-oriented. Therefore, assuming the usual β-orientation for the side-chain at C-7, the hydroxyls at C-3 and C-2 must be β, and the epoxide at C-4α.

Table 1 shows the ^{13}C NMR data of compounds **3**–**5**. The absence of methyl groups in the sesquiterpene moiety makes the assignment of the methyl group in the ester side chain of **4** straightforward, confirming the revision [9] of the value originally reported for the 4-hydroxytigloyl moiety in the germacranolide eupassopilin [10].

Oxygenated guaianolides like **3**–**5** are common in plants of the genus *Centaurea* [11]. Low functionalized eudesmanolides like **1** and **2** are rare not only in this genus, but in the Cynareae as a whole.

Table 1. ^{13}C NMR data for compounds **3**–**5** [50.3 MHz, $CDCl_3$–DMSO-d_6 (9:1), TMS internal standard]

C	3	4	5
1	47.93 *d**	48.05 *d**	49.22 *d**
2	37.62 *t*†	37.57 *t*†	73.14 *d*
3	74.20 *d*‡	74.06 *d*‡	77.16 *d*
4	68.19 *s*	68.24 *s*	65.39 *s*
5	45.68 *d**	45.60 *d**	47.13 *d**
6	76.74 *d*‡	76.91 *d*‡	77.43 *d*‡
7	53.10 *d*	52.95 *d*	51.72 *d*
8	76.27 *d*‡	76.08 *d*‡	78.89 *d*‡
9	36.45 *t*†	36.56 *t*†	36.73 *t*
10	141.36 *s*	141.41 *s*	135.24 *s*
11	136.97 *s*	137.11 *s*	136.89 *s*
12	168.94 *s*	169.13 *s*	168.40 *s*
13	122.74 *t*	122.66 *t*	121.91 *t*
14	118.61 *t*	118.52 *t*	119.36 *t*
15	48.46 *t*	48.45 *t*	47.26 *t*
1′	165.31 *s*	166.53 *s*	166.48 *s*
2′	135.19 *s*	127.97 *s*	128.08 *s*
3′	62.24 *t*	141.71 *d*	138.20 *d*
4′	126.81 *t*	59.76 *t*	11.90 *q*
5′	—	12.70 *q*	14.42 *q*

*†‡Signals with an identical sign in the same column are interchangeable.

EXPERIMENTAL

Plant material. Centaurea uniflora Turra subsp. *nervosa* (Willd.) Bonnier et Layens was collected near Lillaz (Cogne, Valle d'Aosta, Italy) in September 1981 and was identified by P. A. Silvio Stefanelli (Giardino Botanico Alpino Paradisia, Cogne, Italy). A voucher specimen is held at the herbarium of the Giardino Botanico Alpino Paradisia, Cogne, Italy.

Isolation of compounds. Dried powdered leaves (520 g) were extracted with $CHCl_3$ at room temp. and the extract was worked-up by a standard procedure [12] to give 30 g of a black syrup. Part of the latter (18 g) was chromatographed on a silica gel (300 g) column, eluted with $CHCl_3$ containing increasing amounts of MeOH; 200 ml fractions were collected. Fractions 11–13 and 16–17 ($CHCl_3$–MeOH, 49:1) gave 300 mg **1** and 210 mg **2**, respectively. Fractions 40–45 gave 4 g of a mixture of **3** and **4**, and fractions 50–57 gave 2.6 g of a mixture of **3**, **4** and **5**. These mixtures were separated by preparative HPLC. A Perkin–Elmer C-18 10 μm column (25 × 3 cm) was used. The separations were achieved under the following conditions: mobile

Table 2. ^{1}H NMR data for compounds **5**–**7** [200 MHz, $CDCl_3$–DMSO-d_6 (9:1) for **5**, $CDCl_3$ for **6** and **7**; TMS as internal standard]

H	5	6	7
1	2.78 *t*	3.28 *t*	*
2	3.70 *x*	5.41 *dd*	5.29 *t*
3	3.70 *x*	5.08 *dd*	3.86 *d*
5	2.19 *br t*	2.22 *br dd*	*
6	4.18 *dd*	4.42 *dd*	4.48 *t*
7	2.89 *br tt*	3.07 *br tt*	3.18 *m*
8	4.87 *ddd*	5.18 *ddd*	5.04 *m*
9a	2.44 *dd*	2.70 *dd*	*
9b	2.18 *dd*	2.48 *dd*	*
13a	5.91 *d*	6.19 *d*	6.20 *d*
13b	5.52 *d*	5.59 *d*	5.62 *d*
14a	5.01 *br s*	5.19 *br s*	5.23 *br s*
14b	4.79 *br s*	4.97 *br s*	5.03 *br s*
15a	3.08 *d*	3.27 *d*	4.26 *d*
15b	2.84 *d*	2.97 *d*	4.17 *d*
OH a	4.70 *br d*	—	
OH b	4.42 *br d*	—	
3′	6.74 *br qq*	6.91 *br qq*	6.93 *br qq*
4′	1.62 *br d*	1.80 *br d*	1.80 *br d*
5′	1.64 *br s*	1.84 *br s*	1.84 *br s*
OAc	—	2.01 *s*	2.01 *s*
OAc	—	2.06 *s*	2.06 *s*

J (Hz): most coupling constants were virtually the same for **5**–**7**. Those for **6** are given as representative: $J_{1,5} = J_{1,2} = 9.3$; $J_{2,3} = 4.8$; $J_{3,5} = 1.1$; $J_{5,6} = 11.5$; $J_{6,7} = J_{7,8} = 9.3$; $J_{7,13a} = 3.5$; $J_{7,13b} = 3.1$; $J_{8,9a} = 5.2$; $J_{8,9b} = 2.2$; $J_{9a,9b} = 15.2$; $J_{15a,15b} = 4.7$; $J_{3,4'} = 7.0$; $J_{3',5'} = 1.5$. For **5** $J_{1,2} = J_{1,5} = 10$. For **7**: $J_{2,3} = 8.5$; $J_{5,6} = J_{6,7} = 10$; $J_{15a,15b} = 11$.

*The signal of these protons could not be identified owing to overlapping or to the presence of complex non-first order patterns.

phase, MeOH–H_2O (12:13); flow rate 10.5 ml/min; temp. 30°; wavelength 230 nm; attenuation 1024 AUFS.

Known compounds were identified by comparison of their physical and spectral data with those reported in the literature (**3**, **4**; refs [7] and [6] respectively) or by comparison with an authentic sample (**1** and **2**).

8α-Tigloyloxy-2α,3β-dihydroxy-4α-epoxydehydrocostuslactone (**5**). Colourless needless ($CHCl_3$–Me_2CO), mp 162°, $[\alpha]_D^{25}$ + 73° (Me_2CO; *c* 0.50); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1775 (γ-lactone), 1710 (α,β-unsatured ester); UV λ_{max}^{EtOH} nm (log ε): 218 (4.1); EIMS 70 eV, *m/z* (rel. int.): 376 $[M]^+$ ($C_{20}H_{24}O_7$) (0.3), 358 $[M - H_2O]^+$ (0.7), 83 $[C_5H_7O]^+$ (19.00).

Acetylation of **5**. A sample (80 mg) of **5** was treated overnight with 1 ml pyridine and 1 ml Ac_2O. The reaction mixture was diluted with H_2O (10 ml) and extracted with CH_2Cl_2. The organic phase was washed with 5% aq. $NaHCO_3$, dil. HCl and H_2O, and then dried ($MgSO_4$). Purification of the residue through a short column of silica gel (5 g) eluted with petrol (bp 40–60°)–EtOAc (3:1) gave 71 mg **6** as a colourless gum, $[\alpha]_D^{25}$ + 78° ($CHCl_3$; *c* 0.8); IR ν_{max}^{KBr} cm^{-1}: no OH band, 1770 (γ-lactone), 1740 (acetate), 1705 (α,β-unsatured ester); EIMS 70 eV, *m/z* (rel. int.): no molecular ion, 400 $[M - 60]^+$ ($C_{22}H_{24}O_7$) (0.8), 340 $[M - 60 - 60]^+$ (2), 83 $[C_5H_7O]^+$ (100).

Acyl rearrangement of **6** *to* **7**. A sample (60 mg) of **6** in dry CH_2Cl_2 (3 ml) was treated overnight with 5 mg of *p*-toluenesulphonic acid. The reaction mixture was diluted with CH_2Cl_2 and then washed with 5% aq. $NaHCO_3$ and H_2O. Removal of the solvent gave 54 mg of a yellowish gum, which was purified by chromatography through a short column of silica gel (5 g) eluted with petrol (bp 40–60°)–EtOAc (1:3). A white powder (41 mg) was obtained; mp 135–140°; $[\alpha]_D^{25}$ + 76° (Me_2CO; *c* 0.55); IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1760 (γ-lactone), 1740 (acetate), 1710 (α,β-unsatured ester); EIMS 70 eV, *m/z* (rel. int.): no molecular ion, 460 $[M - H_2O]^+$ ($C_{24}H_{28}O_9$), 83 $[C_5H_7O]^+$ (100).

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