Sesquiterpenoids and Phenolics from *Crepis conyzifolia*

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Crepis conyzifolia, Sesquiterpenoids, Phenolics

From the roots of *Crepis conyzifolia*, two new and two known guaianolides were isolated together with three known phenylpropanoids. Structures of the new compounds were established as 8β-hydroxy-4ß, 15-dihydrozaluzanin C and 4ß, 15, 11ß, 13-tetrahydrozaluzanin C-3-O-β-glucopyranoside by spectral methods. The identity of 8-epiisolippidiol and dentalactone was also discussed.

Introduction

Screening tests for potential anticancer agents from natural sources showed that crude alcoholic extracts from plants belonging to the tribe Lactuceae of the Asteraceae exhibited chemoprotective effects on chemical carcinogenesis and differentiation-inducing activities on human leukemia and mause melanoma cell lines. Some bioactive triterpene and sesquiterpene lactone constituents of the plant extracts were isolated and identified (Takasaki *et al.*, 1999; Hata *et al.*, 2000; Lee *et al.*, 2000). Plants of this tribe have been little studied chemically. The present paper deals with root constituents of *Crepis conyzifolia* (Gouan) Kern. which has not been examined so far. From the plant material, four guaianolides, including two new derivatives of zaluzanin C (**1** and **2**, Fig. 1) and three phenylpropanoids have been isolated and characterized.

Results and Discussion

A combination of column and thin layer chromatographies on silica gel followed by semipreparative HPLC of the ethanol extract from the roots of *C. conyzifolia* yielded, in addition to **1** and **2**, the known 8-epiisolippidiol-3-O-β-glucopyranoside (**3**) as the main constituent, its aglycone **4**, 5-methoxyeugenyl-4-O-β-glucopyranoside (**5**), a mixture of the latter with eugenyl-4-O-β-glucopyranoside (**6**) and cichoriin (**7**). Of these, compounds **3** – **6** were identified by direct comparison (^{1}H NMR, EIMS or ESIMS, $[\alpha]_D$) with compounds previously isolated from *Crepis* and *Lactuca* species in our laboratory (Kisiel, 1983; Kisiel and Barszcz, 1995; Kisiel and Barszcz, 1997; Kisiel *et al.*, 2000). The identity of cichoriin (**7**) was established by comparison of its spectral data with those in the literature (Kuwajima *et al.*, 1992). The compound is reported for the first time from *Crepis* species. Since no complete ^{1}H NMR data are available for **4** (Kisiel, 1983), we have included all our assignments in Table I, along with unreported ^{13}C NMR data in $CDCl_3$ and pyridine-d_5.

When proton and carbon chemical shift values of **4** in pyridine-d_5 were compared with those reported for dentalactone (a diastereoisomer of **4** with β methyl groups at C-4 and C-11), first isolated from *Ixeris dentata* (Chung *et al.*, 1994) and then from *Soroseris hookeriana* subsp. e*rysimoides* (Meng *et al.*, 2000), striking similarities were observed. With the exception of the C-13 signal which was shifted slightly upfield to δ 12.5 ($\Delta\delta_C$ – 0.8 ppm) in the ^{13}C NMR spectrum of dentalactone, the remaining proton and carbon chemical shift values were identical to those of **4**. The inversion in the stereochemistry at C-11 in dentalactone should result in an upfield shift of the C-13 signal to higher field ($\Delta_{\delta C}$ *ca.* – 2.0 ppm) and a noticable downfield shift of the H-11 signal ($\Delta_{\delta H}$ *ca.* + 0.4 ppm) (Marco, 1989; Marco *et al.*, 1994; Seto *et al.*, 1986). Taking in mind the sensitivity of the ^{13}C NMR spectroscopy in order to detect configurational changes, it is probable that in spite of different melting points, 8-epiisolippidiol (**4**) and dentalactone could be the same compound. It is

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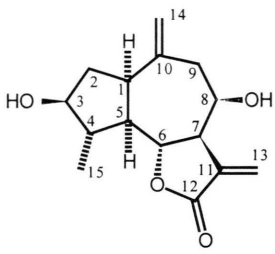

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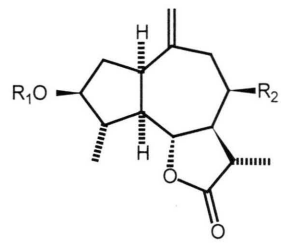

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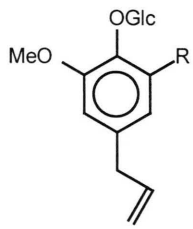
3 R_1 = Glc, R_2 = OH
4 R_1 = H, R_2 = OH



5 R = OMe
6 R = H

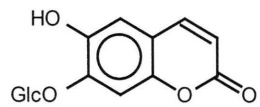


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Fig. 1. Structures of 8β-hydroxy-4β, 15-dihydrozaluzanin C (**1**), 4β, 15, 11β, 13-tetrahydrozaluzanin C-3-O-β-glucopyranoside (**2**), 8-epiisolippidiol-3-O-β-glucopyranoside (**3**), 8-epiisolippidiol (**4**), 5-methoxyeugenyl-4-O-β-glucopyranoside (**5**), eugenyl-4-O-β-glucopyranoside (**6**) and cichoriin (**7**).

Table I. ^{1}H (500.13 MHz) NMR data of **1** and **4** in $CDCl_3$ and ^{13}C (125.76 MHz) NMR data of **4** in $CDCl_3$ and pyridine-d_5[a].

Position	**1**, δ_H, *J* (Hz)	**4**, δ_H, *J* (Hz)	**4**, δ_C, $CDCl_3$	**4**, δ_C, pyridine-d_5
1	2.83 br *ddd* (11.1, 10.6, 6.6)	2.78 br *ddd* (11.5, 10.0, 6.0)	42.04	43.02
2α	2.21 *ddd* (13.0, 6.6, 6.6)	2.17 *ddd* (12.8, 6.0, 6.0)	38.22	39.76
2β	1.79 *ddd* (13.0, 10.6, 8.8)	1.75 *ddd* (12.8, 11.5, 9.0)		
3	3.75 *ddd* (8.8, 8.8, 6.6)	3.71 *ddd* (9.0, 9.0, 6.0)	78.08	78.13
4	1.90 *m*	1.85 *m*	46.98	47.67
5	1.93 *m*	1.85 *m*	50.78	52.14
6	4.27 *dd* (9.7, 9.7)	4.01 *dd* (10.1, 10.1)	79.57	80.96
7	2.80 *m*	1.80 *ddd* (12.2, 10.1, 2.5)	57.77	56.75
8	4.34 br *m*	3.95 br *m*	62.67	63.68
9α	2.37 *dd* (13.5, 3.4)	2.28 *dd* (13.4, 2.1)	43.90	45.53
9β	2.69 *dd* (13.5, 4.9)	2.71 *dd* (13.4, 5.2)		
10	–	–	142.75	144.54
11	–	2.85 *dq* (12.2, 7.0)	36.85	37.08
12	–	–	178.81	179.13
13	5.61 *d* (3.2)	1.23 *d* (7.0)	12.74	13.30
13′	6.37 *d* (3.6)			
14	5.03 br *s*	4.98 br *s*	116.30	115.33
14′	5.16 br *s*	5.14 br *s*		
15	1.22 *d* (6.3)	1.19 *d* (6.3)	18.19	18.51

[a] The assignments were confirmed by ^{1}H–^{1}H COSY and HETCOR experiments.

noteworthy that the configuration of **4** was determined by X-ray diffraction analysis (Rychlewska and Kisiel, 1991).

Compound **1** appeared to be a new natural product and a minor component of the plant material. Its structure was established by mass, 1D and 2D ^{1}H NMR spectral analyses, as well as by direct comparison with the spectra of **4**. From this comparison, it became apparent that 11, 13-dehydro derivative of **4** was present with the molecular formula $C_{15}H_{20}O_4$. The ^{1}H NMR spectrum of **1** (Table I) lacked the doublet for the methyl group at C-11 and instead two exocyclic methylene proton doublets were observed at δ 5.61 (*d*, *J* = 3.2 Hz) and δ 6.37 (*d*, *J* = 3.6 Hz). The relative stereochemistry of **1** was verified by NOESY spectrum. In the spectrum H-1α correlated with H-2α, H-5α and H-9α; H-3α correlated with H-2α and Me-15, while H-7α with H-8α, H-13 and H-13′. Furthermore, both compounds display similar optical rotation values and hence must belong to the configurational series shown in the formulae. Based on the above evidence, compound **1** was characterized as 8β-hydroxy-4β, 15-dihydrozaluzanin C. Its 3-O-β-glucopyranoside was reported earlier from *C. pyrenaica* (Kisiel and Barszcz, 1995).

Table II. ^{1}H (500.13 MHz) and ^{13}C (125.76 MHz) NMR data of **2** in pyridine-d_5[a].

Position	δ_H, *J* (Hz)	δ_C
Aglycone moiety		
1	2.58 br *ddd* (11.0, 10.0, 6.6)	42.71
2α	2.40 *ddd* (13.0, 6.6, 6.6)	38.17
2β	1.99 *ddd* (13.0, 10.0, 9.2)	
3	3.81 *ddd* (9.2, 9.2, 6.6)	87.22
4	2.16 *m*	45.39
5	1.73 *ddd* (11.0, 10.0, 9.2)	50.23
6	3.67 *dd* (10.0, 10.0)	85.99
7	1.65 *m*	52.24
8α	1.83 *dddd* (12.5, 4.2, 4.2, 3.5)	32.81
8β	0.99 *dddd* (12.5, 12.5, 12.5, 3.5)	
9α	1.65 *m*	37.07
9β	2.38 *ddd* (12.5, 4.2, 3.5)	
10	–	148.98
11	2.14 *dq* (11.6, 7.0)	42.19
12	–	178.37
13	1.14 *d* (7.0)	13.26
14	4.78 br *s*	112.34
14′	4.84 br *s*	
15	1.38 *d* (6.6)	18.76
Glucosyl moiety		
1	4.91 *d* (7.8)	105.94
2	4.07 *dd* (8.0, 7.8)	75.50
3	4.27 *m*	78.65[b]
4	4.27 *m*	71.88
5	3.98 *m*	78.42[b]
6	4.37 br *dd* (11.4, 5.2)	63.02
6′	4.57 br *d* (11.4)	

[a] The assignments were confirmed by ^{1}H–^{1}H COSY, NOESY and HETCOR experiments.
[b] Values interchangeable.

Structure **2** for the second new natural product was readily assigned when its ^{1}H NMR and mass spectral data were directly compared with those of 11β,13-dihydroglucozaluzanin C, previously isolated in our laboratory from *Crepis* and *Lactuca* species (Kisiel *et al.*, 2000; Kisiel and Barszcz, 1997). In the ^{1}H NMR spectrum of **2** (Table II) the signals of the exocyclic methylene protons at C-15 were replaced by methyl and methine proton resonances attributed to Me-15 (δ 1.38, *d*, *J* = 6.6 Hz) and H-4 (δ 2.16, *m*), respectively. The NOESY spectrum confirmed proximities of H-5α to H-1α and Me-15, as well as H-7α to H-8α and Me-13. The β linkage of the glucose moiety was deduced from the coupling constant (7.8 Hz) of the anomeric proton signal. Accordingly, compound **2** was presumed to be 4β, 15, 11β, 13-tetrahydrozaluzanin C-3-O-β-glucopyranoside. This structural proposal was in agreement with the mass (positive ESI *m/z*: 435 $[M + Na]^+$) and ^{13}C NMR spectra. The resonances of C-1 to C-4 and C-15 of **2** are compatible with those of **3** (Kisiel *et al.*, 2000), while the resonances of C-7, C-8 and C-11 to C-13 are in agreement with those reported previously for 11β, 13-dihydroglucozaluzanin C (Nishimura *et al.*, 1986), but the C-7 and C-11 chemical shift values should be reversed. The positive sign of optical rotations of the three glycosides mentioned above supports the structure and stereochemistry depicted in the formula **2**.

The composition of the sesquiterpene lactones found in *C. conyzifolia* was reminiscent of those isolated previously from some other *Crepis* species in our laboratory.

Experimental

Merck silica gel was used for CC (Art. 7733 and Art. 7754) and TLC (Art. 5553). Semiprep. HPLC was performed on a Delta-Pak C-18 cartridge column (particle size 15 μm, 25 × 100 mm) coupled to a UV photodiode array detector. The column was eluted with MeOH-H_2O mixtures at a flow rate of 3 ml min^{-1}.

Plant material

Roots of *C. conyzifolia* were collected in June 1999 from plants growing in the Garden of Medicinal Plants of the Institute of Pharmacology, Polish Academy of Sciences, Kraków, where a voucher specimen is deposited. Seeds of the plant collected in the Tatra National Park were provided by the Montain Botanical Garden in Zakopane, Poland.

Extraction and isolation

The dried roots (466 g) were ground and exhaustively extracted with EtOH at room temp. providing a residue (58 g) which was coarsely prefractionated on silica gel using successively hexane, hexane-EtOAc (1 : 1 v/v), EtOAc, EtOAc-MeOH (1 : 1 v/v) and MeOH to give five fractions, 2 l each. Fractions containing phenolic and sesquiterpenoid compounds, eluted with EtOAc and EtOAc-MeOH (1 : 1 v/v), were combined and the solvents were removed giving 39 g of residue which was subjected to column chromatography on silica gel using hexane-EtOAc (up to 100% EtOAc) followed by EtOAc-MeOH (up to 10% MeOH) gradient solvent systems. Fractions from hexane-EtOAc (1 : 1 v/v) elution were further separated by prep. TLC ($CHCl_3$-MeOH, 19 : 1 v/v) followed by semiprep. HPLC (MeOH-H_2O, 2 : 3 v/v) to give **1** (2.0 mg) and **4** (5.6 mg, $[\alpha]^{26}_D$ – 11.2°, $CHCl_3$, *c* 0.74). Fractions eluted with EtOAc and EtOAc-MeOH (19 : 1 v/v) were separated by prep. TLC ($CHCl_3$-MeOH, 17 : 3 or 4 : 1 v/v) to yield a mixture of less polar glycosides, impure **3** and pure **7** (23.2 mg). The mixture was processed by semiprep. HPLC (MeOH-H_2O, 1 : 1 v/v) to afford a mixture of **5** and **6** (*ca.* 2 : 1, 2.5 mg), **5** (1.9 mg) and **2** (7.8 mg), in that order. Compound **3** (30.4 mg, $[\alpha]^{25}_D$ + 9.7 °, MeOH, *c* 2.00) was purified by semiprep. HPLC (MeOH-H_2O, 2 : 3 v/v).

8β-Hydroxy-4β, 15-dihydrozaluzanin C (**1**)

Solid. $[\alpha]^{26}_D$ – 25.2°, MeOH, *c* 0.33. EIMS *m/z* (rel. int.): 264 $[M]^+$ (4.8), 246 $[M - H_2O]^+$ (19.6), 228 $[M - 2 \times H_2O]^+$ (22.8), 218 $[M - H_2O - CO]^+$ (25.3), 202 (35.4), 191 (72.0), 173 (53.2), 166 $[M - C_6H_{10}O]^+$ (100). ^1H and ^{13}C NMR: Table I.

4β, 15, 11β, 13-Tetrahydrozaluzanin C-3-O-β-glucopyranoside (**2**)

Solid. $[\alpha]^{26}_D$ + 7.5°, MeOH, *c* 0.37. ESIMS *m/z* (positive ion mode): 435 $[M + Na]^+$, 847 $[2\ M + Na]^+$. ^1H and ^{13}C NMR: Table II.

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