Polish J. Chem., **76**, 1571–1576 (2002) **ORGANIC CHEMISTRY**

Sesquiterpene Lactones from *Crepis zacintha*

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(Received June 24th, 2002)

A series of guaianolide-type sesquiterpene lactones, including two new natural products, was isolated from the roots of *Crepis zacintha*. The presence in the plant material of three pairs of guaianolide epimers at C-4 was proved by 1D and 2D NMR spectral methods.

Key words: *Crepis zacintha*, Asteraceae, sesquiterpene lactones, guaianolide epimers

In continuation of our chemical studies of *Crepis* species (Asteraceae), we have examined roots of the hitherto unstudied *Crepis zacintha* (L.) Babc. Plants of the genus *Crepis* produce sesquiterpene lactones comprising three types, *i.e.* germacranolides, eudesmanolides and guaianolides. However, guaian-12,6-olides, derivatives of 9α-hydroxy- or 8β-hydroxy-zaluzanin C, are the most representative secondary metabolites of this taxon [1–6]. As a result of the present investigation three further sesquiterpenoids based on the 8β-hydroxy-zaluzanin C structure can be added to the list of *Crepis* constituents, including two new natural products (**1** and **2**, Glc = β-glucopyranosyl) and the known guaianolide ixerin M (**7**), isolated previously from *Ixeris* species [7,8]. The presence in *C. zacintha* of three pairs of guaianolide epimers at C-4, along with other closely related sesquiterpene lactones, is also reported.

RESULTS AND DISCUSSION

The roots of the plant were extracted with ethanol and the extract was subjected to column and thin layer chromatographies on silica gel followed by semipreparative HPLC yielding, in addition to **1**, **2** and **7**, the known 8-epiisolippidiol-3-O-β-glucopyranoside (**3**) and its 11,13-dehydroderivative (**4**), the aglycone of **4** (**5**), 8-epidesacylcynaropicrin-3-O-β-glucopyranoside (**6**), 11β,13-dihydrozaluzanin C-3-O-β-glucopyranoside (**8**), ixerin F (**9**) and its aglycone (**12**), 9α-hydroxy-4α,15; 11β,13-tetrahydrozaluzanin C (**11**) and its epimer at C-4 (**10**), picriside B (**13**), along with benzyl-O-β-glucopyranoside. All the compounds, except **1**, **2** and **7**, were identified by direct comparison (HPLC, ^{1}H NMR, ESIMS and $[\alpha]_D$ wherever possible) with compounds previously isolated from *Crepis* species in our laboratory [1,2,5,6]. The

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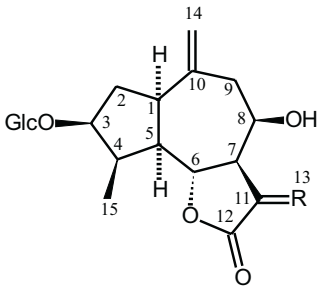
Scheme



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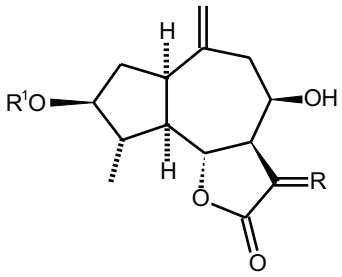
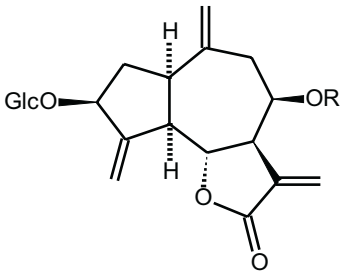
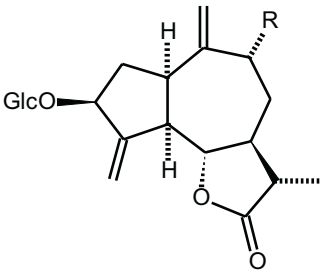
2 R = CH_2



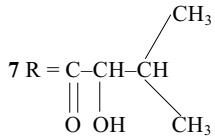
3 R = H, αMe, R^1 = Glc
4 R = CH_2, R^1 = Glc
5 R = CH_2, R^1 = H



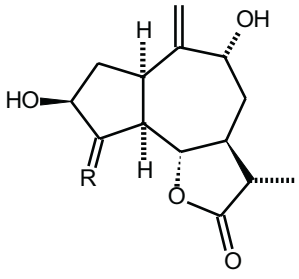
6 R = H



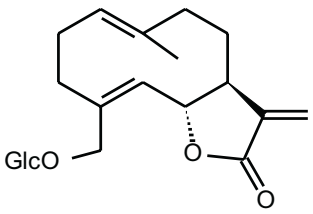
8 R = H
9 R = OH



7 R =



10 R = H, αMe
11 R = H, βMe
12 R = CH_2



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identity of ixerin M (**7**) was established by comparison of its spectral data and $[\alpha]_D$ with those in the literature [7]. Since no complete ^{1}H NMR data are available for **7**, we have included all our assignments, confirmed by ^{1}H - ^{1}H COSY correlations, in Table 1. Ixerin M was a major sesquiterpene lactone of the plant material, while compounds **1** and **2** were obtained in relatively small amounts.

Table 1. ^{1}H NMR (500.13 MHz, C_5D_5N) data of **1**, **2** and **7**.

Position	**1**, δ_H, *J* (Hz)	**2**, δ_H, *J* (Hz)	**7**, δ_H, *J* (Hz)
Aglycone moiety			
1	2.61 *ddd* (11.0, 10.0, 10.0)	2.61 *ddd* (11.0, 10.0, 10.0)	2.87 *m*[a]
2α	2.15 *m*[a]	2.16 *m*[a]	2.15 *ddd* (14.0, 7.0, 7.0)
2β	2.35 *ddd* (13.0, 11.0, 10.0)	2.37 *ddd* (13.0, 11.0, 10.0)	2.41 *ddd* (14.0, 7.7, 7.7)
3	4.66 *m*[b]	4.66 *m*[b]	4.83 br *dd* (7.7, 7.0)
4	2.66 *m*	2.66 *m*	–
5	2.02 *m*[c]	2.16 *m*[a]	2.87 *m*[a]
6	4.85 *dd* (11.0, 9.8)	5.04 *dd* (11.5, 8.7)	4.88 *dd* (9.2, 9.2)
7	2.02 *m*[c]	2.95 *dddd* (8.7, 3.5, 3.1, 1.9)	3.36 *dddd* (9.2, 3.5, 3.0, 2.1)
8	4.16 br *s*	4.52 br *s*	5.79 br *s*
9α	2.15 *m*[a]	2.22 *dd* (13.3, 3.3)	2.56 *m*[b]
9β	2.90 *dd* (13.4, 4.0)	2.89 *dd* (13.3, 3.8)	2.56 *m*[b]
11	3.27 *dt* (11.8, 7.1)	–	–
13	1.30 *d* (7.1)	5.72 *d* (3.1)	5.71 *d* (3.0)
13′		6.51 *d* (3.5)	6.45 *d* (3.5)
14	5.16 br *s*	5.18 br *s*	4.85 br *s*
14′	5.17 br *s*	5.19 br *s*	5.17 br *s*
15	1.23 *d* (7.2)	1.24 *d* (7.2)	5.59 br *s*
15′			5.91 *d* (1.0)
8-OH	6.50 *d* (4.9)	6.54 *d* (4.4)	–
Glucosyl moiety			
1	4.95 *d* (7.8)	4.95 *d* (7.7)	5.03 *d* (7.8)
2	4.11 *dd* (7.8, 8.0)	4.11 *dd* (8.1, 7.7)	4.06 *dd* (8.3, 7.8)
3	4.31 *m*[d]	4.31 *m*[c]	4.24 *m*[c]
4	4.31 *m*[d]	4.31 *m*[c]	4.24 *m*[c]
5	4.06 *ddd* (9.0, 5.6, 2.3)	4.07 *m*	3.95 *m*
6	4.45 *dd* (11.6, 5.6)	4.45 *dd* (11.6, 5.6)	4.38 *dd* (11.7, 5.3)
6′	4.66 *m*[b]	4.66 *m*[b]	4.55 *dd* (11.7, 2.2)
Ester moiety			
2	–	–	4.28 br *d* (4.5)
3	–	–	2.18 *m*
4	–	–	1.03 *d* (6.8)
5	–	–	1.04 *d* (6.8)

[a–d]Signals fully or partially overlapped.

Compound **1** has not been reported previously as a natural product. The structure of **1** was readily assigned when its spectral data were directly compared with those of **3**. Compound **3** was isolated in our laboratory from *Crepis pyrenaica* [1] and *C. mollis* [5]. The ESI mass spectra of both compounds showed quasimolecular ion peaks at *m/z* 451 $[M + Na]^+$ and *m/z* 879 $[2M + Na]^+$ suggesting the same molecular formula $C_{21}H_{32}O_9$. A close comparison of ^{1}H and ^{13}C NMR spectra (Table 1 and 2), including

^{1}H - ^{1}H COSY and HETCOR, pointed to the same base structure and led to the conclusion that **1** was clearly a diastereomer of **3**. The spectra were very similar with the notable exception of distinctive resonances assignable to protons and carbons of the cyclopentane rings. The ^{13}C NMR data of **3** reported previously [5] were added to Table 2 for comparison. In contrast to **3**, the signals of C-3 ($\Delta_{\delta C}$ –9.15), C-4 ($\Delta_{\delta C}$ –8.23) and C-15 ($\Delta_{\delta C}$ –10.41) appeared more upfield, while the signals of H-3 ($\Delta_{\delta H}$ +0.78), H-4 ($\Delta_{\delta H}$ +0.41) and H-15 ($\Delta_{\delta H}$ –0.17) were shifted downfield and upfield, respectively [1]. So, the relative stereochemistries of **1** and **3** were compared using NOESY experiments. The most significant difference was the presence of cross peaks from the 15-methyl to H-6β, H-14/H-14′ and H-2β, indicating that the methyl group is β-oriented. Correlations on the α-face positioned together the 13-methyl, H-5α/H-7α (δ 2.02), H-8α, as well as H-1α, H-2α/H-9α (δ 2.15), H-3α, H-4α and H-5α/H-7α. Furthermore, the NOESY experiment showed proximities of the anomeric sugar proton, H-3α and H-4α, thereby confirming the attachment of the glucose moiety at the 3 position of the aglycone. Thus, compound **1** was proved to be 8β-hydroxy-4α,15; 11β,13-tetrahydrozaluzanin C-3-O-β-glucopyranoside.

Table 2. ^{13}C NMR (125.76 MHz, C_5D_5N) data of **1** and **3**[5].

Position	**1**, δ_C	**3**[5], δ_C
Aglycone moiety		
1	40.56	43.33
2	32.91	38.51
3	78.23[a]	87.38
4	37.27	45.50[a]
5	47.29	51.77
6	76.15	80.70
7	55.61	56.30
8	63.36	63.70
9	47.90	45.25[a]
10	143.28	144.35
11	36.96	37.01
12	178.62	178.99
13	13.07	13.28
14	113.89	115.45
15	8.25	18.66
Glucosyl moiety		
1	102.68	105.83
2	75.10	75.47
3	78.60[a]	78.59[b]
4	71.51	71.89
5	78.31[a]	78.35[b]
6	62.62	63.03

[a,b]Values interchangeable.

Compound **2** was difficult to separate from its mixture with **1**, in view of the small amount available.The structure of **2** was evident from direct comparison of its ^{1}H NMR and mass spectra with those of **1** (Table 1). From this comparison, it became apparent that the 11,13-dehydroderivative of **1** was present. In the ^{1}H NMR spectrum of **2** the signals of the 13-methyl and H-11 were replaced by exocyclic methylene proton

doublets at δ 6.51 (J = 3.5 Hz) and δ 5.72 (J = 3.1 Hz) and the signal due to H-7 was shifted downfield. Other signals closely resembled the corresponding resonances of **1**. The ESIMS confirmed the molecular formula $C_{21}H_{30}O_9$ with quasimolecular ion peaks at *m/z* 449 $[M + Na]^+$ and *m/z* 875 $[2M + Na]^+$. The β-glucosidic linkage deduced from the large coupling constant (J = 7.7 Hz) of the anomeric proton signal finalized the structure and relative stereochemistry of **2** as 8β-hydroxy-4α,15-dihydrozaluzanin C-3-O-β-glucopyranoside, a new natural product. Further comparison of ^{1}H NMR spectra of **2** and **4** [1] showed the same differences in resonances of the cyclopentane ring protons, as mentioned above for **1** and **3**.

The results reported here show that *C. zacintha* contain a range of guaianolides, including three pairs of epimers at C-4, *i.e.* **1** and **3**; **2** and **4**; **10** and **11**. The latter pair of compounds was found previously in *C. rhoeadifolia* [2].

EXPERIMENTAL

General: Merck silica gel was used for CC (Art. 7754) and TLC (Art. 5553). Semipreparative and analytical HPLC were performend on a Delta-Pak C-18 cartridge column (partcle size 15 μm, 25 × 100 mm, flow rate of 3 ml min^{-1}) and μ-Bondapak C-18 column (particle size 10μ, 2 × 300 mm, flow rate of 0.5 ml min^{-1}), respectively, coupled to a UV photodiode array detector. The columns were eluted with MeOH-H_2O mixtures.

Plant Material: A sample of the roots of *C. zacintha* was collected in July 1999 from plants growing in the Garden of Medicinal Plants of the Institute of Pharmacology, Polish Academy of Sciences, Kraków, where a voucher specimen was deposited.

Extraction and Isolation: The dried and finely powdered roots (108 g) were exhaustively extracted with ethanol at room temperature and the residue (12 g), obtained by removal of the solvent at reduced pressure, was chromatographed on a silica gel column, packed in hexane, using hexane-EtOAc followed by EtOAc-MeOH mixtures of increasing polarity, as eluents. Relevant fractions were combined, as shown by TLC, and further separated and purified by preparative TLC ($CHCl_3$-MeOH, 9:1 or 17:3) to give mainly mixtures of structurally closely related compounds. Elution of the column with hexane-EtOAc (1:1) afforded a mixture (3.9 mg) of sesquiterpene lactone aglycones **5**, **10**, **11** and **12** in the ratio *ca.* 1.5:8:0.5:1, respectively. Initial fractions from EtOAc elution afforded benzyl-O-β-glucopyranoside (2.0 mg) and a mixture (19.5 mg) of **7**, **8** and **13** in the ratio *ca.* 9:1:2, respectively. The mixtures were indicated by analytical HPLC and ^{1}H NMR. Further fractions from EtOAc yielded pure **7** (15.5 mg). Later EtOAc fractions gave additional amount of **7** (37.1 mg) and complex mixtures of sesquiterpene lactone glycosides **1**–**4**, **6** and **9**. Final separation of the mixtures was accomplished by repeated semipreparative HPLC (MeOH-H_2O, 2:3) to give **1** (6.3 mg), almost pure **2** (2.4 mg), **3** (7.7 mg), **4** (10.4 mg), **6** (6.0 mg) and **9** (24.7 mg). The initial identity and purity of compounds present in the separated fractions were evaluated by analytical HPLC.

8β-Hydroxy-4α,15; 11β,13-tetrahydrozaluzanin C-3-O-β-glucopyranoside (1): Solid; $[\alpha]_D^{28}$ = –32.3 (c = 0.9, MeOH); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; ESIMS: *m/z* 451 $[M + Na]^+$, 879 $[2M + Na]^+$.

8β-Hydroxy-4α,15-dihydrozaluzanin C-3-O-β-glucopyranoside (2): Solid; ^{1}H NMR, see Table 1; ESIMS: *m/z* 449 $[M + Na]^+$, 875 $[2M + Na]^+$.

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