Root Constituents of *Cichorium pumilum* and Rearrangements of Some Lactucin-like Guaianolides

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Two eudesmanolides, eight lactucin-like guaianolides and five phenolic compounds were isolated for the first time from roots of *Cichorium pumilum*, along with two previously reported eudesmane-type sesquiterpene lactones. Rearrangements of some lactucin-like guaianolides during isolation procedures were also discussed.

Key words: Cichorium pumilum, Sesquiterpenoids, Phenolics

Introduction

Plants of the genus *Cichorium* (Asteraceae, tribe Lactuceae) produce bitter sesquiterpene lactones as their characteristic secondary metabolites, some of which possess interesting biological activities (Pyrek, 1985; Seto *et al.*, 1988; Daniewski *et al.*, 1989; Lee *et al.*, 2000). Our previous phytochemical studies on *Cichorium intybus* L. (Kisiel and Zielińska, 2001) have led to structure revisions of six sesquiterpene lactone aglycones and glycosides reported from some *Cichorium* and *Sonchus* species, including two guaianolides from *Cichorium pumilum* Jacq. [syn. *Cichorium endivia* ssp. *pumilum* (Jacq.) Hegi], named 10β-hydroxy-cichopumilide and its 11β,13-dihydro derivative (El-Masry *et al.*, 1984) which have been reassigned structures **1** (magnolialide, Fig. 1) and **2** (artesin), respectively. Phytochemically, *C. pumilum* has not been analysed in detail and apart from the two above mentioned root constituents, a single flavonoid glycoside (isoquercitrin) has been reported from aerial parts (Saleh *et al.*, 1975). Therefore, we decided to search for additional secondary metabolites that might be present in roots of this plant; this has resulted in the isolation of four eudesmanolides, including **1** and **2**, eight lactucin-like guaianolides and five phenolic compounds. We also comment on the possible rearrangements of these compounds during isolation procedure.

Results and Discussion

Thin layer chromatography of the root extract of *C. pumilum* revealed the presence of several UV-absorbing spots, four of which co-chromatographed with 8-deoxylactucin (**5**), lactucin (**6**), lactucopicrin (**7**) and crepidiaside B (**11**). Following fractionation of the extract by column chromatography on silica gel, a mixture containing lactucin (**6**) was obtained and processed by semiprep. RP-HPLC eluting with 30 % MeOH in H_2O. Four peaks (P1–P4) which showed almost the same on-line UV absorption maxima at *ca.* 260 nm were observed at R_t 25.7 min, R_t 41.0 min, R_t 56.4 min and R_t 65.3 min, respectively; the peaks P3 and P4 could not be completely separated from each other. Relevant fractions (P1–P4) were collected, evaporated and subjected to ^{1}H NMR analyses in pyridine-d_5. Fractions P2 and P3 yielded 11β,13-dihydrolactucin (**9**) and almost pure **6**, respectively. The remaining fractions (P1 and P4) gave mixtures of two compounds, including **6** as a major constituent. Duplication of signals detected in ^{1}H NMR spectra of the mixtures suggested the possible occurrence of two isomeric lactones **6** and **13** with lactone rings *trans*-fused toward C-6 and C-8, respectively. Further confirmation was provided by the same ^{1}H–^{1}H COSY and NOESY correlations. A lactone apparently identical with **13**, called intybulide, was recently isolated from *C. intybus* (Deng *et al.*, 2001). Intybulide was reported to be acid sensitive, slowly rearranging to produce **6**. Its ^{13}C NMR spectrum (in $CDCl_3$) was close to

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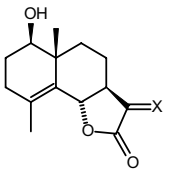
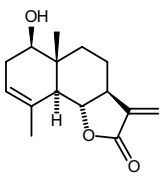


1 X = CH_2
2 X = H, αMe
3 X = H, βMe



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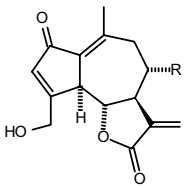
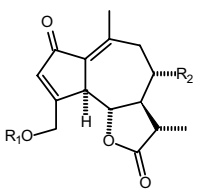
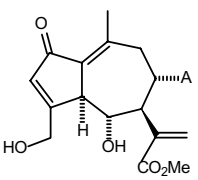


5 R = H
6 R = OH
7 R = A



8 $R_1 = R_2 = H$
9 $R_1 = H$, $R_2 = OH$
10 $R_1 = H$, $R_2 = A$
11 $R_1 = Glc$, $R_2 = H$



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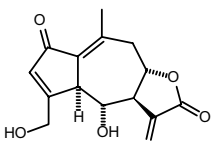

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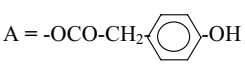
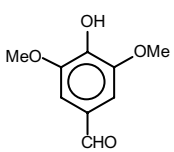


A = -OCO-CH_2-(C6H4)-OH



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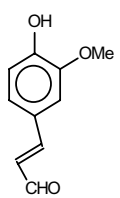

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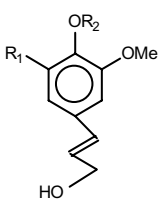

16 $R_1 = R_2 = H$
17 $R_1 = H$, $R_2 = Glc$
18 $R_1 = OMe$, $R_2 = Glc$

Fig. 1. Chemical structures (Glc = β-glucopyranosyl) of magnolialide (**1**), artesin (**2**), 11-epiartesin (**3**), santamarine (**4**), 8-deoxylactucin (**5**), lactucin (**6**), lactucopicrin (**7**), jacquinelin (**8**), 11β,13-dihydrolactucin (**9**), 11β,13-dihydrolactucopicrin (**10**), crepidiaside B (**11**), artifact derived from **7** (**12**), intybulide (**13**), syringaldehyde (**14**), coniferyl aldehyde (**15**), coniferyl alkohol (**16**), coniferin (**17**), syringin (**18**).

that of **6**, even though they were obtained in different solvents. Based on the above observations, we conclude that lactucin and/or intybulide are susceptible to lactone ring opening (peak P1) during the RP-HPLC run. The mixtures of **6** and **13** appeared to be quite stable in pyridine. A comparison of ^1H and ^{13}C NMR spectra of **6** and **13** recorded in the same solvent is not available in the literature, therefore we included all our assignments in Table I.

Similarly, a mixture containing lactucopicrin (**7**) gave rise to three peaks when separated by semiprep. RP-HPLC with 50 % MeOH in H_2O, two of the relevant fractions afforded 11β,13-dihydrolactucopicrin (**10**) and **7**, and the third eluting earlier gave a mixture of **7** and the methyl ester **12** being the known artifact resulting from the facile lactone ring opening of **7** in MeOH (Pyrek, 1985).

It is of interest to note that semiprep. RP-HPLC analyses using MeOH-H_2O mixtures cannot provide the true amounts of compounds **6**, **7** and **13** in the examined fractions due to the facile opening of their lactone rings which allows the formation of the artifact **12** from **7** and re-lactonization of **13** to **6**. On the contrary, the dihydroderivatives **9** and **10** are stable during the run.

Table I. ^{1}H (500.13 MHz)[a] and ^{13}C (125.76 MHz)[b] NMR data of **6** in a mixture with **13** in pyridine-d_5.

Position	**6**, δ_H (J [Hz])	**13**, δ_H (J [Hz])	**6**, δ_C	**13**, δ_C
1	–	–	133.30[d]	132.96
2	–	–	195.08	196.08
3	6.98 br d (1.2)	7.00 br d (1.3)	133.37[d]	135.17
4	–	–	175.36	177.63
5	3.77 d (10.1)	3.7–3.8[c]	49.61[e]	55.74
6	3.65 dd (10.1, 10.1)	3.7–3.8[c]	81.74	72.96
7	3.26[c]	3.28[c]	58.17	58.34
8	4.01 ddd (10.7, 10.1, 2.2)	3.83 br dd (11.0, 11.0)	67.68	77.21
9α	2.95 dd (13.5, 10.7)	2.85 dd (13.8, 11.0)	49.18[e]	41.47
9β	2.60 dd (13.5, 2.2)	2.63 dd (13.8, 1.5)		
10	–	–	146.63	145.87
11	–	–	138.98	139.87
12	–	–	169.34	169.75
13	6.58 dd (3.0, 1.5)	6.47 dd (2.9, 1.4)	122.05	121.54
13′	6.38 dd (3.2, 1.5)	6.36 dd (3.2, 1.4)		
14	2.50 s	2.50 s	21.42	21.12
15	5.33 br d (18.4)	5.51 br d (18.3)	62.57	63.39
15′	4.75 br d (18.4)	4.95[c]		

[a] The assignments were made by ^{1}H-^{1}H COSY and NOESY experiments.
[b] The assignments were based on peak intensities and correlated with those reported for **6** (in pyridine-d_5) and **13** (in $CDCl_3$) by Uchiyama *et al.* (1990) and Deng *et al.* (2001), respectively.
[c] Signals overlapped by others.
[d,e] Values interchangeable.

In addition to the above mentioned lactucin-like compounds also isolated from the extract were 8-deoxylactucin (**5**) in a mixture with jacquinelin (**8**) and crepidiaside B (**11**), the eudesmanolides magnolialide (**1**), artesin (**2**), 11-epiartesin (**3**) and santamarine (**4**) as well as the phenolic compounds syringaldehyde (**14**), coniferyl aldehyde (**15**), coniferyl alkohol (**16**) and a mixture of coniferin (**17**) and syringin (**18**). The compounds, except for **2**–**4** and **14**–**17**, were readily identified by comparing their retention times and ^{1}H NMR spectra with those of reference compounds from our collection isolated from *C. intybus* and other members of the tribe Lactuceae. The identities of compounds **2**–**4** (Marco, 1989; Glasl *et al.*, 1995) and **14**–**17** (Steeves *et al.*, 2001) were established on the basis of their spectral data and comparison with reported values.

Roots of *C. pumilum* exhibited similar but not identical pattern of sesquiterpene lactones to that reported for *C. endivia* L. (Seto *et al.*, 1988); six of the isolated compounds (**1**, **5**–**7**, **9** and **11**) were held in common. Magnolialide (**1**) and lactucopicrin (**7**) appeared to be major sesquiterpene lactone constituents (*ca.* 0.009 % dry weight each). The eudesmanolides **3** and **4**, and the phenolic compounds were not previously found in *Cichorium* species.

Experimental

Merck silica gel was used for CC (Art. 7754) and TLC (Art. 5553). Analytical and semiprep. RP-HPLC analyses were performed on a μ-Bondapack C 18 column (particle size 10 μm, 2 mm × 300 mm, flow rate of 0.5 ml min^{-1}) and a Delta-Pak C-18 column (particle size 15 μm, 25 mm × 100 mm, flow rate of 3 ml min^{-1}), respectively, using MeOH–H_2O mixtures as mobile phases and monitoring with a UV photodiode-array detector.

Plant material

Roots of *C. pumilum* were collected in July 2001 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 were provided by the Botanic Garden of the University of Copenhagen, Denmark.

Extraction and isolation

The dried roots (198 g) were ground and exhaustively extracted with EtOH at room temperature providing a residue (13 g) which was passed through a silica gel column eluted with a hexane-EtOAc gradient solvent system starting with 100 % hexane with increasing amounts of EtOAc to 100 %; the column was finally washed with 5 % and 10 % MeOH in EtOAc. The sesquiterpene lactone and phenolic compounds present in the eluates containing 30 % EtOAc to 100 % EtOAc, after separation and purification by prep. TLC on silica gel, were subjected to analytical RP-HPLC for direct comparison with reference compounds, wherever possible. Elution of the column with hexane-EtOAc (7:3 to 1:1, v/v) followed by prep. TLC (hexane-EtOAc, 3:2 and 1:1, v/v, one or two developments) yielded **15** (1.0 mg), **14** (3.2 mg), a mixture of **1** and **2** (*ca.* 2.7:1, 16.7 mg), a more complex eudesmanolide mixture (16.3 mg), **16** (2.4 mg), a mixture of **5** and **8** (*ca.* 1.5:1, 3.8 mg) and a mixture (32.6 mg) containing **7**, in that order. The eudesmanolide mixture was further separated by semiprep. RP-HPLC (MeOH-H_2O, 1:1, v/v) to give **1** (4.7 mg), **3** (1.0 mg) and **4** (4.7 mg). A portion (26.8 mg) of the latter mixture was processed by semiprep. RP-HPLC using the same solvent system to yield a mixture of **7** and **12** (*ca.* 0.9:1, 8.4 mg), **10** (4.2 mg) and **7** (6.3 mg) in order of decreasing polarity. Initial fractions from EtOAc elution were separated by semiprep. RP-HPLC (MeOH–H_2O, 3:7, v/v) yielding two mixtures of **6** and **13** (P1, *ca.* 1.0:0.5, 1.5 mg and P4, 3.0 mg), **9** (P2, 8.0 mg) and almost pure **6** (P3, 6.5 mg), as described above. Later EtOAc fractions, after prep. TLC purification ($CHCl_3$–MeOH, 9:1, v/v) gave a mixture containing **17** and **18** (3.9 mg) and **11** (5.1 mg). The mixtures, indicated by ^{1}H NMR, were not separated further as the ^{1}H NMR signals could be readily assigned to the respective compounds on the basis of their relative amounts.

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