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# Sesquiterpenoids and phenolics from *Crepis mollis*

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**Abstract**

From the roots of *Crepis mollis*, one new and two known guaianolides were isolated together with eight known guaianolide glycosides, one known germacranolide glycoside and two known phenylpropanoids. The structure and relative configuration of the new compound were established as 9α-hydroxy-4β, 15, 11β, 13-tetrahydro-dehydrozaluzanin C by spectral methods. 

*Keywords:* *Crepis mollis*; Asteraceae; Lactuceae; Sesquiterpene lactones; Phenylpropanoids; Glycosides

## 1. Introduction

Plants of the genus *Crepis* elaborate a variety of guaianolides (for example, see Zidorn et al., 1999), accumulated mainly as glycosides, and some noreudesmanolides (Rossi et al., 1985). In addition to the sesquiterpene lactones, *Crepis foetida* and *C. rhoeadifolia* produce notable amounts of phenolic glycosides isosalicin and helicin (Kisiel and Barszcz, 1999). The present paper deals with root constituents of *C. mollis* (Jacq.) Aschers. which has not been examined so far.

## 2. Results and discussion

The ethanol extract of the roots of *C. mollis* was chromatographed on a silica gel column and relevant fractions were further separated by preparative TLC and semipreparative HPLC to afford twelve sesquiterpene lactone aglycones and glycosides (**1**–**12**), along with two phenylpropanoids (**13** and **14**).

Compound **1** appeared to be a new natural product and a minor component of the plant material. The structure of **1** was readily assigned when its $^1$H NMR (Table 1) and mass spectral data were compared with those reported for 9α-hydroxy-4β, 15, 11β, 13-tetrahydro-dehydrozaluzanin C (Jakupovic et al., 1988), whose absolute configuration was determined by X-ray diffraction analysis (Budesinsky et al., 1989). The EI-mass spectra of the two compounds showed $[M]^+$ ion peaks at $m/z$ 264 suggesting the same molecular formula $C_{15}H_{20}O_4$ and were both characterized by the presence of the same prominent fragments at $m/z$ 246 $[M - H_2O]^+$ and $m/z$ 218 $[M - H_2O - CO]^+$. From comparison of their $^1$H NMR spectra, it became apparent that two guaianolide epimers at C-9 were present. The spectra of both epimers were very similar, with the notable exception of distinctive resonances assignable to protons at the C-1, C-9 and C-14 positions. In the $^1$H NMR spectrum of **1**, the signal of H-9 appeared more downfield as a doublet of doublet at $\delta$ 4.59, whose small couplings ($J$ = 3.6 and 2.6 Hz) were in agreement with the α-orientated hydroxyl group at C-9. The 9α-hydroxyl which is *cis* to H-1 caused a significant downfield shift of the H-1 signal (by 0.76 ppm) and a notable upfield shift of the H-14a signal. The resonances of H-1 and H-9 were comparable to those reported for the structurally related

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guaianolide 9α-hydroxy-dehydrozaluzanin C (Bohlmann et al., 1982). In addition, both epimers under study showed nearly identical coupling constants involving the H-1, H-2α, H-2β, H-6 and H-7 signals, suggesting configurational similarities at the remaining chirality centers. Since the H-4, H-5 and H-11 signals completely overlapped, the relative stereochemistry of **1** was further confirmed using the NOESY spectrum. The following important correlations were observed: H-1α/H-2α, H-1α/H-5α, H-2β/H-4β, H-2β/H-14b, H-7α/Me-13, H-8α/Me-13, H-9β/H-14a. The long-range coupling of H-2β with H-4β was also observed in the $^1$H–$^1$H COSY spectrum which verified the proton assignments, given in Table 1. Thus, the new guaianolide was proved to be 9α-hydroxy-4β, 15, 11β, 13-tetrahydro-dehydrozaluzanin C or its enantiomer. The stereostructure **1** was proposed for the compound by analogy to that of 8-epiisolippidiol (**3**), another guaianolide isolated from the same species. The stereochemistry of compound **3**, first reported from *Crepis capillaris* (Kisiel, 1983), was confirmed by X-ray crystallography (Rychlewska and Kisiel, 1991).

The known compounds isolated were 8-epigrosheimin (**2**) (Barbetti et al., 1979), 8-epiisolippidiol (**3**) (Kisiel, 1983), 11β,13-dihydroglucozaluzanin C (**4**) (Nishimura et al., 1986b), ixerin F (**5**) (Asada et al., 1984), 8-epidesacylcynaropicrin-3-*O*-β-glucopyranoside (**6**), its two esters (**7** and **8**) (Kisiel, 1984) and two hydrogenated derivatives (**9** and **10**) (Massanet et al., 1993; Kisiel and Barszcz, 1995), and the methanol addition product **11** (Marco et al., 1994), as well as picriside B (**12**) (Nishimura et al., 1986a), 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (**13**) (Achenbach et al., 1988) and 5-methoxy-eugenyl-4-*O*-β-glucopyranoside (**14**) (Miyase et al., 1985). Compounds **3**–**8**, **10** and **12** were identified by direct comparison of their $^1$H NMR (500.13 MHz) and mass spectral data with those of compounds isolated previously in our laboratory. The identities of compounds **9** and **11**–**14** were established by comparison of their spectral data with those reported. High resolution $^1$H NMR data of **2**, which until now have not been detailed in the literature, and unreported $^1$H NMR data of **8** as well as $^{13}$C NMR data of **8** and **10** are given in Tables 1 and 2.

The sesquiterpene lactone profile of *C. mollis* is quite similar to those of *C. capillaris* (Kisiel, 1984), *C. tectorum* (Kisiel and Kohlmünzer, 1989) and *C. pyrenaica* (Kisiel and Barszcz, 1995). Roots of the plants accumulate rare esters **7** and **8** accompanied by glycosides **6** and **10** as major constituents. So far, the esters have been reported from the above mentioned species

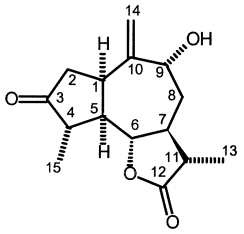
Table 1
$^1$H NMR (500.13 MHz) data of **1** and **2** in $CDCl_3$

| Position | **1**, $\delta_H$, *J* (Hz) | **2**, $\delta_H$, *J* (Hz) |
|---|---|---|
| 1α | 3.72 *ddd* (9.3, 8.0, 1.5) | 3.05 *ddd* (8.4, 8.0, 3.1) |
| 2α | 2.59 *dd* (19.1, 9.3) | 2.60 *dd* (19.3, 8.4) |
| 2β | 2.43 *ddd* (19.1, 1.5, 1.5) | 2.54 *ddd* (19.3, 3.1, 1.4) |
| 4β | 2.19 – 2.26 *m* | 2.33 *ddq* (10.3, 1.4, 6.9) |
| 5α | 2.19 – 2.26 *m* | 2.28 *ddd* (10.3, 9.2, 8.0) |
| 6β | 3.89 *dd* (9.7, 8.3) | 4.55 *dd* (9.2, 9.2) |
| 7α | 2.42 *dddd* (12.0, 11.0, 9.7, 2.6) | 3.16 *dddd* (9.2, 3.5, 3.0, 2.0) |
| 8α | 2.28 *ddd* (14.1, 2.6, 2.6) | 4.46 br *m* |
| 8β | 1.58 *ddd* (14.1, 12.0, 3.6) | – |
| 9α | – | 2.69 *dd* (13.9, 3.0) |
| 9β | 4.59 *dd* (3.6, 2.6) | 2.50 *dd* (13.9, 4.2) |
| 11β | 2.19 – 2.26 *m* | – |
| 13a | 1.28 *d* (7.0) | 6.45 *d* (3.5) |
| 13b | | 5.68 *d* (3.0) |
| 14a | 5.12 br *s* | 5.09 br *s* |
| 14b | 4.75 br *s* | 4.84 br *s* |
| 15 | 1.24 *d* (6.9) | 1.28 *d* (6.9) |

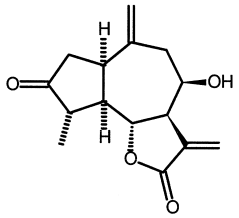
Table 2
$^1$H (500.13 MHz) and $^{13}$C (125.76 MHz) NMR data of **8** and $^{13}$C NMR data of **10** in $C_5D_5N$[a]

| Position | **8**, $\delta_H$, *J* (Hz) | **8**, $\delta_C$ | **10**, $\delta_C$ |
|---|---|---|---|
| Aglycone moiety | | | |
| 1 | 2.84 *m* | 44.3 | 43.3 |
| 2a | 2.40 *ddd* (14.0, 8.0, 8.0) | 38.2 | 38.5 |
| 2b | 2.08 *ddd* (14.0, 7.0, 7.0) | | |
| 3 | 4.82 *m* | 80.6 | 87.3 |
| 4 | – | 150.6 | 45.5[c] |
| 5 | 2.84 *m* | 49.8 | 51.7 |
| 6 | 4.83 *dd* (9.0, 9.0) | 78.9 | 80.7 |
| 7 | 3.37 *dddd* (9.0, 3.4, 2.7, 2.2) | 47.9 | 56.3 |
| 8 | 4.46 *ddd* (5.1, 4.6, 2.2) | 69.7 | 63.7 |
| 9a | 2.63 *dd* (14.0, 4.6) | 40.6 | 45.2[c] |
| 9b | 2.54 *dd* (14.0, 5.1) | | |
| 10 | – | 143.4 | 144.3 |
| 11 | – | 136.0 | 37.0 |
| 12 | – | 169.4 | 178.9 |
| 13a | 6.40 *d* (3.4) | 121.8 | 13.2 |
| 13b | 5.68 *d* (2.7) | | |
| 14a | 5.19 *br s* | 117.4 | 115.4 |
| 14b | 4.98 *br s* | | |
| 15a | 5.88 *br s* | 111.5 | 18.6 |
| 15b | 5.52 *br s* | | |
| Glucosyl moiety | | | |
| 1 | 5.01 *d* (7.8) | 104.7 | 105.8 |
| 2 | 4.07 *dd* (8.3, 7.8) | 75.3 | 75.4 |
| 3 | 4.25 *m* | 78.6[b] | 78.5[d] |
| 4 | 4.25 *m* | 71.7 | 71.8 |
| 5 | 3.95 *m* | 78.5[b] | 78.3[d] |
| 6a | 4.38 *dd* (11.6, 5.4) | 62.8 | 63.0 |
| 6b | 4.56 *dd* (11.6, 1.4) | | |
| Ester moiety | | | |
| 1 | – | 171.6 | |
| 2 | – | 85.3 | |
| 3 | – | 206.7 | |
| 4 | 2.35 *d* (0.9) | 25.7 | |
| 5 | 2.12 *q* (7.3) | 28.9 | |
| 6 | 0.97 *t* (7.3) | 8.0 | |

[a] The assignments were confirmed by HMQC experiments.
[b–d] Values interchangeable.



**1**



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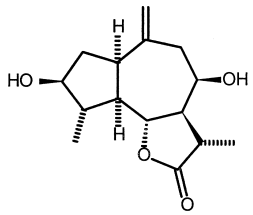

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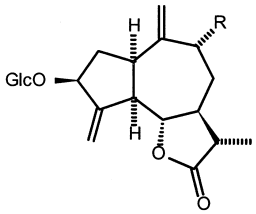
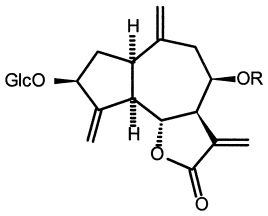
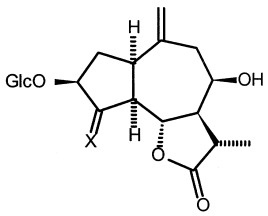


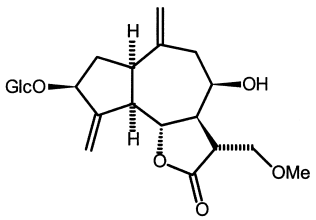
**4 R = H**
**5 R = OH**



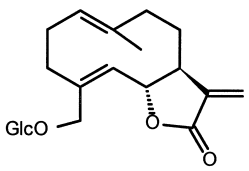
**6 R = H**
**7 R = A**
**8 R = B**



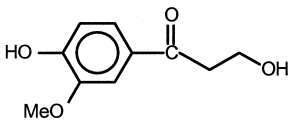
**9 X = $CH_2$**
**10 X = H, αMe**



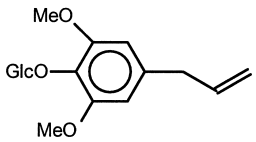
**11**



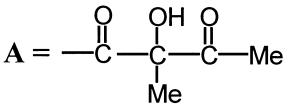
**12**

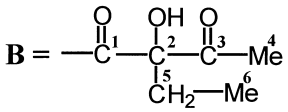


**13**



**14**





only. A survey of the phytochemical literature concerning *Crepis* species indicates that guaianolides, derivatives of 9α-hydroxy- and/or 8β-hydroxy-zaluzanin C, are most common (13 out 14 taxa).

## 3. Experimental

### 3.1. General

Merck silica gel was used for CC (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 $min^{-1}$.

### 3.2. Plant material

Roots of *C. mollis* 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.

### 3.3. Extraction and isolation

The dried and powdered plant material (462 g) was exhaustively extracted with EtOH at room temp. Removal of the solvent at red. pres. provided a residue (26 g) which was subjected to column chromatography on silica gel using as eluents hexane–EtOAc (up to 100% EtOAc), followed by EtOAc–MeOH (up to 15% MeOH). Elution of the column with hexane–EtOAc (1:1) and EtOAc (earlier frs) afforded complex mixtures of **1**–**3** and **13**. The mixtures were subjected to prep. TLC ($CHCl_3$–MeOH, 9:1), followed by semiprep. HPLC (MeOH–$H_2O$, 2:3) to give **13** (2.4 mg), **2** (2.3 mg), **1** (2.6 mg) and **3** (10.5 mg), in that order. Frs eluted with EtOAc–MeOH contained mainly sesquiterpene lactone glycosides. The relevant frs were combined, as shown by TLC, and then purified by prep. TLC ($CHCl_3$–MeOH, 17:3) to yield two crude mixtures of less and more polar compounds. A part (57 mg) of the former mixture (203 mg) was chromatographed on a semiprep. HPLC column (MeOH–$H_2O$, 1:1) to give a mixture of **6** and **7** (ca. 2:1, 11.3 mg), **8** (9.7 mg), **14** (2.1 mg) and a mixture of **4** and **12** (ca. 2:3, 2.5 mg). A part (50 mg) of the latter mixture (135 mg), after semiprep. HPLC separation (MeOH–$H_2O$, 7:13), furnished **5** (2.4 mg), **10** (10.2 mg), **6** (11.0 mg) and a mixture of **9** and **11** (ca. 2.5:1, 6.6 mg). The mixtures were not separated further, as the $^1H$ NMR signals could be assigned to the respective compounds by a careful analysis of the integrals. Due to some difficulties during HPLC separation the isolated amounts do not represent the concentration of pure compounds in the plant material.

#### 3.3.1. 9α-Hydroxy-4β, 15, 11β, 13-tetrahydrodehydrozaluzanin C (*1*)

Solid. EIMS *m/z* (rel. int.): 264 $[M]^+$ (71), 246 (20), 236 (14), 218 (25), 193 (37), 168 (97), 69 (100); $^1H$ NMR: Table 1.

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