Polish J. Chem., **75**, 141–145 (2001) **COMMUNICATION**

A New Taraxastane-Type Triterpenoid and Sesquiterpene Lactones from *Picris evae*

by W. Kisiel and K. Zielińska

Department of Phytochemistry, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland

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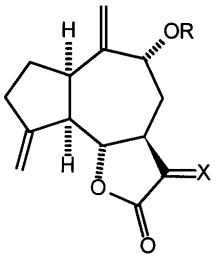
In continuation of our interest in the chemosystematics of the plants belonging to the tribe Lactuceae of the family Asteraceae, we have undertaken an investigation of *Picris evae* Lack., a species originating from Australia. Until now, this plant has not been a subject of phytochemical analysis, but several other *Picris* species, including the most extensively studied *P. hieracioides* and *P. echioides*, have yielded a wide range of sesquiterpene lactones, triterpenoids, ionone derivatives and phenolic compounds [1 and ref. cited in 2 and 3].

Dried roots and aerial parts of *P. evae* were separately extracted with ethanol and the extracts were chromatographed on silica gel columns to give fractions, which contained terpenoids and phenolics. The compounds were further separated and purified by preparative TLC and then subjected to HPLC analysis for direct comparison with authentic samples wherever possible.

The root extract afforded, in addition to methyl and ethyl caffeates, eight guaianolide glycosides and aglycones, which were identified as ixerisoside D (**3**, Glc = β-glucopyranosyl), scorzoside (**4**), ixerin F (**6**) and their aglycones **1**, **2**, **5**, respectively, crepidiaside B (**7**) and 9α-hydroxy-11β(13), 4β(15)-tetrahydrozaluzanin C (**8**). With the exception of **3**, the compounds were characterized by direct comparison (HPLC, ^{1}H NMR, EIMS or ESIMS) with the authentic materials from our collection. The identity of compound **3** was established by comparison of its spectral data with those reported [4]. Up to now, only ixerin F (**6**) [3,5], the major sesquiterpenoid of the plant material, and the aglycone of **7** [5–9] have been previously found in *Picris* species.

The extract from the aerial parts gave a complex mixture of widespread pentacyclic triterpenols, α-amyrin, β-amyrin, lupeol, taraxasterol and ψ-taraxasterol, in relatively good yield, along with 2β, 3β, 22α-trihydroxy-olean-12-ene and loliolide (**9**). The latter compound was isolated from *P. hieracioides* in our laboratory [10] and also reported from *P. echioides* [8]. The triterpene triol was proved to be identical with the compound first reported from *P. hieracioides* on the basis of spectral data [2]. In addition, a new triterpene diol, which has not been described previously, was obtained in a small amount. The EI mass spectrum of this compound displayed a molecular ion peak at *m/z* 442 corresponding to a molecular formula $C_{30}H_{50}O_2$. Its ^{1}H NMR

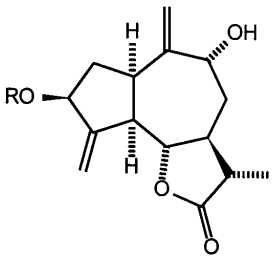


1 R=H, X=CH_2

2 R=H, X=H,αMe

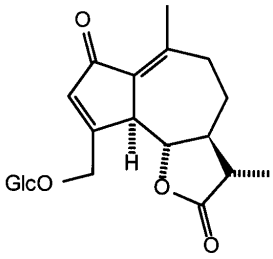
3 R=Glc, X=CH_2

4 R=Glc, X=H,αMe



5 R=H

6 R=Glc



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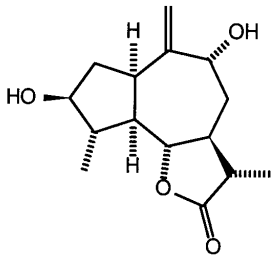

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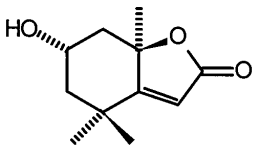

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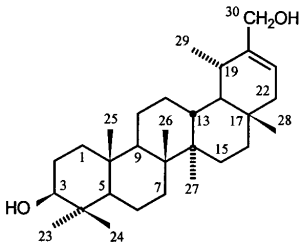

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spectrum revealed the presence of six tertiary methyl singlets, one secondary methyl doublet, an olefinic proton signal at δ 5.58 (br *d*, *J* = 6.2 Hz), a carbinolic methine proton signal at δ 3.20 (*dd*, *J* = 11.5 and 4.8 Hz) and mutually coupled (*J* = 12.7 Hz) hydroxymethylene proton doublets at δ 4.01 and δ 4.12, suggesting an unsaturated pentacyclic triterpenoid containing two hydroxyl groups, one secondary and one allylic primary. A comparison of the ^{1}H NMR spectral data (Table 1) with those recently reported for 21-hydroxy [11] and 21,22-epoxy [12] derivatives of taraxasterol showed that, except for the E-ring protons, the chemical shifts and couplings were almost identical, indicating the location of the secondary hydroxy substituent at the usual 3β (equatorial) position as well as the location of the double bond and the primary hydroxyl in the E-ring. In the ^{1}H - ^{1}H COSY spectrum the olefinic proton signal correlated with two non-equivalent methylene proton signals at δ 1.64 and δ 1.78, while the methyl doublet at δ 1.01 (*J* = 6.5 Hz) correlated with a methine proton signal at δ 1.88, suggesting the fragment -CH(CH_3)-C(CH_2OH)=CH-CH_2- in the E-ring. A structure satisfying these requirements was a ψ-taraxasterol derivative **10**. Firm evidence in support of the above was obtained from the vicinal coupling constants of the key protons and from the ^{1}H - ^{1}H COSY and NOESY spectra, which allowed all proton assignments. The methine proton at C-3 was confirmed to be axially oriented not

only due to the coupling constants noted, but also because of its connectivities with H-5α and Me-23 observed in the NOESY spectrum. The spectrum also verified the proximities of Me-25 to Me-24 and Me-26; Me-27 to H-7α, H-9α and H-16α, as well as Me-28 to H-21, H-13β and H-15β. In the same experiment H-30a and H-30b signals correlated with resonances of Me-29, H-21 and H-19β. The coupling pattern of the olefinic proton at C-21 was comparable to that of other ψ-taraxastane triterpenoids [13,14]. Moreover, the chemical shifts of Me-29 and H-21 signals were in close agreement with those recorded for heliantriol F (3β, 16β, 30-trihydroxy-taraxaster-20-ene) triacetate [14]. The compound was isolated from acetylated triterpene triol fraction of *Helianthus annuus* flower extract. From the foregoing evidence, the new natural product was proved to be 30-hydroxy-ψ-taraxasterol (3β, 30-dihydroxy-taraxaster-20-ene). Derivatives of ψ-taraxasterol oxygenated at C-30 are rarely distributed in nature. So far, only three compounds have been reported, the above mentioned heliantriol F, 3β-hydroxy-taraxaster-20-en-30-al and its acetate [15].

Table 1. ^{1}H NMR spectral data[a] of compound 10 (500.13 MHz, $CDCl_3$).

position	δ_H (*J*)	position	δ_H (*J*)
1α	1.72 *ddd* (13.1, 3.5, 3.5)	15β	1.02 *m*
1β	0.94 *m*	16α	1.29 *m*
2α	1.64 *m*	16β	1.14 *m*
2β	1.64 *m*	18α	1.09 *dd* (11.0, 7.9)
3α	3.20 *dd* (11.5, 4.8)	19β	1.88 *m*
5α	0.70 br *d* (10.3)	21	5.58 br *d* (6.2)
6α	1.54 *m*	22α	1.78 *m*
6β	1.38 *m*	22β	1.64 *m*
7α	1.40 *m*	23	0.975 *s*
7β	1.40 *m*	24	0.769 *s*[b]
9α	1.30 *m*	25	0.851 *s*
11α	1.55 *m*	26	1.041 *s*
11β	1.25 *m*	27	0.961 *s*
12α	1.64 *m*	28	0.761 *s*[b]
12β	1.23 *m*	29	1.01 *d* (6.5)
13β	1.66 *m*	30a	4.01 *d* (12.7)
15α	1.77 *m*	30b	4.12 br *d* (12.7)

[a]The chemical shifts were determined from the position of cross peaks in the COSY and NOESY spectra.
[b]The assignments may be reversed.

Plant material: The aerial parts and roots of *Picris evae* were collected in October 1998 from plants growing in the Garden of Medicinal Plants of the Institute of Pharmacology, Polish Academy of Sciences in Kraków. Seeds of the plant species were obtained from the Botanical Garden in Berlin-Dahlem, Germany.

Extraction and isolation: Dried and finely powdered aerial parts (1 kg) and roots (186 g) were exhaustively extracted with ethanol at room temperature with shaking and the solvent was evaporated under reduced preassure to give 90 g and 23 g of residues, respectively. The latter was chromatographed on a silica gel (Merck, Art. 7754) column using hexane-EtOAc (up to 100% EtOAc), followed by EtOAc-MeOH (up to 10% MeOH) gradient solvent systems and the relevant fractions were combined, as shown by TLC, and further separated and purified by preparative TLC (Merck, Art. 5553) to give mainly mixtures of structurally closely related compounds. Elution of the column with hexane-EtOAc (7:3) afforded a mixture of caffeic acid methyl and ethyl esters (*ca.* 1:1, 5.6 mg) and a mixture of **1** and **2** (*ca.* 1:2, 6.2 mg). Further elution with hexane-EtOAc (1:1) yielded a mixture of **5** and **8** in the ratio *ca.* 3:1 (2.6 mg). More polar fractions, eluted with EtOAc-MeOH (19:1), exhibited three spots on TLC. The upper and the middle bands were purified by TLC ($CHCl_3$-MeOH, 17:3) to give a mixture of **3** and **4** (*ca.* 1:1.5, 8.8 mg), and **7** (8.1 mg), respectively. Purification of the lowest band by TLC using $CHCl_3$-MeOH (4:1) afforded **6** (59.3 mg).
The ethanol extract from the aerial parts was prefractionated on a silica gel (Merck, Art. 7733) column using successively hexane, hexane-EtOAc (1:1), EtOAc and EtOAc-MeOH (1:1) as eluents to give four fractions (2 l each) which contained no sesquiterpene lactones. The hexane-EtOAc (1:1) fraction (29 g) was further chromatographed on a silica gel column using hexane-EtOAc gradient solvent system as described above. Elution of the column with hexane-EtOAc (9:1) gave a crude mixture (7.13 g) of triterpenols (M^+ 426) containing α-amyrin, β-amyrin, lupeol, taraxasterol and ψ-taraxasterol in the ratio *ca.* 1:1:1.8:0.9 (by ^{1}H NMR), respectively, as major constituents. Further elution with hexane-EtOAc mixtures, 4:1 and 1:1, yielded 2β, 3β, 22α-trihydroxy-olean-12-ene (4.0 mg) and **10** (5.8 mg), and **9** (30.6 mg), respectively, after purification by preparative TLC ($CHCl_3$-MeOH, 19:1). The mixtures 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. The HPLC analysis was performed on a μ-Bondapak C-18 column (particle size 10 μm, 2 × 300 mm) coupled to a photodiode array detector using H_2O-MeOH mixtures at a flow rate of 0.5 ml min^{-1}. Compound **10** was obtained as an amorphous powder. EIMS *m/z* (rel. int. %): 442 [M^+] (14), 424 (17), 409 (11), 408 (11), 207 (81), 205 (32), 203 (30), 191 (33), 189 (100), 175 (29), 135 (65), 121 (56), 109 (50), 95 (68), 81 (57), 69 (47), 55 (50). ^{1}H NMR data: see Table 1.

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