PERGAMON

Phytochemistry 50 (1999) 293–296

PHYTOCHEMISTRY

A taraxasterol derivative and phenolic compounds from *Hieracium gymnocephalum*

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Revised 16 July 1998

Abstract

From the aerial parts of *Hieracium gymnocephalum* the new triterpene, 21α-hydroxy-taraxasterol and flavone diglycosides luteolin 7-*O*-α-L-rhamnosyl(1 → 6)-β-D-glucoside and luteolin 7-*O*-β-D-xylosyl(1 → 6)-β-D-glucoside were isolated, as well as further common triterpenes, phenolic acids and flavones. The structures were established from spectral data (UV, MS, ^{1}H and ^{13}C NMR). © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: *Hieracium gymnocephalum*; Compositae; Triterpenes; 21α-Hydroxy-taraxasterol; Flavones; Phenolic acids

1. Introduction

The large genus *Hieracium* (*Compositae*, tribe *Lactucaceae*) consists of over 1000 species. Some species, such as *H. pilosella*, *H. aurantiacum* and *H. murorum*, are used in traditional european medicines as they display diuretic and antiinflammatory effects (Hoppe, 1958; Paris & Moyse, 1971; Constantinescu, Mihele, Vlasie, & Forstner, 1971). In a search for further *Hieracium* species with biological activities, we have studied *H. gymnocephalum* Griseb. ex Pant (Sell & West, 1976) which belongs to the unhybridogenous complex of the endemic section *Pannosa* and has not been investigated to date.

2. Results and discussion

The chloroform extract afforded the known triterpenes α- and β-amyrine which were identified by GC and GC–MS analysis by direct comparison with authentic compounds.

In addition, the new triterpene 21α-hydroxy-taraxasterol (18α,19α-urs-20(30)-en-3β,21α-diol, **1**) was isolated. Until now this taraxasterol derivative has not been found in nature, but has only been synthesized (Pai & Kulkarni, 1979). Here identification was based on GC–MS analysis, and one and two dimensional NMR spectroscopy (^{1}H NMR, ^{1}H–^{1}H COSY, HMQC and HMBC). The EI mass spectrum obtained after GC–MS analysis gave a M^+ peak at m/z 442 equivalent to $C_{30}H_{50}O_2$. The ^{1}H NMR spectrum showed the presence of six tertiary methyl groups on saturated carbons, an exocyclic double bond and two axially oriented secondary carbinolic methine protons (δ_H 4.39 and 3.19 (each dd); δ_C 71.3 and 79.1). A comparison of the ^{13}C NMR data with those of taraxasterol and its 21,22-epoxy derivative showed that, except for the ring E carbons and C-28, the chemical shifts are almost identical (Patra, Mukhopadhyay, & Mitra, 1981; Menichini, Di Benedetto, & Delle Monache, 1996), indicating that the second hydroxyl group must be located on ring E. Its definitive location was established from long-range ^{13}C–^{1}H connectivities. The substitution at C-19 by a methyl group was confirmed by long-range correlations between the protons of this methyl group and C-18, 20 and 19 in the HMBC spectrum, its α-orientation from the coupling constant of 7.1 Hz arising from the diaxial orientation of H-18 and H-19. Thus, as there are long-range correlations between the methine proton at δ_H 4.39 and C-30 the

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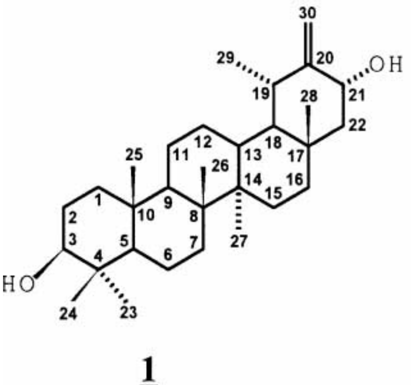
PII: S0031-9422(98)00536-6



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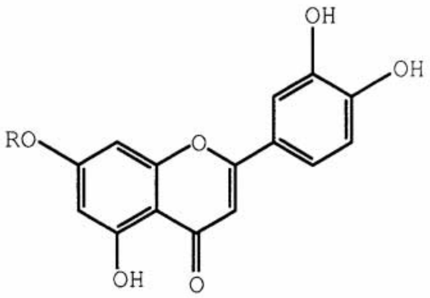

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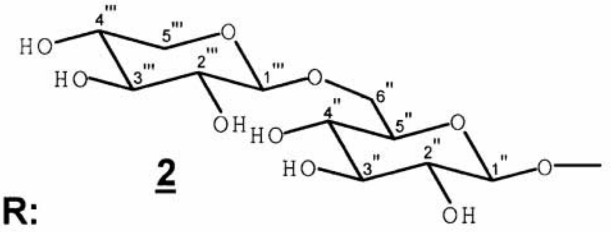

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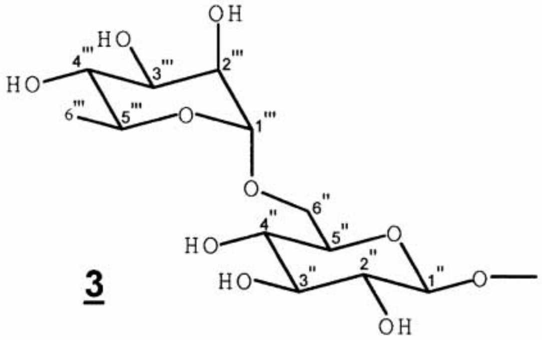

3

second hydroxyl must be located at C-21. Its α-configuration was confirmed by the $^3J_{H,H}$ couplings (4.9 and 8.9 Hz) of the axial proton at C-21. Additionally, the position of the other hydroxyl group at C-3 and the assignments of all further carbons followed from the HMQC and HBMC spectra (see Table 1).

Furthermore, phenolic acids and flavonoids were isolated from the methanolic extract. Comparative TLC and HPLC analyses, as well as partial UV, EI MS and ^{1}H NMR analyses, led to the identification of caffeic acid, chlorogenic acid, 1,5-dicaffeoylquinic acid, and 3,5-dicaffeoylquinic acid. The common flavonoids luteolin, its 7-*O*-β-glucoside, and apigenin 7-*O*-β-glucoside were identified by acid hydrolysis (except of luteolin, FABMS, UV data and comparative TLC and HPLC. Luteolin 7-*O*-α-L-rhamnosyl(1 → 6)-β-D-glucoside (**2**) and the rarely occurring luteolin 7-*O*-β-D-xylosyl(1 → 6)-β-D-glucoside (**3**) were additionally characterized from their ^{1}H NMR (**2** and **3**) and ^{13}C NMR (**2**) data. The (1 → 6)-interglycosidic linkage, as well as that of glucose to the hydroxyl group at C-7, in compound **3** was clearly established from NOE experiments.

The phenolic acids and flavonoids are the main compounds in the methanolic extract of the aerial parts of *H. gymnocephalum*. These may exert diuretic and anti-inflammatory effects, as this has been shown to be the case for many flavonoid containing herbal drugs (Della Loggia, Carle, Sosa, & Tubaro, 1990; Schilcher & Rau, 1988). Moreover, the sole occurrence of flavones underlines the advanced status of *H. gymnocephalum* (Harborne, 1977). However, further studies will show, if this is a characteristic of the large genus *Hieracium*. Preliminary flavonoid studies carried out with three further *Hieracium* species, *H. intybaceum*, *H. compositum* and *H. amplexicaule*, support this view (Giner, Recio, Villalba, Manez, & Rios, 1992; Wollenweber, 1984). The luteolin diglycosides **2** and **3** are reported for the first time in the genus *Hieracium*. To date the occurrence of flavone **2** has only been reported from *Salix repens* as well as from *Dacrydium laxifolium* and *D. intermedium* (Thieme, 1968; Markham, Whitehouse, & Webby, 1987).

3. Experimental

3.1. General

NMR: Bruker DMX-600 and Varian Unity 300 NMR spectrometers, 600/300 MHz (^{1}H NMR) and 150.8/75 MHz (^{13}C NMR), TMS as internal standard; MS: GC–MS: Finnigan 4000, OV-1-Optima capillary (25 m × 0.25 mm i.d.; film: 0.25 μM), carrier He at 0.92 ml/min (total flow), split 27:1, 290°C, FID; FABMS: Finnigan MAT 8230, Xe, 6–7 kV, 0.2–0.3 mA, glycerine.

3.2. Plant material

The aerial parts of *Hieracium gymnocephalum* Griseb. ex Pant were collected at Mount Durmitor, Montenegro (Jugoslavia), in August 1995 and August 1996. The plant was identified by Dr. M. Niketic, Natural History Museum Belgrade. A voucher specimen is deposited at the herbarium of the Natural History Museum of Belgrade, Yugoslavia.

Table 1
NMR data of **1** (600/150.8 MHz, $CDCl_3$)

Position	δ_H, multiplicity (J_{HH})[a]	δ_C, multiplicity[b]	Unambiguous long range ^{1}H–^{13}C correlations from the HMBC spectrum
1	1.70 m, 0.91 m	38.8 t	H-3, H-5, H-25
2	1.61 m	27.5 t	
3	3.19 dd (4.0, 11.3)	79.1 d	H-23, H-24
4	–	39.0 s	H-5, H-23, H-24
5	0.69 bd (~9)	55.4 d	H-23, H-24, H-25
6	1.51 m, 1.37 m	18.3 t	H-5
7	1.38 m	34.1 t	H-5, H-26, H-27
8	–	41.0 s	H-26, H-27
9	1.29 m	50.4 d	H-5, H-25, H-26
10	–	37.2 s	H-5, H-25
11	1.54 m	21.4 t	
12	1.64 m, 1.16 m	26.2 t	
13	1.58 m	38.9 d	H-27
14	–	42.3 s	H-26, H-27
15	1.68 m, 0.96 m	26.4 t	H-27
16	1.31 m, 1.15 m	37.7 t	H-22A, H-28
17	–	34.0 s	H-19, H-21, H-22A, H-28
18	1.17 d (~7, ~12)	48.5 d	H-19, H-22A, H-29
19	2.15 dq (7.0, 7.1)	38.1 d	H-21, H-30A and B, H-29
20	–	156.7 s	H-19, H-30A and B, H-29
21	4.39 dd (4.9, 8.9)	71.3 d	H-19, H-22, H-30A and B
22	1.95 dd (9.1, 13.8), 1.33 dd (~6, ~14)	48.9 t	H-21, H-28
23	0.96 s	28.0 q	H-3, H-5, H-24
24	0.76 s	15.4 q	H-3, H-5, H-24
25	0.84 s	16.3 q	H-5
26	1.01 s	16.0 q	
27	0.94 s	14.8 q	
28	0.76 s	18.2 q	H-22A
29	1.20 d (7.0)	28.5 q	H-19
30	4.98 d (1.4), 4.88 s	113.6 t	H-21

[a]The ^{1}H chemical shifts of H-1, H-2, H-5, H-6, H-7, H-9, H-11, H-12, H-13, H-15, H-16 and H-18 were determined from the position of cross peaks in the COSY and ^{1}H-detected one-bond ^{13}C–^{1}H (HMQC) correlations. [b]From a DEPT-135 ^{13}C spectrum.

3.3. Extraction and isolation

3.3.1. Triterpenes

Powdered aerial parts of *H. gymnocephalum* (1150 g) were extracted with $CHCl_3$ in a Soxhlet apparatus. After evaporation the crude residue was dissolved in 96% EtOH. After addition of $Pb(CH_3COO)_2$ and filtration through cellulose acetate, the filtrate was conc. and extracted with $CHCl_3$. The solvent was removed and a brown, fairly dense extract (15 g) was obtained. Further separation was performed by CC on silica gel (0.063–0.200 mm, Kemika, Zagreb) using petroleum ether to remove waxes, followed by toluene and then toluene–EtOAc (9:1). A mixture of compounds α- and β-amyrine (25 mg) was obtained from toluene and **1** from the last elution. α- and β-amyrine were not separated further. Final purification by TLC analysis on silica gel with toluene–EtOAc (7:3) afforded 1 mg of triterpene **1**.

3.3.2. Phenolic compounds

Powdered aerial parts of *H. gymnocephalum* (1494 g) were exhaustively extracted at room temperature with CH_2Cl_2 followed by methanol. Treatment of the methanolic extract (115 g) with EtOAc and removal of the solvent gave a residue of 80 g. 6 g of this residue were separated by CC on Cellulose (Merck, microcrystalline) using increasingly polar mixtures of petroleum ether–H_2O saturated EtOAc (3:7, 2:8, 1:9), followed by H_2O saturated EtOAc and then H_2O saturated EtOAc–MeOH (9:1). The flavonoid containing fractions were separated from the accompanying phenolic acids using RP18 cartridges (Mega Bond Elut™, Varian). At first the phenolic acids were eluted with H_2O, subsequently the respective flavonoid with MeOH. Further purification was performed by filtration on Sephadex LH-20 with MeOH yielding chlorogenic acid (2.9 mg), luteolin (0.6 mg), luteolin 7-*O*-β-D-glucoside (17 mg), apigenin 7-*O*-β-glucoside (0.4 mg), **2** (6.6 mg), **3** (5.3 mg) and a mixture of 3,5-dicaffeoylquinic acid and luteolin 7-*O*-β-glucoside. 3,5-Dicaffeoylquinic acid (0.5 mg) was purified by prep. TLC on silica gel with EtOAc–CH_3COOH–HCOOH–H_2O–toluene–$HCOOC_2H_5$ (50:5.5:6:13.5:2.5:2). Caffeic acid and 1,5-dicaffeoylquinic acid were not isolated.

Identification followed from fractions by comparative TLC and HPLC analysis.

3.3.3. 21α-Hydroxy-taraxasterol (*1*)

Amorphous powder; EIMS, 70 eV, *m/z* (rel. int.): 442 $[M]^+$ (20), 424 $[M-H_2O]^+$ (10), 372 (13), 357 (9), 207 (55), 189 (30), 173 (13), 159 (11), 145 (15), 134 (31), 125 (37), 109 (38), 95 (45), 81 (49), 69 (72), 55 (80), 43 (100). NMR data, see Table 1.

3.3.4. Luteolin 7-*O*-α-L-rhamnosyl(1 → 6)-β-D-glucoside (*2*)

UV λ_{max}^{MeOH} (nm) 256, 266 sh, 348; +NaOMe 264, 299 sh, 399; $+AlCl_3$ 272, 296 sh, 332 sh, 428; $+AlCl_3$–HCl 272, 296, 360, 388 (Mabry, Markham, & Thomas, 1970; Markham et al., 1987); FABMS (Xe, 6–7 kV, 0.2–0.3 mA, glycerine): neg. ions *m/z* (rel. int.): 593 $[M-H]^-$, 285 $[\text{aglycone}-H]^-$; pos. ions *m/z* (rel. int.): 595 $[M+H]^+$, 287 $[\text{aglycone}+H]^+$; ^1H NMR (300 MHz, CD_3OD): δ 1.18 (3H, d, $J = 6.4$ Hz, H-6‴), 3.36 (2H, m, H-4″, H-4‴), 3.48 (2H, m, H-2″, H-3″), 3.63 (2H, m, H-5″, H-6b″), 3.64 (1H, m, H-5‴), 3.72 (1H, dd, $J_{2''',3'''} = 3.4$ Hz, $J_{3''',4'''} = 9.5$ Hz, H-3‴), 3.90 (1H, dd, $J_{1''',2'''} = 1.2$ Hz, $J_{2''',3'''} = 3.4$ Hz, H-2‴), 4.71 (1H, d, $J_{1''',2'''} = 1.2$ Hz, H-1‴), 4.05 (1H, m, H-6a″), 5.03 (1H, d, $J_{1'',2''} = \sim 7$ Hz, H-1″), 6.51 (1H, d, $J_{6,8} = 2$ Hz, H-6), 6.59 (1H, s, H-3), 6.73 (1H, d, $J_{6,8} = 2$ Hz, H-8), 6.91 (1H, d, $J_{5',6'} = 8.8$ Hz, H-5′), 7.38–7.43 (2H, m, H-2′ and 6′); ^{13}C NMR (75 MHz, CD_3OD, ^{a}assignment may be interchanged): δ 17.8 (C-6‴), 67.5 (C-6″), 69.8 (C-5‴), 71.0 (C-4″), 71.3 (C-3‴), 72.1 (C-2‴), 74.0 (C-4‴), 74.7 (C-2″), 75.1 (C-5″), 77.1 (C-3″), 96.1 (C-8), 101.1 (C-6), 101.6 (C-1‴), 102.1 (C-1″), 104.2 (C-3), 107.1 (C-10), 114.3 (C-5′^{a}), 116.9 (C-2′^{a})), 120.6 (C-6′), 123.5 (C-1′), 147.0 (C-3′), 151.2 (C-4′), 158.9 (C-9), 162.9 (C-5), 164.7 (C-7), 166.9 (C-2); 184.0 (C-4) (Agrawal, 1989).

3.3.5. Luteolin 7-*O*-β-D-xylosyl(1 → 6)-β-D-glucoside (*3*)

$C_{26}H_{28}O_{15}$: UV λ_{max}^{MeOH} (nm) 255, 267 sh, 349; +NaOMe 263, 300 sh, 403; $+AlCl_3$ 274, 299 sh, 331 sh, 430; $+AlCl_3$–HCl 272, 295 sh, 360, 388; FABMS (Xe, 6–7 kV, 0.2–0.3 mA, glycerine): neg. ions *m/z* (rel. int.): 579 $[M-H]^-$, 285 $[\text{aglycone}-H]^-$, pos. ions *m/z* (rel. int.): 581 $[M+H]^+$, 287 $[\text{aglycone}+H]^+$; ^1H NMR (600 MHz, CD_3OD, *multiplicity not determined, partly overlapped by solvent): δ 3.22 (1H, dd, $J_{5a''',5b'''} = 11.3$ Hz, $J_{4''',5a'''} = 10.2$ Hz, H-5a‴), 3.30 (1H, *, H-2‴), 3.36 (1H, *, H-3‴), 3.46 (1H, m, H-4″), 3.50–3.53 (2H, m, H-2″ and H-3″), 3.55 (1H, m, H-4‴), 3.79 (1H, m, H-5″), 3.93 (1H, dd, $J_{4''',5b'''} = 5.2$ Hz, $J_{5a''',5b'''} = 11.3$ Hz, H-5b‴), 4.06 (1H, m, H-6b″), 4.19 (1H, m, H-6a″), 4.36 (1H, d, $J_{1''',2'''} = 7.1$ Hz, H-1‴), 5.07 (1H, m, $J_{1'',2''} = 7.5$ Hz, H-1″), 6.62 (1H, d, $J = 2$ Hz, H-6), 6.64 (1H, s, H-3), 6.84 (1H, d, $J = 2$ Hz, H-8), 6.96 (1H, d, $J_{5',6'} = 8.9$ Hz, H-5′), 7.46 (1H, d, $J_{2',6'} = 2$ Hz, H-2′), 7.47 (1H, dd, $J_{2',6'} = 2$ Hz, $J_{5',6'} = 8.9$ Hz, H-6′).

Acknowledgements

We are grateful to the DAAD for financial support (S.D.P.), to M. Weber, Institute of Pharmaceutical Biology, Freiburg, for taking the mass spectra, to C. Kakoschke, GBF, Braunschweig, for the 600 MHz NMR spectra and to V. Brecht, Institut für Pharmazie, Freiburg, for the 300 MHz NMR spectra.

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