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CHAMAEMELOSIDE, A NEW FLAVONOID GLYCOSIDE FROM *CHAMAEMELUM NOBILE*

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Key Word Index—*Chamaemelum nobile*; Asteraceae; chamaemeloside; apigenin 7-*O*-β-D-glucopyranoside-6″-(3‴-hydroxy-3‴-methyl-glutarate); flavone glycoside.

Abstract—From dried flowers of *Chamaemelum nobile*: a new flavonoid, apigenin 7-glucoside-6″-(3‴-hydroxy-3‴-methyl-glutarate), has been isolated. The structure has been elucidated by interpretation of its spectroscopic data: one- and two-dimensional NMR, mass spectrometry, IR and UV.

INTRODUCTION

Roman chamomile (*Chamaemelum nobile* (L.) All., Asteraceae) is a perennial herb cultivated in western Europe and northern Africa. The double flower-heads (*Chamomillae romanae flos*) are included in several pharmacopoeias (e.g., EP, Ph. Helv. VII, DAB 10). In traditional medicine it is applied in a similar way as German chamomile due mainly to its anti-inflammatory and spasmolytic activity.

The secondary metabolites found in this plant species are volatile oil composed of esters of angelic acid, methacrylic acid and isobutyric acid and C_4- and C_6-alcohols [1, 2], sesquiterpene lactones [3–5], polyacetylenes [6, 7], flavonoids and other phenolics, e.g. caffeic acid. The spasmolytic effect of the plant is reported to be attributed to the presence of flavonoids [8, 9] some of which have been isolated previously [10–14].

During our efforts to develop analytical methods for the qualitative and quantitative determination of the phenolic constituents of the double flower-heads of *C. nobile* a new and unusual apigenin glycoside was isolated as the main component of the aqueous methanol extract. The present paper describes the isolation and structural elucidation of this new apigenin glycoside, chamaemeloside (**1**).

RESULTS AND DISCUSSION

The aqueous methanol extract of Roman chamomile was fractionated using liquid–liquid distribution methods and gelchromatography (see Experimental) to obtain compound **1**. By mass spectrometry (MS), **1** was found to have the molecular formula $C_{27}H_{28}O_{14}$ (FABMS found *m/z* 577 $[M + H]^+$). Comparison of its UV, FABMS, ^{1}H NMR and ^{13}C NMR spectra with published data [15, 16], suggested **1** to be an apigenin derivative (fragment 1, Scheme 1). The aglycone apigenin was clearly deduced by detection of the two *meta*-coupled protons, H-6 and H-8 of ring A ($J_{6,8}$ = 2.1 Hz), a singlet resonance for H-3 (δ6.87) and the typical AA′–XX′ pattern for a *para*-substituted aromatic ring B, in the ^{1}H NMR spectrum of **1** (Table 1). All UV-Vis absorption spectra of **1** indicated the presence of a flavone derivative with free hydroxyl-groups at C-5 and C-4′ (λ_{max} 268 and 333 nm), and the observed shifts in alkaline media indicated the 7-hydroxyl group to be substituted. The chemical shifts of the anomeric proton of what was clearly a glucose moiety (δ5.12), as well as for H_2-6″ [A: δ = 4.08 (*dd*), B: δ = 4.33 (*d*)] indicated it to be disubstituted (fragment 2, Scheme 1). Of the 26 resonances present in the ^{13}C NMR spectra of **1**, 20 were similar to those of apigenin 7-glucoside (**2**) [8] (Table 1). Apigenin 7-glucoside was also identified (chromatographically) after alkaline hydrolysis of **1**. The six remaining ^{13}C NMR signals indicated the presence of two carbonyl groups, one for a free acid [172.5 ppm (*s*)] and one for an ester function [170.6 ppm (*s*)], a tertiary alcohol [69.0 ppm (*s*)], two methylene groups [45.4 (*t*), 45.2 ppm (*t*)] and a methyl group [27.6 ppm (*q*)]. The ^{13}C and ^{1}H NMR data together indicated the methyl group to be tertiary. These data also revealed that there was no coupling between the two methylene functions. These groups can only be assembled to produce a 3-hydroxy-3-methyl-glutaryl acid (fragment 3, Scheme 1). This deduction was also supported by the ^{1}H–^{13}C 2D NMR long range (HMBC, *J* = 8.3 Hz) correlation data for

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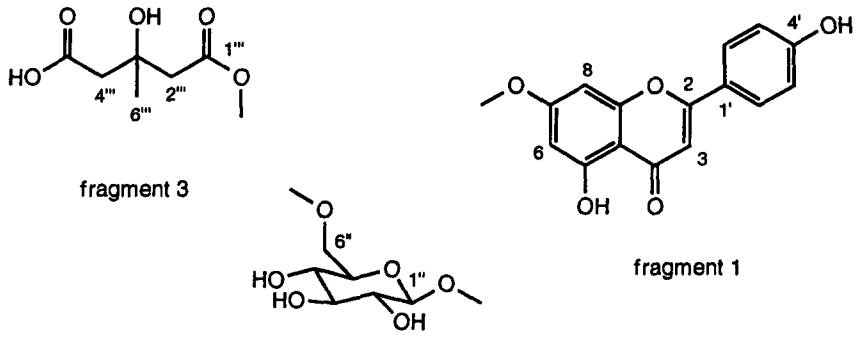
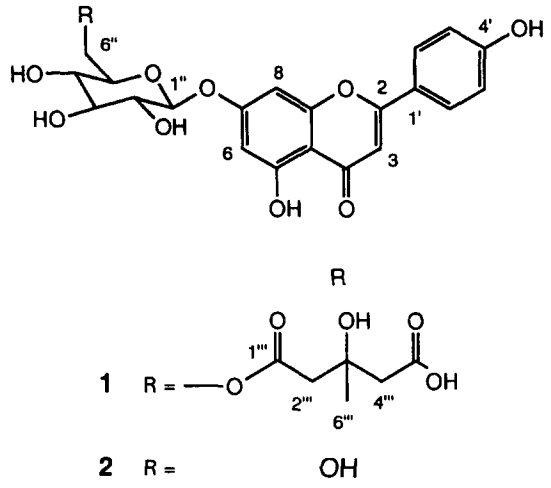


Scheme. 1. Molecular fragments deduced for **1**.

Table 1. ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75.5 MHz, DMSO-d_6) spectral data for **1** and **2**

	^{13}C NMR		^{1}H NMR		Long range ^{1}H–^{13}C correlations
C	**1** δ (ppm)	**2** δ (ppm)	**1** δ (ppm)	J (Hz)	**1** (HMBC, J = 8.3 Hz)
2	164.5 *s**	164.2			H-3, H-2′, H-6′
3	103.3 *d*	103.0	6.87 *s*		
4	182.2 *s*	181.6			H-3
5	161.3 *s*	161.0			H-6, 5-OH
			OH: 12.97 *br s*		
6	99.7 *d*	99.6	6.43 *d*	2.1	H-8, 5-OH
7	162.7 *s*	162.8			H-6, H-8, H-1″
8	94.9 *d*	94.9	6.80 *d*	2.1	H-6
9	157.1 *s*	156.8			H-8
10	105.6 *s*	105.3			H-6, 5-OH
1′	121.2 *s*	121.1			H-3, H-3′, H-5′
2′	128.8 *d*	128.1	7.96 *d*	8.8	H-6′
3′	116.2 *d*	115.8	6.94 *d*	8.8	H-5′
4′	161.5 *s*	160.9			H-2′, H-3′, H-5′, H-6′
5′	116.2 *d*	115.8	6.94 *d*	8.8	H-3′
6′	128.8 *d*	128.1	7.96 *d*	8.8	H-2′
1″	99.7 *d*	100.3	5.12 *d*	7.2	
2″	73.1 *d*	73.1	3.31	8.6/7.2	H-3″
			OH: 5.50 *br s*		
3″	76.3 *d*	77.0	3.36 *dd*	8.6/7.8	H-2″
			OH: 5.28 *br s*		
4″	69.9 *d*	69.9	3.19 *dd*	9.1/7.8	H-6″(A)
			OH: 5.35 *br s*		
5″	74.1 *d*	76.4	3.74 *dd*	9.1/7.0	H-6″(B)
6″	63.4 *t*	60.8	A 4.33 *d*	12.1	
			B 4.08 *dd*	12.1/7.0	
1‴	170.6 *s*				H_2-6″, H_2-2‴
2‴	45.4 *t*		2.64 *d*	14.4	H_2-4‴, H_3-6‴
			2.55 *d*	14.4	
3‴	69.0 *s*				H_2-2‴, H_2-4‴, H_3-6‴
4‴	45.2 *t*		2.48 *d*	14.9	H_2-2‴, H_3-6‴
			2.40 *d*	14.9	
5‴	172.5 *s*				H_2-4‴
6‴	27.6 *q*		1.18 *s*		H_2-2‴, H_2-4‴

*Multiplicities by DEPT.



1 (Table 1). Connectivities between fragments 1, 2 and 3 were established unequivocally from the results of the same experiment (Table 1), which showed correlations from H-1″ to C-7 (fragments 1 and 2, Scheme 1), and from H-6″ to C-1‴ (fragments 2 and 3, Scheme 1). Therefore compound **1** is identified as apigenin 7-*O*-β-D-glucopyranoside-6″-(3‴-hydroxy-3‴-methyl-glutarate) for which the trivial name chamaemeloside is proposed.

All proton and carbon resonances for **1** were unambiguously assigned from the data obtained with its HMBC, HMQC ($J = 150$ Hz) and DQFCOSY 2D NMR spectra (Table 1).

Flavonoids possessing a 3-hydroxy-3-methyl-glutaryl side chain are relatively rare [17–19] and are probably derived from one of two biosynthetic sources, either from 3-hydroxy-3-methyl-glutaryl CoA, an intermediate in the biosynthesis of isopentenyl diphosphate from acetyl CoA [20], or from L-leucin [21].

Attempts to deduce the absolute configuration of **1** by producing a derivative suitable for X-ray crystallography have been unsuccessful.

EXPERIMENTAL

Plant material. Chamomillae romanae flos Ph. Eur. II, Siegfried CH-Zofingen (Batch No. 9007 × 009).

General. Optical rotation in pyridine at 20°; IR: KBr disc; UV spectra in MeOH, using shift reagents; FAB MS in a 3-NOBA matrix at 8.3 keV: 1D and 2D ^{1}H (300 MHz) and ^{13}C NMR (75.5 MHz) spectra in DMSO-d_6. All solvents were HPLC grade.

Extraction and isolation. The plant material (4.5 kg dried flowers) was successively extracted with petrol (105 l), CH_2Cl_2 (98 l), MeOH (20 l) and then 80% MeOH (100 l) employing an ultraturrax. The MeOH and aq. MeOH extracts were evapd to dryness and the residue partitioned between H_2O and *n*-BuOH to yield 585.2 g (13.0%) of BuOH solubles. Part of the *n*-BuOH extract (29.5 g) was fractionated employing a Craig-CCD apparatus [EtOAc–PrOH–H_2O (2:1:2), 120 elements, 25 ml, Labortec, CH-Bubendorf] to yield 120 frs. Frs 106–113 (2.5 g) were combined and subjected to CC on Sephadex LH-20 (4 × 80 cm) using a linear gradient of MeOH and H_2O (50–100% MeOH in 50 hr, flow rate 1 ml min^{-1}, fr. size 20 ml). Frs 115–121 from CC contained mainly **1**. Final purification by pptn from EtOH by addition of petrol afforded 500 mg (0.22%) **1**.

Hydrolysis of **1** with 1 M NaOH under N_2 at ambient temp. during 30 min yielded apigenin 7-*O*-glucoside.

Compound **1** (*chamaemeloside*). Yellow amorphous powder with $[\alpha]_D^{20}$ −74.5° (pyridine; *c* 0.21); IR ν_{max} cm^{-1}: 3400, 2890, 1717 (ester and carboxylic acid), 1655, 1605, 1495, 1440, 1345, 1240, 1145, 1110, 1070; FABMS *m/z* (rel. int.): 577 $[M + H]^+$ (86), 271 [apigenin + H]$^+$ (93), 154 (100), 136 (84). UV λ_{max}^{MeOH} (ε) 268 (20,780), 333 (24,200); + NaOMe 249, 268, 389 (no decomp.); + $AlCl_3$ 275, 300, 348, 385; + $AlCl_3$/HCl 275, 300, 347, 385; + NaOAc 267, 340, 388 (no decomp.) + NaOAc/H_3BO_3 268, 337 nm. ^{1}H NMR and ^{13}C NMR: Table 1.

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