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FLAVONOLS AND ISOFLAVONES FROM *COTONEASTER SIMONSII*

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Key Word Index—*Cotoneaster simonsii*; Rosaceae; leaves and twigs; triterpenes; flavonols and isoflavones; kaempferol 7-methyl ether 4′-glucoside; genistein 5-methyl ether 4′-glucoside; arbutin.

Abstract—Two new derivatives, a flavonol, kaempferol 7-methyl ether-4′-glucoside, and an isoflavone, genistein 5-methyl ether 4′-glucoside, were isolated from the leaves of *Cotoneaster simonsii*. Ursolic, tormentic and euscapic acids, arbutin, genistein 5-methyl ether, catechin, chlorogenic acid and benzyl alcohol glucoside were also identified. The structures of the compounds were determined by spectroscopic methods.

INTRODUCTION

In the course of phytochemical studies on plants of the genus *Cotoneaster* (Rosaceae) [1], we have investigated the species *C. simonsii* Baker, a thorny shrub with deciduous to semi-evergreen leaves, white or rose coloured flowers and red fruits [2].

This species has never been subjected to phytochemical or biological investigation although other *Cotoneaster* species have been studied and many of these have been shown to produce compounds with antiviral, antitumour, and antispasmodic activities [3–5].

RESULTS AND DISCUSSION

The aerial parts of the plant (leaves and twigs) were investigated. The defatted plant material was exhaustively extracted with chloroform, chloroform–methanol (9:1) and methanol. From the chloroform extract, ursolic acid, tormentic acid, euscapic acid, arbutin and genistein 5-methyl ether were isolated. The dried chloroform–methanol extract was purified to give the new derivative (**1**) and benzyl alcohol β-D-glucopyranoside, while from the methanolic residue, catechin, chlorogenic acid and the new derivative (**2**) were isolated. The structures of the known compounds were determined by spectroscopic methods and by comparison with authentic samples.

Compound **1** gave a mass spectrum with a $[M + H]^+$ peak at *m/z* 463 consistent with the formula $C_{22}H_{22}O_{11}$, which was confirmed by ^{13}C NMR and DEPT experiments. The presence of a hexose moiety was determined by the peak at *m/z* 301. The UV spectrum showed absorption maxima at 255, 268 (band II), 301 sh, 320 and 365 nm (band I) typical of a flavonol skeleton. The bathochromic shift of band I with $AlCl_3$/HCl (58 nm) is characteristic of a 3,5-dihydroxyflavone, and the absence of bathochromic shifts of band II with sodium acetate and of band I with sodium methoxide suggested substituted hydroxyl groups at C-7 and C-4′ [6]. The ^1H NMR spectrum confirmed many of the above features and, in addition, revealed the presence of 7-*O*-methylkaempferol and a glucopyranose moiety (see Experimental). Acid hydrolysis of **1** gave 7-*O*-methylkaempferol and D-glucose. The site of methylation of C-7 and the glycosylation at C-4′ were deduced using a combination of UV (see Experimental) and ^{13}C NMR spectral data (Table 1). Thus, in the ^{13}C NMR spectrum the resonance of C-4′ was shifted upfield by 1.1 ppm in comparison with 7-*O*-methylkaempferol, and the *para*-carbon (C-1′) underwent a downfield shift of 3.4 ppm, proving that the glucose is attached at C-4′ [7]. Therefore, **1** was identified as kaempferol 7-methyl ether 4′-β-D-glucopyranoside, a new flavonol glycoside.

Compound **2** gave a mass spectrum with a $[M + H]^+$ peak at *m/z* 447 consistent with the formula $C_{22}H_{22}O_{10}$, which was confirmed by ^{13}C NMR and DEPT experiments and a peak at *m/z* 285 due to the loss of a hexose moiety. The UV spectrum showed absorption maxima at 256 (band II), 280 sh and 320 sh nm (band I), which indicated an isoflavone skeleton. The absence of a bathochromic shift of band II with $AlCl_3$/HCl is characteristic of a substituted 5-hydroxyisoflavone, and the presence of a bathochromic shift of band II with sodium acetate indicated an unsubstituted hydroxyl group at C-7 [6]. The ^1H NMR spectrum confirmed many of the above features and, in addition, revealed the presence of 5-*O*-methylgenistein and a glucopyranose moiety (see Experimental). Acid hy-

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Table 1. ^{13}C NMR data for new compounds **1** and **2** (CD_3OD, 200 MHz)

	Aglycone			Glucose	
Carbons	**1**	**2**	Carbons	**1**	**2**
C-2	146.9	149.4	C-1″	99.5	98.9
C-3	136.0	118.7	C-2″	74.8	73.5
C-4	176.0	173.7	C-3″	77.9	75.7
C-5	161.2	159.6	C-4″	70.3	69.7
C-6	97.4	96.7	C-5″	78.5	76.4
C-7	165.1	161.9	C-6″	62.1	60.4
C-8	91.8	91.9			
C-9	156.1	157.2			
C-10	103.8	105.0			
C-1′	124.7	118.2			
C-2′	131.5	125.8			
C-3′	115.9	110.3			
C-4′	158.0	155.8			
C-5′	115.9	125.8			
C-6′	131.5	110.3			
OMe	55.9	54.4			

drolysis of **2** gave 5-*O*-methylgenistein and D-glucose. The position of attachment at C-4 of this sugar was deduced from the ^{13}C NMR spectral data (see Table 1). The chemical shift of this carbon was shifted upfield by 0.7 ppm in comparison with 5-*O*-methylgenistein, and the *para*-carbon (C-1′) underwent a downfield shift of 3.0 ppm. Therefore, the linkage of glucose was proved to be at the C-4′ of **2** [7], which is characterized as genistein 5-methyl ether 4′-β-D-glucopyranoside, a new isoflavone glycoside.

The results of this investigation could have some chemotaxonomic importance when considered in relation to previous reports of flavonols, catechins and other phenolic constituents from other *Cotoneaster* species. In particular, the presence of the isoflavone **2** in *C. simonsii* correlates this species to *C. serotinus* Hutch. and *C. pannosus* Franc., where the isoflavone biochanin and its 7-glucoside were found [8]. It is notable also that the triterpenes are frequent components in this genus, uvaol having been identified in *C. pannosus* and ursolic acid in both *C. serotinus* and *C. pannosus* [9]. Furthermore, the presence of arbutin is quite unusual, because it is a typical constituent of the genus *Pyrus* [9, 10].

EXPERIMENTAL

Instruments. FAB MS was performed in positive-ion mode with a VG ZAB spectrometer. ^1H and ^{13}C NMR spectra were recorded in CD_3OD on a Bruker AC200 instrument operating at 200.06 MHz for proton and 50.31 MHz for carbon; chemical shifts are given in δ values (ppm) with TMS as int. standard. DEPT, COSY, NOESY and HETCOR spectra were recorded using standard microprogrammes. TLC was carried out on silica gel 60 F_{254} gel precoated aluminium sheets and RP-18 HPTLC plates (Merck). Compounds were visualized under UV (254 nm) and by spraying with $Ce(SO_4)_2$ and NTS/PEG reagents.

Plant material. The aerial parts (leaves and twigs) of *C. simonsii* Baker were collected in the Botanical Garden of Naples in June, 1992. Dried voucher specimens are deposited in the Dipartimento di Chimica Bioorganica, University of Pisa (Italy).

Extraction and isolation of compounds. Dried ground plant material (400 g) was extracted in a Soxhlet apparatus sequentially with *n*-hexane, $CHCl_3$, and $CHCl_3$–MeOH (9:1). The same material was then extracted at room temp. with MeOH. The crude $CHCl_3$ extract (5 g) was subjected to CC on Sephadex LH to give 5 frs using MeOH–$CHCl_3$ (9:1) as eluent. Frs II–IV were further purified by silica gel CC employing MeOH–$CHCl_3$ of increasing polarity (3:13–3:7) to give ursolic acid (15 mg), tormentic acid (25 mg), euscapic acid (18 mg), arbutin (9 mg) and genistein 5-methyl ether (8 mg). The crude $CHCl_3$–MeOH extract (7 g) was subjected to flash CC on silica gel employing MeOH–$CHCl_3$ (3:13) as eluent to give **1** (17 mg) and benzyl alcohol glucoside (30 mg). The crude MeOH extract (8 g) was sepd on a Sephadex LH-20 column by eluting with H_2O–MeOH mixts of increasing polarity (2:3–3:7) to give catechin (15 mg), chlorogenic acid (7 mg) and **2** (23 mg).

Compound **1** (*kaempferol 7-methyl ether 4′-β-D-glucopyranoside*). UV λ_{max}^{MeOH}: 255, 268, 301 sh, 320, 365; +NaOMe: 255, 268, 320, 365; +$AlCl_3$: 256, 320, 423; +$AlCl_3$/HCl: 256, 320, 423; +NaOAc: 255, 268, 320, 365 nm. FAB MS (*m/z*): 485 $[M+Na]^+$, 463 $[M+H]^+$, 301 $[M+H\text{-hexose}]^+$. ^1H NMR: δ 3.05–3.53 (6H, sugar protons), 3.83 (3H, *s*, OMe), 5.03 (1H, *d*, $J=7.2$ Hz, H-1′), 6.53 (1H, *d*, $J=1.8$ Hz, H-6), 6.72 (1H, *d*, $J=1.8$ Hz, H-8), 6.99 (2H, *d*, $J=8.4$ Hz, H-3′, H-5′), 7.49 (2H, *d*, $J=8.4$ Hz, H-2′, H-6′). ^{13}C NMR: see Table 1.

Compound **2** (*genistein 5-methyl ether 4′-β-D-glucopyranoside*). UV λ_{max}^{MeOH} nm: 256, 280 sh, 320 sh; +NaOMe: 256, 280 sh, 320 sh; +$AlCl_3$: 256, 280 sh, 320 sh; +$AlCl_3$/HCl: 256, 280 sh, 320 sh; NaOAc:

261, 280 sh, 322 sh nm. FAB MS (*m/z*): 469 $[M + Na]^+$, 447 $[M + H]^+$, 285 $[M + H - \text{hexose}]^+$. ^1H NMR: δ 3.02–3.48 (6H, sugar protons), 3.78 (3H, *s*, OMe), 5.08 (1H, *d*, $J = 7.7$ Hz, H-1′), 6.46 (1H, *d*, $J = 1.9$ Hz, H-6), 6.72 (1H, *d*, $J = 1.9$ Hz, H-8), 7.00 (2H, *d*, $J = 8.7$ Hz, H-3′, H-5′), 7.51 (2H, *d*, $J = 8.7$ Hz, H-2′, H-6′), 8.41 (1H, *s*, H-2). ^{13}C NMR: see Table 1.

Hydrolysis of **1** *and* **2**. Compounds **1** and **2** (6 mg each) were separately hydrolysed with 5% HCl–MeOH (5 ml) for 3 hr under reflux to yield kaempferol 7-methyl ether and genistein 5-methyl ether, respectively (mmp, co-TLC, ^1H NMR) and D-glucose [co-TLC with an authentic sample using EtOAc–H_2O–MeOH–HOAc (13:3:3:4) as eluent, *p*-anisidine phthalate and naphthoresorcinol as spray reagents].

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