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Phenylethanoid glycosides from *Prostanthera melissifolia*

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Abstract

Six phenylethanoid glycosides were isolated from aerial parts of *Prostanthera melissifolia*, together with apigenin and ursolic acid. The glycosides were characterized by spectral methods as martynoside, isomartynoside, verbascoside, isoverbascoside, betonyoside F and isobetonyoside F, the latter being a new natural product.

Keywords: *Prostanthera melissifolia*; Lamiaceae; Phenylethanoid glycosides; Isobetonyoside F

1. Introduction

Prostanthera melissifolia F. Muell. is a plant species of the family Lamiaceae native to Australia. No chemical studies appear to have been reported for this species. We have examined the constituents of the aerial parts of the plant and this paper describes details of their isolation and characterization.

2. Results and discussion

Repeated chromatographic separation of an ethanolic extract from the aerial parts of *P. melissifolia* yielded, in addition to ursolic acid and apigenin, six phenylethanoid glycosides (Fig. 1), of which one (**6**) proved to be a new compound and the remaining five were identified as martynoside (**1**) (Calis, Lahloub, Rogenmoser & Sticher, 1984), isomartynoside (**2**) (Calis et al., 1984), verbascoside (=acteoside) (**3**) (Andary, Wylde, Laffite, Privat & Winternitz, 1982; Miyase et al., 1982), isoverbascoside (**4**) (Miyase et al., 1982) and betonyoside F (**5**) (Miyase, Yamamoto & Ueno, 1996).

The identities of ursolic acid and apigenin, the former being the main constituent of the extract, were established by direct comparison of their spectra (EIMS, ^{1}H NMR) with those of authentic materials obtained from our earlier studies. The known glycosides were characterized by their electro-spray mass spectra and by comparing their ^{1}H NMR (and ^{13}C NMR in the case of **5**) spectral data with those reported previously (Andary et al., 1982; Budzianowski & Skrzypczak, 1995; Miyase et al., 1982, 1996).

The over-all spectral data and chromatographic behaviour of the new glycoside **6** strongly suggested that the compound was an isomer of **5**. It exhibited a *pseudo*-molecular ion peak at m/z 779, $[M+Na]^+$, in the ESIMS, indicating the same molecular formula as **5** and showed the same UV absorption maxima. From the ^{1}H NMR spectrum of **6** (Table 1), it could be deduced that the compound was also a triglycoside of 2-(3,4-dihydroxyphenyl)-ethyl alcohol with an (*E*)-caffeic acid ester group. Three signals for anomeric protons appeared at δ 4.32 (*d*, $J=7.9$ Hz), δ 5.11 (*d*, $J=2.4$ Hz) and δ 5.35 (*br s*), suggesting the presence of β-glucose, β-apiose and α-rhamnose, respectively, as in **5**, and the same sugar sequence [Api-(1→2)-Rha-(1→3)-Glc]. When comparing directly, further ^{1}H NMR signals of **5** and **6** were almost superimposable, except for some differences in the chemical shift values at the region of sugar protons. The signals assignable to glu-

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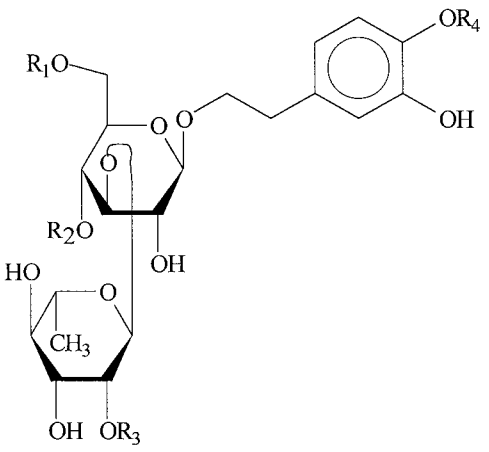

	R_1	R_2	R_3	R_4
1	H	feruloyl	H	Me
2	feruloyl	H	H	Me
3	H	caffeoyl	H	H
4	caffeoyl	H	H	H
5	H	caffeoyl	apiosyl	H
6	caffeoyl	H	apiosyl	H

Fig. 1.

cose protons at C-6 in the ^{1}H NMR spectrum of **6** were significantly shifted downfield from δ 3.53 to δ 4.35 (*dd, J*=11.9 and 5.6 Hz) and from δ 3.64 to δ 4.48 (*dd, J*=11.9 and 2.0 Hz), and the C-4 glucose proton signal was shifted upfield by about 1.6 ppm. The above data indicate that **5** and **6** differ from each other only in the site of acylation and the caffeoyl group in **6** is located at glucose C-6. Thus, the structure of the new product, named isobetonyoside F, was established as **6**.

Betonyoside F (**5**), reported from *Stachys officinalis* (Miyase et al., 1996), and similar compounds having other terminal sugar attached to C-2 of rhamnose are known as constituents of some members of the Lamiaceae family (Budzianowski & Skrzypczak, 1995; Calis, Basaran, Saracoglu & Sticher, 1992; Calis, Ersöz, Tasdemir & Rüedi, 1992; Nishimura, Sasaki, Inagaki, Chin & Mitsuhashi, 1991; Seidel, Bailleul, Libot & Tillequin, 1997).

3. Experimental

3.1. Plant material

Aerial parts of *P. melissifolia* were collected on Mt Dandenong (Australia, Victoria) in February 1995 and were authenticated in the Karwarra Australian Plant Garden, Kalorama, where a voucher specimen No KAP6-295-44 was deposited.

3.2. Extraction and isolation

Dried powdered aerial parts (166 g) were exhaustively extracted with EtOH at room temp. with shaking and the extract was concentrated under reduced pressure. The residue (32 g) was submitted to CC on silica gel (Merck, Art. 7754), using hexane-EtOAc (up to 100% EtOAc) followed by EtOAc-MeOH (up to 20% MeOH) gradient solvent systems. Elution of the column with hexane-EtOAc mixtures gave ursolic acid (451 mg) and apigenin (35 mg). Elution with EtOAc and EtOAc–MeOH (up to 10% MeOH) gradient afforded fr.1 and fr.2, respectively, which were purified by prep. TLC (Merck, Art. 5553, $CHCl_3$–MeOH, 8:2 or 7:3, one or two developments) and the mixtures of isomeric compounds were further separated by prep. PC (Whatman 3 MM, H_2O). A portion (315 mg) of

Table 1
^{1}H NMR spectral data of compound **6** (500.13 MHz, CD_3OD). Chemical shifts in δ relative to TMS, *J* values (Hz) in parentheses

Aglycone moiety	Glucosyl moiety	Rhamnosyl moiety	Apiosyl moiety	Caffeoyl moiety
H-2 6.66 *d* (2.1)	H-1 4.32 *d* (7.9)	H-1 5.35 *br s*	H-1 5.11 *d* (2.4)	H-2 7.02 *d* (2.1)
H-5 6.62 *d* (8.0)	H-2 3.39 *dd* (9.2, 7.9)	H-2 3.91 *m*[a]	H-2 3.95 *d* (2.4)	H-5 6.76 *d* (8.1)
H-6 6.52 *dd* (8.0, 2.1)	H-3 3.52 *m*[a]	H-3 3.64 *m*[a]	H-4 3.73 *d* (9.7)	H-6 6.88 *dd* (8.1, 2.1)
H-α 3.71 *m*	H-4 3.34 *m*[a]	H-4 3.34 *m*	H-4′ 3.96 *d* (9.7)	H-α 6.28 *d* (16.0)
H-α' 3.91 *m*[a]	H-5 3.64 *m*[a]	H-5 3.52 *m*[a]	$H_2$5 3.59 *s*	H-β 7.55 *d* (16.0)
H_2-β 2.77 *br t* (7.0)	H-6 4.35 *dd* (11.9, 5.6)	H_3-6 1.22 *d* (6.2)		
	H-6′ 4.48 *dd* (11.9, 2.0)			

[a] Signal pattern unclear due to overlapping.

the less polar fr.1 (1.578 g) gave **1** (8.6 mg), **2** (3.4 mg), **3** (16.4 mg) and **4** (2.6 mg). The more polar fr. 2 yielded **5** (158.7 mg), **6** (6.1 mg) and additional amounts of **3** (25.0 mg) and **4** (4.2 mg).

Isobetonyoside F (**6**). Solid. UV λ_{max} nm: 203, 220 (sh), 244 (sh), 291, 330. ESIMS m/z: 779 $[M+Na]^+$. ^1H NMR: see Table 1.

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Appendix A

Phenylethanoid glycosides from *Prostanthera melissifolia*

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Isobetonyside F and five other phenylethanoid glycosides were isolated from the aerial parts of *Prostanthera melissifolia* and were characterized by spectral methods.

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