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Phlomisethanoside, a phenylethanoid glycoside from *Phlomis grandiflora* var. *grandiflora*

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Abstract

From the aerial parts of *Phlomis grandiflora* var. *grandiflora*, a new phenylethanoid glycoside, phlomisethanoside, was isolated together with the known compounds, verbascoside, phlomoside A, 8-epiloganin, citroside and benzyl alcohol β-D-glucoside. The structure of the new compound was elucidated by spectral analyses. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Phlomis grandiflora var. *grandiflora*; Labiatae; phlomisethanoside; phenylethanoid glycoside

1. Introduction

The genus *Phlomis* (Labiatae) has been known to contain iridoid glucosides (Maksudov, Maksimov, Umarova, Saatov & Abdullaev, 1996; Al-Hazinn & Alkhathlan, 1996; Kasai et al., 1994), phenylethanoid glycosides (Jimenez & Riguera, 1994), flavonoid glycosides (Harborne, 1988), diterpene glycosyl esters (Katagiri et al., 1994) and nortriterpenes (Kumar, Bhan, Kalla & Dhar, 1992). During the course of our studies on the constituents of Turkish medicinal plants and related species, we examined the glycosidic constituents of *Phlomis grandiflora* H. S. Thompson var. *grandiflora* (Labiatae) and isolated a new phenylethanoid glycoside, phlomisethanoside (**1**), together with the known compounds, verbascoside (Birkofer, Kaiser & Thomas, 1968), phlomoside A (Maksudov, Maksimov, Umarova, Saatov & Abdullaev, 1995), 8-epiloganin (Bianco & Passacantilli, 1981), citroside (Umehara et al., 1988), benzyl alcohol β-D-glucoside (Bonner, Bourne & McNally, 1962), and hattushoside (**2**)(Saracoglu, Kojima, Narput & Ogihara, 1998). This paper deals with the isolation and structure elucidation of the new compound.

2. Results and discussion

Phlomisethanoside (**1**), $[\alpha]_D - 58.6^\circ$ (MeOH), was obtained as an amorphous powder. The molecular formula was determined as $C_{27}H_{34}O_{14}$ based on its negative ion high resolution FAB-mass spectrum. The ^1H– and ^{13}C NMR spectra (see Table 1 and Section 3) are essentially the same as those of hattushoside (**2**) except for the signals due to an acyl group attached to 5–O″ of the apiose moiety, indicating that **1** is (4-hydroxyphenyl) ethyl (5-*O*-acyl-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside. Actually, two anomeric proton signals appeared at 4.29 (*d*, $J = 7.3$ Hz) and 5.44 (*s*) which were assigned to H–1′ and H–1″. Further, the signals due to H_2–5″ and C–5″ resonated downfield at 4.33 and 4.42, and 68.6 compared to those (δ 3.59 and 3.62, and δ 66.0) of salvionoside A (Takeda et al., 1997). In the ^1H NMR spectrum, **1** showed signals at δ 3.84 (OMe), 6.83 (*d*, $J = 8.3$ Hz), 7.55 (*d*, $J = 2.0$

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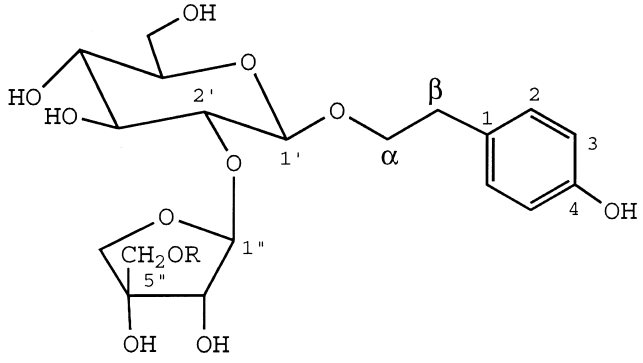
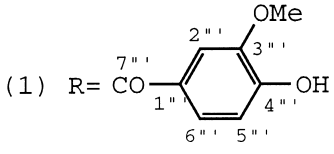
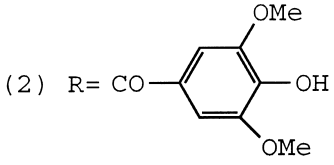
Table 1
^{13}C NMR data for phlomisethanoside (**1**) (CD_3OD)

C		C	
1	130.4	1″	110.1
2,6	130.9	2″	78.4
3,5	116.0	3″	79.3
4	156.7	4″	75.4
α	71.8	5″	68.6
β	36.4	1‴	122.3
1′	103.0	2‴	113.9
2′	77.8	3‴	153.1
3′	78.9	4‴	148.8
4′	71.7	5‴	116.0
5′	77.9	6‴	125.4
6′	62.7	7‴	168.0
		OMe	56.5

Hz) and 7.61 (*dd*, $J = 8.3$ and 2.0 Hz) instead of the signals of the syringoyl moiety in 2. A differential NOE was observed for the signal at δ 7.55 upon irradiation at δ 3.84, leading to the conclusion that the acyl residue in **1** is a vanilloyl group. Thus the structure of phlomisethanoside was elucidated as **1**.







3. Experimental

3.1. General

NMR: ^1H (400 MHz) and ^{13}C (100 MHz). TMS as int. standard; FABMS: PEG 400 as a matrix; CC: silica gel 60 (230–400 mesh), Merck; prep. HPLC (Cosmosil 10 C_{18}, 20×250 mm, Nakarai Tesqui, Kyoto, Japan; solvent: MeOH–H_2O, flow rate, 6.5 ml min^{-1}; detection; 210 or 230 nm.

3.2. Plant material

The plant material was collected in central Anatolia in June, 1995 and identified by the authors (G. H. and E. S.) as *Phlomis grandiflora* var. *grandiflora*. Voucher specimens (95C014) were deposited in the herbaria of the Graduate School of Pharmaceutical Sciences, Kyoto University and the Faculty of Pharmacy, Gazi University.

3.3. Isolation

Dried aerial parts (6.2 kg) of *P. grandiflora* var. *grandiflora* were extracted with MeOH (54 $l \times 2$) at room temperature for two weeks. The combined MeOH extracts were concentrated *in vacuo* and the residue was dissolved in 90% MeOH (2.2 l). The solution was washed with *n*-hexane (2 $l \times 3$) and the aqueous MeOH layer was concentrated *in vacuo*. The residue was suspended in H_2O (1.5 l), and the suspension was extracted with EtOAc (1 $l \times 3$) and *n*-BuOH (1 $l \times 3$), successively. The *n*-BuOH layer was concentrated *in vacuo* to give a residue (180.6 g) which was chromatographed over highly porous synthetic resin Diaion HP–20 (Mitsubishi Kasei, Tokyo) (10 cm diameter × 38 cm length) with a mixture of H_2O and MeOH with increasing MeOH content. [0 (9 l), 10 (5 l), 20 (9 l), 30 (9 l), 40 (9 l), 50 (9 l) and 70 (5 l)% aq. MeOH, and MeOH (9 l)]. Frs of 1 l being collected.

Phlomoside A (15.5 g) [frs 10–14], 8-epilogranin (36 mg), citroside (8.9 mg) benzyl alcohol β-D-glucoside (11.5 mg) [frs 25–32] and verbascoside (702 mg) [frs 35–43] were isolated by combinations of repeated chromatographies including silica gel chromatography and HPLC from the frs mentioned in parentheses.

Frs. 44–57 (67.3 g) were repeatedly separated by silica gel chromatography (solvent: $CHCl_3$–MeOH) and finally by HPLC [solvent: MeOH–H_2O 7: 13] give phlomisethanoside (**1**) (12.8 mg) and hattushoside (**2**) (22.7 mg).

Known compounds, verbascoside (Birkofer et al., 1968), phlomoside A (Maksudov et al., 1995), 8-epiloganin (Bianco & Passacantilli, 1981), citroside (Umehara et al., 1988), benzyl alcohol β-D-glucoside (Bonner et al., 1962) and hattushoside (**2**) (Saracoglu et al., 1998) were identified based on directed comparison or comparison of spectral data with reported data.

3.4. Phlomisethanoside (*1*)

$[\alpha]_D^{25} - 58.6^\circ$ (MeOH, *c*. 0.65). UV λ_{max} (MeOH) nm

(log ε): 222.5 (4.12), 265 (3.88), 286 (3.78), 327 (3.43); ^{1}H NMR (CD_3OD): δ 2.70 (2H, *t*, *J* = 7.6 Hz, H_2–β), 3.24 (1H, *m*, H–5′), 3.30 (overlapped, H–4′), 3.40 (1H, *dd*, *J* = 7.3 and 9.1 Hz, H–2′), 3.48 (1H, *t*, *J* = 9.1 Hz, H–3′), 3.52 (1H, m, Ha–α), 3.64 (1H, *dd*, *J* = 11.7, 5.4 Hz, Ha–6′), 3.78 (1H, *d*, *J* = 9.8 Hz, Ha–4″), 3.83 (overlapped, Hb–6′), 3.84 (3H, *s*, OMe), 3.94 (1H, m, Hb–α), 4.00 (1H, *br.s*, H–2″), 4.16 (1H, *d*, *J* = 9.8 Hz, Hb–4″), 4.29 (1H, *d*, *J* = 7.3 Hz, H–1′), 4.33 and 4.42 (each 1H, *d*, *J* = 11.2 Hz, H_2–5″), 5.44 (1H, *br.s*, H–1″), 6.62 and 6.90 (each 2H, *d*, *J* = 8.5 Hz, H_2–2, 6 and H_2–3, 5), 6.83 (1H, *d*, *J* = 8.3 Hz, H–6‴), 7.55 (1H, *d*, *J* = 2.0 Hz, H–2‴), 7.61 (1H, *dd*, *J* = 8.3, 2.0 Hz, H–5‴), ^{13}C NMR: see Table 1. HR–FABMS (negative): *m*/*z* 581.1864 ($C_{27}H_{33}O_{14}$ requires 581.1870).

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References

Al-Hazinn, H. M. G., & Alkhathlan, H. Z. (1996). *Journal of Chemical Society of Pakistan*, *18,* 336.

Bianco, A., & Passacantilli, A. (1981). *Phytochemistry*, *20,* 1873.

Birkofer, L., Kaiser, C., & Thomas, U. (1968). *Zeitschrift für Naturforschung*, *23B,* 1051.

Bonner, T. G., Bourne, E. J. and McNally, S., *Journal of the Chemical Society*, 1962, 761.

Harborne, J. B. (Ed.) (1988). *Flavonoid: advances in research since 1980*. London: Chapman and Hall.

Jimenez, C., & Riguera, R. (1994). *Natural Products Reports*, *11,* 591.

Kasai, R., Katagiri, M., Ohtani, K., Yamasaki, K., Yang, C. -R., & Tanaka, O. (1994). *Phytochemistry*, *36,* 967.

Katagiri, M., Ohtani, K., Kasai, R., Yamasaki, K., Yang, C. -R., & Tanaka, O. (1994). *Phytochemistry*, *35,* 439.

Kumar, R., Bhan, S., Kalla, A. K., & Dhar, K. L. (1992). *Phytochemistry*, *31,* 2797.

Maksudov, M. S., Maksimov, E. S., Umarova, R. U., Saatov, Z. and Abdullaev, N. D., *Khimiya Prirodnykh Soedinenii*, 1995, 243.

Maksudov, M. S., Maksimov, E. S., Umarova, R. U., Saatov, Z. and Abdullaev, N. D., *Khimiya Prirodnykh*. Soedinenii, 1996, 46.

Saracoglu, I., Kojima, K., Narput, U. S., & Ogihara, Y. (1998). *Chemical Pharmaceutical Bulletin*, *46,* 726.

Takeda, Y., Zhang, H., Matsumoto, T., Otsuka, H., Ooiso, Y., Honda, G., Tabata, M., Fujita, T., Sun, H., Sezik, E., & Yesilada, E. (1997). *Phytochemistry*, *44,* 117.

Umehara, K., Hattori, I., Miyase, T., Ueno, A., Hara, S., & Kageyama, C. (1988). *Chemical Pharmaceutical Bulletin*, *36,* 5004.