

A New Phenylethanoid Glycoside from *Phlomis pungens* WILLD. var. *pungens*

Iclal SARACOGLU,^a Keisuke KOJIMA,^b U. Sebnem HARPUT,^a and Yukio OGIHARA^{*,b}

^aDepartment of Pharmacognosy, Faculty of Pharmacy, Hacettepe University,^a TR-06100 Ankara, Turkey and ^bDepartment of Pharmacognosy, Faculty of Pharmaceutical Sciences, Nagoya City University,^b Tanabe-dori, Mizuho-ku, Nagoya 467, Japan. Received October 9, 1997; accepted November 17, 1997

Three known phenylethanoid glycosides, forsythoside B, alyssonoside and leucosceptoside B, and a known iridoid glycoside, lamiide, were isolated from the methanol extract of the aerial parts of *Phlomis pungens* WILLD. var. *pungens* (Labiatae) along with one new phenylethanoid glycoside, (4-hydroxyphenyl)ethyl (5-*O*-syringyl- β -D-apiofuranosyl)-(1 \rightarrow 2)- β -D-glucopyranoside, termed hattushoside.

Key words *Phlomis pungens*; Labiatae; phenylethanoid glycoside; hattushoside

To date during our systematic phytochemical investigation of *Phlomis* (*P.*) species (Labiatae), which are used as tonics and stimulants in Anatolia,¹⁾ we have studied *P. linearis*^{2–5)} and *P. armeniaca*.⁶⁾ In a continuation of these studies, we have now investigated *P. pungens* WILLD. var. *pungens*. One known iridoid glycoside (**1**), three known phenylethanoid glycosides (**2–4**),⁷⁾ together and one new phenylethanoid glycoside (**5**) were isolated from the MeOH extract of the aerial parts of *P. pungens*.

Compound **1** was identified as lamiide,⁸⁾ **2** as forsythoside B,⁹⁾ **3** as alyssonoside,^{10,11)} **4** as leucosceptoside B,¹²⁾ respectively, by comparison with reported data.

Compound **5**, hattushoside, showed a $[M + Na]^+$ peak at m/z 635.1932 ($C_{28}H_{36}O_{15} + Na$). The ¹H- and ¹³C-NMR spectra of **5** revealed the presence of one syringyl group confirmed by the aromatic protons (δ_H 7.35, 2H, s), two methoxyl signals (δ_H 3.84, 6H, s; δ_C 57.0) and a carbonyl carbon (δ_C 168.0), and one *p*-hydroxyphenethyl alcohol confirmed by A_2B_2 -type aromatic groups (δ_H 6.62, 6.90, each 2H, d, $J = 8$ Hz) and two methylenes which were coupled with each other.

On acidic hydrolysis, **5** provided glucose and apiose (GC) as a sugar moiety. In the ¹H- and ¹³C-NMR spectra, two anomeric signals were observed at δ_H 4.28 (d, $J = 7$ Hz), δ_C 103.0 as a β -linked glucose and δ_H 5.44 (s), δ_C 110.1 as a β -linked apiose. All protons of the two sugar units were assigned unambiguously from the correlation spectroscopy (COSY) spectrum and a heteronuclear multiple quantum coherence (HMQC) experiment correlated all proton resonances with those of the corresponding carbons in each of the sugar units.

The information concerning the location of two sugar units, the syringyl group and the *p*-hydroxyphenethyl alcohol were obtained from the heteronuclear multiple bond correlation (HMBC) spectrum and correlation peaks were observed from the following pairs: H-1'/C-8, H-1''/C-2', H-5''/C-7'''. Therefore, the structure of **5** was identified as (4-hydroxyphenyl)ethyl (5-*O*-syringyl- β -D-apiofuranosyl)-(1 \rightarrow 2)- β -D-glucopyranoside.

Experimental

General Procedures NMR spectra were recorded on a JEOL JNM-A500 spectrometer in methanol-*d*₄ with tetramethylsilane (TMS) as internal standard. FAB-MS were recorded on a JEOL JMS-DX300

spectrometer. UV spectra were recorded on a Shimadzu UV-160A spectrometer. IR spectra were recorded on a Perkin Elmer FTIR 1720X spectrometer. GC was run on a Shimadzu GC-6A gas chromatograph (column, 3 mm \times 2 m; N₂ at 70 ml min⁻¹; 3% SE-30; temperature 170 $^\circ$).

Plant Material The aerial parts of *Phlomis pungens* WILLD. var. *pungens* were collected from the surroundings of Corum, Bogazkale, Hattushas in inner Anatolia. Voucher specimens have been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 91020).

Extraction and Isolation The air-dried aerial parts of the plant (1 kg) were extracted twice with MeOH at 40 $^\circ$ C (each 3.5 l). After evaporation of the MeOH, H₂O (0.5 l) was added and the insoluble material removed by filtration. The filtrate was extracted with petroleum ether and the

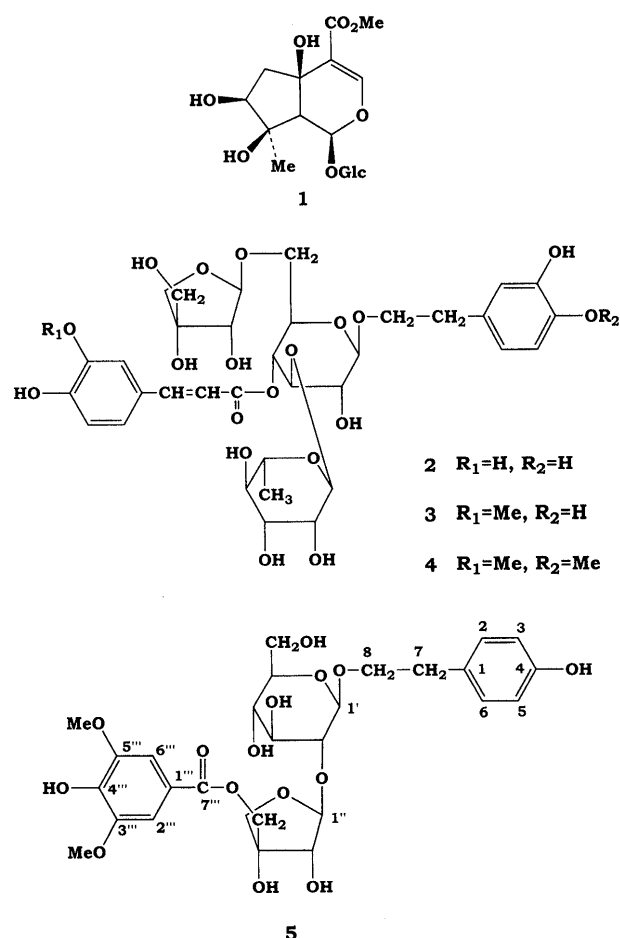


Chart 1

* To whom correspondence should be addressed.

Table 1. ^1H - and ^{13}C -NMR Spectral Data for Compound **5** in Methanol- d_4

	C	H
Aglycone		
1	130.4	
2	130.9	6.90 (d, $J=8$ Hz)
3	116.1	6.62 (d, $J=8$ Hz)
4	156.8	
5	116.1	6.62 (d, $J=8$ Hz)
6	130.9	6.90 (d, $J=8$ Hz)
7	36.5	2.70 (t, $J=8$ Hz)
8	71.8	3.51 (m) 3.93 (m)
Glucose		
1'	103.0	4.28 (d, $J=7$ Hz)
2'	77.9	3.40 (dd, $J=7, 9$ Hz)
3'	79.0	3.48 (t, $J=9$ Hz)
4'	71.8	3.27 ^{a)}
5'	77.9	3.22 (m)
6'	62.7	3.64 (dd, $J=6, 12$ Hz) 3.84 ^{a)}
Apiose		
1''	110.1	5.44 (s)
2''	78.5	4.00 (s)
3''	79.3	
4''	75.5	3.80 (d, $J=9$ Hz) 4.17 (d, $J=9$ Hz)
5''	68.9	4.35 (d, $J=11$ Hz) 4.46 (d, $J=11$ Hz)
Syringyl		
1'''	121.2	
2'''	108.6	7.35 (s)
3'''	149.0	
4'''	142.6	
5'''	149.0	
6'''	108.6	7.35 (s)
7'''	168.0	
OMe	57.0	3.84 (s)

a) Overlapped with other signals.

petroleum ether phase rejected. The aqueous phase was concentrated and chromatographed over polyamide, eluting with water, followed by increasing concentrations of MeOH to yield three main fractions: frs. A—C (fr. A, H_2O ; fr. B, 50% MeOH; fr. C, MeOH).

Fraction A was chromatographed over silica gel by stepwise elution

with CHCl_3 –MeOH– H_2O (80:20:2→60:40:4) and then rechromatographed over Sephadex LH-20 with MeOH to give **1** (16 mg). Fraction B was applied to medium pressure liquid chromatography (MPLC) by using a reversed-phase column. Eluting with increasing amounts of MeOH (20→60%) yielded frs. B_1 – B_3 .

Fraction B_1 was rechromatographed over Sephadex LH-20 with MeOH to give **2** (13 mg). Fraction B_2 was rechromatographed over silica gel by stepwise elution with CHCl_3 –MeOH (9:1→6:4) to yield **3** (16 mg) and **5** (42 mg). Fraction B_3 was rechromatographed over silica gel by stepwise elution with CHCl_3 –MeOH (9:1→6:4) to yield **4** (4 mg).

Hattushoside (5) $[\alpha]_{\text{D}}^{19} -39.3^\circ$ ($c=1.35$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 220, 278. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3394, 1699, 1616–1518. High resolution FAB-MS m/z : 635.1932, $\text{C}_{28}\text{H}_{36}\text{O}_{15} + \text{Na}$ requires 635.1952. ^1H - and ^{13}C -NMR: Table 1.

Acid Hydrolysis **5** was hydrolysed with 2N trifluoroacetic acid (TFA) at 120 °C for 5 h. The reaction mixture was concentrated to yield a residue, which was trimethylsilylated. The trimethylsilyl derivative was examined by GC which showed the presence of glucose (t_{R} 25.1, 40.3) and apiose (t_{R} 7.5, 10.2) in the ratio 1:1.

References

- 1) Baytop T., "Therapy with Medicinal Plants in Turkey (Past and Present)", Publications of Istanbul University, Istanbul, 216–217 (1984).
- 2) Calis I., Basaran A. A., Saracoglu I., Sticher O., Ruedi P., *Phytochemistry*, **29**, 1253–1257 (1990).
- 3) Calis I., Basaran A. A., Saracoglu I., Sticher O., Ruedi P., *Phytochemistry*, **30**, 3073–3075 (1991).
- 4) Basaran A. A., Saracoglu I., Calis I., *Hacettepe University, Journal of The Faculty of Pharmacy*, **11**, 81–83 (1991).
- 5) Basaran A. A., Saracoglu I., Ruedi P., Sticher O., Calis I., Proceedings Book, 8th Symposium on the Plant Originated Pharmaceutical Raw Materials, Istanbul, May 1989, (eds. by Cubukcu B., Sariyar G., Mat A.), 1993, pp. 125–130.
- 6) Saracoglu I., Inoue M., Calis I., Ogihara Y., *Biol. Pharm. Bull.*, **18**, 1396–1400 (1995).
- 7) Saracoglu I., Harput U. S., Kojima K., Ogihara Y., *Hacettepe University, Journal of The Faculty of Pharmacy*, **17**, 63–72 (1997).
- 8) Bianco A., Bonini C., Guiso M., Iavarone C., Tragola C., *Gazz. Chim. Ital.*, **107**, 67–69 (1977).
- 9) Endo K., Takahashi K., Abe T., Hikino H., *Heterocycles*, **19**, 261–264 (1982).
- 10) Calis I., Hosny M., Khalifa T., Ruedi P., *Phytochemistry*, **31**, 3624–3626 (1992).
- 11) Warashina T., Miyase T., Ueno A., *Phytochemistry*, **31**, 961–965 (1992).
- 12) Miyase T., Koizumi A., Ueno A., Noro T., Kuroyanagi M., Fukushima S., Akiyama Y., Takemoto T., *Chem. Pharm. Bull.*, **30**, 2732–2737 (1982).