

Dorycnioside, a New Phenylbutanone Glucoside from *Dorycnium pentaphyllum* subsp. *herbaceum*

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A new phenylbutanone glucoside, dorycnioside, was isolated from the methanol extract of the aerial parts of *Dorycnium pentaphyllum* subsp. *herbaceum* Vill. (Rouy) and identified as 4-(4'-*O*- β -D-glucopyranosyl-3',5'-dimethoxyphenyl)-2-butanone (**1**). In addition, two known phenylbutanone glucosides, five flavonoids, one cyanogenic glucoside, one cyclitol and one hydroquinone glucoside were also isolated and identified. The major constituent of the methanol extract was found to be myricitrin. The structure of **1** was elucidated on the basis of its spectroscopic data. It is the first time that derivatives of phenylbutanone are isolated from the Leguminosae family.

Key words: Dorycnioside, Phenylbutanone, *Dorycnium pentaphyllum*

Introduction

The genus *Dorycnium* belonging to the family Leguminosae is comprised by four species: *Dorycnium hirsutum*, *D. rectum*, *D. graecum* and *D. pentaphyllum*. *D. pentaphyllum* is separated in four subspecies, *pentaphyllum*, *germanicum*, *gracile*, and *herbaceum*. Those four subspecies are treated by many botanists as species, but subspecies *pentaphyllum*, *germanicum* and *gracile* are often difficult to be separated on purely morphological grounds. In contrast, *D. pentaphyllum* subsp. *herbaceum* is well defined and generally considered as a different kind, named by many authors as *D. herbaceum* (Phytochemical Dictionary, 1994). It is a perennial herb or small shrub 10–80 cm high, which can be found in Central and Southeastern Europe.

Previous phytochemical studies on the genus *Dorycnium* have been performed for *D. rectum* (Moreno *et al.*, 2002), *D. pentaphyllum* subsp. *pentaphyllum* (Jay *et al.*, 1978) and *D. hirsutum* (Bell *et al.*, 1978). These studies have shown the presence of several flavonoids and their glycosides.

In continuation of the research on Greek Leguminosae plants (Halabalaki *et al.*, 2000) and based on the generally known broad pharmacological activities of the Leguminosae plants we investigated the chemical constituents of the aerial

parts of *D. pentaphyllum* subsp. *herbaceum* from Greece. This investigation led to the isolation and structure elucidation of dorycnioside (**1**), a new phenylbutanone glucoside. In addition, two known phenylbutanone glucosides, five flavonoids, one cyanogenic glucoside, one cyclitol and one hydroquinone glucoside were identified.

Results and Discussion

Compound **1** was obtained as an amorphous colorless solid. Its molecular formula was determined by HRFABMS as C₁₈H₂₆O₉. The ¹H NMR spectrum of **1** exhibited one signal at 6.54 ppm corresponding to two aromatic protons of a tetra-substituted benzene nucleus, one signal at 3.82 ppm corresponding to six protons of two methoxy groups, one broad singlet at 2.80 ppm corresponding to four protons of two deshielded aliphatic methylene and one deshielded methyl group at 2.13 ppm. Additionally, one anomeric proton with *J* = 7.1 Hz and several overlapped signals corresponding to a typical sugar moiety were observed. The ¹³C NMR and DEPT spectra confirmed the above observations and showed six carbon signals in the region of the sugar carbon atoms, revealing the presence of a hexose, four types of carbon signals in the aromatic region (two of them oxygenated), one signal at 210.9 ppm corresponding to a ketone and also four signals in the aliphatic region

corresponding to two methylene, one methoxy and one methyl group. In the HMBC spectrum (Fig. 1), the anomeric proton was correlated with an oxygenated aromatic carbon which was also correlated with the aromatic protons, while the second type of oxygenated aromatic carbons was correlated only with the protons of the methoxy groups. The aromatic protons were also correlated with the methylene at 30.7 ppm, while the protons of the terminal methyl group showed a 2J correlation with the ketone and a 3J correlation with the second methylene at 45.5 ppm. These data revealed the presence of a symmetrically tetra-substituted aromatic ring with a four-carbon side chain directly attached to a position *ortho* towards the two symmetric aromatic protons. The side chain was a 2-butanone substituted in position 4 because there was only one terminal methyl group which was observed as a clear singlet in the 1H NMR

All these data made obvious that **1** was a glycoside of 4-(4'-hydroxy-3',5'-dimethoxyphenyl)-2-butanone. The highly overlapped 1H NMR spectrum did not permit the direct discrimination of the sugar moiety and thus **1** was further studied in its peracetylated form.

The FABMS spectrum of the peracetylated compound showed a molecular weight increased by four acetoxy groups, relatively to **1**, confirming the presence of a hexose. Its 1H NMR spectrum permitted the clear observation of all the sugar

protons and of their splitting pattern: one anomeric proton (doublet with $J = 7.8$ Hz) and three deshielded protons (due to acetylation), which were observed as one doublet of doublets with $J = 9.5$ and 7.8 Hz and two triplets with $J = 9.5$ Hz. This splitting pattern was compatible only with H-1,2,3,4 of β -glucopyranose.

All these observations made clear that the phenylbutanone was linked with β -glucopyranose. The absolute stereochemistry of the sugar was elucidated after enzymatic hydrolysis using β -D-glucosidase, which afforded D-glucose.

Consequently **1** is 4-(4'-*O*- β -D-glucopyranosyl-3',5'-dimethoxyphenyl)-2-butanone, for which we propose the trivial name dorycnioside.

In addition to dorycnioside, two other known phenylbutanone derivatives were identified: 4-(4'-*O*- β -D-glucopyranosylphenyl)-2-butanone (**2**) (Fan *et al.*, 2000) and 4-(4'-*O*- β -D-glucopyranosyl-3'-methoxyphenyl)-2-butanone (**3**) (Fan *et al.*, 2001).

Apart from phenylbutanone derivatives the plant was found to be very rich in flavonoid glycosides: myricitrin (Mahmoud *et al.*, 2001), quercitrin (Kuroyanagi and Fukushima, 1982; Lawrence *et al.*, 1997), kaempferol 3-*O*- β -glucopyranoside (Strack *et al.*, 1989; Slimestad *et al.*, 1995), kaempferol 3-*O*-(6''-acetyl)- β -glucopyranoside (Slimestad *et al.*, 1995), (+)-dihydromyricetin (Chien-Chang *et al.*, 1993). Finally, (-)-catechin (Chien-Chang *et al.*, 1993), β -sitosterol (Ness *et al.*, 1992), the cyanogenic glucoside lotaustralin (Smith *et al.*, 1980; Jaroszewski *et al.*, 1988), gallic acid methyl ester (Haddoch *et al.*, 1982), the hydroquinone glucoside tachioside (Inoshiri *et al.*, 1987; Zhong *et al.*, 1999), and the typical cyclitol of Leguminosae D-pinitol (Naidoo *et al.*, 1992) were identified.

It should be noted that this is the first report of phenylbutanone derivatives in the Leguminosae family. From a chemotaxonomic point of view, it is noteworthy that the closely related but morphologically clearly distinct *D. pentaphyllum* subsp. *pentaphyllum* has been reported (Jay *et al.*, 1978) to contain flavonoids of different structure, with only two common constituents. Interestingly, in both subspecies the major constituent has been found to be myricitrin.

Experimental

General experimental procedures

Optical rotations were measured with a Perkin-Elmer 341 polarimeter. UV spectra were recorded

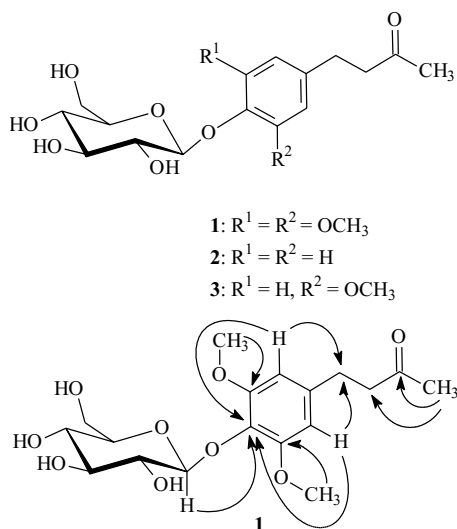


Fig. 1. Structures of dorycnioside (**1**) and the other phenylbutanone glucosides (**2**, **3**) and characteristic HMBC (\rightarrow) correlations for **1**.

on a Shimadzu-160A spectrophotometer. IR spectra were taken on a Perkin-Elmer Paragon 500 instrument. ^1H NMR spectra were measured on a Bruker DRX-400 (400 MHz) spectrometer and ^{13}C NMR spectra on a Bruker AC-200 (50 MHz). Chemical shifts are given in δ values with TMS as an internal standard. Coupling constants (J) are given in Hz. The signals in the ^1H and ^{13}C NMR spectra were assigned unambiguously using 2D NMR techniques: COSY, TOCSY, NOESY, HMQC, and HMBC. These 2D experiments were performed using standard Bruker microprograms. FABMS were obtained using a ZAB HF instrument, in glycerol matrix with NaCl as additive for positive ion mode. HRFABMS were obtained on a AEI MS-902 mass spectrometer. Column chromatography was conducted using silica flash gel 60 Merck (40–63 μm), with an overpressure of 300 mbar. Medium-pressure liquid chromatography (MPLC) was performed with a Büchi model 688 apparatus on columns containing silica gel 60 Merck (20–40 μm) or RP-18 silica gel 60 Merck (20–40 μm).

Plant material

The aerial parts of *D. pentaphyllum* subsp. *herbaceum* were collected from the north side of mount Pindos near Kalpaki village in Epirus region in June 2000. A voucher specimen (No In 026) is deposited in the herbarium of the Division of Pharmacognosy, University of Athens.

Extraction and isolation

The air dried aerial parts (505 g) of *D. pentaphyllum* subsp. *herbaceum* were extracted with CH_2Cl_2 (3 \times 2 l) and then with CH_3OH (4 \times 2 l). The weight of the dried residue of the dichloromethane extract was 9.7 g and the weight of the methanol extract was 59.5 g. A part of methanol extract (35 g) was fractionated by column chromatography over silica gel 60 Merck (40–63 μm), using CH_2Cl_2 and CH_3OH gradient to afford totally 150 fractions. The fractions 6–24 (0.31 g) were rechromatographed by CC [silica gel 60 Merck (20–40 μm), $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ gradient] to afford β -sitosterol (22.3 mg). The fractions 66–89 (3.22 g) were rechromatographed by CC [silica gel 60 Merck (20–40 μm), cyclohexane/EtOAc and

EtOAc/ CH_3OH gradient] to afford (–)-catechin (30.8 mg), lotaustralin (20.3 mg), gallic acid methyl ester (8 mg), (+)-dihydromyricetin (6 mg), 4-(4'-*O*- β -D-glucopyranosylphenyl)-2-butanone (**2**) (11.4 mg), 4-(4'-*O*- β -D-glucopyranosyl-3'-methoxyphenyl)-2-butanone (**3**) (15 mg), dorycnioside (**1**) (7 mg), tachioside (3 mg) and kaempferol 3-*O*-(6''-acetyl)- β -D-glucopyranoside (7.1 mg). The fractions 98–110 (14.94 g) were rechromatographed by MPLC [RP-18 silica gel 60 Merck (20–40 μm), $\text{H}_2\text{O}/\text{CH}_3\text{OH}$ gradient] to afford myricitrin (1.2 g), quercitrin (6 mg), kaempferol 3-*O*- β -D-glucopyranoside (7 mg) and D-pinitol (1.1 g).

Dorycnioside (**1**)

$[\alpha]_{\text{D}}^{25}$: –40.0° (0.05 g/100 ml, MeOH). – UV (MeOH): λ_{max} = 256, 273 nm. – IR (CaF₂): ν_{max} = 1710, 1660, 1514 cm^{-1} . – ^1H NMR (400 MHz, CD_3OD , ppm): δ = 2.13 (3H, s, H-1), 2.8 (4H, s, H-3,4), 3.18 (1H, m, H-5''), 3.38–3.46 (3H, m, H-2'',3'',4''), 3.65 (1H, dd, J = 12.0, 5.4 Hz, H-6_b''), 3.78 (1H, dd, J = 12.0, 2.5 Hz, H-6_a''), 3.82 (6H, s, 3',5'-OMe), 4.79, (1H, d, J = 7.1 Hz, H-1''), 6.54 (2H, s, H-2',6'). – ^{13}C NMR (50 MHz, CD_3OD , ppm): δ = 30.7 (m, C-1,4), 45.5 (t, C-3), 56.7 (m, OMe-3',5'), 62.3 (t, C-6''), 71.0 (d, C-4''), 75.4 (d, C-2''), 77.4 (d, C-5''), 78.0 (d, C-3''), 105.2 (d, C-1''), 107.0 (m, C-2',6'), 110.1 (s, C-1'), 134.6 (s, C-4'), 154.6 (m, C-3',5'), 210.9 (s, C-2). – HRFABMS: found 387.1650 (calcd. for $\text{C}_{18}\text{H}_{26}\text{O}_9$, 386). – FABMS: m/z = 387 $[\text{M}+\text{H}]^+$.

Tetraacetyl dorycnioside

Treatment of **1** (2 mg) with Ac_2O (1 ml) and pyridine (1 ml) at room temperature overnight gave a tetraacetate (95%). – FABMS: m/z = 555 $[\text{M} + \text{H}]^+$.

Enzymatic hydrolysis of dorycnioside

To a solution of **1** (5 mg) in H_2O (3 ml) β -glucosidase (5 mg) was added and the reaction mixture was stirred for 48 h at 37 °C. The solvent was removed under reduced pressure and the residue was chromatographed by MPLC [RP-18 silica gel 60 Merck (20–40 μm), $\text{H}_2\text{O}/\text{MeOH}$ gradient] to afford D-glucose.

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