An Iridoid Glucoside from *Euphrasia pectinata*

Tayfun Ersöz,*,† M. Ziver Berkman,† Deniz Taşdemir,†,§ Chris M. Ireland,§ and İhsan Çalış†

Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara, Turkey, and Department of Medicinal Chemistry, College of Pharmacy, University of Utah, Salt Lake City, Utah 84112

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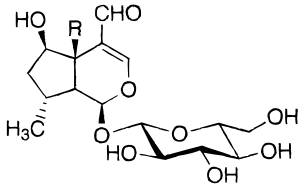
A new iridoid glucoside, 5β,6β-dihydroxyboschnaloside (**1**), was isolated from the aerial parts of *Euphrasia pectinata*. Five known iridoid glucosides, 6β-hydroxyboschnaloside (**2**), aucubin, euphroside, plantarenaloside, and geniposidic acid, and two known phenylethanoid glycosides, verbascoside (= acteoside) and leucosceptoside A, were also obtained and characterized. The structure of compound **1** was established by spectroscopic evidence.

Euphrasia species (Scrophulariaceae) are used in *folk medicine* in some European countries to treate blepharitis, conjunctivitis, eye fatigue, and sties, and also for coughs and hoarseness.[1] Iridoid glucosides,[2−10] a lignan glucoside,[11] flavonoids,[2,3,12] and tannins and phenolic acids[3] have been reported from several *Euphrasia* species. The presence of the iridoid glucosides aucubin and/or catalpol in the genus was suggested as having taxonomic importance.[13,14] The genus *Euphrasia* is represented by 10 species in Turkish flora,[15] with *Euphrasia pectinata* Ten. being widely distributed in Anatolia and its flowering herb used for wound healing in Anatolian folk medicine.[16] In a previous report, the isolation of two iridoids, boschnaloside and 7-hydroxyboschnaloside, was described from the title plant.[10] The current study describes the isolation and structure elucidation of a new iridoid glucoside, 5β,6β-dihydroxyboschnaloside (**1**), together with five known iridoid glucosides, 6β-hydroxyboschnaloside (**2**), aucubin, euphroside, plantarenaloside, and geniposidic acid, along with two known phenylethanoid glycosides, verbascoside (= acteoside) and leucosceptoside A**,** from the aerial parts of *E. pectinata*.



1 R: OH
2 R: H

A concentrated ethanolic extract of the aerial parts of *E. pectinata* was suspended in water and partitioned between $CHCl_3$ and *n*-BuOH. The *n*-BuOH extract was subjected to Si gel vacuum liquid chromatography (VLC) followed by Si gel column chromatography (CC) and C_{18} medium-pressure liquid chromatography (MPLC) to yield **1** and the seven other isolates.

Compound **1** was obtained as an amorphous powder, $[\alpha]^{21}_D$ −140° (*c* 0.27, MeOH). The FABMS exhibited a protonated molecular ion $[M + H]^+$ at *m/z* 377, while the positive ESIMS showed a pseudomolecular ion $[M + Na]^+$ at *m/z* 399 and the negative ESIMS exhibited the ions $[M - H]^-$ at *m/z* 375 and $[2M - H]^-$ at *m/z* 751. All these data were compatible with the molecular formula $C_{16}H_{24}O_{10}$, which was also confirmed by HRFABMS, and in good agreement with the observation of 16 resonances in the ^{13}C NMR spectrum. The FTIR spectrum showed absorption bands at 3370 (br OH), 1665 (C=C−O), and 1625 (C=C) cm^{-1}, and the UV spectrum exhibited a maximum at 242 nm, suggesting a conjugated enol−ether functional group. The ^1H NMR spectrum of **1** exhibited characteristic signals for an iridoid structure with an aldehyde (δ 9.24, s), an oxymethine [δ 4.29, dd (t), J = 3.4 Hz], and a secondary methyl group (δ 0.95 d, J = 6.8 Hz). In addition, an anomeric proton signal at δ 4.59 (d, J = 7.7 Hz) was consistent with the presence of a β-glucopyranose unit in **1**. An HMBC correlation between C-1/H-1′ indicated the attachment of the β-glucopyranose unit at the C-1 position of the iridoid aglycone. The complete interpretation of the remaining NMR data was based on the results of DQF-COSY, HMQC, and HMBC experiments. In the ^1H NMR spectrum, the ^1H proton singlet at δ 9.24 was assigned to an aldehyde function at C-11 (δ_C 193.3 d) on the basis of HMBC cross-peaks observed from C*H*O to C-3 (δ 166.2 d), C-4 (δ 124.7 s), and C-5 (see below). The chemical shift value and the multiplicity of H-3 (δ 7.46 s) were suggestive of an oxygen substitution at C-5. Thus, the quaternary carbon resonance at δ 73.8 showed long-range correlations with H-1, H-3, H-9, and H-11 and was attributed readily to C-5. A phase-sensitive gradient double-quantum COSY experiment allowed the establishment of the spin system sequence from H-1 through H-6. The H-1 signal, which was highly deshielded due to glycosidation (δ 5.92 s), spin coupled to H-9 (δ 2.60, br s), which in turn coupled to a multiplet at δ 2.62 assigned as H-8. The latter proton resonance showed additional homonuclear couplings with a diastereotropic methylene (H_2-7, δ 1.35 ddd and 1.79 ddd) and the secondary methyl function (δ 0.95, d), indicating its attachment at C-8. Further proof for this assignment was provided by HMBC cross-peaks observed between H-7/C-8, H-9/C-8, H_3-10/C-7, H_3-10/C-8, and H_3-10/C-9. The DQF-COSY and gHMBC data also allowed the assignment of the last proton resonance at δ 4.29 to a secondary hydroxyl-bearing sp^3 carbon, specifically C-6 (δ 75.9 d). The magnitude of the coupling constant value of H-6 ($J_{6,7}$) was found to be 3.4 Hz. The determination of the relative stereochemistry of the chiral centers of **1** was based mainly on a NOESY experiment. Dipolar couplings between H-1/H_3-10, H-6/H-7$_\alpha$, and H-7$_\alpha$/H_3-10 indicated these protons to be on the same side (α) of the iridoid skeleton, while NOE correlations between H-7$_\beta$ and H-9 suggested their opposite (β) orientation. The tertiary hydroxyl function at C-5 was assigned as β, by comparison of the ^{13}C NMR data

* To whom correspondence should be addressed. Tel.: +90-312-3051089. Fax: +90-312-3114777. E-mail: tersoz@domi.com.tr.
† Department of Pharmacognosy, Hacettepe University.
§ Department of Medicinal Chemistry, University of Utah.

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with those of iridoid analogues with a 5β,6β-dihydroxy-4-carbonyl partial structure.[17] Final analysis of the NMR data indicated that the structure of **1** was essentially identical to that of 6β-hydroxyboschnaloside (**2**)[18] except for the presence of a β-hydroxyl group at C-5. Therefore, the structure proposed for compound **1** is 5β,6β-dihydroxyboschnaloside.

The remaining isolates were also obtained as amorphous powders. Their structures were determined as 6β-hydroxyboschnaloside (**2**),[18] aucubin,[19] euphroside,[8] plantarenaloside,[20] geniposidic acid,[21] verbascoside (= acteoside),[22] and leucosceptoside A,[23] respectively, on the basis of their UV, IR, and 1D and 2D NMR spectral properties and MS data. The isolation of the iridoid glucosides, aucubin, euphroside, and geniposidic acid from several *Euphrasia* species[2−5,7,8] has been described previously. However, 6β-hydroxyboschnaloside (**2**) and plantarenaloside and the phenylethanoid glycosides verbascoside and leucosceptoside A are being reported from *Euphrasia* species for the first time.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a Perkin-Elmer 2000 FTIR spectrometer using KBr pellets. NMR measurements in CD_3OD were performed on a Varian Unity 500 spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. High- and low-resolution FABMS were performed on a Finnigan MAT95 spectrometer. ESIMS were recorded in the positive and negative ion modes on a Finnigan LCQDECA ion trap mass spectrometer. Silica gel 60 (0.063−0.200 mm, Merck) was used for VLC (column 5.2 × 20 cm) and open CC. MPLC separations were performed on a Labomatic glass column (1.8 × 35.2 cm, i.d.) packed with LiChroprep C_{18} (Merck), using a Lewa M5 peristaltic pump. TLC analyses were carried out on precoated silica gel 60F_{254} aluminum sheets (Merck). Compounds were detected by UV fluorescence absorption and/or spraying with vanillin−H_2SO_4 reagent followed by heating at 100 °C for 5 min.

Plant Material. *Euphrasia pectinata* Ten. (Scrophulariaceae) was collected between Bolu and Kartalkaya, Northern Anatolia, Turkey, in August 1996. A voucher specimen has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University (HUEF 96-006).

Extraction and Isolation. The air-dried and powdered aerial parts of *E. pectinata* (250 g) were extracted with EtOH (3 × 1 L) at 45 °C. The EtOH extracts were combined and evaporated to dryness in vacuo. The resultant crude extract (41 g) was dissolved in H_2O and partitioned between $CHCl_3$ and *n*-BuOH, sequentially. Half of the *n*-BuOH extract (14 g) was fractionated by Si gel VLC employing gradient $CHCl_3$−MeOH−H_2O mixtures (90:10:0.5 to 60:40:4) and MeOH as eluents. This yielded 24 fractions, which were combined into four main fractions, A (0.318 g), B (1.097 g), C (4.42 g), and D (1.39 g). A 2 g amount of fraction C was rechromatographed over Si gel. Elution with $CHCl_3$−MeOH−H_2O (80:20:2 and 70:30:3) yielded fractions C_1 (517 mg), C_2 (939 mg), and C_3 (110 mg). Fraction C_1 was subjected to C_{18} MPLC using H_2O−MeOH gradients (5% to 50% MeOH) to give **1** (37.7 mg), **2** (11.1 mg), verbascoside (44.9 mg), and leucosceptoside A (16.3 mg). An aliquot of fraction C_2 (400 mg) was subjected to C_{18} MPLC using H_2O−MeOH gradients (5% to 12.5% MeOH) to give aucubin (2.8 mg) and euphroside (6.0 mg). Fraction B was applied to a Si gel column employing $CHCl_3$−MeOH mixtures (9:1 and 8:2) to yield fractions B_1 (124 mg) and B_2 (542 mg). Fraction B_2 was then subjected to C_{18} MPLC using H_2O−MeOH gradients (10% to 25% MeOH) to afford three fractions, B_{1a} (33 mg), B_{1b} (40 mg), and B_{1c} (104 mg). Fraction B_{1c} was rechromatographed on a Si gel column eluting with $CHCl_3$−MeOH (8:2) to give plantarenaloside (6.0 mg). Fraction D was fractionated over Si gel using $CHCl_3$−MeOH−H_2O (70:30:3 and 60:40:4) as eluent to yield four fractions, D_1 (77 mg), D_2 (534 mg), D_3 (396 mg), and D_4 (189 mg). Fraction D_2 was rechromatographed on a Si gel column ($CHCl_3$−MeOH−H_2O, 80:20:2) to afford geniposidic acid (12.0 mg) together with a crude fraction of the same compound (120 mg). The latter fraction was purified over Si gel eluting with $CHCl_3$−MeOH (8:2) to give additional amounts of pure geniposidic acid (14.3 mg).

5β,6β-Dihydroxyboschnaloside (1): amorphous powder, $[\alpha]^{21}_D$ −140° (*c* 0.27, MeOH); UV (MeOH) λ_{max} 242 (4.10) nm; IR (KBr) ν_{max} 3370, 2924, 1665, 1625, 1258, 1139, 1077 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 5.92 (1H, s, H-1), 7.46 (1H, s, H-3), 4.29 [1H, dd (t), J = 3.4 Hz, H-6], 1.79 (1H, ddd, J = 12.8, 6.8, 2.6 Hz, H-7$_\beta$), 1.35 (1H, ddd, J = 12.8, 7.6, 4.6 Hz, H-7$_\alpha$), 2.62 (1H, m, H-8), 2.60 (1H, br.s, H-9), 0.95 (3H, d, J = 6.8 Hz, H-10), 9.24 (1H, s, H-11), 4.59 (1H, d, J = 7.7 Hz, H-1′), 3.17 (1H, dd, J = 7.7, 8.5 Hz, H-2′), 3.34 (1H, t, J = 8.5 Hz, H-3′), 3.24 (1H, t, J = 9.4 Hz, H-4′), 3.32 (1H, m, H-5′), 3.91 (1H, dd, J = 11.9, 1.7 Hz, H-6′$_b$), 3.65 (1H, dd, J = 11.9, 5.9 Hz, H-6′$_a$); ^{13}C NMR (125 MHz, CD_3OD) δ 97.2 (d, C-1), 166.2 (d, C-3), 124.7 (s, C-4), 73.8 (s, C-5), 75.9 (d, C-6), 40.4 (t, C-7), 31.3 (d, C-8), 49.6 (d, C-9), 16.7 (q, C-10), 193.3 (d, C-11), 100.1 (d, C-1′), 74.2 (d, C-2′), 77.3 (d, C-3′), 71.5 (d, C-4′), 78.3 (d, C-5′), 62.6 (t, C-6′); LRFABMS *m/z* 399 [M + Na]$^+$ (0.9), 377 [M + H]$^+$ (3); HRFABMS *m/z* 377.1429 (calcd for $C_{16}H_{25}O_{10}$ 377.1447); positive-ion ESIMS *m/z* 399 [M + Na]$^+$ (100); negative-ion ESIMS *m/z* 751 [2M − H]$^-$ (23), 375 [M − H]$^-$ (100).

6β-Hydroxyboschnaloside (2): IR (KBr) ν_{max} 3392, 2926, 1669, 1629, 1260, 1183, 1078 cm^{-1}; LRFABMS *m/z* 383 [M + Na]$^+$ (10), 361 [M + H]$^+$ (16); positive-ion ESIMS *m/z* 383 [M + Na]$^+$ (100); $[\alpha]^{21}_D$ UV, ^{1}H NMR, and ^{13}C NMR data were superimposable with those reported in the literature.[18]

The UV, ^{1}H NMR, and ^{13}C NMR data were superimposable with those reported in the literature for aucubin,[19] euphroside,[8] plantarenaloside,[20] geniposidic acid,[21] verbascoside (= acteoside),[22] and leucosceptoside A.[23]

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