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MEGASTIGMANE GLUCOSIDES FROM *STACHYS BYZANTINA*

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Key Word Index—*Stachys byzantina*; Labiatae; byzantionosides A and B; megastigmane glucoside.

Abstract—From the aerial parts of *Stachys byzantina*, two new megastigmane glucosides, byzantionosides A and B, were isolated, together with the known glycosides, icariside B_2, blumeol C glucoside, (6*R*, 9*R*)- and (6*R*, 9*S*)-3-oxo-α-ionol glucosides and verbascoside. The structures of the new compounds have been elucidated by spectroscopic means. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

To the best of our knowledge, no reports have appeared on the constituents of *Stachys byzantina* C. Koch. This paper deals with the isolation of two new megastigmane glucosides, byzantionosides A (**1**) and B (**3**), together with the known compounds, icariside B_2 [1], blumeol C glucoside [2], (6*R*, 9*R*)- and (6*R*, 9*S*)-3-oxo-α-ionol glucosides [3] and verbascoside [4] from the aerial parts of the title plant collected in northern Anatolia.

RESULTS AND DISCUSSION

Compound **1**, $[\alpha]_D$ +74.9° (methanol) was obtained as an amorphous powder, and the molecular formula was determined to be $C_{19}H_{30}O_7$ based on its high resolution negative ion FAB mass spectrum. The ^1H and ^{13}C NMR spectra showed the presence of a methyl ketone group conjugated with a *trans*-double bond [δ_H 2.26 (3H, *s*), δ_C 27.1 (*q*) and 201.0 (*s*); δ_H 6.12 (1H, *d*, *J* = 15.6 Hz) and 6.67 (1H, *dd*, *J* = 15.6 and 9.8 Hz), δ_C 134.4 and 149.7 (each *d*)], a methine group [δ_H 2.56 (1H, *d*, *J* = 9.8 Hz), δ_C 55.7 (*d*)] which is connected with the *trans*-double bond, a trisubstituted double bond [δ_H 5.74 (1H, *d*, *J* = 1.5 Hz), δ_C 125.9 (*d*) and 136.7 (*s*)] and a secondary oxymethine group [δ_H 4.36 (1H, *m*), δ_C 73.9 (*d*)] in addition to the signals due to a methylene group, a methyl group on the latter double bond, two tertiary methyl groups and a β-glucopyranosyl moiety (Table 1) in the structure of **1**. These spectral data, coupled with the UV data, suggested that **1** has an ionol glucoside structure having the same side chain structure as that of alangionoside L [5]. The orientation of the glucosyloxy group was inferred as pseudo-α-equatorial judged

Table 1. ^{13}C NMR data* for byzantionosides A (**1**) and B (**3**) and blumeol C glucoside (**4**)

C	1	3	4
1	34.6	37.2	37.3
2	40.7	48.0	48.0
3	73.9	202.3	202.2
4	125.9	125.3	125.4
5	136.7	170.0	169.7
6	55.7	52.3	52.4
7	149.7	26.7	26.6
8	134.4	37.7	37.3
9	201.0	75.0	77.5
10	27.1	19.8	21.9
11	29.6	27.5	27.5
12	25.4	29.0	29.0
13	22.9	25.0	25.0
1′	103.1	102.0	103.9
2′	75.1	75.4	75.2
3′	77.9	78.0	78.1
4′	71.6	71.7	71.5
5′	78.2	77.7	77.7
6′	62.8	62.8	62.7

*Measured at 100 MHz for CD_3OD solution.

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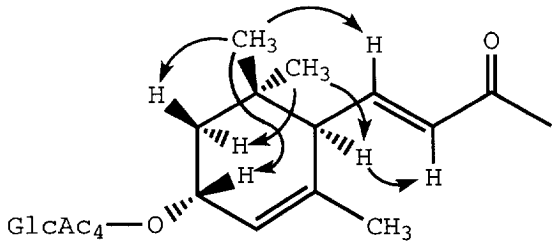


Fig. 1. NOE correlations for byzantionoside A tetraacetate (2) detected by differential NOE experiments.

from the coupling pattern of H_2-2 [δ_H 1.57 (1H, *dd*, J = 13.7 and 5.9 Hz) and 1.83 (1H, *dd*, J = 13.7 and 6.4 Hz)] [6]. The relative stereochemistry in the aglycone portion was elucidated on the basis of the results of differential NOE experiments for the tetraacetate (**2**) as shown in Fig. 1. The absolute stereochemistry at C-6 was determined to be 6*R* by comparing the circular dichroic (CD) spectrum ($\Delta\varepsilon_{239}$ +8.04) to that reported for (3*R*, 6*R*)-3-methoxy-α-ionone [6].

Compound **3**, $[\alpha]_D$ +48.0° (methanol), was also obtained as an amorphous powder, and the elemental composition was determined as $C_{19}H_{32}O_7$ based on its high resolution negative ion FAB mass spectrum, which is the same as that of blumeol C glucoside (**4**). Usual acetylation gave the tetraacetate (**5**). The ^1H and ^{13}C NMR (Table 1) spectra of **3** were very similar to those of **4** except for the resonance due to C-9 (δ_C 75.0). The corresponding signals were observed at δ_C 77.5 and 76.8 in **4** and dihydroalangionoside A [7], respectively, which have the *R*-configuration at C-9, whereas the C-9 signal was observed at δ_C 74.6 in salvionoside C [8], which has the *S*-configuration at C-9. These observations indicated that **3** has the same structure as that of **4** except for the stereochemistry at C-9, i.e. 9*S*. The stereochemistry at C-6 was confirmed as 6*R* by the fact that the CD spectrum showed a positive extreme at 237 nm [$\Delta\varepsilon$ +3.28], which was essentially the same as that [$\Delta\varepsilon_{236}$ +3.02] of **4**. Thus, the structure of byzantionoside B was elucidated as **3**, an epimer of **4** at C-9.

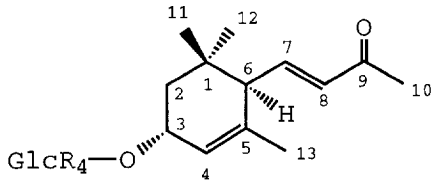
EXPERIMENTAL

General. ^1H (400 MHz) and ^{13}C (100 MHz) NMR, TMS as int. standard. FABMS: matrix PEG-400. CC: silica gel 60 (230–400 mesh, Merck). TLC and prep. TLC: precoated silica gel plates 60 F_{254} (0.25 and 0.5 mm). HPLC: column, ODS (Cosmosil 10C_{18}); solvent, H_2O–MeOH (5 ml min^{-1}); detection, 230 nm.

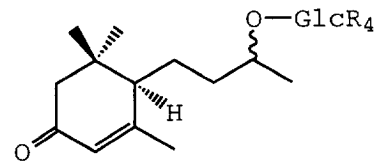
Plant materials. Plant material, *S. byzantina*, was collected in the suburbs of Tokat, Turkey, on 4 July 1991 and identified by the authors (G. H. and E. S.). Voucher specimens (91D 106) are deposited in the Herbaria of the Faculty of Pharmaceutical Sciences, Kyoto University, and Faculty of Pharmacy, Gazi University.

Isolation. Dried aerial parts (4.8 kg) of *S. byzantina* were extracted with MeOH (47 l) for 2 weeks at room temp. The plant was extracted again with the same vol. of MeOH for 3 weeks. The combined MeOH extracts were evapd *in vacuo*. The residue was dissolved in 90% MeOH (1.1 l), and the soln was washed with *n*-hexane (1 l × 3). The aq. MeOH soln was concd *in vacuo*. The residue was suspended in H_2O (1 l) and partitioned with EtOAc (1 l × 3). The aq. layer was extracted with *n*-BuOH (1 l × 3). After being washed with H_2O, the *n*-BuOH extract was evapd *in vacuo* to give a residue (32.4 g). The residue was subjected to CC over silica gel (1.2 kg) with $CHCl_3$–MeOH as eluent with increasing MeOH content. 2 l each of $CHCl_3$, $CHCl_3$–MeOH (19:1), $CHCl_3$–MeOH (93:7), $CHCl_3$–MeOH (9:1), $CHCl_3$–MeOH (17:3), $CHCl_3$–MeOH (4:1) and $CHCl_3$–MeOH (7:3) were passed successively through the column and 500-ml frs were collected. Fr. 9 gave a residue (1.027 g) on evapn which was repeatedly sepd by HPLC MeOH–H_2O, 9:11, then 7:13) to give icariside B_2 [1] (15.4 mg), **3** (35.8 mg) and **4** [2] (25.8 mg). Fr. 10 gave a residue (860 mg) on evapn which was sepd by HPLC (MeOH–H_2O, 7:13, then 1:3) to give **1** (9.9 mg), (6*R*, 9*S*)-3-oxo-α-ionol glucoside (4.6 mg) [3] and (6*R*, 9*R*)-3-oxo-α-ionol glucoside (10.5 mg) [3]. Fr. 16 gave a residue (1.85 g) which was repeatedly sepd by HPLC (MeOH–H_2O, 9:11, then 7:13) to give verbascoside (31.2 mg) [4]. The known compounds were identified by comparisons of spectral data with those reported.

Byzantionoside A (**1**). Amorphous powder, $[\alpha]_D^{24}$ +74.9° (MeOH, *c* 0.44,). UV λ_{max}^{MeOH} nm (ε): 226.5 (8830). IR ν_{max}^{KBr} cm^{-1}: 3392, 1670, 1076. ^1H NMR (CD_3OD): δ 0.91 (3H. *s*, H_3-11), 1.04 (3H, *s*, H_3-12), 1.57 (1H, *dd*, J = 13.7, 5.9 Hz, H_α-2), 1.63 (3H, *br s*, H_3-13), 1.83 (1H, *dd*, J = 13.7, 6.4 Hz, H_β-2), 2.26 (3H, *s*, H_3-10), 2.56 (1H, *d*, J = 9.8 Hz, H-6), 3.15 (1H, *dd*, J = 9.5, 8.5 Hz, H-2′), 3.67 (1H, *m*, H_1-6′), 3.87 (1H, *br d*, J = 11.7 Hz, H_1-6′), 4.36 (1H, *m*, H-



1 R=H
2 R=Ac



3 9*S* ; R=H
4 9*R* ; R=H
5 9*S* ; R=Ac

3), 4.39 (1H, *d*, $J = 7.8$ Hz, H-1′), 5.74 (1H, *d*, $J = 1.5$ Hz, H-4), 6.12 (1H, *d*, $J = 15.6$ Hz, H-8), 6.67 (1H, *dd*, $J = 15.6$, 9.8 Hz, H-7). CD $\Delta\varepsilon_{239} + 8.04$ (MeOH, 2.67×10^{-5} M). Negative ion FABMS *m/z* 369.1907 $[M-H]^-$ ($C_{19}H_{29}O_7$ requires 369.1913).

Byzantionoside B (**3**). Amorphous powder, $[\alpha]_D^{24} + 48.0°$ (MeOH, *c* 1.53,). UV λ_{max}^{MeOH} nm (ε): 240 (12500). IR ν_{max}^{KBr} cm^{-1}: 3368, 1648. ^1H NMR (CD_3OD): δ 1.01, 1.09 (each 3H, *s*, H_3-11, H_3-12), 1.18 (3H, *d*, $J = 6.4$ Hz, H_3-10), 1.50 (1H, *m*), 1.64 (2H, *m*), 1.96 (2H, *m*), 1.97 (1H, *d*, $J = 17.6$ Hz, H_1-2), 2.05 (3H, *d*, $J = 1.5$ Hz), 2.46 (1H, *d*, $J = 17.6$ Hz, H_1-2), 3.14 (1H, *dd*, $J = 8.8$, 7.8 Hz, H-2′), 3.64 (1H, *dd*, $J = 11.7$, 5.4 Hz, H_1-6′), 3.87 (2H, H-9, H_1-6′), 4.32 (1H, *d*, $J = 7.8$ Hz, H-1′), 5.80 (1H, *br s*, H-4). ^{13}C NMR: Table 1. CD $\Delta\varepsilon_{237} + 3.28$ (MeOH, 2.49×10^{-3} M). Negative ion FABMS *m/z*: 371.2068 $[M-H]^-$ ($C_{19}H_{31}O_7$ requires 371.2070).

Byzantionoside A tetraacetate (**2**). Compound **1** (2.2 mg) was acetylated with a mixt. of Ac_2O (0.5 ml) and pyridine (0.5 ml) for 18 h at room temp. Excess of MeOH was added to the mixt. and the soln was concd *in vacuo* to give a residue which was purified by prep. TLC (Et_2O) to give **2** (2.3 mg). ^1H NMR ($CDCl_3$): δ 0.88 (3H, *s*, H_3-11), 0.97 (3H, *s*, H_3-12), 1.44 (1H, *dd*, $J = 14.2$, 4.9 Hz, H_α-2), 1.62 (1H, *br s*, H_3-13), 1.71 (1H, *dd*, $J = 14.2$, 6.1 Hz, H_β-2), 2.00 (3H), 2.03 (6H), 2.10 (3H) (each *s*, $4 \times$ OAc), 2.25 (3H, *s*, H_3-10), 2.45 (1H, *d*, $J = 9.8$ Hz, H-6), 3.69 (1H, *m*, H-5′), 4.16 (1H, *dd*, $J = 12.2$, 2.7 Hz, H_1-6′), 4.22 (1H, *m*, H-3), 4.26 (1H, *dd*, $J = 12.2$, 5.4 Hz, H_1-6′), 4.62 (1H, *d*, $J = 8.1$ Hz, H-1′), 4.97 (1H, *dd*, $J = 9.8$, 8.1 Hz, H-2′), 5.08 (1H, *dd*, $J = 9.8$ and 9.8 Hz, H-4′), 5.21 (1H, *dd*, $J = 9.8$, 9.8 Hz, H-3′), 5.66 (1H, *m*, H-4), 6.08 (1H, *d*, $J = 15.9$ Hz, H-8), 6.52 (1H, *dd*, $J = 15.9$, 9.8 Hz, H-7). Negative ion FABMS *m/z*: 537.2298 $[M-H]^-$ ($C_{27}H_{37}O_{11}$ requires 537.2336).

Byzantionoside B tetraacetate (**5**). Compound **3** (2.5 mg) was acetylated and purified as before to give **5** (2.3 mg) as an amorphous powder. ^1H NMR ($CDCl_3$): δ 1.01, 1.04 (each 3H, *s*, H_3-11 and 12), 1.11 (3H, *d*, $J = 6.4$ Hz, H_3-10), 1.40 (1H, *m*), 1.55 (1H, *m*), 1.86 (1H, *m*), 1.98 (3H, *d*, $J = 1.0$ Hz, H_3-13), 2.01 (3H), 2.03 (6H), 2.07 (3H) (each *s*, $4 \times$ OAc), 2.36 (1H, *d*, $J = 17.1$ Hz, H_1-2), 3.36 (1H, *m*, H-5′), 3.69 (1H, *m*, H-9), 4.12 (1H, *dd*, $J = 12.2$, 2.4 Hz, H_1-6′), 4.23 (1H, *dd*, $J = 12.2$, 4.9 Hz, H_1-6′), 4.53 (1H, *d*, $J = 7.8$ Hz, H-1′), 4.94 (1H, *dd*, $J = 9.8$, 7.8 Hz, H-2′), 5.08 (1H, *dd*, $J = 9.8$, 9.8 Hz, H-4′), 5.20 (1H, *dd*, $J = 9.8$, 9.8 Hz, H-3′), 5.82 (1H, *br s*, H-4). Negative ion FABMS *m/z*: 539.2506 $[M-H]^-$ ($C_{27}H_{39}O_{11}$ requires 539.2493).

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