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MEGASTIGMANE GLYCOSIDES FROM *SALVIA NEMOROSA*

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Key Word Index—*Salvia nemorosa*; Labiatae; megastigmane glycosides; salvionosides A–C.

Abstract—From the aerial parts of *Salvia nemorosa*, three new megastigmane glycosides, salvionosides A–C, were isolated, along with the known compounds, (6*S*,9*R*)- and (6*S*,9*S*)-roseosides, (6*R*,9*R*)- and (6*R*,9*S*)-3-oxo-α-ionol glucosides and blumeol C glucoside. The structures of the new compounds were elucidated on the basis of spectral and chemical evidence. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Leaves of *Salvia nemorosa* have been used in Turkish medicine to stop bleeding by applying externally [1]. Despite this, only two diterpenic compounds, nemorone and deacetylnemorone, have been described as constituents [2, 3]. In continuation of our studies on the constituents of Turkish medicinal and related plants, we examined the glycosidic constituents of the title species and isolated three new megastigmane glycosides, salvionosides A (**1**), B (**2**) and C (**5**), together with the known compounds, (6*R*,9*R*)- and (6*R*,9*S*)-3-oxo-α-ionol glucosides (**3**, **4**) [4], blumeol C glucosides (**6**) [5], and (6*S*,9*R*)- and (6*S*,9*S*)-reseosides (**7**, **8**) [6]. The present paper deals with the isolation and structural elucidation of the new compounds.

RESULTS AND DISCUSSION

The new megastigmane glycosides, **1**, **2** and **5**, were isolated from the methanolic extract according to the procedures described in the Experimental.

Salvionoside A (**1**), $[\alpha]_D$ +67.7° (MeOH), was obtained as an amorphous powder and the molecular formula was determined as $C_{24}H_{38}O_{11}$ by negative-ion high resolution FAB-mass spectrometry. The ^1H and ^{13}C NMR spectra suggested that **1** was a megastigmane glycoside and the chemical shifts due to the aglycone portion were essentially the same as those of (6*R*,9*R*)-3-oxo-α-ionol glucoside (**3**) [4]. GC analysis of the methanolysis product of compound **1** revealed the presence of glucose and apiose moieties in the structure. The ^{13}C NMR spectrum (Table 1) showed the presence of a terminal β-apiofuranose moiety [7] and inspection of the remaining ^{13}C NMR signals of the sugar portion revealed that the structure of the sugar portion of compound **1** was β-D-apiofuranosyl (1″→2′)-β-D-glucopyranose, which was confirmed by analyses of the ^1H–^1H COSY spectrum of the hexaacetate. In addition, the CD spectrum of compound **1** showed an extreme value for $\Delta\varepsilon$ (nm) +16.5 (243), which supports the assignment of the absolute stereochemistry at C-6 as *R* [4]. Thus, the structure of salvionoside A was elucidated to be formula **1**.

Salvionoside B (**2**), $[\alpha]_D$ +49.2° (MeOH) was also isolated as an amorphous powder and the elemental composition, determined by negative high-resolution FAB-mass spectrometry, is the same as that of salvionoside A (**1**). The ^1H and ^{13}C NMR spectra of the aglycone portion were essentially the same as those of (6*R*,9*S*)-3-oxo-α-ionol glucoside (**4**) [4] and the ^{13}C NMR spectra also showed the presence of a β-D-apiofuranosyl (1″→2′)-β-D-glucopyranose moiety in the structure, which was further confirmed by the ^1H NMR spectrum of the hexaacetate. Based on the above-mentioned spectral data, together with the CD spectrum ($\Delta\varepsilon$ (nm) +19.6 (243)), the structure of salvionoside B was elucidated to be formula **2**.

Salvionoside C (**5**), $[\alpha]_D$ −23.3° (MeOH), was obtained as an amorphous powder and the elemental composition, determined by negative-ion high resolution FAB-mass spectrometry to be $C_{24}H_{40}O_{11}$, is two mu more than that of salvionosides A (**1**) and B (**2**). The ^1H and ^{13}C NMR spectra lacked the signals due to

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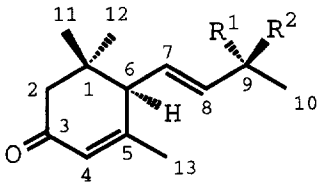
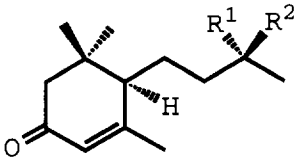
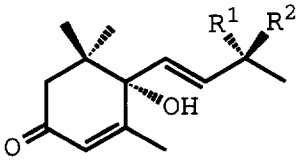


1: R^1=-O-Glc(2'-1")Api; R^2=H
2: R^1=H; R^2=-O-Glc(2'-1")Api
3: R^1=-O-Glc; R^2=H
4: R^1=H; R^2=-O-Glc



5: R^1=H; R^2=-O-Glc(2'-1")Api
6: R^1=-O-Glc; R^2=H



7: R^1=-O-Glc; R^2=H
8: R^1=H; R^2=-O-Glc

Glc: β-D-Glucopyranosyl
Api: β-D-Apiofuranosyl

a *trans* double bond which were observed in the spectra of compounds **1** and **2**, and instead two more methylene groups were observed at δ_C 26.7 and 37.8 (each *t*). Thus, salvionoside C (**5**) has the same planar structure as blumeol C glucoside (**6**) [5] in the aglycone moiety.

Table 1. ^{13}C NMR data of salvionosides A–C (**1**, **2** and **5**) (measured in CD_3OD)

C	1	2	5
1	37.1	37.2	37.3
2	48.2	48.3	48.1
3	202.0	202.1	202.4
4	126.0	126.3	125.4
5	165.8	165.6	170.1
6	56.7	56.8	52.3
7	128.7	131.5	26.7
8	138.1	136.8	37.8
9	76.5	74.8	74.6
10	20.9	22.2	19.6
11	27.7	27.5	27.6
12	28.0	28.0	29.1
13	23.8	24.0	25.0
1'	100.9	99.7	100.4
2'	79.2	78.8	78.8
3'	77.8	78.0	77.9
4'	71.4	71.7	71.9
5'	77.9	78.5	78.5
6'	62.6	62.8	62.9
1"	110.7	110.6	110.5
2"	78.5	78.1	77.7
3"	80.6	80.7	80.5
4"	75.3	75.3	75.4
5"	66.0	66.2	66.3

The structure of the sugar moiety proved to be identical to those of compounds **1** and **2** from inspection of the ^{13}C NMR spectra and analyses of the ^{1}H NMR spectrum of the hexaacetate. The stereochemistry at C-6 was determined to be *R* based on the fact that the CD spectrum showed a positive extreme at 239 nm ($\Delta\varepsilon$ + 1.6), which is qualitatively the same as that of blumeol C glucoside (**6**) [5]. The remaining chiral centre at C-9 was elucidated to be *S*-configuration by comparing the ^{13}C NMR chemical shift of C-9 (δ_C 74.6) with those of dihydroalangionoside A (δ_C 76.8) and I (δ_C 76.4), which are known to have the *R*-configuration [6, 8]. Thus, the structure of salvionoside C was elucidated to be formula **5**.

EXPERIMENTAL

General. NMR: ^{1}H (400 MHz) and ^{13}C (100 MHz), TMS as int. standard. HR-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: Cosmosil 10 C_{18}, detection 230 nm, solvent MeOH–H_2O, 5 ml min^{-1}.

Plant material. Collected in Merkaya, north eastern Anatolia on 14 July, 1991, and identified as *S. nemorosa* by G. H. and E. S. Voucher specimens (91 E 076 F) are deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Kyoto University, and the Faculty of Pharmacy, Gazi University.

Isolation. Dried aerial parts (1.73 kg) were extracted (×2) with MeOH (18 l) at room temp. for 2 weeks. The comb. MeOH extracts were concd *in vacuo*. The

residue was dissolved in 90% MeOH (1 l) and the soln washed with *n*-hexane (0.9 l × 3). The 90% MeOH layer was concd *in vacuo*. The resultant residue was suspended in H_2O (1 l) and the suspension extracted with EtOAc (0.9 l × 3). The aq. layer was extracted with *n*-BuOH (0.8 l × 3). The *n*-BuOH layer was concd *in vacuo* to give a residue (36 g), which was chromatographed over silica gel (1 kg). Elution was carried out with 2.5 l portions of $CHCl_3$, then $CHCl_3$–MeOH at 97:3, 19:1, 93:7, 9:1, 17:3, 4:1, 3:1 and 7:3, successively; 500 ml frs were collected. Fr. 29 gave a residue (1.79 g) which was repeatedly sepd by HPLC (MeOH–H_2O (9:11) and then MeOH–H_2O (7:13) and MeOH–H_2O (1:3)) to give six known megastigmane glucosides, **3** (31.1 mg), **4** (32.5 mg), **6** (10.6 mg), **7** (17.0 mg) and **8** (8.8 mg) [4–6]. Frs 32–34 gave a residue (2.72 g), a portion (1.90 g) of which was sepd by repeated HPLC [MeOH–H_2O (9:11) and then MeOH–H_2O (7:13)] to give the three new megastigmane glycosides, salvionosides A (**1**) (22.6 mg), B (**2**) (10.5 mg) and C (**5**) (10.0 mg) as amorphous powders. The known compounds (**3, 4, 6–8**) were identified by comparisons with reported physical data.

Salvionoside A (**1**). Amorphous powder. $[\alpha]_D^{24}$ +67.7° (MeOH, *c* 1.13). UV λ_{max}^{MeOH} nm (ε): 237.5 (13550). IR ν_{max}^{KBr} cm^{-1}: 3392, 1651, 1073. ^1H NMR (CD_3OD): δ 1.00 (3H, *s*, H_3-12), 1.03 (3H, *s*, H_3-11), 1.28 (3H, *d*, J = 6.4 Hz, H_3-10), 1.94 (3H, *d*, J = 1.0 Hz, H_3-13), 2.04 (1H, *ABd*, J = 16.6 Hz, H-2α), 2.43 (1H, *ABd*, J = 16.6 Hz, H-2β), 2.67 (1H, *d*, J = 9.3 Hz, H-6), 3.18 (1H, *m*, H-5′), 3.29 (1H, *dd*, J = 7.8 and 9.3 Hz, H-2′), 3.34 (1H, *br t*, J = 7.8 Hz, H-4′), 3.46 (1H, *t*, J = 8.8 Hz, H-3′), 3.59 (1H, *d*, J = 11.2 Hz, Ha-5″), 3.62 (1H, *d*, J = 11.2 Hz, Hb-5″), 3.64 (1H, *dd*, J = 12.2 and 5.9 Hz, Ha-6′), 3.71 (1H, *d*, J = 9.8 Hz, Ha-4″), 3.81 (1H, *dd*, J = 12.2 and 2.4 Hz, Hb-6′), 3.93 (1H, *d*, J = 1.5 Hz, H-2″), 4.04 (1H, *Abd*, J = 9.3 Hz, Hb-4″), 4.40 (1H, *m*, H-9), 4.41 (1H, *d*, J = 7.8 Hz, H-1′), 5.36 (1H, *d*, J = 1.5 Hz, H-1″), 5.63 (1H, *dd*, J = 15.6 and 9.3 Hz, H-7), 5.77 (1H, *dd*, J = 15.6 and 6.3 Hz, H-8), 5.87 (1H, *br s*, H-4). ^{13}C NMR: see Table 1. CD: $\Delta\varepsilon_{243}$ +16.5 (MeOH, 2.66 × 10^{-5} M). Negative ion FABMS *m/z*: 501.2356 [M-H]$^-$ ($C_{24}H_{37}O_{11}$ requires: 501.2336).

Salvionoside B (**2**). Amorphous powder. $[\alpha]_D^{24}$ +49.2° (MeOH, *c* 0.49). UV λ_{max}^{MeOH} nm (ε): 237 (11470). IR ν_{max}^{KBr} cm^{-1}: 3392, 1654, 1648, 1072. ^1H NMR (CD_3OD): δ 0.99 (3H, *s*, H_3-12), 1.03 (3H, *s*, H_3-11), 1.28 (3H, *d*, J = 6.4 Hz, H_3-10), 1.98 (3H, *s*, H_3-13), 2.05 (1H, *ABd*, J = 16.6 Hz, H-2α), 2.48 (1H, *ABd*, J = 16.6 Hz, H-2β), 2.70 (1H, *d*, J = 9.3 Hz, H-6), 3.11 (1H, *m*, H-5′), 3.23 (1H, *br t*, J = 8.8 Hz, H-2′), 3.35 (1H, *br t*, J = 7.8 Hz, H-3′), 3.54–3.67 (3H, Ha-6′, H_2-5″), 3.69 (1H, *ABd*, J = 9.8 Hz, Ha-4″), 3.84 (1H, *dd*, J = 12.2 and 2.4 Hz, Ha-6′), 3.87 (1H, *d*, J = 1.5 Hz, H-2″), 4.03 (1H, *ABd*, J = 9.8 Hz, Hb-4″), 4.33 (1H, *d*, J = 7.3 Hz, H-1′), 4.45 (1H, *m*, H-9), 5.32 (1H, *d*, J = 1.5 Hz, H-1″), 5.57 (1H, *dd*, J = 15.1 and 7.8 Hz, H-8), 5.72 (1H, *dd*, J = 15.1 and 9.3 Hz, H-7), 5.90 (1H, *s*, H-4). ^{13}C NMR see Table 1. CD: $\Delta\varepsilon_{243}$ +19.6 (MeOH, 2.09 × 10^{-5} M). Negative ion FABMS *m/z*: 501.2354 [M − H]$^-$ ($C_{24}H_{37}O_{11}$ requires: 501.2336).

Salvionoside C (**5**). Amorphous powder. $[\alpha]_D^{24}$ −23.3° (MeOH, *c* 0.40). UV λ_{max}^{MeOH} nm (ε): 238 (10670). IR ν_{max}^{KBr} cm^{-1}: 3386, 1648, 1073. ^1H NMR (CD_3OD): δ 1.01 (3H, *s*, H_3-11), 1.09 (3H, *s*, H_3-12), 1.17 (3H, *d*, J = 5.9 Hz, H_3-10), 1.53 (1H, *m*), 1.63 (2H, *m*), 1.97 (1H, *d*, J = 17.6 Hz, Ha-2), 1.99 (2H, *m*), 2.05 (3H, *s*, H_3-13), 2.46 (1H, *d*, J = 17.6 Hz, Hb-2), 3.23–3.68 (7H, 2′, 3′, 4′, 5′, 6′-Ha and H_2-5″), 3.69 (1H, *d*, J = 9.8 Hz, Ha-4″), 3.84 (1H, *br d*, J = 11.7 Hz, Hb-6′), 3.86 (1H, *m*, H-9), 3.90 (1H, *s*, H-2″), 4.04 (1H, *d*, J = 9.3 Hz, Hb-4″), 4.39 (1H, *d*, J = 7.8 Hz, H-1′), 5.73 (1H, *s*, H-1″), 5.80 (1H, *br s*, H-4). ^{13}C NMR: see Table 1. CD $\Delta\varepsilon_{239}$ + 1.6 (MeOH, 4.48 × 10^{-5} M. Negative ions FABMS *m/z*: 503.2503 [M − H]$^-$ ($C_{24}H_{39}O_{11}$ requires: 503.2492).

GC analysis of sugar portion of salvionoside A (**1**). A few mg of the glycoside was treated with 5% HCl in MeOH at 95° for 3 hr. The reaction mixt. was neutralized by addition of Ag_2CO_3 and filtered. The filtrate was concd and the residue silylated with a few drops of TMS–imidazole for 15 min at 60°. The reaction mixt. was partitioned between *n*-hexane (2 ml) and H_2O (2 ml) and the concd organic layer subjected to GC analysis; Shimadzu CPB-20, 0.22 mm × 25 m, layer thickness 0.25 μm, temp. 160°, carrier gas N_2 at 1.5 kg cm^{-2}. Standard sugars, apiose 2.71, 2.84, 2.96 and 3.15 min; glucose 8.18 and 8.87 min (standard apiose was from a previous expt [8]). Compound **1**: 2.73, 2.84, 2.98 and 3.15 min (apiose) and 8.12 and 8.81 min (glucose).

Acetylation of salvionosides. Salvionosides A (**1**) (4.5 mg), B (**2**) (2.1 mg) and C (**5**) (2.3 mg) were acetylated with a mixt. of Ac_2O (0.1 ml) and pyridine (0.1 ml) at 60° for 18 hr. After addition of excess MeOH, solvent was removed *in vacuo*. The residues were purified by prep. TLC (Et_2O) to give the hexaacetates (6.2, 3.5 and 2.8 mg, respectively) as amorphous powders.

Salvionoside A hexaacetate (3′,4′,6′,2″,3″,5″-O-*acetyl*). ^1H NMR ($CDCl_3$): δ 0.98 and 1.04 (each 3H, *s*, H_3-11 and 12), 1.29 (3H, *d*, J = 6.8 Hz, H_3-10), 1.89 (3H, *d*, J = 1.0 Hz, H_3-13), 2.00, 2.03, 2.06 (each 3H, *s*, 3 × OAc), 2.08 (9H, *s*, 3 × OAc), 2.10 and 2.35 (each 1H, *d*, J = 16.6 Hz, H_2-2), 2.53 (1H, *d*, J = 9.1 Hz, H-6), 3.59 (1H, *m*, H-5′), 3.66 (1H, dd, J = 9.8 and 7.7 Hz, H-2′), 4.06 (1H, *dd*, J = 12.2 and 2.7 Hz, Ha-6′), 4.08 (1H, *d*, J = 10.0 Hz, Ha-4″), 4.21 (1H, *dd*, J = 12.2 and 4.4 Hz, Hb-6′), 4.30 (1H, *m*, H-9), 4.31 (1H, *d*, J = 10.0 Hz, Hb-4″), 4.46 (1H, *d*, J = 7.7 Hz, H-1′), 4.58 and 4.63 (each 1H, *d*, J = 12.2 Hz, H_2-5″), 4.98 (1H, *dd*, J = 9.8 and 9.8 Hz, H-4′), 5.11 (1H, *s*, H-2″), 5.16 (1H, *s*, H-1″), 5.16 (1H, *dd*, J = 9.8 and 9.8 Hz, H-3′), 5.56 (1H, *dd*, J = 15.6 and 9.1 Hz, H-7), 5.70 (1H, *dd*, J = 15.6 and 6.6 Hz, H-8) and 5.90 (1H, *br s*, H-4). Negative ion FABMS *m/z*: 753.2949 [M − H]$^-$ ($C_{36}H_{49}O_{17}$ requires: 753.2969).

Salvionoside B hexaacetate (3′,4′,6′,2″,3″,5″-O-

acetyl). ^{1}H NMR ($CDCl_3$): δ 0.95 and 1.054 (each 3H, *s*, H_3-11 and 12), 1.33 (3H, *d*, $J = 6.4$ Hz, H_3-10), 1.93 (3H, *br s*, H_3-13), 2.01, 2.03, 2.04, 2.076, 2.084, 2.10 (each 3H, *s*, 6 × OAc), 2.12 and 2.33 (each 1H, *d*, $J = 16.6$ Hz, H_2-2), 2.59 (1H, *d*, $J = 8.3$ Hz, H-6), 3.51 (1H, *m*, H-5′), 3.71 (1H, *dd*, $J = 9.8$ and 7.8 Hz), 4.082 (1H, *d*, $J = 10.5$ Hz, Ha-4″), *ca* 4.086 (1H, Ha-6′, overlapped), 4.20 (1H, *d*, $J = 12.2$ and 4.9 Hz, Hb-6′), 4.32 (1H, *d*, $J = 10.5$ Hz, Hb-4″), 4.387 (1H, *m*, H-9), 4.389 (1H, *d*, $J = 7.8$ Hz, H-1′), 4.60 (2H, *s*, H_2-5″), 4.95 (1H, *dd*, $J = 9.8$ and 9.8 Hz, H-4′), 5.105 (1H, *dd*, $J = 9.8$ and 9.8 Hz, H-3′), 5.114 (1H, *s*, H-2″), 5.16 (1H, *s*, H-1″), 5.93 (1H, *br s*, H-4) and 5.53 (2H, H-7 and H-8). Negative ion FABMS *m/z*: 753.2968 $[M-H]^-$ ($C_{36}H_{49}O_{17}$ requires: 753.2969).

Salvionoside C hexaacetate (3′,4′,6″,2″,3″,5″-O-*acetyl*). ^{1}H NMR ($CDCl_3$): δ 1.02 and 1.06 (each 3H, *s*, H_3-11 and 12), 1.16 (3H, *d*, $J = 6.4$ Hz), 1.99 (3H, *d*, $J = 1.5$ Hz, H_3-13), 2.01, 2.02, 2.05, 2.07 (each 3H, *s*, 4 × OAc), 2.08 (6H, *s*, 2 × OAc), 2.37 (1H, *d*, $J = 17.1$ Hz, H_1-2), 3.64 (2H, H-2′, H-5′), 3.81 (1H, *m*, H-9), 4.07 (1H, *d*, $J = 10.3$ Hz, Ha-4″), 4.10 (1H, *dd*, $J = 12.2$ and 2.4 Hz, Ha-6′), 4.21 (1H, *dd*, $J = 12.2$ and 4.9 Hz, Hb-6′), 4.30 (1H, *d*, $J = 10.3$ Hz, Hb-4″), 4.45 (1H, *d*, $J = 7.3$ Hz, H-1′), 4.57 and 4.64 (each 1H, *d*, $J = 12.5$ Hz, H_2-5″), 4.96 (1H, *dd*, $J = 9.8$ and 9.8 Hz, H-4′), 5.09 (1H, *s*, H-2″), 5.15 (1H, *s*, H-1″), 5.18 (1H, *dd*, $J = 9.8$ and 9.8 Hz, H-3′) and 5.83 (1H, *br s*, H-4). Negative ion FABMS *m/z*: 755.3139 $[M-H]^-$ ($C_{36}H_{51}O_{17}$ requires: 755.3126).

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