Pergamon

PII: S0031-9422(96)00359-7

Phytochemistry, Vol. 44, No. 1, pp. 117–120, 1997

0031-9422/97 $17.00 + 0.00

MEGASTIGMANE GLYCOSIDES FROM *SALVIA NEMOROSA*

YOSHIO TAKEDA,* HONGJIE ZHANG,† TAKASHI MATSUMOTO, HIDEAKI OTSUKA,‡ YASUSHI OOSIO, GISHO HONDA,§ MAMORU TABATA,§ TETSURO FUJITA,§ HANDONG SUN,¶ EKREM SEZIK†† and ERDEM YESILADA††

Faculty of Integrated Arts and Sciences, The University of Tokushima, Tokushima 770, Japan; ‡Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Minami-ku, Hiroshima 734, Japan; §Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan; ¶Kunming Institute of Botany, Academia Sinica, Kunming, Yunnan 650204, China; ††Faculty of Pharmacy, Gazi University, Ankara 06330, Turkey

(*Received* 28 *March* 1996)

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.

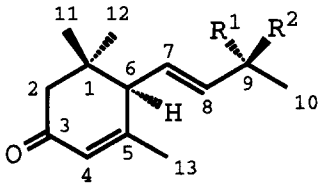
Salvionoside A (**1**), $[\alpha]_D$ +67.7° (MeOH), was obtained as an amorphous powder and the molecular formula was determined as $C_{24}H_{38}O_{11}$ by negative-ion high resolution FAB-mass spectrometry. The ^1H and ^{13}C NMR spectra suggested that **1** was a megastigmane glycoside and the chemical 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**.

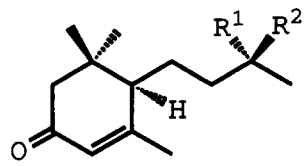
Salvionoside C (**5**), $[\alpha]_D$ −23.3° (MeOH), was obtained as an amorphous powder and the elemental composition, determined by negative-ion high resolution FAB-mass spectrometry to be $C_{24}H_{40}O_{11}$, is two mu more than that of salvionosides A (**1**) and B (**2**). The ^1H and ^{13}C NMR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

*Author to whom correspondence should be addressed.

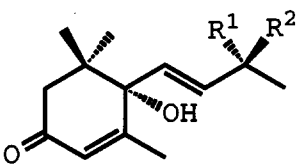
†On leave from Kunming Institute of Botany, Academia Sinica.



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 ^1H 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: ^1H (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).

Acknowledgements—The authors thank the Cooperative Center of the University of Tokushima for opportunities to record NMR spectra. H.Z. is grateful to the Gohou Life Science International Fund for financial support. This work was supported in part by a grant-in-aid from the Ministry of Education, Science, Sports and Culture, Japan (No. 02041048).

REFERENCES

1. Tabata, M., Honda, G., Sezik, E. and Yesilada, E. (1993) A Report on Traditional Medicine and Medicinal Plants in Turkey (1990, 1991), p. 176. Faculty of Pharmaceutical Sciences, Kyoto University.
2. Romanova, A. S., Pribylova, G. S., Zakharov, P. I., Sheichenko, V. I. and Ban'lovskii, A. I. (1971) *Khim. Prir. Soedin*, 199.
3. Romonova, A. S., Pribylova, G. S., Zakhalov, P. I., Sheichenko, V. I. and Ban'lovskii, A. I. (1972) *Khim. Prir. Soedin*, 237.
4. Pabst, A., Barron, D., Semon, E. and Schreier, P. (1992) *Phytochemistry* **31**, 1649.
5. Miyase, T., Ueno, A., Takizawa, N., Kobayashi, H. and Oguchi, H. (1988) *Chem. Pharm. Bull.* **36**, 2475.
6. Otsuka, H., Yao, M., Kamada, K. and Takeda, Y. (1995) *Chem. Pharm. Bull.* **43**, 754 (and refs cited therein).
7. Yahara, S., Shigeyama, C., Ura, T., Wakamatsu, K., Yasuhara, T. and Nohara, T. (1993) *Chem. Pharm. Bull.* **41**, 703.
8. Otsuka, H., Kamada, K., Ogimi, C., Hirata, E., Takushi, A. and Takeda, Y. (1994) *Phytochemistry* **35**, 1331.