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HYDROQUINONE GLYCOSIDES FROM LEAVES OF *MYRSINE SEGUINII*

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Key Word Index—*Myrsine seguinii*; Myrsinaceae; leaves; arbutin; arbutin 2′-*O*-*β*-apiofuranoside; arbutin 6′-*O*-*β*-apiofuranoside; arbutin 2′-*O*-*β*-apiofuranoside 5″-*O*-acyl ester; seguinosides A–F.

Abstract—From leaves of *Myrsine seguinii*, seven hydroquinone glycosides were isolated. By spectroscopic analyses, their structures were elucidated to be arbutin, arbutin 2′- and 6′-*O*-*β*-apiofuranosides (seguinosides A and B, respectively), and the benzoyl, *p*-hydroxybenzoyl, 3-methoxy-4-hydroxybenzoyl and 3,5-dimethoxy-4-hydroxybenzoyl esters of the alcohol hydroxyl group on C-5″ of arbutin 2′-*O*-*β*-apiofuranoside (seguinosides C-F, respectively).

INTRODUCTION

Myrsine seguinii is a perennial tree which grows in moderate and subtropical climate areas. The isolation of cytotoxic saponins has been reported from a New Zealand *Myrsine* species [1].

Five flavonol glycosides were isolated on phytochemical investigation of *M. seguinii*, collected in Okinawa Prefecture [2]. Further investigation furnished seven phenolic glycosides, one of which was identified as a known compound, namely arbutin (**1**) [3]. This paper deals with the structural elucidation of the six new compounds, named seguinosides A–F (**2**–**7**).

RESULTS AND DISCUSSION

Phenolic glycosides were isolated from the *n*-BuOH soluble fraction of a MeOH extract of leaves by a combination of various kinds of chromatography (see Section 3). The structure of the known compound (**1**) was confirmed by comparison of its spectral data with those of the authentic compound and those of the new compounds (**2**–**7**) were elucidated mainly by spectroscopic methods.

Seguinoside A (**2**), $[\alpha]_D$ −83.6°, was isolated as an amorphous powder whose elemental composition was determined to be $C_{17}H_{24}O_{11}$ by negative ion HR-FAB mass spectrometry. The ^1H and ^{13}C NMR spectra were similar to those of arbutin. On methanolysis, **2** yielded one mole each of methyl apioside and methyl glucoside as sugar components. From the upfield shift of the anomeric carbon signal of the glucopyranose unit from **1** to **2** (Table 1), the *β*-apiofuranose was expected to be linked to the hydroxyl group at the 2′-position of the glucose. This was confirmed by an acetylation experiment on **2**. The heptaacetate (**2a**) was prepared and the H–H COSY spectrum was examined. Since the H-1′ signal (δ_H 4.94) showed a cross-peak with the H-2′ proton (δ_H 3.94), which essentially remained intact on acetylation, the structure of **2** was elucidated to be 2′-*O*-*β*-D-apiofuranosylarbutin.

Seguinoside B (**3**) was analyzed to have the same elemental composition as that of **2**. The ^{13}NMR spectrum indicated that it was an isomer with regard to the position of the apiofuranosyl unit of **2**. A significant downfield shift of C-6′ (δ_C 68.8) led to the conclusion that **3** is 6′-*O*-*β*-apiofuranosylarbutin.

Seguinoside C (**4**) was isolated as colourless needles, whose elemental composition was determined to be $C_{24}H_{28}O_{11}$. The IR spectrum indicated the

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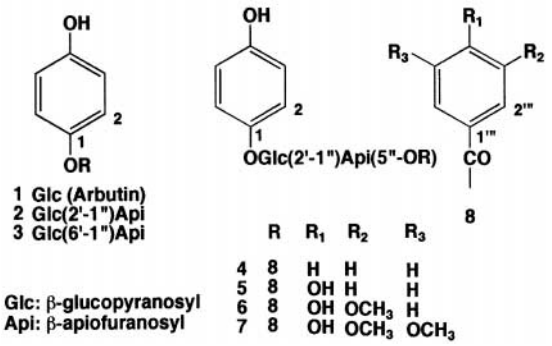

presence of aromatic ring(s) (1600 and 1510 cm^{-1}) and an ester linkage (1705 cm^{-1}). The NMR data indicated that 4 contained seguinoside A (**2**) as a partial structure. Taking into account the results of HR-FAB-mass spectrometry, the acyl moiety must comprise of seven carbons. However, the ^{13}C NMR spectrum for the acyl moiety showed only three aromatic signals with hydrogen, two of which had double strength, one without hydrogen and a carbonyl carbon. From this evidence, the acyl moiety was expected to be a symmetrical compound, namely benzoic acid. Methanolysis gave methyl apioside and methyl glucoside as sugar units and the ^{13}C NMR chemical shifts of the glucopyranose portion were the same as those of **2**. While those of C-5″ (66.1 → 68.4) and C-3″ (80.8 → 79.3) were shifted downfield and upfield, respectively, by acylation. Those of **2**, isolated by alkaline hydrolysis, coincided with the reported values for *β*-D-apiofuranosyl(1-2)-*β*-D-glucopyranoside [4, 5]. Therefore, the structure of **4** was presumed to be 2′-*O*-*β*-apiofuranosylarbutin 5″-*O*-benzoic acid ester. Further confirmation of the esterified position will be discussed later.

Seguinosides D–F (**5**–**7**) are analogous compounds to **4** with substituted benzoates. The NMR spectral data of **5** showed that the benzoyl portion was symmetrically substituted with one hydroxyl group. Those of **6** showed that the acyl portion has three protons in a ABX-coupling system and one hydroxyl and one methoxyl group. The position of the methoxyl substituent was determined by means of a difference NOE experiment. On irradiation of the methoxyl signal (δ_H 3.86), the intensity of the doublet aromatic signal (δ_H 7.49) was enhanced. Thus, the methoxyl function was placed at the 3‴-position. Those of **7** showed that the acyl portion was symmetrically substituted by one hydroxyl group and two 3‴,5‴-methoxyl groups. This evidence led to the conclusion that the structures of **5**, **6** and **7** were the *p*-hydroxbenzoic, 3‴-methoxy-4‴-hydroxybenzoic and 3‴,5‴-dimethoxy-4‴-hydroxybenzoic acid esters of the alcoholic hydroxyl group on C-5″ of 2′-*O*-*β*-apiofuranosylarbutin, respectively.

The ^{13}C NMR data for the *β*-apiofuranosyl moiety in compounds **4**–**7** were not identical with those for **2** presumably due to acylation on the hydroxyl group at C-5″. The HMBC spectrum of **6** finally confirmed that the ester linkage is on the hydroxyl group on C-5″, since significant cross-peaks were observed between δ_C 167.8 and δ_H 4.27 and 4.37.

Table 1. ^{13}C NMR data for arbutin (1) and seguinosides A–F (**2**–**7**) (CD_3OD, 100 MHz)

Carbon number	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	152.5	152.4	152.5	152.1	152.2	152.1	152.0
2, 6	119.4	119.2	119.5	118.8	118.9	118.8	118.8
3, 5	116.7	116.7	116.7	116.7	116.7	116.7	116.7
4	153.8	153.8	153.9	153.6	153.6	153.6	153.5
1′	103.1	102.3	103.8	101.8	101.9	101.8	101.8
2′	75.0	78.8[a]	75.0	78.9[a]	78.8[a]	78.8[a]	78.8[a]
3′	78.1[a]	78.2	78.1	78.4	78.6[a]	78.4	78.4
4′	71.5	71.5	71.7	71.6	71.6	71.6	71.5
5′	78.0[a]	78.7[a]	76.9	78.7[a]	78.7[a]	78.7[a]	78.7[a]
6′	62.6	62.6	68.8	62.6	62.6	62.6	62.5
1″		110.8	111.1	110.5	110.6	110.5	110.5
2″		77.9	78.0	78.0	78.0	78.0	78.0
3″		80.8	80.6	79.2	79.3	79.3	79.3
4″		75.5	75.0	75.4	75.5	75.5	75.4
5″		66.1	65.7	68.4	68.0	68.3	68.5
1‴				131.1	122.0	122.3	121.1
2‴				129.6	133.0	113.8	108.5
3‴				130.7	116.2	153.0	148.9
4‴				134.3	163.6	148.7	142.1
5‴				130.7	116.2	116.0	148.9
6‴				129.6	133.0	125.3	108.5
7‴				167.8	167.9	167.8	167.8
-O*Me*						56.5	56.9

[a]Exchangeable in each column.

EXPERIMENTAL

General

Instrumentation and isolation techniques used, plant material and a part of the extraction and isolation procedures were the same as those reported previously [1]. EI-MS: 70 eV.

Isolation

An *n*-BuOH-sol. fr. (200 g) was obtained from a MeOH extract of leaves of *M. seguinii* Lév. (5.95 kg) by solvent partition. A portion (50 g) of the *n*-BuOH-sol. fr. was separated by CC on a highly porous synthetic resin, Diaion HP-20 with MeOH–H_2O [(1:4, 3.5 l), (2:3, 3 l), (3:2, 3 l) and (4:1, 3 l), and MeOH (3 l)], 500 ml frs being collected. The residue (5.56 g in frs 3–8) of the 20% MeOH eluate was separated by silica gel (200 g) CC with $CHCl_3$ (2 l) and $CHCl_3$–MeOH [(99:1, 3 l), (97:3, 3 l), (19:1, 3 l), (37:3, 3 l), (90:1, 3 l), (17:3, 3 l), (4:1, 3 l), (3:1, 3 l) and (7:3, 3 l)], 500 ml frs being collected. The residue (1.02 g in frs 27–34) of the 10% MeOH eluate was subjected to reverse-phase silica gel column chromatography (RPCC). From frs 16–28, 655 mg of arbutin (**1**) was isolated. A portion of **1** was recrystallized from EtOAc to give colourless needles. The residue (1.17 g in frs 35–45) of the 15–20% MeOH eluate obtained on silica gel CC was similarly subjected to RPCC (86 mg in frs 31–37) and then droplet counter-current chromatography (DCCC). The residue (39 mg in frs 15–20 of DCCC) was finally purified by reverse-phase HPLC (10% MeOH in H_2O) to afford 5.0 mg of **3** (17.5 min) and 24 mg of **2** (19.5 min). A further amount (13 mg) of **2** was isolated from the successive frs (21–26) obtained on DCCC. The residue (14.8 g in frs 9–15) of the 20–40% MeOH eluate obtained by Diaion HP-20 CC was subjected to silica gel (450 g) CC with $CHCl_3$ (3 l) and $CHCl_3$–MeOH [(99:1, 6 l), (97:3, 6 l), (19:1, 6 l), (37:3, 6 l), (9:1, 6 l), (17:3, 6 l), (4:1, 6 l), (3:1, 6 l) and (7:3, 6 l)], 500 ml frs being collected. The residue (761 mg in frs 41–54) of the 7.5% MeOH eluate was subjected to RPCC (91 mg in frs 121–132) and then DCCC to give 72 mg (in frs 100–121) of 4 in a crystalline state. The residue (1.18 g in frs 70–80) of the 15% MeOH eluate obtained on silica gel CC was subjected to RPCC (116 mg in frs 105–119) and then DCCC. The residues (11 mg in frs 26–30, 31 mg in frs 31–37 and 37 mg in frs 38–45) were finally purified by HPLC to give 8 mg (26 min, 30% MeOH in H_2O), 17 mg (18 min, 25% MeOH) and 26 mg (22 min, 25% MeOH) of **5**, **6** and **7**, respectively. Further amounts of **5** (31 mg) and **6** (67 mg) were isolated in a similar manner from the residue (857 mg in frs 55–64) of the 10% MeOH eluate obtained by silica gel CC.

*Arbutin (**1**)*

Colourless needles (EtOAc), m.p. 199–200°C. $[\alpha]_D^{25}$ −51.9° (MeOH, *c* 0.67). ^{13}C NMR (CD_3OD): Table 1 [1].

*Seguinoside A (**2**)*

Amorphous powder. $[\alpha]_D^{25}$ −83.6° (MeOH, *c* 1.54). IR ν_{max}^{KBr} cm^{-1}: 3300, 1510, 1210, 1080–990. UV λ_{max}^{MeOH} nm (log ε): 224 (3.79), 287 (3.28). ^1H NMR (CD_3OD): δ 3.56 (H, *d*, *J* = 11 Hz, H-5″a), 3.59 (H, *d*, *J* = 11 Hz, H-5″b), 3.68 (H, *dd*, *J* = 6 and 12 Hz, H-6′a), 3.78 (H, *dd*, *J* = 10 Hz, H-4″a), 3.87 (H, *dd*, *J* = 2 and 12 Hz, H-6′b), 3.97 (H, *d*, *J* = 2 Hz, H-2″), 4.07 (H, *d*, *J* = 10 Hz, H-4″b), 4.97 (H, *d*, *J* = 8 Hz, H-1′), 5.46 (H, *d*, *J* = 2 Hz, H-1″), 6.70 (2H, *d*, *J* = 9 Hz, H-3 and 5), 6.94 (2H, *d*, *J* = 9 Hz, H-2 and 6). ^{13}C NMR (CD_3OD): Table 1. HR-FAB-MS (negative centroid) *m*/*z*: 403.1251 $[M–H]^-$ ($C_{17}H_{23}O_{11}$ requires 403.1241).

*Seguinoside B (**3**)*

Amorphous powder. $[\alpha]_D^{25}$ −68.9° (MeOH, *c* 0.33). UV λ_{max}^{MeOH} nm (log ε): 224 (3.81), 286 (3.31). ^1H NMR (CD_3OD): δ 3.58 (2H, *s*, H-5″a and 5″b), 3.61 (H, *dd*, *J* = 6 and 11 Hz, H-6′a), 3.75 (H, *d*, *J* = 10 Hz, H-4″a), 3.91 (H, *d*, *J* = 2 Hz, H-2″), 3.96 (H, *d*, *J* = 10 Hz, H-4″a), 4.01 (H, *dd*, *J* = 2 and 11 Hz, H-6′b), 4.68 (H, *d*, *J* = 8 Hz, H-1′), 4.98 (H, *d*, *J* = 2 Hz, H-1″), 6.70 (2H, *d*, *J* = 9 Hz, H-3 and 5), 6.96 (2H, *d*, *J* = 9 Hz, H-2 and 6). ^{13}C NMR (CD_3OD): Table 1. HR-FAB-MS (negative centroid) *m*/*z*: 403.1246 $[M–H]^-$ ($C_{17}H_{23}O_{11}$ requires 403.1241).

*Seguinoside C (**4**)*

Colourless needles (MeOH), m.p. 234–236°C. $[\alpha]_D^{27}$ −86.3° (pyridine, *c* 0.58). IR ν_{max}^{KBr} cm^{-1}: 3300, 1705, 1600, 1510, 1280, 1225, 1115, 1060, 990, 825. UV λ_{max}^{MeOH} nm (log ε): 227 (4.21), 282 (3.41). ^1H NMR (CD_3OD): δ 3.55–3.65 (2H, *m*), 3.63 (H, *dd*, *J* = 6 and 12 Hz, H-6′a), 3.86 (H, *dd*, *J* = 2 and 12 Hz, H-6′b), 3.92 (H, *d*, *J* = 10 Hz, H-4″a), 4.02 (H, *d*, *J* = 1 Hz, H-2″), 4.33 (H, *d*, *J* = 11 Hz, H-5″a), 4.33 (H, *d*, *J* = 10 Hz, H-4″b), 4.40 (H, *d*, *J* = 11 Hz, H-5″b), 4.80 (H, *d*, *J* = 8 Hz, H-1′), 5.51 (H, *d*, *J* = 1 Hz, H-1″), 6.54 (2H, *d*, *J* = 9 Hz, H-3 and 5), 6.85 (2H, *d*, *J* = 9 Hz, H-2 and 6), 7.42 (2H, *t*, *J* = 8 Hz, H-3‴ and 5‴), 7.58 (H, *tt*, *J* = 1 and 8 Hz, H-4‴), 7.96 (2H, *dd*, *J* = 1 and 8 Hz, H-2‴ and 6‴). ^{13}C NMR (CD_3OD): Table 1. HR-FAB-MS (negative centroid) *m*/*z*: 507.1478 $[M–H]^-$ ($C_{24}H_{27}O_{12}$ requires 507.1503).

*Seguinoside D (**5**)*

Amorphous powder. $[\alpha]_D^{25}$ −67.1° (MeOH, *c* 0.39). IR ν_{max}^{KBr} cm^{-1}: 3350, 1690, 1610, 1510, 1220, 1110–1000. UV λ_{max}^{MeOH} nm (log ε): 212 (4.07), 224 (3.91) *sh*, 258 (4.08), 273 (3.91) *sh*, 290 (3.41) *sh*. ^1H NMR (CD_3OD): δ 3.57–3.65 (2H, *m*), 3.63 (H, *dd*, *J* = 8

and 9 Hz, H-2′), 3.67 (H, *dd*, *J* = 6 and 12 Hz, H-6′a), 3.87 (H, *dd*, *J* = 2 and 12 Hz, H-6′b), 3.90 (H, *d*, *J* = 10 Hz, H-4″a), 4.01 (H, *d*, *J* = 1 Hz, H-2″), 4.27 (H, *d*, *J* = 11 Hz, H-5″a), 4.31 (H, *d*, *J* = 10 Hz, H-4″b), 4.35 (H, *d*, *J* = 11 Hz, H-5″b), 4.79 (H, *d*, *J* = 8 Hz, H-1′), 5.50 (H, *d*, *J* = 1 Hz, H-1″), 6.57 (2H, *d*, *J* = 9 Hz, H-3 and 5), 6.74 (2H, *d*, *J* = 9 Hz, H-3‴ and 5‴), 6.86 (2H, *d*, *J* = 9 Hz, H-2 and 6), 7.83 (2H, *d*, *J* = 9 Hz, H-2‴ and 6‴). ^{13}C NMR (CD_3OD): Table 1. HR-FAB-MS (negative centroid) *m*/*z*: 523.1437 $[M–H]^-$ ($C_{24}H_{27}O_{13}$ requires 523.1452).

*Seguinoside E (***6***)*

Amorphous powder. $[\alpha]_D^{27}$ −66.8° (MeOH, *c* 0.48). IR ν_{max}^{KBr} cm^{-1}: 3350, 1690, 1600, 1510, 1280, 1210, 1100–1000. UV λ_{max}^{MeOH} nm (log ε): 221 (4.22), 264 (3.95), 290 (3.80). ^{1}H NMR (CD_3OD): δ 3.63 (H, *dd*, *J* = 8 and 9 Hz, H-2′), 3.67 (H, *dd*, *J* = 6 and 12 Hz, H-6′a), 3.87 (3H, *s*, C*H*$_3$O–), 3.87 (H, *dd*, *J* = 2 and 12 Hz, H-6′b), 3.91 (H, *d*, *J* = 10 Hz, H-4″a), 4.02 (H, *d*, *J* = 1 Hz, H-2″), 4.27 (H, *d*, *J* = 11 Hz, H-5″a), 4.32 (H, *d*, *J* = 10 Hz, H-4″b), 4.37 (H, *d*, *J* = 11 Hz, H-5″b), 4.78 (H, *d*, *J* = 8 Hz, H-1′), 5.51 (H, *d*, *J* = 1 Hz, H-1″), 6.54 (2H, *d*, *J* = 9 Hz, H-3 and 5), 6.80 (H, *d*, *J* = 8 Hz, H-5‴), 6.84 (2H, *d*, *J* = 9 Hz, H-2 and 6), 7.49 (H, *d*, *J* = 2 Hz, H-2‴), 7.52 (H, *dd*, *J* = 2 and 8 Hz, H-6‴). ^{13}C NMR (CD_3OD): Table 1. HR-FAB-MS (negative centroid) *m*/*z*: 553.1543 $[M–H]^-$ ($C_{25}H_{29}O_{14}$ requires 553.1558).

*Seguinoside F (***7***)*

Amorphous powder. $[\alpha]_D^{27}$ −67.0° (MeOH, *c* 0.51). IR ν_{max}^{KBr} cm^{-1}: 3350, 2900, 1690, 1610, 1510, 1460, 1330, 1215, 1100–990. UV λ_{max}^{MeOH} nm (log ε): 219 (4.30), 280 (4.30). ^{1}H NMR (CD_3OD): δ 3.55–3.62 (2H, *m*), 3.63 (H, *dd*, *J* = 6 and 12 Hz, H-6′a), 3.86 (6H, *s*, $CH_3O–$ ×2), 3.92 (H, *d*, *J* = 10 Hz, H-4″a), 4.05 (H, *d*, *J* = 1 Hz, H-2″), 4.28 (H, *d*, *J* = 11 Hz, H-5″a), 4.30 (H, *d*, *J* = 10 Hz, H-4″b), 4.42 (H, *d*, *J* = 11 Hz, H-5″b), 4.78 (H, *d*, *J* = 8 Hz, H-1′), 5.52 (H, *d*, *J* = 1 Hz, H-1″), 6.53 (2H, *d*, *J* = 9 Hz, H-3 and 5), 6.82 (2H, *d*, *J* = 9 Hz, H-2 and 6), 7.28 (2H, *s*, H-2‴ and 6‴). ^{13}C NMR (CD_3OD): Table 1. HR-FAB-MS (negative centroid) *m*/*z*: 583.1658 $[M–H]^-$ ($C_{26}H_{31}O_{15}$ requires 583.1663).

GC analysis of sugar portions of **2** *and* **4**

A few mg each of **2** and **4** was hydrolyzed with 5% HCl in MeOH at 95°C for 3 h in a sealed tube. The reaction mixture was then neutralized by the addition of Ag_2CO_3 and filtered. The filtrate was evaporated to dryness and then treated with several drops of trimethylsilylimidazole at 60°C for 15 min. After partitioning between *n*-hexane and H_2O, the concentrated organic layer was subjected to GC analysis (FID; Shimadzu CPB-20, 0.22 mm × 20 m, 0.25 μm film thickness; temperature, 160°C; carrier gas, N_2 at 1.5 kg cm^{-2}). Standard sugars: apiose, 2.73, 2.84, 2.98 and 3.15 min; glucose, 8.12 and 8.79 min (standard sugars were from a previous experiment [6]). Seguinoside A (**2**): apiose, 2.73, 2.84, 2.98 and 3.15 min and glucose, 8.13 and 8.81 min, seguinoside C (**4**): apiose, 2.72, 2.85, 2.97 and 3.16 and glucose, 8.15 and 8.82 min.

Acetylation of **2**

Seguinoside A (**2**, 11 mg) was acetylated with 250 μl each of $(Ac)_2O$ and pyridine at 50°C for 13 h. The reagents were removed by a N_2 stream and then the residue was recrystallized from MeOH to give 15 mg (79%) of the heptaacetate (**2a**) as colourless needles (MeOH), m.p. 200–202°C. $[\alpha]_D^{27}$ −32.9° ($CHCl_3$, *c* 0.85). ^{1}H NMR ($CDCl_3$): δ 1.98, 2.02, 2.03, 2.05, 2.09, 2.11, (each 3H, each *s*, CH_3CO-×7 on alcoholic OH), 2.28 (3H, *s*, CH_3CO- on phenolic OH), 3.79 (H, *ddd*, *J* = 2, 6 and 9 Hz, H-5′), 3.94 (H, *dd*, *J* = 8 and 9 Hz, H-2′), 4.12 (H, *dd*, *J* = 2 and 12 Hz, H-6′a), 4.13 (H, *d*, *J* = 10 Hz, H-4″a), 4.29 (H, *dd*, *J* = 6 and 12 Hz, H-6′b), 4.33 (H, *d*, *J* = 10 Hz, H-4″b), 4.57 (2H, *s*, H_2-5″), 4.94 (H, *d*, *J* = 8 Hz, H-1′), 5.05 (H, *t*, *J* = 9 Hz, H-4′), 5.18 and 5.20 (each H, each *s*, H-1″ and 2″), 5.27 (H, *t*, *J* = 9 Hz, H-3′), 7.13 and 7.05 (each 2H, each *d*, *J* = 9 Hz, H-2 and 6 and H-3 and 5). EI-MS *m*/*z* (rel. int.): 547 (45) [Api(OAc)$_3$Glc(OAc)$_3$ oxonium ion]$^+$, 278 (35), 259 (100) [Api(OAc)$_3$ oxonium ion]$^+$, 139 (95). FAB-MS (positive centroid) *m*/*z*: 721 $[M + Na]^+$ (+NaI), 737 $[M + K]^+$ (+KI); HR-FAB-MS (negative centroid) *m*/*z*: 655.1873 $[M–H–CH_2C{=}O]^-$ ($C_{29}H_{35}O_{17}$ requires 655.1874).

*Alkaline hydrolysis of seguinoside E (***6***)*

Seguinoside E (**6**) (24 mg) was treated with 0.1 N NaOH in MeOH at 25°C for 6 h under a N_2 stream. The reaction mixture was neutralized by the addition of Amberlite IR-120B (H^+) and then concentrated. The residue was purified by CC [silica gel (27 g), *L* = 15 cm, *Φ* = 15 mm, $CHCl_3$ (100 ml), $CHCl_3$–MeOH (9:1, 100 ml), (4:1, 100 ml), (7:3, 300 ml) and $CHCl_3$–MeOH–H_2O (35:15:2, 500 ml), frs of 10 ml being collected] afford 3-methoxy-4-hydroxybenzoic acid methyl ester (**6a**, 3.5 mg, 64%) in frs 6–8. Meanwhile, the starting material (**6**) was recovered in frs 27–35, 6 mg) and the hydrolyzed compound in frs 37–110. The latter was further purified by HPLC (MeOH–H_2O, 1:9) to give 8.8 mg (67%) of compound **6b** (=**2**). Me ester (**6a**), ^{1}H NMR ($CDCl_3$): δ 3.89 and 3.95 (each 3H, each *s*, -OMe and -COOMe), 6.93 (H, *d*, *J* = 8 Hz, H-5‴) 7.55 (H, *d*, *J* = 2 Hz, H-2‴), 7.64 (H, *dd*, *J* = 2 and 8 Hz, H-6‴).

Compound **6b**: An amorphous powder. $[\alpha]_D^{27}$ − 95.5° (MeOH, *c* 0.59). All other physico–chemical properties were essentially the same as those of seguinoside A (**2**).

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