PERGAMON

Phytochemistry 52 (1999) 923–927

PHYTOCHEMISTRY

Hydroquinone diglycoside acyl esters from the leaves of *Myrsine seguinii*

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Received 4 January 1999; received in revised form 10 May 1999

Abstract

From the leaves of *Myrsine seguinii*, five phenolic glycosides, seguinosides G–K, were isolated. Their structures were elucidated as comprising tiglic, furoic, menthiafolic and 2-E-hexenoic acid esters attached to the 5″-hydroxyl group of seguinoside A [=arbutin(2′-1″)apiofuranoside], and a 3-methoxy-4-hydroxybenzoic acid ester linked to the 5″-hydroxyl group of methoxyseguinoside A. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Myrsine seguinii*; Myrsinaceae; Tachioside; Isotachioside; Arbutin apiofuranoside acyl ester; Seguinosides G–K

1. Introduction

Previously, we reported the isolation of flavonol glycosides (Zhong et al., 1997) and hydroquinone glycosides (Zhong et al., 1998) from the leaves of *Myrsine seguinii* Lev. (Fam. Myrsinaceae), collected in the Okinawa Prefecture. Further investigation has furnished the known phenolic glucosides; tachioside (**1**) and isotachioside (**2**), and five new hydroquinone diglycoside acyl esters, seguinosides G–K (**3**–**7**), whose structure are now reported.

2. Results and discussion

Phenolic glycosides were isolated using a combination of various chromatographic methods, with the structures of known compounds elucidated by comparison of previously reported spectral data.

Seguinoside G (**3**), $[\alpha]_D$ −85.1°, 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 suggested that **3** is a derivative of seguinoside A (**8**), with an ester moiety on the 5″-hydroxy group. The ester moiety was also shown by NMR spectroscopy to comprise one trisubstituted double bond, two methyls on the double bond and an ester carbonyl functionality. Therefore, the acyl moiety was expected to the be either tiglic (**3a**), angelic (**3b**), or senecioic (**3c**) acid. Comparison of the NMR data of authentic acid methyl esters with those of the acyl moiety of **3** led to the conclusion that **3** is the 5″-*O*-tiglate ester of seguinoside A (**8**) (Table 1).

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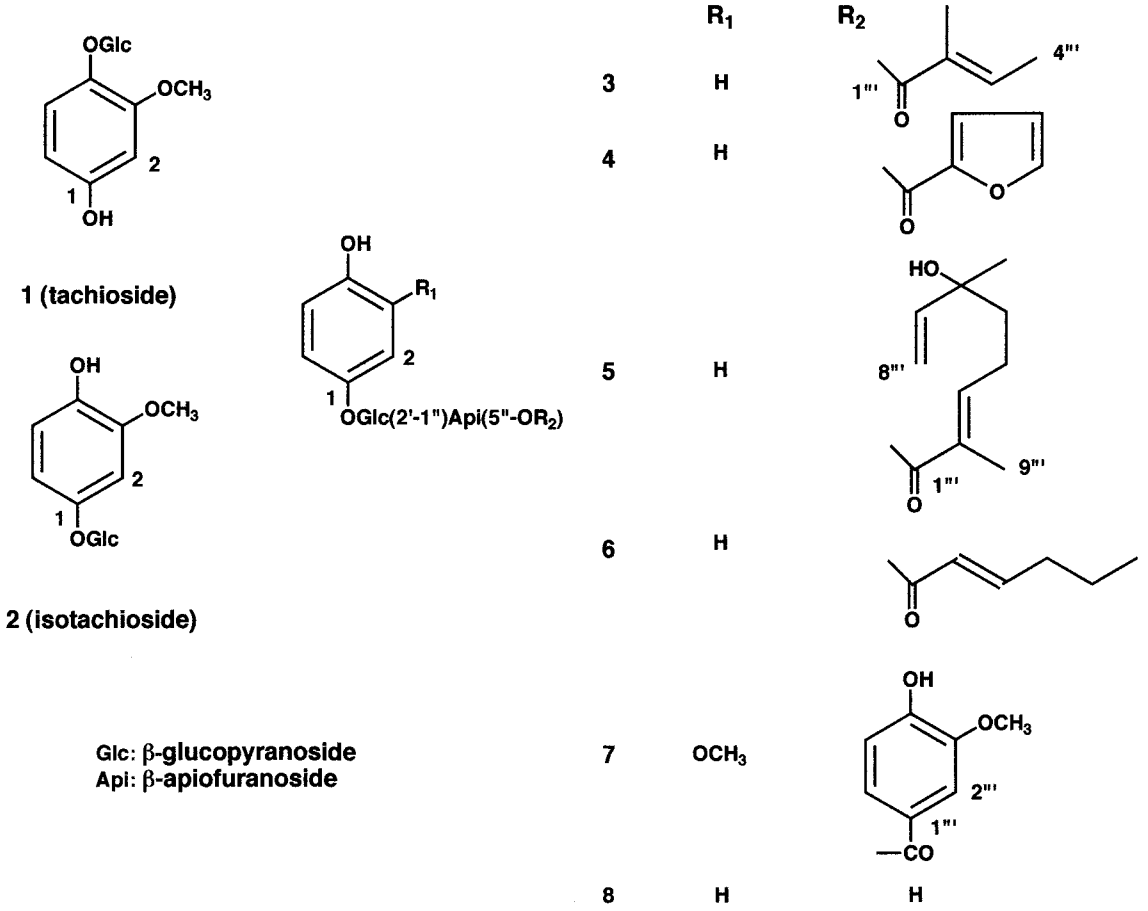
0031-9422/99/$ - see front matter
PII: S0031-9422(99)00334-9



Seguinoside H (**4**) was isolated as an amorphous powder, whose elemental composition was found to be $C_{22}H_{26}O_{13}$. The ^{13}C NMR spectral data indicated that **4** contained all the carbon signals of **8** along with five additional signals. The additional carbon signals were due to: one disubstituted and one trisubstituted double bond, and a carbonyl carbon. Evaluation of the HR-FAB-MS data suggested that the acyl portion must contain two oxygen atoms with three degrees of unsaturation. Therefore, 2- or 3-furoic acid was the candidate, and the presence of the singlet carbon signal at δ_C 145.8 led to the conclusion that 2-furoic acid was the acyl moiety. This was confirmed by comparison of the ^{13}C NMR spectral data of methyl 2-furoate (**4a**) with those of the acyl portion of 4, and **4** was elucidated to be the 5″-*O*-furoate of seguinoside A (**8**).

Seguinoside I (**5**) was obtained as an amorphous powder, and the analysis of the HR-FAB-MS and NMR spectra indicated that it was similar to compound **4**, albeit with a different acyl moiety. The acyl portion composed of ten carbon atoms, and the ^{13}C NMR spectral data for the menthiafolic acid portion reported for ambiguuside were essentially identical to seguinoside I (**5**) (Arslanian, Anderson & Stermitz, 1990). Therefore, the structure was elucidated to be the 5″-*O*-menthiafolate of seguinoside A. Due to the low yield of the sample, the absolute configuration of the quartenary carbon atom (C-6‴) was not determined.

Seguinoside **J** (**6**) was also obtained as an amorphous powder, and the spectroscopic data indicated that **6** is an anlogous compound to the preceding ones. From the NMR data, the structure of the acyl moiety was concluded to be *E*-hex-2-enoic acid, and seguinoside **J** is shown as that of **6**.

Seguinoside K (**7**) was isolated as an amorphous powder, and its elemental composition was revealed as $C_{24}H_{28}O_{13}$ by HR-FAB mass spectrometry. The ^1H NMR spectrum showed the presence of two sets of three aromatic protons coupled in an ABX system and two methoxyl signals. The ^{13}C NMR spectrum indicated that the sugar portion was the same as that of the preceding compounds, such as β-apiofuranosyl(1″-2′)-β-glucopyranoside, and the acyl moiety is also the same as that of seguinoside E (Zhong et al., 1998). Therefore, the core unit must have the tachioside (**1**) or isotachioside (**2**) skeleton (Inoshiri, Sasaki, Kohda, Otsuka & Yamasaki, 1987). In the NOESY experiment, the anomeric proton of the glucose moiety (δ_H 4.79) was observed to cross two aromatic protons at

Table 1
^{13}C NMR spectral data for tachioside (**1**), isotachioside (**2**), seguinoside A (**8**), seguinosides G–K (**3**–**7**), tiglic, angelic and senecioic acids methyl esters (**3a**, **3b**, and **3c**, respectively), and 2-furoic acid methyl ester (**4a**) (CD_3OD, 100 MHz)

Carbon number	**1**	**2**	**8**[a]	**3**	**3a**	**3b**	**3c**	**4**	**4a**	**5**	**6**	**7**
1	152.9	141.1	152.4	152.2				152.1		152.2	152.2	152.5
2	104.0	152.0	119.2	118.8				118.8		118.9	119.0	103.1
3	149.3	104.4	116.7	116.7				116.7		116.7	116.8	149.2
4	143.0	155.0	153.8	153.7				153.6		153.7	153.8	142.7
5	116.0	107.6	116.7	116.7				116.7		116.7	116.8	116.0
6	110.0	120.6	119.2	118.8				118.8		118.9	119.0	109.4
1′	103.8	101.9	102.8	101.8				101.8		101.9	102.0	101.9
2′	75.1	75.0	78.8[b]	78.9[b]				78.8[b]		78.8[b]	78.8[b]	78.9[b]
3′	78.2[b]	78.2	78.2	78.3				78.3		78.3	78.4	78.5
4′	71.6	71.4	71.5	71.6				71.6		71.5	71.6	71.7
5′	77.9[b]	78.1	78.7[b]	78.7[b]				78.5[b]		78.6[b]	78.7[b]	78.8[b]
6′	62.7	62.6	62.6	62.6				62.5		62.5	62.6	62.7
1″			110.8	110.4				110.3		110.4	110.4	110.6
2″			77.9	78.0				78.0		78.0	78.0	78.1
3″			80.8	79.2				79.2		79.2	79.1	79.3
4″			75.5	75.3				75.3		75.3	75.4	75.7
5″			66.1	67.9				68.3		68.0	67.8	68.0
1‴				169.2	170.0	170.0	168.6	159.9	160.7	169.3	168.0	167.8
2‴				129.3	129.6	129.0	116.5	145.5	145.8	128.3	121.8	122.3
3‴				139.3	138.6	138.9	158.4	113.1	113.0	146.0	151.5	113.8
4‴				12.1	12.1	15.9	27.4	119.7	119.2	24.6	35.2	153.0
5‴				14.4	14.4	20.7	20.3	148.4	148.3	41.7	22.4	148.7
6‴										73.7	14.0	125.3
7‴										144.6		115.9
8‴										112.5		
9‴										12.4		
10‴										27.9		
–OMe	56.4	56.6										56.3 × 2
–COOMe					52.2	51.7	51.2		52.4			

[a] Data taken from Zhong et al. (1998).
[b] The assignments in each column may be interchangeable.

δ_H 6.44 (*dd, J* = 2, 8 Hz) and 6.66 (*d, J* = 2 Hz), and the methoxyl proton at δ_H 3.73 crossed δ_H 6.66. This confirmed that the core portion has the tachioside skeleton, and the structure of seguinoside K is shown as that of **7**.

3. Experimental

The instrumentation and isolation techniques used were as reported previously (Zhong et al., 1997). Methyl tiglate and methyl angelicate were purchased from Tokyo Kasei Co., Ltd (Tokyo, Japan), and methyl furoate was obtained from Wako Pure Chemical Ind. Ltd (Osaka, Japan). Senecioic acid methyl ester (methyl, 3,3-dimethylacrylate) was purchased from Aldrich Chem. Co. (Milwaukee, WI).

3.1. Extraction and isolation

M. seguinii leaves (5.95 kg) were extracted according to the previous report (Zhong et al., 1997). The residue (5.56 g, frs 3–8) of the 20% MeOH eluate obtained from the HP-20 column chromatography was subjected to silica gel (200 g) column chromatography with $CHCl_3$ (2 l) and $CHCl_3$–MeOH (3 l each of 99:1, 97:3, 19:1, 37:3, 9:1, 17:3, and 4:1) as elements, with frs of 500 ml were collected. The residue (1.02 g, frs 24–34) of the 10% MeOH eluate was then subjected to reversed-phase silica gel column chromatography (RPCC) [ODS (Cosmosil, Nacaral Tesque, Kyoto, Japan), MeOH–H_2O (1:9, 1 l → 1:1, 1 l, with frs of 10 g being collected]. Finally, the residue (56 mg in frs 29–38) was purified by preparative HPLC [ODS (Inertsil, GL Science, Tokyo, Japan), $\Phi = 20$ mm, L = 25 cm, flow rate: 6 ml min^{-1}, detection: UV at 278 nm, MeOH–H_2O–CH_3CN (1:97:2)] to give 16 mg of **1** and 28 mg of **2**.

The residue (14.8 g in frs 9–15) of the 40% MeOH eluate obtained from the Diaion HP-20 column chromatography was subjected to silica gel (450 g) column chromatography with $CHCl_3$ (3 l) and $CHCl_3$–MeOH (6 l each of 99:1, 97:3, 19:1, 37:3, 9:1, 17:3, 4:1, 3:1 and 7:3) with frs totalling 500 ml being collected. The residue (761 mg in frs 41–54) of the 7.5% MeOH eluate was subjected to RPCC under the same conditions. The residue (49 mg) of frs 88–94 was purified by droplet counter-current chromatography (DCCC) ($CHCl_3$–MeOH–H_2O-1-PrOH, 9:12:8:2, ascending method, with frs of 5 g being collected and numbered according to the elution of the mobile phase) to give 10 mg of **4**. Another 12 mg of **4** was obtained from a different fr. obtained on silica gel column chromatography. From the residue (66 mg in frs 112–120) of RPCC eluate, 9 mg of compound **3** was isolated by DCCC, followed by preparative HPLC [detection: UV at 210 nm, MeOH–H_2O (7:13)].

The residue (857 mg in frs 55–64) of the 10% MeOH eluate obtained on silica gel column chromatography was subjected to RPCC. The residue (129 mg in frs 110–117) was purified by DCCC (29 mg in frs 61–73) and then preparative HPLC [MeOH–H_2O (3:7), followed by MeOH–H_2O–CH_3CN (1:17:2)] to give 5 mg of **7**. Another 9 mg of **7** was isolated from a different RPCC fr. The residue (94 mg in frs 144–156) was purified by DCCC to afford 15 mg of **5** in frs 57–64.

The residue (3.26 g) of the 60% MeOH eluate obtained on the Diaion HP-20 column chromatography was similarly separated by silica gel, RPCC, DCCC and preparative HPLC to give 6 mg of **6**.

3.2. Known compounds isolated

Tachioside (**1**), mp 210–212° (MeOH), $[\alpha]_D^{24}$ −55.6° (MeOH, *c* 0.86), ^{13}C NMR: Table 1 (Inoshiri et al., 1987). Isotachioside (**2**), mp 190–192° (MeOH), $[\alpha]_D^{24}$ −52.5° (MeOH, *c* 0.36), ^{13}C NMR: Table 1 (Inoshiri et al., 1987).

3.3. Seguinoside G (*3*)

Amorphous powder, $[\alpha]_D^{22}$ −85.1° (MeOH, *c* 0.48); IR ν_{max} (film) cm^{-1}: 3354, 2936, 1699, 1509, 1267, 1074, 831, 777; UV λ_{max} (MeOH) nm (log ϵ): 221 (4.11), 286 (3.29); ^{1}H NMR (CD_3OD): δ 1.73 (3H, *qd, J* = 1, 5 Hz, H_3-4‴), 1.74 (3H, *br s*, H_3-5‴), 3.57 (1H, *t, J* = 9 Hz, H-3′), 3.62 (1H, *dd, J* = 7, 9 Hz, H-2′), 3.67 (1H, *dd, J* = 6, 12 Hz, H-6′a), 3.82 (1H, *d, J* = 10 Hz, H-4″a), 3.87 (1H, *dd, J* = 2, 12 Hz, H-6′b), 3.93 (1H, *d, J* = 1.5 Hz, H-2″), 4.11 (1H, *d, J* = 12 Hz, H-5″a), 4.20 (1H, *d, J* = 12 Hz, H-5″b), 4.23 (1H, *d, J* = 10 Hz, H-4″b), 4.80 (1H, *d, J* = 7 Hz, H-1′), 5.47 (1H, *d, J* = 1.5 Hz, H-1″), 6.64 (2H, *d, J* = 9 Hz, H_2-3, 5), 6.87 (2H, *d, J* = 9 Hz, H_2-2, 6), 6.80 (1H, *m*, H-3‴); ^{13}C NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) *m/z*: 485.1651 [M–H]$^-$ ($C_{22}H_{29}O_{12}$ requires 485.1659).

3.4. Seguinoside H (*4*)

Amorphous powder, $[\alpha]_D^{22}$ −72.1° (MeOH, *c* 0.89); IR ν_{max} (film) cm^{-1}: 3353, 2948, 1717, 1509, 1306, 1221, 1116, 1073, 1017, 831, 772; UV λ_{max} (MeOH) nm (log ϵ): 224 (3.97), 252 (4.03) 285 (3.35); ^{1}H NMR (CD_3OD): δ 3.58 (1H, *t, J* = 9 Hz, H-4′), 3.62 (1H, *dd, J* = 8, 9 Hz, H-2′), 3.67 (1H, *dd, J* = 5, 12 Hz, H-6′a), 3.87 (1H, *dd, J* = 2, 12 Hz, H-6′b), 3.88 (1H, *d, J* = 10 Hz, H-4″a), 3.96 (1H, *d, J* = 1 Hz, H-2″), 4.29 (1H, *d, J* = 11 Hz, H-5″a), 4.30 (1H, *d, J* = 10 Hz, H-4″b), 4.37 (1H, *d, J* = 11 Hz, H-5″b), 4.81 (1H, *d, J* = 8 Hz, H-1′), 5.50 (1H, *d, J* = 1 Hz, H-1″), 6.56 (1H, *dd, J* = 2, 4 Hz, H-4‴), 6.58 (2H, *d, J* = 9 Hz, H_2-3, 5), 6.87 (2H, *d, J* = 9 Hz, H_2-2, 6), 7.17 (1H, *dd, J* = 1, 4 Hz, H-3‴), 7.72 (1H, *dd, J* = 1, 2 Hz, H-5‴); ^{13}C NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) *m/z*: 497.1275 [M–H]$^-$ ($C_{22}H_{25}O_{13}$ requires 497.1295).

3.5. Seguinoside I (*5*)

Amorphous powder, $[\alpha]_D^{22}$ −53.7° (MeOH, *c* 0.99); IR ν_{max} (film) cm^{-1}: 3378, 2934, 1699, 1509, 1277, 1221, 1074, 1019, 930, 831, 777; UV λ_{max} (MeOH) nm (log ϵ): 222 (4.14), 287 (3.23); ^{1}H NMR (CD_3OD): δ 1.26 (3H, *s*, H_3-10‴), 1.55 (1H, *td, J* = 6, 13 Hz, H-5‴a), 1.59 (1H, *td, J* = 6, 13 Hz, H-5‴b), 1.74 (3H, *d, J* = 1 Hz, H_3-9‴), 2.18 (2H, *m*, H_2-4‴), 3.61 (1H, *dd, J* = 7, 9 Hz, H-2′), 3.67 (1H, *dd, J* = 5, 12 Hz, H-6′a), 3.83 (1H, *d, J* = 10 Hz, H-4″a), 3.86 (1H, *dd, J* = 2, 12 Hz, H-6′b), 3.93 (1H, *d, J* = 1 Hz, H-2″), 4.11 (1H, *d, J* = 11 Hz, H-5″a), 4.21 (1H, *d, J* = 11 Hz, H-5″b), 4.23 (1H, *d, J* = 10 Hz, H-4″b), 4.79 (1H, *d, J* = 7 Hz, H-1′), 5.06 (1H, *dd, J* = 1.5, 11 Hz, H-8‴a *cis* to H-7‴), 5.22 (1H, *dd, J* = 1.5, 17 Hz, H-8‴b *trans* to H-7‴), 5.47 (1H, *d, J* = 1 Hz, H-1″), 5.90 (1H, *dd, J* = 11, 17 Hz, H-7‴), 6.64 (2H, *d, J* = 9 Hz, H_2-3, 5), 6.73 (1H, *qt, J* = 1, 8 Hz, H-3‴), 6.87 (2H, *d, J* = 9 Hz, H_2-2, 6); ^{13}C NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) *m/z*: 569.2247 [M–H]$^-$ ($C_{27}H_{37}O_{13}$ requires 569.2234).

3.6. Seguinoside J (*6*)

Amorphous powder, $[\alpha]_D^{23}$ −70.2° (MeOH, *c* 0.41); IR ν_{max} (film) cm^{-1}: 3393, 2961, 1709, 1510, 1223, 1074, 829, 777; UV λ_{max} (MeOH) nm (log ϵ): 223 (4.04), 286 (3.30); ^{1}H NMR (CD_3OD): δ 0.94 (3H, *t, J* = 7 Hz, H_3-6‴), 1.46 (2H, *sextet, J* = 7 Hz, H_2-5‴), 2.15 (2H, *ddd, J* = 2, 7 Hz, H_2-4‴), 3.57 (1H, *t,*

J = 8 Hz, H-2′), 3.61 (1H, *t*, J = 9 Hz, H-4′), 3.67 (1H, *dd*, J = 5, 12 Hz, H-6′a), 3.83 (1H, *d*, J = 10 Hz, H-4″a), 3.87 (1H, *dd*, J = 2, 12 Hz, H-6′b), 3.91 (1H, *d*, J = 1 Hz, H-2″), 4.15 (1H, *d*, J = 12 Hz, H-5″a), 4.20 (1H, *d*, J = 12 Hz, H-5″b), 4.21 (1H, *d*, J = 10 Hz, H-4″b), 4.81 (1H, *d*, J = 8 Hz, H-1′), 5.48 (1H, *d*, J = 1 Hz, H-1″), 5.75 (1H, *td*, J = 2, 16 Hz, H-2‴), 6.66 (2H, *d*, J = 9 Hz, H_2-3, 5), 6.89 (2H, *d*, J = 9 Hz, H_2-2, 6), 6.92 (1H, *td*, J = 7, 16 Hz, H-3‴); ^{13}C NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) m/z: 499.1835 $[M-H]^-$ ($C_{24}H_{27}O_{13}$ requires 499.1816).

3.7. Seguinoside K (7)

Amorphous powder, $[\alpha]_D^{22}$ −52.4° (MeOH, *c* 0.21); IR ν_{max} (film) cm^{-1}: 3355, 1701, 1601, 1285, 1219, 1072, 1028, 762; UV λ_{max} (MeOH) nm (log ϵ): 220 (4.10), 264 (3.78), 288 (3.67); ^{1}H NMR (CD_3OD): δ 3.66 (1H, *dd*, J = 6, 12 Hz, H-6′a), 3.73 (3H, *s*, –OMe on C-3), 3.83 (3H, *s*, –OMe on C-3‴), 3.88 (1H, *dd*, J = 2, 12 Hz, H-6′b), 3.90 (1H, *d*, J = 10 Hz, H-4″a), 4.05 (1H, *d*, J = 1 Hz, H-2′), 4.29 (1H, *d*, J = 11 Hz, H-5″a), 4.30 (1H, *d*, J = 10 Hz, H-4″b), 4.39 (1H, *d*, J = 11 Hz, H-5″b), 4.79 (1H, *d*, J = 8 Hz, H-1′), 5.50 (1H, *d*, J = 1 Hz, H-1″), 6.44 (1H, *dd*, J = 2, 8 Hz, H-6), 6.54 (1H, *d*, J = 8 Hz, H-5), 6.66 (1H, *d*, J = 2 Hz, H-2), 6.78 (1H *d*, J = 8 Hz, H-5‴), 7.47 (1H, *d*, J = 2 Hz, H-2‴), 7.50 (1H, *dd*, J = 2, 8 Hz, H-6‴); ^{13}C NMR (CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) m/z: 583.1671 $[M-H]^-$ ($C_{24}H_{27}O_{13}$ requires 583.1663).

Acknowledgements

The authors are grateful for access to the superconducting NMR instrument in the Research Center for Molecular Medicine of Hiroshima University School of Medicine.

Appendix

Hydroquinone diglycoside acyl esters from the leaves of *Myrsine seguinii*

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From *M seguinii*, five hydroquinone diglycoside acyl esters, seguinosides G–K (**1**–**5**), were isolated. The structures were elucidated by spectroscopic analyses. (R, **1**: tigloyl, **2**: 2-furoyl, **3**: menthiafolyl, **4**: *E*-hex-2-enoyl.)

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