Constituents from the Bark of *Tabebuia impetiginosa*

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Thirteen new phenolic glycosides were obtained by further study of constituents from the bark of *Tabebuia impetiginosa* (MART. ex DC) Standley (Bignoniaceae). The structures of these compounds were determined based on NMR, mass spectral and chemical evidence. Most of them have a glycosyl unit esterified by a benzoic acid derivative.

Key words *Tabebuia impetiginosa* Standley; Bignoniaceae; phenolic glycoside; (*R*)-6-hydroxymellein; apiofuranose; glucopyranose

Tabebuia spp. (Bignoniaceae) can be found throughout central and south America. The bark of *Tabebuia impetiginosa* has been used as a folk medicine for treating diabetes, ulcers, and syphilis.[1)] In connection with a study on the constituents of Brazilian plants, we reported some new compounds obtained from the bark of *T. impetiginosa*.[2,3)] The present paper describes the isolation and structural elucidation of additional constituents from this plant.

Extraction of the bark of *T. impetiginosa* was described in a previous paper.[2)] The 50% MeOH and MeOH eluates from a porous polymer gel column were concentrated, and the residue was chromatographed on a silica gel column, followed by semi-preparative HPLC to give compounds **1**—**16**.

Compounds **1**—**3** were known phenylethanoid and phenolic glycosides, and identified as osmanthuside H (**1**),[4)] 3,4-dimethoxyphenyl 1-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**2**)[2)] and 3,4,5-trimethoxyphenyl 1-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**3**).[5)]

Compound **4** was proposed to have the molecular formula $C_{23}H_{30}O_{11}$ based on high resolution (HR)-FAB-MS [*m/z*: 505.1679 $[M+Na]^+$]. The ^{13}C-NMR spectrum of **4** exhibited 17 carbon signals due to the aglycone, in addition to six carbon signals of a *β*-D-glucopyranosyl group. These signals consisted of twelve aromatic signals (*δ* 150.2, 148.4, 147.0, 146.3, 140.1, 131.9, 123.3, 120.3, 117.4, 115.7, 114.8, 112.6), two methoxyl signals (*δ* 56.6, 56.5), and three aliphatic signals (*δ* 56.6, 64.5, 75.0). The ^{1}H-NMR spectrum of **4** revealed the presence of two aromatic ABX systems [*δ* 7.06 (1H, d, *J*=8.0 Hz), 6.76 (1H, dd, *J*=8.0, 2.0 Hz), 6.69 (1H, d, *J*=2.0 Hz) and *δ* 6.68 (1H, d, *J*=8.0 Hz), 6.66 (overlapping), 6.58 (1H, dd, *J*=8.0, 2.0 Hz)], and two methoxyl groups [*δ* 3.75 (3H, s) and 3.69 (3H, s)]. In the aliphatic region, ABMX-type signals were observed [*δ* 5.00 (1H, d, *J*=5.5 Hz), 3.89 (1H, dd, *J*=10.5, 6.5 Hz), 3.72 (1H, dd, *J*=10.5, 6.5 Hz) and 2.91 (1H, td, *J*=6.5, 5.5 Hz)]. The ^{1}H-detected heteronuclear multiple-bond connectivity (HMBC) experiment exhibited long-range correlations from H-1 (*δ* 5.00) to C-2 (*δ* 56.6), C-3 (*δ* 64.5), C-1′ (*δ* 140.1), C-2′ (*δ* 112.6), C-6′ (*δ* 120.3) and C-1″ (*δ* 131.9); and from H-2 (*δ* 2.91) to C-1 (*δ* 75.0), C-3 (*δ* 64.5), C-1′ (*δ* 140.1), C-1″ (*δ* 131.9) and C-2″ (*δ* 114.8), and C-6″ (*δ* 123.3). In addition, long-range correlations were observed between one methoxyl signal (*δ* 3.69) and the C-3′ signal (*δ* 150.2), and between another methoxyl signal (*δ* 3.75) and the C-3″ signal (*δ* 148.4). Thus, the aglycone of **4** was believed to be 1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol.[6)] The relative stereochemistry of C-1 and C-2 of **4** was determined to be *erythro* by comparing the coupling constant between H-1 and H-2 (*J*=5.5 Hz) with those of the *erythro* and *threo* isomers.[6—9)] Enzymatic hydrolysis of **4** afforded **4a**, which was confirmed to be (+)-*erythro*-1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propandiol by comparison of its ^{13}C- and ^{1}H-NMR spectroscopic data with the reported ones.[6)]

In the difference nuclear Overhauser effect (NOE) experiment, irradiation of the anomeric proton signal of the *β*-D-glucopyranosyl group [*δ* 4.94 (1H, d, *J*=8.0 Hz)] showed a NOE on the H-5′ signal (*δ* 7.06). Based on this observation, it was suggested that the *β*-D-glucopyranosyl group was attached to the C-4′ position of the aglycone. And this suggestion was supported by a long-range correlation between the anomeric proton signal of the *β*-D-glucopyranosyl group and the C-4′ signal (*δ* 147.0) in the HMBC experiment. Accordingly, the structure of **4** was determined to be *erythro*-1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol 4′-*O*-*β*-D-glucopyranoside.

The molecular formula of compound **5** was also $C_{23}H_{30}O_{11}$ on HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectroscopic data of **5** were similar to those of **4**. Enzymatic hydrolysis of **5** yielded **5a**. The ^{13}C- and ^{1}H-NMR spectroscopic data of **5a** were also similar to those of **4a**. However, the large coupling constant between H-1 and H-2 (*J*=8.5 Hz) suggested that the relative stereochemistry of C-1 and C-2 was *threo* by comparing the reported ^{1}H-NMR spectroscopic data.[6—9)] Thus, **5a** was determined to be (−)-*threo*-1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propandiol, and **5** was elucidated to be *threo*-1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol 4′-*O*-*β*-D-glucopyranoside.

The absolute stereochemistry of the aglycone moiety of **4** was considered to be C-1(*S*) and C-2(*R*) by the optical rotation value of **4a** (see Experimental section).[6)] But the absolute stereochemistry of the aglycone moiety of **5** remains to be determined.

The molecular formula of compound **6** was indicated to be $C_{23}H_{28}O_{12}$ from the results of HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectroscopic data of **6** corresponded to those of **17**,[3)] except for the data of the ester moiety. Alkaline hydrolysis of **6** afforded 4-hydroxybenzoic acid and 2-methoxy-4-[(1*S*,2*S*)-1,2,3-trihydroxypropyl]phenyl 1-*O*-*β*-D-glucopyranoside (**6a**),[3)] which were identified by HPLC analyses with the authentic samples. Thus, **6** was established to be 2-

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methoxy-4-[(1*S*,2*S*)-1,2,3-trihydroxypropyl]phenyl 1-*O*-*β*-D-[6-*O*-(4-hydroxybenzoyl)]-glucopyranoside.

Compound **7** had the molecular formula $C_{19}H_{28}O_{12}$, based on the HR-FAB-MS measurement. The ^{13}C- and ^{1}H-NMR spectra of the aglycone of **7** showed the presence of six aromatic carbon signals (δ 157.6, 152.1, 142.1, 120.2, 105.6, 101.7), two methoxyl carbon signals (δ 56.7, 56.1), an aromatic AMX system [δ 7.10 (1H, d, J=8.5 Hz), 6.58 (1H, d, J=2.5 Hz), 6.46 (1H, dd, J=8.5, 2.5 Hz)] and two methoxyl groups [δ 3.84 (3H, s) and 3.76 (3H, s)]. In the difference NOE experiments, NOEs were observed as follows: δ 3.84 and 6.58 (H-3); δ 3.76 and 6.58 (H-3), and 6.46 (H-5). These observations suggested that the aglycone of **7** was 2,4-dimethoxyphenol. Because of the observations of two anomeric carbon and proton signals at δ 104.1, 111.0 and δ 4.69 (1H, d, J=8.0 Hz), 4.98 (1H, d, J=2.0 Hz) in the ^{13}C- and ^{1}H-NMR spectra and the result of acid hydrolysis, **7** had D-glucose and D-apiose as the sugar moiety. By comparison of the ^{1}H- and ^{13}C-NMR spectroscopic data of **7** with those of **1**—**3**, the signals at δ 104.1 and 4.69 were assigned to the anomeric carbon and proton signals of *β*-D-glucopyranose, and the signals at δ 111.0 and 4.98 belonged to the anomeric carbon and proton signals of *β*-D-apiofuranose. In difference NOE experiments, NOEs between the anomeric proton signal of *β*-D-apiofuranose (δ 4.98) and the H-6 signal of *β*-D-glucopyranose (δ 3.98, 3.61) suggested that its sugar moiety was the *β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranosyl group. Moreover, a NOE between δ 4.69 (H-1 of *β*-D-glucopyranose) and 7.10 (H-6) revealed that this sugar chain was linked to C-1 of the aglycone. Thus, the structure of **7** was determined to be 2,4-dimethoxyphenyl 1-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside.

Compounds **8** and **9** were suggested to have the same molecular formulae, $C_{21}H_{28}O_{13}$, based on HR-FAB-MS. The ^{1}H-, ^{13}C-NMR, CD spectra,[2,10] and the result of acid hydrolysis suggested that these compounds were (*R*)-6-hydroxymellein diglycosides. The sugar chain of **8** was also identified as a *β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranosyl group by comparing the ^{13}C- and ^{1}H-NMR spectroscopic data with those of **1**—**3** and **7**. On the other hand, the sugar chain of **9** was identified as a *β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranosyl group, according to the result of acid hydrolysis (see Experimental) and the observation of a NOE between the anomeric proton signal of *β*-D-xylopyranose [δ 4.93 (1H, d, J=8.0 Hz)] and the H-6 signal of *β*-D-glucopyranose [δ 4.78 (1H, m)] in the difference NOE experiment. The attached position of the sugar moiety of each compound was determined to be the C-6 of (*R*)-6-hydroxymellein (**8a**) based on observation of NOEs to the H-5 and H-7 signals by irradiation of the anomeric proton signals of *β*-D-glucopyranose. Thus, the structures of **8** and **9** were elucidated as shown in Chart 1.

The ^{13}C- and ^{1}H-NMR spectra revealed that compounds **10** and **11** were 6-hydroxymellein 6-*O*-glycosides, with molecular formulae of $C_{29}H_{34}O_{15}$ and $C_{24}H_{26}O_{11}$ by the HR-FAB-MS measurements. Both compounds showed the presence of a 4-methoxybenzoyl group by the ^{1}H- and ^{13}C-NMR spectra (see Table 1 and Experimental). Alkaline hydrolysis of **10** afforded 4-methoxybenzoic acid and **8**, which were identified by HPLC comparison with the authentic samples. Similarly, compound **11** was hydrolyzed to 4-methoxybenzoic acid and (*R*)-hydroxymellein 6-*O*-*β*-D-glucopyranoside (**11a**),[11] which were also confirmed by HPLC comparison with the authentic samples. The positions of esterification in **10** and **11** were C-5 of *β*-D-apiofuranose and C-6 of *β*-D-glucopyranose, respectively, based on each HMBC measurement. Namely, long-range correlations were observed between the carbonyl carbon signal of the 4-methoxybenzoyl group (δ 167.7) and the H-5 signals of *β*-D-apiofuranose [δ 4.38 (1H, d, J=11.5 Hz), 4.33 (1H, d, J=11.5 Hz)] in **10**, and between the carbonyl carbon signal of the 4-methoxybenzoyl group (δ 167.7) and the H-6 signals of *β*-D-glucopyranose [δ 4.68 (1H, dd, J=12.0, 2.0 Hz), 4.32 (1H, dd, J=12.0, 8.0 Hz)] in **11**. Therefore, the structures of **10** and **11** were determined to be (*R*)-6-hydroxymellein 6-*O*-*β*-D-[5-*O*-(4-methoxybenzoyl)]-apiofuranosyl-(1→6)-*β*-D-glucopyranoside and (*R*)-6-hydroxymellein 6-*O*-*β*-D-[6-*O*-(4-methoxybenzoyl)]-glucopyranoside.

Compound **12** had the molecular formula $C_{28}H_{36}O_{15}$ (HR-FAB-MS), a decrease in CH_2O relative to **18**.[3] Consistency of the ^{1}H- and ^{13}C-NMR spectroscopic data of **12** with those of **18**, except for the data of the ester moiety, revealed that **12** consists of the 4-(1,2-dihydroxyethyl)-2-methoxyphenyl 1-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside and another ester moiety. The above decrease in the molecular formula of **12** was derived from a difference in the ester moiety, and this ester moiety was identified to be a 4-methoxybenzoyl group by HPLC analysis following alkaline hydrolysis. Thus, the structure of **12** was proposed to be as shown in Chart 1.

The molecular formula of compound **13** was considered to be $C_{37}H_{44}O_{18}$ on the basis of HR-FAB-MS analysis. Alkaline hydrolysis of **13** yielded 3,4-dimethoxybenzoic acid and 4-hydroxymethyl-2-methoxyphenyl 1-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**13a**).[3] The ^{1}H- and ^{13}C-NMR spectra showed signals due to two sets of 3,4-dimethoxybenzoyl groups (see Table 1 and Experimental), and the HMBC experiment exhibited long-range correlations between the carbonyl carbon signals of the 3,4-dimethoxybenzoyl groups (δ 167.6, 167.7) and the H-7 signal of vanillyl alcohol [δ 5.15 (2H, s)] and H-5 signals of *β*-D-apiofuranose [δ 4.32 (1H, d, J=11.5 Hz), 4.37 (1H, d, J=11.5 Hz)]. These observations suggested that the 3,4-dimethoxybenzoyl groups were attached at C-7 of the aglycone and C-5 of *β*-D-apiofuranose. Based on the above results, the structure of **13** was established to be 4-[[(3,4-dimethoxybenzoyl)oxy]-methyl]-2-methoxyphenyl 1-*O*-*β*-D-[5-*O*-(3,4-dimethoxybenzoyl)]-apiofuranosyl-(1→6)-*β*-D-glucopyranoside.

The molecular formula of compound **14** was indicated to be $C_{35}H_{40}O_{17}$ by HR-FAB-MS. Alkaline hydrolysis of **14** afforded 3,4-dimethoxybenzoic acid and 4-hydroxybenzoic acid, together with **13a**. The HMBC experiment of **14** suggested that the esterified positions in **14** also included C-7 of the aglycone and C-5 of *β*-D-apiofuranose. But this HMBC experiment could not illustrate which esters were attached at either position, because the carbonyl carbon signals of the two ester moieties resonated at the same chemical shift (δ 167.8). However, the production of odontoside trimethyl ether (**13b**)[12] by acid hydrolysis of **14** (see Experimental) revealed that the C-7 position of the aglycone was esterified by the 3,4-dimethoxybenzoyl group, and the remaining 4-hydroxybenzoyl group was attached at C-5 of *β*-D-apiofuranose. Therefore, **14** was determined to be 4-[[(3,4-dimethoxybenzoyl)oxy]methyl]-2-methoxyphenyl 1-*O*-*β*-D-

Table 1. ^{13}C-NMR Spectral Data of Compounds **7**—**16**

No	**7**	**8**	**9**[a)]	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Aglycone moiety										
C-1	142.1	171.3	167.0	171.3	171.3	147.3	147.9	147.9	147.9	147.9
-2	152.1	—	—	—	—	150.8	150.9	150.9	150.9	150.9
-3	101.7	77.4	75.8	77.3	77.2	112.1	114.0	114.0	114.0	114.0
-4	157.6	35.5	34.7	35.5	35.4	138.4	132.5	132.6	132.6	132.6
-5	105.6	108.5	107.7	108.5	108.2	120.2	122.3	122.4	122.3	122.3
-6	120.2	165.1[b)]	164.6[b)]	165.2[b)]	165.1[b)]	118.2	118.1	118.2	118.2	118.2
-7	—	103.6	103.4	103.6	103.7	75.6	67.4[b)]	67.5[b)]	67.3[b)]	67.0
-8	—	165.0[b)]	164.5[b)]	165.0[b)]	164.9[b)]	68.6[b)]	—	—	—	—
-9	—	104.0	103.4	103.8	103.9	—	—	—	—	—
-10	—	143.3	142.2	143.1	143.3	—	—	—	—	—
-11	—	20.8	20.5	20.8	20.8	—	—	—	—	—
-OMe	56.7	—	—	—	—	56.7	56.8	56.8	56.8	56.8
	56.1	—	—	—	—	—	—	—	—	—
Sugar moiety										
Glc-1	104.1	101.3	101.7	101.3	101.1	103.0	102.8	102.8	102.8	102.8
-2	75.0	74.7	74.7	74.7	74.7	74.9[c)]	74.9[c)]	74.9[c)]	74.9[c)]	74.9[b)]
-3	77.9	77.9	78.5	78.0	77.8	77.9	77.9	77.9	77.9	78.0
-4	71.7	71.6	71.4	71.7	72.0	71.7	71.8	71.8	71.8	71.9
-5	72.1	77.2	77.7	77.1	75.8	77.1	77.0	77.1	77.1	77.0
-6	68.7	68.8	70.1	68.8	65.3	68.7[b)]	68.9	68.8	68.8	69.0
	Api	Api	Xyl	Api		Api	Api	Api	Api	Api
-1	111.0	111.0	106.0	110.9	—	110.8	110.9	110.8	110.8	111.0
-2	78.0	78.2	75.0	78.7	—	78.6	78.7	78.6	78.6	78.7
-3	80.5	80.4	78.2	79.0	—	79.0	79.0	79.0	79.0	79.0
-4	75.0	75.0	71.1	75.1	—	75.1[c)]	75.1[c)]	75.1[c)]	75.1[c)]	75.1[b)]
-5	65.7	65.8	67.1	67.6	—	67.5	67.6[b)]	67.4[b)]	67.4[b)]	67.6
Ester moiety attached at C-5 of β-D-apiofuranose or C-6 of β-D-glucopyranose										
-1′	—	—	—	167.7	167.7	167.7	167.6[d)]	167.8	167.8[d)]	167.6
-2′	—	—	—	123.4	123.5	123.3	123.4	122.0	122.0	123.5
-3′	—	—	—	132.8	132.8	132.9	113.7	133.0	133.0	113.8
-4′	—	—	—	114.9	114.9	114.9	150.2	116.2	116.3	150.2
-5′	—	—	—	165.3	165.3	165.3	155.0[e)]	163.7	163.7	155.0
-6′	—	—	—	114.9	114.9	114.9	112.0	116.2	133.0	111.9[c)]
-7′	—	—	—	132.8	132.8	132.9	125.1[f)]	132.0	122.0	125.2
OMe	—	—	—	56.0	56.0	56.1	56.5×2	—	—	56.3×2
Ester moiety attached at C-7 of the aglycone moiety										
-1″	—	—	—	—	—	—	167.7[d)]	167.8	167.9[d)]	169.0
-2″	—	—	—	—	—	—	123.7	123.8	123.6	115.4
-3″	—	—	—	—	—	—	113.5	113.6	132.7	147.0
-4″	—	—	—	—	—	—	150.2	150.2	114.9	127.7
-5″	—	—	—	—	—	—	154.9[e)]	154.9	165.2	112.0[c)]
-6″	—	—	—	—	—	—	112.0	112.1	114.9	149.4
-7″	—	—	—	—	—	—	124.9[f)]	125.0	132.7	150.7
-8″	—	—	—	—	—	—	—	—	—	116.5
-9″	—	—	—	—	—	—	—	—	—	124.2
OMe	—	—	—	—	—	—	56.5×2	56.8×2	56.0	56.3

Measured in MeOH-d_4 solution at 35 °C. *a*) Measured in pyridine-d_5 solution at 35 °C. Glc: β-D-glucopyranosyl, Api: β-D-apiofuranosyl, Xyl: β-D-xylopyranosyl. *b*—*f*) Interchangeable in each column.

[5-*O*-(4-hydroxybenzoyl)]-apiofuranosyl-(1→6)-β-D-glucopyranoside.

Compounds **15** and **16** had the molecular formulae $C_{34}H_{38}O_{16}$ and $C_{38}H_{44}O_{18}$, respectively, on the basis of HR-FAB-MS analyses. Alkaline hydrolysis yielded 4-hydroxybenzoic acid and 4-methoxybenzoic acid from **15**, and 3,4-dimethoxybenzoic acid and ferulic acid from **16**, together with **13a**. Compounds **15** and **16** were considered to be the congeners of **14** and **13**, according to ^{1}H- and ^{13}C-NMR spectroscopic data. Compound **15** was decomposed into **15b** by acid hydrolysis (see Experimental). The ^{1}H-NMR spectrum of **15b** showed signals due to the 4-hydroxymethyl-2-methoxyphenyl 1-*O*-β-D-glucopyranose and 4-methoxybenzoyl group. Similarity of the ^{1}H-NMR spectroscopic data of **15b** with those of **13b** corroborated that **15b** was 4-[[(4-methoxybenzoyl)oxy]methyl-2-methoxyphenyl 1-*O*-β-D-glucopyranoside. Thus, the structure of **15** was concluded to be 4-[[(4-methoxybenzoyl)oxy]methyl]-2-methoxyphenyl 1-*O*-β-D-[5-*O*-(4-hydroxybenzoyl)]-apiofuranosyl-(1→6)-β-D-glucopyranoside. The ^{1}H-detected heteronuclear multiple quantum coherency (HMQC) and HMBC experiments of **16** confirmed that the signals at δ 169.0 and 167.6 were assigned to the carbonyl carbons of the feruloyl and 3,4-dimethoxybenzoyl groups, respectively. Moreover, the HMBC spectrum showed long-range correlations between the carbonyl carbon signal of the feruloyl group (δ 169.0) and the H-7 signal of the aglycone [δ 5.05 (2H, s)], and between the carbonyl carbon signal of the 3,4-dimethoxybenzoyl group (δ 167.6) and the H-5 signals of apiofuranose [δ 4.38 (1H, d, J=11.0 Hz), 4.33 (1H, d, J=11.0 Hz)]. Thus, the

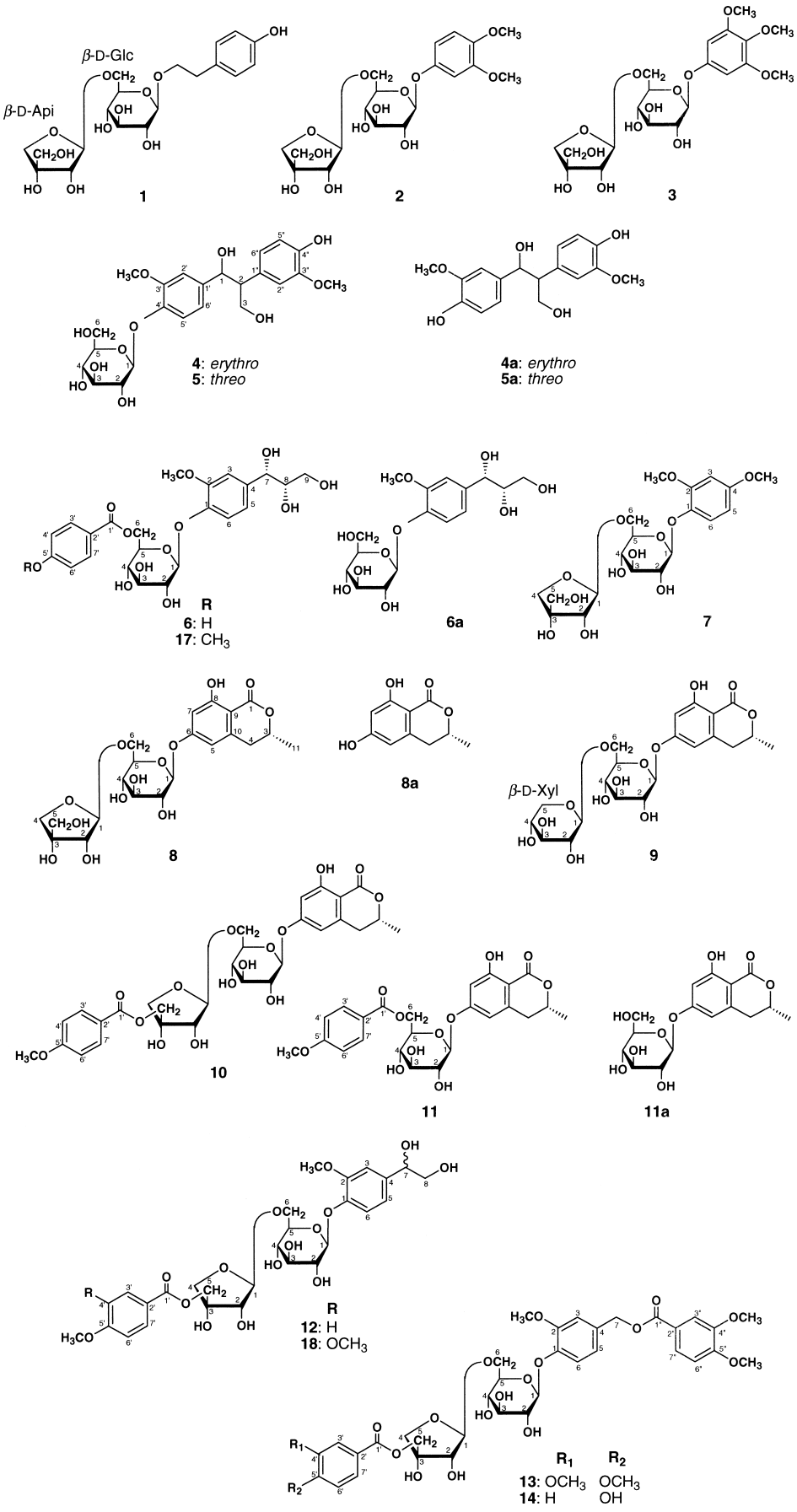
β-D-Glc
β-D-Api
1
2
3
4: erythro
5: threo
4a: erythro
5a: threo
R
6: H
17: CH3
6a
7
8
8a
β-D-Xyl
9
10
11
11a
12: H
18: OCH3
R1 R2
13: OCH3 OCH3
14: H OH

Chart 1

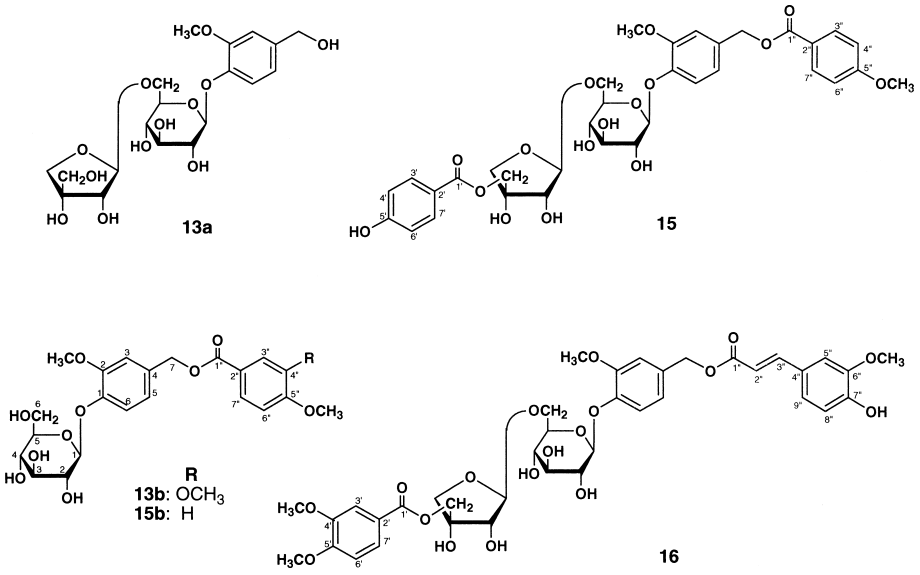

Chart 1 (continued)

established structure of **16** was as shown in Chart 1.

In present and previous papers,[2,3] the polar fractions of the MeOH extract of the bark of *T. impetiginosa* afforded many kinds of constituents: iridoid glucosides, phenylethanoide glycosides, phenolic glycosides, isocoumarine glycosides and lignan glycosides. *Tabebuia* spp. has been shown to contain many iridoid glucosides: ajugol and its acylated derivatives. From the results of a series of investigations, the main compounds of the bark of *T. impetiginosa* were also presumed to be phenolic glycosides, as well as iridoid glucosides. The antitumor, antiparasitic, antibacterial, antifungal and antiviral activities of the naphthoquinone derivatives from *Tabebuia* spp. were reported previously.[13] Because a decoction of the bark of *T. impetiginosa* has been used as a folk medicine for treating diabetes, ulcers and syphilis,[1] we are interested in the biological activities of not only the naphthoquinone derivatives but also other constituents yielded from the polar fractions of the MeOH extract of this plant.

Experimental

General Procedure Instrumental analysis was carried out as described previously.[2]

Extraction and Isolation Extraction of the bark of *T. impetiginosa* is described in the previous paper.[2] The MeOH–H_2O (1 : 1) eluate from the porous polymer gel (Mitsubishi Diaion HP-20) column was concentrated, and the residue (27.0 g) chromatographed on a silica gel column with a $CHCl_3$–MeOH–EtOAc–H_2O system (46 : 15 : 35 : 2) to be separated into six fractions (A (0.9 g), B (5.5 g), C (3.1 g), D (4.5 g), E (3.1 g), and F (10.5 g)). Using semi-preparative HPLC (Develosil-ODS-15/30, -C8 and YMC-ODS: 7.5—17% MeCN in water and 15—25% MeOH in water), fraction D (2.3 g) afforded compounds **2** (137 mg), **3** (109 mg), **7** (7 mg), **8** (18 mg) and **12** (5 mg), and fraction E (2.0 g) afforded compounds **1** (16 mg), **4** (3 mg), **5** (5 mg), **6** (5 mg) and **9** (67 mg).

Similarly, the MeOH eluate from the porous polymer gel column was concentrated and the residue (2.9 g) chromatographed on a silica gel column with a $CHCl_3$–MeOH system (95 : 5—9 : 1) to be separated into four fractions (A (0.35 g), B (0.19 g), C (0.53 g) and D (1.33 g)). Using semi-preparative HPLC (YMC-ODS: 30—32.5% MeCN in water and 50—57.5% MeOH in water), fraction B afforded compounds **11** (5 mg) and **13** (7 mg), and fraction C afforded compounds **10** (5 mg), **13** (16 mg), **14** (9 mg), **15** (7 mg) and **16** (5 mg).

Compound **4**: Amorphous powder. $[\alpha]_D^{22}$ +23° (c=0.33, MeOH). UV λ_{max} (MeOH) nm (log ε): 203 (4.66), 225 (sh), 278 (3.66). FAB-MS m/z: 505 $[M+Na]^+$. HR-FAB-MS m/z: 505.1679 (Calcd for $C_{23}H_{30}O_{11}Na$: 505.1686). ^{13}C-NMR (MeOH-d_4 at 35°C) δ: 150.2 (C-3′), 148.4 (C-3″), 147.0 (C-4′), 146.3 (C-4″), 140.1 (C-1′), 131.9 (C-1″), 123.3 (C-6″), 120.3 (C-6′), 117.4 (C-5′), 115.7 (C-5″), 114.8 (C-2″), 112.6 (C-2′), 103.0 (C_{Glc}-1), 78.2, 77.9 (C_{Glc}-3, C_{Glc}-5), 75.0, 74.9 (C-1, C_{Glc}-2), 71.4 (C_{Glc}-4), 64.5 (C-3), 62.6 (C_{Glc}-6), 56.6×2 (C-2, C-3′-OMe), 56.5 (C-3″-OMe). ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 7.06 (1H, d, 8.0, H-5′), 6.76 (1H, dd, 8.0, 2.0, H-6′), 6.69 (1H, d, 2.0, H-2′), 6.68 (1H, d, 8.0, H-5″), 6.66 (overlapping, H-2″), 6.58 (1H, dd, 8.0, 2.0, H-6″), 5.00 (1H, d, 5.5, H-1), 4.94 (1H, d, 8.0, H_{Glc}-1), 3.89 (1H, dd, 10.5, 6.5, H-3), 3.86 (1H, dd, 12.0, 1.5, H_{Glc}-6), 3.75 (3H, s, C-3″-OMe), 3.72 (1H, dd, 10.5, 6.5, H-3), 3.69 (3H, s, C-3′-OMe), 3.69 (overlapping, H_{Glc}-6), 2.91 (1H, td, 6.5, 5.5, H-2).

Compound **5**: Amorphous powder. $[\alpha]_D^{22}$ −96° (c=0.51, MeOH). UV λ_{max} (MeOH) nm (log ε): 202 (4.67), 223 (sh), 278 (3.66). FAB-MS m/z: 505 $[M+Na]^+$. HR-FAB-MS m/z: 505.1678 (Calcd for $C_{23}H_{30}O_{11}Na$: 505.1686). ^{13}C-NMR (MeOH-d_4 at 35 °C) δ: 150.1 (C-3′), 148.5 (C-3″), 147.0 (C-4′), 146.2 (C-4″), 139.7 (C-1′), 132.6 (C-1″), 122.6 (C-6″), 120.7 (C-6′), 117.3 (C-5′), 115.8 (C-5″), 114.4 (C-2″), 113.0 (C-2′), 103.0 (C_{Glc}-1), 78.2, 77.8 (C_{Glc}-3, C_{Glc}-5), 78.0 (C-1), 74.9 (C_{Glc}-2), 71.4 (C_{Glc}-4), 65.7 (C-3), 62.5 (C_{Glc}-6), 56.6 (C-3′-OMe), 56.5 (C-3″-OMe), 56.3 (C-2). ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 7.00 (1H, d, 8.0, H-5′), 6.73 (1H, dd, 8.0, 2.0, H-6′), 6.66 (1H, d, 2.0, H-2′), 6.61 (1H, d, 8.0, H-5″), 6.50 (overlapping, H-2″), 6.50 (1H, dd, 8.0, 2.0, H-6″), 4.84 (1H, d, 8.5, H-1), 4.80 (1H, d, 8.0, H_{Glc}-1), 4.09 (1H, dd, 10.5, 6.5, H-3), 3.91 (1H, dd, 10.5, 6.5, H-3), 3.84 (1H, dd, 12.0, 2.0, H_{Glc}-6), 3.70 (3H, s, C-3″-OMe), 3.69 (3H, s, C-3′-OMe), 3.67 (dd, 12.0, 5.5, H_{Glc}-6), 2.99 (1H, td, 6.5, 8.5, H-2).

Compound **6**: Amorphous powder. $[\alpha]_D^{22}$ −18° (c=0.33, MeOH). λ_{max} (MeOH) nm (log ε): 201 (4.80), 226 (4.03), 257 (4.13). FAB-MS m/z: 519 $[M+Na]^+$. HR-FAB-MS m/z: 519.1469 (Calcd for $C_{23}H_{28}O_{12}Na$: 519.1479). ^{13}C-NMR (MeOH-d_4 at 35 °C) δ: 168.0 (C-1′), 163.9 (C-5′), 150.7 (C-2), 147.2 (C-1), 138.5 (C-4), 132.9×2 (C-3′, C-7′), 122.0 (C-2′), 120.5 (C-5), 117.8 (C-6), 116.4×2 (C-4′, C-6′), 112.5 (C-3), 102.8 (C_{Glc}-1), 77.8 (C_{Glc}-3), 77.4 (C-8), 75.7 (C_{Glc}-5), 75.0, 74.9 (C-7, C_{Glc}-2), 72.0 (C_{Glc}-4), 64.9 (C_{Glc}-6), 64.3 (C-9), 56.7 (C-2-OMe). ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 7.89 (2H, d, 9.0, H-3′, H-7′), 7.07 (1H, d, 8.0, H-6), 7.06 (1H, d, 2.0, H-3), 6.85 (2H, d, 9.0, H-4′, H-6′), 6.73 (1H, dd, 8.0, 2.0, H-5), 4.88 (1H, d, 8.0, H_{Glc}-1), 4.65 (1H, dd, 12.0, 2.5, H_{Glc}-6), 4.54 (1H, d, 6.0, H-7), 4.35 (1H, dd, 12.0, 7.5, H_{Glc}-6), 3.85 (3H, s, C-2-OMe), 3.73 (1H, m, H_{Glc}-5), 3.64 (1H, td, 6.0, 4.0, H-8), 3.54 (1H, t, 8.0, H_{Glc}-2), 3.50 (1H, t, 8.0, H_{Glc}-3), 3.49 (1H, dd, 11.5, 4.0, H-9), 3.43 (1H, t, 8.0, H_{Glc}-4), 3.37 (1H, dd, 11.5, 6.0, H-9).

Compound **7**: Amorphous powder. $[\alpha]_D^{22}$ −81.3° (c=1.36, MeOH). UV λ_{max} (MeOH) nm (log ε): 202 (4.42), 223 (3.85), 279 (3.42). FAB-MS m/z: 471 $[M+Na]^+$. HR-FAB-MS m/z: 471.1461 (Calcd for $C_{19}H_{28}O_{12}Na$: 471.1479). ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 7.10 (1H, d, 8.5, H-6), 6.58 (1H, d, 2.5, H-3), 6.46 (1H, dd, 8.5, 2.5, H-5), 4.98 (1H, d, 2.0, H_{Api}-1), 4.69 (1H, d, 8.0, H_{Glc}-1), 3.98 (1H, dd, 11.5, 2.0, H_{Glc}-6), 3.93 (1H, d, 9.5, H_{Api}-4), 3.88 (1H, d, 2.0, H_{Api}-2), 3.84 (3H, s, C-2-OMe), 3.76 (3H, s, C-4-OMe), 3.74 (1H, d, 9.5, H_{Api}-4), 3.61 (1H, dd, 11.5, 6.5, H_{Glc}-6), 3.57 (2H, s, H_{Api}-5), 3.48 (1H, m, H_{Glc}-5), 3.44 (1H, t, 8.0, H_{Glc}-2), 3.42 (1H, t, 8.0, H_{Glc}-3),

3.33 (overlapping, H_{Glc}-4).

Compound **8**: Amorphous powder. $[\alpha]_D^{22}$ −139° (c=1.78, MeOH). UV λ_{max} (MeOH) nm (log ε): 214 (4.35), 261 (4.05), 303 (3.62). FAB-MS m/z: 511 $[M+Na]^+$. HR-FAB-MS m/z: 511.1417 (Calcd for $C_{21}H_{28}O_{13}Na$: 511.1427). CD $\Delta\varepsilon$ (nm): −5.59 (226), −5.59 (264), −0.86 (302) (c=0.0532, MeOH). ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 6.55 (1H, d, 2.0, H-7), 6.50 (1H, br s, H-5), 4.96 (1H, d, 2.0, H_{Api}-1), 4.95 (1H, d, 8.0, H_{Glc}-1), 4.70 (1H, m, H-3), 4.02 (1H, br d, 11.5, H_{Glc}-6), 3.99 (1H, d, 9.5, H_{Api}-4), 3.91 (1H, d, 2.0, H_{Api}-2), 3.76 (1H, d, 9.5, H_{Api}-4), 3.64 (1H, m, H_{Glc}-5), 3.60 (overlapping, H_{Glc}-6), 3.59 (2H, s, H_{Api}-5), 2.98 (1H, dd, 16.5, 4.0, H-4), 2.90 (1H, dd, 16.5, 10.5, H-4), 1.47 (3H, d, 6.5, H-11).

Compound **9**: Amorphous powder. $[\alpha]_D^{22}$ −109° (c=0.83, MeOH). UV λ_{max} (MeOH) nm (log ε): 215 (4.37), 261 (4.07), 301 (3.65). FAB-MS m/z: 489 $[M+H]^+$, 511 $[M+Na]^+$. HR-FAB-MS m/z: 489.1603 (Calcd for $C_{21}H_{29}O_{13}$: 489.1608). CD $\Delta\varepsilon$ (nm): −4.77 (226), −5.30 (264), −0.90 (302) (c=0.0559, MeOH). ^{1}H-NMR (pyridine-d_5 at 35 °C) δ: 6.97 (1H, d, 2.0, H-7), 6.70 (1H, br s, H-5), 5.61 (1H, d, 8.0, H_{Glc}-1), 4.93 (1H, d, 8.0, H_{Xyl}-1), 4.78 (1H, m, H_{Glc}-6), 4.53 (1H, m, H-3), 4.32 (overlapping, H_{Glc}-6), 4.31 (1H, dd, 11.5, 5.5, H_{Xyl}-5), 4.28 (1H, t, 8.0, H_{Glc}-3), 4.24 (1H, t, 8.0, H_{Glc}-2), 4.20 (1H, m, H_{Xyl}-4), 4.19 (1H, t, 8.0, H_{Glc}-4), 4.08 (1H, t, 8.0, H_{Xyl}-3), 4.02 (1H, t, 8.0, H_{Xyl}-2), 3.61 (1H, dd, 11.5, 10.0, H_{Xyl}-5), 2.75 (overlapping, H-4), 1.24 (3H, d, 6.0, H-11).

Compound **10**: Amorphous powder. $[\alpha]_D^{25}$ −109° (c=0.47, MeOH). UV λ_{max} (MeOH) nm (log ε): 201 (4.88), 208 (4.55), 257 (4.43), 302 (3.66). FAB-MS m/z: 645 $[M+Na]^+$. HR-FAB-MS m/z: 645.1798 (Calcd for $C_{29}H_{34}O_{15}Na$: 645.1795). ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 7.95 (2H, d, 9.0, H-3′, H-7′), 6.96 (2H, d, 9.0, H-4′, H-6′), 6.53 (1H, d, 2.0, H-7), 6.42 (1H, br s, H-5), 5.00 (1H, d, 2.0, H_{Api}-1), 4.94 (1H, d, 8.0, H_{Glc}-1), 4.62 (1H, m, H-3), 4.38 (1H, d, 11.5, H_{Api}-5), 4.33 (1H, d, 11.5, H_{Api}-5), 4.14 (1H, d, 9.5, H_{Api}-4), 4.05 (1H, br d, 11.0, H_{Glc}-6), 4.02 (1H, d, 2.0, H_{Api}-2), 3.87 (1H, d, 9.5, H_{Api}-4), 3.86 (3H, s, C-5′-OMe), 3.62 (1H, dd, 11.0, 6.0, H_{Glc}-6), 3.66 (1H, m, H_{Glc}-5), 2.88 (1H, dd, 16.5, 4.0, H-4), 2.82 (1H, dd, 16.5, 10.5, H-4), 1.43 (3H, d, 6.5, H-11).

Compound **11**: Amorphous powder. $[\alpha]_D^{24}$ −84° (c=0.44, MeOH). UV λ_{max} (MeOH) nm (log ε): 213 (4.50), 256 (4.41), 302 (3.63). FAB-MS m/z: 513 $[M+Na]^+$. HR-FAB-MS m/z: 513.1367 (Calcd for $C_{24}H_{26}O_{11}Na$: 513.1373). ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 7.97 (2H, d, 9.0, H-3′, H-7′), 6.95 (2H, d, 9.0, H-4′, H-6′), 6.53 (1H, d, 2.0, H-7), 6.41 (1H, br d, 2.0, H-5), 5.03 (1H, d, 8.0, H_{Glc}-1), 4.68 (1H, dd, 12.0, 2.0, H_{Glc}-6), 4.60 (1H, m, H-3), 4.32 (1H, dd, 12.0, 8.0, H_{Glc}-6), 3.87 (3H, s, C-5′-OMe), 3.86 (1H, m, H_{Glc}-5), 3.41 (1H, t, 8.0, H_{Glc}-4), 2.73 (1H, dd, 16.5, 3.5, H-4), 2.54 (1H, dd, 16.5, 11.5, H-4), 1.40 (3H, d, 6.5, H-11).

Compound **12**: Amorphous powder. $[\alpha]_D^{22}$ −71° (c=0.49, MeOH). UV λ_{max} (MeOH) nm (log ε): 225 (4.05), 257 (4.25). FAB-MS m/z: 635 $[M+Na]^+$. HR-FAB-MS m/z: 635.1950 (Calcd for $C_{28}H_{36}O_{15}Na$: 635.1952). ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 8.01 (2H, d, 9.0, H-3′, H-7′), 7.09 (1H, d, 8.0, H-6), 7.03 (1H, d, 2.0, H-3), 6.99 (2H, d, 9.0, H-4′, H-6′), 6.84 (1H, dd, 8.0, 2.0, H-5), 5.00 (1H, d, 2.0, H_{Api}-1), 4.80 (1H, d, 8.0, H_{Glc}-1), 4.59 (1H, dd, 7.0, 5.0, H-7), 4.38 (1H, d, 11.5, H_{Api}-5), 4.34 (1H, d, 11.5, H_{Api}-5), 4.06 (1H, d, 9.5, H_{Api}-4), 4.02 (1H, dd, 11.5, 2.0, H_{Glc}-6), 3.98 (1H, d, 2.0, H_{Api}-2), 3.86 (3H, s, C-5′-OMe), 3.85 (overlapping, H_{Api}-4), 3.85 (3H, s, C-2-OMe), 3.61 (1H, dd, 11.0, 6.0, H_{Glc}-6), 3.59 (1H, dd, 11.5, 5.0, H-8), 3.55 (1H, dd, 11.5, 7.0, H-8), 3.52 (1H, m, H_{Glc}-5), 3.49 (1H, t, 8.0, H_{Glc}-2), 3.44 (1H, t, 8.0, H_{Glc}-3), 3.35 (1H, t, 8.0, H_{Glc}-4).

Compound **13**: Amorphous powder. $[\alpha]_D^{23}$ −51.8° (c=1.60, MeOH). UV λ_{max} (MeOH) nm (log ε): 203 (5.01), 218 (4.91), 261 (4.60), 286 (4.31). FAB-MS m/z: 799 $[M+Na]^+$. HR-FAB-MS m/z: 799.2401 (Calcd for $C_{37}H_{44}O_{18}Na$: 799.2425). ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 7.65 (1H, dd, 8.0, 2.0, H-7′), 7.61 (1H, dd, 8.0, 2.0, H-7″), 7.51 (1H, d, 2.0, H-3′), 7.50 (1H, d, 2.0, H-3″), 7.13 (1H, d, 8.0, H-6), 7.04 (1H, d, 2.0, H-3), 6.93 (1H, d, 8.0, H-6′), 6.95 (1H, d, 8.0, H-6″), 6.90 (1H, dd, 8.0, 2.0, H-5), 5.15 (2H, s, H-7), 5.00 (1H, d, 2.0, H_{Api}-1), 4.83 (1H, d, 8.0, H_{Glc}-1), 4.37 (1H, d, 11.5, H_{Api}-5), 4.32 (1H, d, 11.5, H_{Api}-5), 4.06 (1H, d, 10.0, H_{Api}-4), 4.03 (1H, br d, 10.5, H_{Glc}-6), 4.00 (1H, d, 2.0, H_{Api}-2), 3.86 (overlapping, H_{Api}-4), 3.86 (3H, s, C-5″-OMe), 3.84 (3H, s, C-2-OMe), 3.84 (3H, s, C-5′-OMe), 3.82 (3H, s, C-4″-OMe), 3.79 (3H, s, C-4′-OMe), 3.60 (overlapping, H_{Glc}-6), 3.57 (1H, m, H_{Glc}-5), 3.50 (1H, t, 8.0, H_{Glc}-2), 3.45 (1H, t, 8.0, H_{Glc}-3), 3.32 (1H, t, 8.0, H_{Glc}-4).

Compound **14**: Amorphous powder. $[\alpha]_D^{24}$ −61° (c=0.82, MeOH). λ_{max} (MeOH) nm (log ε): 201 (4.96), 213 (sh), 259 (4.46), 290 (sh). FAB-MS m/z: 755 $[M+Na]^+$. HR-FAB-MS m/z: 755.2128 (Calcd for $C_{35}H_{40}O_{17}Na$: 755.2163). ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 7.88 (2H, d, 9.0, H-3′, H-7′), 7.65 (1H, dd, 8.0, 2.0, H-7″), 7.53 (1H, d, 2.0, H-3″), 7.14 (1H, d, 8.0, H-6), 7.06 (1H, d, 2.0, H-3), 6.98 (1H, d, 8.0, H-6″), 6.92 (1H, dd, 8.0, 2.0, H-6), 6.79 (2H, d, 9.0, H-4′, H-6′), 5.19 (2H, s, H-7), 4.99 (1H, d, 2.0, H_{Api}-1), 4.85 (1H, d, 8.0, H_{Glc}-1), 4.33 (1H, d, 11.5, H_{Api}-5), 4.30 (1H, d, 11.5, H_{Api}-5), 4.04 (1H, d, 10.0, H_{Api}-4), 4.03 (1H, br d, 11.0, H_{Glc}-6), 3.97 (1H, d, 2.0, H_{Api}-2), 3.84 (1H, d, 10.0, H_{Api}-4), 3.87 (3H, s, C-5″-OMe), 3.85 (3H, s, C-2-OMe), 3.84 (3H, s, C-4″-OMe), 3.61 (1H, dd, 11.0, 6.5, H_{Glc}-6), 3.57 (1H, m, H_{Glc}-5), 3.51 (1H, t, 8.0, H_{Glc}-2), 3.45 (1H, t, 8.0, H_{Glc}-3).

Compound **15**: Amorphous powder. $[\alpha]_D^{24}$ −60° (c=0.73, MeOH). UV λ_{max} (MeOH) nm (log ε): 202 (4.99), 256 (4.50). FAB-MS m/z: 725 $[M+Na]^+$. HR-FAB-MS m/z: 725.2050 (Calcd for $C_{34}H_{38}O_{16}Na$: 725.2058). ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 7.95 (2H, d, 8.0, H-3″, H-7″), 7.89 (2H, d, 8.0, H-3′, H-7′), 7.13 (1H, d, 8.0, H-6), 7.05 (1H, d, 2.0, H-3), 6.95 (2H, d, 8.0, H-4″, H-6″), 6.92 (1H, dd, 8.0, 2.0, H-6), 6.80 (2H, d, 8.0, H-4′, H-6′), 5.18 (2H, s, H-7), 5.00 (1H, d, 2.0, H_{Api}-1), 4.85 (1H, d, 8.0, H_{Glc}-1), 4.35 (1H, d, 11.5, H_{Api}-5), 4.30 (1H, d, 11.5, H_{Api}-5), 4.05 (1H, d, 10.0, H_{Api}-4), 4.03 (1H, br d, 11.5, H_{Glc}-6), 3.98 (1H, d, 2.0, H_{Api}-2), 3.85 (3H, s, C-2-OMe), 3.84 (1H, d, 10.0, H_{Api}-4), 3.83 (3H, s, C-5″-OMe), 3.61 (1H, dd, 11.5, 6.0, H_{Glc}-6), 3.56 (1H, m, H_{Glc}-5), 3.50 (1H, t, 8.0, H_{Glc}-2), 3.45 (1H, t, 8.0, H_{Glc}-3), 3.33 (1H, t, 8.0, H_{Glc}-4).

Compound **16**: Amorphous powder. $[\alpha]_D^{23}$ −52° (c=0.48, MeOH). UV λ_{max} (MeOH) nm (log ε): 201 (4.82), 218 (4.56), 253 (4.16), 261 (4.17), 295 (4.20), 325 (4.20). FAB-MS m/z: 811 $[M+Na]^+$. HR-FAB-MS m/z: 811.2427 (Calcd for $C_{38}H_{44}O_{18}Na$: 811.2425). ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 7.68 (1H, dd, 8.0, 2.0, H-7′), 7.60 (1H, d, 16.0, H-3″), 7.54 (1H, 2.0, H-3′), 7.17 (1H, d, 2.0, H-5″), 7.11 (1H, d, 8.0, H-6), 7.05 (1H, dd, 8.0, 2.0, H-9″), 7.00 (1H, d, 2.0, H-3), 6.96 (1H, d, 8.0, H-6′), 6.85 (1H, dd, 8.0, 2.0, H-5), 6.79 (1H, d, 8.0, H-8″), 6.35 (1H, d, 16.0, H-2″), 5.05 (2H, s, H-7), 5.00 (1H, d, 2.0, H_{Api}-1), 4.83 (1H, d, 8.0, H_{Glc}-1), 4.38 (1H, d, 11.0, H_{Api}-5), 4.33 (1H, d, 11.0, H_{Api}-5), 4.06 (1H, d, 9.5, H_{Api}-4), 4.04 (1H, dd, 10.5, 1.5, H_{Glc}-6), 4.00 (1H, d, 2.0, H_{Api}-2), 3.86 (overlapping, H_{Api}-4), 3.87 (3H, s, C-6″-OMe), 3.85 (3H, s, C-5′-OMe), 3.84 (3H, s, C-2-OMe), 3.80 (3H, s, C-4′-OMe), 3.60 (overlapping, H_{Glc}-6), 3.57 (1H, m, H_{Glc}-5), 3.50 (1H, t, 8.0, H_{Glc}-2), 3.44 (1H, t, 8.0, H_{Glc}-3).

Enzymatic Hydrolysis of Compounds 4 and 5 Compounds **4** (2 mg) and **5** (3 mg) were dissolved in EtOH (50 μl) and H_2O (0.50 ml), respectively, then cellulase (Sigma Chem. Co.) (*ca.* 20 mg) was added into each solution. The mixtures were stirred at 40 °C for 1 d. After hydrolysis, the reaction mixtures were diluted with H_2O and extracted with EtOAc. Compounds **4a** (1 mg) and **5a** (1 mg) were purified from the residue of each EtOAc layer using HPLC (column, YMC-ODS 10 mm×25 cm; solvent, 10% MeCN in water). Compounds **4a** and **5a** were identified to be (+)-*erythro*-1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propandiol and (−)-*threo*-1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propandiol, respectively, on the basis of the ^{13}C-, ^{1}H-NMR spectroscopic data and the optical rotation values.[6)]

Compound **4a**: $[\alpha]_D^{22}$ +60° (c=0.12, MeOH). (lit: $[\alpha]_D^{25}$ +41° (c=0.7, MeOH)[6)]) UV λ_{max} (MeOH) nm (log ε): 207 (4.80), 229 (4.02), 280 (3.63). FAB-MS m/z: 343 $[M+Na]^+$. HR-FAB-MS m/z: 343.1161 (Calcd for $C_{17}H_{20}O_6Na$: 343.1158). ^{13}C-NMR (pyridine-d_5+D_2O) δ: 148.2, 148.1 (C-3′, C-3″), 146.9×2 (C-4′, C-4″), 137.1 (C-1′), 132.7 (C-1″), 120.3 (C-6′), 115.9, 115.7 (C-5′, C-5″), 115.0 (C-2″), 111.9 (C-2′), 74.6 (C-1), 64.6 (C-3), 57.0 (C-2), 56.0, 55.9 (C-3′-OMe, C-3″-OMe). The signal of C-6″ was overlapped with the signal of pyridine-d_5. ^{1}H-NMR (pyridine-d_5+D_2O) δ: 7.28 (1H, d, 2.0, H-2″), 7.26 (1H, d, 1.5, H-2′), 7.20 (2H, overlapping, H-5′, H-6′). 7.19 (1H, d, 8.0, H-5″), 7.15 (1H, dd, 8.0, 2.0, H-6″), 5.73 (1H, d, 5.5, H-1), 4.58 (1H, dd, 10.5, 6.5, H-3), 4.34 (1H, dd, 10.5, 6.5, H-3), 3.68 (3H, s, C-3″-OMe), 3.64 (3H, s, C-3′-OMe), 3.60 (1H, td, 6.5, 5.5, H-2). The reported ^{13}C- and ^{1}H-NMR spectroscopic data were described in ref. 6.

Compound **5a**: $[\alpha]_D^{22}$ −74° (c=0.14, MeOH). UV λ_{max} (MeOH) nm (log ε): 209 (4.82), 225 (4.09), 280 (3.71). FAB-MS m/z: 343 $[M+Na]^+$. HR-FAB-MS m/z: 343.1150 (Calcd for $C_{17}H_{20}O_6Na$: 343.1158). ^{13}C-NMR (pyridine-d_5+D_2O) δ: 148.2, 148.1 (C-3′, C-3″), 146.9, 146.6 (C-4′, C-4″), 137.8 (C-1′), 133.1 (C-1″), 122.5 (C-6″), 120.6 (C-6′), 116.1, 115.7 (C-5′, C-5″), 114.3 (C-2″), 112.0 (C-2′), 77.7 (C-1), 65.7 (C-3), 56.3 (C-2), 56.0, 55.9 (C-3′-OMe, C-3″-OMe). ^{1}H-NMR (pyridine-d_5+D_2O) δ: 7.23 (overlapping with the signal of pyridine-d_5, H-2′ or H-2″), 7.16 (1H, dd, 8.0, 2.0, H-6′ or H-6″), 7.12 (2H, d, 8.0, H-5′, H-5″). 7.08 (1H, d, 2.0, H-2″ or H-2′), 7.07 (1H, dd, 8.0, 2.0, H-6″ or H-6′), 5.52 (1H, d, 8.0, H-1), 4.69 (1H, dd, 11.0, 6.5, H-3), 4.55 (1H, dd, 11.0, 6.5, H-3), 3.66 (6H, s, C-3′-OMe, C-3″-OMe), 3.66 (overlapping, H-2). A part of the ^{13}C- and ^{1}H-NMR spectroscopic data were reported in ref. 6.

Mild Acid Hydrolysis of Compounds 13—15 Compounds **13** (4 mg) and **15** (3 mg) were dissolved in dioxane (400 μl) and 2 M HCl (400 μl), and heated at 100 °C for 15 min. The reaction mixture was diluted with H_2O and

extracted with EtOAc. The EtOAc layer was dried with Na_2SO_4 anhydride overnight, and evaporated off *in vacuo* after removing Na_2SO_4 anhydride by filtration. When the residue of the EtOAc layer was chromatographed by HPLC (column, YMC-ODS 10 mm×25 cm; solvent, 47.5% MeOH in water and 50% MeOH in water), **13b** (0.9 mg) and **15b** (0.6 mg) were afforded. Compounds **13b** and **15b** were identified to be odontoside trimethyl ether[12] and 4-[[(4-methoxybenzoyl)oxy]methyl]-2-methoxyphenyl 1-*O*-*β*-D-glucopyranoside, respectively, on the basis of the ^{1}H-NMR spectroscopic data.

Compound **13b**: $[\alpha]_D^{23}$ −37° (*c*=0.09, MeOH) (lit. $[\alpha]_D^{24}$ −62° (*c*=0.13, MeOH)[11]). FAB-MS *m/z*: 503 $[M+Na]^+$. HR-FAB-MS *m/z*: 503.1538 (Calcd for $C_{23}H_{28}O_{11}Na$: 503.1529). λ_{max} (MeOH) nm (log ε): 203 (4.68), 219 (4.47), 262 (4.12), 282 (3.88). ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 7.68 (1H, dd, 8.0, 2.0, H-7″), 7.56 (1H, d, 2.0, H-3″), 7.18 (1H, d, 8.0, H-6), 7.12 (1H, d, 2.0, H-3), 7.02 (1H, d, 8.0, H-6″), 7.01 (1H, dd, 8.0, 2.0, H-5), 5.28 (2H, s, H-7), 4.91 (1H, d, 8.0, H_{Glc}-1), 3.89 (3H, s, C-5″-OMe), 3.88 (3H, s, C-2-OMe), 3.87 (overlapping, H_{Glc}-6), 3.86 (3H, s, C-4″-OMe), 3.68 (1H, dd, 12.0, 5.5, H_{Glc}-6), 3.50 (1H, t, 8.0, H_{Glc}-2), 3.46 (1H, t, 8.0, H_{Glc}-3).

Compound **15b**: $[\alpha]_D^{23}$ −51° (*c*=0.06, MeOH). FAB-MS *m/z*: 473 $[M+Na]^+$. HR-FAB-MS *m/z*: 473.1415 (Calcd for $C_{22}H_{26}O_{10}Na$: 473.1423). UV λ_{max} (MeOH) nm (log ε): 202 (4.80), 229 (4.11), 256 (4.35). ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 7.98 (2H, d, 9.0, H-3″, H-7″), 7.18 (1H, d, 8.0, H-6), 7.11 (1H, d, 2.0, H-3), 7.01 (1H, dd, 8.0, 2.0, H-5), 6.98 (2H, d, 9.0, H-4″, H-6″), 5.27 (2H, s, H-7), 4.91 (1H, d, 8.0, H_{Glc}-1), 3.87 (3H, s, C-2-OMe), 3.87 (overlapping, H_{Glc}-6), 3.86 (3H, s, C-5″-OMe), 3.68 (1H, dd, 12.0, 5.5, H_{Glc}-6), 3.50 (1H, t, 8.0, H_{Glc}-2), 3.46 (1H, t, 8.0, H_{Glc}-3).

Compound **14** (*ca* 0.5 mg) was dissolved in dioxane and 2 M HCl (50 μl each), then heated at 100 °C for 15 min. After hydrolysis, the reaction mixture was partitioned between EtOAc and H_2O. HPLC analysis detected **13b** from the residue of the EtOAc layer. HPLC conditions: column, YMC-ODS 4.6 mm×25 cm; flow rate, 1.0 ml/min; solvent, 50% MeOH in water; t_R 11.8 min (**13b**).

Acid Hydrolysis of Compounds 4, 5, 7, 8 and 9 Compounds **4**, **5**, **7**, **8** and **9** (*ca.* 0.5 mg) were dissolved in dioxane and 2 M HCl (50 μl each). The solution was heated at 100 °C for 1 h, and the reaction mixture was partitioned between EtOAc and H_2O. By HPLC analysis, (*R*)-6-hydroxymellein (**8a**) was detected from the EtOAc layers of **8** and **9**. HPLC conditions: column, YMC-ODS 4.6 mm×25 cm; flow rate, 1.0 ml/min; solvent, 27.5% MeCN in water; t_R 19.8 min ((*R*)-6-hydroxymellein). The H_2O layer was passed through an Amberlite IRA-60E column. The eluate was concentrated to dryness and the residue was stirred with D-cysteine methyl ester hydrochloride, hexamethyldisilazane and trimethylsilylchloride in pyridine using the same procedures as in previous reports.[14,15] After the reactions, the supernatant was subjected to GC analysis. GC conditions: column, GL capillary column TC-1 (GL Science, Inc.) 0.25 mm×30 m; carrier gas, N_2; column temperature, 230 °C; t_R 21.6 min (D-glucose), 20.8 min (L-glucose); column temperature 215 °C; t_R 18.8 min (D-xylose), 17.4 min (L-xylose). The t_R for L-xylose was obtained from its enantiomer (D-xylose+L-cysteine). D-Glucose was detected from **4**, **5**, **7**, **8** and **9**, and D-xylose was found from **9**.

Compounds **7** and **8** were dissolved in dioxane and 2 M HCl, then heated at 100 °C for 5 min. The procedures following hydrolysis were the same as described above. The neutralized H_2O layer was reduced with $NaBH_4$ (*ca.* 1 mg) for 1 h at room temperature. The procedures to obtain alditol acetate were described in a previous paper.[16] Apiitol acetate was detected from **7** and **8** by GC analysis. GC conditions: column, Supelco SP-2380^{TM} capillary column 0.25 mm×30 m; carrier gas, N_2; column temperature, 250 °C; t_R 7.8 min (apiitol acetate).

Alkaline Hydrolysis and Acid Hydrolysis of Compounds 6 and 10—16 Compounds **6** and **10**—**16** (*ca.* 0.5 mg) were dissolved in 0.05 M NaOH, then stirred for 2.5 h at room temperature under a N_2 gas atmosphere. The procedures after alkaline hydrolysis were carried out as in previous report.[2] 4-Hydroxybenzoic acid was detected from the residue of the EtOAc layers of **6**, **14** and **15** by HPLC analysis. HPLC conditions: column, YMC-ODS 4.6 mm×25 cm; flow rate, 1.0 ml/min; solvent, 17.5% MeCN+0.05% trifluoroacetic acid (TFA); t_R 8.4 min (4-hydroxybenzoic acid). Similarly, HPLC analyses detected 4-methoxybenzoic acid from **10**, **11**, **12** and **15**, 3,4-dimethoxybenzoic acid from **13**, **14** and **16**, and ferulic acid from **16**. HPLC conditions: column, YMC-ODS 4.6 mm×25 cm; flow rate, 1.0 ml/min; solvent, 27.5% MeCN+0.05% TFA; t_R 12.8 min (4-methoxybenzoic acid); solvent, 17.5% MeCN+0.05% TFA; t_R 19.8 min (3,4-dimethoxybenzoic acid), 18.8 min (ferulic acid). Additionally, **6a**[3] and **8** were identified from the residue of the H_2O layers of **6** and **10** by HPLC analysis, respectively. In the same way, **11a**[11] was detected from **11**, and **13a**[3] was confirmed from **13**, **14**, **15** and **16**. HPLC conditions: column, YMC-ODS 4.6 mm×25 cm; flow rate, 1.0 ml/min; solvent, 2% MeOH in water; t_R 14.6 min (**6a**); solvent, 15% MeCN in water; t_R 12.6 min (**8**), 15.2 min (**11a**); solvent, 7.5% MeCN in water; t_R 8.4 min (**13a**).

The residues of the H_2O layer of **12** were hydrolyzed with 2 M HCl and dioxane (50 μl each). The solution was heated at 100 °C for 1 h, then subjected to the same procedures described above. D-Glucose was detected from **12**. (Conditions of the GC analysis are described above.) Moreover, another solution of the residue of H_2O layer of **12** in 2 M HCl and dioxane (50 μl each) was heated at 100 °C for 5 min. The following procedures were the same as described above. Apiitol acetate was detected from **12**. (Conditions of the GC analysis are described above.)

References and Notes

1) Hashimoto G., "Illustrated Cyclopedia of Brazilian Medical Plants," Aboc-sha, Kamakura, 1996, p. 158.
2) Warashina T., Nagatani Y., Noro T., *Phytochemistry*, **65**, 2003—2011 (2004).
3) Warashina T., Nagatani Y., Noro T., *Phytochemistry*, **66**, 589—597 (2005).
4) Sugiyama M., Kikuchi M., *Phytochemistry*, **32**, 1553—1555 (1993).
5) Kanchanapoom T., Kasai R., Yamasaki K., *Phytochemistry*, **59**, 551—556 (2002).
6) Yoshikawa K., Mimura N., Arihara S., *J. Nat. Prod.*, **61**, 1137—1139 (1998).
7) Lee T.-H., Kuo Y.-C., Wang G.-J., Kou Y.-H., Chang C.-I., Lu C.-K., Lee C.-K., *J. Nat. Prod.*, **65**, 1497—1500 (2002).
8) Miki K., Takehara T., Sasaya T., Sakakibara A., *Phytochemistry*, **19**, 449—453 (1980).
9) Hsiao J.-J., Chiang H.-C., *Phytochemistry*, **39**, 899—902 (1995).
10) Antus S., Snatzke G., Steinke I., *Liebigs Ann. Chem.*, **1983**, 2247—2261 (1983).
11) (*R*)-6-Hydroxymellein 6-*O*-*β*-D-glucopyranoside (**11a**) was afforded from (*R*)-6-hydroxymellein 6-*O*-*β*-D-[5-*O*-(3,4-dimethoxybenzoyl)]-apiofuranosyl-(1→6)-*β*-D-glucopyranoside[2] by acid hydrolysis with 0.2 M HCl. (see ref. 2). Its spectroscopic and physical data are described below. Compound **11a**: Amorphous powder. $[\alpha]_D^{22}$ −93° (*c*=0.16, MeOH). UV λ_{max} (MeOH) nm (log ε): 214 (4.30), 261 (4.00), 302 (3.56). FAB-MS *m/z*: 379 $[M+Na]^+$. HR-FAB-MS *m/z*: 379.0995 (Calcd for $C_{16}H_{20}O_9Na$: 379.1005). CD $\Delta\varepsilon$ (nm): −4.17 (223), −3.94 (265), −0.37 (300) (*c*=0.0438, MeOH). ^{13}C-NMR (MeOH-d_4 at 35 °C) δ: 171.3 (C-1), 165.1×2 (C-6, C-8), 143.4 (C-10), 108.4 (C-5), 103.6 (C-7), 101.5 (C_{Glc}-1), 78.4 (C_{Glc}-3), 77.9 (C_{Glc}-5), 77.4 (C-3), 74.8 (C_{Glc}-2), 71.3 (C_{Glc}-4), 62.5 (C_{Glc}-6), 35.5 (C-4), 20.8 (C-11). The C-9 signal was not detected. ^{1}H-NMR (MeOH-d_4 at 35 °C) δ: 6.52 (overlapping, H-5, H-7), 5.00 (1H, d, 8.0, H_{Glc}-1), 4.70 (1H, m, H-3), 3.89 (1H, dd, 12.0, 2.5, H_{Glc}-6), 3.70 (1H, dd, 12.0, 5.5, H_{Glc}-6), 2.98 (1H, dd, 16.5, 3.5, H-4), 2.87 (1H, dd, 16.5, 11.0, H-4), 1.48 (3H, d, 6.5, H-11).
12) Hori K., Satake T., Yamaguchi H., Saiki Y., Murakami T., Chen C.-H., *Yakugaku Zasshi*, **107**, 774—779 (1987).
13) Mills S., Born K., "Principles and Practice of Phytotherapy: Modern Herbal Medicine," Churchill Livingstone, London, 2000, pp. 499—506.
14) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull.*, **35**, 501—506 (1987).
15) Zhang D., Miyase T., Kuroyanagi M., Umehara K., Ueno A., *Chem. Pharm. Bull.*, **44**, 173—179 (1996).
16) Nagatani Y., Warashina T., Noro T., *Chem. Pharm. Bull.*, **49**, 1388—1394 (2001).