New Phenolic Constituents from *Smilax bracteata*

Shu Yuan Li,[†] Hiroyuki Fuchino,*[,‡] Nobuo Kawahara,[§] Setsuko Sekita,[‡] and Motoyoshi Satake[§]

Guangzhou Institute for Drug Control, 23 Xizeng Road, Xicun Guangzhou, 510160, People's Republic of China, Tsukuba Medicinal Plant Research Station, National Institute of Health Sciences, Hachimandai 1, Tsukuba, Ibaraki 305-0843, Japan, and National Institute of Health Sciences, Kamiyoga 1-18-1, Setagaya-ku, Tokyo 158-8501, Japan

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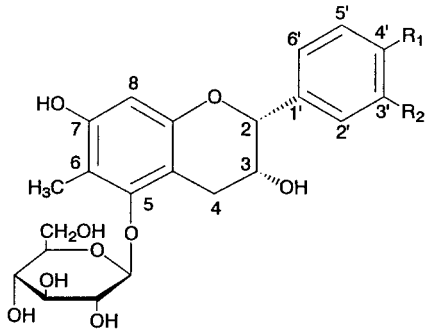
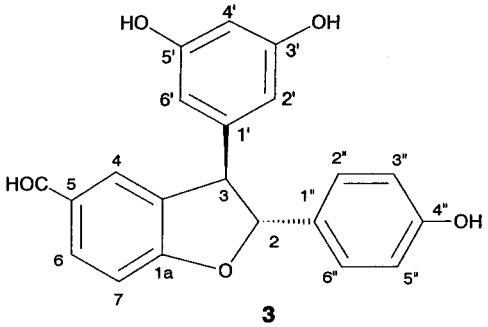
From the methanol extract of *Smilax bracteata* rhizomes, six new phenolic compounds, (2*S*,3*S*)-5-*O*-*β*-D-glucopyranosyloxy-6-methyl-3′-methoxy-3,7,3′-trihydroxyflavan (**1**), (2*S*,3*S*)-5-*O*-*β*-D-glucopyranosyloxy-6-methyl-4′-methoxy-3,7,4′-trihydroxyflavan (**2**), 3*β*-(3′,5′-dihydroxyphenyl)-2α-(4″-hydroxyphenyl)-dihydrobenzofuran-5-carbaldehyde (**3**), (1-*p*-*O*-coumaroyl-6-*O*-feruroyl)-*β*-D-fructofuranosyl-α-D-glucopyranoside (**4**), (1-*p*-*O*-coumaroyl-3,6-di-*O*-feruroyl)-*β*-D-fructofuranosyl-α-D-glucopyranoside (**5**), and (6-*O*-feruroyl)-*β*-D-fructofuranosyl-(6-*O*-acetyl)-α-D-glucopyranoside (**6**) were isolated together with five known compounds. Their structures were established by spectral data interpretation.

The genus *Smilax* is known to contain smilax saponins, and several species in this genus are used as folk medicines. For example, the rhizomes of *Smilax glabra* have been used in the treatment of syphilis and are listed in the Japanese Pharmacopoeia.[1] *Smilax bracteata* Presl. (Liliaceae) grows in Taiwan, mainland China, The Philippines, and the southern part of Kyushyu, Japan,[2] but its chemical constituents have yet to be investigated thoroughly. From the methanol extract of the rhizomes of *S. bracteata*, two new flavan-3-ol glycosides (**1** and **2**), a new stilbene (**3**), and three new phenylpropanoid glycosides (**4**−**6**) were isolated together with five known compounds. Herein we report the isolation and structure determination of six new phenolic constituents (**1**−**6**) from *S. bracteata*.



1 R_1=OH, R_2=OCH_3
2 R_1=OCH_3, R_2=OH



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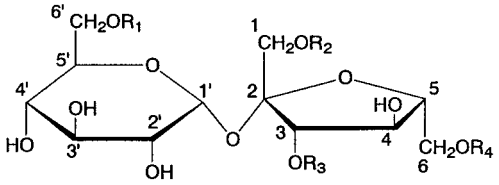
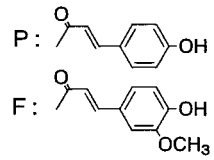


4 R_1=R_3=H, R_2=P, R_4=F
5 R_1=H, R_2=P, R_3=R_4=F
6 R_1=CH_3CO, R_2=R_3=H, R_4=F



Results and Discussion

The methanol extract of the air-dried rhizomes of *S. bracteata* was purified as described in the Experimental Section to obtain compounds **1**−**6** together with five known compounds, methyl 3,4-dihydroxybenzoate,[3] methyl caffeate,[4] 1,4-dihydroxy-3-methoxy-4-*O*-*β*-D-glucopyranoside,[5] adenosine,[6] and 5,7,4′-trihydroxyflavanone.[7]

Compound **1**, a colorless amorphous powder, was formulated as $C_{23}H_{28}O_{11}$ by HRFABMS. The IR spectrum of **1** suggested the presence of a hydroxyl group (3402 cm^{-1}) and an aromatic ring (1518 cm^{-1}). ^{13}C NMR analysis revealed the presence of two aromatic rings, a methoxy group (*δ* 56.4), a methyl group (*δ* 9.8), a hexosyl moiety (*δ* 105.3, 78.0, 78.0, 75.8, 71.9, 62.9), two methine groups (*δ* 67.6, 80.0), and a methylene group (*δ* 30.4). The presence of a −CH(O−)−CH(O−)−CH_2− substructure was confirmed by DQFCOSY NMR. Therefore, **1** could be proposed as a flavan-3-ol monoglycoside. Enzymatic hydrolysis of **1** was unsuccessful, probably because of steric hindrance around the sugar moiety. The hexosyl moiety was determined to be a glucopyranose by comparison with the ^{13}C NMR data for methyl *β*-D-glucopyranoside.[8] In the ^1H NMR spectrum, 1,2,4-trisubstituted aromatic signals [*δ* 6.78 (1H, d, *J* = 8.2 Hz), 6.89 (1H, dd, *J* = 8.2, 1.8 Hz), 7.11 (1H, d, *J* = 1.8 Hz)] and a noncoupled aromatic signal (*δ* 6.23, 1H, s) were observed at low field. The signals observed at 5.22 ppm (brs), 4.56 ppm [t-like (*J* = 3.5 Hz)], 3.30 ppm [dd (*J* = 16.5, 3.7 Hz)], and 4.38 ppm [dd (*J* = 16.5, 3.0 Hz)] in

* To whom correspondence should be addressed. Tel: +81 298 38 0572. Fax: +81 298 38 0575. E-mail: fuchino@nihs.go.jp.
† Guangzhou Institute for Drug Control.
‡ Tsukuba Medicinal Plant Research Station, National Institute of Health Sciences.
§ National Institute of Health Sciences.

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Table 1. ^{13}C NMR Data for **1** and **2**[a]

position	**1**[b]	**1**[c]	**2**[b]	**2**[c]
2	80.0	80.0	79.7	79.7
3	67.6	66.6	67.4	66.6
4	30.4	30.6	30.2	30.5
5	155.7	156.1	155.7	156.0
6	111.8[d]	111.6	112.0	111.5
7	156.0	156.5	156.1	156.5
8	100.5	100.5	100.5	100.4
9	154.2	154.1	154.1	154.0
10	106.9	106.9	106.9	106.9
1′	132.3	131.8	133.7	134.0
2′	112.1[d]	112.2	119.2	118.5
3′	148.7	148.3	147.3	147.9
4′	147.1	147.8	148.5	148.1
5′	115.7	120.7	112.4	112.1
6′	120.6	131.8	115.2	116.0
CH_3-6	9.8	10.4	9.8	10.4
OCH_3	56.4	55.7	56.5	56.0
Glc-1	105.3	106.1	105.3	106.0
2	75.8	75.9	75.8	75.9
3	78.0	78.6	77.9	78.5
4	71.9	72.2	71.9	72.2
5	78.0	78.1	77.9	78.1
6	62.9	63.4	62.9	63.3

[a] Assignments were made from the HMQC and HMBC spectra. [b] In methanol-d_4. [c] In pyridine-d_5. [d] Assignments may be interchanged.

pyridine-d_5 were assigned as H-2, H-3, H-4a, and H-4b, respectively, by DQFCOSY and HMBC correlations. Bearing in mind the coupling constants for H-2, H-3, and H-4, the relative stereochemistry of the phenyl group at C-2 and hydroxyl group at C-3 were both determined as α. The positions of the hydroxyl, methyl, and methoxyl groups and the glucose moiety were determined by 2D NMR (DQFCOSY, HMQC, and HMBC). Specifically, a correlation between an anomeric proton of glucose [δ_H 4.64 (d J = 7.6 Hz)] and an oxygenated carbon (δ_C 155.7) was observed in the HMBC spectrum, and the latter carbon was designated as C-5 because a correlation to H-4 was also observed. Thus, the glucose moiety was determined to be substituted at C-5. As a NOE correlation between an anomeric proton and a methyl group [δ_H 2.13 s] was observed, the methyl group was determined to be situated at C-6. A correlation between a methyl proton at C-6 and an oxygenated aromatic carbon (δ_C 156.0) was observed in the HMBC spectrum; therefore, a hydroxyl group was determined to be substituted at C-7. Since a NOE correlation between a methoxyl group and a proton [δ 7.11 (1H, d, J = 1.8 Hz)] in the 1,2,4-trisubstituted aromatic ring was observed, the methoxyl group was determined to be located at C-3′. The absolute configurations of C-2 and 3 were deduced from the CD spectral measurement in comparison with CD data reported for (−)-epicatechin.[9] Opposite Cotton effects [**1**: $\Delta\epsilon$ +13.9 (281), −5.9 (238), (−)-epicatechin: $\Delta\epsilon$ −0.6 (280), +1.5 (238)] were observed; thus, the absolute configurations of C-2 and -3 were both determined as *S*. Accordingly, the structure of **1** was established as (2*S*,3*S*)-5-*O*-*β*-D-glucopyranosyloxy-6-methyl-3′-methoxy-3,7,4′-trihydroxyflavan.

Compound **2**, a colorless amorphous powder, was formulated as $C_{23}H_{28}O_{11}$ by HRFABMS. Its ^1H and ^{13}C NMR data were similar to those of **1** except for the presence of the B-ring signals. In the ^1H NMR spectrum, a 1,2,4-trisubstituted aromatic signal pattern was assigned for the B-ring. As a NOE correlation between a methoxy (δ_H 3.69 s) and an aromatic proton (δ_H 6.92 d J = 8.3 Hz) was observed, the methoxy group was determined to be substituted at C-4′. In the CD spectrum, the Cotton effects for **2** were similar to those of **1** [**2**: $\Delta\epsilon$ +9.5 (285), −16.7 (237)]. Thus, the structure of **2** was established as (2*S*,3*S*)-5-*O*-*β*-D-glucopyranosyloxy-6-methyl-4′-methoxy-3,7,3′-trihydroxyflavan. 2D NMR spectra (DQFCOSY, HMBC) were used in making this structural assignment.

Compound **3**, a pale yellow amorphous powder, was formulated as $C_{21}H_{16}O_5$ by HREIMS. The IR spectrum of **3** suggested the presence of a hydroxyl group (3358 cm^{-1}) and a conjugated carbonyl (1672 cm^{-1}). The ^1H and ^{13}C NMR spectral data revealed the presence of three aromatic rings, an aldehyde group (δ_C 192.7, δ_H 9.77), and two methine groups (δ_C 96.0, δ_H 5.53, d, J = 8.2 Hz; δ_C 57.8, δ_H 4.46, d, J = 8.2 Hz). Bearing in mind the coupling constants for aromatic signals in the ^1H NMR spectrum, the presence of a 1,4-disubstituted [δ_H 6.78 (2H, d, J = 8.6 Hz); 7.17 (2H, d, J = 8.6 Hz)], a 1,3,5-trisubstituted [δ_H 6.09 (1H, d, J = 2.1 Hz); 6.18 (1H, t, J = 2.1 Hz)], and a 1,2,4-trisubstituted [δ_H 7.03 (1H, d, J = 8.2 Hz); 7.57 (1H, d, J = 1.5 Hz); 7.83 (1H, dd, J = 8.2, 1.5 Hz)] aromatic ring was deduced. The linkage of two methine groups was confirmed by DQFCOSY NMR spectroscopy. The planar structure of **3** was established from a combination of its DQFCOSY, HMQC, and HMBC spectra. The NOE enhancement observed for **3** in the difference NOE (DIFNOE) NMR spectrum was similar to that of aropecuron A,[10] as shown in Figure 1, indicating that the stereochemistry for C-2 and 3 are both *trans*-oriented. In the CD spectrum, no

Table 2. ^1H NMR Data for **1** and **2**[a]

position	**1**[b]	**2**[b]	**1**[c]	**2**[c]
2	4.87 br s	4.85 brs	5.22 s	5.23 s
3	4.16 ddd (4.3, 2.8, 1.2)	4.16 ddd (4.3, 3.1, 1.2)	4.56 brt	4.58 br t
4	2.91 dd (16.5, 4.3)	2.90 dd (16.8, 4.3)	3.30 dd (16.5, 3.7)	3.30 dd (16.3, 3.6)
	3.33 dd (16.5, 2.8)	ca. 3.3 NC[e]	4.38 dd (16.5, 3.0)	4.35 dd (16.3, 3.1)
8	6.23 s	6.23 s	6.85 s	6.78 s
2′	7.11 d (1.8)	6.908[d] s	7.51 d (1.8)	7.66 d (2.0)
5′	6.78 d (8.2)	6.906[d] s	7.21 d (8.2)	6.92 d (8.3)
6′	6.89 dd (8.2, 1.8)	6.98 s	7.29 dd (8.2, 1.8)	7.24 dd (8.3, 2.0)
Glc-1″	4.64 d (7.6)	4.64 d (7.6)	5.43 d (7.6)	5.43 d (7.5)
2″	3.49 dd (9.2, 7.6)	3.48 dd (9.2, 7.6)	4.43 dd (8.5, 7.6)	4.43 NC[e]
3″	3.42 dd (9.2, 8.9)	3.42 dd (9.2, 7.9)	4.34 dd (8.9, 8.5)	4.25 NC[e]
4″	3.33 NC[e]	3.31 dd (9.7, 7.9)	4.18 dd (9.5, 8.9)	4.19 dd (9.5, 8.7)
5″	3.13 ddd (9.8, 6.1, 2.4)	3.13 ddd (9.7, 6.1, 2.4)	3.90 ddd (9.5, 6.4, 2.4)	3.91 ddd (9.5, 6.4, 2.8)
6″	3.61 dd (12.2, 6.1)	3.60 dd (12.2, 6.1)	4.24 dd (11.9, 6.4)	
	3.74 dd (12.2, 2.4)	3.74 dd (12.2, 2.4)	4.45 dd (11.9, 2.4)	
CH_3-6	2.13 s	2.13 s	2.84 s	2.84 s
OCH_3	3.86 s	3.84 s	3.55 s	3.69 s

[a] Assignments were made from the DQF COSY, HMQC, and HMBC spectra. [b] In methanol-d_4. [c] In pyridine-d_5. [d] Assignments may be interchanged. [e] Coupling patterns were not confirmed because of overlapping signals.

Table 3. ^{13}C and ^{1}H NMR Data for **3** in CD_3OD[a]

position	δ_C	δ_H (J in Hz)
C-1a	166.7	
2	96.0	5.53 d (8.2)
3	57.8	4.46 d (8.2)
3a	144.6	
4	128.1	7.57 dd (1.5, 1.2)
5	132.3	
6	134.1	7.83 dd (8.2, 1.5)
7	110.9	7.03 d (8.2)
1′	133.6	
2′,6′	107.6	6.09 d (2.1)
3′,5′	160.1	
4′	102.7	6.18 t (2.1)
1″	132.0	
2″,6″	128.7	7.17 d (8.6)
3″,5″	116.4	6.78 d (8.6)
4″	159.0	
CHO	192.7	9.77 s

[a] Assignments were made from the HMQC and HMBC spectra.

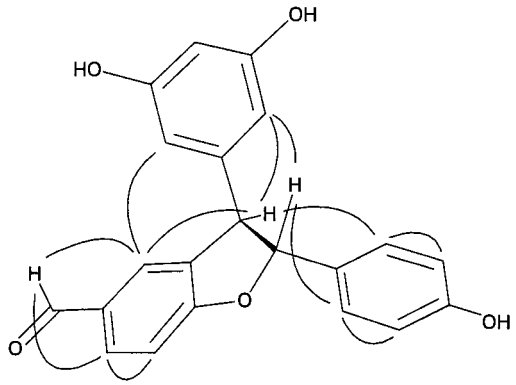

Figure 1. Key NOE correlations for compound **3**.

Cotton effect was observed, suggesting that **3** is a mixture of the 2*R*,3*S* and 2*S*,3*R* forms. Thus, the structure of **3** was determined to be 3*β*-(3′,5′-dihydroxyphenyl)-2α-(4″-hydroxyphenyl)dihydrobenzofuran-5-carbaldehyde, and this compound can be classified as a stilbene. Interestingly, **3** has a structure similar to viniferin, which has been isolated as a phytoalexin from grapevine (*Vitis vinifera*) leaves.[11]

Compound **4**, a colorless amorphous powder, exhibited a $[M + Na]^+$ peak at 687 in the positive FABMS and was

Table 4. ^{13}C NMR Data for **4**−**6** in CD_3OD[a]

	4		**5**		**6**	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
β-D-fructose-1	63.9	4.31 d (12.2) 4.43 d (12.2)	66.2	4.33 s	64.0	3.54 s
2	104.5		103.5		105.4	
3	78.6	4.18 d (8.5)	79.1	5.61 d (8.5)	78.8	4.03 d (8.3)
4	76.0	4.13 dd (8.5, 8.2)	74.1	4.51 t (8.5)	76.7	3.99 dd (8.3, 7.9)
5	80.8	4.02 ddd (8.2, 7.6, 2.8)	81.1	4.19 ddd (8.5, 7.0, 3.4)	80.9	4.00 NC[c]
6	66.4	4.45 dd (11.9, 2.8) 4.50 dd (11.9, 7.6)	65.9	4.53 dd (12.2, 3.4) 4.57 dd (12.2, 7.0)	66.7	4.36 NC[c]
α-D-glucose-1′	94.0	5.45 d (3.9)	93.5	5.51 d (3.7)	93.2	5.30 d (3.7)
2′	73.2	3.42 dd (10.1, 3.9)	73.0	3.42 dd (9.8, 3.7)	73.2	3.34 dd (9.8, 3.7)
3′	74.7	3.72 dd (10.1, 9.8)	74.4	3.66 dd (9.8, 9.2)	74.7	3.62 dd (9.8, 9.2)
4′	71.5	3.37 dd (9.8, 9.2)	73.0	3.41 dd (9.8, 9.2)	72.0	3.15 dd (9.2, 9.2)
5′	74.3	3.91 ddd (10.1, 4.9, 2.1)	75.0	4.00 ddd (9.8, 4.6, 2.8)	71.9	4.00 NC[c]
6′	62.5	3.74 dd (11.9, 4.9) 3.86 NC	62.7	3.82 dd (11.9, 4.6) 3.92 dd (11.9, 2.8)	65.6	3.90 dd (11.3, 4.9) 4.36 d (11.3)
p-coumaroyl-α	168.4		168.5			
β	114.7	6.36 d (15.9)	114.7	6.33 d (15.9)		
γ	147.2	7.65 d (15.9)	147.1	7.64 d (15.9)		
1	127.1		127.1			
2, 6	131.3	7.45 d (8.9)	131.3	7.39 d (8.9)		
3, 5	116.8	6.793 d (8.9)	116.8	6.73 d (8.9)		
4	161.4		161.3			
feruloyl-α	169.0		169.0		169.0	
β	115.2	6.39 d (16.2)	115.1	6.41 d (15.9)	115.1	6.28 d (15.9)
γ	147.2	7.62 d (16.2)	147.3	7.64 d (15.9)	147.1	7.54 d (15.9)
1	127.7		127.7		127.5	
2	111.6	7.16 d (1.8)	111.7	7.17 d (1.8)	111.7	7.09 s
3	149.3		149.3[b]		149.5	
4	150.7		150.7		151.1	
5	116.4	6.790 d (8.6)	116.5	6.796 d (8.2)	116.6	6.71 d (8.2)
6	124.3	7.05 dd (8.6, 1.8)	124.3	7.07 dd (8.2, 1.8)	124.2	6.98 d (8.2)
−OCH_3	56.5	3.85 s	56.5	3.87 s	56.5	3.80 s
feruloyl-α′			168.3			
β′			114.7	6.45 d (15.9)		
γ′			148.1	7.71 d (15.9)		
1′			127.6			
2′			112.1	7.18 d (2.1)		
3′			149.4[b]			
4′			150.8			
5′			116.5	6.798 d (7.9)		
6′			124.4	7.10 dd (7.9, 2.1)		
−$OCH_{3'}$			56.5	3.87 s		
CH_3CO−					20.9	2.00 s
CH_3CO−					173.0	

[a] Assignments were made by HMQC and HMBC spectra. [b] Assignments may be interchanged. [c]NC: coupling patterns were not confirmed because of overlapping signals.

formulated as $C_{31}H_{36}O_{16}Na$ by HRFABMS. In the ^{13}C NMR spectrum, 18 sp^2 carbons including two ester carbonyl carbons, three oxygen-bearing methylenes, nine oxygen-bearing methines, and a methoxy carbon were observed. The coupling constants in the ^1H NMR spectrum *suggested* the presence of two *trans*-olefins [δ_H 6.36, d, J = 15.9 Hz; 7.65, d, J = 15.9 Hz; 6.39, d, J = 16.2 Hz; 7.62, d, J = 16.2 Hz], a 1,4-disubstituted aromatic ring [δ_H 7.45, d, J = 8.9 Hz; 6.79, d, J = 8.9 Hz], and a 1,2,4-trisubstituted aromatic ring [δ_H 7.16, d, J = 1.8 Hz; 6.79, d, J = 8.6 Hz; 7.05, dd, J = 8.6 Hz, 1.8 Hz]. In addition, the ^{13}C NMR chemical shifts due to sp^2 carbons were in good agreement with those of *para*-coumaroyl and feruloyl moieties; therefore, **4** was found to contain both a *para*-coumaroyl group and a feruloyl group. On alkaline and acid hydrolysis, **4** afforded sucrose and a mixture of glucose and fructose, respectively. Moreover, chemical shifts due to sucrose were observed, which indicated that **4** is phenylpropanoid glycoside. The NMR assignments for the sugar protons were completed using the DQFCOSY and HMQC spectra. HMBC correlations between the ester carbonyl carbon of the *para*-coumaroyl group (δ_C 168.4) and H-1 of fructose (δ_H 4.31, 4.43), the ester carbonyl carbon of the feruloyl group (δ_C 169.0) and H-6 of fructose (δ_H 4.45, 4.50), were observed. Thus, the structure of **4** was determined as shown.

Compound **5**, a colorless amorphous powder, exhibited a $[M + Na]^+$ peak at 863 in the positive FABMS and was formulated as $C_{41}H_{44}O_{19}Na$ by HRFABMS. The ^{13}C NMR data of **5** were in good agreement with those of **4** except for the presence of additional feruroyl group signals. Considering the molecular formula obtained from HR-FABMS, **5** was assigned as the feruroyl ester of **4**. The linkages between H-1 and H-6 of glucose and H-3 and H-5 of fructose were confirmed from the DQFCOSY NMR spectrum. In the HMBC spectrum, correlations between the carbonyl carbon (δ_C 168.3) and H-3 of fructose (δ_H 5.61), the carbonyl carbon (δ_C 169.0) and H-6 of fructose (δ_H 4.57), and the carbonyl carbon (δ_C 168.5) and H-1 of fructose (δ_H 4.33) were observed. Therefore, the structure of **5** was determined as shown.

Compound **6**, a colorless amorphous powder, exhibited a $[M + Na]^+$ peak at 583 in the positive FABMS and was formulated as $C_{24}H_{32}O_{15}Na$ by HRFABMS. Its ^1H and ^{13}C NMR data were similar to those of **4** and **5**. In contrast to the ^{13}C NMR data for **4**, signals for the *para*-coumaroyl moiety were lacking. Furthermore, additional signals due to an acetyl group were observed. This indicated that **6** possesses both a feruroyl group and an acetyl group. On comparison of the ^{13}C NMR chemical shifts of **4** and **6**, glycosylation shifts[12] were observed for C-5 and C-6 of glucose (+3.1 ppm for C-6, −2.4 ppm for C-5). Furthermore, correlations between the acetyl carbonyl carbon signal (δ_C 173.0) and H-6 of glucose (δ_H 4.36) were observed in the HMBC spectrum. Consequently, the acetyl group was determined to be located at C-6 of the glucose unit. The structure of **6** was thus established as shown, by the DQFCOSY, HMQC, and HMBC NMR spectra.

In conclusion, 11 phenolic constituents including two new flavan-3-ol glucosides, a new stilbene, and three new phenylpropanoid glycosides were isolated from a methanol extract of the rhizomes of *S. bracteata*, and their chemical structures were determined. However, steroidal saponins, the so-called "smilax saponin",[13,14] were not isolated from this plant. Among the members of the genus *Smilax*, *S. bracteata* is the most subject to lignification and is therefore considered to be abundant in phenolic constituents, which are presumably biosynthesized via the shikimate pathway.

Experimental Section

General Experimental Procedures. Optical rotations were taken with a JASCO DIP-370 automatic polarimeter. Ultraviolet (UV) spectra were recorded on a Hitachi U-2000 spectrometer and infrared (IR) spectra on a JASCO FTIR-5300 spectrometer. CD spectra were measured on a JASCO J720. The ^1H and ^{13}C NMR spectra were measured with a JEOL Alpha 500 spectrometer (multiplicity, s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, NC: coupling patterns were not confirmed because of overlapping signals). Mass (MS) spectra were measured with a JEOL JMS-D300 spectrometer (FABMS) or a JEOL JMS-HX-110 spectrometer (EIMS).

Plant Material. The air-dried rhizomes of *S. bracteata* were collected in the city of Nakatane, Kagoshima Prefecture, Japan, and identified by M.S. A voucher specimen (No. S-003) is on file at the National Institute of Health Sciences, Japan.

Extraction and Isolation. Air-dried and sliced rhizomes (3 kg) were extracted with MeOH at room temperature over 24 h. The extract was filtered and evaporated under reduced pressure. The residue obtained (59.4 g) was resuspended in water and partitioned between CH_2Cl_2, EtOAc, and BuOH, successively. The CH_2Cl_2 extract (8.9 g) was subjected to column chromatography over Si gel (170 g) and eluted with a gradient of CH_2Cl_2−MeOH (from 50:1 to 5:1) to obtain 36 fractions (each 100 mL). These fractions were pooled into nine fractions (Fr. 1−9) on the basis of similar TLC profiles. Fr. 7 (2.8 g) was rechromatographed on Si gel (53 g) and eluted with *n*-hexane−EtOAc (2:1) and then EtOAC−MeOH (5:1) to afford seven further fractions (Fr. 7-1−Fr. 7-7). Fr. 7-4 (609 mg) was rechromatographed on Si gel (16 g) and eluted with *n*-hexane−EtOAc (20:1) and then EtOAc−MeOH (1:2), to give six fractions (Fr. 7-4-1−Fr. 7-4-6). Fr. 7-4-2 (238.2 mg) was further chromatographed on Si gel (8 g) by elution with a gradient of *n*-hexane−acetone (from 10:1 to 0:100) to produce four fractions (Fr. 7-4-2-1−Fr. 7-4-2-4). Further purification of Fr. 7-4-2-2 (59.5 mg) was carried out by medium-pressure liquid chromatography (MPLC) with 30% CH_3CN−H_2O as the eluent to yield methyl 3,4-dihydroxy benzoate[3] (16.8 mg) and methyl caffeate[4] (4.8 mg).

The *n*-butanol extract (19.6 g) was subjected to column chromatography over Si gel (242 g) and eluted with a gradient of $CHCl_3$−MeOH (from 100:0 to 0:100) to yield 18 fractions (each 200 mL). These fractions were pooled into seven fractions (Fr. B-1−Fr. B-7) on the basis of similar TLC profiles. Further chromatography of Fr. B-1 (8.27 g) on a Sephadex LH-20 column with 90% MeOH−H_2O as eluent generated three fractions (Fr. B-1-1−Fr. B-1-3). Fr. B-1-2 (1.54 g) was rechromatographed over Sephadex LH-20 and eluted with a gradient of H_2O−MeOH (from 100:0 to 0:100) to obtain 14 fractions (Fr. B-1-2-1−Fr. B-1-2-14). Fr. B-1-2-3 was then chromatographed on ODS and eluted with a gradient of MeOH−H_2O (from 10% to 50%) to yield 3-methoxy-1,4-dihydroxy-4-*O*-*β*-D-glucopyranoside[5] (6.4 mg) and adenosine[6] (1.1 mg). Fr. B-1-2-6 (150.2 mg) was chromatographed on Si gel (15 g) and eluted with $CHCl_3$−MeOH−H_2O (100:2:1) to obtain three fractions (Fr. B-1-2-6-1−Fr. B-1-2-6-3). Fr. B-1-2-6-1 (31.5 mg) was rechromatogtaphed on ODS and eluted with 40% MeOH−H_2O to yield **1** (2.9 mg), **2** (3.2 mg), and **6** (7.5 mg). Fr. B-1-2-14 was purified by ODS column chromatography to afford **4** (55.6 mg). Fr. B-2 (6.07 g) was chromatographed on ODS to yield two fractions (Fr. B-2-1−Fr. B-2-2). Fr. B-2-1 was chromatographed on Sephadex LH-20 with a gradient of MeOH−H_2O (from 50% to 70%) and 14 fractions (Fr. B-2-1-1−Fr. B-2-1-14) were obtained. Fr. B-2-1-9 was rechromatographed on ODS with a gradient of MeOH−H_2O (from 50% to 80%) to yield **3** (9.3 mg) and 5,7,4′-trihydroxyflavanone[7] (14.4 mg). Fr. B-2-2 was rechromatographed on Sephadex LH-20 and eluted with a gradient of MeOH−H_2O (from 50% to 90%) to obtain two fractions (Fr. B-2-2-1−Fr.B-2-2-2). In turn, Fr. B-2-2-1 was subjected to MPLC to afford eight fractions (Fr. B-2-2-1-1−Fr. B-2-2-1-8), with Fr. B-2-2-1-8 (115.2 mg) being further purified by MPLC to yield **5** (32.7 mg).

Compound 1: amorphous powder; $[\alpha]_D$ +14.5° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (4.71), 224sh (4.67), 282

(3.90) nm; IR (KBr) ν_{max} 3402, 2920, 1622, 1518, 1068 cm^{-1}; CD (*c* 0.001, MeOH) $\Delta\epsilon$ +13.9 (281), −5.9 (238); HRFABMS (positive mode) *m*/z 481.1725 (calcd for $C_{23}H_{29}O_{11}$, 481.1710).

Compound 2: amorphous powder; $[\alpha]_D$ +14.5° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (4.72), 224sh (4.67), 282 (3.90) nm; IR (KBr) ν_{max} 3402, 2926, 1620, 1514, 1074 cm^{-1}; CD (*c* 0.001, MeOH) $\Delta\epsilon$ +9.5 (285), −16.7 (237); HRFABMS (positive mode) *m*/z 481.1700 (calcd for $C_{23}H_{29}O_{11}$, 481.1710).

Compound 3: amorphous powder; $[\alpha]_D$ +10.0° (*c* 0.3, MeOH); UV(MeOH) λ_{max} (log ϵ) 233sh (4.39), 280 (4.14), 302sh (4.01) nm; IR (KBr) ν_{max} 3358, 2930, 1672, 1601, 1242 cm^{-1}; HREIMS *m*/z 348.0984 (calcd for $C_{21}H_{16}O_5$, 348.0996).

Compound 4: amorphous powder; $[\alpha]_D$ −30° (*c* 0.2, MeOH); UV (MeOH) λ_{max}(log ϵ) 231sh (4.58), 292sh (4.52), 318 (4.62) nm; IR (KBr) ν_{max} 3378, 2942, 1699, 1632, 1603, 1516, 1273, 1161 cm^{-1}; HRFABMS (positive mode) *m*/z 687.1895 $[M + Na]^+$ (calcd for $C_{31}H_{36}O_{16}Na$, 687.1904).

Compound 5: amorphous powder; $[\alpha]_D$ +36° (*c* 0.2, MeOH); UV (MeOH) λ_{max} 233sh (log ϵ 4.68), 296sh (4.68), 320 (4.77) nm; IR (KBr) ν_{max} 3405, 2944, 1698, 1632, 1603, 1516, 1271, 1159 cm^{-1}; HRFABMS (positive mode) *m*/z 863.2389 $[M + Na]^+$ (calcd for $C_{41}H_{44}O_{19}Na$, 863.2372).

Compound 6: amorphous powder; $[\alpha]_D$ +85° (*c* 0.1, MeOH); UV(MeOH) λ_{max} 237sh (log ϵ 4.33), 291sh (4.23), 324 (4.33) nm; IR (KBr) ν_{max} 3405, 2940, 1707, 1632, 1601, 1516, 1273 cm^{-1}; HRFABMS (positive mode) *m*/z 583.1653 $[M + Na]^+$ (calcd for $C_{24}H_{32}O_{15}Na$, 583.1629).

Acid Hydrolysis of 4. A solution of **4** (1 mg) in 3% HCl (2 mL) was heated under reflux for 4 h. Then, the solution was evaporated in vacuo and the residue was subjected to TLC analysis. Glucose and fructose were identified by their TLC behavior [Si gel, developed with $CHCl_3$−MeOH−H_2O (6:4:1)].

Alkaline Hydrolysis of 4. Compound **4** (1 mg) was dissolved in 3% KOH−MeOH (2 mL), and the solution was stirred at room temperature for 6 h. The reaction mixture was neutralized with 1 N HCl and then extracted with $CHCl_3$. From the H_2O layer, sucrose was identified by its TLC behavior [Si gel, developed with $CHCl_3$−MeOH−H_2O (6:4:1)].

References and Notes

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