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NEW PHENOLIC GLYCOSIDES FROM THE LEAVES OF *SALACIA CHINENSIS*

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Abstract — Seven new phenolic glycosides named foliachinenosides A_1 (**1**), A_2 (**2**), A_3 (**3**), B_1 (**4**), B_2 (**5**), C (**6**), and D (**7**), were isolated from the leaves of *Salacia chinensis* (Hippocrateaceae) collected in Thailand. Their structures were elucidated on the basis of chemical and physicochemical evidence.

Salacia chinensis Linn. (Hippocrateaceae) is widely distributed in Thailand, Myanmar, and India. The decoctions of the stems and leaves are given to cure diabetes mellitus in the Ayurvedic system of Indian traditional medicine. In the course of our characterization studies on bioactive constituents from *Salacia* species,[1–13] we have reported the isolation and absolute stereostructure elucidation of thirteen megastigmane glycosides, foliasalaciosides A_1, A_2, B_1, B_2, C, D, E_1, E_2, E_3, F, G, H, and I from the leaves of *S. chinensis* together with twenty known constituents.[14,15] As a continuing study on the leaves of *S. chinensis*, we have isolated seven new phenolic glycosides, foliachinenosides A_1 (**1**), A_2 (**2**), A_3 (**3**), B_1 (**4**), B_2 (**5**), C (**6**), and D (**7**), from this herbal medicine. In this paper, we describe the isolation and structure elucidation of these seven new constituents.

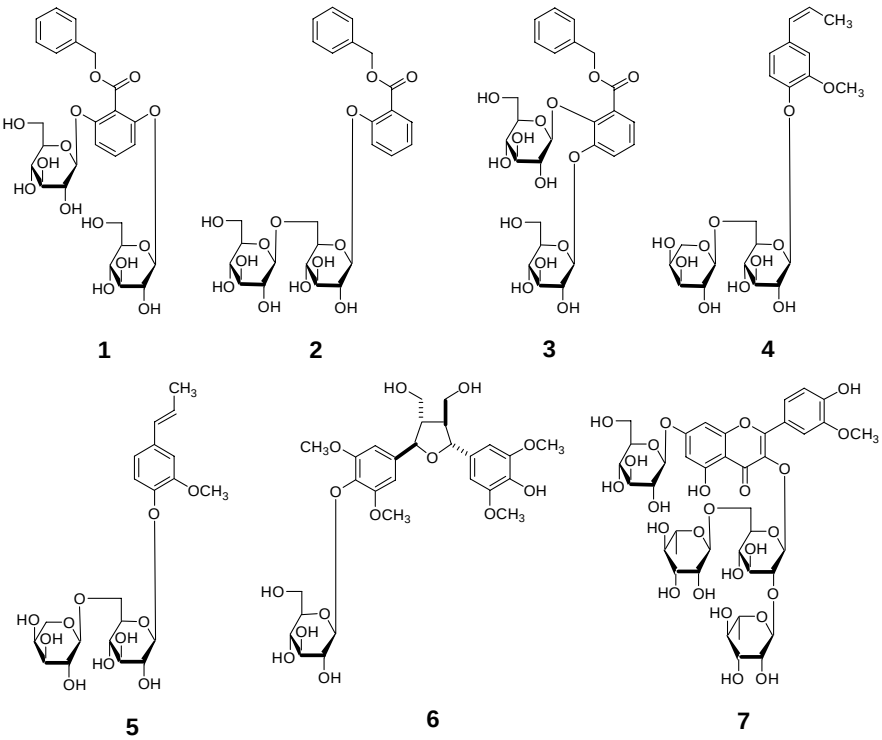
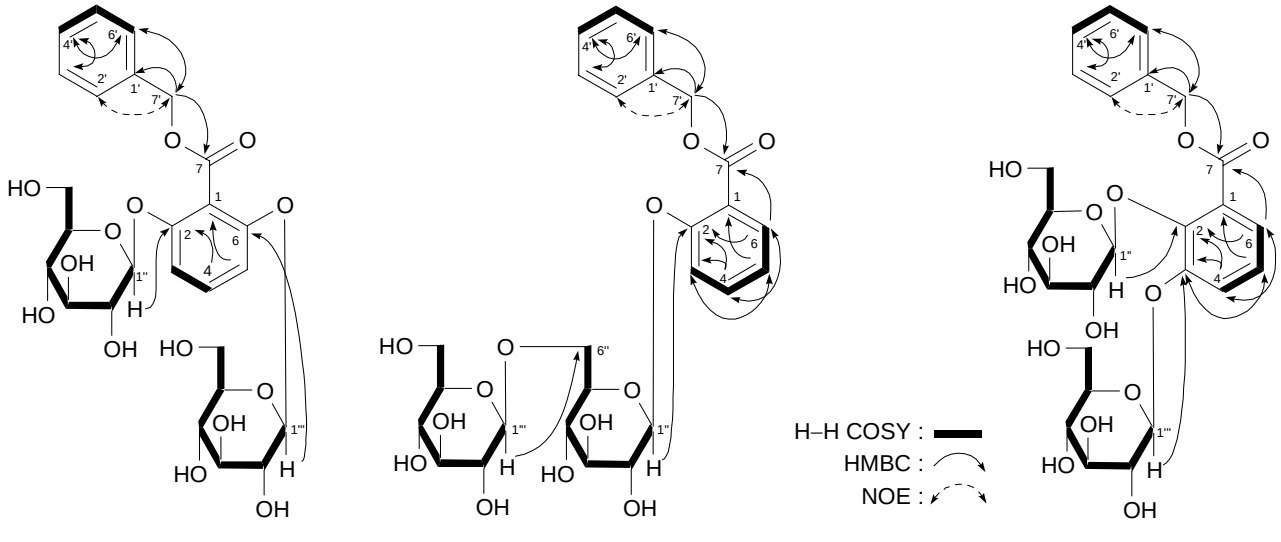


Chart 1

The leaves of *S. chinensis* were finely cut and treated with methanol (MeOH) to furnish a methanolic extract (13.0%). The MeOH extract was partitioned into an EtOAc–H_2O (1:1, v/v) mixture to furnish an

EtOAc-soluble fraction (4.1%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH- and a H_2O-soluble fraction (2.4% and 6.6%, respectively) as previously reported.[14] The *n*-BuOH-soluble fraction was subjected to Diaion HP-20 column chromatography (H_2O $\rightarrow$ MeOH $\rightarrow$ acetone) to give H_2O-, MeOH-, and acetone-eluted fractions (1.19%, 0.93%, and 0.26%, respectively). From the MeOH-eluted fraction, foliachinenosides A_1 (**1**, 0.0010%), A_2 (**2**, 0.00013%), A_3 (**3**, 0.00003%), B_1 (**4**, 0.00006%), B_2 (**5**, 0.00047%), C (**6**, 0.00044%), and D (**7**, 0.00044%) were isolated using normal-, reverse-phase silica gel column chromatography, and finally HPLC. (Chart 1).

Foliachinenoside A_1 (**1**) was isolated as an amorphous powder with negative optical rotation ($[\alpha]_D^{27}$ – 23.5° in MeOH). The molecular formula, $C_{26}H_{32}O_{14}$, of **1** was clarified from the positive- and negative-ion FABMS [(*m/z* 591 (M+ Na)$^+$, *m/z* 567 (M–H)$^-$) and by HRFABMS measurement. The UV and IR spectra showed the presence of hydroxyl (3397 cm^{-1}), ether (1073 cm^{-1}), aromatic ring and an aromatic ester bond (274 nm; 1719, 1655, 1561, 1458 and 1260 cm^{-1}). Acid hydrolysis **1** with 1 M HCl liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[16–18] The ^{1}H- and ^{13}C-NMR (CD_3OD, Table 1) spectra[19] indicated the presence of a 1,2,6-trisubstituted aromatic ring and a benzyl group [δ5.29, 5.44 (1H each, both d, *J* = 12.2 Hz, 7'-H_2), 6.97 (2H, d, *J* = 8.6 Hz, 3,5-H), 7.32 (1H, m, 4'-H), 7.35 (2H, m, 3',5'-H), 7.37 (1H, m, 4-H), 7.48 (2H, dd, *J* = 1.3, 8.6 Hz, 2',6'-H)], and two β-D-glucopyranosyl moieties [δ4.94 (2H, d, *J* = 7.6 Hz, 1',1''-H). As shown in Figure 1, the ^{1}H–^{1}H COSY experiment on **1** indicated the presence of partial structure written in bold lines. The carbon skeleton and the positions of functional groups were characterized by the HMBC experiment, which showed long-range correlations between the following proton and carbon pairs: 3,5-H and 1-C; 4-H and 2,6-C; 4'-H and 2',6'-C; 7'-H_2 and 7-C, 1'-C, 2',6'-C; 1'', 1'''-H and 2,6-C. Furthermore, the NOESY spectrum showed NOE correlations between the 7'-protons and 2', 6'- protons. On the basis of this evidence, the structure of **1** was elucidated as shown.



1 **2** **3**

Figure 1

Table 1. ^{1}H- and ^{13}C-NMR (CD_3OD, 500/125 MHz) data for **1–3**

	1		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	117.3		122.5		131.0	
2	156.4		158.7		145.8	
3	111.6	6.97 (d, 8.6)	119.2	7.45 (br d, *ca*. 8)	151.9	
4	132.8	7.37 (m)	135.5	7.56 (ddd, 1.4, 8.2, 8.2)	122.5	7.48 (dd, 1.6, 8.2)
5	111.6	6.97 (d, 8.6)	123.7	7.12 (br dd, *ca*. 8, 8)	126.5	7.19 (dd, 8.2, 8.2)
6	156.4		132.1	7.78 (dd, 1.4, 8.2)	124.4	7.28 (dd, 1.6, 8.2)
7	168.2		168.0		169.9	
1'	137.4		137.5		137.3	
2'	129.3	7.48 (dd, 1.3, 8.6)	129.4	7.47 (br d, *ca*. 8)	129.7	7.49 (dd, 1.3, 7.6)
3'	129.5	7.35 (m)	129.7	7.39 (dd, 8.2, 8.2)	129.6	7.39 (dd, 7.6, 7.6)
4'	129.2	7.32 (m)	129.4	7.33 (m)	129.4	7.34 (m)
5'	129.5	7.35 (m)	129.7	7.39 (dd, 8.2, 8.2)	129.6	7.39 (dd, 7.6, 7.6)
6'	129.3	7.48 (dd, 1.3, 8.6)	129.4	7.47 (br d, *ca*. 8)	129.7	7.49 (dd, 1.3, 7.6)
7'	68.4	5.29 (d, 12.2)	68.1	5.32 (d, 12.4)	68.5	5.21 (d, 12.2)
		5.44 (d, 12.2)		5.37 (d, 12.4)		5.42 (d, 12.2)
1"	102.9	4.94 (d, 7.6)	103.8	4.91 (d, 7.5)	105.3	4.75 (d, 7.7)
2"	74.9	3.38 (m)	75.0	3.51 (dd, 7.5, 8.9)	75.4	3.38 (m)
3"	77.9	3.44 (dd, 8.9, 8.9)	77.5	3.47 (dd, 8.9, 8.9)	77.8	3.39 (m)
4"	71.2	3.35 (m)	71.3	3.40 (dd, 8.9, 8.9)	71.8	3.20 (dd, 9.0, 9.0)
5"	78.3	3.36 (m)	77.7	3.68 (m)	78.1	3.13 (m)
6"	62.5	3.66 (dd, 5.5, 11.7)	69.9	3.81 (dd, 6.2, 11.7)	63.1	3.50 (m)
		3.85 (dd, 2.2, 11.7)		4.17 (dd, 1.8, 11.7)		3.70 (dd, 2.8, 12.2)
1"'	102.9	4.94 (d, 7.6)	104.8	4.38 (d, 7.6)	103.5	4.91 (d, 7.7)
2"'	74.9	3.38 (m)	75.2	3.21 (dd, 7.6, 8.9)	74.8	3.54 (dd, 7.7, 9.2)
3"'	77.9	3.44 (dd, 8.9, 8.9)	78.0	3.32 (dd, 8.9, 8.9)	77.6	3.47 (dd, 9.2, 9.2)
4"'	71.2	3.35 (m)	71.7	3.27 (dd, 8.9, 8.9)	71.3	3.41 (m)
5"'	78.3	3.36 (m)	78.0	3.21 (m)	78.4	3.43 (m)
6"'	62.5	3.66 (dd, 5.5, 11.7)	62.8	3.64 (dd, 6.2, 12.4)	62.5	3.71 (dd, 5.2, 11.6)
		3.85 (dd, 2.2, 11.7)		3.84 (dd, 2.6, 12.4)		3.89 (dd, 1.6, 11.6)

Foliachinenoside A_2 (**2**) was isolated as an amorphous powder with negative optical rotation ($[\alpha]_D^{27}$ – 33.3° in MeOH). In the positive- and negative-ion FABMS of **2**, quasimolecular ion peaks were observed at *m/z* 575 $(M+Na)^+$ and *m/z* 551 $(M–H)^–$, respectively. Its elemental composition was determined to be $C_{26}H_{32}O_{13}$ by HRFABMS (positive-ion mode) analysis. The IR spectra indicated the presence of hydroxyl (3400 cm^{-1}), ether (1073 cm^{-1}), an aromatic ring and aromatic ester (1723, 1653, 1559, 1456, and 1260 cm^{-1}). The acid hydrolysis of **2** liberated D-glucose.[16–18] The ^{1}H- and ^{13}C-NMR (CD_3OD, Table 1) spectra[19] showed the signals caused by a benzyl group and a 1,2-disubstituted aromatic ring [δ 7.12 (1H, br dd, *J* = *ca*. 8, 8 Hz, 5-H), 7.45 (1H, br d, *J* = *ca*. 8 Hz, 3-H), 7.56 (1H, ddd, *J* = 1.4, 8.2, 8.2 Hz, 4-H), 7.78 (1H, dd, *J* = 1.4, 8.2 Hz, 6-H)], and two β-D-glucopyranosyl moiety [δ 4.38 (1H, d, *J* = 7.6 Hz, 1"'-H) and 4.91 (1H, d, *J* = 7.5 Hz, 1"-H). As shown in Figure 1, the ^{1}H–^{1}H COSY experiment on **2** indicated the presence of partial structure written in bold lines. The carbon skeleton and the positions of functional groups were characterized by the HMBC experiment, which showed long-range correlations between the following proton and carbon pairs: 4-H and 6-C; 5-H and 1-C; 6-H and 2-C, 4-C, 7-C; 4'-H and 2',6'-C; 7'-H_2 and 7-C, 1'-C, 2',6'-C; 1"-H and 2-C; 1"'-H and 6"-C. Furthermore, the NOESY spectrum showed NOE correlations between the 7'-protons and 2',6'- protons. On the basis of this evidence, the structure of **2** was elucidated as shown.

Foliachinenoside A_3 (**3**) $[\alpha]_D^{27}$ –14.0° (MeOH), was isolated as an amorphous powder. In the positive- and negative-ion FABMS of **3**, quasimolecular ion peaks were observed at *m/z* 591 $(M+Na)^+$ and *m/z* 567 $(M–H)^-$, respectively. HRMS analysis of the quasimolecular ion peak in the positive-ion FABMS indicated the molecular formula of **3** to be $C_{26}H_{32}O_{14}$, which was the same as that of **1**. The ^{1}H- and ^{13}C-NMR (CD_3OD, Table 1) spectra[19] showed the signals caused by a benzyl group [δ 5.21, 5.42 (1H each, both d, J = 12.2 Hz, 7'-H_2), 7.34 (1H, m, 4'-H), 7.39 (2H, dd, J = 7.6, 7.6 Hz, 3',5'-H), 7.49 (2H, dd, J = 1.3, 7.6 Hz, 2',6'-H)], one 1,2,3-trisubstituted aromatic protons unit [δ 7.19 (1H, dd, J = 8.2, 8.2 Hz, 5-H), 7.28 (1H, dd, J = 1.6, 8.2 Hz, 6-H), 7.48 (1H, dd, J = 1.6, 8.2 Hz, 4-H)], and two β-D-glucopyranosyl moieties [δ 4.75 (1H, d, J = 7.7 Hz, 1"-H) and 4.91 (1H, d, J = 7.7 Hz, 1'"-H)]. The acid hydrolysis **3** with 1 M HCl liberated D-glucose.[16–18] The connectivities of sugar parts in **3** were characterized by HMBC experiment, which showed long-range correlations between the 1"-proton and the 2-carbon and between the 1'"-proton and the 3-carbon. Thus, **3** was elucidated as shown in Figure 1.

Table 2. ^{1}H- and ^{13}C-NMR (CD_3OD, 500/125 MHz) data for **4** and **5**

	4		**5**	
	δ_C	δ_H	δ_C	δ_H
1	134.2		134.7	
2	114.5	6.91 (d, 2.0)	110.0	6.98 (d, 1.8)
3	150.4		150.9	
4	146.5		146.9	
5	117.9	7.16 (d, 8.2)	118.3	7.10 (d, 8.6)
6	123.1	6.89 (dd, 2.0, 8.2)	120.3	6.90 (dd, 1.8, 8.6)
7	130.6	6.36 (dd, 1.4, 11.1)	131.8	6.34 (br d, *ca*. 16)
8	126.7	5.72 (m)	125.3	6.17 (dq, 6.1, 15.6)
9	14.9	1.89 (dd, 1.4, 6.8)	18.6	1.84 (dd, 0.9, 6.1)
1'	102.6	4.90 (d, 7.7)	102.6	4.87 (d, 7.8)
2'	75.0	3.50 (m)	74.9	3.48 (m)
3'	77.7	3.45 (m)	77.7	3.47 (m)
4'	71.5	3.39 (dd, 9.2, 9.2)	71.5	3.37 (m)
5'	77.4	3.61 (m)	77.5	3.60 (m)
6'	69.3	3.75 (m)	69.3	3.75 (m)
		4.09 (dd, 2.0, 10.8)		4.08 (dd, 1.9, 11.6)
1"	104.9	4.28 (d, 6.8)	104.8	4.27 (d, 6.8)
2"	72.5	3.56 (dd, 6.8, 8.9)	72.5	3.56 (dd, 6.8, 8.1)
3"	74.2	3.48 (m)	74.1	3.46 (m)
4"	69.5	3.75 (m)	69.5	3.73 (m)
5"	66.7	3.42 (m)	66.7	3.35 (m)
		3.82 (dd, 2.7, 12.4)		3.79 (m)
C*H*$_3$O-3	56.7	3.86 (s)	56.7	3.86 (s)

Foliachinenoside B_1 (**4**) was obtained as an amorphous powder with negative optical rotation ($[\alpha]_D^{28}$ –53.9° in MeOH). The IR spectrum showed absorption bands assignable to hydroxyl, phenyl and ether functions (3372, 1653, 1509, 1456 and 1073 cm^{-1}). The molecular formula, $C_{21}H_{30}O_{11}$, of **4** was determined from the positive- and negative-ion FABMS [*m/z* 481 $(M+Na)^+$, *m/z* 457 $(M–H)^-$] and by HRFABMS (positive-ion mode) measurements. Acid hydrolysis of **4** with 1 M HCl liberated D-glucose and L-arabinose, which were identified by HPLC analysis using an optical rotation detector.[16–18] The ^{1}H (CD_3OD) and ^{13}C NMR (Table 2) spectra[19] indicated the presence of the following functions: a methyl [δ

1.89 (3H, dd, J = 1.4, 6.8 Hz, 9-H_3)], a methoxyl [δ 3.86 (3H, s, 3-OCH_3)], a *cis*-double bond [δ 5.72 (1H, m, 8-H), 6.36 (1H, dd, J = 1.4, 11.1 Hz, 7-H)] and an ABX-type aromatic protons [δ 6.89 (1H, dd, J = 2.0, 8.2 Hz, H-6), 6.91 (1H, d, J = 2.0 Hz, 2-H), 7.16 (1H, d, J = 8.2 Hz, 5-H)] together with a β-D-glucopyranosyl moiety [δ 4.90 (1H, d, J = 7.7 Hz, 1'-H)] and an α-L-arabinopyranosyl moiety [δ 4.28 (d, J = 6.8 Hz, 1''-H)]. On the basis of above-mentioned evidence, the aglycon of **4** was confirmed as *cis*-isoeugenol. As shown in Figure 2, the positions of the glycoside linkages in **4** were determined by a HMBC experiment, which showed long-range correlations between the following protons and carbons: 1'-H and 4-C; 1''-H and 6'-C. Furthermore, the geometry of the 7-double bond and the position of a methoxyl group were determined by NOESY experiments, in which NOE correlations were observed between the following proton pairs: 2-H and 3-OCH_3; 7-H and 8-H. Thus, the structure of **4** was characterized as *cis*-isoeugenyl *O*-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside.

Foliachinenoside B_2 (**5**) was obtained as an amorphous powder with negative optical rotation ($[\alpha]_D^{28}$ –55.7° in MeOH). The same molecular formula, $C_{21}H_{30}O_{11}$, as **4** was determined from the positive- and negative-ion FABMS and by HRFABMS (positive-ion mode) measurements. As shown in Table 2, the sugar parts of the proton and carbon signals of **5** were identical with those of **4**. The acid hydrolysis of **5** liberated D-glucose and L-arabinose.[16–18] Except for the signals of an oligosugar moiety, ten carbon signals were assignable to the *trans*-isoeugenol moiety assigned by various NMR experiments[19] as shown in Figure 2. Additionally, in the ^{1}H-NMR spectrum of **5**, the coupling constant between 7-H and 8-H (J = 15.6 Hz) established that the geometric structure of the double bond is *trans*. Consequently, the structure of **5** was characterized as *trans*-isoeugenyl *O*-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside.

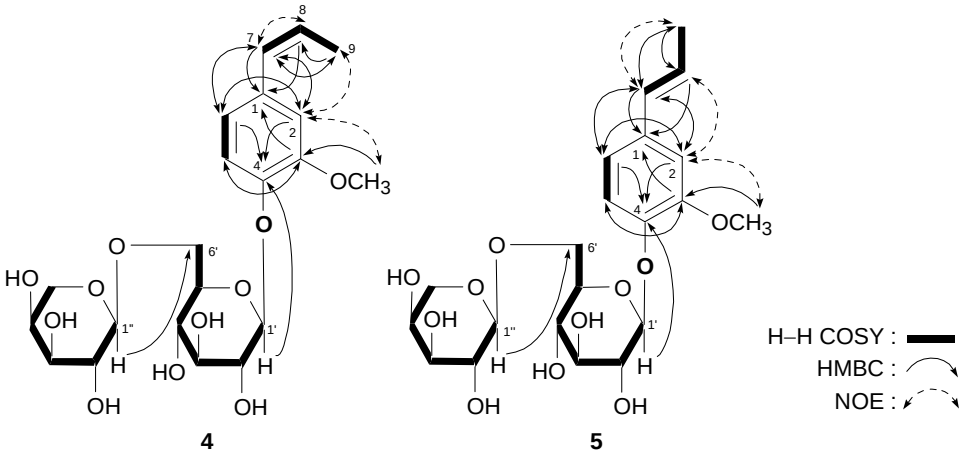


Figure 2

Foliachinenoside C (**6**), $[\alpha]_D^{25}$ –0.2° (MeOH), was isolated as an amorphous powder. HRFABMS revealed the molecular formula of **6** to be $C_{28}H_{38}O_{14}$. The UV and IR spectra indicated the presence of hydroxyl (3389 cm^{-1}), phenolic hydroxyl (238 and 271 nm, 1655 and 1561 cm^{-1}), and ether (1123 cm^{-1}) functions. Analysis of the ^{1}H- and ^{13}C-NMR (CD_3OD, Table 3) spectra[19] suggested that **6** was a lignan, exhibiting two methines [δ 2.305, 2.308 (both m, H-8', 8)], two oxymethylenes {[δ 3.60 (1H, dd, J = 4.9, 11.2 Hz), 3.70 (1H, m), H_2-9'], [δ 3.66, 3.73 (1H each, both m, H_2-9)]}, four methoxyl groups [δ 3.86, 3.88 (6H each, both s, CH_3O-3',5', 3,5)], two oxymethines [δ 4.97 (1H, d, J = 8.3 Hz, 7'-H), 5.01 (1H, d, J = 7.9 Hz, 7-H)], four aromatic protons [δ 6.74, 6.80 (2H each, both s, 2',6', 2,6-H)] together with a β-D-glucopyranosyl moiety [δ 4.86 (1H, d, J = 7.6 Hz, 1''-H)].

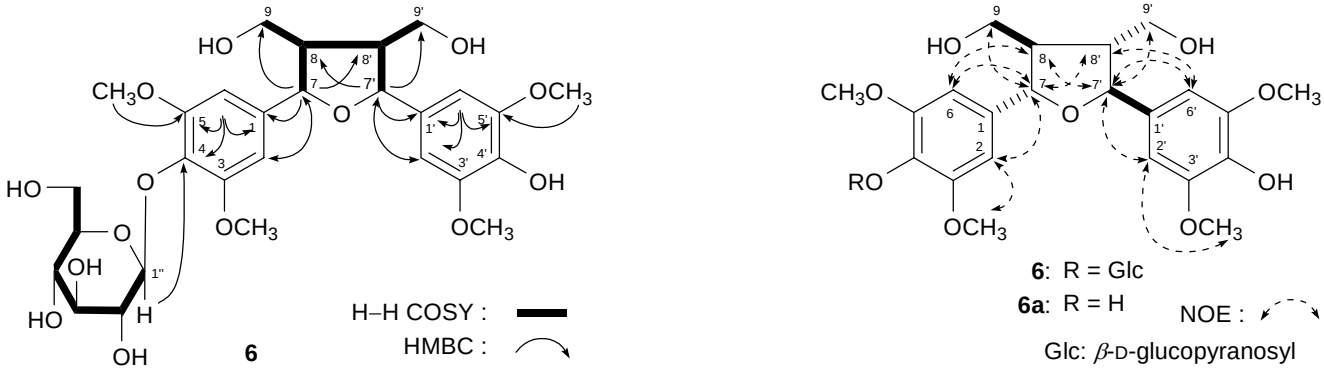


Figure 3

Table 3. ^{1}H- and ^{13}C-NMR (CD_3OD, 500/125 MHz) data for **6** and **6a**

	6		**6a**	
	δ_C	δ_H	δ_C	δ_H
1	140.8		136.4	
2	105.2	6.80 (s)	104.9	6.74 (s)
3	154.4		149.4	
4	135.7		134.3	
5	154.4		149.4	
6	105.2	6.80 (s)	104.9	6.74 (s)
7	84.2	5.01 (d, 7.9)	84.6	4.96 (d, 8.2)
8	55.10	2.308 (m)	55.2	2.32 (m)
9	61.8	3.66 (m)	61.7	3.62 (dd, 4.8, 11.0)
		3.73 (m)		3.71 (dd, 3.4, 11.0)
1'	136.3		136.4	
2'	104.9	6.74 (s)	104.9	6.74 (s)
3'	149.4		149.4	
4'	134.1		134.3	
5'	149.4		149.4	
6'	104.9	6.74 (s)	104.9	6.74 (s)
7'	84.7	4.97 (d, 8.3)	84.6	4.96 (d, 8.2)
8'	55.14	2.305 (m)	55.2	2.32 (m)
9'	61.6	3.60 (dd, 4.9, 11.2)	61.7	3.62 (dd, 4.8, 11.0)
		3.70 (m)		3.71 (dd, 3.4, 11.0)
1"	105.5	4.86 (d, 7.6)		
2"	75.8	3.48 (m)		
3"	77.9	3.41 (m)		
4"	71.4	3.42 (m)		
5"	78.4	3.21 (m)		
6"	62.6	3.67 (m)		
		3.78 (dd, 2.5, 11.9)		
C*H*$_3$O-3,5	57.1	3.88 (s)	56.8	3.87 (s)
C*H*$_3$O-3',5'	56.9	3.86 (s)	56.8	3.87 (s)

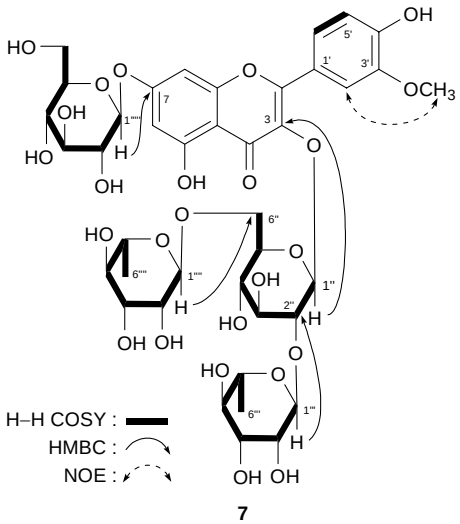
The acid hydrolysis of **6** liberated D-glucose.[16–18] The ^{1}H–^{1}H COSY experiment on **6** indicated the presence of partial structure written in bold lines, and the carbon skeleton and the positions of functional groups were characterized by the HMBC experiment as shown in Figure 3. The *trans*-configurations between the 7- and 8-postions, and between the 7'- and 8'-postions were established by the coupling constants of the 7-proton [δ 5.01 (d, J = 7.9 Hz)] and 7'-proton [δ 4.97 (d, J = 8.3 Hz)], respectively. The relative stereostructure of **6** was characterized by a NOESY experiment, which showed correlations

between the following proton pairs: 2,6-H and 7-H, 8-H, 3,5-OCH_3; 7-H and 9-H_2, 8'-H; 8-H and 7'-H; 2',6'-H and 7'-H, 8'-H, 3',5'-OCH_3; 7'-H and 9'-H_2. Next, enzymatic hydrolysis of **6** with β-glucosidase and cellulase (1:3) furnished a new aglycon, **6a**. The absolute stereostructures of the 7- and 7'-positions in **6** and **6a** were determined by CD spectra.[20,21] Namely, the CD spectra (MeOH) of known phenolic glycosides, 7*R*,7'*R*,8*S*,8'*S*-(+)-neo-olivil-4-*O*-β-D-glucopyranoside[20] and 7*S*,7'*S*,8*R*,8'*R*-icariol A_2-9-*O*-β-D-glucopyranoside[21] having the same fundamental skeleton as **6** showed a positive Cotton effect [236 nm ($\Delta\varepsilon$ +6.7)] and a negative Cotton effects [246 nm ($\Delta\varepsilon$ –4.4)], respectively. The CD spectra (MeOH) of **6** and **6a** showed positive Cotton effects [**6**: 243 nm ($\Delta\varepsilon$ +6.0); **6a**: 246 nm ($\Delta\varepsilon$ +6.3)], respectively. Thus, the absolute configurations of **6** and **6a** were determined to be 7*R*,7'*R*,8*S*,8'*S* orientation as shown.

Table 4. ^{1}H- and ^{13}C-NMR (DMSO-d_6, 500/125 MHz) data for **7**

	δ_C	δ_H		δ_C	δ_H
2	155.9		6"	66.7	3.28 (m)
3	132.6				3.71 (m)
4	177.2		1'''	100.7	5.03 (br s)
5	160.8		2'''	70.5	3.22 (m)
6	99.2	6.45 (br s)	3'''	70.2	3.43 (m)
7	162.8		4'''	71.7	31.2 (m)
8	94.6	6.77 (br s)	5'''	68.2	3.69 (m)
9	156.9		6'''	17.0	0.71 (d, 6.1)
10	105.5		1''''	100.6	4.38 (br s)
1'	120.7		2''''	70.5	3.36 (m)
2'	113.2	7.86 (br s)	3''''	70.3	3.25 (m)
3'	146.8		4''''	71.7	3.05 (m)
4'	149.4		5''''	68.2	3.22 (m)
5'	115.1	6.91 (d, 8.3)	6''''	17.6	0.96 (d, 6.1)
6'	122.2	7.53 (br d, *ca*. 8)	1'''''	99.7	5.07 (d, 7.3)
CH_3O-3'	55.5	3.86 (s)	2'''''	73.0	3.27 (m)
1"	98.4	5.65 (d, 7.7)	3'''''	76.3	3.28 (m)
2"	77.4	3.44 (m)	4'''''	69.5	3.17 (m)
3"	76.9	3.46 (m)	5'''''	77.1	3.27 (m)
4"	70.5	3.74 (m)	6'''''	60.5	3.47 (m)
5"	75.8	3.30 (m)			3.72 (m)

Foliachinenoside D (**7**) was isolated as a yellow powder with negative optical rotation ($[\alpha]_D^{27}$ –66.7° in MeOH). The IR spectrum of **7** showed absorption bands assignable to hydroxyl (3423 cm^{-1}), ester carbonyl (1655 cm^{-1}), aromatic ring (1608, 1509, and 1452 cm^{-1}), and ether (1073 cm^{-1}) functions. In the positive- and negative-ion FABMS of **7**, quasimolecular ion peaks were observed at *m/z* 955 $(M+Na)^+$ and *m/z* 931 $(M-H)^-$, respectively. HRMS analysis of the quasimolecular ion peaks in the positive-ion FABMS indicated the molecular formula of **7** to be $C_{40}H_{52}O_{25}$. The ^{1}H- and ^{13}C-NMR (DMSO-d_6, Table 4) spectra[19] of **7**, which were assigned by various NMR experiments, showed signals ascribable to a isorhamnetin part [δ 3.86 (3H, s, 3'-



7

Figure 4

OC*H*$_3$), 6.45, 6.77 (1H each, both br s, 6, 8-H), 6.91 (1H, d, *J* = 8.3 Hz, 5'-H), 7.53 (1H, br d, *J* = *ca*. 8, 6'-H), 7.86 (1H, br s, 2'-H)] together with two β-D-glucopyranosyl moieties and two α-L-rhamnopyranosyl moieties [δ 0.71, 0.96 (3H each, both d, *J* = 6.1 Hz, 6'''-H, 6''''- H), 4.38, 5.03 (both br s, 1''''-H, 1'''-H), 5.07 (d, *J* = 7.3 Hz, 1'''''-H), 5.65 (d, *J* = 7.7 Hz, 1'''-H)]. Treatment **7** with 1 M HCl liberated D-glucose and L-rhamnose, which was identified by HPLC analysis using an optical rotation detector.[16–18] Furthermore, in the HMBC experiment, long-range correlations were observed between the 1''-H and 3-C, 1'''-H and 2''-C, 1''''-H and 6''-C, 1'''''-H and 7-C. Thus the structure of **7** was determined as shown.

EXPERIMENTAL

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (*l* = 5 cm); CD spectra, JASCO J-720WI spectrometer; UV spectra, Shimadzu UV-1600; IR spectra, Shimadzu FTIR-8100 spectrophotometer; FAB-MS and high-resolution FAB-MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) and JNM-LA500 (500 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) and JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV-VIS detectors; and HPLC column, Cosmosil 5C$_{18}$-MS-II (250 × 4.6 mm i.d.) and (250 × 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: normal-phase column chromatography; Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh), reversed-phase column chromatography; Diaion HP-20 (Nippon Rensui): TLC, pre-coated TLC plates with Silica gel 60F$_{254}$ (Merck, 0.25 mm) (normal-phase) and Silica gel RP-18 F$_{254S}$ (Merck, 0.25 mm) (reversed-phase); HPTLC, pre-coated TLC plates with Silica gel RP-18 WF$_{254S}$ (Merck, 0.25 mm) (reversed-phase) and detection was achieved by spraying with 1% Ce(SO$_4$)$_2$-10% aqueous H$_2$SO$_4$, followed by heating.

Plant Material

The dried leaves of *S. chinensis* were collected at Thailand in 2006 and identified by one of authors (Rajamangala University of Technology Srivijaya, Pongpiriyadacha Y.). A voucher of the plant is on file in our laboratory (2006. Thai-06).

Isolation of Foliachinenosides A$_1$ (1), A$_2$ (2), A$_3$ (3), B$_1$ (4), B$_2$ (5), C (6), and D (7)

The dried leaves of *S. chinensis* Linn. (5.8 kg) were finely cut and extracted 3 times with methanol (MeOH) under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (756 g, 13.0%). The MeOH extract (712 g) was partitioned into an EtOAc–H$_2$O (1:1, v/v) mixture to furnish an EtOAc-soluble fraction (222 g, 4.1%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (130 g, 2.4%) and a H$_2$O-soluble fraction (361 g, 6.6%). The *n*-BuOH-soluble fraction (100.0 g) was subjected to Diaion HP-20 column chromatography (1.5 kg, H$_2$O→MeOH→acetone) to give H$_2$O-eluted fraction (49.8 g, 1.19%), MeOH-eluted fraction (39.2 g, 0.93%) and acetone-eluted fraction (11.0 g, 0.26%), respectively. The MeOH-eluted fraction (39.2 g) was subjected to ordinary-phase silica gel column chromatography {480 g,

$CHCl_3$–MeOH (10:1, v/v) → $CHCl_3$–MeOH–H_2O [(10:3:1, v/v/v, lower layer) → (7:3:1, v/v/v, lower layer) → (6:4:1, v/v/v, lower layer)] → MeOH} to give ten fractions [Fr. 1 (0.5 g), Fr. 2 (0.6 g), Fr. 3 (1.3 g), Fr. 4 (7.3 g), Fr. 5 (3.0 g), Fr. 6 (6.7 g), Fr. 7 (1.6 g), Fr. 8 (2.4 g), Fr. 9 (9.3 g), Fr. 10 (3.5 g)]. Fraction 4 (7.3 g) was subjected to reversed-phase silica gel column chromatography [220 g, H_2O → MeOH–H_2O (10:90 → 20:80 → 30:70 → 40:60 → 60:40, v/v) → MeOH → $CHCl_3$] to afford ten fractions [Fr. 4-1 (783 mg), Fr. 4-2 (821 mg), Fr. 4-3 (1122 mg), Fr. 4-4 (577 mg), Fr. 4-5 (288 mg), Fr. 4-6 (652 mg), Fr. 4-7 (1295 mg), Fr. 4-8 (413 mg), Fr. 4-9 (253 mg), Fr. 4-10 (760 mg)]. Fraction 4-4 (577 mg) was isolated by HPLC [MeOH–H_2O (25:75, v/v)] to give seven fractions [Fr. 4-4-1 (25.2 mg), Fr. 4-4-2 (13.8 mg), Fr. 4-4-3 (35.6 mg), Fr. 4-4-4 (18.5 mg), Fr. 4-4-5 (8.9 mg), Fr. 4-4-6 (12.1 mg), Fr. 4-4-7 (138.2 mg)]. Fractions 4-4-1 (25.2 mg), 4-4-4 (18.5 mg) were subjected to HPLC [MeOH–H_2O–CH_3CN (10:8:82, v/v/v)] to furnish foliachinenoside C (**6**, 18.2 mg, 0.00042%) Fraction 4-7 (1295 mg) was separated by HPLC [MeOH–H_2O (40:60, v/v)] and [CH_3CN–MeOH–H_2O (15:8:77, v/v/v)] to furnish foliachinenosides B_1 (**4**, 2.6 mg, 0.00006%) and B_2 (**5**, 16.6 mg, 0.00039%). Fraction 4-8 (413 mg) was subjected to HPLC [MeOH–H_2O (40:60, v/v)] to afford nine fractions [Fr. 4-8-1 (11.9 mg), Fr. 4-8-2 (22.2 mg), Fr. 4-8-3 (47.6 mg), Fr. 4-8-4 (16.2 mg), Fr. 4-8-5 (14.2 mg), Fr. 4-8-6 (11.4 mg), Fr. 4-8-7 (14.6 mg), Fr. 4-8-8 (16.8 mg), Fr. 4-8-9 (16.2 mg)]. Fraction 4-8-3 (47.6 mg) was further purified by HPLC [CH_3CN–MeOH–H_2O (15:8:77, v/v/v)] to give foliachinenoside B_2 (**5**, 3.3 mg, 0.00008%). Fraction 4-8-8 (16.8 mg) was separated by HPLC [CH_3CN–MeOH–H_2O (17:8:75, v/v/v)] to furnish foliachinenoside A_2 (**2**, 5.4 mg, 0.00013%). Fraction 6 (6.7 g) was subjected to reversed-phase silica gel column chromatography [220 g, MeOH–H_2O (10:90 → 20:80 → 30:70 → 40:60 → 50:50 → 60:40, v/v) → MeOH → $CHCl_3$] to give eleven fractions [Fr. 6-1 (809 mg), Fr. 6-2 (859 mg), Fr. 6-3 (118 mg), Fr. 6-4 (103 mg), Fr. 6-5 (480 mg), Fr. 6-6 (300 mg), Fr. 6-7 (250 mg), Fr. 6-8 (1277 mg), Fr. 6-9 (239 mg), Fr. 6-10 (335 mg), Fr. 6-11 (1247 mg)]. Fraction 6-2 (859 mg) was separated by HPLC [MeOH–H_2O (25:75, v/v)] to afford thirteen fractions [Fr. 6-2-1 (18.2 mg), Fr. 6-2-2 (28.5 mg), Fr. 6-2-3 (36.3 mg), Fr. 6-2-4 (16.7 mg), Fr. 6-2-5 (42.5 mg), Fr. 6-2-6 (32.1 mg), Fr. 6-2-7 (38.9 mg), Fr. 6-2-8 (70.6 mg), Fr. 6-2-9 (37.5 mg), Fr. 6-2-10 (58.3 mg), Fr. 6-2-11 (21.2 mg), Fr. 6-2-12 (24.6 mg), Fr. 6-2-13 (18.9 mg)]. Fraction 6-2-5 (42.5 mg) was identified as foliachinenoside A_1 (**1**, 42.5 mg, 0.0010%). Fraction 6-6 (300 mg) was isolated with HPLC [MeOH–H_2O (40:60, v/v)] to afford foliachinenoside A_3 (**3**, 12.8 mg, 0.00031%). Fraction 9 (9.3 g) was subjected to reversed-phase silica gel column chromatography [50 g, MeOH–H_2O (20:80 → 30:70 → 40:60 → 50:50 → 60:40, v/v) → MeOH] to afford eleven fractions [Fr. 9-1 (1187 mg), Fr. 9-2 (322 mg), Fr. 9-3 (230 mg), Fr. 9-4 (295 mg), Fr. 9-5 (511 mg), Fr. 9-6 (954 mg), Fr. 9-7 (189 mg), Fr. 9-8 (1299 mg), Fr. 9-9 (484 mg), Fr. 9-10 (981 mg), Fr. 9-11 (1662 mg)]. Fraction 9-4 (295 mg) was separated by HPLC [MeOH–H_2O (24:76, v/v)] and HPLC [CH_3CN–MeOH–H_2O (7:7:86, v/v/v)] to furnish foliachinenoside D (**7**, 9.3 mg, 0.00022%). Fraction 9-5 (511 mg) was isolated with HPLC [MeOH–H_2O (24:76, v/v)] and HPLC [CH_3CN–MeOH–H_2O (8:8:84, v/v/v)] to afford foliachinenoside D (**7**, 9.3 mg, 0.00022%).

Foliachinenoside A_1 (1): an amorphous powder, $[\alpha]_D^{27}$ –23.5° (*c* 2.30, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{26}H_{32}O_{14}Na$ $(M+Na)^+$: 591.1690. Found: 591.1696. UV [MeOH, nm, (logε)]:

274 (3.27). IR (KBr): 3397, 2932, 2889, 1719, 1655, 1561, 1458, 1073 cm^{-1}. ^1H- and ^{13}C-NMR data (500/125 MHz, CD_3OD): given in Table 1. Positive-ion FAB-MS: *m/z* 591 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 567 $(M–H)^–$.

Foliachinenoside A_2 (2): an amorphous powder, $[\alpha]_D^{27}$ –33.3° (*c* 0.11, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{26}H_{32}O_{13}Na$ $(M+Na)^+$: 575.1741. Found: 575.1745. UV [MeOH, nm, (logε)]: 288 (3.40), 232 (3.94), 204 (4.57). IR (KBr: 3400, 2926, 1771, 1653, 1559, 1456, 1260, 1073 cm^{-1}. ^1H- and ^{13}C-NMR data (500/125 MHz, CD_3OD): given in Table 1. Positive-ion FAB-MS: *m/z* 575 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 551 $(M–H)^–$.

Foliachinenoside A_3 (3): an amorphous powder, $[\alpha]_D^{27}$ –14.0° (*c* 0.64, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{26}H_{32}O_{14}Na$ $[M + Na]^+$: 591.1690. Found: 591.1696. UV [MeOH, nm, (logε)]: 283 (3.35), 204 (4.42). IR (KBr): 3420, 2934, 1715, 1651, 1559, 1507, 1456, 1073, 1047, 756 cm^{-1}. ^1H- and ^{13}C-NMR data (500/125 MHz, CD_3OD): given in Table 1. Positive-ion FAB-MS: *m/z* 591 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 567 $(M–H)^–$.

Foliachinenoside B_1 (4): an amorphous powder, $[\alpha]_D^{28}$ –53.9° (*c* 0.13, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{21}H_{30}O_{11}Na$ $[M + Na]^+$: 481.1686. Found: 481.1679. UV [MeOH, nm, (logε)]: 253 (4.15), 210 (4.43). IR (KBr): 3372, 2926, 1653, 1638, 1561, 1509, 1456, 1073 cm^{-1}. ^1H- and ^{13}C-NMR data (500/125 MHz, CD_3OD): given in Table 2. Positive-ion FAB-MS: *m/z* 481$(M+Na)^+$. Negative-ion FAB-MS: *m/z* 457 $(M–H)^–$.

Foliachinenoside B_2 (5): an amorphous powder, $[\alpha]_D^{25}$ –55.7° (*c* 0.88, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{21}H_{30}O_{11}Na$ $[M + Na]^+$: 481.1686. Found: 481.1692. UV [MeOH, nm, (logε)]: 256 (4.15), 209 (4.32). IR (KBr): 3422, 2924, 2855, 1655, 1637, 1561, 1458, 1339, 1117 cm^{-1}. H- and ^{13}C-NMR data (500/125 MHz, CD_3OD): given in Table 2. Positive-ion FAB-MS: *m/z* 481$(M+Na)^+$. Negative-ion FAB-MS: *m/z* 457 $(M–H)^–$.

Foliachinenoside C (6): an amorphous powder, $[\alpha]_D^{25}$ –0.2° (*c* 0.91, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{28}H_{38}O_{14}Na$ $[M + Na]^+$: 621.2159. Found: 621.2162. UV [MeOH, nm, (logε)]: 271 (3.28), 238 (4.06), 213 (4.62). CD [MeOH, nm, ($\Delta\varepsilon$)]: 243 (+6.00). IR (KBr): 3389, 2932, 1655, 1561, 1123 cm^{-1}. ^1H- and ^{13}C-NMR data (500/125 MHz, CD_3OD): given in Table 3. Positive-ion FAB-MS: *m/z* 621 $(M+Na)^+$.

Foliachinenoside D (7): a yellow powder, $[\alpha]_D^{27}$ –66.7° (*c* 0.14, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{40}H_{52}O_{25}Na$ $[M + Na]^+$: 955.2695. Found: 955.2697. UV [MeOH, nm, (logε)]: 343 (4.14), 263 (4.23). IR (KBr): 3423, 2931, 1655, 1509, 1452, 1347, 1181, 1073 cm^{-1}. ^1H- and ^{13}C-NMR data (500/125 MHz, DMSO-d_6): given in Table 4. Positive-ion FAB-MS: *m/z* 955 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 931$(M–H)^–$.

Enzymatic hydrolysis of foliachinenoside C (6) with β-glucosidase and cellulase (1:3)

To a solution of **6** (5.1 mg) in 2.0 mL of 0.2 M acetate buffer (pH 3.8), was added β-glucosidase (5.0 mg, 200 U) and cellulase (15.0 mg, 4.5 U, from Sigma Chemical Co., USA) and stirred at 37°C for 48 h. Work up the reaction mixture as described above to give a residue, which was separated by HPLC [MeOH–H_2O (30:70, v/v)] to afford **6a** (0.8 mg, 22%).

Compound 6a: an amorphous powder, $[\alpha]_D^{28}$ +14.5° (*c* 0.04, MeOH). High-resolution EI-MS: Calcd for

$C_{22}H_{28}O_9$ $[M]^+$: 436.1733. Found: 436.1729. UV [MeOH, nm, (logε)]: 271 (3.40), 239 (4.09), 213 (4.61). CD [MeOH, nm, ($\Delta\varepsilon$)]: 246 (+6.30). IR (KBr): 3368, 2938, 1655, 1615, 1520, 1462, 1329, 1217, 1116, 754 cm^{-1}. ^1H- and ^{13}C-NMR data (500/125 MHz, CD_3OD): given in Table 3. EIMS: *m/z* 436 $(M)^+$ (5), 418 (24), 400 (51), 319 (100).

Acid Hydrolysis of New Compounds 1–7

A solution of compounds **1–7** (each 1.5 mg) in 1 M HCl (1.0 mL) was heated under reflux for 3 h. After cooling, the reaction mixture was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the following conditions, respectively: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d. × 250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan); mobile phase, CH_3CN-H_2O (85:15, v/v); flow rate 0.8 mL/min]. Identification of L-rhamnose (i) from **7**; L-arabinose (ii) from **4** and **5**; and D-glucose (iii) from **1–7** present in the aqueous layer was carried out by comparison of its retention time and optical rotation with those of authentic sample, t_R: (i) 7.5 min (L-rhamnose, negative optical rotation), (ii) 10.2 min (L-arabinose, positive optical rotation), and (iii) 12.8 min (D-glucose, positive optical rotation).

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