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Symplocosionosides A-C, Three Megastigmane Glycosides, a Neolignan Glucoside, and Symplocosins A and B, Two Triterpene Glycosyl Esters from the Leaves of *Symplocos cochinchinensis* var. *Philippinensis*

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ABSTRACT

From the 1*-BuOH-soluble fraction of a MeOH extract of the leaves of Symplocos cochinchinensis var. philippinensis*, 12 *compounds were isolated. Spectroscopic analyses of compounds* 1 - 3 *established their structures to be megastigmane glycosides*, *named symplocosionosides A-C. The absolute structure of* 1 *was determined by the modified Mosher's method. Compound* 4 *was found to be a neolignan glucoside and named symplocosneolignan. The structures of compounds* 5 *and* 6, *named symplocosins A and B*, *were elucidated to be the saponins of hederagenin sugar esters. The structures of the remaining known compounds* (7 - 12) *were identified by comparison of spectroscopic data with those reported in the literature*.

***Keywords*:** *Symplocos Cochinchinensis var. Philippinensis*, *Symplocaceae*, *Megastigmane Glycoside*, *Neolignan Glucoside*, *Triterpene Glycosyl Ester*, *Modified Mosher's Method*

1. Introduction

Genus *Symplocos* comprises about 300 species, which are mainly found in tropical, except for Africa, and subtropical areas, with a small number of species in the temperate zone. *Symplocos cochinchinensis* (Loureiro) Spencer Le Marchant Moore var. *philippinensis* (Brand) Nootboom (Symplocaceae) is an evergreen tall tree, which is distributed in the Amami and Okinawa Islands, Taiwan, Southern China and Indochina. It grows up to about 15 m in height and bears white flowers in spikes [1]. So far as we know, no chemical investigation has been performed on this plant. Even for its elementary species, *S. cochinchinensis* (Loureiro) Spencer Le Marchant Moore, only few pharmacological works have been performed on its extract [2-4]. Thus, we were promted to invetigate the chemical constituents in the title plant. From the 1-BuOH-soluble fraction of a MeOH extract of the leaves of *S. cochinchinensis* var. *philippinensis*, three new megastigmane glycosides, named symplocosionosides A-C (1-3), a new neolignan glucoside, named symplocosneolignan A (4), and two new triterpene glycosyl esters, named symplocosins A and B (5,6), together with six known compounds, dendranthemoside A (7) [5], 4,5-dihydroblumenol (8) [6], alangionoside B (9) [7], ampelopsisionoside (10) [8], citroside A (11) [9], and nigaichigoside F1 (12) [10] were isolated. This paper deals with structural elucidation of the new compounds.

2. Results and Discussion

From the 1-BuOH-soluble fraction of a MeOH extract of the leaves of *S. cochinchinensis* var. *philippinensis*, six new compounds (1-6) and six known (7-12) compounds were isolated by means of a combination of various chromatographic technique.

Symplocosionoside A (1), $[\alpha]_D^{25}$ –44.0, was isolated as an amorphous powder and its elemental composition was determined to be $C_{24}H_{42}O_{11}$ by high-resolution (HR)-electrospray ionization (ESI)-mass spectrometry (MS).

Strong absorption bands at 3394 cm^{-1} and 1055 cm^{-1} in the IR spectrum indicated that symplocosionoside A (1) was a glycoside and in the NMR spectra, two anomeric carbon and proton signals (δ_C 102.9 with δ_H 4.33 and δ_C 110.9 with δ_H 5.00) were observed. The ^{1}H-NMR spectrum exhibited signals for two singlet and two doublet methyls, and one *trans* double bond. Of the 24 carbon NMR signals, 11 were assigned to those for a *β*-D-(6-*O*-*β*-D-apiofuranosyl) glucopyranoside moiety (**Table 1**) [11]. The absolute configurations of apiose and glucose were determined to be of the D-series by HPLC analysis of a hydrolyzate of 1. The remaining 13 signals comprised of those of four methyls, two methylenes, two oxymethines, two methines, one quaternary carbon and a double bond. From the above evidence, the aglycone of symplocosionoside A (1) was assumed to have a megastigmane skeleton, and the proton spin-spin coupling sequence from H_2-2 through H_3-10 was revealed by the ^{1}H-^{1}H correlation spectroscopy (COSY) spectrum. Further detailed inspection of two-dimensional NMR spectra established the structure of the aglycone moiety. Since the H-3 proton (δ_H 3.83) was coupled with the axial protons at H-2 and H-4 with a coupling constant of 12 Hz, the hydroxy substituent at the 3-position was placed in the equatorial position, and the similar coupling constants for H-4ax and H-5, and H-5 and H-6 also are favorable for placement of both of the methyl group at the 5-position and the side chain at the 6-position in the equatorial position. The site of sugar attachment was established to be to the hydroxy group at the 3-position by the HMBC correlation cross peak between H-1' (δ_H 4.33) and C-3 (δ_C 76.0). The absolute configuration at the 3-position was first expected to be *S* from the empirical *β*-D-glucopyranosylation-induced shift-trend rule for glycoside (1) and the aglycone (1a) (**Table 1**) [12], and then the final confirmation of that of the aglycone (1a) was achieved by the modified Mosher's method (**Figure 2**) [13]. Therefore, the structure of symplocosionoside A (1) was elucidated to be (3*S*, 5*R*, 6*R*, 7*E*, 9*R*)-megastigman-7-ene-3, 9-diol 3-*O*-*β*-D-(6'-*O*-*β*-D-apiofuranosyl) glucopyranoside, as shown in **Figure 1**.

Table 1. ^{13}C-NMR Data for Symplocosionoside A–C (1–3) and 1a (100 MHz, CD_3OD).

c	1	1a	2	3
1	35.8	35.9	44	37.0 (36.4)[b]
2	48.0	51.3 (–3.3)[a]	52.5	48.8 (50.3)
3	76.0	67.4 (+8.6)	215.0	63.8 (62.5)
4	43.9	45.6 (–1.7)	46.2	48.0 (47.4)
5	32.2	32.2	37.9	78.8 (78.3)
6	58.6	58.6	78.2	119.0 (118.6)
7	131.2	131.2	134.7	200.9 (197.6)
8	138.4	138.5	134.3	101.4 (100.9)
9	69.3	69.3	78.1	213.1 (211.6)
10	24.0	24.0	21.5	26.7 (26.6)
11	21.7	21.69	25.1	30.1 (29.8)
12	31.9	31.9	25.4	32.5 (32.3)
13	21.7	21.72	16.5	26.7 (26.6)
1'	102.9		102.7	98.7 (98.5)
2'	75.0		75.4	75.3 (75.1)
3'	78.0		78.0	78.4 (79.1)
4'	71.8		71.7	71.7 (71.8)
5'	76.7		77.0	76.5 (76.6)
6'	68.6		68.6	69.2 (68.8)
1"	110.9		110.0	105.0 (105.1)
2"	78.0		77.6	72.3 (72.2)
3"	80.5		80.5	74.1 (74.2)
4"	75.1		75.0	69.9 (69.8)
5"	65.8		65.7	66.3 (66.1)

a) $\Delta\delta_{1\text{-}1a}$. *b*) Data for C_5D_5N solution.

Symplocosionoside B (2), $[\alpha]_D^{26}$ –63.0, was isolated as an amorphous powder and its elemental composition was determined to be $C_{24}H_{40}O_{12}$ by HR-ESI-MS. The IR

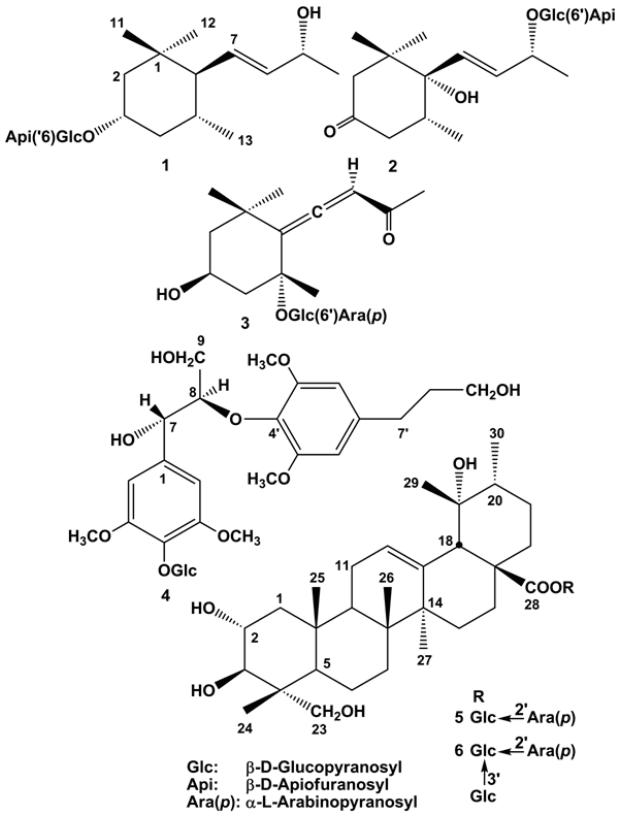

Figure 1. Structures of isolated new compounds.

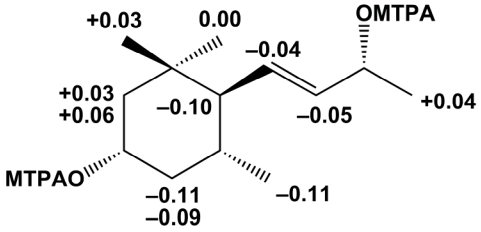

Figure 2. Results of modified Mosher's method for symplocosionoside A (1).

spectrum exhibited a glycosidic feature (3399 cm^{-1} and 1056 cm^{-1}) and the presence of a ketonic functional group (1694 cm^{-1}). In the ^{1}H-NMR spectrum, the signals for two singlet methyls, two doublet methyls, one *trans* double bond and two anomeric protons were observed. The ^{13}C-NMR spectrum exhibited 24 signals, 11 of which were assignable to those of a 9-*O*-*β*-D-(6-*O*-*β*-D-apiofur-anosyl) glucopyranoside moiety. The absolute configurations of apiose and glucose were determined to be of the D-series by HPLC analysis of the hydrolyzate of 2. The remaining 13 signals comprised those of four methyls, two methylenes, one oxymethine, one methine, one double bond, two quaternary carbons and a ketone functional group. From the above evidence together with the information obtained from two dimensional spectra, the structure of symplocosonoside B (2) was determined to be 3-keto-6-hydro- xymegastigmane glycoside, as shown in **Figure 1**. From the proton-proton coupling constant, $J_{4ax\text{-}5}$ = 14 Hz, the methyl group at the 5-position was placed in an equatorial position, and then the correlation cross peak between H-5 (δ_H 2.28) and H-7 (δ_H 5.74) observed on phase-sensitive (PS)-nuclear Overhauser effect spectroscopy (NOESY) allowed placement of the side chain also in an equatorial position. Based on the octant rule, the absolute configuration at the 5-position was determined to be *R* from the positive Cotton effect at 289 nm in the CD spectrum and thus the 6-position must have the *S* configuration. By comparing the reported data, the absolute configuration at the 9-position was determined to be *R*, and the linkage of the sugar unit to the hydroxy group at the 9-positon was confirmed by the HMBC correlation cross peak between H-1' (δ_H 4.34) and C-9 (δ_C 78.1). Therefore, the structure of symplocosionoside B (2) was elucidated to be (5*R*, 6*S*, 7*E*, 9*R*)-megastigman-7-en-9-ol-3-one 9-*O*-*β*-D-(6'-*O*-*β*-D-apiofuranosyl) glucopyranoside.

Symplocosonoside C (3), $[\alpha]_D^{25}$ –57.9, was isolated as an amorphous powder and its elemental composition was determined to be $C_{24}H_{38}O_{12}$ by HR-ESI-MS. The IR spectrum exhibited a characteristic absorption band for an allenic part (1938 cm^{-1}), and the presence of this functional group was supported by the ^{13}C-NMR spectrum (δ_C 118.6, 197.6 and 100.9 in C_5D_5N). Other ^{13}C-NMR signals showed good similarity to those of citrosides A and B, isolated from *Citrus unshiu* [9], except for the presence of signals for a terminal *α*-arabinopyranose, and the upfield and downfield shifts of the C-5' (δ_C 76.6 in C_5D_5N) and C-6' (δ_C 68.8 in C_5D_5N) signals. A significant cross peak, observed between H-1" (δ_H 5.05 in C_5D_5N) and C-6' in the HMBC spectrum, confirmed the sugar linkage. The absolute configurations of arabinose and glucose were determined to be of the L- and D-series, respectively, by HPLC analysis of the hydrolyzate of 3 using the chiral detector. The axis chirality of the allene part was determined to be the same as that of citroside A, tentatively *R*, as judged on comparison of the ^{13}C-NMR chemical shift of C-7 (δ_C 197.6 in C_5D_5N) with those for citrosides A (C-7: δ_C 197.6) and B (C-7: δ_C 199.0). Therefore, the structure of symplocosionoside C (3) was elucidated to be citroside A 6'-*O*-*α*-L-arabinopyranoside, as shown in **Figure 1**.

Symplocosneolignan (4), $[\alpha]_D^{25}$ –13.5, was isolated as an amorphous powder and its elemental composition was determined to be $C_{28}H_{40}O_{14}$ by HR-ESI-MS. The IR spectrum showed that symplocosneolignan (4) was a glycosidic compound (3395 cm^{-1}) with aromatic ring (s) (1592 cm^{-1}). A UV absorption band (269 nm) also supported the presence of the aromatic ring (s). In the ^{1}H-NMR spectrum, signals for two singlet aromatic protons (δ_H 6.53 and 6.75), two methoxy protons (δ_H 3.79 and 3.84), and an anomeric proton (δ_H 4.83) were observed (**Table 2**). Each of the aromatic signals accounted for two protons and that of the methoxy signals six protons. The ^{13}C-NMR spectrum exhibited 22 signals, of which six were assignable to *β*-glucopyranose. As to the remaining 16 signals, two methoxy signals were expected to include those of four carbons from their peak heights and each of the four aromatic signals was obviously for two carbons. Thus, the two aromatic rings must be substituted symmetrically and the other signals comprised those of two primary alcohols, two methylenes and two oxymethines. The proton chains from H-7 to H-9 and H-7' to H-9' were assigned by inspection of the ^{1}H-^{1}H COSY spectrum, and the correlation cross peaks between H-8 (δ_H 4.18), and C-1 (δ_C 139.5) and C-4' (δ_C 134.9) in the HMBC spectrum established the structure of symplocosneolignan (4), as shown in **Figure 1**. Other HMBC correlations shown in **Figure 3** also supported the structure. The site of the sugar linkage was determined to be to the hydroxy group at the 4-position, judging from the cross peak between H-1' and C-4 (δ_C 135.6) in the HMBC spectrum, and the mode of linkage to be *β* from the coupling constant (*J* = 8 Hz) of the anomeric proton.

Table 2. NMR spectral data for symplocosneolignan 4 and 4a [CD_3OD, 100 MHz (^{13}C) and 400 MHz (^1H)].

	4		4a	
	C	H	C	H
1	139.5		132.4	
2, 6	106.1	6.75 (2H, s)	105.4	6.67 (2H, s)
3, 5	153.9		149.1	
4	135.6		133.2	
7	74.1	4.93 (1H, d, 5)	74.3	4.91 (1H, d, 5)
8	87.2	4.18 (1H, ddd, 6, 6, 5)	87.5	4.18 (1H, ddd, 5, 5, 5)
9	61.5	3.86 (1H, dd, 12, 6)	61.7	3.88 (1H, dd, 12, 5)
		3.56 (1H, dd, 12, 6)		3.55 (1H, dd, 12, 5)
$-OCH_3$	57.0	3.79 (6H, s)	56.9	3.80 (6H, s)
1'	140.0		140.0	
2', 6'	107.0	6.53 (2H, s)	107.1	6.54 (2H, s)
3', 5'	154.4		154.4	
4'	134.9		135.0	
7'	33.4	2.64 (2H, t, 7)	33.5	2.63 (2H, t, 7)
8'	35.4	1.83 (2H, tt, 7, 7)	35.5	1.83 (2H, tt, 7, 7)
9'	62.3	3.59 (2H, t, 7)	62.2	3.57 (2H, t, 7)
$-OCH_3$	56.7	3.84 (6H, s)	56.7	3.85 (6H, s)
1"	105.6	4.83 (1H. d, 8)		
2"	75.8	3.58 (1H, m)		
3"	77.9	3.43 (1H, m)		
4"	71.4	3.43 (1H, m)		
5"	78.4	3.21 (1H, m)		
6"	62.7	3.77 (1H, dd, 12, 2)		
		3.66 (1H, dd, 12, 6)		

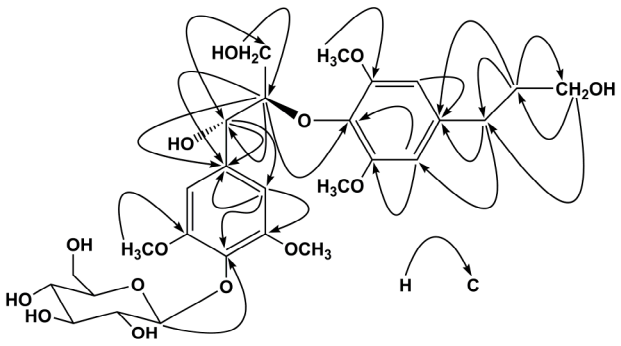

Figure 3. Diagnostic HMBC correlations for symplocosneolignan (4).

Judging from the coupling constant (J = 5 Hz) for H-7 and 8, symplocosneolignan (4) was concluded to possess the *erythro* relative stereochemistry [15]. Enzymatic hydrolysis of symplocosneolignan (4) gave its aglycone (4a), whose $J_{7\text{-}8}$ remained as 5 Hz [16]. Judging from the negative Cotton effect at 242 nm, the absolute configuration of 4 was determined to be 7*S*, 8*R* [16-18]. Therefore, the structure of symplocosneolignan (4) was elucidated to be (7*S*, 8*R*)-4,7,9,9'-tetrahydroxy-3,5,3',5'-tetramethoxy-8,4'-oxyneolignan 4-*O*-*β*-D-glucopyranoside, as shown in **Figure 1**.

Symplocosin A (5), $[\alpha]_D^{25}$ +14.8, was isolated as an amorphous powder and its elemental composition was determined to be $C_{41}H_{66}O_{15}$ by HR-ESI-MS. The IR spectrum showed that 5 was a glycosidic compound (3382 cm^{-1}) and the ^{13}C-NMR spectroscopic data were similar to those of niga-ichigoside F1 (13) (**Table 3**) [10], which co-occurred in this plant, except for the appearance of five signals assignable for *α*-arabinopyranoside (**Table 3**). Arabinose and glucose were similarly determined by HPLC to be of the L- and D-series, respectively. The position of arabinose was determined to be the hydroxy group at the 2'-position from the HMBC correlation between H-1" (δ_H 5.14) and C-2' (δ_C 80.9). Therefore, the structure of 5 was elucidated to be 2*α*, 3*α*, 19*α*, 23-tetrahydroxyurs-12-en-28-oic acid (= 19*α*-hydroxyasiatic acid) *β*-D-(2'-*O*-*β*-L-arabinopyranosyl) glucopyranosyl ester, namely niga-ichigoside F_1 2'-*O*-*β*-L-arabinopyranoside, as shown in **Figure 1**.

Symplocosin B (6), $[\alpha]_D^{25}$ +9.1, was also isolated as an amorphous powder and its elemental composition was determined to be $C_{47}H_{76}O_{20}$ by HR-ESI-MS. The NMR spectral data of 6 showed high similarity to those of 5, and the presence of two terminal sugars, *α*-arbinopyranose and *β*-glucopyranose (**Table 3**). The positions of the sugars were established from the HMBC cross peaks between H-1' (δ_H 6.07) and C-28 (δ_C 177.0), H-1" (δ_H 5.38) and C-2' (δ_C 77.4), and H-1'" (δ_H 5.28) and C-3' (δ_C 88.1). Therefore, the structure of symplocosin B (6) was elucidated to be 2*α*, 3*β*, 19*α*, 23-tetrahydroxyurs-12-en-28-oic acid *β*-D-(2'-*O*-*α*-L-arabinopyranosyl, 3'-*O*-*α*-D-glucopy-ranosyl) glucopyranosyl ester, as shown in **Figure 1**.

From the leaves of *S. cochinchiensis* var. *philippinensis*, three new megastigmane diglycosides, named symploco- sionosides A-C (1-3), neolignan glucoside (4) and sym- plocosins A and B, two ursane-type triterpenoid glycosyl esters (5,6) were isolated. Their structures were eluci- dated by the spectroscopic evidence. The absolute struc- ture of 1 was finally determined by the modified Mosher's method and that of 4 by the Cotton effect, observed in the CD spectrum. Three species of *Symplocos* were medicinally used as "lodhra" in Indian system of traditional medicine, Ayurveda. For treatment of diabetes mellitus in Ayuveda, *Symplocos* species were given along with the juice of cucumber [19]. It is of interest for further works to define the compounds responsible for this treatment.

Table 3. ^{13}C-NMR data for compounds 5, 6 and 12 (C_5D_5N + one drop of D_2O, 150 MHz).

c	5		6	12[a)]
1	47.6	(48.0)[b)]	47.6	47.9
2	68.7	(69.0)	68.7	68.8
3	78.1	(78.4)	78.3	78.2
4	43.4	(43.6)	43.3	43.5
5	47.9	(48.2)	48.0	48.5
6	18.5	(18.8)	18.6	18.7
7	32.9	(33.1)	33.1	33.1
8	40.5	(40.7)	40.5	40.6
9	47.7	(47.9)	47.7	47.9
10	38.2	(38.4)	38.2	38.3
11	24.1	(124.3)	24.1	24.2
12	128.1	(128.3)	128.0	128.3
13	139.3	(139.5)	139.4	139.2
14	42.0	(42.2)	42.1	42.1
15	28.9	(29.2)	29.0	29.1
16	25.9	(26.2)	25.7	26.0[c)]
17	48.6	(48.7)	48.6	48.5
18	54.3	(54.5)	54.3	54.4
19	72.5	(72.7)	72.5	72.5
20	41.9	(42.1)	41.9	42.1
21	26.6	(26.8)	26.6	27.0[c)]
22	37.3	(37.4)	37.1	37.7
23	66.4	(66.9)	66.6	66.6
24	14.0	(14.3)	14.0	14.2
25	17.2[c)]	(17.4)	17.3	17.5[d)]
26	17.3[c)]	(17.3)	17.3	17.5[d)]
27	24.3	(24.3)	24.3	24.5
28	177.1	(177.1)	177.2	176.8
29	26.8	(27.1)	26.9	27.0
30	16.5	(16.7)	16.6	16.7[d)]
1'	94.0	(94.2)	93.5	95.7
2'	80.9	(81.4)	77.4	73.9
3'	77.9	(78.6)	88.1	78.8[e)]
4'	70.6	(71.1)	69.5[c)]	71.2
5'	78.7	(78.9)	78.3	79.9[e)]
6'	62.0	(62.4)	62.1	62.3
1"	106.0	(106.5)	104.6	
2"	73.0	(73.5)	72.8	
3"	74.0	(74.4)	74.5	
4"	69.0	(69.4)	69.6[c)]	
5"	67.0	(67.4)	67.2	
1"'			104.3	
2"'			75.1	
3"'			78.3[d)]	
4"'			71.3	
5"'			78.2[d)]	
6"'			61.7	

a) Data taken from ref. 10. Data for C_5D_5N without D_2O at 25 MHz. *b*) Data for C_5D_5N without D_2O at 100 MHz. c), d), e). The signals with the same superscripts in each column may be interchangeable.

3. Materials and Methods

3.1. Plant Material

Leaves of *S. cochinchinensis* var. *philippinensis* were collected in Taketomi-cho, Yaeyama-gun, Okinawa, Japan, in November, 2003, and a voucher specimen was deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (03-SC-Okinawa-1105).

3.2. General Experimental Procedure

Optical rotations were measured on a JASCO P-1030 digital polarimeter. IR and UV spectra were measured on Horiba FT-710 and JASCO V-520 UV/Vis spectrophotometers, respectively. ^{1}H- and ^{13}C-NMR spectra were taken on JEOL JNM α-400 and ECA-600 spectrometers at 400 MHz or 600 MHz and 100 MHz or 150 MHz, respectively, with tetramethylsilane as an internal standard. CD spectra were obtained with a JASCO J-720 spectropolarimeter. Positive-ion HR-ESI-MS was performed with an Applied Biosystems QSTAR® XL NanoSprayTM System.

A highly-porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel CC was performed on silica gel 60 (E. Merck, Darmstadt, Germany), and ODS open CC on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto) [Φ = 50 mm, L = 25 cm, linear gradient: MeOH-H_2O (1:9, 1 l) → (1:1, 1 l), fractions of 10 g being collected]. The DCCC (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ = 2 mm, L = 40 cm), the lower and upper layers of a solvent mixture of $CHCl_3$-MeOH-H_2O-*n*-PrOH (9:12:8:2) being used as the stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on an ODS column (Inertsil; GL Science, Tokyo, Japan; Φ = 20 mm, L = 250 mm, 6 ml/min), and the eluate was monitored with a UV detector at 254 nm, and a refractive index monitor. Crude hesperidinase was a generous gift from Tanabe Pharmaceutical Company Ltd. (*R*)-(+)- and (*S*)-(–)-α-methoxy-α-trifluoromethylpheylacetic acids (MTPAs) were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Authentic D-apiose [$[\alpha]_D^{23}$ +9.4 (c = 0.84, H_2O)] was obtained by chromatographic separation of the hydrolyzate of apiin, isolated from commercial parsley (*Petroselinum crispum*). D-Apiose was identified by NMR spectroscopy [17].

3.3. Extraction and Isolation

Leaves of *S. cochinchinensis* var. *phillipinensis* (10.8 kg) were extracted three times with MeOH (45 l × 3) at room

temperature for one week and then concentrated to 6 l *in vacuo*. The concentrated extract was washed with *n*-hexane (6 l, 150 g) and then the MeOH layer was concentrated to a gummy mass. The latter was suspended in water (6 l) and then extracted with EtOAc (6 l) to give 107 g of an EtOAc-soluble fraction. The aqueous layer was extracted with 1-BuOH (6 l) to give a 1-BuOH-soluble fraction (247 g), and the remaining water-layer was concentrated to furnish 494 g of a water-soluble fraction. The 1-BuOH-soluble fraction (119 g) was subjected to Diaion HP-20 CC (Φ = 50 mm, L = 50 cm), using H_2O-MeOH (4:1, 4 l), (3:2, 4 l), (2:3, 4 l), and (1:4, 4 l), and MeOH (3 l), 500 ml fractions being collected. The residue (12.5 g in fractions 11 - 18) of the 20% - 40% MeOH eluate obtained on HP-20 CC was subjected to silica gel (500 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (3 l), and $CHCl_3$-MeOH (99:1, 3 l), (97:3, 3 l), (19:1, 3 l), (37:3, 3 l), (9:1, 3 l), (7:1, 3 l), (17:3, 3 l), (33:7, 3 l), (4:1, 3 l), (3:1, 3 l) and (7:3, 3 l)], and $CHCl_3$-MeOH-H_2O (70: 30: 4, 3 l), 500 ml fractions being collected. The residue (2.52 g) in fractions 39 - 49 was separated by ODS open CC and then the residue (996 mg) in fractions 56 - 81 was subjected to DCCC. The residue (130 mg) in fractions 75 - 88 was purified by HPLC (MeOH:H_2O, 1:3) to give 85.7 mg of 11 from the peak at 10 min. The residue (2.18 g) in fractions 50 - 63, obtained on silica gel CC, was separated by ODS open CC to give three subfractions (1.02 g in fractions 42 - 67, 347 mg in fractions 68 - 87 and 138 mg in fractions 110 - 140). The first subfraction was separated again by DCCC to give a residue (877 mg) in fractions 39 - 60, which was then purified by HPLC (MeOH:H_2O, 1:4) to afford 6.2 mg of 9 and 11.2 mg of 3 from the peaks at 35 min and 45 min, respectively. The second subfraction was separated by DCCC to give a residue (105 mg) in fractions 34 - 42, which was purified by HPLC (MeOH:H_2O, 2:5) to yield 23.9 mg of 2 from the peak at 14 min. The last subfraction was separated by DCCC to give a residue (88.1 mg) in fractions 16 - 32, which was purified by HPLC (MeOH:H_2O, 9:20) to give 12.3 mg of 1 from the peak at 22 min.

The residue (19.7 g in fractions 19 - 25) of the 40% - 60% MeOH eluate obtained on HP-20 CC was subjected to silica gel (500 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (3 l), and $CHCl_3$-MeOH (99:1, 3 l), (97:3, 3 l), (19:1, 3 l), (37:3, 3 l), (9:1, 3 l), (7:1, 3 l), (17:3, 3 l), (33:7, 3 l), (4:1, 3 l), (3:1, 3 l) and (7:3, 3 l)], and $CHCl_3$-MeOH-H_2O (70:30:4, 3 l), 500 ml fractions being collected. The residue (221 mg) in fractions 8 - 14 was purified by HPLC (MeOH:H_2O, 3:10), to give 2.0 mg of 8 from the peak at 44 min. The residue (115 mg) in fractions 16 - 25 was purified by HPLC (MeOH:H_2O, 3:10) to give 4.8 mg of 7. The residue (2.94 g) in fractions 17 - 43 was separated by ODS open CC to give two subfractions (332 mg in fractions 61 - 63 and 541 mg in fractions 84 - 99). The former was separated by DCCC and then the residue (16.8 mg) in fractions 47 - 54 was purified by HPLC (MeOH:H_2O, 1:4) to give 2.8 mg of 10 from the peak at 16 min. The latter was separated by DCCC and then the residue (194 mg) in fractions 67 - 84 was purified by HPLC (MeOH:H_2O, 7:20) to afford 11.9 mg of 4 from the peak at 13 min. The residue (2.92 g) in fractions 44 - 60, obtained on silica gel CC, was subjected to ODS open CC to give a residue (495 mg) in fractions 130 - 170. This residue was separated by DCCC to give two subfactions (136 mg in fractions 16 - 21 and 189 mg in fractions 22 - 41). The former was purified by HPLC (MeOH:H_2O, 2:3) to give 20.0 mg of 12 from the peak at 6 min. The latter was also purified by HPLC (MeOH:H_2O, 9:20) to give 23.0 mg of 5 at 10 min. The residue (2.37 g) in fractions 61 - 72, obtained on silica gel CC, was subjected to ODS open CC to give a residue (492 mg) in fractions 154 - 172, which was then separated by DCCC to give 82.9 mg of 6 in fractions 29 - 42.

3.4. Symplocosionoside A (1)

Amorphous powder, $[\alpha]_D^{25}$ –44.0 (c = 0.82, MeOH); IR ν_{max} (film): 3394, 2962, 2927, 2877, 1650, 1055, 1014 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.45 (1H, dd, J = 15, 6 Hz, H-8), 5.28 (1H, dd, J = 15, 10 Hz, H-7), 5.00 (1H, d, J = 2 Hz, H-1''), 4.33 (1H, d, J = 8 Hz, H-1'), 4.21 (1H, qd, J = 6, 6 Hz, H-9), 3.96 (1H, J = 10Hz, H-4''a), 3.94 (1H, dd, J = 12, 2 Hz, H-6'a), 3.87 (1H, d, J = 2 Hz, H-2''), 3.83 (1H, dddd, J = 12, 12, 4, 4 Hz, H-3), 3.78 (1H, d, J = 10 Hz, H-4''b), 3.59 (1H, dd, J = 12, 6 Hz, H-6'b), 3.54 (2H, s, H_2-5''), 3.39 (1H, ddd, J = 9, 6, 2 Hz, H-5'), 3.34 (1H, dd, J = 9, 9 Hz, H-3'), 3.25 (1H, dd, J = 9, 9 Hz, H-4'), 3.11 (1H, dd, J = 9, 8 Hz, H-2'), 2.12 (1H, dddd, J = 12, 4, 4, 2 Hz, H-4eq), 1.83 (1H, ddd, J = 12, 4, 2 Hz, H-2eq), 1.52 (1H, m, H-5), 1.30 (1H, dd, J = 10, 10 Hz, H-6), 1.21 (3H, d, J = 6 Hz, H_3-10), 1.16 (1H, dd, J = 12, 12 Hz, H-2ax), 1.10 (1H, ddd, J = 12, 12, 12 Hz, H-4ax), 0.91 (3H, s, H_3-11), 0.87 (3H, s, H_3-12), 0.83 (3H, d, J = 7 Hz, H_3-13); ^{13}C-NMR (CD_3OD, 100 MHz): **Table 1**; HR-ESI-MS (positive-ion mode) m/z: 529.2620 $[M + Na]^+$ (Calcd for $C_{24}H_{42}O_{11}Na$: 529.2619).

3.5. Symplocosionoside B (2)

Amorphous powder, $[\alpha]_D^{26}$ –63.0 (c = 1.59, MeOH); IR ν_{max} (film): 3399, 2969, 2931, 2880, 1694, 1651, 1150, 1056 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.89 (1H, dd, J = 16, 6 Hz, H-8), 5.74 (1H, d, J = 16 Hz, H-7), 4.98 (1H, d, J = 2 Hz, H-1''), 4.43 (1H, qd, J = 6, 6 Hz, H-9), 4.34 (1H, d, J = 8 Hz, H-1'), 3.97 (1H, d, J = 10 Hz, H-

4"a), 3.94 (1H, dd, *J* = 12, 2 Hz, H-6'a), 3.91 (1H, d, *J* = 2 Hz, H-2"), 3.75 (1H, d, *J* = 10 Hz, H-4"b), 3.58 (1H, dd, *J* = 12, 6 Hz, H-6'b), 3.57 (2H, s, H_2-5"), 3.34 (1H, m, H-5'), 3.32 (1H, m, H-3'), 3.29 (1H, dd, *J* = 9, 9, H-4'), 3.18 (1H, dd, *J* = 9, 8 Hz, H-2'), 2.87 (1H, d, *J* = 14 Hz, H-2ax), 2.44 (1H, dd, *J* = 14, 14 Hz, H-4ax), 2.28 (1H, dqd, *J* = 14, 7, 4 Hz, H-5), 2.12 (1H, ddd, *J* = 14, 4, 2 Hz, H-4eq), 1.82 (1H, dd, *J* = 14, 2 Hz, H-2eq), 1.32 (3H, d, *J* = 6 Hz, H_3-10), 0.99 (3H, s, H_3-11), 0.93 (3H, s, H_3-12), 0.90 (3H, d, *J* = 7 Hz, H_3-13); ^{13}C-NMR (CD_3OD, 100 MHz): **Table 1**; CD (MeOH) nm ($\Delta\varepsilon$): 289 (+0.89), 246 (+0.63) ($c = 2.51 \times 10^{-5}$ M); HR-ESI-MS (positive-ion mode) *m/z*: 543.2404 $[M + Na]^+$ (Calcd for $C_{24}H_{40}O_{12}Na$: 543.2411).

3.6. Symplocosionoside C (3)

Amorphous powder, $[\delta]_D^{25}$ – 57.9 (*c* = 0.61, MeOH); IR ν_{max} (film): 3399, 3366, 2958, 2927, 1938, 1669, 1515, 1366, 1243, 1069, 1042 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 321 (4.53), 283 (3.91), 220 (4.04); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.96 (1H, s, H-8), 4.52 (1H, d, *J* = 8 Hz, H-1'), 4.31 **(**1H, dddd, *J* = 12, 12, 4, 4 Hz, H-3), 4.27 (1H, d, *J* = 7 Hz, H-1"), 4.00 (1H, dd, *J* = 11, 2 Hz, H-6'a), 3.85 (1H, dd, *J* = 12, 4 Hz, H-5"a), 3.81 (1H, m, H-4"), 3.69 (1H, dd, *J* = 11, 5 Hz, H-6'b), 3.64 (1H, dd, *J* = 8, 7 Hz, H-2"), 3.54 (1H, m, H-3"), 3.53 (1H, dd, *J* = 12, 3 Hz, H-5"b), 3.37 (1H, m, H-5'), 3.35 (1H, dd, *J* = 9, 9 Hz, H-4'), 3.33 (1H, dd, *J* = 9, 9 Hz, H-3'), 3.14 (1H, dd, *J* = 9, 8 Hz, H-2'), 2.48 (1H, ddd, *J* = 12, 4, 2 Hz, H-4eq), 2.19 (3H, s, H_3-10), 1.91 (1H, ddd, *J* = 12, 4, 2 Hz, H-2eq), 1.47 (3H, s, H_3-13), 1.37 (3H, s, H_3-11), 1.36 (1H, dd, *J* = 12, 12 Hz, H-4ax), 1.34 (1H, dd, *J* = 12, 12 Hz, H-2ax), 1.16 (3H, s, H_3-12); ^{13}C-NMR (CD_3OD and C_5D_5N, 100 MHz): **Table 1**; CD (MeOH) nm ($\Delta\varepsilon$): 319 (–0.47), 263 (–2.87), 207 (–23.1) ($c = 3.41 \times 10^{-5}$ M). HR-ESI-MS (positive-ion mode) *m/z*: 541.2257 $[M + Na]^+$ (Calcd for $C_{24}H_{38}O_{12}Na$: 541.2255).

3.7. Symplocosneolignan (4)

Amorphous powder, $[\alpha]_D^{25}$ –13.5 (*c* = 0.79, MeOH); IR ν_{max} (film): 3395, 2939, 1592, 1419, 1328, 1229, 1123, 1061 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 269 (3.59), 213 (4.31); ^{1}H-NMR (CD_3OD, 400 MHz): **Table 2**; ^{13}C-NMR (CD_3OD, 100 MHz): **Table 2**; CD (MeOH) nm ($\Delta\varepsilon$): 304 (+1.71), 242 (–4.67) ($c = 4.00 \times 10^{-5}$ M); HR-ESI-MS (positive-ion mode) *m/z*: 623.2294 $[M + Na]^+$ (Calcd for $C_{28}H_{40}O_{14}Na$: 623.2310).

3.8. Symplocoside A (5)

Amorphous powder, $[\alpha]_D^{22}$ +14.8 (*c* = 0.54, MeOH); IR ν_{max} (film): 3382, 2930, 2882, 1730, 1635, 1140, 1074, 1036 cm^{-1}; ^{1}H-NMR (C_5D_5N + one drop of D_2O, 600 MHz) δ: 6.07 (1H, d, *J* = 8 Hz, H-1'), 5.50 (1H, dd, *J* = 4, 4 Hz, H-12), 5.14 (1H, d, *J* = 7 Hz, H-1"), 4.44 (1H, dd, *J* = 9, 7 Hz, H-2"), 4.40 (2H, dd, *J* = 14, 2 Hz, H-6'a), 4.28 (1H, dd, *J* = 9, 9 Hz, H-3'), 4.27 (1H, m, H-6'b and 5"a), 4.26 (2H, m, H-2' and 4"), 4.19 (2H, m, H-2 and 4'), 4.14 (1H, dd, *J* = 9, 3 Hz, H-3"), 4.10 (1H, d, *J* = 10 Hz, H-3), 4.05 (1H, d, *J* = 11 Hz, H-23a), 3.94 (1H, ddd, *J* = 9, 5, 2 Hz, H-5'), 3.71 (1H, dd, *J* = 13, 2 Hz, H-5"b), 3.58 (1H, d, *J* = 11 Hz, H-23b), 2.99 (1H, ddd, *J* = 13, 13, 5 Hz, H-16a), 2.86 (1H, br s, H-18), 2.24 (1H, dd, *J* = 12, 4 Hz, H-1a), 2.30 (1H, ddd, *J* = 14, 13, 5 Hz, H-15a), 2.07 (2H, m, H_2-11), 2.02 (1H, m, H-16b), 1.97 (3H, m, H-21a and H_2-22), 1.96 (1H, m, H-9), 1.78 (1H, m, H-7a), 1.70 (1H, br d, *J* = 12 Hz, H-5), 1.60 (2H, m, H-6a and 7b), 1.57 (3H, s, H_3-27), 1.43 (1H, m, H-20), 1.38 (3H, s, H_3-29), 1.34 (1H, m, H-6b), 1.32 (1H, m, H-15b), 1.24 (1H, m, H-21b), 1.30 (1H, m, H-1b), 1.09 (3H, s, H_3-26), 1.06 (3H, d, *J* = 7 Hz, H_3-30), 1.05 (3H, s, H_3-25), 0.96 (3H, s, H_3-24); ^{13}C-NMR (C_5D_5N, 100 MHz): **Table 3**; HR-ESI-MS (positive-ion mode) *m/z*: 821.4277 $[M + Na]^+$ (Calcd for $C_{41}H_{66}O_{15}Na$: 821.4293).

3.9. Symplocoside B (6)

Amorphous powder, $[\alpha]_D^{22}$ +9.1 (*c* = 1.22, MeOH); IR ν_{max} (film): 3395, 2931, 2883, 1730, 1647, 1156, 1076, 1035cm^{-1}; ^{1}H-NMR (C_5D_5N + one drop of D_2O, 600 MHz) δ: 6.07 (1H, d, *J* = 8 Hz, H-1'), 5.49 (1H, dd, *J* = 4, 4 Hz, H-12), 5.38 (1H, d, *J* = 8 Hz, H-1"), 5.28 (1H, d, *J* = 8 Hz, H-1'"), 4.48 (1H, dd, *J* = 12, 2 Hz, H-6'a), 4.35 (1H, dd, *J* = 9, 8 Hz, H-2'), 4.32 (1H, dd, *J* = 12, 2 Hz, H-6'"a), 4.31 (1H, m, H-2"), 4.28 (1H, dd, *J* = 9, 9 Hz, H-3'), 4.20 (2H, m, H-5"a and H-6'"b), 4.18 (1H, m, H-2), 4.16 (3H, m, H-4', 4" and 3'"), 4.15 (1H, m, H-6'b), 4.10 (1H, dd, *J* = 9, 3 Hz, H-3"), 4.09 (1H, d, *J* = 9 Hz, H-3), 4.04 (1H, dd, *J* = 9, 9 Hz, H-4'"), 4.03 (1H, d, *J* = 11 Hz, H-23a), 3.98 (1H, dd, *J* = 9, 8 Hz, H-2'"), 3.94 (1H, ddd, *J* = 9, 6, 2 Hz, H-5'"), 3.89 (1H, ddd, *J* = 9, 5, 2 Hz, H-5'), 3.64 (1H, br d, *J* = 12 Hz, H-5"), 3.54 (1H, d, *J* = 11 Hz, H-23b), 2.99 (1H, ddd, *J* = 13, 13, 5 Hz, H-16a), 2.87 (1H, br s, H-18), 2.27 (1H, m, H-15a), 2.24 (1H, dd, *J* = 12, 4 Hz, H-1a), 2.16 (1H, br d, *J* = 13 Hz, H-16b), 2.07 (2H, m, H_2-11), 1.98 (1H, m, H-21a), 1.97 (2H, m, H_2-22), 1.96 (1H, m, H-9), 1.77 (1H, ddd, *J* = 13, 13, 4 Hz, H-7a), 1.67 (1H, br d, *J* = 12 Hz, H-5), 1.65 (1H, ddd, *J* = 13, 2, 2 Hz, H-7b), 1.57 (3H, s, H_3-27), 1.56 (1H, m, H-6a), 1.48 (1H, m, H-15b), 1.46 (1H, m, H-20), 1.37 (3H, s, H_3-29), 1.31 (1H, m, H-6b), 1.29 (1H, m, H-1b), 1.26 (1H, m, H-21b), 1.12 (3H, s, H_3-26), 1.06 (3H, d, *J* = 7 Hz, H_3-30), 1.04 (3H, s, H_3-25), 0.95 (3H, s, H_3-24); ^{13}C-NMR (C_5D_5N, 100 MHz): **Table 3**; HR-ESI-MS (positive-ion mode) *m/z*: 983.4823 $[M + Na]^+$ (Calcd for $C_{47}H_{76}O_{20}Na$: 983.4822).

3.10. Enzymatic Hydrolysis of Symplocosionoside A (1)

Symplocosionoside A (**1**) (6.6 mg) in 2 ml of H_2O was hydrolyzed with emulsin (14.2 mg) and crude hesperidinase (6.0 mg) for 15 h at 37˚C. The reaction mixture was evaporated to dryness, and then the methanolic solution was absorbed on silica gel and subjected to silica gel CC (30 g, Φ = 18 mm, L = 21 cm) with $CHCl_3$ (100 ml) and $CHCl_3$-MeOH (19:1, 100 ml, 9:1, 100 ml, 17:3, 100 ml and 7:3, 300 ml), 12 ml fractions being collected. An aglycone (**1a**) (1.8 mg, 65%) was recovered in fractions 19 - 23. Aglycone (megastigman-7-ene-3,9-diol) (**1a**): Colorless syrup, $[\alpha]_D^{29}$ –20.0 (c = 0.12, MeOH); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 5.45 (1H, dd, J = 15, 6 Hz, H-8), 5.29 (1H, dd, J = 15, 10, H-7), 4.21 (1H, qd, J = 6, 6 Hz, H-9), 3.71 (1H, dddd, J = 12, 12, 4, 4 Hz, H-3), 1.96 (1H, dddd, J = 12, 12, 4, 2 Hz, H-4eq), 1.70 (1H, ddd, J = 12, 4, 2 Hz, H-2eq), 1.52 (1H, ddq, J = 12, 10, 7 Hz, H-5), 1.30 (1H, dd, J = 10 , 10 Hz, H-6), 1.21 (3H, d, J = 6 Hz, H_3-10), 1.10 (1H, dd, J = 12, 12 Hz, H-2ax), 0.89 (1H, ddd, J = 12, 12, 12 Hz, H-4ax), 0.88 (3H, s, H_3-12), 0.86 (3H, s, H_3-11), 0.82 (3H, d, J = 7 Hz, H_3-13); ^{13}C-NMR (CD_3OD, 100 MHz): **Table 1**; HR-ESI-MS (positive-ion mode) m/z: 235.1661 $[M + Na]^+$ (Calcd for $C_{13}H_{24}O_2Na$: 235.1668).

3.11. Preparation of (R)- and (S)-MPTA Diesters (1b and 1c) from 1a

A solution of **1a** (0.7 mg) in 1 ml of dehydrated CH_2Cl_2 was reacted with (*R*)-MTPA (42 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (31 mg) and *N*,*N*-dimethyl-4-aminopyridine (4-DMAP) (31 mg), and then the mixture was occasionally stirred at 25˚C for 30 min. After the addition of 1 ml of CH_2Cl_2, the solution was washed with H_2O (1 ml), 5% HCl (1 ml), $NaHCO_3$-saturated H_2O, and then brine (1 ml), successively. The organic layer was dried over Na_2SO_4 and then evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25 mm thickness), being applied for 18 cm, developed with $CHCl_3$-$(CH_3)_2CO$ (19:1) for 9 cm, and then eluted with $CHCl_3$-MeOH (9:1)] to furnish an ester, **1b** (1.5 mg, 71%). Through a similar procedure, **1c** (1.1 mg, 61%) was prepared from **1a** (0.6 mg) using (*S*)-MTPA (53 mg), EDC (41 mg), and 4-DMAP (24 mg). (3*S*, 5*R*, 6*R*, 7*E*, 9*R*)-Megastigman-7-ene-3,9-diol 3,9-*O*-(*R*)-MTPA diester (**1b**) An amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.47 - 7.43 (4H, m, aromatic protons), 7.35 - 7.30 (6H, m, aromatic protons), 5.56 (1H, qd, J = 6, 6 Hz, H-9), 5.43 (1H, m, H-8), 5.42 (1H, m, H-7), 5.15 (1H, dddd, J = 12, 12, 4, 4 Hz, H-3), 3.48 (3H, br s, -OCH_3), 3.47 (3H, br s, -OCH_3), 2.13 (1H, dddd, J = 12, 4, 4, 2 Hz, H-4eq), 1.76 (1H, ddd, J = 12, 4, 2 Hz, H-2eq), 1.68 (1H, m, H-5), 1.43 (1H, dd, J = 10, 10 Hz, H-6), 1.36 (3H, d, J = 6 Hz, H_3-10), 1.16 (1H, dd, J = 12, 12 Hz, H-2ax), 0.99 (1H, ddd, J = 12, 12, 12 Hz, H-4ax), 0.88 (3H, d, J = 6 Hz, H_3-13), 0.84 (3H, s, H_3-11), 0.82 (3H, s, H_3-12); HR-ESI-TOF-MS (positive-ion mode) m/z: 667.2472 $[M+Na]^+$ (Calcd for $C_{33}H_{38}O_6F_6Na$, 667.2464). (3*S*, 5*R*, 6*R*, 7*E*, 9*R*)- Megastigman-7-ene-3,9-diol 3,9-*O*-(*S*)-MTPA diester (**1c**) An amorphous powder; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.47 - 7.43 (4H, m, aromatic protons), 7.35 - 7.30 (6H, m, aromatic protons), 5.56 (1H, qd, J = 6, 6 Hz, H-9), 5.39 (1H, m, H-8), 5.38 (1H, m, H-7), 5.14 (1H, dddd, J = 12, 12, 4, 4 Hz, H-3), 3.48 (3H, br s, -OCH_3), 3.46 (3H, br s, -OCH_3), 2.04 (1H, dddd, J = 12, 4, 4, 2 Hz, H-4eq), 1.82 (1H, ddd, J = 12, 4, 2 Hz, H-2eq), 1.60 (1H, m, H-5), 1.41 (3H, d, J = 6 Hz, H_3-10), 1.37 (1H, dd, J = 10, 10 Hz, H-6), 1.19 (1H, dd, J = 12, 12 Hz, H-2ax), 0.88 (1H, ddd, J = 12, 12, 12 H-4ax), 0.87 (3H, s, H_3-11), 0.82 (3H, s, H_3-12), 0.77 (3H, d, J = 6 Hz, H_3-13); HR-ESI-MS (positive-ion mode) m/z: 667.2471 $[M+Na]^+$ (Calcd for $C_{33}H_{38}O_6F_6Na$, 667.2464).

3.12. Enzymatic Hydrolysis of Symplocosneolignan (4)

Symplocosneolignan (4) (4.7 mg) in 2 ml of H_2O was hydrolyzed with emulsin (12 mg) and crude hesperidinase (6.0 mg) for 15 h at 37˚C. The reaction mixture was evaporated to dryness, and then the methanolic solution was absorbed on silica gel and subjected to silica gel CC (30 g, Φ = 18 mm, L = 21 cm) with $CHCl_3$ (100 ml) and $CHCl_3$-MeOH (19:1, 100 ml, 9:1, 100 ml, 17:3, 100 ml and 7:3, 300 ml), 12 ml fractions being collected. An aglycone (**1a**) (0.9 mg, 26%) was recovered in fractions 23 - 27. Aglycone (**4a**): Colorless syrup, $[\alpha]_D^{25}$ –21.0 (c = 0.06, MeOH); ^{1}H-NMR (CD_3OD, 400 MHz): **Table 2**; ^{13}C-NMR (CD_3OD, 100 MHz): **Table 2**; HR-ESI-MS (positive-ion mode) m/z: 461.1786 $[M+Na]^+$ (Calcd for $C_{22}H_{30}O_9Na$, 461.1788).

3.13. Sugar Analysis

About 350 μg each of 1 and 2 was hydrolyzed with 1N HCl (0.2 ml) at 90˚C for 2 h. The reaction mixtures were washed with an equal amount of EtOAc and then passed through Amberlite MB-3. The pass-through fractions were evaporated to dryness to give residues. The residues were dissolved in 0.1 ml of dry pyridine and then 0.5 mg of L-cysteine methyl ester was added. To these mixtures, 1.4 mg of *o*-tolylthioisocyanate in 70 μl of pyridine was added, followed by standing at 60˚C for 1 h. The reaction

mixtures were analyzed by HPLC [ODS: Cosmosil 5C18ARII (4.6 mm × 250 mm), CH_3CN-50 mM H_3PO_4 (1:3), 0.8 ml/min, UV detector at 250 nm] to give peaks for thiocarbamoylthiazolidine derivatives of D-glucose and D-apiose at 18.0 min and 31.0 min, respectively [21]. The peaks were identified by co-chromatography with thiocarbamoyl-thiazolidine derivatives of authentic D-glucose and D-apiose.

About 500 μg of each compound, except for 1 and 2, was hydrolyzed with 1N HCl (0.1 ml) at 90˚C for 2 h. The reaction mixtures were partitioned with an equal amount of EtOAc (0.1 ml), and the water layers were analyzed with a chiral detector (JASCO OR-2090*plus*) on an amino column [Asahipak NH_2P-50 4E, CH_3CN-H_2O (3:1), 1 ml/min]. A hydrolyzate of 4 gave a peak for D-glucose at 7.9 min, and ones of 3, 5 and 6 gave peaks for L-arabinose and D-glucose at 6.1 min and 13.7 min, respectively, with positive optical rotation signs. The peaks were identified by co-chromatography with authentic samples.

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