Megastigmane, Benzyl and Phenethyl Alcohol Glycosides, and 4,4′-Dimethoxy-β-truxinic Acid Catalpol Diester from the Leaves of *Premna subscandens* MERR.

Hirokazu SUDO,[a] Toshinori IDE,[a] Hideaki OTSUKA,*,[a] Eiji HIRATA,[b] Anki TAKUSHI,[c] Takakazu SHINZATO,[d] and Yoshio TAKEDA[e]

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[a] 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8551, Japan, Faculty of Agriculture, University of the Ryukyus,[b] 1 Senbaru, Nishihara-cho, Nakagami-gun, Okinawa 905–0213, Japan, 134 Furugen, Yomitan-son, Nakagami-gun, Okinawa 904–0014, Japan,[c] University Forest, Faculty of Agriculture, University of the Ryukyus,[d] 685 Aza Yona, Kunigami-son, Kunigami-gun, Okinawa 905–1427, Japan, and Faculty of Integrated Arts and Sciences, Tokushima University,[e] Minamijosanjima-cho, Tokushima 770–8502, Japan.

Received October 27, 1999; accepted December 18, 1999

Extensive isolation work on the *n*-BuOH-soluble fraction obtained from the leaves of *Premna subscandens*, collected on Ishigaki island, Okinawa, afforded six compounds. Two were identified as megastigmane glucosides, 7-(3,5-dihydroxy-1,1,5-trimethylcyclohexylidene)-9-methylprop-8-enyl 9-*O*-β-D-glucopyranoside and 3-hydroxy-5,6-epoxy-β-ionol 9-*O*-β-D-glucopyranoside. The structures of the remaining four new compounds were elucidated to be a 2′-*O*-β-D-apiofuranosyl derivative of 3-hydroxy-5,6-epoxy-β-ionol 9-*O*-β-D-glucopyranoside, named premnaionoside, benzyl alcohol β-D-(2′-*O*-β-D-xylopyranosyl)glucopyranoside, phenethyl alcohol β-D-(2′-*O*-β-D-glucopyranosyl)glucopyranoside, and 4,4′-dimethoxy-β-truxinic acid catalpol diester by spectroscopic analyses.

Key words *Premna subscandens*; Verbenaceae; megastigmane glycoside; benzyl alcohol glycoside; phenethyl alcohol glycoside; 4,4′-dimethoxy-β-truxinic acid catalpol ester

In previous papers, we reported the isolation of a number of iridoid glucoside derivatives and phenylethanoids from the leaves of *Premna subscandens*.[1] In continuing work on this plant, together with two known megastigmane glycosides (**1**, **2**), and four new compounds (**3**—**6**) were isolated by means of various chromatographic techniques. The structures of the new compounds were elucidated from spectroscopic evidence and the two known compounds were identified as megastigmane glucosides, 7-(3,5-dihydroxy-1,1,5-trimethylcyclohexylidene)-9-methylprop-8-enyl 9-*O*-β-D-glucopyranoside (**1**),[2] and 3-hydroxy-5,6-epoxy-β-ionol 3-*O*-β-D-glucopyranoside (**2**).[3] This paper mainly deals with structural elucidation of the new compounds, although spectroscopic data for the glucoside (**1**) is also included in the text.

Compound **1**, an amorphous powder, was formulated as $C_{19}H_{32}O_8$ on the basis of high-resolution (HR) FAB-MS. The ^{1}H- and ^{13}C-NMR spectra indicated that **1** is a derivative of grasshopper ketone, except for the missing ketone function on C-9, which should have been reduced to an alcohol function. The planar structure of **1** was identical with that of the megastigmane glucoside isolated from *Lycium halimifolium* as its pentaacetate.[2] Therefore, compound **1** was acetylated and the ^{1}H-NMR data ($CDCl_3$) for the pentaacetate (**1a**) were superimposable on those reported. Accordingly, the relative orientations of substituents on the six-membered ring must be the same as those in the case of **1**, isolated from *L. halimifolium*, and the absolute stereochemistry of C-9 was tentatively deduced to be *R* from the ^{13}C-NMR chemical shifts of the C-9 and 10 carbon atoms (δ_C 76.0 and 20.7, respectively).[4] However, the absolute stereochemistries at C-3 and C-5, and the axis chirality of the allenic system remain to be determined.

Compound **2** was identified as 3-hydroxy-5,6-epoxy-β-ionol 3-*O*-β-D-glucopyranoside, which has been isolated from flue-cured tobacco,[3] on the basis of NMR data. The ab-

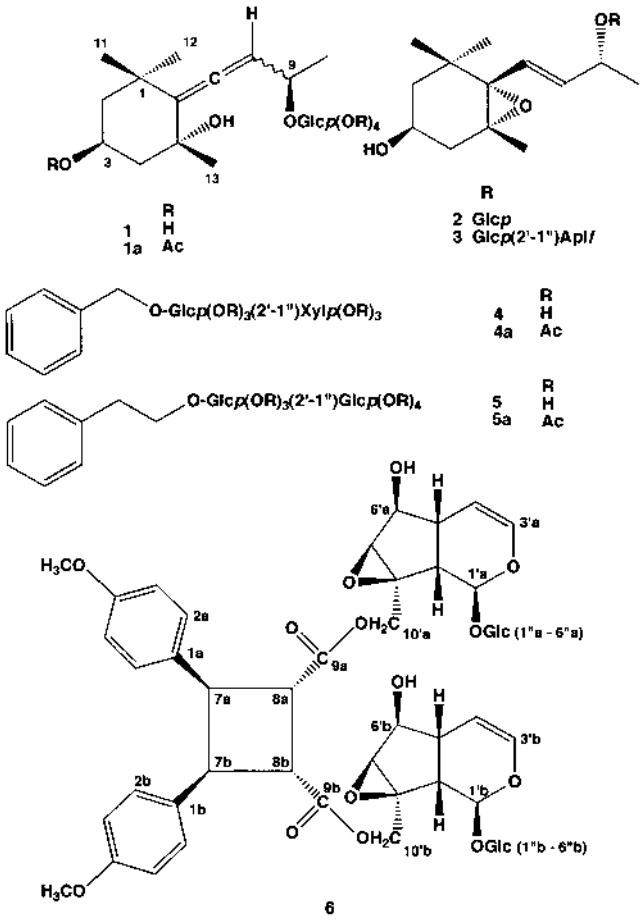

Fig. 1. Structures of Compounds **1**—**6**

* To whom correspondence should be addressed.

Table 1. ^{13}C-NMR Data for Compounds **2** and **3** (100 MHz, CD_3OD)

	2	**3**
1	36.0	36.0
2	48.0	48.0
3	64.6	64.6
4	41.6	41.6
5	68.0	68.1
6	71.2	71.2
7	127.8	127.7
8	137.2	137.1
9	76.9	76.4
10	21.0	20.9
11	30.1	30.2
12	25.2	25.2
13	20.3	20.3
1′	102.7	101.1
2′	75.3	79.3
3′	78.1	78.0
4′	71.4	71.5
5′	77.9	77.8
6′	62.6	62.6
1″		110.9
2″		78.7
3″		80.7
4″		75.4
5″		66.1

solute configuration at C-9 was confirmed to be *R* from the ^{13}C-NMR chemical shifts at C-9 and 10.[4)]

Premnaionoside (**3**) was isolated as an amorphous powder, with the formula $C_{24}H_{40}O_{12}$, as judged by HR-FAB-MS. Spectroscopic data indicated that the structure of **3** is an apiofuranosyl derivative of **2**. Acidic methanolysis gave glucose and apiose, which were identified by GLC analysis. The position of the apiofuranosyl residue was determined to be at the hydroxyl group on C-2 of the glucose moiety by comparison with reported data.[4b)] Therefore, the structure of **3** was elucidated to be a 2′-*O*-β-D-apiofuranosyl derivative of **2**.

Compound **4** was obtained as colorless needles, with a formula of $C_{18}H_{26}O_{10}$, on the basis of HR-FAB-MS. The ^{13}C-NMR spectrum showed the presence of monosubstituted-aromatic and primary carbinol carbon signals, along with ones due to substituted hexopyranose and terminal β-xylopyranose moieties. Two anomeric proton signals, observed by ^{1}H-NMR, and GLC analysis revealed the presence of xylose and glucose as sugar units. The xylopyranose linkage was analyzed as its hexaacetate (**4a**), for which the H-2′ signal remained almost intact (δ_H 3.77) by ^{1}H–^{1}H correlation spectroscopy (COSY). Therefore, the structure of **4** was elucidated to be benzyl alcohol β-D-(2′-*O*-β-D-xylopyranosyl)glucopyranoside.[5)]

Compound **5**, isolated as colorless needles and with $[\alpha]_D$ −37.5°, was similar to compound **4**, except for the presence of an additional methylene carbon and β-glucopyranose as the terminal sugar, as deduced by ^{1}H- (see Experimental) and ^{13}C-NMR spectra (Table 1). The inner sugar was also confirmed to be glucose by GLC analysis and the linkage of the sugar moiety was similarly determined from the ^{1}H–^{1}H COSY spectrum of its heptaacetate (**5a**). Thus, the structure of **5** was concluded to be phenethyl alcohol β-D-(2′-*O*-β-D-glucopyranosyl)glucopyranoside. This compound has independently been isolated from fruits of *Bupleurum falcatum* as an amorphous powder with $[\alpha]_D$ −17.7°.[6)]

Table 2. ^{13}C-NMR Data for Compounds **4** and **5** (100 MHz, CD_3OD)

	4	**5**
1	139.2	139.8
2,6	129.0	129.4
3,5	129.3	130.1
4	128.6	127.3
7	72.1	36.8
8		71.8
1′	102.4	102.5
2′	83.9	81.9
3′	77.9	77.2
4′	71.2	70.9
5′	77.9	77.4
6′	62.8	62.3
1″	106.3	104.1
2″	75.9	75.5
3″	77.4	77.7
4″	71.5	71.1
5″	67.2	77.6
6″		62.2

Compound **6** was isolated as an amorphous powder. Elemental composition was determined to be $C_{50}H_{60}O_{24}$ by negative-ion HR-FAB-MS. The IR spectrum indicated the presence of ester groups, hydroxyl groups and aromatic rings. Many of the ^{13}C-NMR signals appeared as two close sets, with four neat signals at δ_C 45.9 (d), 46.2 (d), 44.6 (d) and 45.1 (d). Signals assignable to two dihydropyran rings with two hemiacetalic carbons, one set of one primary and one secondary alcohol, one set of tertiary and quarternary carbons with oxygen substituents, and two glucopyranoses were presumably due to the presence of two units of an iridoid glucoside, namely catalpol, in the molecule. The ^{1}H–^{1}H COSY and heteronuclear single quantum correlation spectroscopy (HSQC) spectra showed that four protons on δ_C 45.9, 46.2, 44.6 and 45.1 were coupled in series. Thus, these carbons must form a rare four-membered ring. Compound **6** was then hydrolyzed under methanolic alkaline condition to give a methyl ester of the acid moiety (**6a**) and catalpol (**6b**). Spectroscopic analysis revealed that the acid moiety was composed of the four-membered ring and two 4-methoxyphenyl and carbomethoxy units. From these data, the acid moiety was deduced to be truxinic acid (**6**) or the truxillic acid skeleton. Since the ^{1}H–^{1}H COSY spectrum of **6** showed correlations between H-2a (H-6a) and H-7a, H-7a and H-7b, and H-7b and H-2b (H-6b), truxillic acid was ruled out as a candidate (**7**) (Fig. 2). Six stereogenic isomers are known for truxinic acid, two meso forms and two sets of enantiomers. Since the methyl ester of the acid moiety of **6**, obtained by alkaline hydrolysis, was a symmetrical compound by NMR, and from optical rotation, circular dichroism (CD) and optical rotatory dispersion (ORD) data, it was optically inactive, it was clearly one of the meso forms. Finally, phase-sensitive rotating frame nuclear Overhauser effect (NOE) spectroscopy (ROESY) of **6** showed significant NOE correlations between H-2a (H-6a) and H-7a, 8a and 8b, and between H-2b (H-6b) and H-7b, H-8b and H-8a, leading to the structure of the acid moiety of **6** as shown in Fig. 2. On the basis of the above data, the structure of **6** was determined to be 4,4′-dimethoxy-β-truxinic acid catalpol diester, as shown in Fig. 1.

Truxinic and truxillic acid diesters are relatively rare in na-

ture. Recently, they were found as stachysetin (truxinic acid apigenin glucoside diester) in *Stachys aegyptiaca* (Labiatae),[7] truxillic acid tropane alkaloid diesters in *Erythroxylum moonii* (Erythroxylaceae),[8] incarvillateine and its congeners, truxillic acid N-containing iridoid diesters, in *Incarvillea sinensis* (Bignoniaceae),[9] and sagerinic acid, μ-truxinic acid 3,4-dihydroxyphenyl-1-propanoic acid-8-ol diester in *Salvia officinalis*.[10]

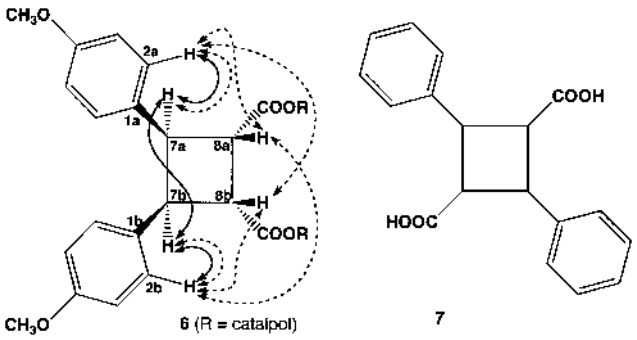

Fig. 2. Skeletons of Truxinic (**6**) and Truxillic Acids (**7**)

Solid lines indicate correlations indicated by the ^{1}H–^{1}H COSY spectrum and dotted lines indicate correlations observed in the phase-sensitive ROESY experiment (mixing time, 500 ms).

Experimental

A highly porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo). Silica gel column chromatography (CC) and reversed phase [octadecyl silica gel (ODS)] open CC (RPCC) were performed on Silica gel 60 (Merck) and Cosmosil 75C$_{18}$-OPN (Nacalai Tesque, Kyoto) [Φ=50 mm, L=25 cm, linear gradient: MeOH–H$_2$O (1 : 9, 1 l)→(1 : 1, 1 l), fractions of 10 g being collected], respectively. Droplet counter-current chromatography (DCCC) (Tokyo Rikakikai, Tokyo) was equipped with 500 glass columns (Φ=2 mm, L=40 cm), and the lower and upper layers of a solvent mixture of CHCl$_3$–MeOH–H$_2$O–*n*-PrOH (9 : 12 : 8 : 2) were used as the stationary and mobile phases, respectively. Five gram fractions were collected and numbered according to the order of elution of the mobile phase. HPLC was performed on ODS (Inertsil, GL Science, Tokyo; Φ=6 mm, L=250 mm) and the eluate was monitored with UV and refractive index detectors.

All melting points were determined with a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured on a Union Giken PM-101 digital polarimeter. IR spectra were measured on a Shimadzu FTIR-4200 spectrophotometer and UV spectra on a Shimadzu UV-160A spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM α-400 spectrometer at 400 MHz and 100 MHz, respectively, with tetramethylsilane (TMS) as an internal standard. Negative ion HR-FAB-MS were taken on a JEOL JMS SX-102 spectrometer with PEG-400 or 600 as a matrix. CD and ORD spectra were obtained on a JASCO J-720 spectropolarimeter.

Plant Material The leaves of *Premna subscandens* were collected in Okinawa, Japan, in August 1990, and identified by one of the authors (A.T.). A voucher specimen was deposited in the Herbarium of the Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine (PS-92-Okinawa).

Extraction and Fractionation The air-dried leaves (840 g) of *P. sub-*

Table 3. ^{13}C (100 MHz)- and ^{1}H (400 MHz)-NMR Data for Compounds **6** and **6a** (CD_3OD)[a]

6						**6a**		
	C	H		C	H		C	H
1a	132.3	—	1′a	95.6	5.01 (d, 10)	1a	131.9	—
1b	132.4	—	1′b	95.9	5.03 (d, 10)	1b		
2a	130.1	6.93 (d, 9)	3′a	141.9	6.32 (dd, 2, 6)	2a	129.8	6.99 (d, 9)
2b	130.4	6.96 (d, 9)	3′b		6.35 (dd, 2, 6)	2b		
3a	114.4	6.65 (d, 9)	4′a	103.9	5.04 (dd, 4, 6)	3a	114.0	6.69 (d, 9)
3b	114.5	6.67 (d, 9)	4′b	104.1	5.06 (dd, 4, 6)	3b		
4a	159.5	—	5′a	39.0	2.23 (ddt, 2, 4, 8)	4a	158.9	—
4b	159.6	—	5′b		2.25 (ddt, 2, 4, 8)	4b		
5a	114.4	6.65 (d, 9)	6′a	79.5	=	5a	114.0	6.69 (d, 9)
5b	114.5	6.67 (d, 9)	6′b		=	5b		
6a	130.1	6.93 (d, 9)	7′a	62.8	3.44 (d, 1)	6a	129.8	6.99 (d, 9)
6b	130.4	6.96 (d, 9)	7′b		3.45 (d, 1)	6b		
			8′a	63.3	—	7a	45.1	4.26 (d, 6)
(Cyclobutane ring)			8′b	63.4	—	7b		
7a	45.9	4.39 (dd, 9, 10)	9′a	43.4	2.53 (dd, 8, 10)	8a	44.0	3.88 (d, 6)
7b	46.2	4.17 (dd, 6, 10)	9′b		2.58 (dd, 8, 10)	8b		
8a	44.6	3.99 (dd, 9, 10)	10′a	65.2	4.20 (d, 13)	9a	173.7	—
8b	45.1	3.83 (dd, 6, 10)			5.00 (d, 13)	9b		
(Carboxyl carbon)			10′b	65.4	4.24 (d, 13)			
9a	174.3	—			5.07 (d, 13)	$COOCH_3$	52.1	3.68 (s)
9b	175.1	—				$COOCH_3$		
			1″a	100.2	4.75 (d, 8)	OCH_3	55.3	3.69 (s)
OCH_3	55.6	3.65 (s)	1″b	100.6	4.73 (d, 8)	OCH_3		
OCH_3	55.6	3.66 (s)	2″a	74.8	3.25 (dd, 8, 9)			
			2″b	74.9	=			
			3″a	78.64	=			
			3″b	78.62	=			
			4″a	71.5	=			
			4″b	71.7	=			
			5″a	77.9	=			
			5″b		=			
			6″a	63.1	=, =			
			6″b		=, =			

a) Letters and figures in parentheses are multiplicities and coupling constants in Hz.
=: Signals could not be assigned due to overlap of many signals.

sandens were extracted with MeOH (12 l×2). The MeOH extracts were concentrated to 1.5 l, and then 75 ml of H_2O was added to give a 95% aqueous solution, which was washed with 1.5 l of *n*-hexane, and then the MeOH layer was concentrated to give a residue. The residue was suspended in 1.5 l of H_2O, and then extracted with EtOAc (1.5 l) and *n*-BuOH (1.5 l), successively. The *n*-BuOH-soluble fraction (56.3 g) thus obtained was subjected to Diaion HP-20 CC (Φ=5.5 cm, L=40 cm) with H_2O–MeOH mixtures as eluent [H_2O–MeOH (4 : 1, 3 l, 20%a: fractions 1—3, and 20%b: fractions 4—7), (3 : 2, 2.5 l, 40%a: fractions 8—11), (2 : 3, 2.5 l, 60%a: fractions 12—13, and 60%b: fractions 14—16), and (1 : 4, 2.5 l, 80%a: fractions 17—18, and 80%b: fractions 19—23), and MeOH (2.5 l), 500 ml fractions being collected]. The residue (1.85 g) of the 20%b eluate was subjected to RPCC. The residue (198 mg) of fractions 44—59 was separated by DCCC, and purification of the residue (24 mg) of fractions 27—31 by preparative HPLC (H_2O–MeOH, 3 : 1) gave 2.0 mg of **1**. The residue (144 mg) of fractions 80—92 was separated by DCCC, and purification of the residue (16 mg) of these fractions by HPLC afforded 11 mg of **4** in a crystalline state. The residue of fractions 93—109 was similarly separated by DCCC, and HPLC furnished 15 mg of **2** and 7 mg of **5**. Compound **3** (8 mg) was purified from the residue (57 mg) of fractions 110—118 in a similar manner.

A truxinic acid derivative (**6**) was isolated from the residue (5.14 g) of the 80%a eluate obtained on RPCC by similar chromatographic methods in a yield of 29 mg.

7-(3,5-Dihydroxy-1,1,5-trimethylcyclohexylidene)-9-methylprop-8-enyl 9-*O*-β-D-Glucopyranoside (**1**): Amorphous powder, UV λ_{max} (MeOH) nm (log ε): 204 (3.67), 324 (3.06). ^{1}H-NMR (CD_3OD) δ: 1.10 (3H, s H_3-11eq), 1.24 (1H, dd, J=11, 12 Hz, H-2ax), 1.30 (3H, s, H_3-12ax), 1.31 (1H, dd, J=11, 13 Hz, H-4ax), 1.34 (3H, s, H_3-13), 1.85 (1H, ddd, J=2, 4, 12 Hz, H-2eq), 2.14 (1H, ddd, J=2, 4, 13 Hz, H-4eq), 3.16 (1H, dd, J=8, 9 Hz, H-2′), 3.20-3.40 (3H, m, H-3′, 4′, 5′), 3.67 (1H, dd, J=5, 12 Hz, H-6a), 3.84 (1H, dd, J=2, 12 Hz, H-6′b), 4.17 (1H, tt, J=4, 11 Hz, H-4), 4.39 (1H, d, J=8 Hz, H-1′), 4.41 (1H, quintet, J=6 Hz, H-9), 5.46 (1H, d, J=6 Hz, H-8). ^{13}C-NMR (CD_3OD) δ: 20.7 (C-10), 29.6 (C-12ax), 31.6 (C-13), 33.1 (C-11eq), 36.3 (C-1), 50.0 (C-4), 50.6 (C-2), 62.8 (C-1′), 64.8 (C-4), 71.6 (C-4′), 72.9 (C-5), 75.3 (C-2′), 76.0 (C-9), 78.0 (C-5), 78.3 (C-3), 99.1 (C-8), 103.1 (C-1′), 117.3 (C-6), 200.2 (C-7) (NMR signals were assigned by ^{1}H–^{1}H COSY, HSQC and heteronuclear multiple bond correlation spectroscopies). CD (c=1.42×10^{-3} M, MeOH) $\Delta\varepsilon$ (nm): +1.12 (226), −0.24 (286). ORD (c=1.42×10^{-3} M, MeOH) [α] (nm): +143° (243). HR-FAB-MS (negative-ion mode) *m/z*: 387.2003 [M−H]$^-$ (Calcd for $C_{19}H_{31}O_8$: 387.2019).

3-Hydroxy-5,6-epoxy-β-ionol 3-*O*-β-D-glucopyranoside (**2**): Amorphous powder. $[\alpha]_D^{19}$ −35.2° (c=1.08, MeOH). ^{1}H-NMR (CD_3OD) δ: 0.96 (3H, s, H_3-12ax), 1.12 (3H, s, H_3-11eq), 1.19 (3H, s, H_3-13), 1.21 (1H, dd, J=11, 13 Hz, H-2ax), 1.27 (3H, d, J=7 Hz, H_3-10), 1.55 (1H, ddd, J=2, 3, 13 Hz, H-2eq), 1.60 (1H, dd, J=9, 14 Hz, H-4ax), 2.26 (1H, ddd, J=2, 5, 14 Hz, H-4eq), 3.17 (1H, dd, J=8, 9 Hz, H-2′), 3.21 (1H, ddd, J=2, 5, 10 Hz, H-5′), 3.25—3.35 (2H, m, H-3′,4′), 3.67 (1H, dd, J=5, 12 Hz, H-6′a), 3.74 (1H, dddd, J=3, 5, 9, 11 Hz, H-4), 3.81 (1H, dd, J=2, 12 Hz, H-6′b), 4.35 (1H, d, J=8 Hz, H-1′), 4.40 (1H, dquintet, J=1, 7 Hz, H-9), 5.72 (1H, dd, J=7, 16 Hz, H-8), 5.96 (1H, dd, J=1, 16 Hz, H-7). ^{13}C-NMR (CD_3OD): Table 1. HR-FAB-MS (negative-ion mode) *m/z*: 387.2018 [M−H]$^-$ (Calcd for $C_{19}H_{31}O_8$: 387.2019).

Premnaionoside (**3**): Amorphous powder. $[\alpha]_D^{19}$ −82.6° (c=0.56, MeOH). ^{1}H-NMR (CD_3OD) δ: 0.96 (3H, s, H_3-12ax), 1.12 (3H, s, H_3-11eq), 1.19 (3H, s, H_3-13), 1.21 (1H, dd, J=11, 13 Hz, H-2ax), 1.27 (3H, d, J=6 Hz, H_3-10), 1.55 (1H, ddd, J=2, 4, 13 Hz, H-2eq), 1.61 (1H, dd, J=9, 14 Hz, H-4ax), 2.26 (1H, ddd, J=2, 5, 14 Hz, H-4eq), 3.19 (1H, ddd, J=2, 5, 10 Hz, H-5′), 3.20—3.35 (3H, m, H-2′, 3′, 4′), 3.60 (1H, d, J=11 Hz, H-5″a), 3.63 (1H, d, J=11 Hz, H-5″b), 3.66 (1H, dd, J=5, 12 Hz, H-6′a), 3.72 (1H, d, J=11 Hz, H-4″a), 3.74 (1H, dddd, J=3, 5, 9, 11 Hz, H-4), 3.80 (1H, dd, J=2, 12 Hz, H-6′b), 3.94 (1H, d, J=2 Hz, H-2″), 4.05 (1H, d, J=11 Hz, H-4″b), 4.41 (1H, d, J=8 Hz, H-1′), 4.42 (1H, dquintet, J=1, 7 Hz, H-9), 5.38 (1H, d, J=2 Hz, H-1″), 5.71 (1H, dd, J=6, 16 Hz, H-8), 5.98 (1H, dd, J=1, 16 Hz, H-7). ^{13}C-NMR (CD_3OD): Table 1. HR-FAB-MS (negative-ion mode) *m/z*: 519.2441 [M−H]$^-$ (Calcd for $C_{24}H_{39}O_{12}$: 519.2441).

Benzyl alcohol β-D-(2′-*O*-β-D-xylopyranosyl)glucopyranoside (**4**): Colorless needles, mp 197—198 °C (MeOH). $[\alpha]_D^{19}$ −40.5° (c=0.67, MeOH). UV λ_{max} (MeOH) nm (log ε): 208 (3.75), 258 (2.46). ^{1}H-NMR (CD_3OD) δ: 3.09 (1H, dd, J=10, 12 Hz, H-5″a), 3.20—3.40 (6H, m, H-3′, 4′, 5′, 2″, 3″, 4″), 3.45 (1H, dd, J=8, 9 Hz, H-2′), 3.68 (1H, dd, J=6, 12 Hz, H-6′a), 3.83 (1H, dd, J=5, 12 Hz, H-5″b), 3.89 (1H, dd, J=2, 12 Hz, H-6′b), 4.50 (1H, d, J=8 Hz, H-1′), 4.51 (1H, d, J=7 Hz, H-1″), 4.65 (1H, d, J=12 Hz, H-7a), 4.94 (1H, d, J=12 Hz, H-7b), 7.26 (2H, t, J=7 Hz, H-3, 5), 7.32 (1H, tt, J=2, 7 Hz, H-4), 7.42 (2H, dd, J=2, 7 Hz, H-2, 6). ^{13}C-NMR (CD_3OD): Table 2. HR-FAB-MS (negative-ion mode) *m/z*: 401.1431 [M−H]$^-$ (Calcd for $C_{18}H_{25}O_{10}$: 401.1448).

Phenethyl alcohol β-D-(2′-*O*-β-D-glucopyranosyl)glucopyranoside (**5**): Colorless needles, mp 185—186 °C (MeOH). $[\alpha]_D^{19}$ −37.5° (c=0.40, MeOH). UV λ_{max} (MeOH) nm (log ε): 208 (3.73), 259 (2.45). ^{1}H-NMR (CD_3OD) δ: 2.95 (2H, td, J=2, 7 Hz, H_2-7), 3.27 (1H, dd, J=8, 9 Hz, H-2″), 3.30—3.45 (6H, m, H-3′, 4′, 5′, 3″, 4″, 5″), 3.52 (1H, dd, J=8, 9 Hz, H-2′), 3.68 (1H, dd, J=5, 12 Hz, H-6′a or 6″a), 3.70 (1H, dd, J=8, 9 Hz, H-6″a or 6′a), 3.84 (1H, dd, J=2, 12 Hz, H-6′b or 6″b), 3.88 (1H, dd, J=2, 12 Hz, H-6″b or 6′b), 4.11 (1H, dd, J=7, 10 Hz, H-8a), 4.13 (1H, dd, J=7, 10 Hz, H-8b), 4.51 (1H, d, J=8 Hz, H-1′), 4.69 (1H, d, J=8 Hz, H-1″), 7.23 (2H, m, H-3, 5). ^{13}C-NMR (CD_3OD): Table 2. HR-FAB-MS (negative-ion mode) *m/z*: 445.1713 [M−H]$^-$ (Calcd for $C_{20}H_{29}O_{11}$: 445.1710).

4,4′-Dimethoxy-β-truxinic acid catalpol diester (**6**): Amorphous powder. $[\alpha]_D^{22}$ −71.9° (c=1.77, MeOH). IR ν_{max} (KBr) cm^{-1} 3300, 2875, 1715, 1645, 1605, 1505, 1245, 1075, 1030, 920. UV λ_{max} (MeOH) nm (log ε): 207 (4.30), 230 (4.33), 279 (3.70), 285 (3.67). ^{1}H- and ^{13}C-NMR (CD_3OD): Table 3. HR-FAB-MS (negative-ion mode) *m/z*: 1043.3400 [M−H]$^-$ (Calcd for $C_{50}H_{59}O_{24}$: 1043.3396).

Acetylation of Compound 1 About 500 μg of **1** was acetylated with 25 μl each of acetic anhydride and pyridine at 20 °C for 14 h. Reagents were removed with a stream of N_2 and then the residue was dried *in vacuo*. Pentaacetate (**1a**), ^{1}H-NMR ($CDCl_3$): essentially the same as the reported data.[2] FAB-MS (positive-ion mode) *m/z*: 621 [M+Na]$^+$ (+NaI).

Acetylation of Compounds 4 and 5 to Their Hexaacetate (4a) and Heptaacetate (5a) About 2 mg each of **3** and **4** was acetylated with 50 μl each of acetic anhydride and pyridine at 20 °C for 15 h. The reagents were removed with a stream of N_2 and the residue was dried *in vacuo*. Benzyl alcohol β-D-(2′-*O*-β-D-xylopyranosyl)glucopyranoside hexaacetate (**4a**), ^{1}H-NMR ($CDCl_3$) δ: 2.00, 2.02, 2.03, 2.04, 2.07, 2.08 (each 3H, s, Ac×6), 3.20 (1H, dd, J=8, 12 Hz, H-5″a), 3.66 (1H, ddd, J=2, 5, 10 Hz, H-5′), 3.77 (1H, d, J=8, 9 Hz, H-2′), 4.10 (1H, dd, J=5, 12 Hz, H-5″a), 4.14 (1H, dd, J=2, 12 Hz, H-6′a), 4.25 (1H, dd, J=5, 12 Hz, H-6′b), 4.52 (1H, d, J=8 Hz, H-1′), 4.66 (1H, d, J=12 Hz, H-7a), 4.72 (1H, d, J=6 Hz, H-1″), 4.86 (1H, dd, J=6, 8 Hz, H-2″), 4.91 (1H, td, J=8, 5 Hz, H-4″), 4.93 (1H, d, J=12 Hz, H-7b), 4.98 (1H, t, J=10 Hz, H-4′), 5.07 (1H, t, J=8 Hz, H-3″), 5.17 (1H, t, J=9 Hz, H-3′), 7.26—7.37 (5H, m, H on benzene ring). Electron impact (EI)-MS *m/z* (rel. int.): 259 (86) [xylose triacetate oxonium ion]$^+$, 91 (100) [aglycone tropoloniumion]$^+$. Phenethyl alcohol β-D-(2′-*O*-β-D-glucopyranosyl)glucopyranoside heptaacetate (**5a**), ^{1}H-NMR ($CDCl_3$) δ: 1.989, 1.995, 2.00, 2.02, 2.04, 2.05, 2.07 (each 3H, Ac×7), 2.90 (1H, td, J=7, 14 Hz, H-7a), 2.95 (1H, td, J=7, 14 Hz, H-7b), 3.21 (1H, ddd, J=2, 4, 10 Hz, H-5″), 3.65 (1H, ddd, J=2, 5, 10 Hz, H-5′), 3.70 (1H, dd, J=8, 9 Hz, H-2′), 3.74 (1H, td, J=7, 9 Hz, H-8a), 4.01 (1H, dd, J=2, 12 Hz, H-6″a), 4.09 (1H, dd, J=2, 12 Hz, H-6′a), 4.13 (1H, ddd, J=6, 7, 9 Hz, H-8b), 4.15 (1H, dd, J=4, 12 Hz, H-6″b), 4.26 (1H, dd, J=5, 12 Hz, H-6′b), 4.49 (1H, d, J=8 Hz, H-1′), 4.70 (1H, d, J=8 Hz, H-1″), 4.90 (1H, dd, J=8, 9 Hz, H-2″), 4.97 (1H, t, J=9 Hz, H-4′), 5.05 (1H, t, J=8 Hz, H-3″), 5.16 (1H, t, J=9 Hz, H-3′), 7.26—7.38 (5H, m, H on benzene ring). EI-MS *m/z* (rel. int.): 331 (100) [glucose tetraacetate oxonium ion]$^+$, 105 (99) [aglycone methyltropolonium ion]$^+$.

GLC Analysis of the Sugar Portions of Compounds 3, 4 and 5 About 2 mg of each sample was treated with 5% HCl in MeOH at 95 °C for 3 h in a sealed tube. The reaction mixture was then neutralized by the addition of Ag_2CO_3. After filtering off the Ag_2CO_3, the solvent was evaporated and the residue dried *in vacuo*. The residue was then silylated with several drops of trimethylsilylimidazole at 60 °C for 15 min. The reaction mixture was partitioned between H_2O and *n*-hexane (2 ml each). The separated *n*-hexane layer was evaporated to dryness and then subjected to GLC analysis with flame ionization detector; Shimadzu CPB-20 capillary column, 0.22 mm×20 m, 0.25 μm film thickness; temperature, 160 °C (isothermal); carrier gas, N_2 at 1.5 kg cm^{-2}). Standard sugars: apiose, 2. 54, 2.65, 2.79 and 2.96 min; xylose, 5.36 and 5.94 min; glucose, 8.31 and 9.01 min. Compound **3**: apiose, 2.53, 2.66, 2.78 and 2.97 min and glucose, 8.35 and 9.04 min. Compound **4**: xylose, 5.36 and 5.93 min and glucose, 8.29 and 8.99 min. Compound **5**: glucose, 8.34 and 9.03 min.

Alkaline Hydrolysis of 6 to 4,4′-Dimethoxy-β-truxinic Acid Dimethyl Ester (6a) and Catalpol (6b) Compound **6** (17 mg) was treated with 0.1 N methanolic NaOH at 18 °C for 5 h under an N_2 atmosphere. The reaction mixture was neutralized with Amberlite IR-120B (H^+) and then the solvent was removed. The residue was partitioned with $CHCl_3$ (1 ml) and H_2O (1 ml). The residue in the organic layer was purified by preparative TLC [Silica gel GF_{254}, Merck, 0.25 mm thickness, 10 cm×10 cm, developed with C_6H_6–$(CH_3)_2CO$ (9 : 1) and eluted with $CHCl_3$–MeOH (9 : 1)] to give 3.8 mg

of 4,4′-dimethoxy-β-truxinic acid dimethyl ester (**6a**) (64%). The residue in the aqueous layer was purified by silica gel CC [Φ=2.6 cm, L=13 cm, $CHCl_3$ (50 ml) and $CHCl_3$–MeOH (9 : 1, 100 ml and 7 : 3, 200 ml), fractions of 12.4 ml being collected] in fractions 30—38, and then by DCCC to give 8.4 mg of catalpol in fractions 15—22 (**6b**) (68%). 4,4′-Dimethoxy-β-truxinic acid dimethyl ester (**6a**), amorphous solid, $[\alpha]_D^{21}$ ±0° (c=0.25, MeOH). UV λ_{max} (MeOH) nm (log ε): 207 (4.16), 229 (4.27), 278 (3.57), 285 (3.54). CD ($c=7.81\times10^{-5}$ M, MeOH): no significant absorption between 210 and 400 nm. ORD ($c=7.81\times10^{-5}$ M, MeOH): no significant absorption between 220 and 600 nm. HR-FAB-MS (negative-ion mode) m/z: 383.1481 $[M-H]^-$ (Calcd for $C_{22}H_{23}O_6$: 383.1494). Catalpol (**6b**), amorphous powder, $[\alpha]_D^{22}$ −94.1° (c=0.56, MeOH). ^{13}C-NMR data were essentially the same as reported values.[11)]

Acknowledgements The authors are grateful for access to the superconducting NMR instrument in the Analytical Center of Molecular Medicine in the Hiroshima University School of Medicine. Thanks are also due to the Okinawa Foundation for financial support with an Okinawan Research Promotion Award (H.O.).

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