Premnafolioside, a New Phenylethanoid, and Other Phenolic Compounds from Stems of Premna corymbosa var. obtusifolia

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PREMNAFOLIOSIDE, A NEW PHENYLETHANOID, AND OTHER PHENOLIC COMPOUNDS FROM STEMS OF *PREMNA CORYMBOSA* VAR. *OBTUSIFOLIA*

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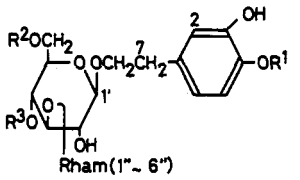
ABSTRACT.—From an MeOH extract of the stems of *Premna corymbosa* var. *obtusifolia*, thirteen compounds were isolated. The structure of a new compound **1** was determined to be 3-hydroxy-4-methoxyphenethyl alcohol β-D-(3′-*O*-α-L-rhamnopyranosyl,-4′-*O*-β-D-glucopyranosyl,-6′-*O*-feruloyl) glucopyranoside. Confirmation of the stereochemical structure of 4-*epi*-gummadiol-4-*O*-β-D-glucopyranoside [**6**] was also performed in this study.

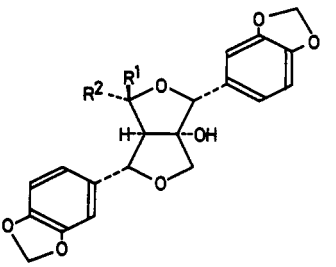
From the leaves of *Premna corymbosa* (Burm. fil.) Rottb. et Willd. var. *obtusifolia* (R. Br.) Flecher (Verbenaceae), harvested on an Okinawan island, premcoryoside, a conjugate of verbascoside and mussaenosidic acid, was isolated (1). From the stems of the same plant, a new phenyl ethanoid, named premnafolioside [**1**], and twelve known compounds have now been isolated from the *n*-BuOH soluble fraction of the MeOH extract.

Premnafolioside [**1**], $[\alpha]_D$ −35.8°, was obtained as an amorphous pale yellow powder whose elemental composition was determined to be $C_{37}H_{50}O_{20}$ by negative hrfabms. The uv λ max at 206, 289, and 326 nm, and ir absorption at 3399 (ν_{OH}), 1696 (a conjugated ester), 1632 (a double bond), and 1595 and 1516 (aromatic rings) cm^{-1} were very similar to those of verbascoside [**2**] and martynoside [**3**] (2), which co-exist in this plant. The ^1H nmr spectrum showed the presence of two units of aromatic protons coupled in an ABX system, a trans double bond [δ 6.40 (d, J=16 Hz) and 7.26 (d, J=16 Hz)], anomeric protons for β-glucopyranose [δ 4.34 (d, J=8 Hz)] and for rhamnopyranose [δ 5.35 (d, J=2 Hz)] and two MeO signals. The ^{13}C-nmr spectrum indicated that, besides the presence of the above-mentioned moieties, there was one more molecule of non-substituted β-glucopyranose [$\delta_{H-1''}$ 4.32 (d, J=9 Hz), $\delta_{C-1''}$ 104.34] in the skeleton. These results indicated that premnafolioside [**1**] was a derivative of martynoside [**3**] (see Table 1), having one more glucopyranose unit.

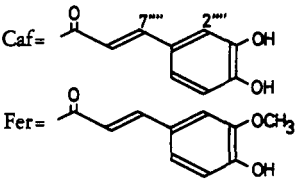
A phenylethanoid **4**, which has an extra glucose unit at the 6 position of the glucopyranoside in martynoside [**3**], was isolated from other plant sources (3, 4), but the ^{13}C-nmr chemical shifts of **1** were not superimposable with those of 6′-*O*-β-D-glucopyranosyl martynoside (see **1** and **4** in Table 1). Namely, significant differences in the chemical shifts of C-4′ and C-6′ between **4** (δ 70.47 and 69.47, respectively) and premnafolioside [**1**] (δ 76.2 and 63.7, respectively) suggested that the extra glucose and acyl moieties in **4** have been exchanged. This was supported by the fact that the ^{13}C-nmr chemical shift of the C-6′ position of isoacteoside [**5**] (δ 64.6) (5–7) was close to that of **1**. Similarly, the ^1H-nmr spectrum suggested acylation-induced downfield shifts at the H-6′ protons by 0.93 and 0.72 ppm from **3** to **1**. On irradiation of the anomeric proton of rhamnopyranose at δ 5.53 in the long-range selective decoupling experiment, the sharpening of the signal δ_C 79.36 (C-3′) in the coupled ^{13}C-nmr spectrum ruled out the possibility of exchange of the two sugar moieties attached to the core glucopyranose. Thus, the structure of **1** is 3-hydroxy-4-methoxyphenethyl alcohol β-D-(3′-*O*-α-L-rhamnopyranosyl-4′-*O*-β-D-glucopyranosyl-6′-*O*-feruloyl) glucopyranoside.



	R^1	R^2	R^3
1	Me	Fer	Glu (1‴~6‴)
2	H	H	Caf
3	Me	H	Fer
4	Me	Glu	Fer
5	H	Caf	H



6 R^1=OGlu, R^2=H
6a R^1=H, R=OH
6b R^1=OH, R^2=H



Glu=β-D-glucopyranosyl
Rham=α-L-rhamnopyranosyl

Isoacteoside (acteoside isomer) [**5**] was considered as an artifact, which was formed through acyl migration from the 4′ to the 6′ position of verbascoside during the extraction and purification procedure (5–7). However, isolation of premnafolioside [**1**] indicated the possibility that trans esterification of the acyl moiety in verbascoside or similar compounds occurred in the plants themselves.

4-*epi*-Gummadiol-4-*O*-β-D-glucopyranoside [**6**], $[\alpha]_D$ +44.8°, was obtained as a white amorphous powder. From spectroscopic evidence, this compound was proven to be the same as first isolated from the heartwood of *Gmelia arborea* by Anjaneyulu *et al.* (8). In their report, they proposed the structure of **6**, in which the orientation of the sugar moiety at the C-4 position was tentatively assigned based on the direct field effect of the glucose residue onto the chemical shift of the benzylic proton at C-6. However, the chemical shift of H-6 of 4-*epi*-gummadiol [**6b**] (δ 5.40) (minor) was close to that of **6** (δ 5.54), which simply indicated that the aglycone may not be gummadiol, but 4-*epi*-gummadiol (Table 2).

To confirm this, nOe experiments were performed. On irradiation of the anomeric proton (δ 4.56), the 11% increase of the signal intensity at H-4 confirmed that the sugar was linked to the OH group at the 4 position, and 11% and 14% nOe effects from H-4 (δ 5.63) to the anomeric proton and H-5 (δ 3.16), and 10% from H-5 to H-4 were also observed. Therefore, the sugar moiety must be oriented in the axial position, and the aglycone was proven to be 4-*epi*-gummadiol [**6b**], as mentioned in the literature (8). NOe enhancements of the aromatic protons, on irradiation of H-2 (δ 5.14) and H-6 (δ 5.54), helped us to assign the aromatic carbon signals.

On enzymatic hydrolysis, **6** gave D-glucose and an aglycone, a mixture of gummadiol [**6a**] and 4-*epi*-gummadiol [**6b**]. Although Anjaneyulu *et al.* (8) expected the aglycone to exist as a thermodynamically stable form, namely **6a**, our spectroscopic data revealed that it was a mixture of two compounds at the acetalic center, in the ratio of 6:4. ^{13}C- and ^1H-nmr assignments of the glucoside and aglycone, some of which have not been available before, are summarized in Table 2.

EXPERIMENTAL

INSTRUMENTATION.—^{13}C- (100 MHz) and ^1H- (400 MHz) nmr spectra were recorded on a JEOL JNM-GSX 400 spectrometer. Ir and uv spectra were recorded on Shimadzu IR-408 and UV-160A spectropho-

TABLE 1. ^{13}C nmr Spectral Data for Premnafolioside [**1**], Martynoside [**3**], 6′-*O*-β-D-Glucopyranosyl Martynoside [**4**], and Acteoside Isomer **5** (CD_3OD).

Carbon	Compound			
	1[a]	**3**	**4**[b]	**5**[c]
Aglycone				
C-1	132.7	132.9	132.96	131.4
C-2	112.8	112.9	112.96	117.1
C-3	147.5	147.6	147.59	146.0
C-4	147.4	147.4	147.38	144.6
C-5	117.0	117.1	117.15	116.3
C-6	121.1	121.2	121.25	121.3
C-7	36.7	36.6	36.57	36.6
C-8	72.3	72.1	72.11	72.3
Inner Glucose				
C-1′	104.5	104.2	104.24	104.3
C-2′	76.6	76.0	76.17	75.4
C-3′	79.4	81.5	81.55	84.0
C-4′	76.2	70.6	70.47	70.0
C-5′	74.9	76.2	74.84	75.6
C-6′	63.7	62.4	69.47	64.6
Rhamnose				
C-1″	101.9	103.0	103.05	102.7
C-2″	72.4	72.4	72.26	72.3
C-3″	72.1	72.0	72.38	72.3
C-4″	74.3	73.8	73.80	74.0
C-5″	69.3	70.4	70.71	70.4
C-6″	18.0	18.5	18.46	17.8
Outer Glucose				
C-1‴	104.3		104.74	
C-2‴	75.3		75.13	
C-3‴	78.0		77.95	
C-4‴	70.7		71.50	
C-5‴	78.3		77.86	
C-6‴	62.1		62.69	
Feruloyl (Caffeoyl)				
C-1⁗	127.7	127.7	127.69	127.7
C-2⁗	111.8	111.8	111.89	115.1
C-3⁗	150.7	150.8	150.88	146.7
C-4⁗	149.4	149.4	149.42	149.5
C-5⁗	116.5	116.5	116.56	116.5
C-6⁗	124.4	124.4	124.42	123.1
C-7⁗	147.3	147.9	148.13	147.2
C-8⁗	115.2	115.1	115.19	114.9
C-9⁗	168.7	168.3	168.47	169.1
−OMe	56.4	56.46	56.54	
	56.5	56.50	56.55	

[a]Assignments for **1** were performed by means of 1D and 2D nmr spectroscopies.
[b]Data in this column are from Otsuka (4).
[c]Data in this column are from Miyase *et al.* (2).

tometers, respectively. Optical rotations and cd spectra were measured with a Union Giken PM-101 automatic digital polarimeter and a JASCO J-720 spectrometer. Ms spectra were recorded on a JEOL JMS-SX 102 mass spectrometer with glycerol as a matrix.

EXTRACTION AND ISOLATION.—The plant material, *P. corymbosa* var. *obtusifolia*, was collected on an Okinawan island in August, 1989. A voucher specimen (89-PCO-Okinawa) is deposited at the Hebarium of Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine.

TABLE 2. ^{13}C-nmr and ^{1}H-nmr Assignments for 4-*epi*-Gummadiol-4-*O*-β-D-Glucopyranoside [**6**] and Gummadiols.[a]

Position	Compound						
	6[b]		Aglycones[c]				
			gummadiol (major) [**6a**]			4-*epi*-gummadiol (minor) [**6b**]	
1	92.7	—		89.2	—	92.0	—
2	86.4	5.14 (br s)		88.1	5.20 (s)	84.3	5.22 (s)
4	104.02[d]	5.63 (d, 6)		101.12[g]	5.49 (br s)	96.5	5.79 (dd, 3', 6)
5	67.4	3.16 (dd, 5, 6)		66.4	2.89 (dd, 1, 7)	64.9	3.11 (dd, 5, 6)
6	81.3	5.54 (br d, 5)		83.4	4.91 (d, 7)	79.8	5.40 (d, 5)
8	76.6	3.83 (d, 9)		75.1	4.00 (d, 9)	75.2	3.85 (d, 10)
		4.04 (d, 9)			4.07 (d, 9)		4.03 (d, 10)
1'	131.1	—	1'	129.71		128.57	
2'	109.7	6.94 (br d, 2)	1"	135.11		135.14	
3'	148.9[e]	—	2', 2"	106.38		107.18	
4'	149.0[e]	—	5', 5"	107.62		107.52	
5'	108.7	6.79 (d, 8)		108.28		108.14	
6'	122.5	6.86 (ddd, 1[h], 2, 8)		108.51		108.57	
			3', 3"	147.42		147.03	
			4', 4"	147.96		147.86	
1"	137.3	—		148.13		148.02	
2"	108.2	7.03 (br d, 2)		148.01		148.19	
3"	149.3	—	6', 6"	119.38		119.91	
4"	148.4[e]	—		120.30		120.30	
5"	108.9	6.76 (d, 8)					
6"	121.1	6.97 (ddd, 1[h], 2, 8)					
OCH_2O	102.33	5.920 (s)		101.17[g]		101.04	
	102.37	5.923 (br s)		101.31		101.31	
G-1	104.00[d]	4.56 (d, 7)					
2	75.3						
3	78.33[f]						
4	71.2						
5	78.28[f]						
6	62.6	3.66 (dd, 5, 12)					
		3.78 (dd, 2, 12)					

[a]Letters and figures in parentheses are multiplicities and coupling constants in Hz.
[b]Measured for CD_3OD solution.
[c]Measured for $CDCl_3$ solution.
[d–g]Assignments may be interchanged.
[h]Couplings were between those and H-2 and H-6, respectively. Cross peaks were observed in the H-H COSY spectrum.
[i]This coupling disappeared on addition of D_2O.

Pulverized stems (9.70 kg) were exhaustively extracted with MeOH. The MeOH extract was concentrated to 2 liters, and 100 ml of H_2O was added to obtain 95% aqueous MeOH solution. This solution was extracted with *n*-hexane (1 liter×2), and the MeOH layer was concentrated. This was dispersed in H_2O (1.5 liters), and then extracted with EtOAc (1.5 liters) and *n*-BuOH (1.5 liters), successively. The *n*-BuOH extract (182 g) was separated by highly porous synthetic resin, Diaion HP-20 cc (20% aqueous MeOH→100% MeOH). The residue of the 60% MeOH eluate was subjected to Si gel cc with increasing amounts of MeOH in $CHCl_3$. The 10–12.5% MeOH eluate (1.37 g) was further separated by open cc [Cosmosil, 10% aqueous MeOH (1 liter)→70% aqueous MeOH (1 liter), linear gradient], followed by dccc [$CHCl_3$–MeOH–H_2O–*n*-PrOH (9:12:8:2) to give 115 mg of premnafolioside [**1**]-enriched fraction. This was finally purified by preparative hplc [Inertsil, MeOH-H_2O (45:55)] to afford 29 mg of **1**.

The residue (410 mg) of the 4% MeOH eluate of Si gel cc was subjected to open cc with the same conditions as above. From the 50% MeOH eluate, 201 mg of 4-*epi*-gummadiol-4-*O*-β-D-glucopyranoside [**6**] was obtained.

KNOWN COMPOUNDS ISOLATED.—Verbascoside (=acteoside) [**2**]: $[\alpha]_D$ −95.9° (MeOH, *c*=0.75) (2). Martynoside [**3**]: $[\alpha]_D$ −60.1° (MeOH, *c*=0.75); ^{13}C nmr see Table 1 (2). 6-α-L-(2"-*O*-*p*-Coumaroyl)-rhamnopyranosylcatalpol (=saccatoside): $[\alpha]_D$ −103.9° (MeOH, *c*=0.83) (9). 6-α-L-(4"-*O*-Feruloyl)-rhamnopyranosylcatalpol: $[\alpha]_D$ −122.2° (MeOH, *c*=0.70) (10). Premnoside C, $[\alpha]_D$ +23.6° (MeOH, *c*=0.72) (11). Premnoside D: $[\alpha]_D$ +18.5° (MeOH, *c*=0.70) (11). *erythro*-(4-Hydroxy-3-methoxyphenyl)-2-{4-[2-formyl-(*E*)-vinyl]-2-methoxyphenoxy}-propan-1,3-diol: $[\alpha]_D$ +0.59° (MeOH, *c*=0.51) (12,13). *threo*-(4-Hydroxy-3-methoxyphenyl)-2-{4-[2-carbinyl-(*E*)-vinyl]-2-methoxyphenoxy}-propan-1,3-diol: $[\alpha]_D$ +1.91° (MeOH, *c*=1.25) (12, 13). (+)-Lyoniresinol-2a-*O*-β-D-glucopyranoside: $[\alpha]_D$ +45.7° (MeOH,

c=0.72); cd (MeOH, c=0.00183) nm ($\Delta\epsilon$) 206 (+20.4), 217 (−14.2), 244 (+11.6), 272 (+3.34), 287 (−1.09) (14, 15). Plucheoside D_1: $[\alpha]_D$ −82.3° (MeOH, c=0.69); cd (MeOH, c=0.00346) nm ($\Delta\epsilon$) 212 (−2.17), 236 (+6.48), 257 (−2.98), 280 (+0.11), 342 (−2.66), 399 (−0.40) (16). (−)-Olivil: $[\alpha]_D$ −34.0° (MeOH, c=0.79) (17, 18).

Premnafolioside [**1**].—Amorphous powder: $[\alpha]_D$ −35.8° (MeOH, c=0.67); ir (KBr) ν max 3399, 2926, 1696, 1632, 1595, 1516, 1273, 1070–1030, 812 cm^{-1}; uv λ max (log ϵ) (MeOH) 206 (4.49), 217 sh (4.31), 231 sh (4.23), 289, (4.13), 326, (4.26) nm; ^{13}C nmr see Table 1; ^1H nmr (CD_3OD) δ 1.22 (3H, d, J=6 Hz, H-6″), 2.79 (2H, t, J=7 Hz, H-8), 3.17 (ddd, J=2, 5, 9 Hz, H-5‴), 3.20 (dd, J=8, 9 Hz, H-2‴), 3.85 (2H, H-6‴), 3.39 (dd, J=8, 9 Hz, H-2′), 3.45 (t, J=9 Hz, H-4‴), 3.74 (3H, s, $-OCH_3$), 3.87 (3H, s, $-OCH_3$), 4.32 (d, J=9 Hz, H-1‴), 4.34 (d, J=8 Hz, H-1′), 4.47 (qd, J=6, 10 Hz, H-5″), 4.58 (dd, J=5, 12 Hz, H_a-6′), 4.66 (dd, J=2, 12 Hz, H_b-6′), 5.35 (d, J=2 Hz, H-1″), 6.40 (d, J=16 Hz, H-8‴), 6.60 (dd, J=2, 8 Hz, H-6), 6.67 (d, J=8 Hz, H-5), 6.68 (d, J=2 Hz, H-2), 6.80 (d, J=8 Hz, H-5‴), 7.04 (dd, J=2, 8 Hz, H-6‴), 7.17 (d, J=2 Hz, H-2‴), 7.62 (d, J=16 Hz, H-7‴); hrfabms (negative centroid) m/z $[M-H]^-$ 813.2819 ($C_{37}H_{49}O_{20}$ requires 813.2817).

4-epi-*Gummadiol-4*-O-β-D-*glucopyranoside* [**6**].—Amorphous powder: $[\alpha]_D$ +44.8° (MeOH, c=0.79); uv λ max (log ϵ) (MeOH) 207 (4.44), 236 (4.05), 286 (3.93) nm; ^1H nmr (Me_2CO-d_6) δ 3.16 (dd, J=5, 6 Hz, H-5), 3.86 (d, J=9 Hz, H_a-8), 4.06 (d, J=9 Hz, H_b-8), 5.17 (s, H-2), 5.53 (d, J=5 Hz, H-4), 5.79 (d, J=6Hz, H-6) [these data showed no discrepancy from the reported values (8)], ^{13}C and ^1H nmr (CD_3OD) see Table 2.

ENZYMATIC HYDROLYSIS OF **6**.—Compound **6** (59 mg) in 5 ml of H_2O was treated with an equal amount of crude hesperidinase for 1.5 h at 37°, and the reaction mixture was partitioned between EtOAc (100 ml) and H_2O (100 ml). The residue of the organic layer was purified by Sephadex LH-20 cc to give 20 mg (48%) of a mixture of aglycones **6a** and **6b**: colorless powder; $[\alpha]_D$ +14.2° (MeOH, c=0.70); uv λ max (log ϵ) (MeOH) 208 (4.20), 237 (3.80), 286 (3.74) nm; ^{13}C and ^1H nmr ($CDCl_3$) see Table 2.

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