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PREMNOSIDES A–D: DIACYL 6-*O*-α-L-RHAMNOPYRANOSYLCATALPOLS FROM *PREMNA ODORATA*

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Key Word Index—*Premna odorata*; Verbenaceae; iridoid; diacylrhamnopyranosylcatalpol; premnoside; NMR.

Abstract—Premnosides A–D were isolated from the leaves of *Premna odorata*. Their structures were determined to be (2″-*O*-, 3″-*O*-dicaffeoyl), [2″-*O*-, 3″-*O*-(or 3″-*O*-, 2″-*O*-)caffeoyl, feruloyl], [2″-*O*-, 3″-*O*-(or 3″-*O*-, 2″-*O*-)caffeoyl, *p*-*trans*-coumaroyl] and [2″-*O*-, 3″-*O*-(or 3″-*O*-, 2″-*O*-)feruloyl, *p*-*trans*-coumaroyl]-6-*O*-α-L-rhamnopyranosylcatalpols, respectively, by means of NMR spectroscopy and chemical methods.

INTRODUCTION

To date, the isolation of 6-*O*-α-L-rhamnopyranosylcatalpol from *Scrophularia nodosa* [1] has been reported. Several acylated 6-*O*-α-L-rhamnopyranosylcatalpols have been isolated from other species of plants, i.e. diacylrhamnopyranosylcatalpol [2″-*O*-, 3″-*O*-(or 3″-*O*-, 2″-*O*-)acetyl, *p*-methoxy-*trans*-cinnamoyl-] from *Verbascum sinuatum* [2], Monoacyl rhamnopyranosylcatalpols, from two other species of Verbascum plants [3, 4], and triacyl derivatives from *Scrophuraria scopolii* [5].

In a previous paper [6], we reported the isolation of 6-*O*-α-L-(2″-*O*- and 3″-*O*-caffeoyl)rhamnopyranosylcatalpols from a Philippine medicinal plant, *Premna odorata* Blanco. Further investigation of the methanol extract of this plant has given four new diacylrhamnopyranosylcatalpols which we have named as premnosides A–D.

RESULTS AND DISCUSSION

Premnosides A–D were isolated from the methanol extract of the leaves of *P. odorata* by a combination of highly porous polymer Diaion HP-20 and silica gel CC, preparative HPLC and DCCC.

Premnoside A(**1**), $C_{39}H_{44}O_{20}$, was obtained as an amorphous powder, whose M_r was confirmed by the observation of an ion peak at *m/z* 855 $[M+Na]^+$, and *m/z* 871 $[M+K]^+$ in FABMS. The IR spectrum of **1** showed the presence of conjugated esters (1695 and 1625 cm^{-1}) and aromatic rings (1600 and 1510 cm^{-1}). The UV maxima at 219, 245, 303sh and 328 nm are very similar to those of caffeic acid [6, 7] and the extinction coefficient of the maximum suggested that two caffeoyl moieties were present in the molecule. This was also supported by the observation by ^1H NMR of four acetyl signals (δ2.28 × 2 and 2.31 × 2) on the phenolic hydroxyl groups on acetylation of **1**. The ^1H NMR spectrum of **1** also showed the presence of two sets of *trans* double bonds [δ6.23(*d*, *J* = 16 Hz), 6.34 (*d*, *J* = 16 Hz), 7.54 (*d*, *J* = 16 Hz) and 7.59 (*d*, *J* = 16 Hz)] and six aromatic protons. In the ^{13}C NMR spectrum, six typical signals for β-glucopyranose and six signals for a substituted α-rhamnopyranose were observed, and the presence of these sugars was confirmed by GC analysis of TMS derivatives of methanolysates of **1**. Eighteen carbon signals which can be attributed to two caffeoyl moieties were reasonably assigned as shown in Table 1. The remaining nine signals fitted very well with a 6-substituted 6-*O*-α-L-rhamnopyranosylcatalpol (**5**) [6] and suggested **1** to be this compound with two caffeoyl units linked to the rhamnosyl moiety. The points of attachment were shown to be the 2″- and 3″-oxygen atoms in the following way. The anomeric carbon signal of rhamnose in **1** was shifted 2.4 ppm upfield when compared to that in **5**, proving acylation in the 2″-position. However, the signals from C-5″ showed no significant difference when comparing **1** and **5**, and thus the second caffeoyl group is not esterified with the 4″-hydroxyl group.

To confirm this, the chemical shifts of rhamnosyl carbons in related compounds were compared. On going from 6-*O*-α-L-(2″-*O*-caffeoyl) rhamnopyranosylcatalpol, previously isolated from the same plant [6], the C-3″ signal was significantly shifted downfield by δ2.6 and the C-2″ and C-4″ signals were shifted upfield by δ2.7 and 2.5, respectively. Even if the assignments of C-2″ and C-4″ signals are interchanged, upfield shifts of more than 2 ppm are still to be expected. Therefore the structure of premnoside A was definitely elucidated to be 6-*O*-α-L-(2″, 3″-di-*O*-caffeoyl) rhamnopyranosylcatalpol.

Premnoside B(**2**), $C_{39}H_{44}O_{19}$, was obtained as a colourless powder and FABMS showed the $[M+Na]^+$ ion peak at *m/z* 816 i.e. 16 mass units smaller than **1**. The ^1H NMR spectrum showed close resemblance to **1**. However, an obvious difference was seen in the aromatic region, which showed an A_2B_2 coupling system at δ6.79 (2H, *d*, *J* = 8 Hz) and 6.81 (2H, *d*, *J* = 8 Hz). The ^{13}C NMR spectrum of **2** was also similar to that of **1** (Table 1). Nine

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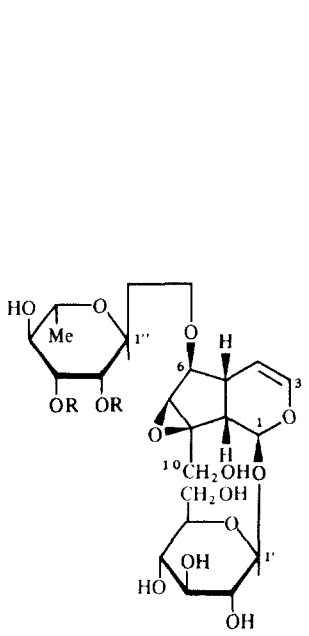

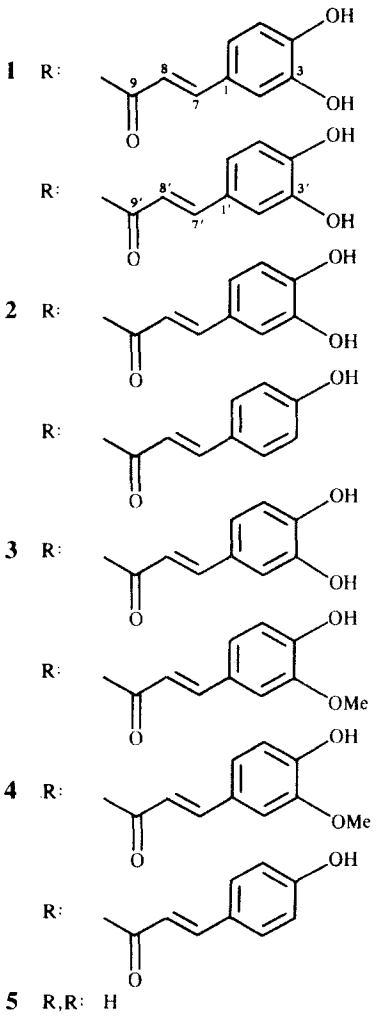

signals in the low field were reasonably assigned to the caffeoyl moiety and the remaining seven signals, two of which have double intensity, were essentially the same as reported for *p-trans*-coumaric acid [8]. This was also supported by the ^{1}H NMR spectrum of the nonaacetate of **2** which showed three acetyl signals (δ2.27 × 2 and 2.31) on phenolic hydroxyl groups. Since other ^{13}C NMR signals were essentially the same as that of **1**, including the rhamnose moiety, the structure of premnoside B (**2**) was determined to be 6-*O*-α-L-[2″-*O*-, 3″-*O*-(or 3″-*O*-, 2″-*O*-) caffeoyl, *p-trans*-coumaroyl] rhamnopyranosylcatalpol.

The disposition of the acyl groups in this compound cannot be determined by partial hydrolysis of the acyl groups in alkaline or acidic media due to facile acyl migration between the 2- and 3-hydroxyl groups of the rhamnose residue [9]. Sticher *et al.* determined the acyl positions of trisubstituted 6-*O*-α-L-rhamnopyranosylcatalpols by a selective long range C-H decoupling method. In their case, two of the three acyl moieties were the same and the carbonyl carbon signals were easily assigned because the substituents were acetic and *p*-methoxycinnamic acids (or cinnamic acids) [5]. In the case of **2**, the chemical shifts of the two carbonyl carbon signals are too close (δ168.0 and 168.4) to be unconditionally assigned. Furthermore, the proton signals at the 2′- and 3′-positions are not well enough resolved in the ^{1}H NMR spectrum to perform a long range proton selective decoupling experiment. Thus the structure of premnoside B is tentatively presented as two alternatives. The same is the case for premnosides C and D.

Premnoside C(**3**), $C_{40}H_{46}O_{20}$, was obtained as a colourless amorphous powder, M_r 846 (FABMS), i.e. 14 mass units larger than **1**. The ^{1}H and ^{13}C NMR spectra showed the presence of a methoxyl group (δ3.76 and 56.4, respectively). On comparing the ^{13}C NMR spectrum with that of **1**, the structure of **3** is seen to be that of **1** in which one of the caffeoyl moieties is replaced by ferulic acid [10]. The other physical and chemical data supported the structure 6-*O*-α-L-[2″-*O*-, 3″-*O*-(or 3″-*O*-, 2″-*O*-)caffeoyl, feruloyl] rhamnopyranosylcatalpol for premnoside C.

Premnoside D(**4**), $C_{40}H_{46}O_{19}$, was obtained as a slight yellow powder, M_r 830 (FABMS). The ^{1}H NMR spectrum showed the presence of a methoxyl signal (δ3.76) and four protons in an A_2B_2 system δ7.00 (2H, *d*, J = 8 Hz) and 7.46 (2H, *d*, J = 8 Hz). The ^{13}C NMR spectrum also showed a methoxyl signal at δ56.3. From other physical and chemical data, premnoside D was deter-

Table 1. ^{13}C NMR data of premnoside A (1), B (2), C (3) and D (4) and 5 (25 MHz, CD_3OD)

	1	2	3	4	5†
Aglycone 1	95.2	95.2	95.2	95.2	95.1
3	142.3	142.3	142.4	142.3	142.1
4	103.4	103.4	103.5	103.4	103.6
5	37.2	37.2	37.2	37.2	37.2
6	84.4	84.4	84.5	84.4	83.5
7	59.5	59.5	59.5	59.5	59.3
8	66.5	66.5	66.6	66.5	66.5
9	43.3	43.3	43.3	43.3	43.2
10	61.5	61.5	61.6	61.5	61.4
Glucose 1′	99.7	99.7	99.8	99.7	99.7
2′	74.8	74.8	74.9	74.8	74.8
3′	78.6	78.6	78.6	78.5	78.5
4′	71.7	71.7	71.8	71.7	71.7
5′	77.6	77.6	77.7	77.6	77.6
6′	62.9	62.9	63.0	62.9	62.9
Rhamnose 1″	97.9	97.9	97.9	97.8	100.3
2″	71.4	71.4	71.5	71.4	72.2
3″	73.1	73.1	73.1	73.0	72.2
4″	71.7	71.7	71.8	71.7	73.8
5″	70.3	70.3	70.3	70.3	70.1
6″	18.1	18.0	18.1	18.1	18.0
Acyl* 1, 1′	127.5, 127.6	1 127.0, 1′ 127.6	1 127.6, 1′ 127.6	1 127.6, 1′ 127.0	
2, 2′	114.3, 114.8	2 114.3, 2′ 131.4	2 114.3, 2′ 111.9	2 111.8, 2′ 131.4	
3, 3′	149.6, 149.8	3 149.5, 3′ 116.9	3 149.9, 3′ 150.6	3 150.6, 3′ 116.9	
4, 4′	146.7	4 146.7, 4′ 161.4	4 146.8, 4′ 149.3	4 149.2, 4′ 161.5	
5, 5′	116.5, 116.6	5 116.5, 5′ 116.9	5 116.5, 5′ 116.5	5 116.4, 5′ 116.9	
6, 6′	123.0	6 123.2, 6′ 131.4	6 123.3, 6′ 124.0	6 124.0, 6′ 131.4	
7, 7′	147.4, 148.1	7 147.4, 7′ 147.7	7 147.3, 7′ 148.1	7 147.3, 7′ 147.7	
8, 8′	115.0, 115.3	8 115.1, 8′ 114.8	8 115.4, 8′ 115.2	8 115.2, 8′ 114.4	
9, 9′	168.0, 168.5	9 168.0, 9′ 168.4	9 168.4, 9′ 168.1	9 168.0, 9′ 168.6	
–OMe			56.4	56.3	

*Assignments of C-7, 7′, C-8, 8′ and C-9, 9′ of acyl moieties in each compound may be interchanged.
†Data taken from ref. [1].

mined to be 6-*O*-α-L-[2″-*O*-, 3″-*O*-(or 3″-*O*-, 2″-*O*-) feruloyl, *p-trans*-coumaroyl] rhamnopyranosylcatalpol.

EXPERIMENTAL

^1H and ^{13}C NMR: 100 and 25 MHz, respectively. Chemical shifts are given as δ values (ppm) with TMS as internal standard. MS: 70 eV.

Plant material. Premna odorata was cultivated and harvested at the Department of Horticulture, University of Philippines at Los Baños, Philippines.

Extraction and isolation. Dried and powdered leaves of *P. odorata* (2.12 kg) were extracted with *n*-hexane followed by MeOH. The MeOH extract (222 g) was suspended in H_2O and then extracted with EtOAc followed by *n*-BuOH. The *n*-BuOH extract (105.5 g) was chromatographed on a highly porous polymer (Diaion, HP-20; Mitsubishi Chemical Ind. Co.) with stepwise increase of MeOH content in H_2O (20, 40, 60, 80 and 100%). The 60% MeOH fraction (25 g) was chromatographed over silica gel with $CHCl_3$–MeOH as solvent. From the 15 and 17.5% MeOH in $CHCl_3$ eluent, 5.0 g of premnoside A-rich fraction was obtained. This fraction was further purified over silica gel (EtOAc–EtOH–H_2O 100:10:1) to give 466 mg of pure premnoside A. From the 80% MeOH fraction from Diaion chromatography, 2.5 g of a premnoside B, C and D-rich fraction was obtained by silica gel CC with 15% MeOH in $CHCl_3$ as eluent. This fraction was further purified over silica gel (EtOAc–EtOH–H_2O) and then DCCC ($CHCl_3$–MeOH–H_2O–*n*-PrOH, 9:12:8:2, ascending method) to give 89, 91 and 61 mg of premnoside B, C and D, respectively. Final purification of premnoside D (61 mg) was performed by prep. HPLC on an ODS column with 55% MeOH in H_2O (16 mg).

Premnoside A (1). Slight yellow amorphous powder, $[\alpha]_D^{20}$ +24.8° (MeOH; *c* 0.27); IR ν_{max}^{KBr} cm^{-1}: 3350, 1695, 1620, 1600, 1510, 1442, 1270, 1155, 1110, 1065, 849, 810; UV λ_{max}^{MeOH} nm (log ε): 219 (4.40), 245 (4.27), 303 (4.43) sh, 328 (4.54); FABMS *m/z*: 855 $[M+Na]^+$ (+NaI), 871 $[M+K]^+$ (+KI); ^1H NMR (MeOH-d_4): δ 1.37 (3H, *d*, *J* = 6 Hz, H-6″), ~2.5 (2H, *m*, 5-H, 9-H), 6.23 (H, *d*, *J* = 16 Hz), 6.34 (H, *d*, *J* = 16 Hz), 6.39 (H, *d*, *J* = 6 Hz, H-3), 6.6–7.0 (6H, aromatic protons), 7.54 (H, *d*, *J* = 16 Hz), 7.59 (H, *d*, *J* = 16 Hz); ^{13}C NMR: see Table 1. (Found: C, 53.9; H, 5.63. $C_{39}H_{44}O_{20}\cdot 2H_2O$ requires: C, 53.91; H, 5.57%).

Alkaline hydrolysis of premnoside A (1). Premnoside A (150 mg) (1) was hydrolysed with a stoichiometric amount of 0.01 M NaOH at 20°. The reaction was followed by TLC (silica gel, precoated, EtOAc–EtOH–H_2O, 8:2:1). After disappearance of the starting material, the reaction mixture was neutralized with Amberlite MB-6, and then concd *in vacuo*. The 6-α-rhamnopy-

ranosylcatalpol formed in the reaction was purified by DCCC ($CHCl_3$–MeOH–H_2O–*n*-PrOH 9:12:8:2, ascending method) and Sephadex LH-20 CC (MeOH) (**5**, 64 mg). Small amounts of premnosides B, C and D were also hydrolysed to give **5** on TLC (silica gel, precoated, Merck, $CHCl_3$–MeOH–H_2O 15:6:1 and EtOAc–EtOH–H_2O 8:2:1). Colourless amorphous powder, $[\alpha]_D$ −150° (MeOH; *c* 0.41); IR ν_{max}^{KBr} cm^{-1}: 3320, 1645, 1050; UV: no absorption between 210 and 360 nm; FABMS *m/z*: 531 $[M+Na]^+$, 323, 173; ^1H NMR (DMSO-d_6): δ1.14 (3H, *d*, *J* = 5 Hz, H-6″), ~2.3 (2H, *m*, H-5, 9), 4.79 (H, *s*, H-1″), 4.96 (H, *d*, *J* = 8 Hz, H-1′), 6.47 (H, *d*, *J* = 6 Hz, H-3); ^{13}C NMR (DMSO-d_6) [1] δ17.7 (C-6″), 35.5 (C-5), 41.8 (C-9), 57.3 (C-7), 58.7 (C-10), 61.2 (C-6′), 65.2 (C-8), 68.7 (C-5″), 70.1 (C-3″), 70.4 (C-4′), 71.8 (C-4″), 73.3 (C-2′), 76.3 (C-5′), 77.3 (C-3′), 81.2 (C-6), 93.0 (C-1), 97.7 (C-1′), 98.7 (C-1″), 102.3 (C-4), 140.8 (C-3). (Found: C, 46.0; H, 6.25. Calcd. for $C_{21}H_{32}O_{14}\cdot 2H_2O$: C, 46.32; H, 6.66%).

Premnoside A decaacetate. Premnoside A (40 mg) was treated with a mixture of Ac_2O and pyridine at 25° overnight. Usual work-up gave 46 mg of the expected decaacetate. Colourless amorphous powder, $[\alpha]_D$ +11.6° ($CHCl_3$; *c* 0.50); IR ν_{max}^{KBr} cm^{-1}: 1750, 1630, 1500, 1425, 1370, 1215, 1040, 905; UV λ_{max}^{MeOH} nm (log ε): 219 (4.58), 277 (4.47); EIMS *m/z*: 575, 503, 429, 331, 169, 136; FABMS *m/z*: 1275 $[M+Na]^+$ (+NaI), 1291 $[M+K]^+$ (+KI); ^1H NMR ($CDCl_3$): δ1.28 (3H, *d*, *J* = 4 Hz), 2.04 (Ac × 4), 2.11 (Ac), 2.14 (Ac), 2.28 (Ac × 2), 2.31 (Ac × 2), ~2.5 (2H, *m*), 5.49 (H, *br s*), 6.29 (H, *d*, *J* = 16 Hz), 6.34 (H, *d*, *J* = 6 Hz), 6.52 (H, *d*, *J* = 16 Hz), 7.1 ~ 7.4 (6H, aromatic protons), 7.58 (H, *d*, *J* = 16 Hz), 7.68 (H, *d*, *J* = 16 Hz); ^{13}C NMR ($CDCl_3$): δ17.4, 20.6 (Ac × 10), 58.1, 61.1, 62.1, 62.4, 66.9, 67.0, 68.3, 69.2, 70.3, 70.6, 71.0, 72.3, 72.6, 83.6, 94.3, 96.6 (× 2), 102.4, 118.2 (× 2), 122.8, 123.0, 124.0 (× 2), 126.8 (× 2), 132.9 (× 2), 141.4, 142.5 (× 3), 143.8, 143.9, 144.1, 144.4, 165.3, 165.5, 167.9 (Ac × 3), 168.0 (Ac), 169.0 (Ac), 169.3 (Ac), 170.0 (Ac), 170.2 (Ac), 170.6 (Ac × 2). (Found: C, 54.8; H, 5.24. $C_{59}H_{64}O_{30}\cdot 2H_2O$ requires: C, 54.97; H, 5.31%).

Premnoside B(**2**). Colourless amorphous powder, $[\alpha]_D$ +19.4° (MeOH; *c* 0.38); IR ν_{max}^{KBr} cm^{-1}: 3325, 1694, 1629, 1604, 1514, 1444, 1260, 1155, 1063, 831; UV λ_{max}^{MeOH} nm (log ε): 222 (4.27), 236 (4.21) sh, 309 (4.52) inf, 321 (4.57); FABMS *m/z*: 839 $[M+Na]^+$, 855 $[M+K]^+$(+KI); ^1H NMR (MeOH-d_4): δ1.38 (3H, *d*, *J* = 5 Hz), ~2.5 (2H, *m*), 5.43 (H, *br s*), 6.23 (H, *d*, *J* = 16 Hz), 6.40 (H, *d*, *J* = 16 Hz), 6.40 (H, *d*, *J* = 6 Hz), ~6.65 (2H, aromatic protons), 6.79 (H, *d*, *J* = 8 Hz), 6.81 (2H, *d*, *J* = 8 Hz), 7.00 (H, *br s*, H-$2_{caffeoyl}$), 7.56 (2H, *d*, *J* = 8 Hz), 7.59 (H, *d*, *J* = 16 Hz), 7.69 (H, *d*, *J* = 16 Hz); ^{13}C NMR: see Table 1. (Found: C, 54.9; H, 5.56. $C_{39}H_{44}O_{19}\cdot 2H_2O$ requires C, 54.92; H, 5.67%).

Premnoside B nonaacetate. Premnoside B (35 mg) was treated with a mixture of Ac_2O and pyridine at 25° overnight. Usual work-up gave 36 mg of the expected nonaacetate. Colourless amorphous powder, $[\alpha]_D$ +3.9° ($CHCl_3$; *c* 0.44); IR ν_{max}^{KBr} cm^{-1}: 1745, 1630, 1500, 1420, 1370, 1215, 1040, 905; UV λ_{max}^{MeOH} nm (log ε) 218 (4.58), 279 (4.77); EIMS *m/z*: 419, 377, 331, 169, 147; FABMS *m/z*: 1217 $[M+Na]^+$ (+NaI), 1233 $[M+K]^+$ (+KI); ^1H NMR ($CDCl_3$): δ1.28 (3H, *d*, *J* = 6 Hz), 2.03 (Ac × 4), 2.11 (Ac), 2.13 (Ac), 2.27 (Ac × 2), 2.31 (Ac), ~2.5 (2H, *m*), 5.49 (H, *br s*), 6.29 (H, *d*, *J* = 16 Hz), 6.34 (H, *d*, *J* = 5 Hz), 6.53 (H, *d*, *J* = 16 Hz), 7.57 (H, *d*, *J* = 16 Hz), 7.60 (2H, *d*, *J* = 8 Hz), 7.1 ~ 7.3 (5H, aromatic protons), 7.73 (H, *d*, *J* = 16 Hz); ^{13}C NMR ($CDCl_3$) δ17.4, 20.6 (Ac × 8), 21.1 (Ac), 35.5, 41.7, 58.1, 61.1, 62.1, 67.0, 68.3, 69.3, 70.2, 70.6, 71.1, 72.6, 72.3, 83.6, 94.3, 96.6 (× 2), 102.4, 117.2, 118.2, 122.2 (× 2), 122.8, 123.9, 126.7, 129.5 (× 2), 131.6, 131.8, 133.0, 141.1, 142.5, 143.7, 144.0, 152.5, 165.3, 165.8, 167.9 (Ac), 168.0 (Ac), 169.0 (Ac × 2), 169.3 (Ac × 2), 170.0 (Ac), 170.2 (Ac), 170.6 (Ac). (Found: C, 56.4; H, 5.14. $C_{57}H_{62}O_{28}\cdot H_2O$ requires: C, 56.44; H, 5.32%).

Premnoside C(**3**). Colourless amorphous powder, $[\alpha]_D$ +25.9° ($CDCl_3$; *c* 0.36); IR ν_{max}^{KBr} cm^{-1}: 3350, 1693, 1630, 1600, 1514, 1444, 1262, 1155, 1120, 1065, 846, 811; UV λ_{max}^{MeOH} nm (log ε): 222 (4.34), 245 (4.23), 315 (4.45) inf, 336 (4.55); FABMS *m/z*: 869 $[M+Na]^+$(+NaI), 885 $[M+K]^+$(+KI); ^1H NMR (MeOH-d_4): δ1.38 (3H, *d*, *J* = 5 Hz), ~2.5 (2H, *m*), 3.76 (3H, *s*), 5.43 (H, *br s*), 6.31 (H, *d*, *J* = 16 Hz), 6.35 (H, *d*, *J* = 16 Hz), 6.40 (H, *d*, *J* = 5 Hz), 6.75 (H, *d*, *J* = 8 Hz), 6.78 (H, *d*, *J* = 8 Hz); 6.9 ~ 7.0 (4H, aromatic protons), 7.58 (2H, *d*, *J* = 16 Hz); ^{13}C NMR: see Table 1. (Found: C, 54.6; H, 5.60. $C_{40}H_{46}O_{20}\cdot 2H_2O$ requires: C, 54.42; H, 5.70%).

Premnoside C nonaacetate. Premnoside C (34 mg) was treated with a mixture of Ac_2O and pyridine at 25° overnight. Usual work-up afforded 39 mg of the expected nonaacetate. Colourless amorphous powder, $[\alpha]_D$ +4.2° ($CHCl_3$; *c* 0.40); IR ν_{max}^{KBr} cm^{-1}: 1750, 1630, 1500, 1420, 1370, 1215, 1035, 900; UV λ_{max}^{MeOH} nm (log ε): 218 (4.59), 279 (4.69); EIMS *m/z*: 357, 289, 160, 159, 118; FABMS *m/z*: 1247 $[M+Na]^+$(+NaI), 1263 $[M+K]^+$ (+KI); ^1H NMR ($CDCl_3$): δ1.27 (3H, *d*, *J* = 6 Hz), 2.04 (Ac × 4), 2.11 (Ac), 2.13 (Ac), 2.31 (Ac × 3), ~2.5 (2H, *m*), 3.79 (3H, *s*), 6.29 (H, *d*, *J* = 16 Hz), 6.34 (H, *d*, *J* = 5 Hz), 6.53 (H, *d*, *J* = 16 Hz), 7.0 ~ 7.4 (6H, aromatic protons), 7.58 (H, *d*, *J* = 16 Hz), 7.68 (H, *d*, *J* = 16 Hz); ^{13}C NMR ($CDCl_3$) δ17.4, 20.6 (Ac × 9), 35.4, 41.7, 56.0, 58.0, 61.1, 62.1, 62.3, 67.0, 68.2, 69.1, 70.3, 70.6, 71.3, 72.2, 72.6, 83.6, 94.3, 96.5 (× 2), 102.4, 111.3, 117.2, 118.2, 121.5, 122.9, 123.2, 124.0, 126.7, 132.9, 133.0, 141.1, 141.2, 141.7, 142.5, 143.9, 144.3, 145.2, 151.4, 165.4 (× 2), 167.9 (Ac), 168.6 (Ac), 169.0 (Ac), 169.2 (Ac), 170.0 (Ac), 170.2 (Ac × 2), 170.5 (Ac × 2). (Found: C, 56.3; H, 5.19. $C_{58}H_{64}O_{29}\cdot H_2O$ requires: C, 56.04; H, 5.35%).

Premnoside D(**4**). Slight yellow amorphous powder, $[\alpha]_D$ +14.0° (MeOH; *c* 0.30); IR ν_{max}^{KBr} cm^{-1}: 3325, 1693, 1625, 1601, 1510, 1433, 1260, 1155, 1120, 1065, 830; UV λ_{max}^{MeOH} nm (log ε): 220 (4.24), 235 (4.20)sh, 310 (4.44) inf, 336 (4.55); FABMS *m/z*: 853 $[M+Na]^+$ (+NaI), 869 $[M+K]^+$(+KI); ^1H NMR (MeOH-d_4): δ1.38 (H, *d*, *J* = 7 Hz), ~2.6 (2H), 3.76 (3H, *s*), 6.31 (H, *d*, *J* = 16 Hz), 6.40 (H, *d*, *J* = 16 Hz), 6.40 (H, *d*, *J* = 5 Hz), ~6.8 (3H, aromatic protons), 7.00 (2H, *d*, *J* = 8 Hz), 7.46 (2H, *d*, *J* = 8 Hz), 7.58 (H, *d*, *J* = 16 Hz), 7.64 (H, *d*, *J* = 16 Hz); ^{13}C NMR: see Table 1. (Found: C, 55.3; H, 5.65. $C_{40}H_{46}O_{19}\cdot 2H_2O$ requires: C, 55.42; H, 5.81%).

Premnoside D octaacetate. Premnoside D (*ca* 5 mg) was treated with a mixture of a few drops of Ac_2O and pyridine at 25° overnight. The reaction mixture was evapd to dryness. EIMS *m/z*: 331, 169, 43; FABMS *m/z*: 1189 $[M+Na]^+$(+NaI), 1205 $[M+K]^+$(+KI); ^1H NMR ($CDCl_3$): δ1.28 (3H, *d*, *J* = 6 Hz), 2.02 (Ac), 2.34 (Ac), 2.05 (Ac × 2), 2.11 (Ac), 2.13 (Ac), 2.30 (Ac), 2.32 (Ac), ~2.6 (2H, *m*), 3.78 (3H, *s*), 6.29 (H, *d*, *J* = 16 Hz), 6.33 (H, *d*, *J* = 6 Hz), 6.53 (H, *d*, *J* = 16 Hz), ~7.0 (3H, aromatic protons), 7.14 (2H, *d*, *J* = 9 Hz), 7.57 (H, *d*, *J* = 16 Hz), 7.58 (H, *d*, *J* = 9 Hz), 7.71 (H, *d*, *J* = 16 Hz).

GC analysis of sugar portion. Ca 2 mg of each sample was treated with 2 ml of 5% HCl in dry MeOH in a sealed tube at 95° for 3 hr. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evapd to dryness and several drops of TMS-imidazole added. After 15 min at 60°, 1 ml of H_2O was added and the mixture partitioned with 2 ml of *n*-hexane. After concn, the hexane layer was subjected to GC analysis. GC: column 1.5% OV-1 (3 mm × 2 m), N_2 40 ml/min, 180° (isothermal)., rham, 2.80 min; glc, 9.07 and 9.97 min. Premnoside A: rham, 2.80 min; glc 9.96 and 10.00 min. Premnoside B: rham, 2.79 min; glc, 9.09 and 9.99 min. Premnoside C: rham, 2.79 min; glc, 9.18 (overlapped by methyl ferulate-4-*O*-TMS) and 9.98 min. Premnoside D: rham, 2.81 min; glc, 9.16 (overlapped by methyl ferulate-4-*O*-TMS) and 9.96 min.

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