Five New Ocotillone-Type Saponins from *Gynostemma pentaphyllum*

Xin Liu,[†,‡] Wencai Ye,[†] Ziyao Mo,[§] Biao Yu,[‡] Shouxun Zhao,[†] Houming Wu,[‡] Chuntao Che,[∥] Renwang Jiang,[#] Thomas C. W. Mak,[#] and W. L. Wendy Hsiao*[,⊥]

Department of Phytochemistry, China Pharmaceutical University, Nanjing 210009, People's Republic of China, State Key Laboratory of Bio-organic Chemistry, Shanghai Institute of Organic Chemistry, Shanghai 200032, People's Republic of China, Department of Biology, Hong Kong University of Science and Technology, Hong Kong, People's Republic of China, School of Chinese Medicine and Institute of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong, People's Republic of China, and Biomedical Science, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, People's Republic of China

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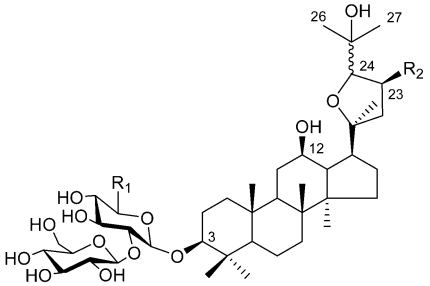
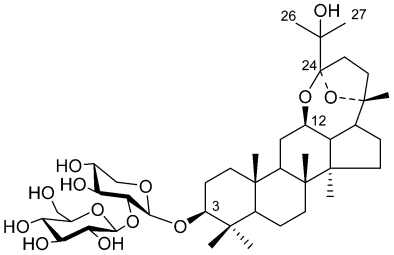
Five new ocotillone-type saponins, gynosides A−E (**1**−**5**), along with 10 known dammarane-type saponins, were isolated from the aerial parts of *Gynostemma pentaphyllum*. The structures of these new compounds were determined by NMR analysis and acid hydrolysis. The structure and stereochemistry of gynoside A (**1**) were confirmed by X-ray crystallography.

Gynostemma pentaphyllum (Thunb.) Makino, a perennial creeping herb belonging to the Cucurbitaceae, is distributed widely in mainland China, particularly south of the Qinling Mountains and the Yangtze River.[1] It is used as a folk medicine for lowering cholesterol levels, regulating blood pressure, strengthening the immune system, treating chronic bronchitis and gastritis, and reducing inflammation. The main constituents of the aerial parts of this plant are a series of dammarane-type saponins. They include seven glycosides, gypenosides III, IV, VIII, and XII, 6′-malonylginsenosides III and VIII, and 6′-malonylginsenoside V, which are structurally identical to the known ginsenosides Rb_1, Rb_3, Rd, and F_2 and malonylginsenosides Rb1 and Rd, respectively.[2,3] This is the first example of ginseng saponins ever found from a plant not belonging to the Araliaceae. These findings have earned the herb the name "Southern Ginseng" and have stimulated keen interest in the biochemical investigation of the pharmacologically active components of *G. pentaphyllum*.

In a search for additional constituents of *G. pentaphyllum*, we have isolated 15 dammarane-type saponins. Ten of them, gynosaponin TN-1,[4] ginsenosides Rb_3 and Rd,[3] and gypenosides XLII,[5] XLV 4-Ed,[6] XLVI,[6] LVI,[5] LVII,[5] LX,[5] and LXXVII,[7] were previously isolated from the same species, while the other five compounds, gynosides A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), are new ocotillone-type saponins, which are a kind of dammarane-type triterpenoids bearing a side chain at C-17 with an epoxy ring. At present, there are few X-ray crystallographic data on saponins due to the difficulty in obtaining suitable single crystals for X-ray diffraction analysis.[8] We successfully obtained single crystals of **1**, and the structure was confirmed by X-ray crystallography.



1	R_1 = H	R_2 = H	24 *S*
2	R_1 = CH_2OH	R_2 = H	24 *S*
3	R_1 = H	R_2 = H	24 *R*
4	R_1 = H	R_2 = OH	24 *S*



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Results and Discussion

Gynoside A (**1**) was obtained as colorless crystals. Its molecular formula was determined as $C_{41}H_{70}O_{13}$ by ^{13}C NMR and FABMS (*m*/*z* 771 $[M + H]^+$ and 793 $[M + Na]^+$) data analysis, which was confirmed by HRESIMS. Positive results from both Liebermann−Burchard and Molish reactions indicated that **1** is a saponin. The EIMS displayed a base peak at *m*/*z* 143 ($C_{18}H_{15}O_2$) assignable to a hydroxyisopropyl-tetrahydrofuran ion. Its ^1H NMR spectrum showed signals for eight tertiary methyl groups, an axial oxymethine (H-3) proton at δ 3.21 (dd, J = 4.2, 12.0 Hz), a hydroxyl proton (OH-12) at δ 6.20 (s) undergoing hydrogen bonding to the oxygen of the tetrahydrofuran ring, and two anomeric protons at δ 4.77 (d, J = 6.6 Hz) and 5.30 (d, J = 7.2 Hz). These results suggested **1** to be an ocotillone-type of triterpenoid saponin.[9] Acid hydrolysis of **1** with 1 M HCl gave an aglycon identical to (20*S*,24*S*)-20,24-epoxydammarane-3β,12β,25-triol (**1a**),[9] as well as D-glucose and D-xylose. The coupling constants of the anomeric protons of these sugar moieties indicated a β-configuration for the sugar units. In determining the interglycosidic linkage, the HMBC correlations were observed between H-1 of the Xyl

* To whom correspondence should be addressed. Tel: 852-3411-2959. Fax: 852-3411-2461. E-mail: bowhsiao@hkbu.edu.hk.
[†] China Pharmaceutical University.
[‡] Shanghai Institute of Organic Chemistry.
[§] Hong Kong University of Science and Technology.
[∥] The Chinese University of Hong Kong.
[#] Institute of Chinese Medicine, The Chinese University of Hong Kong.
[⊥] School of Chinese Medicine, Hong Kong Baptist University.

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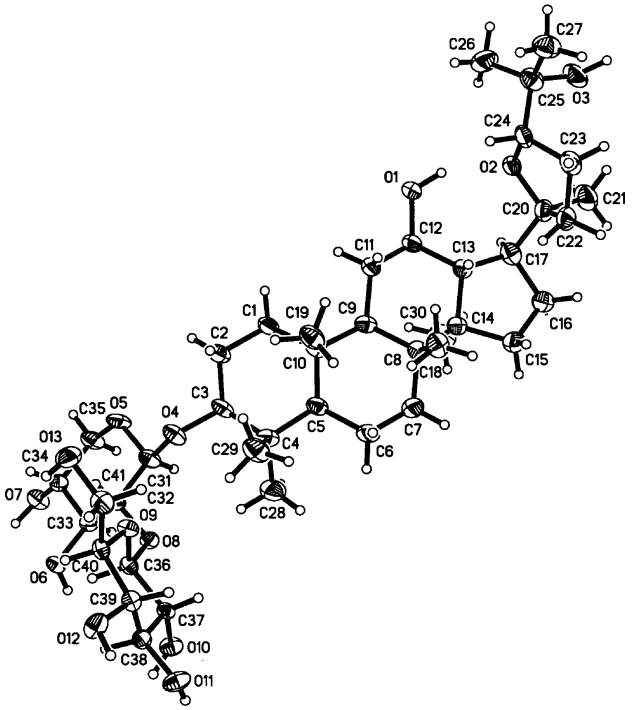

Figure 1. ORTEP drawing of gynoside A (**1**).

and C-3 of the aglycon, as well as between H-1 of the Glc and C-2 of the Xyl. Furthermore, NOESY correlations were observed between H-1 of Xyl and H-3 of the aglycon, and H-1 of the Glc and H-2 of the Xyl. The above analysis led to the conclusion that **1** has the structure (20*S*,24*S*)-20,-24-epoxy-12,25-dihydroxydammaran-3-yl *O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-xylopyranoside, which was subsequently confirmed by an X-ray crystallographic experiment (Figure 1).[10]

Gynoside B (**2**) was obtained as an amorphous powder. Its molecular formula was determined as $C_{42}H_{72}O_{14}$ from the ^{13}C NMR spectrum and FABMS (*m*/*z* 801 $[M + H]^+$ and 783 $[801 - H_2O]^+$) and was confirmed by HRESIMS. The EIMS gave a base peak at *m*/*z* 143 ($C_{18}H_{15}O_2$), assignable to a hydroxyisopropyltetrahydrofuran ion. The ^1H and ^{13}C NMR spectra of the aglycon part were similar to those of **1**. In the ^1H NMR spectrum, two anomeric proton signals appeared at *δ* 4.86 (d, *J* = 7.2 Hz) and 5.32 (d, *J* = 7.8 Hz), corresponding to the anomeric carbons appearing at *δ* 105.3 and 106.0. Upon acid hydrolysis, compound **1a** was obtained as the aglycon. The saccharide unit of **2** was identified as D-glucose, and the coupling constant of the anomeric protons suggested a *β*-configuration. In determining the interglycosidic linkage, HMBC correlations were observed as follows: H-1 of inner Glc and C-3 of the aglycon, and H-1 of the terminal Glc and C-2 of the inner Glc. The NOESY spectrum displayed correlations between H-1 of the inner Glc and H-3 of the aglycon, as well as between H-1 of the terminal Glc and H-2 of the inner Glc. On the basis of the above data, the structure of **2** was determined as (20*S*,24*S*)-20,24-epoxy-12,25-dihydroxydammaran-3-yl *O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside.

Gynoside C (**3**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{41}H_{70}O_{13}$ from the ^{13}C NMR spectrum and FABMS (*m*/*z* 771 $[M + H]^+$ and 793 $[M + Na]^+$) data and was confirmed by HRESIMS. Although the ion peak at *m*/*z* 143 ($C_{18}H_{15}O_2$) was absent in EIMS, its ^1H NMR spectrum still displayed a hydroxyl proton at *δ* 5.75 (s) undergoing hydrogen bonding to the tetrahydrofuran ring oxygen. Also observed were an axial oxymethine (H-3) proton at *δ* 3.25 (dd, *J* = 4.4, 11.8 Hz), and two anomeric protons at *δ* 4.84 (1H, d, *J* = 6.7 Hz) and 5.36 (1H, d, *J* = 7.8 Hz), as well as signals for eight tertiary methyl groups. The ^{13}C NMR and DEPT spectra revealed the same number of methyl, methylene, and methine groups and tertiary carbons as in **1**. All of the evidence indicated that **3** is an ocotillone-type saponin. As in the case of **1**, the following NOESY correlations supported *β*-configurations of OH-3 and OH-12 and H-21/H-17, as well as an *S* configuration of C-20. Due to the change in the C-24 configuration when compared to **1**, no NOE effect could be seen between H-13 and H-24. The shift in the C-24 signal observed between **1** and **3** (*δ* 88.8 in **1** and *δ* 85.8 in **3**) may be due to the greater steric hindrance between OH-12 and the hydroxyisopropyl group. By comparing with a reference compound, the aglycon was proved to be (20*S*,24*R*)-20,24-epoxydammaran-3*β*,12*β*,25-triol.[9] The ^{13}C NMR pattern of the sugar chain was similar to that of **1**. Thus, HMBC correlations were observed between the H-1 of Xyl and C-3 of the aglycon, and the H-1 of Glc and C-2 of the Xyl. Similarly, NOESY correlations were observed between the H-1 of Xyl and H-3 of the aglycon, and the H-1 of Glc and H-2 of the Xyl. Consequently, **3** was elucidated as (20*S*,24*R*)-20,24-epoxy-12,25-dihydroxydammaran-3-yl *O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-xylopyranoside.

Gynoside D (**4**) was obtained as an amorphous powder. Its molecular formula was determined to be $C_{41}H_{70}O_{14}$ from the ^{13}C NMR spectrum and FABMS (*m*/*z* 787 $[M + H]^+$) and was confirmed by HRESIMS. The EIMS displayed a base peak at *m*/*z* 159 ($C_{18}H_{15}O_3$) assignable to a 1-hydroxyisopropyl-2-hydroxyltetrahydrofuran ion. The ^1H NMR spectrum showed the presence of eight tertiary methyl groups, an axial oxymethine proton (H-3) at *δ* 3.24 (dd, *J* = 4.2, 11.4 Hz), a hydroxyl proton (OH-12) at *δ* 5.75 (s) undergoing hydrogen bonding to the oxygen of the tetrahydrofuran ring, and an oxymethine proton (H-23) at *δ* 5.03, which coupled with H-24 and two anomeric protons at *δ* 4.81 (d, *J* = 7.2 Hz) and 5.33 (d, *J* = 7.2 Hz). These results suggested **4** to be an ocotilone-type saponin. NOESY correlations supported a *β*-configuration of OH-3 and OH-12 and an *S* configuration of C-20. When the ^1H NMR spectrum of **4** was compared with that of **1**, the H-13 signal of **4** showed a shift from *δ* 1.80 to 1.97. A strong NOE effect between H-23α and Me-21 suggested that OH-23 is in *β* position and that the steric repulsion of OH-23*β* caused the downfield shift of H-13. Acid hydrolysis of **4** yielded an aglycon identified as (20*S*,24*S*)-epoxydammaran-3*β*,12*β*,-23,24-tetrol (**4a**), together with D-glucose and D-xylose.[9] The HMBC correlations observed between the H-1 of Xyl and C-3 of the aglycon, and the H-1 of Glc and the C-2 of Xyl, together with NOESY correlations between H-1 of Xyl and H-3 of aglycon, and H-1 of Glc and H-2 of Xyl, led to the elucidation of **4** as (20*S*,24*S*)-20,24-epoxy-12,23*β*,25-trihydroxydammaran-3*β*-yl *O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-xylopyranoside.

Gynoside E (**5**) was obtained as an amorphous powder. The molecular formula was determined as $C_{41}H_{70}O_{13}$ from the ^{13}C NMR, FABMS (*m*/*z* 769 $[M + H]^+$), and HRESIMS data. The EIMS gave a base peak at *m*/*z* 143 ($C_{18}H_{15}O_2$) assignable to a hydroxyisopropyltetrahydrofuran ion. Its ^1H NMR spectrum indicated the presence of eight tertiary methyl groups, an axial oxymethine proton (H-3) at *δ* 3.26 (dd, *J* = 4.3, 11.7 Hz), and two anomeric protons at *δ* 4.75 (d, *J* = 6.9 Hz) and 5.38 (d, *J* = 7.6 Hz). However, a hydrogen-bonded OH group was absent. An acetal signal

was observed at δ 113.2. Given the eight degrees of unsaturation of **5**, it suggests that a ring was formed between C-12 and -24. Such an assumption was supported by the observation of downfield shifts of C-12 and C-24 (δ 71.2 to 73.9 for C-12, and δ 88.8 to 113.2 for C-24). The NOESY correlation between H-12 (δ 4.47) and Me-26 (δ 1.57) in **5** was stronger than that observed in compounds **1**, **2**, and **4**. Other NOESY correlations supported a β-configuration of OH-3 and OH-12 and an *S* configurations of C-20. A comparison with reference data led to the identification of the aglycon of **5** as (12*R*,20*S*,24S)-20,24;20,12-diepoxydammarane-3β,25-diol.[10] The ^{13}C NMR pattern of the sugar chain of **5** was similar to that of **1**. HMBC correlations were observed between H-1 of Xyl and C-3 of the aglycon, and H-1 of Glc and C-2 of the Xyl. NOESY correlations were observed between H-1 of Xyl and H-3 of the aglycon, and H-1 of Glc and H-2 of Xyl. On the basis of the above data, **5** was determined to be (12*R*,20*S*,24*S*)-20,-24;20,12-diepoxy-25-hydroxydammaran-3β-yl *O*-β-D-glucopyranosyl-(1→2)-β-D-xylopyranoside.

Experimental Section

General Experimental Procedures. Melting points were determined with a X-4 melting point apparatus (Taike Instruments Co.) and are uncorrected. Optical rotations were taken with a Perkin-Elmer 241 polarimeter (Perkin-Elmer). IR data were obtained using a Perkin-Elmer 16 PC FT-IR spectrometer (Perkin-Elmer). ^{1}H, ^{13}C, and 2D NMR spectra were recorded in pyridine-d_5 on an Avance-500 (Bruker) or an INOVA-600 (Varian) NMR spectrometer. Chemical shifts are expressed in δ (ppm) with reference to pyridine-d_5. EIMS were determined with an HP 5989A instrument (Hewlett-Packard), and HRESIMS on a FTMS-7 instrument (Bruker Daltonics). GC experiments were carried out on a HP-1 TCD instrument (Hewlett-Packard), using an HP-Chiral column (30 × 0.25 × 1.0, 20% permethylated β-cyclodextrin). The conditions selected for analysis were as follows: front inlet 250 °C, column 80 °C → 230 °C, 5 °C/min. Open column chromatography was carried out using silica gel (200−300 mesh, Qingdao Marine Chemical Co., Qingdao, People's Republic of China), octadecyl silica gel (ODS, 40−60 μm, Merck), or Sephadex LH-20 (Pharmacia) as stationary phase. TLC was conducted on Si gel 60 F_{254} S plates (Merck). All chemical reagents (AR grade) were purchased from Shanghai Reagent Co. Ltd.

Plant Material. The leaves of *G. pentaphyllum* were collected in November 1998 from Huadu (a suburb of Guangzhou), Guangdong Province, People's Republic of China, and authenticated by Dr. Minjian Qin. A voucher specimen (No. SIOC-Bio-20020829) has been deposited in the State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, People's Republic of China.

Extraction and Isolation. Dried leaves of *G. pentaphyllum* (10 kg) were extracted with EtOH (95%). The extract was concentrated, defatted with cyclohexane, and partitioned sequentially with $CHCl_3$ and *n*-BuOH. The *n*-BuOH layer was dried in vacuo to yield 81 g of crude total saponins. It was then repeatedly separated by silica gel column chromatography using $CHCl_3$−CH_3OH as solvent to yield a series of fractions. Each of the fractions was then subjected to ODS silica gel column chromatography with MeOH−H_2O (30:70−70:30) to afford **1** (0.2 g), **2** (25 mg), **3** (12 mg), **4** (77 mg), **5** (10 mg), gynosaponin TN-1 (3.6 g), ginsenoside Rb_3 (0.7 g), ginsenoside Rd (0.9 g), gypenoside XLII (21 mg), gypenoside XLII (86 mg), gypenoside XLVI (5.1 g), gypenoside LVI (63 mg), gypenoside LVII (5.1 g), gypenoside LX (23 mg), and gypenoside LXXVII (6.1 g).

Gynoside A (1): colorless plates, mp 203−205 °C, $[\alpha]^{20}_D$ −0.47° (*c* 0.9, MeOH); IR (KBr) ν_{max} 3420, 2969, 2943, 1639, 1456, 1381, 1164, 1080, 1042, 880, 645 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 771 $[M + H]^+$ and 793 $[M + Na]^+$; HRESIMS *m*/*z* found 771.4889 (calcd for $C_{41}H_{71}O_{13}$, 770.4895).

Table 1. ^{13}C NMR Spectral Data for Compounds **1**−**5**[a]

carbon	1	2	3	4	5
1	39.8	39.6	39.4	39.6	39.4
2	27.2	27.1	26.9	27.2	27.1
3	89.4	89.4	88.8	89.0	88.7
4	40.2	40.0	39.9	40.1	40.0
5	57.0	56.8	56.6	56.8	56.5
6	19.0	18.8	18.6	18.8	18.7
7	35.6	35.5	35.3	35.5	35.2
8	40.5	40.3	40.1	40.2	40.1
9	51.1	50.9	50.9	50.9	50.2
10	37.5	37.3	37.1	37.3	37.3
11	32.8	32.8	32.6	32.8	30.3
12	71.2	71.2	71.2	71.1	73.9
13	49.7	49.7	49.9	49.8	49.0
14	52.7	52.5	52.3	52.5	49.5
15	33.0	32.9	32.9	32.8	32.2
16	29.1	28.9	28.9	28.9	23.7
17	49.9	49.8	48.5	50.2	53.4
18	16.1	15.9	15.7	15.9	16.4
19	17.1	16.9	16.7	16.9	16.3
20	87.6	87.4	86.8	85.4	88.2
21	29.4	29.3	27.3	30.0	27.8
22	32.6	32.5	31.8	42.4	29.9
23	26.3	26.1	25.6	70.7	36.4
24	88.8	88.6	85.8	91.6	113.2
25	70.8	70.5	70.4	70.5	74.1
26	27.2	27.2	27.0	28.0	25.9
27	26.9	26.8	27.8	26.8	25.4
28	28.5	28.4	28.2	28.3	28.4
29	16.9	16.9	16.6	17.0	16.9
30	18.5	18.4	18.4	18.4	17.8
Xyl or Glc					
1	105.9	105.3	105.8	105.8	105.8
2	82.9	83.3	83.4	83.2	83.5
3	78.3	78.5	78.1	78.1	78.1
4	71.3	71.8	71.1	71.1	71.1
5	67.0	78.1	66.8	66.9	66.8
6		63.1			
Glc					
1	106.0	106.0	106.2	106.1	106.2
2	77.1	77.3	77.2	77.2	77.2
3	78.3	78.3	78.1	78.2	78.1
4	72.0	71.8	71.8	71.8	71.8
5	78.6	78.5	78.4	78.5	78.4
6	63.0	63.1	62.8	62.9	62.9

[a] Measured in pyridine-d_5 at 500 MHz.

Gynoside B (2): amorphous powder, mp 194−196 °C, $[\alpha]^{20}_D$ +0.10° (*c* 0.05, MeOH); IR (KBr) ν_{max} 3413, 2968, 2944, 1642, 1455, 1380, 1163, 1078, 1043, 880, 646 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 801 $[M + H]^+$; HRESIMS *m*/*z* found 823.4814 (calcd for $C_{42}H_{72}NaO_{14}$, 823.4820).

Gynoside C (3): amorphous powder, mp 194−196 °C, $[\alpha]^{20}_D$ −8.0° (*c* 0.1,MeOH); IR (KRr) ν_{max} 3354, 2967, 2933, 2877, 1665, 1601, 1467, 1388, 1313, 1233, 1165, 1080, 1042, 903; ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 770 $[M]^+$; HRESIMS *m*/*z* found 793.4709 (calcd for $C_{41}H_{70}NaO_{13}$, 793.4714).

Gynoside D (4): amorphous powder, mp 195−197 °C, $[\alpha]^{20}_D$ +0.61° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3401, 2969, 2944, 1643, 1455, 1382, 1164, 1081, 1039, 923, 898, 646 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 787 $[M + H]^+$; HRESIMS *m*/*z* 809.4658 (calcd for $C_{41}H_{70}NaO_{14}$, 809.4663).

Gynoside E (5): amorphous powder, mp 207−209 °C, $[\alpha]^{20}_D$ +2.0° (*c* 0.15, MeOH); IR (KBr) ν_{max} 3429, 2949, 2876, 1639, 1467, 1385, 1161, 1081, 1038, 899, 646, 596 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 787 $[M + H]^+$; HRESIMS *m*/*z* 791.4552 $[M + Na]^+$ (calcd for $C_{41}H_{68}NaO_{13}$, 791.4558).

Compound 1a: amorphous powder, mp 135−137 °C, $[\alpha]^{20}_D$ +2.9° (*c* 2.7, MeOH); IR (KBr) ν_{max} 3395, 2970, 2876, 1466, 1380, 1314, 1163, 1125, 1080, 1044, 1017, 879 cm^{-1}; ^{1}H NMR

Table 2. ^{1}H NMR Spectral Data for Compounds **1**−**5**[a]

position	1	2	3	4	5
1	0.80	0.72	0.86	0.86	0.83,
	1.58 (br d, *J* = 12.6 Hz)	1.45	1.59	1.64 (m)	1.56
2	1.82	1.77	1.88	1.87	1.88,
	2.02	2.13	2.11	2.09	2.11 (m)
3	3.21 (dd, *J* = 4.2, 12.0 Hz)	3.24 (dd, *J* = 4.2, 11.6 Hz)	3.25 (dd, *J* = 11.8, 4.4 Hz)	3.24 (dd, *J* = 4.2, 11.4 Hz)	3.26 (dd, *J* = 4.3, 11.7 Hz)
5	0.70 (br d, *J* = 10.8 Hz)	0.65 (br d, *J* = 11.2 Hz)	0.73 (d, *J* = 10.4 Hz)	0.72 (br d, *J* = 12.0 Hz)	0.72 (br d, *J* = 11.6 Hz)
6	1.34	1.33	1.38 (m)	1.32	1.37
	1.45	1.47	1.49	1.46	1.49
7	1.19	1.19	1.24	1.17 (m)	1.21 (br d, *J* = 12.3 Hz),
	1.36	1.35	1.38	1.37	1.51
9	1.45	1.38	1.45	1.47	1.43
11	1.33	1.31	1.29	1.33	1.42
	2.04	1.99	2.02	2.07	1.93
12	3.71 (dt, *J* = 4.8, 10.2 Hz)	3.71 (dt, *J* = 4.2, 10.2 Hz)	3.70	3.76 (dt, *J* = 4.8, 10.8 Hz)	4.47
OH-12	6.20 (s)	6.20 (s)	5.75 (s)	6.05 (s)	
13	1.80	1.81	1.82 (m)	1.97	1.72
15	0.98	0.99	1.04	0.99 (m)	1.09
	1.47	1.47	1.53	1.49	1.66
16	1.23	1.23	1.28	1.28	1.09
	1.81	1.83	1.90	1.85	1.65
17	2.23 (m)	2.24 (m)	2.22 (m)	2.27 (dt, *J* = 4.2, 10.8 Hz)	2.21 (m)
18	0.99 (3H, s)	0.98 (3H, s)	0.98 (3H, s)	0.86 (3H, s)	0.99 (3H, s)
19	0.83 (3H, s)	0.79 (3H, s)	0.81 (3H, s)	0.83 (3H, s)	0.82 (3H, s)
21	1.26 (3H, s)	1.29 (3H, s)	1.27 (3H, s)	1.41 (3H, s)	1.30 (3H, s)
22	1.68 (dd, *J* = 7.8, 11.4 Hz)	1.68 (dd, *J* = 7.2, 12.0 Hz)	1.59	2.33 (dd, *J* = 6.6, 12.0 Hz)	1.44
	1.93	1.94	1.86	2.41	1.48
23	1.89	1.89	1.86	5.03	2.02
	2.13 (m)	2.13	2.15		2.84
24	4.12	4.12 (dd, *J* = 10.8, 4.8 Hz)	3.95 (*m*)	4.22	
26	1.42 (3H, s)	1.43 (3H, s)	1.47 (3H, s)	1.61 (3H, s)	1.57 (3H, s)
27	1.29 (3H, s)	1.29 (3H, s)	1.26 (3H, s)	1.58 (3H, s)	1.54 (3H, s)
28	1.21 (3H, s)	1.23 (3H, s)	1.27 (3H, s)	1.26 (3H, s)	1.29 (3H, s)
29	1.05 (3H, s)	1.06 (3H, s)	1.08 (3H, s)	1.09 (3H, s)	1.12 (3H, s)
30	0.88 (3H, s)	0.88 (3H, s)	0.90 (3H, s)	0.91 (3H, s)	0.92 (3H, s)
1′	4.77 (d, *J* = 6.6 Hz)	4.86 (d, *J* = 7.2 Hz)	4.84 (1H, d, *J* = 6.7 Hz)	4.81 (d, *J* = 7.2 Hz)	4.75 (d, *J* = 6.9 Hz)
2′	4.15	4.19	4.21	4.19	4.23
3′	4.17	4.26	4.22	4.21	4.24
4′	4.10	4.07	4.16	4.14	4.18
5′	3.87 (t, *J* = 10.9 Hz)	3.88	3.67	3.68 (dd, *J* = 11.4, 10.2 Hz)	4.31 (dd, *J* = 11.6, 5.3 Hz)
	4.27 (dd, *J* = 11.4, 4.8 Hz)		4.31	4.29	3.69 (t, *J* = 10.6 Hz)
6′		4.51 (br d, *J* = 10.8 Hz)			
		4.30 (dd, *J* = 12.0, 6.0 Hz)			
1″	5.30 (d, *J* = 7.2 Hz)	5.32 (d, *J* = 7.8 Hz)	5.36 (1H, d, *J* = 7.8 Hz)	5.33 (d, *J* = 7.2 Hz)	5.38 (d, *J* = 7.6 Hz)
2″	4.04	4.06	4.12	4.09	4.13
3″	4.19	4.18	4.25	4.22	4.27
4″	4.22	4.22	4.35	4.31	4.37 (t, *J* = 9.2 Hz)
5″	3.87	3.86	3.94	3.91 (dt, *J* = 9.6, 3.6 Hz)	3.95 (dd, *J* = 9.5, 3.8 Hz)
6″	4.37 (dd, *J* = 11.4, 3.6 Hz)	4.38 (dd, *J* = 11.4, 3.6 Hz)	4.48 (2H, br s)	4.43 (dd, *J* = 11.4, 3.6 Hz)	4.49 (br s, 2H)
	4.44 (br d, *J* = 10.2 Hz)	4.45 (br d, *J* = 10.8 Hz)		4.48 (dd, *J* = 11.4, 2.4 Hz)	

[a] These assignments were based largely on DQF-COSY, HMQC, and HMBC experiments. Signals overlapping are not labeled.

(pyridine-d_5, 500 MHz) δ 6.06 (1H, s, OH-12), 4.18 (1H, dd, *J* = 5.4, 10.9 Hz, H-24), 3.77 (1H, dt, *J* = 4.6, 10.1 Hz, H-12), 3.45 (1H, dd, *J* = 4.7, 10.5 Hz, H-3), 2.31 (1H, m, H-17), 2.18 (1H, H-23a), 2.15 (1H, H-11a), 2.01 (1H, H-22a), 1.96 (1H, H-23b), 1.89 (2H, H-13, -16a), 1.85 (2H, H_2-2), 1.75 (1H, H-22b), 1.72 (1H, H-1a), 1.62 (1H, H-6a), 1.56 (1H, H-9), 1.55 (1H, H-15a), 1.51 (1H, H-6b), 1.50 (1H, H-7a), 1.46, 1.25, 1.08, 1.06, 0.95, 0.94 (each 3H, s, H_3-26, -28, -18, -29, -19, -30), 1.43 (1H, H-11b), 1.33 (6H, s, H_3-21, -27), 1.31 (1H, H-7b), 1.30 (1H, H-16b), 1.06 (1H, H-15b), 0.99 (1H, H-1b), 0.86 (1H, br d, *J* = 8.8 Hz, H-5); ^{13}C NMR (pyridine-d_5, 125 MHz) δ 88.6 (CH, C-24), 87.2 (C, C-20), 78.2 (CH, C-3), 71.0 (CH, C-12), 70.2 (C, C-25), 56.7 (CH, C-5), 52.5 (C, C-14), 50.9 (CH, C-9), 49.7 (CH, C-13), 49.7 (CH,C-17), 40.2 (C, C-8), 39.7 (C, C-4), 39.7 (CH_2, C-1), 37.6 (C, C-10), 35.4 (CH_2, C-7), 32.9 (CH_2, C-11), 32.8 (CH_2, C-15), 32.4 (CH_2, C-22), 29.2 (CH_3, C-21), 28.8 (CH_2, C-16), 28.8 (CH_3, C-28), 28.4 (CH_2, C-2), 27.1 (CH_3, C-26), 26.7 (CH_3, C-27), 26.0 (CH_2, C-23), 19.0 (CH_2, C-6), 18.3 (CH_3, C-30), 16.8 (CH_3, C-19), 16.4 (CH_3, C-29), 15.9 (CH_3, C-18); ESIMS *m*/*z* 499 $[M + Na]^+$, 477 $[M + H]^+$.

Compound 4a: amorphous powder, mp 154−156 °C, $[\alpha]^{20}_D$ +36.9° (*c* 0.75, MeOH); IR (KBr) ν_{max} 3401, 2970, 2877, 1466, 1381, 1277, 1167, 1126, 1082, 1033, 923, 896 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 6.06 (1H, s, OH-12), 5.06 (1H, m, H-23), 4.25 (1H, d, *J* = 7.96 Hz, H-24), 3.81 (1H, dt, *J* = 4.5, 10.1 Hz, H-12), 3.44 (1H, m, H-3), 2.45 (1H, m, H-22a), 2.37 (1H, m, H-22b), 2.31 (1H, m, H-17), 2.14 (1H, H-11a), 2.03 (1H, t, *J* = 9.9 Hz, H-13), 1.89 (1H, H-16a), 1.85 (2H, H_2-2), 1.71 (1H, br d, *J* = 2.9 Hz, H-1a), 1.63, 1.61, 1.43, 1.23, 1.05, 0.90 (each 3H, s, H_3-27, -26, -21, -28, -29, -19), 1.58 (1H, H-6a), 1.55 (1H, H-9), 1.53 (1H, H-15a), 1.47 (1H, H-6b), 1.46 (1H, H-7a), 1.39 (1H, m, H-11b), 1.32 (1H, H-16b), 1.25 (1H, H-7b), 1.02 (1H, H-15b), 0.97 (1H, H-1b), 0.92 (6H, s, H_3-18, 30), 0.84 (1H, br d, *J* = 8.8 Hz, H-5); ^{13}C NMR (pyridine-d_5, 125 MHz) δ 91.7 (CH, C-24), 85.3 (C, C-20), 78.2 (CH, C-3), 71.0 (CH, C-12), 70.7 (CH, C-23), 70.3 (C, C-25), 56.6 (CH, C-5), 52.4 (C, C-14), 50.8 (CH, C-9), 50.2 (CH, C-17), 49.9 (CH, C-13), 42.3 (CH_2, C-22), 40.2 (C, C-8), 39.7 (C, C-4), 39.7 (CH_2, C-1), 37.6 (C, C-10), 35.4 (CH_2, C-7), 32.8 (CH_2, C-11), 32.6 (CH_2, C-15), 29.9 (CH_3, C-21), 28.8 (CH_3, C-28), 28.7 (CH_2, C-2), 28.4 (CH_2, C-16), 27.7 (CH_3, C-26), 26.8 (CH_3, C-27), 18.9 (CH_2, C-6), 18.3 (CH_3, C-30), 16.8 (CH_3, C-19), 16.4 (CH_3, C-29), 15.7 (CH_3, C-18); ESIMS *m*/*z* 515 $[M + Na]^+$, 493 $[M + H]^+$.

Acid Hydrolysis of Compounds 1, 2, and 4. Gynoside A (**1**) (30 mg) was heated under reflux in 50 mL of 1 M HCl (MeOH−H_2O, 1:1) for 3 h. After removal of the solvent, the residue was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble portion was evaporated and subjected to ODS silica gel purification and eluted with 90% MeOH to yield 12 mg of protopanaxodiol oxide (**1a**). The aqueous layer was neutralized with Dowex (HCO_3^-), then filtered. The filtrate was evaporated down to 2 mL, then treated with $NaBH_4$ (40 mg) at room

temperature for 3 h. Excessive $NaBH_4$ was removed with 30% AcOH. After evaporation at 60 °C and washing with 0.1% hydrochloride acid (in MeOH) repeatedly until the BO_3^{3-} was removed, the reaction mixture was heated to dryness at 105 °C for 15 min, followed by the addition of pyridine (anhydrous, 0.5 mL) and Ac_2O (0.5 mL). The mixture was incubated in a water bath at 100 °C for 1 h and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated for GC analysis. Saccharides were identified as D-glucose and D-xylose (1:1). By the same method, we derived **1a** (5 mg) from **2** (12 mg) and derived **4a** (6 mg) from **4** (21 mg). The monosaccharides were identified as D-glucose for **2** and D-glucose and D-xylose (1:1) for **4**.

X-ray Structure Analysis of 1. Crystal data: $C_{41}H_{70}O_{13} \times CH_3OH \times 3H_2O$; $M_r = 871.09$, dimensions 0.45 × 0.38 × 0.16 mm, monoclinic, $P2_1$, $a = 11.6591(7)$ Å, $b = 8.0596(5)$ Å, $c = 23.5397(14)$ Å, $\alpha = 90°$, $\beta = 95.4900(10)°$, $\gamma = 90°$, $V = 2201.8(2)$ Å^3, $Z = 2$, $D_{calc} = 1.444$ g/cm^3, $F_{000} = 936.00$, μ(Mo Ka) = 0.099 mm^{-1}. Data collection was performed on a SMART CCD using graphite-monochromated radiation ($\lambda = 0.71073$ Å); 9503 unique reflections were collected to $\theta_{max} = 28.25°$, in which 3995 reflections were observed [$F^2 > 4\sigma(F^2)$]. The structure was solved by direct methods (SHELXTL version 5.1) and refined by full-matrix least-squares on F^2. In the structure refinements, non-hydrogen atoms were refined anisotropically. Hydrogen atoms bonded to carbons were placed on the geometrically ideal positions by the "ride on" method. Hydrogen atoms bound to oxygen were located by the difference Fourier method and were included in the calculation of structure factors with isotropic temperature factors. The final indices were $R = 0.0569$, $R_w = 0.1295$ and $S = 0.859$.

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References and Notes

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(10) Crystallographic data for compound **1** reported in this paper have been deposited with the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 200550. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

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