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Flavan-4-ol Glycosides from the Rhizomes of *Abacopteris penangiana*

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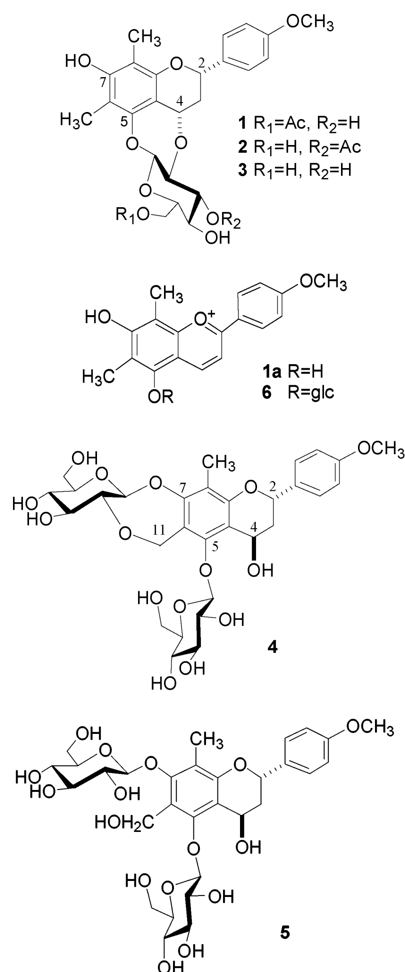
Four new flavan-4-ol glycosides, abacopterins A−D (**1**−**4**), were isolated from a methanol extract of the rhizomes of *Abacopteris penangiana*, together with two known compounds, triphyllin A (**5**) and 6,8-dimethyl-7-hydroxy-4′-methoxyanthocyanidin-5-*O*-*β*-D-glucopyranoside (**6**). Their structures were elucidated by extensive spectroscopic analysis and chemical methods. The cytotoxic activity of **1**−**5** against HepG2 human hepatoma cells was investigated.

The genus *Abacopteris* (Thelypteridaceae) comprises approximately 35 species that are found in tropical and subtropical zones.[1] *Abacopteris penangiana* (Hook.) Ching is a fern widely distributed in the south of mainland China. It has been used as a folk medicine to treat upper respiratory tract infections and dysentery.[2,3] Previous phytochemical investigations on thelypteridaceous ferns showed the presence of some unusual flavan-4-ol glycosides.[4−6] No chemical studies have been performed on *A. penangiana*. As a part of an investigation on *Abacopteris* species, we examined the rhizomes of *A. penangiana*. This study has resulted in four new flavan-4-ol glycosides (**1**−**4**), together with a known flavan-4-ol glycoside (**5**) and a known anthocyanidin glycoside (**6**). Herein, we report the isolation and structural elucidation of **1**−**4**, as well as the cytotoxic activity of **1**−**5** against HepG2 human hepatoma cells.

The air-dried rhizomes of *A. penangiana* (5.0 kg) were extracted with MeOH. The extract was concentrated under vacuum to leave a residue, which was suspended in H_2O and extracted with $CHCl_3$, EtOAc, and *n*-BuOH, sequentially. After being chromatographed on silica gel, Sephadex LH-20, and RP C_{18} columns, the EtOAc fraction afforded **1**−**3** and **6**, and the *n*-BuOH fraction afforded **4** and **5**. The structures of the new compounds were elucidated on the basis of spectroscopic data (^{1}H and ^{13}C NMR, HSQC, HMBC, ^{1}H−^{1}H COSY, and ROESY) interpretation and chemical evidence.

Compound **1** was obtained as white needles. Its molecular formula was assigned as $C_{26}H_{30}O_{10}$ on the basis of its ^{13}C DEPT and HREIMS (*m/z* 502.1854 [M]$^+$) data. The ^{1}H NMR spectrum of **1** (Table 1) showed signals characteristic for three methyls as singlets at δ 2.01, 2.05, and 2.14, a methoxy as a singlet at δ 3.80, an anomeric proton at δ 4.38 (d, J = 7.2 Hz), and four aromatic protons as two doublets at δ 6.96 (2H, d, J = 8.8 Hz) and 7.44 (2H, d, J = 8.8 Hz), suggesting a *para*-substituted phenyl group (Table 1). The ^{13}C NMR spectrum of **1** (Table 1) analyzed with the aid of the DEPT and HSQC spectra revealed there are one *para*-substituted and one fully substituted phenyl group and two aromatic methyl groups in the aglycon. A substructure, −CH−CH_2−CH−, was also confirmed in the aglycon through a ^{1}H−^{1}H COSY experiment, from the correlations of H-2 (δ 4.93) with H_2-3 (δ 2.11, 2.55), and H-4 (δ 5.00) with H_2-3 (δ 2.11, 2.55). Analysis of these structural characteristics indicated **1** is a flavan-4-ol-type glycoside. Alkaline hydrolysis of **1** with 1% KOH gave a deacetylated glycoside, **3**, which was identified by TLC analysis. On hydrolysis with acid, **1** provided a degraded aglycon, **1a**, which is



a known anthocyanidin,[5] and D-glucose. The β-configuration of the glycosidic bond was deduced from the ^{1}H and ^{13}C NMR data of the sugar moiety. The sequence of the sugar and binding sites to the aglycon were determined by 2D NMR experiments. As observed in the HMBC NMR spectrum (Figure 1), correlations of H-1″ of the sugar unit with C-5 (δ 151.9) and H-4 with C-2″ (δ 86.0) were used to establish that the sugar has two binding sites to the aglycon, with C-1″ attached to C-5 and C-2″ attached to C-4. Thus, C-5, C-10, C-4, C-1″, and C-2″ formed a heptacyclic ring, inclusive of two oxygen atoms. The acetyl group was attached to C-6″ of the sugar, and its binding site was also confirmed from the HMBC spectrum according to the correlations of the carbonyl carbon (δ 171.0) with H-6″ (δ 4.27, 4.40).

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Table 1. ^{13}C and ^{1}H NMR Data (δ values) of Compounds **1**−**3**[a]

	1		**2**		**3**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	77.0 d	4.93 brd (11.0)	77.0 d	4.93 brd (12.0)	76.0 d	4.92 brd (10.0)
3	38.2 t	2.55 dd (13.7, 7.8) 2.11 m	38.3 t	2.43 dd (13.5, 7.8) 2.03 m	37.3 t	2.47 dd (10.0, 8.0) 2.03 m
4	73.1 d	5.00 brd (7.8)	72.9 d	4.97 brd (7.8)	72.1 d	4.97 brd (8.0)
5	151.9 s		151.7 s		151.1 s	
6	110.3 s		110.4 s		110.0 s	
7	154.1 s		154.2 s		153.4 s	
8	108.5 s		108.6 s		107.9 s	
9	152.4 s		152.4 s		151.5 s	
10	113.0 s		112.8 s		112.4 s	
Me-6	9.0 q	2.01 s	9.0 q	2.01 s	9.5 q	1.94 s
Me-8	8.5 q	2.14 s	8.5 q	2.13 s	8.9 q	2.07 s
1′	133.6 s		133.5 s		132.7 s	
2′,6′	127.9 d	7.44 d (8.8)	127.9 d	7.42 d (8.8)	127.5 d	7.41 d (8.8)
3′,5′	114.2 d	6.96 d (8.8)	114.2 d	6.96 d (8.8)	113.8 d	6.97 d (8.8)
4′	159.9 s		160.0 s		159.0 s	
OMe	55.2 q	3.80 s	55.2 q	3.80 s	55.1 q	3.77 s
1″	102.2 d	4.38 d (7.2)	102.1 d	4.47 d (7.4)	101.7 d	4.30 d (6.6)
2″	86.0 d	3.43 m	83.9 d	3.49 m	85.6 d	3.29 m
3″	74.8 d	3.49 m	75.4 d	5.02 m	73.8 d	3.27 m
4″	70.3 d	3.49 m	68.4 d	3.68 m	69.6 d	2.36 m
5″	75.3 d	3.63 m	78.2 d	3.54 m	77.9 d	3.27 m
6″	63.8 t	4.27 dd (5.7, 11.9) 4.40 dd (2.2, 11.9)	61.5 t	3.77 dd (5.6, 11.9) 3.90 dd (2.2, 11.9)	60.6 t	3.54 dd (3.8, 11.5) 3.73 br d (11.5)
CO	171.0 s		170.3 s			
Me	20.4 q	2.05 s	20.6 q	2.05 s		

[a] Compounds **1** and **2** were measured in acetone-d_6 and compound **3** in DMSO-d_6. Coupling constants (*J* in Hz) are in parentheses. Multiplicities were determined by DEPT experiments.

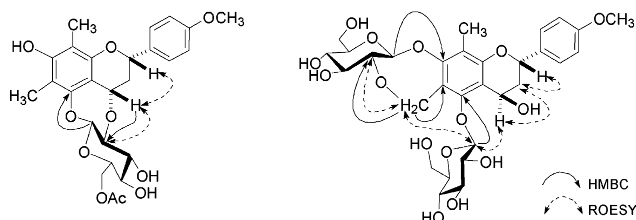

Figure 1. Selected HMBC and ROESY correlations for compounds **1** and **4**.

Comparison of the above NMR spectroscopic data of **1** with those of eruberin A, which is a flavan-4-ol glycoside isolated from *Glaphyropteridopsis erubescens* (Wall.) Ching (Thelypteridaceae),[5] showed that **1** has a very similar structure, with an additional acetyl group. Since the optical rotation of **1** (−15°) is different from that of eruberin A (+88°),[5] it was proposed that the configurations at C-2 and C-4 of **1** are different from the C-2 (*R*) and C-4 (*S*) configurations of eruberin A. The coupling constants of H-2 (brd, J = 11.0 Hz) and H-4 (brd, J = 7.8 Hz) suggested that the stereochemical relationship between H-2 and H-4 was *cis*.[7] To further determine the configurations at C-2 and C-4, a ROESY experiment was performed, where cross-peaks at H-4/H-2″ and H-4/H-2 were observed (Figure 1). On the basis of the above spectroscopic evidence, the configurations at C-2 and C-4 were established as 2*S* and 4*S*. Thus, the structure of **1** was elucidated as (2*S*,4*S*)-6,8-dimethyl-7-hydroxy-4′-methoxy-4,2″-oxidoflavan-5-*O*-β-D-6-*O*-acetyl-glucopyranoside.

Compound **2** was obtained as white needles. The molecular formula ($C_{26}H_{30}O_{10}$) was deduced from the ^{13}C NMR data and the ESITOFMS (*m*/*z* 503.1928 [M + H]$^+$). Compounds **1** and **2** have the same elemental formula, and their ^{1}H and ^{13}C NMR spectra (Table 1) for the aglycon moiety are very similar, suggesting that they have the same aglycon. The EIMS of **2** further confirmed this, in which **2** had the same base peak *m*/*z* 299 (100) as **1**. The differences between their structures are in their sugar moieties. Alkaline hydrolysis of **2** gave **3**, and acid hydrolysis of **2** gave **1a** and D-glucose. The β-configuration of the glycosidic bond was deduced from the coupling constant (J = 7.4 Hz) of the anomeric proton and the ^{13}C NMR data of the sugar moiety. The ^{1}H and ^{13}C NMR signals of the sugar moiety were assigned by a combination of HSQC, HMBC, and ^{1}H−^{1}H COSY experiments. The attachment of the acetyl group at C-3″ of the sugar was evidenced from the correlation of the carbonyl carbon (δ 170.3) with H-3″ (δ 5.02) in the HMBC spectrum. The configurations at C-2 and C-4 were determined as 2*S* and 4*S* by comparing the CD spectrum of **2** with that of **1**, in which the negative Cotton effects at 222 (Δε −6.17) and 278 nm (Δε −1.30) were very similar to 226 (Δε −6.83) and 279 nm (Δε −1.69) of **1**. From these results, **2** was characterized as (2*S*,4*S*)-6,8-dimethyl-7-hydroxy-4′-methoxy-4,2″-oxidoflavan-5-*O*-β-D-3-*O*-acetyl-glucopyranoside.

Compound **3** was isolated as white needles and was assigned the molecular formula $C_{24}H_{28}O_9$, which was determined from its HREIMS (*m*/*z* 460.1725 [M]$^+$) and confirmed by its ^{13}C NMR and DEPT data. The IR, EIMS, and NMR spectroscopic data indicated that **3** is the deacetylated derivative of **1** or **2**, which was confirmed by the 2D NMR (HSQC, ^{1}H−^{1}H COSY, and HMBC) spectral analysis of **3**. The configurations at C-2 and C-4 were determined as 2*S* and 4*S* by comparing the optical rotation, CD spectrum, and coupling constants of key protons of **3** with those of **1**. Thus compound **3** was elucidated as (2*S*,4*S*)-6,8-dimethyl-7-hydroxy-4′-methoxy-4,2″-oxidoflavan-5-*O*-β-D-glucopyranoside.

Compound **4** was isolated as white needles, and its molecular formula was deduced as $C_{30}H_{38}O_{15}$ on the basis of the ESITOFMS (*m*/*z* 661.2103 [M + Na]$^+$). On comparing the ^{1}H and ^{13}C NMR spectroscopic data of **4** with those of triphyllin A (**5**), it was evident that their aglycons were similar. The ^{1}H NMR spectrum of **4** exhibited two anomeric proton signals at δ 4.33 (d, J = 7.2 Hz) and 4.44 (d, J = 7.6 Hz). On acid hydrolysis of **4**, D-glucose was detected. In conjunction with the ^{1}H and ^{13}C NMR data of the sugar moiety (Table 2), the absolute configuration of the sugar was thus determined as β-D-glucose by GC. As observed in the HMBC spectrum, the correlation of H-11a (δ 5.43, brd, J = 13.0 Hz) with C-6 (δ 119.8) suggested that C-11 (δ 64.1) was bonded to C-6, and the correlations of H-1″ (δ 4.44) with C-5 (δ 150.3) and H-1‴ (δ 4.33) with C-7 (δ 153.9) suggested that two D-glucoses were attached to C-5 and C-7, respectively. After complete assignment

Table 2. ^{13}C and ^{1}H NMR Data (δ values) of Compound **4** in DMSO-d_6

position	δ_C	δ_H
2	72.4 d	5.25 dd (1.6, 12.0)
3	36.5 t	2.06 m
		1.86 brt (12.0)
4	56.7 d	4.95 brs
5	150.3 s	
6	119.8 s	
7	153.9 s	
8	114.8 s	
9	152.7 s	
10	115.5 s	
11	64.1 t	5.43 brd (13.0)
		4.34 brd (13.0)
Me-8	9.1 q	2.02 s
1′	133.3 s	
2′,6′	127.7 d	7.38 d (8.8)
3′,5′	114.8 d	6.96 d (8.8)
4′	159.0 s	
OMe	55.2 q	3.76 s
O-glc-5		
1″	105.3 d	4.44 d (7.6)
2″	73.9 d	3.22−3.26 m
3″	76.2 d	3.22 m
4″	70.1 d	3.11 m
5″	76.7 d	3.16 m
6″	61.0 t	3.43 dd (6.6, 11.5)
		3.64 brd (11.5)
O-glc-7		
1‴	102.2 d	4.33 d (7.2)
2‴	85.8 d	3.18 m
3‴	73.9 d	3.22−3.26 m
4‴	69.6 d	3.20 m
5‴	78.0 d	3.21 m
6‴	60.5 t	3.50 dd (2.9, 11.7)
		3.68 brd (11.7)

of the protons and carbons in the sugar moiety from the HSQC, $^{1}H-^{1}H$ COSY, and HMBC spectra, it was found that the chemical shift of C-2‴ of the sugar unit shifted downfield about 12 ppm, as observed for C-2″ of the sugar moiety of compound **1**. This suggested that C-2‴ might be the binding site to the aglycon. The correlation of H-11a with C-2‴ (δ 85.8) in the HMBC spectrum (Figure 1) confirmed that C-2‴ of the sugar (located at C-7) formed an ether linkage to C-11. Thus, between the sugar and the aglycon, an unusual heptacyclic ring was formed. A ROESY experiment was performed to determine the configurations at C-2 and C-4, in which correlations of H-2 with H-3 and H-4 with H-3 were observed (Figure 1), while a correlation between H-2 and H-4 was not observed. The results of the ROESY experiment were not enough to establish the configurations at C-2 and C-4. Therefore, the CD spectrum and chemical shifts and coupling constants of H-2, H-3, and H-4 were compared with those of **5** to further confirm the configurations at C-2 and C-4. A negative Cotton effect at 224 nm ($\Delta\epsilon$ −1.80) and positive Cotton effects at 239 ($\Delta\epsilon$ +0.89) and 282 nm ($\Delta\epsilon$ +1.32) were observed in the CD spectrum of **4**, which were quite comparable to those of **5**, and the H-2 (δ 5.25, dd, J = 1.6 Hz, J = 12.0 Hz), H-3 (δ 2.06, m; δ 1.86, brt, J = 12.0 Hz), and H-4 (δ 4.95, brs) signals of **4** were also consistent with those of **5**. The results showed that **4** has the same configuration (2*S*,4*R*) as **5**. Thus, the structure of **4** was established as (2*S*,4*R*)-4-hydroxy-4′-methoxy-8-methyl-11,2‴-oxidoflavan-5,7-di-*O*-β-D-glucopyranoside.

Compound **5** was identified as triphyllin A and compound **6** as 6,8-dimethyl-7-hydroxy-4′-methoxyanthocyanidin-5-*O*-β-D-glucopyranoside, respectively, by comparison with their spectroscopic data reported in the literature.[4,5]

In compounds **1**−**4**, besides the glycosidic bond of the anomeric carbon, the C-2 of the sugar also formed an ether linkage to the aglycon, and thus an unusual seven-membered ring was formed between the sugar and the aglycon. Due to the ring strain of the seven-membered ring and sugar ring, the stability of this newly formed ring is poor. When compound **3** was treated with weak acid, the ether linkage was broken, and it mainly converted to compound **6**.

Table 3. Cytotoxicity of Compounds **1**−**5** against HepG2 Human Hepatoblastoma Cells

	1	**2**	**3**	**4**	**5**	5-fluorouracil
IC_{50} (μg/mL)	3.5	4.1	4.0	3.1	5.7	1.3

Compounds **1**−**5** were tested for in vitro cytotoxicity against a human hepatoblastoma cell line (HepG2) using the MTT method.[9] The IC_{50} values of **1**−**5** are listed in Table 3. They all showed weak cytotoxicity against HepG2 cells.

Experimental Section

General Experimental Procedures. Melting points were determined on an X4 micro melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer Model 341 polarimeter, and CD spectra were recorded on a JASCO J-810 spectropolarimeter. UV spectra were measured with a Shimadzu UV-260 UV−visible spectrophotometer. IR spectra were determined on a Perkin-Elmer Spectrum One FT-IR spectrometer, as KBr pellets. NMR spectra were taken with TMS as internal standard on a Bruker AM-400 spectrometer. HREIMS were obtained on a Finnigan-MAT 95 instrument. HRESITOFMS were obtained on a Mariner spectrometer, and LRESIMS on a Finnigan LCQ-DECA spectrometer. The GC was performed on a GC-14C gas chromatograph (Shimadzu, Japan) with a DB-17 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) (J & W Scientific); detection, FID; carrier gas, N_2; temperature for injector and detector, 230 °C; temperature gradient system for the oven, 150 °C for 1 min and then raised to 230 °C at the rate of 5 °C/min. Silica gel plates for TLC and silica gel for column chromatography were produced by Qingdao Marine Chemical Company, Qingdao, People's Republic of China. Solvents and chemicals were of analytical grade.

Plant Material. The rhizomes of *Abacopteris penangiana* (Hook.) Ching were collected in October 2004 from Wufeng County of Hubei Province, People's Republic of China. The rhizomes were air-dried at room temperature. A specimen (PZX0310) was deposited in the College of Pharmacy, Tongji Medical Center, Huazhong University of Science and Technology.

Extraction and Isolation. The air-dried rhizomes (5.0 kg) were ground and extracted with MeOH (5 × 10 L) at room temperature. The MeOH extract was concentrated under vacuum to leave a residue, which was suspended in H_2O (3 L) and extracted with $CHCl_3$ (3 L × 3), EtOAc (3 L × 3), and *n*-BuOH (3 L × 3), sequentially. A part of the EtOAc extract (20 g) was chromatographed on a silica gel (200−300 mesh, 800 g) column, eluting with a $CHCl_3$−MeOH gradient system (50:1 → 20:1 → 15:1 → 10:1 → 5:1), to yield 80 fractions. Each fraction (500 mL) was examined by TLC, and fractions with similar TLC patterns were combined to yield 10 major fractions (A_1−A_{10}). Fraction A_3 (0.8 g) was separated over a Sephadex LH 20 column eluted with MeOH−$CHCl_3$ (3:2), then further purified on a silica gel (300−400 mesh, 80 g) column, eluted with a petroleum−acetone gradient (4:1 → 2:1 → 1:1), to yield **1** (62 mg) and **2** (15 mg). Purification of fraction A_4 (1.2 g) was carried out on a Sephadex LH 20 column, eluted with MeOH−$CHCl_3$ (3:2), to yield 15 subfractions designated as $A_{4.1}$−$A_{4.15}$. Subfraction $A_{4.8}$ (0.4 g) was further purified by RP C_{18} (230−400 mesh, 100 g) column chromatography, eluted with MeOH−H_2O (4:3), to yield **3** (40 mg). Compound **6** (25 mg) was isolated from fraction A_8 by chromatography on silica gel columns, eluted with MeOH−$CHCl_3$ (6:1). A part of the *n*-BuOH extract (15 g) was chromatographed on silica gel (200−300 mesh, 750 g), using $CHCl_3$−MeOH−H_2O (4:1:0.1) for elution, to yield 20 fractions, B_1−B_{20} (each 500 mL). Compound **5** (200 mg) was obtained from fraction B_{15-17} (1.0 g) by chromatography on a Sephadex LH-20 column (MeOH−H_2O, 2:1). Fraction B_{9-11} (0.5 g) was further separated over a Sephadex LH-20 column (MeOH−H_2O, 2:1) and finally purified on RP C_{18} (230−400 mesh, 100 g) (MeOH−H_2O, 1:2) to yield **4** (22 mg).

Abacopterin A (1): white needles, mp 188−190 °C; $[\alpha]^{25}_{D}$ −15 (*c* 0.25, MeOH); CD (*c* 0.0032, MeOH), λ ($\Delta\epsilon$) 226 (−6.83), 279 (−1.69) nm; UV (MeOH) λ_{max} (log ϵ) 227 (4.35), 274 (3.45), 280 (3.42)

nm; IR (KBr) ν_{max} 3420, 2879, 1724, 1613, 1516, 1466, 1251, 1136, 824 cm^{-1}; ^{1}H (400 MHz in acetone-d_6) and ^{13}C NMR (100 MHz in acetone-d_6), see Table 1; EIMS *m/z* 502 [M]$^+$ (5), 299 (100), 267 (4), 207 (2), 191 (6), 180 (6), 145 (12), 121 (15), 91 (2); HREIMS *m/z* 502.1854 [M]$^+$ (calcd for $C_{26}H_{30}O_{10}$, 502.1839).

Abacopterin B (2): white needles, mp 160−163 °C; $[\alpha]^{25}_D$ −18 (*c* 0.24, MeOH); CD (*c* 0.0070, MeOH), λ ($\Delta\epsilon$) 222 (−6.17), 278 (−1.30) nm; UV (MeOH) λ_{max} (log ϵ) 227 (4.32), 274 (3.46), 280 (3.43) nm; IR (KBr) ν_{max} 3436, 2927, 1726, 1614, 1517, 1466, 1256, 1134, 828 cm^{-1}; ^{1}H (400 MHz in acetone-d_6) and ^{13}C NMR (100 MHz in acetone-d_6), see Table 1; EIMS *m/z* 502 [M]$^+$ (6), 299 (100), 267 (4), 207 (3), 191 (7), 180 (5), 145 (12), 121 (13), 91 (2); ESITOFMS *m/z* 503.1928 [M + H]$^+$ (calcd for $C_{26}H_{31}O_{10}$, 503.1917).

Abacopterin C (3): white needles, 194−196 °C; $[\alpha]^{25}_D$ −16 (*c* 0.26, MeOH); CD (*c* 0.0034, MeOH), λ ($\Delta\epsilon$) 226 (−5.09), 278 (−1.59) nm; UV (MeOH) λ_{max} (log ϵ) 227 (4.24), 274 (3.52), 280 (3.50) nm; IR (KBr) ν_{max} 3411, 2929, 1613, 1517, 1467, 1250, 1133, 1080, 826 cm^{-1}; ^{1}H (400 MHz in DMSO-d_6) and ^{13}C NMR (100 MHz in DMSO-d_6), see Table 1; EIMS *m/z* 460 [M]$^+$ (7), 299 (100), 267 (5), 207 (2), 191 (7), 180 (6), 145 (11), 121 (15), 91 (2); HREIMS *m/z* 460.1725 [M]$^+$ (calcd for $C_{24}H_{28}O_9$, 460.1733).

Abacopterin D (4): white needles, mp 278−280 °C; $[\alpha]^{25}_D$ +18 (*c* 0.20, MeOH); CD (*c* 0.0048, MeOH), λ ($\Delta\epsilon$) 224 (−1.80), 233 (0), 239 (+0.89), 282 (+1.32) nm; UV (MeOH) λ_{max} (log ϵ) 227 (4.32), 275 (3.42), 281 (3.40) nm; IR (KBr) ν_{max} 3422, 2926, 1610, 1517, 1461, 1252, 1133, 1085, 834 cm^{-1}; ^{1}H (400 MHz in DMSO-d_6) and ^{13}C NMR (100 MHz in DMSO-d_6), see Table 2; EIMS *m/z* 638 [M]$^+$ (1), 458 (18), 297 (100), 282 (20), 267 (7), 181 (30), 134(58), 121 (23), 91 (7), 83 (9); ESITOFMS *m/z* 661.2103 [M + Na]$^+$ (calcd for $C_{30}H_{38}O_{15}$ + Na, 661.2108).

Triphyllin A (5): colorless prisms, mp 197−200 °C; $[\alpha]^{25}_D$ +19 (*c* 0.26, MeOH); CD (*c* 0.0038, MeOH), λ ($\Delta\epsilon$) 224 (−3.22), 233 (0), 239 (+1.71), 281 (+1.43) nm; UV (MeOH) λ_{max} (log ϵ) 227 (4.33), 276 (3.36), 281 (3.36) nm; IR (KBr) ν_{max} 3401, 2920, 1602, 1517, 1458, 1250, 1152, 1074, 835 cm^{-1}; ^{1}H NMR (400 MHz in DMSO-d_6) δ 7.40 (2H, d, J = 8.4 Hz, H-2′ and H-6′), 6.98 (2H, d, J = 8.4 Hz, H-3′ and H-5′), 5.25 (1H, brd, J = 13.0 Hz, H-2), 5.08 (1H, d, J = 7.6 Hz, H-1″), 4.95 (1H, brs, H-4), 4.70, 4.56 (each 1H, d, J = 12.5 Hz, C*H*$_2$OH-6), 4.60 (1H, d, J = 7.6 Hz, H-1‴), 3.77 (3H, s, OC*H*$_3$-4′), 2.14 (3H, s, C*H*$_3$-8), 2.08 (1H, brd, J = 13.0 Hz, H-3a), 1.86 (1H, brt, J = 12.0 Hz, H-3b); ^{13}C NMR (100 MHz in DMSO-d_6) δ 72.2 (CH, C-2), 36.5 (CH_2, C-3), 56.9 (CH, C-4), 152.0 (C, C-5), 120.9 (C, C-6), 154.1 (C, C-7), 115.7 (C, C-8), 152.8 (C, C-9), 115.5 (C, C-10), 53.0 (CH_2, *C*H$_2$OH-6), 9.5 (CH_3, *C*H$_3$-8), 133.3 (C, C-1′), 127.5 (CH, C-2′ and C-6′), 113.9 (CH, C-3′ and C-5′), 158.9 (C, C-4′), 55.1 (CH_3, 4′-O*C*H$_3$), 104.1 (CH, C-1″), 73.9 (CH, C-2″), 76.2 (CH, C-3″), 70.0 (CH, C-4″), 77.0 (CH, C-5″), 60.9 (CH_2, C-6″), 103.7 (CH, C-1‴), 73.9 (CH, C-2‴), 76.1 (CH, C-3‴), 70.0 (CH, C-4‴), 76.5 (CH, C-5‴), 60.9 (CH_2, C-6‴); ESIMS *m/z* 679 [M + Na]$^+$, 477 [M − Glc + H]$^+$, 297 [M − 2Glc + H]$^+$.

Acid Hydrolysis of 1. A solution of **1** (30 mg) in 9% HCl (2 mL) was stirred at 90 °C for 5 h. After being cooled to 2−4 °C, the reaction mixture was filtered. The product was chromatographed on a silica gel (300−400 mesh, 10 g) column ($CHCl_3$−MeOH, 15:1) to yield **1a** (5 mg).

Alkaline Hydrolysis of 1 and 2. Compounds **1** and **2** (0.5 mg) were each hydrolyzed with 1% aqueous KOH (0.5 mL) for 1 h at room temperature. The reaction mixture was adjusted to pH 6 with dilute 1% HCl and then extracted with EtOAc (3 × 0.5 mL). Evaportation of the EtOAc gave compound **3**, analyzed by co-TLC (petroleum−acetone, 1:1, R_f 0.30).

Weak Acid Hydrolysis of 3. Compound **3** (1 mg) was treated with 90% HOAc (1 mL) in 90 °C for 4 h, and then the reaction mixture was freeze-dried. The reaction residue was finally dissolved in MeOH (0.5 mL) and examined by co-TLC ($CHCl_3$−MeOH, 5:1), in which compound **6** was detected (R_f 0.50).

Determination of the Absolute Configuration of Sugars.[8] Compounds **1**−**4** (3 mg) were each subjected to acid hydrolysis as described for **1**. The filtrate was examined by TLC with two solvent systems for sugar analysis (A: $CHCl_3$−MeOH−H_2O, 16:9:2; B: EtOAc−*n*-BuOH−H_2O−HOAc, 4:4:1:1), with R_f values of D-glucose being 0.60 in solvent A and 0.35 in solvent B, respectively. Each remaining filtrate was freeze-dried to give a residue and dissolved in 100 μL of dry pyridine, to which was added 200 μL of L-cysteine methyl ester hydrochloride (0.1 M). The mixture was stirred at 60 °C for 1 h, then 150 μL of hexamethyldisilazane−trimethylchlorosilane (2:1) was added, and the mixture was stirred at 60 °C for another 30 min. After centrifugation, the supernatant was directly subjected to GC analysis. The sugar derivatives obtained from compounds **1**−**4** showed a single peak (t_R at 24.10 min) comparable with that of a D-glucose derivative, and the retention time of the L-glucose derivative was 25.06 min.

Cytotoxicity Bioassay. Cytotoxic activity was determined against a human hepatoblastoma cell line (HepG 2) using the MTT method,[9] according to a previously described procedure.[10] The OD value was read on a plate reader at a wavelength of 570 nm. 5-Fluorouracil was used as the positive control.

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Supporting Information Available: CD spectra of compounds **1**−**5** and spectroscopic data of compound **6**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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