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Homoflavonoid Glucosides from *Ophioglossum pedunculosum* and Their Anti-HBV Activity

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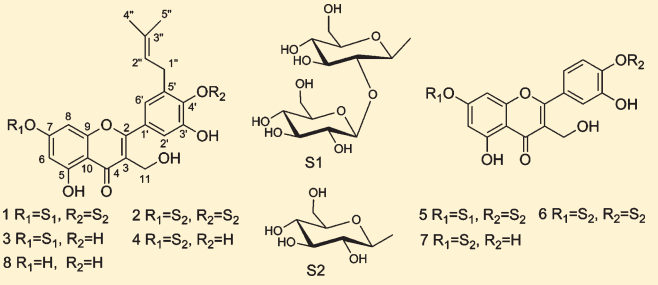
Supporting Information

ABSTRACT: Chemical investigation of the ethanolic extracts of the whole plant of *Ophioglossum pedunculosum* afforded seven new homoflavonoid glucosides, pedunculosumosides A−G (**1**−**7**). Pedunculosumosides A and C exhibit modest activity of blocking HBsAg secretion with IC_{50} values of 238.0 and 70.5 μM, respectively.



Homoflavonoids, characterized by one more carbon atom directly added to the $C_6-C_3-C_6$ backbone of flavonoids, are rare secondary metabolites of plants. The genus *Ophioglossum* (Ophioglossaceae) was shown to be a rich source of homoflavonoids. A previous study on *O. petiolatum* afforded six homoflavonoids, with one showing antihepatitis B virus (HBV) surface antigen (HBsAg) activity.[1] The closely related species *O. pedunculosum* is a small fern distributed in the Asian tropical and subtropical regions.[2] It has been used to treat hepatitis in traditional Chinese medicine, however, with no report on its constituents as yet. Herein, we describe the isolation and identification of seven new homoflavonoid glucosides (**1**−**7**) from the ethanolic extracts of *O. pedunculosum* and their HBsAg secretion blocking effect on HBV-infected HepG2 2.2.15 cells.

RESULTS AND DISCUSSION

Seven new homoflavonoid glycosides, pedunculosumosides A−G (**1**−**7**), together with four known homoflavonoids and four quercetin derivatives were isolated from the 75% EtOH extracts of the whole plant of *O. pedunculosum* by successive open column chromatography over silica gel, Sephadex LH-20, MCI gel, and ODS, and by preparative HPLC (pHPLC).

Pedunculosumoside A (**1**), $[\alpha]_D^{27}$ −60 [*c* 0.15, MeOH−H_2O (1:1)], was obtained as a pale yellow, amorphous powder with a chemical formula of $C_{39}H_{50}O_{22}$ from its negative HRESIMS *m*/*z* 869.2740 $[M - H]^-$ (calcd for $C_{39}H_{49}O_{22}$, 869.2721). The IR spectrum displayed hydroxy (3407 cm^{-1}), conjugated carbonyl (1656 cm^{-1}), and aromatic (1608 and 1493 cm^{-1}) absorptions. Its UV absorption maxima at 306, 262, and 206 nm (MeOH−H_2O) are attributed to a flavone skeleton and are similar to those of ophioglonol 4′-*O*-β-D-glucopyranoside.[1] The ^{13}C NMR spectrum revealed the presence of 39 carbons, including a carbonyl group (δ_C 181.7), three anomeric carbon (δ_C 98.3, 104.6, 105.4) resonances due to saccharides, and two methyl groups, corresponding to a prenylated flavonoid glycoside. Analysis of the ^1H NMR spectrum revealed the presence of one oxygenated methylene [δ_H 4.28 (1H, d, J = 11.1 Hz) and 4.33 (1H, d, J = 11.1 Hz)], two pairs of *meta*-coupled [δ_H 6.52 and 6.74 (1H each, d, J = 2.0 Hz), δ_H 7.29 and 7.21 (1H each, d, J = 2.0 Hz)] aromatic protons, one hydrogen-bonded hydroxy group [δ_H 12.94 (1H, br s)], an additional hydroxy group [δ_H 9.47(1H, br s)], and one 3-methyl-2-buten-1-yl group [δ_H 1.69 (6H, s), 5.32 (1H, t, J = 7.4 Hz), 3.45 (1H, d, J = 7.4 Hz), 3.60 (1H, d, J = 7.4 Hz)].[3] The analysis of its NMR data (Table 1), including HMQC and HMBC spectra, allowed for an unambiguous assignment of all proton and carbon signals. The HMBC cross-peaks (Figure 1) from H-11 (δ_H 4.28, 4.33) to C-2 (δ_C 164.4), C-3 (δ_C 118.3), and C-4 (δ_C 181.7) suggested the location of the oxygenated methylene at C-3; cross-peaks from H-1″ (δ_H 3.60, 3.45) to C-4′ (δ_C 145.6), C-5′ (δ_C 136.2), and C-6′ (δ_C 120.7) and from H-6′ (δ_H 7.21) to C-1″ (δ_C 28.2) placed the 3-methyl-2-buten-1-yl group at C-5′ of the B-ring; cross-peaks from the phenolic hydroxy group at δ_H 9.47 to C-2′ (δ_C 115.1), C-3′ (δ_C 149.4), and C-4′ (δ_C 145.6) located it at C-3′. Thus, the aglycone of **1** was established to be 5′-(3-methyl-2-buten-1-yl)ophioglonol. In addition, hydrolysis of compound **1** yielded a new aglycone (**8**), whose structure was established as 5′-(3-methyl-2-buten-1-yl)ophioglonol by ^1H NMR, ^{13}C NMR, HSQC, and HMBC spectra as well as HRESIMS *m*/*z* 383.1149 $[M - H]^-$ (calcd for $C_{21}H_{19}O_7$, 383.1136), and sugars, which were determined to be D-glucose based on GC-MS analysis of chiral derivatives.

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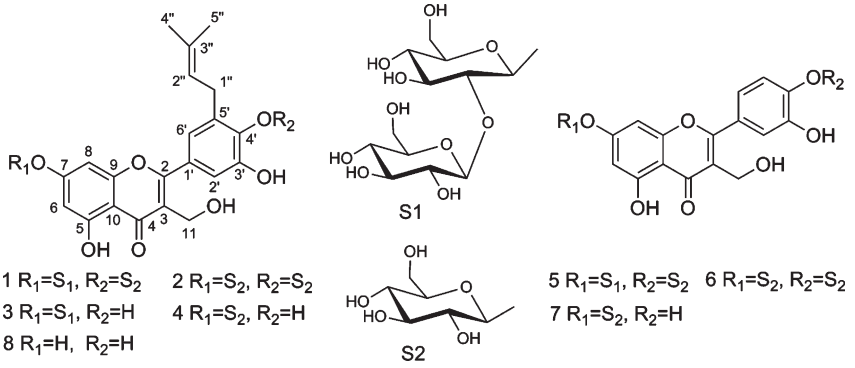
Chart 1. Structures of Compounds 1−8



Besides the signals of the aglycone, the ^{1}H and ^{13}C NMR spectra of **1** (Table 1) showed three anomeric protons at δ_H 5.21 (1H, d, J = 7.0 Hz), 4.48 (1H, d, J = 8.0 Hz), and 4.62 (1H, d, J = 7.8 Hz), correlating with carbon signals at δ_C 98.3, 104.6, and 105.4 in the HSQC spectrum, respectively. All the sugars were determined to be glucose after full assignments of the proton and carbon signals by the analysis of the HSQC and HMBC spectra. The ^3J coupling constants (7.0, 7.8, and 8.0 Hz) of the anomeric proton signals suggested a β-configuration for the three glucosyl moieties. The HMBC correlations from H-1‴ (δ_H 5.21) to C-7 (δ_C 163.0) and H-1⁗ (δ_H 4.62) to C-4′ (δ_C 145.6) suggested the linkage of one glucosyl unit to C-7 at ring A and the second to C-4′ of the B-ring, which was also supported by the NOESY relationships between H-6 (δ_H 6.53), H-8 (δ_H 6.74), and H-1‴ (δ_H 5.21) as well as H-1″ (δ_H 3.60, 3.45), OH-3′ (δ_H 9.47), and H-1⁗ (δ_H 4.62). The interglycosidic linkage of the third glucosyl residue to C-2‴ of the glucosyl moiety at C-7 was suggested by the observation of significantly up-shifted signals of C-2‴ (δ_C 82.6) and evidenced by the HMBC correlation between H-1⁗ (δ_H 4.48) and C-2‴ (δ_C 82.6) and by the NOESY cross-peak from H-2‴ (δ_H 3.51) to H-1⁗ (δ_H 4.48).[4] Therefore, the structure of **1** was determined to be 5′-(3-methyl-2-buten-1-yl)ophioglonol 7-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-4′-*O*-β-D-glucopyranoside.

The similarity between compounds **1** and **2** in their NMR spectra and the determined molecular formula of **2** as $C_{33}H_{40}O_{17}$ by HRESIMS suggested the loss of a glucosyl moiety compared to **1**. The ^{1}H NMR spectrum of **2** showed only two anomeric protons at δ_H 5.07 (1H, d, J = 7.5 Hz) and 4.63 (1H, d, J = 7.8 Hz), correlating to C-7 (δ_C 163.2) and C-4′ (δ_C 145.5) in the HMBC spectrum. This indicated the positions of the two glucosyl moieties at C-7 and C-4′, respectively, which was confirmed by the NOESY correlations from H-1‴ (δ_H 5.07) to H-6 (δ_H 6.47) and H-8 (δ_H 6.70) and from H-1⁗ (δ_H 4.63) to H-1″ (δ_H 3.60, 3.45) and OH-3′ (δ_H 9.47). Compound **2** was thus identified as 5′-(3-methyl-2-buten-1-yl)ophioglonol 7-*O*-β-D-glucopyranosyl-4′-*O*-β-D-glucopyranoside.

Inspection of the NMR data (Table 1) of compound **3** indicated that the aglycone unit was the same as that of **1**. Compound **3** was different from **1** as far as the absence of the signals of one glucosyl moiety and the presence of an additional hydroxy signal at δ_H 9.74. HRESIMS of **3** displayed an ion at m/z 707.2193 $[M - H]^-$, corresponding to a molecular formula of $C_{33}H_{40}O_{17}$, 162 mass units less than **1**. Therefore, the structure of **3** was elucidated as 5′-(3-methyl-2-buten-1-yl)ophioglonol 7-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

The HRESIMS (negative-ion mode) of **4** exhibited a pseudomolecular ion at m/z 545.1641 $[M - H]^-$ (calcd 545.1665), consistent with a molecular formula of $C_{27}H_{30}O_{12}$, 162 mass units less than **3**. The ^{1}H NMR spectrum of **4** was similar to that of **3** except for the absence of the signals of a glucosyl group and the chemical shift of C-2‴ (δ_C 73.1) of one glucosyl residue. The structure of compound **4** was thus elucidated as 5′-(3-methyl-2-buten-1-yl)ophioglonol 7-*O*-β-D-glucopyranoside.

Compound **5** was obtained as a pale yellow, amorphous powder. Its molecular formula was assigned to be $C_{34}H_{42}O_{22}$ as determined from negative HRESIMS m/z 801.2095 $[M - H]^-$ (calcd for $C_{34}H_{41}O_{22}$, 801.2112). The IR spectrum showed absorption bands at 3422 cm^{-1} (OH), 1658 cm^{-1} (C=O), 1608, and 1498 cm^{-1} (phenyl group). The ^{1}H and ^{13}C NMR spectra of **5** were similar to those of **1**, except for the absence of the signals of the 3-methyl-2-buten-1-yl moiety (Tables 1, 2). Analysis of the ^{1}H NMR spectrum revealed the presence of one oxygenated methylene [δ_H 4.33 (2H, br s)], one phenolic hydroxy [δ_H 9.01(1H, br s)], one hydrogen-bonded hydroxy group [δ_H 12.97 (1H, br s)], two *meta*-coupled aromatic protons [δ_H 6.51 and 6.76 (1H each, d, J = 2.0 Hz)], and an aromatic ABX system [δ_H 7.43 (1H, d, J = 2.0 Hz), δ_H 7.40 (1H, dd, J = 8.5, 2.0 Hz), and δ_H 7.29 (1H, d, J = 8.5 Hz)], indicating a 1,3,4-trisubstituted phenyl group in the B-ring of the homoflavone skeleton. The HMBC spectrum of **5** showed a cross-peak from H-11 (δ_H 4.33) to C-3 (δ_C 53.4), which suggested the location of the oxygenated methylene at C-3. The aglycone of compound **5** was thus determined as ophioglonol.[1] Hydrolysis of **5** afforded ophioglonol as the aglycone and D-glucose as the sugar, confirming the above conclusion. The ^{1}H and ^{13}C NMR data of the glucosyl moieties of **5** and **1** are identical (Tables 1, 2). Their positions were determined by the HMBC correlations between the anomeric H-1″ (δ_H 5.22) and C-7 (δ_C 162.9), H-1⁗ (δ_H 4.87) and C-4′ (δ_C 147.9), and H-1‴ (δ_H 4.47) and C-2″ (δ_C 82.6), as well as the NOESY cross-peak between H-2″ (δ_H 3.51) and H-1‴ (δ_H 4.47). Therefore, the structure of compound **5** was elucidated as ophioglonol 7-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-4′-*O*-β-D-glucopyranoside.

Compounds **6** and **7** possessed the same aglycone moiety as **5** according to their ^{1}H and ^{13}C NMR data (Tables 1, 2). The sugar moieties of **6** and **7** were the same as those of **2** and **4**, respectively (Tables 1, 2). The structures of **6** and **7** were confirmed by 2D NMR spectra. Thus, compounds **6** and **7** were identified as ophioglonol 7-*O*-β-D-glucopyranosyl-4′-*O*-β-D-glucopyranoside (**6**) and ophioglonol 7-*O*-β-D-glucopyranoside (**7**), respectively.

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Table 1. NMR Spectroscopic Data for Compounds 1−4 (500 MHz, DMSO-d_6)[a]

	1		2		3		4	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	164.4		164.4		165.3		165.3	
3	118.3		118.4		117.4		117.4	
4	181.7		181.6		181.7		181.7	
5	161.1		161.1		161.2		161.2	
6	99.6	6.52 d (2.0)	99.4	6.47 d (2.0)	99.5	6.50 d (2.0)	99.3	6.46 d (2.0)
7	163.0		163.2		162.8		163.1	
8	94.7	6.74 d (2.0)	94.5	6.70 d (2.0)	94.5	6.70 d (2.0)	94.3	6.67 d (2.0)
9	156.8		156.9		156.8		156.8	
10	104.9		104.9		104.7		104.7	
11	53.4	4.28 d (11.1)	53.4	4.30 br s	53.7	4.36 d (11.0)	53.6	4.40 d (11.0)
		4.33 d (11.1)				4.30 d (11.0)		4.31 d (11.0)
1′	128.3		128.3		128.2		128.1	
2′	115.1	7.29 d (2.0)	115.0	7.28 d (2.0)	113.6	7.25 d (2.0)	113.5	7.25 d (2.0)
3′	149.4		149.4		144.6		144.6	
4′	145.6		145.5		146.5		146.5	
5′	136.2		136.2		121.7		121.7	
6′	120.7	7.21 d (2.0)	118.4	7.20 d (2.0)	121.6	7.21 d (2.0)	121.6	7.22 d (2.0)
1″	28.2	3.60 d (7.4)	28.2	3.60 d (7.4)	28.1	3.28 d (7.4)	28.1	3.27 d (7.4)
		3.45 d (7.4)		3.45 d (7.4)				
2″	122.4	5.32 t (7.4)	122.4	5.32 t (7.4)	122.3	5.30 t (7.4)	122.3	5.30 t (7.4)
3″	132.0		132.0		131.7		131.7	
4″	25.5	1.69 s	25.4	1.69 s	25.4	1.69 s	25.4	1.69 s
5″	17.7	1.69 s	17.7	1.69 s	17.6	1.68 s	17.6	1.69 s
7-Glc								
1‴	98.3	5.21 d (7.0)	99.8	5.07 d (7.5)	98.2	5.22 d (7.0)	99.9	5.07 d (7.5)
2‴	82.6	3.51 dd (7.0, 9.0)	73.0	3.25 dd (7.5, 9.0)	82.6	3.51 dd (7.0, 9.0)	73.1	3.26 dd (7.5, 9.0)
3‴	75.6	3.52 t (9.0)	76.3	3.28 t (9.0)	75.6	3.52 t (9.0)	76.3	3.28 t (9.0)
4‴	69.1	3.24 t (9.0)	69.5	3.17 t (9.0)	69.1	3.24 t (9.0)	69.5	3.17 t (9.0)
5‴	76.9	3.49 m	77.1	3.43 m	76.9	3.49 m	77.1	3.43 m
6‴	60.4	3.70 br d (10.0)	60.5	3.70 br d (12.0)	60.4	3.71 br d (10.0)	60.6	3.70 br d (10.0)
		3.47 dd (5.7, 10.0)		3.48 dd (5.7, 12.0)		3.48 dd (5.7, 10.0)		3.48 dd (5.7, 10.0)
2‴-Glc								
1⁗	104.6	4.48 d (8.0)			104.6	4.47 d (8.0)		
2⁗	74.6	2.99 dd (8.0, 9.0)			74.6	2.99 dd (8.0, 9.0)		
3⁗	76.2	3.20 t (9.0)			76.2	3.20 t (9.0)		
4⁗	69.6	3.13 t (9.0)			69.6	3.13 t (9.0)		
5⁗	76.8	3.14 m			76.8	3.14 m		
6⁗	60.6	3.70 br d (10.0)			60.6	3.71 br d (10.0)		
		3.47 dd (5.7, 10.0)				3.48 dd (5.7, 10.0)		
4′-Glc								
1⁗′	105.4	4.62 d (7.8)	105.4	4.63 d (7.8)				
2⁗′	73.9	3.36 dd (7.8, 9.0)	73.9	3.36 dd (7.8, 9.0)				
3⁗′	76.2	3.28 t (9.0)	76.1	3.28 t (9.0)				
4⁗′	69.7	3.21 t (9.0)	69.6	3.21 t (9.0)				
5⁗′	77.4	3.23 m	77.4	3.22 m				
6⁗′	60.8	3.70 br d (10.0)	60.8	3.70 br d (12.0)				
		3.47 dd (5.7, 10.0)		3.48 dd (5.7, 12.0)				
5-OH		12.94 br s		12.94 br s		13.07 br s		13.08 br s
3′-OH		9.47 br s		9.47 br s		9.00 br s		9.00 br s
4′-OH						9.74 br s		9.74 br s

[a] All assignments are based on HSQC and HMBC experiments.

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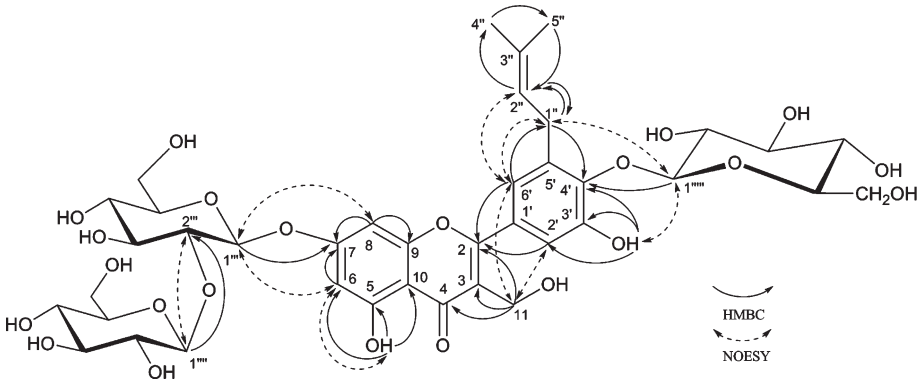

Figure 1. Key HMBC and NOESY correlations of pedunculosumoside A (**1**).

The known compounds were identified as ophioglonol,[1] ophioglonol 4′-*O*-*β*-D-glucopyranoside,[1] ophioglonin,[1] ophioglonin 7-*O*-*β*-D-glucopyranoside,[1] quercetin,[5] quercetin 3-*O*-methyl ether,[6] 3-*O*-methylquercetin 7-*O*-*β*-D-glucopyranoside,[7] and 3-*O*-methylquercetin 7-*O*-*β*-D-glucopyranosyl-4′-*O*-*β*-D-glucopyranoside[8] by comparing their UV, ESIMS, and ^{1}H NMR and ^{13}C NMR data with those reported.

Some of the isolates were investigated for their cytotoxicity and ability to block HBsAg secretion in HBV-infected HepG2 2.2.15 cells at a nontoxic concentration (Table 3). Pedunculosumosides A and C, with low cytotoxicity, showed significant inhibitory effects on the secretion of HBsAg in a dose-dependent manner, which suggested the key role of the 3-methyl-2-buten-1-yl group of the homoflavonoid glycosides. With the only structural difference in the number of glucosyl moieties, pedunculosumosides A and C showed a relatively large difference in their ability to inhibit HBsAg secretion, with IC_{50} values of 238.0 and 70.5 μM, respectively, which suggested that the number of glucosyl groups also influences their anti-HBV activity. In terms of cytotoxicity, most of the compounds showed high toxicity to HepG2 2.2.15 cells except for pedunculosumosides A, C, and E. Glycosylation seemed to decrease the cytotoxicity of compounds markedly,[9] as evidenced by the CC_{50} values of ophioglonol 7-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-4′-*O*-*β*-D-glucopyranoside (CC_{50} > 100.0 μM), ophioglonol 7-*O*-*β*-D-glucopyranosyl-4′-*O*-*β*-D-glucopyranoside (CC_{50} = 56.7 μM), ophioglonol 4′-*O*-*β*-D-glucopyranoside (CC_{50} = 19.7 μM), and ophioglonol (CC_{50} = 15.9 μM).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined with a JASCO P-1020 digital polarimeter (Shimadzu). UV spectra were recorded using a UV-2450 UV−visible spectrophotometer (Shimadzu). IR spectra were performed on a Bruker Tensor 27 spectrometer. NMR spectra were measured in DMSO-d_6 at 303 K on a Bruker AV-500 NMR (^{1}H NM, 500 MHz; ^{13}C NMR, 125 MHz) spectrometer with TMS as internal standard. ESIMS and HRESIMS data were acquired on an Agilent 1100 series LC/MSD Trap mass spectrometer (ESIMS) and a Mariner ESI TOF spectrometer (HRESIMS), respectively. Silica gel (Qingdao Haiyang Chemical Co. Ltd.), MCI gel CHP-20 (Mitsubishi), Sephadex LH-20 (Pharmacia), and ODS (40−63 μm, Fuji) were used for open column chromatography. Preparative HPLC was carried out using an Agilent 1100 Series coupled with a Shim-pack RP-C_{18} column (200 × 20 mm i.d., Shimadzu) and an 1100 Series multiple wavelength detector.

Plant Material. The whole plants of *O. pedunculosum* were purchased in August 2008 from the local traditional Chinese medicine market in Bozhou, Anhui Province, China, and authenticated by Professor Mian Zhang, Department of Pharmacognosy, China Pharmaceutical University. Voucher specimens (No. 200808101) are deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation. The dried materials (4.0 kg) were homogenized and successively extracted three times with 75% EtOH under reflux. After concentration, the combined EtOH extract (692.0 g) was subjected to silica gel column chromatography (CC) using a MeOH−CH_2Cl_2 solvent system in gradient (0, 10, 50, 70, and 100% MeOH) to obtain four subfractions (I−IV). Subfraction II (10% MeOH−CH_2Cl_2) was separated by another silica gel CC with acetone−petroleum ether (from 25% to 75% acetone). Subfractions of 33−50% acetone−petroleum ether were then followed by repeated Sephadex LH-20 CC (MeOH) to give ophioglonol (99.8 mg), ophioglonin (6.0 mg), quercetin (20.0 mg), and quercetin 3-*O*-methyl ether (196.0 mg). Subfraction III (50% MeOH−CH_2Cl_2) was purified by MCI gel CC with MeOH−H_2O (from 10% to 80% MeOH). Subfractions of 50−70% MeOH−H_2O were further isolated by passage over a Sephadex LH-20 column (MeOH) and repeated ODS column (40−60% MeOH−H_2O) and finally purified by repeated pHPLC (30−40% MeOH−H_2O) to afford compounds **2** (5.0 mg), **3** (27.2 mg), **4** (3.3 mg), **7** (4.8 mg), ophioglonol 4′-*O*-*β*-D-glucopyranoside (9.1 mg), ophioglonin 7-*O*-*β*-D-glucopyranoside (11.8 mg), and 3-*O*-methylquercetin 7-*O*-*β*-D-glucopyranoside (88.0 mg). Subfraction IV (70−100% MeOH−CH_2Cl_2) was purified by MCI gel CC with MeOH−H_2O (from 0% to 50% MeOH). Subfractions of 30% MeOH−H_2O were followed by Sephadex LH-20 CC (50% MeOH−H_2O) and by repeated pHPLC (10−28% MeOH−H_2O) to obtain compounds **1** (36.0 mg), **5** (15.5 mg), **6** (62.6 mg), and 3-*O*-methylquercetin 7-*O*-*β*-D-glucopyranosyl-4′-*O*-*β*-D-glucopyranoside (185.3 mg).

Pedunculosumoside A (***1***): pale yellow, amorphous powder; $[\alpha]_D^{27}$ −60 [*c* 0.15, MeOH−H_2O (1:1)]; UV [MeOH−H_2O (1:1)] λ_{max} (log ε) 306 (2.45), 262 (2.74), 206 (3.02) nm; IR (KBr) ν_{max} 3407, 1656, 1608, 1493, 1453, 1349, 1298, 1183, 1078, 1027, 893, 814 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 1; ESIMS *m*/*z* 869 $[M - H]^-$; HRESIMS *m*/*z* 869.2740 $[M - H]^-$ (calcd for $C_{39}H_{49}O_{22}$, 869.2721).

Pedunculosumoside B (***2***): yellow, amorphous powder; $[\alpha]_D^{27}$ −57 [*c* 0.15, MeOH−H_2O (1:1)]; UV [MeOH−H_2O (1:1)] λ_{max} (log ε) 306 (2.44), 262 (2.72), 204 (3.04) nm; IR (KBr) ν_{max} 3414, 1656, 1610, 1496, 1450, 1345, 1296, 1185, 1073, 1024, 893, 816 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 1; ESIMS *m*/*z* 707 $[M - H]^-$; HRESIMS *m*/*z* 707.2213 $[M - H]^-$ (calcd for $C_{33}H_{39}O_{17}$, 707.2193).

Table 2. NMR Spectroscopic Data for Compounds 5−7 (500 MHz, DMSO-d_6)[a]

	5		6		7	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	164.5		164.5		165.7	
3	117.9		118.0		117.8	
4	181.6		181.6		182.2	
5	161.1		161.1		161.7	
6	99.6	6.51 d (2.0)	99.4	6.46 d (2.0)	99.9	6.46 d (2.0)
7	162.9		163.1		163.6	
8	94.6	6.76 d (2.0)	94.5	6.73 d (2.0)	94.9	6.71 d (2.0)
9	156.8		156.9		157.4	
10	104.5		104.8		105.3	
11	53.4	4.33 br s	53.4	4.30 d (11.0)	54.1	4.33 br s
				4.34 d (11.0)		
1′	125.7		125.7		123.0	
2′	116.3	7.43 d (2.0)	116.4	7.43 d (2.0)	116.7	7.37 d (2.0)
3′	146.4		146.4		145.7	
4′	147.9		147.9		149.4	
5′	115.7	7.29 d (8.5)	115.7	7.29 d (8.5)	115.9	6.92 d (8.0)
6′	120.8	7.40 dd (8.5, 2.0)	120.8	7.40 dd (8.5, 2.0)	121.8	7.33 dd (8.0, 2.0)
7-Glc						
1″	98.2	5.22 d (7.5)	99.8	5.07 d (7.5)	100.3	5.08 d (7.5)
2″	82.6	3.51 dd (7.5, 9.0)	73.1	3.26 dd (7.5, 9.0)	73.6	3.26 dd (7.5, 9.0)
3″	75.6	3.52 t (9.0)	76.3	3.28 t (9.0)	76.8	3.29 t (9.0)
4″	69.1	3.25 t (9.0)	69.5	3.16 t (9.0)	70.0	3.18 t (9.0)
5″	76.9	3.50 m	77.1	3.44 m	77.6	3.44 m
6″	60.7	3.70 br d (12.5)	60.6	3.70 br d (10.0)	61.1	3.70 br d (10.0)
		3.48 br d (12.5)		3.48 br d (10.0)		3.48 br d (10.0)
2″-Glc						
1‴	104.8	4.47 d (8.0)				
2‴	74.6	2.99 dd (8.0, 8.0)				
3‴	76.1	3.21 m				
4‴	69.6	3.14 m				
5‴	76.8	3.14 m				
6‴	60.5	3.70 br d (12.5)				
		3.48 br d (12.5)				
4′-Glc						
1⁗	101.5	4.87 d (7.5)	101.5	4.87 d (7.0)		
2⁗	73.2	3.36 dd (7.5,9.0)	73.2	3.35 dd (7.0,9.0)		
3⁗	75.8	3.32 m	75.8	3.32 t (9.0)		
4⁗	69.8	3.19 m	69.8	3.20 t (9.0)		
5⁗	77.2	3.40 m	77.3	3.40 m		
6⁗	60.4	3.70 br d (12.5)	60.7	3.70 br d (10.0)		
		3.48 br d (12.5)		3.48 br d (10.0)		
7-OH						
5-OH		12.97 br s		12.98 br s		13.08 br s
4′-OH						9.79 br s
3′-OH		9.01 br s		9.02 br s		9.38 br s

[a] All assignments are based on HSQC and HMBC experiments.

Pedunculosumoside C (**3**): yellow, amorphous powder; $[\alpha]_D^{27}$ −47 [*c* 0.15, MeOH−H_2O (1:1)]; UV [MeOH−H_2O (1:1)] λ_{max} (log ε) 335 (2.19), 259 (2.38), 204 (2.75) nm; IR (KBr) ν_{max} 3441, 1654, 1606, 1493, 1442, 1337, 1183, 1077, 1028, 893, 813 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 1; ESIMS *m*/*z* 707 $[M - H]^-$; HRESIMS *m*/*z* 707.2171 $[M - H]^-$ (calcd for $C_{33}H_{39}O_{17}$, 707.2193).

Pedunculosumoside D (**4**): yellow, amorphous powder; $[\alpha]_D^{27}$ −19 [*c* 0.15, MeOH−H_2O (1:1)]; UV [*c* 0.05, MeOH−H_2O (1:1)] λ_{max} (log ε) 335 (2.24), 259 (2.41), 204 (3.08) nm; IR (KBr) ν_{max} 3445,

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Table 3. Anti-HBV Activity[a] and Cytotoxicity of the Compounds from *O. pedunculosum*

compound	CC_{50} (μM)[b]	IC_{50} (μM)[c]
5′-(3-methyl-2-buten-1-yl)ophioglonol 7-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-4′-*O*-*β*-D-glucopyranoside	>200.0	238.0
5′-(3-methyl-2-buten-1-yl)ophioglonol 7-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside	>100.0	70.5
ophioglonol 7-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-4′-*O*-*β*-D-glucopyranoside	>100.0	NE[d]
ophioglonol 7-*O*-*β*-D-glucopyranosyl-4′-*O*-*β*-D-glucopyranoside	56.7	NE
ophioglonol	15.9	NE
ophioglonol 4′-*O*-*β*-D-glucopyranoside	19.7	NE
ophioglonin	20.4	NE
quercetin	38.1	NE
quercetin 3-*O*-methyl ether	2.1	NE
3-*O*-methylquercetin 7-*O*-*β*-D-glucopyranoside	5.4	NE
3-*O*-methylquercetin 7-*O*-*β*-D-glucopyranosyl-4′-*O*-*β*-D-glucopyranoside	7.9	NE
lamivudine	2400.0	58.0

[a] Anti-HBV activity, the activity of anti-HBV surface antigen (HBsAg) secretion. [b] CC_{50}, 50% cytotoxicity concentration. [c] IC_{50}, 50% effective concentration. [d] NE, there was no effect at a nontoxic concentration.

1652, 1603, 1495, 1454, 1305, 1180, 1075 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 1; ESIMS m/z 545 $[M - H]^-$; HRESIMS m/z 545.1641 $[M - H]^-$ (calcd for $C_{27}H_{29}O_{12}$, 545.1665).

*Pedunculosumoside E (**5**):* pale yellow, amorphous powder; $[\alpha]_D^{27}$ −69 [*c* 0.15, MeOH−H_2O (1:1)]; UV [MeOH−H_2O (1:1)] λ_{max} (log ε) 322 (2.59), 261 (2.81), 203 (3.09) nm; IR (KBr) ν_{max} 3422, 1658, 1608, 1498, 1454, 1396, 1358, 1297, 1253, 1190, 1078, 1044, 895, 816 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; ESIMS m/z 801 $[M - H]^-$; HRESIMS m/z 801.2095 $[M - H]^-$ (calcd for $C_{34}H_{41}O_{22}$, 801.2112).

*Pedunculosumoside F (**6**):* yellow, amorphous powder; $[\alpha]_D^{27}$ −82 [*c* 0.15, MeOH−H_2O (1:1)]; UV [MeOH−H_2O (1:1)] λ_{max} (log ε) 322 (2.52), 261 (2.74), 203 (3.03) nm; IR (KBr) ν_{max} 3418, 1655, 1609, 1498, 1453, 1351, 1294, 1256, 1209, 1182, 1075, 995, 814 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; ESIMS m/z 639 $[M - H]^-$; HRESIMS m/z 639.1540 $[M - H]^-$ (calcd for $C_{28}H_{31}O_{17}$, 639.1567).

*Pedunculosumoside G (**7**):* yellow, amorphous powder; $[\alpha]_D^{27}$ −36 [*c* 0.15, MeOH−H_2O (1:1)]; UV [MeOH−H_2O (1:1)] λ_{max} (log ε) 334 (3.34), 254 (2.53), 204 (2.81) nm; IR (KBr) ν_{max} 3427, 1653, 1607, 1497, 1454, 1374, 1298, 1182, 1078, 820 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; ESIMS m/z 477[M − H]$^-$; HRESIMS m/z 477.1058 $[M - H]^-$ (calcd for $C_{22}H_{21}O_{12}$, 477.1038).

*Acid Hydrolysis of **1** and **5**.* Compounds **1** and **5** (8.0 mg each) were separately refluxed with 10 mL of 2 M HCl (MeOH−H_2O, 1:1) at 100 °C for 4 h. After the MeOH was removed, the residue was diluted with H_2O and extracted with EtOAc (10 mL × 3). The EtOAc extracts were purified by chromatography on Sephadex LH-20 to obtain **8** (3.8 mg) from **1** and ophioglonol (3.5 mg) from **5**, respectively. The aqueous layer was concentrated under vacuum to give a residue of the sugar fraction. The residue was dissolved in pyridine (0.1 mL), and then 0.08 M L-cysteine methyl ester hydrochloride (0.15 mL) was added. After reacting at 60 °C for 1.5 h, the reaction mixture was dried under vacuum and trimethylsilylated with l-trimethylsilylimidazole (0.1 mL) for 2 h. Then the mixture was partitioned between *n*-hexane and H_2O (each 0.3 mL), and the *n*-hexane extract was subjected to GC-MS (Varian 3800 GC, Varian 2200 MS, 70 eV) analysis under the following conditions: capillary column, SE30 (30 m × 0.25 mm × 0.25 μm); column temperature, 170−250 °C with a rate of 5 °C·min^{-1}; carrier gas, N_2 (30 mL·min^{-1}). In the acid hydrolysates of **1** and **5**, only D-glucose was detected by comparison of the retention time (t_R 8.0 min) of their derivatives with that of the authentic D-glucose derivative prepared in the same way.[10]

*5′-(3-Methyl-2-buten-1-yl)ophioglonol (**8**):* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 337 (2.93), 256 (3.07), 210 (3.39) nm; IR (KBr) ν_{max} 3423, 1657, 1612, 1500, 1441, 1355, 1305, 1176, 1022, 828 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 13.08 (1H, br s, OH-5), 10.82 (1H, br s, OH-7), 9.74 (1H, br s, H-4′), 9.01 (1H, br s, H-3′), 7.24 (1H, d, *J* = 2.0 Hz, H-2′), 7.22 (1H, d, *J* = 2.0 Hz, H-6′), 6.34 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 5.30 (1H, m, H-2″), 4.30 (2H, s, H-11), 3.28 (2H, d, *J* = 7.5 Hz, H-1″), 1.68 (6H, s, H-4″, H-5″); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 181.3 (C, C-4), 164.6 (C, C-2), 164.2 (C, C-7), 161.5 (C, C-5), 157.1 (C, C-9), 146.3 (C, C-4′), 144.5 (C, C-3′), 131.6 (C, C-3″), 128.0 (C, C-1′), 122.3 (CH, C-2″), 121.8 (C, C-5′), 121.5 (CH, C-6′), 116.9 (C, C-3), 113.3 (CH, C-2′), 103.0 (C, C-10), 98.6 (CH, C-6), 93.4 (CH, C-8), 53.5 (CH_2, C-11), 28.0 (CH_2, C-1″), 25.4 (CH_3, C-4″), 17.6 (CH_3, C-5″); HRESIMS m/z 383.1149 $[M - H]^-$ (calcd for $C_{21}H_{19}O_7$, 383.1136).

Antihepatitis B Virus (HBV) Assay. HepG2 2.2.15 is a human hepatoblastoma cell line stably transfected with cloned HBV DNA, which was used as the model system.[11−13] The HepG2 2.2.15 cells were routinely cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10% (v/v) fetal calf serum (Gibco, USA), penicillin G 100 units·mL^{-1}, streptomycin 100 μg·mL^{-1}, amphotericin B 0.25 μg·mL^{-1}, L-glutamine 2 mmol·mL^{-1}, and G418 (Sigma) 200 μg·mL^{-1} under 5% CO_2 atmosphere at 37 °C. Sequential dilutions of the purified compounds and lamivudine as a positive control dissolved in DMSO were added to the medium after cells were plated at a density of 2 × 10^4 cells·mL^{-1} on 24-well plates. Cells were grown in the presence or absence of the tested compounds for 8 days with changing the medium on the fourth day. After cultivation, the viability of the cultured cells with tested compounds was measured by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method as previously described.[14] Briefly, 20 μL of MTT (5 g·mL^{-1}) was added to each well and further incubated for 4 h. Then, the culture medium was removed from each well and washed twice with phosphate-buffered saline, and then 150 μL of DMSO was added to dissolve the purple formazan of MTT. The absorbance at 570 nm was read, and the percent of cell death calculated. The 50% toxicity concentration (CC_{50}) of the samples was estimated from the graphical interpolation. For the

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HBsAg assay, the levels of HBV surface antigen in the supernatant of the HepG2 2.2.15 cells were determined by the enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (Shanghai Kehua Biotech Co., Ltd.).[9] The inhibiting rates (%) were calculated by comparing the treatment group with the tested compounds and the solvent control group with DMSO. The percent of inhibition (%) = [1 − OD value of sample well/OD value of DMSO well] × 100.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR, ^{13}C NMR, and HRESIMS spectra of **1**−**7** are available free of charge via the Internet at http://pubs.acs.org.

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