Tetranorclerodanes and Clerodane-Type Diterpene Glycosides from *Dicranopteris dichotoma*

Xiao-Li Li,[†,⊥] Liu-Meng Yang,[§] Yu Zhao,[†,⊥] Rui-Rui Wang,[§] Gang Xu,[†] Yong-Tang Zheng,[§] Lin Tu,[†,⊥] Li-Yan Peng,[†] Xiao Cheng,[†] and Qin-Shi Zhao*,[†]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, People's Republic of China, Key Laboratory of Animal Models and Human Disease Mechanisms and Laboratory of Molecular Immunopharmacology, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, Yunnan, People's Republic of China, and Graduate School of the Chinese Academy of Sciences, Beijing 100039, People's Republic of China

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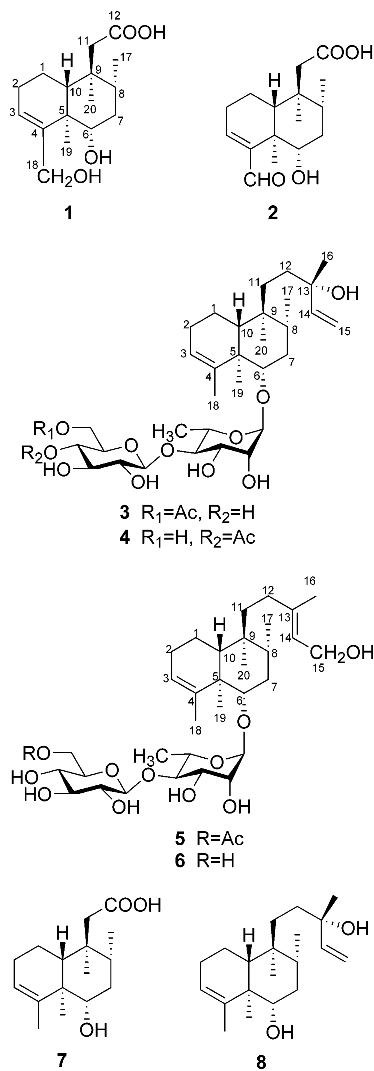
The acetone extract of *Dicranopteris dichotoma* afforded two new tetranorclerodanes, 18-hydroxyaylthonic acid (**1**) and 18-oxo-aylthonic acid (**2**), and four new clerodane-type diterpene glycosides, (6*S*,13*S*)-6-*O*-[6-*O*-acetyl-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl]cleroda-3,14-dien-13-ol (**3**), (6*S*,13*S*)-6-*O*-[4-*O*-acetyl-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl]cleroda-3,14-dien-13-ol (**4**), 6-*O*-[6-*O*-acetyl-β-D-glucopyranos-yl-(1→4)-α-L-rhamnopyranosyl]-(13*E*)-cleroda-3,13-dien-15-ol (**5**), and 6-*O*-[β-D-glucopyranosyl]-(1→4)-α-L-rhamnopyranosyl-(13*E*)-cleroda-3,13-dien-15-ol (**6**), together with two known compounds, aylthonic acid (**7**) and (6*S*,13*S*)-cleroda-3,14-diene-6,13-diol (**8**). The structures of these new compounds were established by a combination of 1D and 2D NMR techniques, MS, and acid hydrolysis. Compound **8** showed modest anti-HIV-1 activity.

Dicranopteris species are very common ferns and grow as large communities containing no other plant species. Previous studies of *Dicranopteris* species have reported flavonoids,[1,2] phenols,[3] proanthocyanidins,[4] and clerodane-type glycosides.[2,5] Recent research showed that some clerodane-type glycosides isolated from *Dicranopteris* species accelerated the growth of the stems of lettuce or inhibited the root growth.[6] To find bioactive secondary metabolites from this species, we chemically investigated the fronds of *D. dichotoma* Bernb. and reported the isolation and structural elucidation of two new highly oxygenated phenolic derivatives, dichotomains A and B.[6] Continuous screening of the fronds of *D. dichotoma* led to the discovery of two new tetranorclerodanes, 18-hydroxyaylthonic acid (**1**) and 18-oxo-aylthonic acid (**2**), and four new clerodane-type diterpene glycosides, (6*S*,13*S*)-6-*O*-[6-*O*-acetyl-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl]cleroda-3,14-diene-13-ol (**3**), (6*S*,13*S*)-6-*O*-[4-*O*-acetyl-β-D-glucopy-ranosyl-(1→4)-α-L-rhamnopyranosyl]cleroda-3,14-dien-13-ol (**4**), 6-*O*-[6-*O*-acetyl-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl]-(13*E*)-cleroda-3,13-dien-15-ol (**5**), and 6-*O*-[β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl]-(13*E*)-cleroda-3,13-dien-15-ol (**6**), together with two known compounds, aylthonic acid (**7**)[7] and (6*S*,13*S*)-cleroda-3,14-diene-6,13-diol (**8**).[6] In the present paper, we report the structural characterization of compounds **1**−**8** and their anti-HIV-1 activities.



Results and Discussion

Compound **1** was obtained as colorless crystals. The molecular formula of $C_{16}H_{26}O_4$ was established on the basis of HRESIMS $[M - H]^-$ (found 281.1755, calcd 281.1752). The ^{13}C NMR spectrum of **1** displayed the signals of one carboxylic acid group (δ_C 174.4), two quaternary carbons (δ_C 45.0 and 40.5), two olefinic carbons (δ_C 147.6 and 126.0), three methines (δ_C 74.9, 47.3, and 36.2), five methylenes (δ_C 66.3, 43.8, 37.2, 27.1, and 19.4), and three methyls (δ_C 17.5, 17.0, and 16.4). Its IR spectrum showed the absorption bands typical for the hydroxyl group (3483 cm^{-1}) and carboxylic acid group (1700, 3251−2857 cm^{-1}). The ^1H and ^{13}C NMR spectra of **1** were similar to those of aylthonic acid (**7**).[7] The key difference was that the methyl group (Me-18) in **7** was replaced by a hydroxymethyl group (δ_C 66.3) in **1**, which was confirmed by the HMBC correlations of H_2-18 (δ_H 4.56, 4.41) with C-3 (δ_C 126.0), C-4 (δ_C 147.6), and C-5 (δ_C 45.0).

The relative configuration of **1** was determined by a ROESY experiment. The ROESY correlations of H-6/H-8 and H-10 and of

* Corresponding author. Tel: 86-871-5223058. Fax: 86-871-5215783. E-mail: Qinshizhaosp@yahoo.com.
† Kunming Institute of Botany.
§ Kunming Institute of Zoology.
⊥ Graduate School of the Chinese Academy of Sciences.

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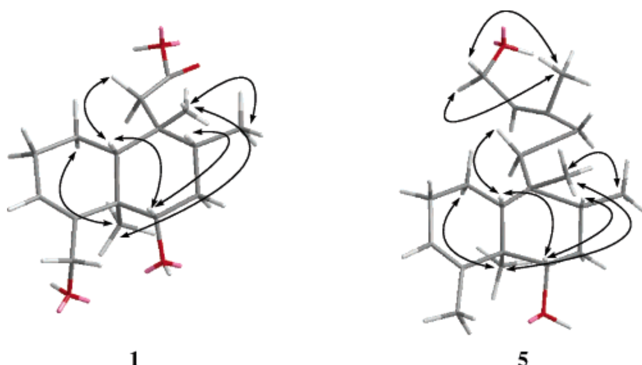

Figure 1. ROESY correlations of compound **1** and the aglycone of **5**.

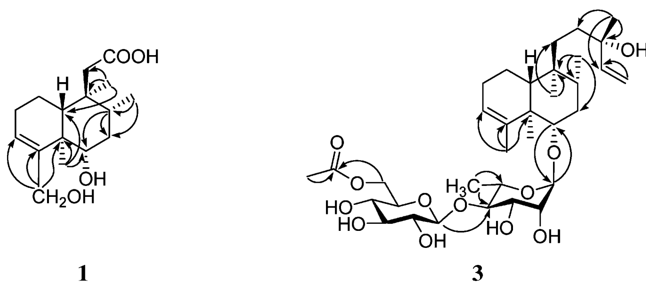

Figure 2. HMBC correlations of compounds **1** and **3**.

H-10/H-11β indicated that H-6, H-8, and H-10 were β-oriented and, accordingly, the hydroxyl group at C-6 was α-oriented. The ROESY correlation of Me-19/Me-20 and H-1α and of Me-20/Me-17 suggested that Me-17, Me-19, and Me-20 possessed α-orientations (Figure 1). The structure of **1** was 18-hydroxyaylthonic acid.

Compound **2**, colorless crystals, had the molecular formula $C_{16}H_{24}O_4$ on the basis of the HRESIMS (found 279.1596 and calcd 279.1596). The similarity of the ^{1}H and ^{13}C NMR spectra of **1** and **2** (Tables 1 and 2) suggested that **2** was a related tetranorclerodane derivative. The only difference between the two compounds was that the hydroxymethylene moiety (δ_C 66.3, C-18) in **1** was replaced by an aldehydic function (δ_C 198.2) in **2**. This was confirmed by the HMBC correlations between the aldehydic proton (δ_H 9.42) and C-3 (δ_C 157.8), C-4 (δ_C 151.4), and C-5 (δ_C 45.2) and supported by the downfield shift of C-3 and C-4 from δ_C 123.0 and 147.6 in **1** to δ_C 157.8 and 151.4 in **2**. The relative configuration was established via a ROESY experiment. Similar ROESY correlations to those in **1** confirmed that H-6, H-8, and H-10 were β-oriented, while Me-17, M-19, and Me-20 were α-oriented. Thus, compound **2** was identified as 18-oxo-aylthonic acid.

Compound **3** was obtained as a pale yellow powder, and its molecular formula was indicated as $C_{34}H_{56}O_{12}$ by HRESIMS (found 655.3691, calcd 655.3693), corresponding to seven unsaturation degrees. The ^{1}H and ^{13}C NMR spectra of **3** showed resonances characteristic of a diterpene, two hexoses, and an acetyl group (Tables 1 and 2). Assignment of each glycosidic proton system was achieved by analysis of ^{1}H−^{1}H COSY and HMQC-TOCSY experiments. The diterpene possessed a secondary methyl, three teriary methyls, four olefinic carbons, an oxygenated methine, and an oxygenated quaternary carbon, which suggested that it was similar to (6*S*,13*S*)-cleroda-3,14-diene-6,13-diol (**8**),[5] a common aglycone of the clerodane-type glycosides of *Dicranopteris*.[2,5] The sugar moieties comprised a rhamnopyranosyl and a glucopyranosyl unit by similarity of their spectroscopic data with literature data.[2,5,8] Acid hydrolysis of **3** could not afford aglycone, which was decomposed under acid condition, but gave D-glucose and L-rhamnose. The two monosaccharides were identified by comparison of their R_f and specific rotation with those of authentic samples.[9,10] HMBC correlation between the anomeric proton (δ_H 5.19) of the glucopyranosyl unit and C-4 (δ_C 85.2) of the rhamnosyl unit identified a glucopyranosyl (1→4) rhamnosyl linkage. The C-6 location of the acetyl group in the glucopyranosyl unit was confirmed by HMBC correlations of H-6″ (δ_H 4.85 and 4.75) with the carbonyl carbon (δ_C 170.9) of the acetyl group. Furthermore, the sugar chain was linked to C-6 of the aglycone by HMBC correlation of the anomeric proton (δ_H 5.35) of the rhamnopyranosyl unit and C-6 (δ_C 86.5) of the aglycone. The rhamnopyranosyl and glucopyranosyl units were in α- and β-configurations, respectively, by the coupling constants of their anomeric protons (Table 1).[5]

The relative configuration was established by a ROESY experiment. The ROESY correlations confirmed that H-6, H-8, and H-10 were β-oriented, while Me-17, Me-19, and Me-20 were α-oriented. (6*S*,13*S*)-Cleroda-3,14-diene-6,13-diol (**8**) was also isolated from this plant, and its specific rotation ($[\alpha]^{25}_D$ −6.7) coincided with the reported value.[5] From a biogenetic perspective, compound **8** should be the aglycone of **3** because it was isolated from the same genus. Thus, the structure of **3** was determined as (6*S*,13*S*)-6-*O*-[6-*O*-acetyl-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl]cleroda-3,14-dien-13-ol.

Compound **4** was obtained as a pale yellow powder. Its molecular formula $C_{34}H_{56}O_{12}$ was determined by analysis of ^{1}H, ^{13}C, and DEPT NMR data and verified by HRESIMS (found 655.3700, calcd 655.3693), which revealed 11 unsaturation degrees. The resemblance of the NMR spectra (Tables 1 and 2) to those of **3** suggested that **4** was a related clerodane-type diterpene glycoside. The acetyl group in compound **4** was determined to be at C-4″ (δ_C 72.3) of the glucosyl moiety from the HMBC correlation of H-4″ (δ_H 5.66) to the carbonyl carbon (δ_C 170.5). Sugar analysis of **4** was carried out using the same method as for **3**. Therefore, compound **4** was identified as (6*S*,13*S*)-6-*O*-[4-*O*-acetyl-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl]cleroda-3,14-dien-13-ol.

Compound **5** showed a molecular ion at m/z 655 $[M - H]^-$, and the molecular formula $C_{34}H_{56}O_{12}$ was established by HRESIMS (found 655.3703, calcd 655.3693). The ^{1}H and ^{13}C NMR spectra of **5** (Tables 1 and 2) indicated the presence of a diterpene, two hexoses, and an acetyl group. The ^{1}H and ^{13}C NMR spectra of the aglycone of **5** were similar to those of **3**, except for the resonances of the side chain (Tables 1 and 2). HMBC correlations from Me-16 (δ_H 1.76) to C-14 (δ_C 126.6) and from H_2-15 (δ_H 4.46) to C-13 (δ_C 138.0) confirmed that the side chain had a C-13−C-14 double bond and C-15 hydroxymethyl moiety. The acetyl group was located at C-6″ of the glucosyl moiety by HMBC correlations of H_2 (δ_H 4.85 and 4.74) with the carbonyl carbon (δ_C 170.9) of the acetyl group. Acid hydrolysis of **5** by the same method used for **3** led to decomposition of the aglycone, but gave D-glucose and L-rhamnose. The two monosaccharides were identified by comparison of their R_f and specific rotation with those of authentic samples.

The ROESY correlations of Me-16/H_2-15 established the 13*E* configuration of the C-13−C-14 double bond. The β-orientation of H-6, H-8, and H-10 and the α-orientation of Me-17, M-19, and Me-20 were also confirmed by ROESY correlations (Figure 1). Thus, the structure of **5** was defined as 6-*O*-[6-*O*-acetyl-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl]-(13*E*)-cleroda-3,13-dien-15-ol.

Compound **6** was formulated as $C_{32}H_{54}O_{11}$ from HRESIMS (found 613.3578, calcd 613.3587). The ^{1}H and ^{13}C NMR spectra of **6** (Tables 1 and 2) were co-incident with **5** except for the absence of an acetyl group in **6**. Sugar analysis of **6** was also carried out as for **5**. The ROESY correlations of Me-16 (δ_H 1.77) with H_2-15 (δ_H 4.48) suggested that the double bond between C-13 and C-14 was also *E*-configured. Accordingly, compound **6** was determined to be 6-*O*-[β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl]-(13*E*)-cleroda-3,13-dien-15-ol.

Compounds **1**−**8** were tested for cytotoxicity against C8166 cells (CC50), and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC50), using AZT as a positive control. Compound **8** exerted modest cytotoxic activity

Table 1. ^{1}H NMR Assignments of Compounds **1**−**6**[a]

no.	1	2	3	4	5	6
1 α	1.77 (m)	1.55 (m)	1.50 (m)	1.48 (2H, overlapped)	1.48 (m)	1.50 (m)
1 β	2.10 (m)	1.87 (m)	1.61 (m)		1.52 (m)	1.56 (m)
2 α	2.16 (m)	2.11 (m)	1.92 (2H, m)	1.89 (2H, m)	1.93 (2H, m)	1.95 (2H, m)
2 β	2.30 (m)	2.31 (m)				
3	5.66 (br s)	6.64 (d, 3.2)	5.13 (m)	5.11 (s)	5.18 (overlapped)	5.13 (s)
6	3.98 (t, 4.3)	3.71 (dd, 11.1, 4.5)	3.39 (m)	3.39 (m)	3.39 (m)	3.40 (m)
7 α	1.82 (2H, m)	1.72 (2H, m)	1.60 (overlapped)	1.72 (2H, m)	2.15 (m)	2.15 (m)
7 β			1.51 (overlapped)		1.57 (m)	1.57 (m)
8	2.40 (m)	2.36 (m)	2.15 (m)	2.10 (m)	1.44 (m)	1.45 (m)
10	2.05 (m)	1.82 (m)	1.31 (m)	1.29 (m)	1.23 (m)	1.26 (m)
11 α	2.52 (d, 13.6)	2.51 (2H, m)	1.58 (overlapped)	1.52 (m)	1.23 (m)	1.30 (m)
11 β	2.62 (d, 13.6)		1.51 (overlapped)	1.60 (m)	1.29 (m)	1.39 (m)
12 α			1.58 (m)	1.60 (m)	1.85 (2H, m)	1.87(2H, m)
12 β			1.55 (m)	1.52 (m)		
14			6.18 (m)	6.18 (m)	5.74 (m)	5.75 (m)
15 α			5.24 (overlapped)	5.17 (m)	4.46 (2H, m)	4.48 (2H, m)
15 β			5.57 (dd, 6.5, 17.3)	5.57 (m)		
16			1.51 (3H, s)	1.51 (3H, s)	1.76 (3H, s)	1.77 (3H, s)
17	1.00 (3H, d, 6.7)	0.98 (3H, d, 6.7)	0.79 (3H, d, 6.3)	0.74 (3H,overlapped)	0.72 (3H, d, 6.6)	0.73 (3H, d, 6.7)
18	4.41 (d., 12.7) 4.56 (d., 12.7)	9.42 (s)	1.74 (3H, overlapped)	1.74 (3H, s)	1.74 (3H, s)	1.74 (3H, s)
19	1.80 (3H, s)	1.21 (3H, s)	1.07 (3H, s)	1.07 (3H, s)	1.04 (3H, s)	1.08 (3H, s)
20	0.79 (3H, s)	0.73 (3H, s)	0.74 (3H, s)	0.74 (3H, overlapped)	0.44 (3H, s)	0.67 (3H, s)
Rha						
1			5.35 (br s)	5.33 (br s)	5.35 (br s)	5.34 (br s)
2			4.50 (m)	4.49 (m)	4.48 (m)	4.50 (m)
3			4.54 (m)	4.55 (m)	4.52 (m)	4.54 (m)
4			4.38 (t, 8.3)	4.44 (t, 8.3)	4.40 (t, 8.2)	4.45 (t,8.3)
5			4.20 (m)	4.20 (m)	4.21 (m)	4.24 (m)
6			1.74 (3H, overlapped)	1.74 (3H, overlapped)	1.72 (3H, overlapped)	1.69 (3H, d, 6.3)
Glc						
1			5.19 (d, 7.3)	5.28 (d, 7.3)	5.20 (d, 7.2)	5.26 (d, 7.2)
2			4.12 (t, 7.3)	4.29 (m)	4.16 (m)	4.16 (t, 7.2)
3			4.16 (m)	4.15 (m)	4.17 (m)	4.25 (m)
4			4.00 (t, 7.3)	5.66 (m)	4.00 (t, 7.2)	4.29 (t, 7.2)
5			3.86 (m)	3.84 (m)	3.85 (m)	3.80 (m)
6			4.85 (d, 11.3) 4.74 (m)	4.15 (m) 4.06 (m)	4.85 (d, 11.3) 4.75 (m)	4.47 (m) 4.38 (m)
Ac						
CH_3			1.95 (3H, s)	1.98 (3H, s)	1.93 (3H, s)	

[a] Spectra were recorded in C_5D_5N; chemical shifts (δ) are in ppm and J in Hz.

against C8166 cells with CC_{50} > 653.04 μM and showed anti-HIV-1 activity with EC_{50} = 59.43 μM and a selectivity index (CC_{50}/EC_{50}) more than 10.99.

Experimental Section

General Experimental Procedures. Both 1D and 2D NMR experiments were performed on a Bruker AM-400 or a DRX-500 spectrometer. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. MS was recorded on a VG Auto Spec-3000 or a Finnigan MAT 90 intrument. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer for KBr pellets. UV data were obtained on a UV 210A spectrometer. Optical rotations were measured with a Horiba SEPA-300 polarimeter or a Perkin-Elmer model 241 polarimeter. Column chromatography was performed either on silica gel (200−300 mesh, Qingdao Marine Chemical, China), silica gel H (10−40 μm, Qingdao Marine Chemical, China), or MCI gel CHP20P (75−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Semipreparative HPLC was performed on a Hewlett-Packard instrument (column: Zorbax SB-C18, 250 × 9.4 mm; UV detector). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The fronds of *D. dichotoma* were collected from Xiaowei Mountain, Nanxi, Hekou County, Yunnan Province, P. R. China, in March 2005. A specimen (CZ 007) was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences, and was identified by one of the authors (X.C.).

Extraction and Isolation. The dry fronds of *D. dichotoma* (32 kg) were powdered and extracted with acetone (3 × 30 L) for 24 h at room temperature. The acetone extract was concentrated in vacuo to give a crude extract (2.3 kg), which was then suspended in H_2O and subjected to column chromatography over DM 130 eluting with H_2O and 95% EtOH, respectively. The fraction eluting by 95% EtOH was concentrated in vacuo to give a residue (1.5 kg), which was then subjected to column chromatography over silica gel (200−300 mesh) eluting with $CHCl_3$−MeOH (from 1:0 to 0:1) to afford fractions A−H. Fraction B was subjected to column chromatography over silica gel eluting with $CHCl_3$−MeOH (100:1) to yield **1** (30 mg), **2** (10 mg), **7** (20 mg), and **8** (47 mg). Fraction D was subjected to RP-18 and silica gel column chromatography, repeatedly, to afford **6** (100 mg). Further purification on Sephadex LH-20, RP-18, and semipreparation HPLC (Agilent 1100 HPLC system; Zorbax SB-C18, 250 × 9.4 mm; UV detector) yielded **3** (25 mg), **4** (7.8 mg), and **5** (6 mg).

Compound 1: colorless crystals from MeOH; mp 207−208 °C; $[\alpha]^{18}_{D}$ −51.9 (c 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 nm (2.1); IR (KBr) ν_{max} 3483, 3251, 2960, 2857, 1700, 1252 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; negative ESIMS m/z 281 $[M - H]^-$; HRESIMS m/z 281.1755 (calcd for $C_{16}H_{26}O_4$ 281.1752).

Compound 2: colorless crystals from MeOH; mp 161−162 °C; $[ga]^{17}_{D}$ −43.3 (c 0.8, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 nm (2.5); IR (KBr) ν_{max} 3435, 2963, 2889,1709, 1228, 991 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; negative ESIMS m/z 279 $[M - H]^-$; HRESIMS m/z 279.1596 (calcd for $C_{16}H_{24}O_4$ 279.1596).

Compound 3: pale yellow powder; $[\alpha]^{18}_{D}$ −50.5 (c 3.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 nm (2.4); IR (KBr) ν_{max} 3424, 2960,

Table 2. ^{13}C NMR Assignments of **1−6**[a]

no.	1	2	3	4	5	6
1	19.4 t	18.7 t	18.1 t	18.1 t	17.9 t	17.9 t
2	27.2 t	29.0 t	27.0 t	27.0 t	27.0 t	27.0 t
3	126.0 d	157.8 d	123.1 d	123.0 d	122.9 d	123.0 d
4	147.6 s	151.4 s	143.6 s	143.6 s	143.6 s	143.6 s
5	45.0 s	45.2 s	44.2 s	44.2 s	44.1 s	44.0 s
6	74.9 d	74.1 d	86.5 d	86.4 d	86.3 d	86.2 d
7	37.2 t	36.7 t	35..3 t	35.3 t	35.2 t	35.2 t
8	36.2 d	35.6 d	34.4 d	34.4 d	34.3 d	34.3 d
9	40.5 s	40.4 s	38.1 s	38.1 s	38.4 s	38.6 s
10	47.3 d	46.7 d	46.0 d	46.1 d	45.9 d	45.9 d
11	43.8 t	43.6 t	32.7 t	32.7 t	37.2 t	37.2 t
12	174.4 s	174.3 s	35.9 t	35.9 t	25.6 t	25.6 t
13			72.6 s	72.6 s	138.0 s	140.0 s
14			147.2 d	147.3 d	126.6 d	126.6 d
15			111.5 t	111.4 t	58.7 t	58.7 t
16			28.6 q	28.7 q	23.8 q	23.4 q
17	16.4 q	16.3 q	16.0 q	16.0 q	15.9 q	15.9 q
18	66.3 t	198.2 d	23.0 q	22.9 q	23.0 q	22.9 q
19	17.0 q	16.8 q	16.1 q	16.4 q	16.3 q	16.3 q
20	17.5 q	17.0 q	18.4 q	18.3 q	18.0 q	18.0 q
Rha						
1			103.4 d	103.3 d	103.4 d	103.3 d
2			72.3 d	72.7 d	72.2 d	72.2 d
3			72.8 d	72.8 d	72.8 d	72.8 d
4			85.2 d	84.9 d	85.2 d	85.2 d
5			68.3 d	68.3 d	68.3 d	68.4 d
6			18.2 q	18.2 q	18.1 q	18.2 q
Glc						
1			106.6 d	106.5 d	106.7 d	106.9 d
2			76.4 d	76.0 d	76.4 d	76.6 d
3			78.4 d	76.6 d	78.4 d	78.6 d
4			71.6 d	72.3 d	71.6 d	71.5 d
5			75.3 d	76.0 d	75.4 d	78.6 d
6			64.6 t	62.2 t	64.6 t	62.7 t
Ac						
CH_3			20.8 q	21.1 q	20.8 q	
CO			170.9 s	170.5 s	170.9 s	

[a] Spectra were recorded in C_5D_5N; chemical shifts (δ) are in ppm.

Table 3. Cytotoxicity and Anti-HIV-1 Activity of Compounds **1−8**

compound	cytotoxicity, CC_{50} (μM)	anti-HIV-1 activity, EC_{50} (μM)	selectivity index, CC_{50}/EC_{50}
1	708.52	301.51	>2.34
2	713.37	325.30	>2.19
3	120.93	110.29	1.10
4	304.51	145.40	>2.09
5	135.7	76.31	1.78
6	325.33	85.06	>3.82
7	750.81	197.01	>3.81
8	653.04	59.43	>10.99
AZT	5746.1	0.0147	390 406.06

2924, 2877,1731, 1642, 1451, 1384, 1053 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; negative ESIMS *m/z* 655 [M − H]$^-$; HRESIMS *m/z* 655.3691 (calcd for $C_{34}H_{56}O_{12}$ 655.3693).

Compound 4: pale yellow powder; $[\alpha]^{18}_{D}$ −16.2 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 nm (2.2); IR (KBr) ν_{max} 3441, 2959, 2922, 2852,1728, 1634, 1451, 1384, 1059 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; negative ESIMS *m/z* 655 [M − H]$^-$; HRESIMS *m/z* 655.3700 (calcd for $C_{34}H_{56}O_{12}$ 655.3693).

Compound 5: pale yellow powder; $[\alpha]^{18}_{D}$ −47.8 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 nm (2.0); IR (KBr) ν_{max} 3426, 2961, 2927, 2876,1741, 1633, 1450, 1384, 1054 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; negative ESIMS *m/z* 655 [M − H]$^-$; HRESIMS *m/z* 655.3703 (calcd for $C_{34}H_{56}O_{12}$ 655.3693).

Compound 6: pale yellow powder; $[\alpha]^{18}_{D}$ −62.1 (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 nm (2.7); IR (KBr) ν_{max} 3419, 2961, 2934, 2877, 1636, 1450, 1384, 1058 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; negative ESIMS *m/z* 613 [M − H]$^-$; HRESIMS *m/z* 613.3578 (calcd for $C_{32}H_{54}O_{11}$ 613.3587).

Acid Hydrolysis of 3, 4, 5, and 6. A solution of **3** (10 mg) in 2 M HCl (3 mL) was heated in a water bath at 70 °C for 6 h. After cooling, the reaction mixture was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. Through TLC comparison with an authentic sample using $CHCl_3$−MeOH (8:2) as a developing system, D-glucose and L-rhamnose were detected in the water layer (R_f = 0.16 and 0.43, respectively). The aqueous solution was further concentrated to dryness and subjected to silica gel chromatography eluting with $CHCl_3$−MeOH (9:1) to give D-glucose (2 mg), $[\alpha]^{18}_{D}$ +40 (*c* 0.2, MeOH), and L-rhamnose (1.3 mg), $[\alpha]^{18}_{D}$ +10 (*c* 0.15, MeOH). The aglycone of **3** was not obtained, because the TLC of the $CHCl_3$ part indicated that there were at least four products, and was not subjected to further isolation and identification due to the limited amount. Acid hydrolysis of **4**, **5**, and **6** by the same method used for **3** led to decomposition of the aglycones, but gave D-glucose and L-rhamnose. The two monosaccharides were identified by comparison of their R_f and specific rotation with those of authentic samples.

Anti-HIV-1 Assay. Cytotoxicity against C8166 cells (CC_{50}) was assessed using the MTT method, and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}).[11]

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