Chemical Studies on an Endemic Philippine Plant: Sulfated Glucoside and *seco*-A-Ring Triterpenoids from *Dillenia philippinensis*

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The leaves of the endemic Philippine plant, *Dillenia philippinensis* yielded 11 compounds including one new sulfated glucoside and a new *seco*-A-ring oleanane-type triterpenoid. The molecular structures of these compounds were elucidated by means of NMR, MS and other spectroscopic techniques, as well as by comparison with literature data. Anti-Leishmania activity and cytotoxic activity against A549 human lung adenocarcinoma cells were also examined.

Key words *Dillenia philippinensis*; Dilleniaceae; sulfated glucoside; *seco*-A-ring triterpenoid

Dillenia philippinensis Rolfe, known as katmon in most of the local Philippine languages, belongs to the family Dilleniaceae of which there are approximately 120 species. The species *D. philippinensis* is endemic to the Philippine islands, common in forests at low and medium altitudes.[1,2] Many *Dillenia* species are used in traditional medicine for various conditions, such as cancer, diarrhea and arthritis.[3,4] In the Philippines, the acidic juice of the fruit is used traditionally as a cure for coughs and is also used as a hair cleanser. The fruit is used for cooking to impart a sour flavor to some native dishes.[5] Phytochemical studies on *Dillenia* species have shown the presence of flavonoids such as isorhamnetin and naringenin and triterpenoids mainly of the lupane and oleanane types.[6—8] In the present study, the isolation and structure elucidation of sulfated glucoside and *seco*-A-ring oleanane-type triterpenoids is described.

Table 1. ^{13}C- and ^{1}H-NMR Spectral Data for Compound **1** (100 and 400 MHz, Respectively, CD_3OD) δ ppm and J in Hz

	C	H
1	139.0	—
2, 6	129.3	7.42 dd 7.1, 1.4
3, 5	129.2	7.31 dd 7.1, 7.1
4	128.7	7.25 tt 7.1, 1.4
7	71.8	4.63 d 11.8
		4.88 d 11.8
1′	103.2	4.33 d 7.7
2′	75.1	3.25 dd 9.4, 7.7
3′	77.9	3.35 m
4′	71.5	3.35 m
5′	76.1	3.46 m
6′	68.3	4.15 dd 11.0, 5.8
		4.35 dd 11.0, 2.0

m: multiplet or overlapped signal.

Results and Discussion

The air-dried and powdered leaves of *D. philippinensis* were extracted with MeOH at ambient temperature. The MeOH extract was concentrated to 6 l *in vacuo* and washed with *n*-hexane. The methanolic layer was concentrated, suspended in water and then extracted with EtOAc and 1-BuOH, successively. The 1-BuOH- and EtOAc-soluble fractions were subjected to Diaion HP-20, silica gel, octadecylsilanized silica gel (ODS) column chromatographies, droplet counter-current chromatography (DCCC), and HPLC to afford two new compounds (**1**, **2**). The spectral assignments for a previously described triterpenoid (**3**) were also established as well as the structures of two triterpenoids (**4**, **5**) which might be considered as artifacts from the extraction process.

Compound **1**, $[\alpha]_D^{26}$ −27.9, was isolated as an amorphous solid with a molecular formula of $C_{13}H_{18}O_9S$ as determined by high resolution-electrospray ionization-mass spectrometry (HR-ESI-MS). The IR spectrum showed absorption bands characteristic for hydroxyl groups (3390 cm^{-1}), aromatic absorptions (1651, 1508 cm^{-1}) and a sulfonyl functional group (1456 cm^{-1}). The ^{1}H-NMR spectrum showed an anomeric proton signal at δ_H 4.33 (1H, d, J=7.7 Hz) corresponding to a β-glucopyranosyl moiety, oxymethylene proton signals at δ_H 4.63 (1H, d, J=11.8 Hz) and δ_H 4.88 (1H, d, J=11.8 Hz) and aromatic resonances for five protons (Table 1). The ^{13}C-NMR spectral data displayed 13 resonances (Table 1), six of which were attributable to a β-glucopyranosyl moiety. The remaining seven signals consisting of an oxymethylene carbon signal at δ_C 71.8, one quaternary aromatic carbon at δ_C 139.0 and five aromatic methine signals at δ_C 129.3 (2C), δ_C 129.2 (2C) and δ_C 128.7 (1C) were assigned to a benzyl moiety. The attachment of the glucosyl moiety onto the hydroxyl group at the 7-position was deduced from the observation of a long-range correlation between the anomeric proton (δ_H 4.33) and the oxymethylene carbon (δ_C 71.8) establishing **1** to be a benzyl glucoside. Similarly to **6**, the downfield-shift displayed by the 6′-position of the β-glucopyranosyl moiety suggested the attachment of sulfonyl moiety at that position, which was substantiated by the measurement of the mass in a deuterium labile solvent. The ESI-MS showed a single peak corresponding to $C_{13}H_{14}D_3O_9S$ $[M-H]^-$, consistent with the structure of a sulfonated benzyl glucoside. Therefore, the structure of **1** elucidated as shown in Fig. 1. Compound **1** was known as a synthetic material.[9] However, **1** was isolated as a natural compound for the first time.

Compound **2**, $[\alpha]_D^{26}$ +84.3, was isolated as an amorphous solid with a molecular formula of $C_{30}H_{46}O_6$ as determined by HR-ESI-MS. The IR spectrum showed absorption bands characteristic for an olefinic group (1436 cm^{-1}) and carbonyl moieties (1690, 1712 cm^{-1}). The ^{1}H-NMR spectrum showed seven singlet methyl signals at δ_H 0.75, 0.93, 0.97, 1.07, 1.10

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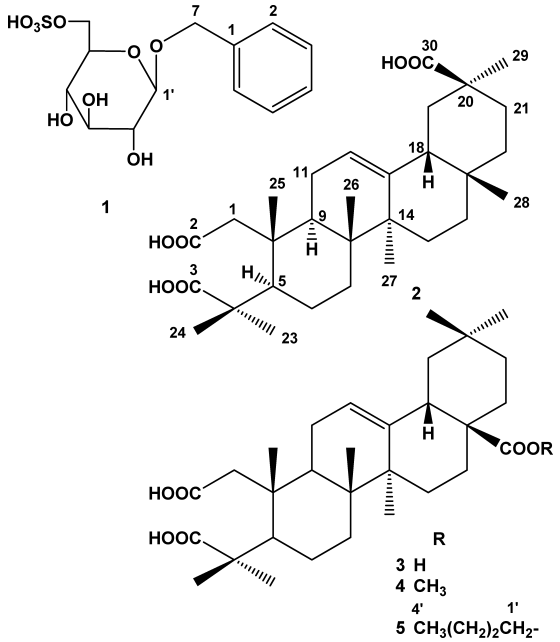

Fig. 1. Structures of the Isolated Compounds

and 1.15 (×2), an olefinic proton signal at δ_H 5.19 (1H, br s), as well as a pair of methylene signals at δ_H 2.17 and 2.50 each (each doublet, J=16.5 Hz) (Table 2). The ^{13}C-NMR spectrum displayed 30 resonances consisting of ten quaternary signals one of which was for an olefinic group and three were for carbonyl moieties, four methine signals of which one was for an olefinic group, nine methylene signals and seven methyl signals corresponding to the seven methyl resonances in the ^{1}H-NMR spectrum (Table 2). Inspection of both ^{1}H- and ^{13}C-NMR spectra suggested a triterpenoid structure of the oleanane-type skeleton. The correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond connectivity (HMBC) experiments and comparison with literature data confirmed the structure to be a *seco*-A ring oleanane-type triterpenoid with three carboxylic acid groups at positions 2, 3 and 30.[10—13] The presence of a carbonyl moiety at the 2-position is also consistent with the splitting pattern of the methylene resonances at δ_H 2.17 and δ_H 2.50, assigned to the protons at the 1-position.[10—13] The positions of two carboxylic acid groups were substantiated by the observation of the the HMBC correlation between the methylene protons (H_2-1) and the carbonyl resonance (C-2) at δ_C 172.7, between the methyl protons (H_3-23) at δ_H 1.15 (3H, s) and the carbonyl resonance (C-3) at δ_C 181.1 (Fig. 2). The position of the third carboxylic acid group was established by the presence long-range cross-peaks between the methyl protons (H_3-29) at δ_H 1.07 (3H, s) and the carbonyl resonance at δ_C 177.9. The position of the olefinic moiety was confirmed by the presence of the long-range correlations between the olefinic proton (δ_H 5.19) and C-9 (δ_C 39.0), C-14 (δ_C 41.8) and C-18 (δ_C 47.8), as well as a COSY correlation between the olefinic proton and the methylene protons (H_2-11) at δ_H 1.83.

The configuration at the 20-position was established by careful interpretation of the nuclear Overhauser effect spectroscopy (NOESY) spectrum which showed correlations between the methyl protons at δ_H 1.07 (H_3-29) and the protons at δ_H 1.71 and 1.78 (H-19*eq*, H-21*eq*, respectively) as well as between the methine proton at δ_H 1.87 (H-18) and the δ_H proton at 1.59 (H-19*ax*) and between the H-19*ax* proton and the H-21*ax* proton at δ_H 1.28. The methyl protons at δ_H 0.75 (H_3-28) also displayed NOE correlations with the H-18 and H-19*ax* protons establishing the structure of **2** (Fig. 3).

Table 2. ^{13}C- and ^{1}H-NMR Spectral Data for Compounds **2** and **3** (100 and 400 MHz, Respectively, DMSO-d_6) δ ppm and J in Hz

	2		**3**	
	C	H	C	H
1	42.8	2.17 d 16.5 2.50 d 16.5	42.0	2.25 d 17.9 2.45 d 17.9
2	172.7	—	172.6	—
3	181.1	—	177.9	—
4	46.0	—	43.1	—
5	48.0	2.23 m	47.3	2.42 br d 9.9
6	21.0	1.41 m 1.48 m	21.2	1.48 m
7	31.5	1.48 m	31.5	1.48 m
8	39.2	—	39.2	—
9	39.0	2.45 m	38.9	2.61 dd 8.6, 8.6
10	41.3	—	41.1	—
11	23.2	1.83 m	23.2	1.85 m
12	122.3	5.19 br s	122.1	5.18 br s
13	143.7	—	143.9	—
14	41.8	—	41.8	—
15	25.7	1.48 m 1.82 m	25.7	1.48 m 1.74 m
16	26.4	1.92 ddd 13.4, 13.4, 4.5	26.5	1.95 ddd 13.4, 13.4, 3.5
17	31.6	—	46.1	—
18	47.8	1.87 m	47.8	1.88 m
19	42.4	1.59 br dd 13.2, 13.2 1.71 ddd 13.2, 3.2, 3.2	42.4	1.61 br dd 13.5, 13.5 1.72 ddd 13.5, 3.3, 3.3
20	43.1	—	31.6	—
21	30.6	1.28 m 1.78 m	30.6	1.28 m 1.78 m
22	38.0	1.25 m	38.0	1.26 m
23	24.3	1.15 s	24.9	1.20 s
24	27.2	1.15 s	28.1	1.07 s
25	18.8	0.97 s	18.9	0.98 s
26	16.5	0.93 s	16.5	0.92 s
27	25.1	1.10 s	25.2	1.12 s
28	28.1	0.75 s	180.2	—
29	28.1	1.07	25.7	1.16 s
30	177.9	—	28.1	0.74 s

m: multiplet or overlapped signal.

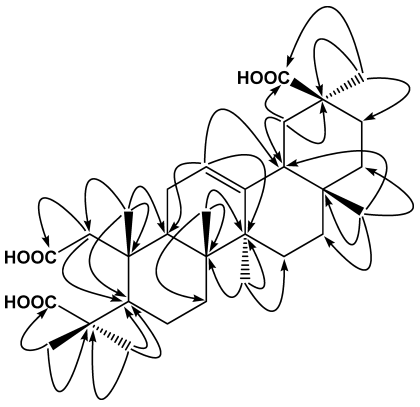

Fig. 2. Diagnostic HMBC Correlations for **2**

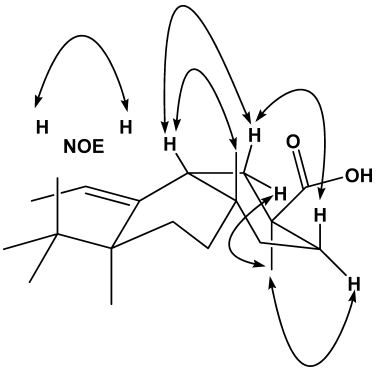

Fig. 3. Diagnostic NOE Correlations for **2**

Table 3. Growth Inhibition against A549 and *Leishmania major*

Cpd	A549	*L. major*
1	na	na
2	na	na
3	na	na
4	44.6±4.1	46.6±4.0
5	15.4±1.7	56.7±6.1
6	na	na
7	na	na
8	48.0±2.8	67.1±1.8
9	25.2±2.5	45.0±3.0
10	na	na
11	na	na
Dox	0.6±0.1	—
Amp	—	7.1±0.8

na: no activity. Values: IC_{50}±S.D. in μM. Dox: doxorubicin. Amp: amphotericin B.

Compound **3**, $[\alpha]_D^{26}$ −79.1, was isolated as an amorphous solid with a molecular formula of $C_{30}H_{46}O_6$ as determined by HR-ESI-MS. Inspection of both ^{1}H- and ^{13}C-NMR spectra suggested a triterpenoid structure of the oleanane-type skeleton. COSY, HSQC and HMBC experiments and comparison with literature data confirmed the structure to be a *seco*-A ring derivative of oleanolic acid with two more carboxylic acid groups at positions 2 and 3.[10—13] Compound **3** has been described previously by Crowley as 2,3-*seco*-olean-12-ene-2,3,28-trioic acid, isolated from the heartwood of *Bursera graveolens* var. *villosula*.[13] The spectroscopic assignments for **3** are described here unambiguously for the first time.

Compound **4**, $[\alpha]_D^{26}$ +145, and compound **5**, $[\alpha]_D^{26}$ +100, were analogous compounds to compound **3**. In the ^{13}C-NMR spectrum, two carboxyl signals at C-2 and C-3 positions remained intact, whereas that of C-28 was obviously shifted upfield by esterification. The ester moieties of compounds **4** and **5** were found to be methyl and butyl groups, respectively, from NMR data. These compounds were probably formed during extraction and purification.

Five known compounds (**6**—**11**) were also isolated and identified as corchoionoside C6′-*O*-sulfate (**6**),[14] betulinic acid (**7**),[15] messagenic acid (**8**),[16] 2α,3β-dihydroxyolean-12-en-28-oic acid (**9**),[17] (3*S*,5*R*,6*R*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetraol 3-*O*-β-D-glucopyranoside (**10**),[18,19] and tiliroside (**11**).[20—22]

Results of activity assay revealed that compounds **4**, **5**, **8** and **9** exhibited moderate growth inhibition activity against *Leishmania major* parasites and A549 human lung adenocarcinoma cells (Table 3). Correlation of the results for both assays suggested that these compounds had a general cytotoxic activity and were not specific against the parasites. Interestingly, of the *seco*-A ring oleanane derivatives, only the acylated compounds (**4**, **5**) were active, whereas the tricarboxylic acid forms (Compounds **2**, **3**) were found to be inactive.

Conclusion

The structures of one new sulfated glucoside and a new *seco*-A-ring oleanane-type triterpenoid were elucidated by means of NMR, MS and other spectroscopic techniques, as well as comparison with literature data. The isolated compounds were also tested against anti-*Leishmania major* and cytotoxicity assays.

Experimental

General Experimental Procedures IR spectra were obtained on a Horiba Fourier transform infrared spectrophotometer FT-710. Optical rotation data were measured on a JASCO P-1030 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM α-400 spectrometer at 400 MHz and 100 MHz, respectively, with tetramethylsilane as an internal standard. HR-FAB mass spectra (negative-ion mode) and HR-ESI mass spectra (positive-ion mode) were taken on a JEOL JMS-SX 102 mass spectrometer and an Applied Biosystems QSTAR XL System, respectively.

Highly-porous synthetic resin Diaion HP-20 was purchased from Mitsubishi Chemical Co., Ltd. (Tokyo, Japan). Silica gel column chromatography (CC) was performed on silica gel 60 [(E. Merck, Darmstadt, Germany), 70—230 mesh]. Reversed-phase ODS open CC (RPCC) was performed on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) [Φ=2 cm, L=40 cm, linear gradient: MeOH–H_2O (1 : 9, 1.5 l)→(7 : 3, 1.5 l), 10-g fractions being collected]. The DCCC (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ=2 mm, L=40 cm), and the lower and upper layers of a solvent mixture of $CHCl_3$: MeOH : H_2O : 1-PrOH (9 : 12 : 8 : 2) were used as the mobile and stationary phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on an ODS (Inertsil; GL Science, Tokyo, Japan; Φ=6 mm, L=25 cm, flow rate: 1.6 ml/min) column, using refractive index and/or UV detectors. Precoated silica gel 60 F_{254} plates (E. Merck; 0.25 mm in thickness) were used for TLC analyses, visualized by spraying with a 10% solution of H_2SO_4 in ethanol and heated to around 150 °C on a hotplate.

Plant Material The leaves of *D. philippinensis* were collected in Santa Cruz, Laguna, the Philippines in 2008 and identified by Dr. Inocencio E. Buot, Jr., a professor of ecology and systematics at the Institute of Biological Sciences, University of the Philippines at Los Banos, Laguna, the Philippines. A voucher specimen was deposited in the Herbarium of Ateneo de Manila University, the Philippines (Accession No. 198).

Extraction and Isolation Air-dried leaves of *D. philippinensis* (4.00 kg) were extracted with MeOH (45 l×3) for a week at ambient conditions, and then the MeOH extract was concentrated to 6 l *in vacuo*. The extract was washed with *n*-hexane (6 l) and then the methanolic layer was concentrated to a viscous gum (*n*-hexane-soluble fraction: 65.5 g). The residue was suspended in H_2O (6 l) and then extracted with EtOAc (6 l) and 1-BuOH (6 l), successively to afford 213 g of EtOAc-soluble and 144 g of 1-BuOH-soluble fractions. Evaporation of the H_2O layer left 100 g of residue.

The 1-BuOH-soluble fraction was applied to Diaion HP-20 CC (Φ=60 mm, L=50 cm) and eluted with a stepwise-gradient of MeOH–H_2O (1 : 4, 2 : 3, 3 : 2 and 4 : 1, 3 l each) and MeOH (3 l), 500-ml fractions being collected. The residue (12.2 g) eluted with 20% MeOH in fraction 1 obtained on HP-20 CC was subjected to silica gel CC (600 g) using $CHCl_3$ (2 l), $CHCl_3$: MeOH (49 : 1, 24 : 1, 23 : 2, 9 : 1, 17 : 3, 4 : 1, 3 : 1 and 7 : 3, 3 l each), and $CHCl_3$: MeOH : H_2O (70 : 30 : 4, 3 l), 500-ml fractions being collected. The residue (109 mg) from fractions 24—28 was then subjected to DCCC to afford 14.6 mg of **10** from fractions 41—49. The residue (4.64 g) from fractions 43—63 was then subjected to RPCC to afford 607 mg of a residue (fractions 14—30) which was further subjected to DCCC. The residue from fractions 15—21 (188 mg) was subsequently purified by HPLC (20% MeOH in H_2O) to yield 12.7 mg of **1** from the peak at 6.5 min and 35.9 mg of **6** from the peak at 7.4 min.

The residue (10.0 g) eluted with 80% MeOH in fraction 5 obtained on HP-20 CC was subjected to silica gel CC (750 g) using $CHCl_3$ (3 l),

$CHCl_3$: MeOH (49 : 1, 24 : 1, 23 : 2, 9 : 1, 17 : 3, 4 : 1, 3 : 1 and 7 : 3, 4.5 l each), and $CHCl_3$: MeOH : H_2O (70 : 30 : 4, 4.5 l), 500-ml fractions being collected. A white precipitate (3.51 g) formed in fractions 8—16 of the 8% MeOH in $CHCl_3$ eluate was separated and identified as betulinic acid (**7**). The remaining supernatant liquid was dried and the residue (6.22 g) was further subjected to silica gel CC (400 g) using $CHCl_3$ (1 l), $CHCl_3$: MeOH (99 : 1, 49 : 1, 19.5 : 0.5, 19 : 1, 9 : 1, 1 l each), and MeOH (1 l), 250-ml fractions being collected. From the second silica gel experiment, the residue (726 mg) from fractions 174—350 was subsequently subjected to RPCC (Φ=30 mm, L=20 cm) yielding 12.0 mg of **8** from fractions 95—98, 14.6 mg of **9** from fractions 109—110 and 47.1 mg of **5** from fractions 117—118. From the same RPCC experiment, a white solid (4.70 mg) that crystallized out from the tenth fraction (140—175) was identified as **5**. The remaining filtrate from that fraction was dried to yield 186 mg of a residue which was then subjected to silica gel (Φ=25 mm, L=20 cm) to yield 38.6 mg of **4** from fractions 38—40.

From the first silica gel experiment, the residue (327 mg) from fractions 49—55 was subsequently subjected to RPCC (Φ=30 mm, L=20 cm) yielding 179 mg of a residue (191—240) which was subsequently purified by DCCC. The residue (96.4 mg) from the last fraction was then applied to a silica gel column (100 g) using $CHCl_3$: MeOH : H_2O (15 : 6 : 1), 20-ml fractions being collected, to yield 32.8 mg of **2** from fractions 23—24.

The EtOAc-soluble fraction was applied to silica gel CC (750 g) using hexane : EtOAc (9 : 1, 4 : 1, 7 : 3, 3 : 2, 1 : 1, 6 l each), EtOAc (6 l), EtOAc : MeOH (9 : 1, 4 : 1, 7 : 3, 3 : 2, 1 : 1, 6 l each), 1-l fractions being collected. The residue (10.6 g) in fractions 40—43 of the 10% MeOH in EtOAc eluate was further subjected to silica gel CC (400 g) using $CHCl_3$: MeOH : H_2O (17 : 6 : 1, 4 l), and MeOH (1 l), 20-ml fractions being collected. The residue (1.14 g) from fractions 44—100 was then subjected to RPCC to afford 53.0 mg of **11** from fractions 181—210. A white solid (49.7 mg) that precipitated out from the 13th fraction (229—242) was identified as **3**.

Compound **1**: Amorphous solid. $[\alpha]_D^{26}$ −27.9 (c=1.27, MeOH). IR ν_{max} (film) cm^{-1}: 3390, 1651, 1508, 1456, 1225, 1066, 1003. UV λ_{max} (MeOH) nm (log ε): 343 (3.14), 324 (3.13), 242 (3.33). ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD): Table 1. HR-ESI-MS (negative-ion mode) m/z: 349.0591 $[M-H]^-$ (Calcd for $C_{13}H_{17}O_9S$: 349.0598); m/z: 352.0794 $[M-D]^-$ (in D_2O) (Calcd for $C_{13}H_{14}D_3O_9S$: 352.0784).

Compound **2**: Amorphous solid. $[\alpha]_D^{26}$ +84.3 (c=3.03, dimethyl sulfoxide (DMSO)). IR ν_{max} (film) cm^{-1}: 2953, 2919, 1712, 1690, 1436, 1176, 1025, 953. ^{1}H-NMR (400 MHz, DMSO-d_6) and ^{13}C-NMR (100 MHz, DMSO-d_6): Table 2. HR-ESI-MS (negative-ion mode) m/z: 501.3225 $[M-H]^-$ (Calcd for $C_{30}H_{45}O_6$: 501.3221).

Compound **3**: Amorphous solid. $[\alpha]_D^{26}$ −79.0 (c=1.00, C_5H_5N). IR ν_{max} (film) cm^{-1}: 2970, 2865, 1720, 1700, 1455, 1177, 1028, 953. ^{1}H-NMR (400 MHz, DMSO-d_6) and ^{13}C-NMR (100 MHz, DMSO-d_6): Table 2. HR-ESI-MS (positive-ion mode) m/z: 525.3180 $[M+Na]^+$ (Calcd for $C_{30}H_{46}O_6Na$: 525.3192).

Compound **4**: Amorphous solid. $[\alpha]_D^{26}$ +145 (c=0.21, DMSO). IR ν_{max} (film) cm^{-1}: 2972, 2867, 1723, 1696, 1448, 1136, 1029, 955. δ: 0.74 (3H, s, H_3-30), 0.92 (3H, s, H_3-26), 0.94 (3H, s, H_3-25), 1.06 (3H, s, H_3-24), 1.11 (3H, s, H_3-27), 1.18 (3H, s, H_3-29), 1.19 (3H, s, H_3-23), 1.26 (3H, m, H-7a, H_2-22), 1.28 (1H, m, H-21a), 1.41 (1H, m, H-6a), 1.50 (3H, m, H-6b, 7b, 15a), 1.60 (1H, d, J=13.4 Hz, H-19a), 1.69 (1H, m, H-15b), 1.71 (1H, ddd, J=13.4, 3.2, 3.2 Hz, H-19b), 1.77 (1H, m, H-21b), 1.82 (1H, m, H-11a), 1.87 (1H, dd, J=9.0, 3.8 Hz, H-18), 1.93 (1H, m, H-16b), 1.97 (1H, ddd, J=9.3, 9.3, 4.3 Hz, H-11b), 2.16 (1H, d, J=18.3 Hz, H-1a), 2.25 (1H, d, J=18.3 Hz, H-1b), 2.40 (1H, d, J=8.6 Hz, H-5), 2.67 (1H, dd, J=10.3, 7.4 Hz, H-9), 3.55 (3H, s, $COOCH_3$), 5.18 (1H, br s, H-12). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 16.4 (CH_3-26), 18.7 (CH_3-25), 20.5 (CH_2-6), 23.2 (CH_2-11), 24.0 (CH_3-23), 25.1 (CH_3-27), 25.7 (CH_2-15), 26.4 (CH_2-16), 26.8 (CH_3-29), 28.1 (CH_3-24/CH_3-30), 30.6 (CH_2-21), 31.4 (CH_2-7), 31.6 (C-20), 38.0 (CH_2-22), 38.4 (CH-9), 39.1 (C-8), 40.8 (C-10), 41.3 (CH_2-1), 41.8 (C-14), 42.3 (CH_2-19), 43.1 (C-4), 45.8 (C-17), 47.8 (CH-18), 51.5 ($COOCH_3$), 122.0 (CH-12), 143.9 (C-13), 172.2 (C-2), 177.8 (C-3), 178.8 (C-28). HR-ESI-MS (positive-ion mode) m/z: 539.3330 $[M+Na]^+$ (Calcd for $C_{31}H_{48}O_6Na$: 539.3349).

Compound **5**: Amorphous solid. $[\alpha]_D^{26}$ +100 (c=0.47, DMSO). IR ν_{max} (film) cm^{-1}: 2982, 2913, 1712, 1649, 1436, 1313, 1046, 1030, 954. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.74 (3H, s, H_3-30), 0.89 (3H, t, J=7.4 Hz, H_3-4′), 0.92 (3H, s, H_3-26), 0.95 (3H, s, H_3-25), 1.06 (3H, s, H_3-24), 1.11 (3H, s, H_3-27), 1.18 (3H, s, H_3-29), 1.20 (3H, s, H_3-23), 1.26 (3H, m, H-7a, H_2-22), 1.28 (1H, m, H-21a), 1.38 (1H, m, H-6a), 1.52 (3H, m, H-6b, 7b, 15a), 1.53 (2H, m, H_2-2′), 1.60 (1H, d, J=13.6 Hz, H-19a), 1.69 (3H, m, H_2-3′, H-15b), 1.70 (1H, ddd, J=13, 3, 3 Hz, H-19b), 1.77 (1H, m, H-21b), 1.82 (1H, m, H-11a), 1.88 (1H, m, H-18), 1.92 (1H, dd, J=13.4, 3.9 Hz, H-16b), 1.97 (1H, ddd, J=13.5, 13.5, 4.2 Hz, H-11b), 2.25 (2H, br s, H_2-1), 2.42 (1H, d, J=9.5 Hz, H-5), 2.63 (1H, dd, J=9.2, 9.2 Hz, H-9), 3.85 (1H, ddd, J=11.0, 6.3, 6.3 Hz, H-1′a), 4.06 (1H, ddd, J=11.0, 6.3, 6.3 Hz, H-1′b), 5.18 (1H, br s, H-12). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 13.4 (CH_3-4′), 16.4 (CH_3-26), 18.6 (CH_2-3′), 18.8 (CH_3-25), 20.9 (CH_2-6), 23.2 (CH_2-11), 24.5 (CH_3-23), 25.1 (CH_3-27), 25.7 (CH_2-15), 25.9 (CH_3-29), 26.4 (CH_2-16), 28.1 (CH_3-24/CH_3-30), 29.9 (CH_2-2′), 30.6 (CH_2-21), 31.3 (CH_2-7), 31.6 (C-20), 37.9 (CH_2-22), 38.7 (CH-9), 39.1 (C-8), 40.9 (C-10), 41.6 (CH_2-1), 41.3 (C-14), 42.3 (CH_2-19), 43.1 (C-4), 46.3 (C-17), 47.4 (CH-18), 63.6 (CH_2-1′), 122.0 (CH-12), 143.9 (C-13), 172.3 (C-2), 177.8 (C-3), 178.2 (C-28). HR-ESI-MS (positive-ion mode) m/z: 581.3804 $[M+Na]^+$ (Calcd for $C_{34}H_{54}O_6Na$: 581.3818).

Analysis of Sugar A solution of the compound **1** (6.0 mg) 1.5 ml 2 M HCl was stirred at 80 °C for 3 h. The cooled reaction mixture was washed with $CHCl_3$ (1.5 ml, three times) and the aqueous layer was neutralized with $Ba(OH)_2$, precipitating $BaSO_4$. The resulting mixture was centrifuged to separate the precipitate which was then washed with 2 M HCl to confirm the presence of sulfates. The supernatant was further neutralized with Amberlite IRA-400 (OH^- form) and filtered. The resulting solution was subjected to HPLC analysis using a Shodex Asahipak NH 2P-50 4E column and an optical rotation detector (JASCO 2090*Plus* chiral detector) with CH_3CN–H_2O (4 : 1, 1 ml/min) as the mobile phase. The retention time of D-glucose (11.7 min) was compared with that of an authentic sample.

***Leishmania major* Growth Inhibition Assay** The parasites were cultured in M199 medium supplemented with 10% heat-inactivated fetal bovine serum and 100 μg/ml of kanamycin. Into a 96-well plate, aliquots of the DMSO solution of the test compounds (1% final concentration) were incubated with L. major cells (1×10^5 cells/well) in a CO_2 incubator at 27 °C for 48 h. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added into each well and further incubated overnight. Absorbance was measured at 540 nm using a Molecular Devices Versamax tunable microplate reader. DMSO was used as a negative control and Amphotericin B as positive control. The viability was compared to that of control cells incubated in the same medium sans the test compounds. Inhibition activity was calculated as:

$$\% \text{ inhibition} = [1-(A_{test}-A_{blank})/(A_{control}-A_{blank})]\times 100$$

where $A_{control}$ is the absorbance of the control (DMSO) well, A_{test} is the absorbance of the test wells and A_{blank} is the absorbance the cell-free wells.

A549 Growth Inhibition Assay The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum and 100 μg/ml of kanamycin and 0.5 μg/ml amphotericin. Into a 96-well plate, aliquots of the DMSO solution of the test compounds (1% final concentration) were incubated with A549 cells (5×10^3 cells/well) in a CO_2 incubator at 37 °C for 72 h. MTT was added into each well and the plate was further incubated for another hour. Absorbance was measured at 540 nm using a Molecular Devices Versamax tunable microplate reader. DMSO was used as a negative control and Doxorubicin as positive control. The viability was compared to that of control cells incubated in the same medium sans the test compounds. Cytotoxic activity was calculated as:

$$\% \text{ inhibition} = [1-(A_{test}-A_{blank})/(A_{control}-A_{blank})]\times 100$$

where $A_{control}$ is the absorbance of the control (DMSO) well, A_{test} is the absorbance of the test wells and A_{blank} is the absorbance the cell-free wells.

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