Bioactive Constituents of the Leaves of *Phyllanthus polyphyllus* var. *siamensis*

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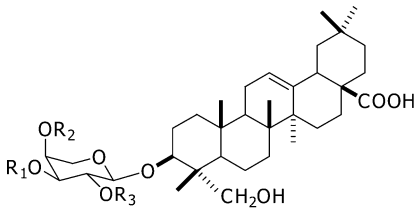
A dichloromethane extract of the leaves of *Phyllanthus polyphyllus* var. *siamensis* afforded two new monoacetylated triterpene arabinosides (**1** and **2**) and 15 known compounds. The structures of the new compounds were elucidated on the basis of spectroscopic data interpretation. The isolates and some chemical transformation products were evaluated biologically, and the presence of an arabinosyl moiety among the hederagenin glycosides evaluated was found to be important for the modulation of cytotoxic activity against cancer cells.

In an ongoing search for biologically active compounds from the genus *Phyllanthus* (Euphorbiaceae), which produces terpenes,[1−3] alkaloids,[4,5] lignans,[6,7] flavonoids,[8] and tannins,[9] *Phyllanthus polyphyllus* Willd. var. *siamensis* was chosen for study and is known in Thailand as "Sieo Yai". This plant is a deciduous shrub or small tree that grows up to 6−12 m in height. There have been no reports on the medicinal uses, chemical constituents, or bioactivity of *P. polyphyllus*. The use of the brine shrimp lethality test[10] as an in-house bioassay-guided method for screening plant extracts showed that the CH_2Cl_2 extract of the leaves of this plant caused 93.3 and 50% mortality of the brine shrimp nauplii at a 250 and 100 ppm concentration, respectively. The CH_2Cl_2 extract was chosen therefore for further investigation. Systematic fractionation of this extract led to the separation of two new acetylated arabinosyl olean-12-en-28-oic acid derivatives (**1** and **2**) together with 15 known compounds. The structures of the new compounds were elucidated by spectroscopic methods. The known compounds were identified as 2′-*O*-acetyl-3-*O*-α-L-arabinosyl-23-hydroxyolea-12-en-28-oic acid (**3**),[11] seco-isolariciresinol,[12] scopoletin,[13] meridinol,[14] dihydrocubebin,[15] menisdaurilide,[16] aquilegiolide,[17] 2,3-dihydromenisdaurilide,[16] 2,3-dihydroaquilegiolide,[16] blumenol B,[18] boscialin,[19] 7-megastigmen-3,6,9-triol,[20] 3,23-dihydroxyolean-12-en-28-oic acid (hederagenin),[21] 3-*O*-α-L-arabinosyl oleanolic acid (**4**),[22] and 3-*O*-α-L-arabinosyl hederagenin (**5**),[21] by comparison with previously reported data.



1 $R_1 = COCH_3$, $R_2 = R_3 = H$

2 $R_2 = COCH_3$, $R_1 = R_3 = H$

3 $R_3 = COCH_3$, $R_1 = R_2 = H$

Results and Discussion

Compound **1** was obtained as a colorless crystalline solid. The FT-IR spectrum showed the presence of a carboxyl group at ν_{max} 3446 and 1717 cm^{-1} as well as C=C stretching at ν_{max} 1653 cm^{-1}. The HRFABMS revealed a $[M + H]^+$ ion peak at *m/z* 645.4008 corresponding to the elemental formula $C_{37}H_{57}O_9$. The ^{13}C NMR spectrum exhibited 37 carbon signals, comprising seven methyls, 12 methylenes, nine methines, and nine quaternary carbon signals, including two carbonyl carbons. The ^1H and ^{13}C NMR spectra showed evidence for a trisubstituted olefinic group [δ_H 5.25 and δ_C 143.8 (s) and 122.4 (d)], an oxymethine group [δ_H 3.58; δ_C 84.9 (d)], and a hydroxymethylene group [δ_H 3.60 and 3.39 and δ_C 66.4 (t)] and indicated a 3,23-dihydroxy-olean-12-en skeleton.[23] The presence of an OH group at C-23 was confirmed by a NOE interaction between H-3/H_2-23 in the NOESY spectrum. The ^{13}C NMR signal at δ_C 182.4, having a ^3J correlation with the ^1H NMR signal at δ_H 2.79 (H-18) in particular, confirmed the placement of the carboxyl group at C-28. The connection of the aglycon and the sugar unit between C-3 and C-1′ was evidenced from the long-range $^1H-^{13}C$ NMR correlations between the ^1H NMR signal at δ_H 3.58 (m, H-3) and the acetal ^{13}C NMR signal at δ_C 104.8 (d, C-1′). The presence of an acetyl group [δ_H 2.15 (s) and δ_C 21.0 9 (q) and 171.1 (s)], a doublet of doublets signal at δ_H 4.75 (J = 9.9, 3.2 Hz), and the $^1H-^1H$ COSY cross-peak between H-1′ (δ_H 4.33)/H-2′ (δ_H 3.76), which was further correlated with the less shielded signal at δ_H 4.75 (H-3′), indicated the connectivity of an *O*-acetyl group at C-3′ of the sugar residue. The ^1H and ^{13}C NMR signals of the sugar residue closely resembled those of arabinopyranose.[24] Further ^1H and ^{13}C NMR chemical shift assignments were secured from the overall $^1H-^1H$ COSY, HMQC, and HMBC NMR correlations (Tables 1 and 2). Accordingly, compound **1** was concluded to be 3′-*O*-acetyl-3-*O*-α-L-arabinosyl-23-hydroxy-olean-12-en-28-oic acid.

Compound **2** was obtained as a colorless powder. The FT-IR spectrum showed absorption bands of carboxyl and olefinic groups at ν_{max} 3436, 1729, and 1696 cm^{-1}. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) showed chemical shift values similar to **1**. However, the appearance of one acetate methyl singlet at δ_H 2.07 and a low-field oxymethine proton resonating as a broad singlet at δ_H 4.98 indicated that the acetyl group is at different position from **1**, and the $^1H-^1H$ COSY and HMBC NMR spectra suggested the attachment of the acetoxyl group to be at C-4′. Thus, compound **2** was assigned as 4′-*O*-acetyl-3-*O*-α-L-arabinosyl-23-hydroxyolean-12-en-28-oic acid.

Compound **3** was isolated as a colorless solid. The FT-IR spectrum showed absorption bands for carboxyl and olefinic groups (ν_{max} 3418, 1731, and 1693 cm^{-1}, respectively). The ^1H and ^{13}C NMR spectra also showed patterns

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds **1**−**3** (in $CDCl_3$)[a]

position	**1**	**2**	**3**	HMBC of **1** (^{1}H→^{13}C)
1	0.92,1.59	0.87, 1.55	0.92, 1.56	C-10, C-25
2	1.09, 1.78	1.78	1.67	
3	3.58	3.54	3.63 dd (11.6, 4.8)	C-23, C-24, C-1′
5	1.04	1.0	1.13	C-4, C-6, C-10, C-23, C-25
6	1.31, 1.44	1.28,1.42	n.o.	
7	1.24, 1.42	1.22, 1.43	1.25, 1.44	
9	1.50	1.52	1.54	C-1, C-5, C-8, C-10, C-14, C-25, C-26
11	1.84	1.82	1.83	
12	5.25 br s	5.20 br s	5.23 br s	C-9, C-11, C-14, C-18, C-27
15	1.05, 1.63	0.99	1.03, 1.67	C-27
16	1.57, 1.90	1.54, 1.88	1.93	C-28
18	2.79 br d (12.6)	2.76 dd (12.3, 3.0)	2.78 br d (13.6)	C-12, C-13, C-14, C-16, C-17, C-28
19	1.06, 1.56	1.08, 1.55	1.10, 1.57	C-18, C-20, C-29, C-30
21	1.16,1.30	1.12, 1.27	1.16, 1.31	C-17, C-20, C-30
22	1.53, 1.72	1.51, 1.69	1.53, 1.73	C-16, C-17, C-18, C-21, C-28
23	0.76 s	0.72 s	0.61 s	C-3, C-4, C-5, C-24
24	3.39 d (11.2) 3.6	3.31 d (11.3) 3.53	3.34	C-3, C-5, C-23
25	0.94 s	0.89 s	0.91 s	C-1, C-5, C-9, C-10
26	0.73 s	0.70 s	0.73 s	C-7, C-8, C-9, C-14
27	1.10 s	1.07 s	1.08 s	C-8, C-13, C-14, C-15
29	0.88 s	0.83 s	0.86 s	C-19, C-20, C-21, C-30
30	0.90 s	0.86 s	0.89 s	C-19, C-20, C-21, C-29
1′	4.33 d (7.5)	4.31 d (7.0)	4.57 brd (4.8)	C-3, C-3′
2′	3.76 dd (9.8, 7.6)	3.56 dd (9.5, 6.9)	4.87 dd (6.9, 5.0)	C-1′, C-3′
3′	4.75 dd (9.9, 3.2)	3.62 dd (9.4, 3.3)	3.70	C-2′, C-3′-*CO*CH_3
4′	3.99 br s	4.98 br s	3.84	C-1, C-2′
5′	3.6, 3.95 dd (12.9, 2.1)	3.51, 3.92 dd (13.4, 2.3)	3.54, 3.95	C-1′, C-3′, C-4′
CO*CH*$_3$	3′, 2.15 s	4′, 2.07 s	2′, 2.08 s	C-3′, C-3′-*CO*CH_3

[a] Assignments were based on COSY, HMQC, and HMBC NMR experiments; coupling constants are listed in parentheses in Hz. Only the HMBC NMR correlations of compound **1** are illustrated.

of signals similar to those of compounds **1** and **2**. The acetate methyl protons and a less shielded oxymethine proton signal resonated at δ_H 2.08 and 4.87, respectively. The ^{1}H−^{1}H COSY and HMBC NMR spectra permitted the placement of the acetyl group at the 2′-position of the arabinopyranose unit. Compound **3** was concluded to be 2′-*O*-acetyl-3-*O*-α-L-arabinosyl-23-hydroxyolean-12-en-28-oic acid. This compound was documented earlier as a constituent of *Patrinia scabiosaefolia*, but no detailed ^{1}H and ^{13}C NMR spectral data were reported.[11] The use of HMQC and HMBC experiments allowed full assignments of the ^{1}H and ^{13}C NMR resonances of **3** as shown in Tables 1 and 2.

All isolates and chemical transformation products obtained, except for scopoletin, meridinol, aquilegiolide, boscialin, and 7-megastigmen-3,6,9-triol, were evaluated for their cytotoxic activity against three human cancer cell lines [human oral carcinoma (KB), breast cancer (BC), and small cell lung cancer (NCI-H187)]. Seco-isolariciresinol, dihydrocubebin, menisdaurilide, 2,3-dihydromenisdaurilide, 2,3-dihydroaquilegiolide, blumenol B, and 3,23-dihydroxyolean-12-en-28-oic acid (hederagenin) were found to be inactive (IC_{50} > 5 μg/mL) against all cell lines. Compounds **1**, **2**, **4**, **5**, and **4a** were inactive against the KB cell line, but compound **5a** showed IC_{50} values of 3.7 μg/mL. With the BC cell line, compounds **2**, **4**, **4a**, and **5a** exhibited IC_{50} values of 3.6, 1.7, 3.3, and 3.6 μg/mL, respectively. Compounds **1**, **2**, **3**, **4a**, and **5** were inactive, but compounds **4** and **5a** showed IC_{50} values of 3.2 and 2.2 μg/mL, respectively, using the NCI-H187 cell line. Due to the scarcity of the isolated materials, only compounds **2**, **4**, and **4a** were further tested for their cytotoxicity using Vero cells and showed IC_{50} values of 10.7, 2.8, and 3.2 μg/mL, respectively. Since the aglycon hederagenin exhibited no cytotoxic activity with all the cell lines used, while its arabinosides and their acetates showed weak cytotoxic activity, these results suggest that the presence of an arabinosyl moiety among these hederagenin glycosides is important for the modulation of cytotoxic activity against cancer cells.

Experimental Section

General Experimental Procedures. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP 1020 polarimeter. The IR spectra were obtained on a Perkin-Elmer 1760x FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker AVANCE 400 MHz spectrometer with the solvent signal as internal reference. EIMS and HRFABMS were recorded on a Finnigan MAT 90 mass spectrometer, and HRESIMS was recored on a Bruker Daltonics microTOF instrument.

Plant Material. The leaves of *P. polyphyllus* Willd. var. *siamensis* (Euphorbiaceae) were collected from Ban Talad Village, Tare District, Ampur Uthumpornpisai, Srisagate Province, Thailand, in June, 1999. Botanical identification was kindly provided by Mr. Winai Somprasong, Sirindhorn Museum, Botanical Section, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok 10903, Thailand. A voucher specimen (SSPP/1999) is kept at the Department of Chemistry, Faculty of Science, Ramkhamhaeng University.

Extraction and Isolation. The dried leaves of *P. polyphyllus* were milled to obtain 12.56 kg of a powder. The pulverized leaves were extracted successively with *n*-hexane and CH_2Cl_2 in a Soxhlet extraction apparatus. After extraction

Table 2. ^{13}C NMR Spectroscopic Data of Compounds **1**−**3** (in $CDCl_3$)[a]

position	**1**	**2**	**3**
1	38.1 t	38.0 t	38.1 t
2	24.8 t	24.7 t	25.0 t
3	84.9 d	85.2 d	82.5 d
4	42.5 s	42.4 s	42.6 s
5	48.1 d	48.0 d	47.0 d
6	18.0 t	18.0 t	17.8 t
7	32.4 t	32.3 t	32.5 t
8	39.3 s	39.2 s	39.2 s
9	47.6 d	47.6 d	47.6 d
10	36.8 s	36.7 s	36.5 s
11	23.4 t	23.4 t	23.4 t
12	122.4 d	122.2 d	122.2 d
13	143.8 s	143.8 s	143.8 s
14	41.6 s	41.6 s	41.7 s
15	27.7 t	27.6 t	27.7 t
16	22.9 t	22.9 t	23.0 t
17	46.4 s	46.4 s	46.3 s
18	41.0 d	41.0 d	41.2 d
19	45.9 t	45.9 t	46.0 t
20	30.6 s	30.6 s	30.6 s
21	33.8 t	33.8 t	33.8 t
22	32.4 t	32.4 t	32.5 t
23	12.5 q	12.4 q	12.8 q
24	66.4 t	66.3 t	64.4 t
25	16.0 q	15.9 q	15.8 q
26	17.2 q	16.9 q	16.8 q
27	25.9 q	25.8 q	25.8 q
28	182.4 s	181.6 s	180.4 s
29	33.0 q	33.0 q	33.0 q
30	23.6 q	23.5 q	23.5 q
1′	104.8 d	104.4 d	100.6 d
2′	69.8 d	72.0 d	72.1 d
3′	75.5 d	71.7 d	70.5 d
4′	67.1 d	70.7 d	66.4 d
5′	66.2 t	63.8 t	62.8 t
CO*CH*$_3$	3′, 21.0 q	4′, 21.0 q	2′, 20.9 q
*CO*CH$_3$	171.1 s	171.3 s	170.8 s

[a] Multiplicities were obtained from DEPT experiments.

with CH_2Cl_2, the marc was soaked in MeOH for 15 days. The extracts were filtered and concentrated to remove solvent under reduced pressure on a rotary evaporator to obtain dark green *n*-hexane (472.33 g), dark green CH_2Cl_2 (83 g), and reddish brown MeOH (24 g) residues.

The CH_2Cl_2 extract of the leaves of *P. polyphyllus* (83 g) was subjected to silica gel column chromatography with a gradient of *n*-hexane−CH_2Cl_2 (20:80) to CH_2Cl_2−MeOH (100:0 to 0:100) to afford 19 major fractions. Fraction 10 was column chromatographed (silica gel, *n*-hexane−EtOAc, 90:10 to EtOAc−MeOH, 1:100) to obtain 26 subfractions (10.1 to 10.26). Subfraction 10.17 was further separated by column chromatography (silica gel, CH_2Cl_2−MeOH, 99.5:0.5 to 80:20) to yield 17 additional subfractions (10.17.1 to 10.17.17). Subfraction 10.17.12 after further purification by column chromatography (silica gel, CH_2Cl_2−MeOH, 100:0 to 90:10) yielded secoisolariciresinol (9.4 mg). Subfraction 10.20 was column chromatographed (silica gel, CH_2Cl_2−MeOH, 100:0 to 90:10) to yield 16 subfractions (10.20.1 to 10.20.16). Subfraction 10.20.9 was further purified using reversed-phase column chromatography (RP C_{18}, H_2O−MeOH, 70:30 to 0:100) to give scopoletin (6.2 mg). Fraction 11 was subjected to column chromatography (silica gel, *n*-hexane−CH_2Cl_2, 10:90, to CH_2Cl_2−MeOH, 30:70) to yield 26 subfractions (11.1 to 11.26). Subfraction 11.14 was rechromatographed (silica gel, CH_2Cl_2−MeOH, 99.5:0.5 to 50:50) and yielded 12 subfractions (11.14.1 to 11.14.12). Subfraction 11.14.4 was further purified by column chromatography (silica gel, *n*-hexane−EtOAc, 85:15 to 50:50) to yield 11 subfractions (11.14.4.1 to 11.14.4.11). Subfraction 11.14.4.9 after column chromatography using reversed-phase C_{18} (H_2O−MeOH, 40:60 to 0:100) gave meridinol (15 mg). Subfraction 11.19 was purified by column chromatography (silica gel, *n*-hexane−EtOAc, 50:50 to 0:100) to obtain 14 subfractions (11.19.1 to 11.19.14). Subfraction 11.19.8 was column chromatographed (silica gel, *n*-hexane−EtOAc, 80:20 to 75:25) to obtain eight subfractions (11.19.8.1 to 11.19.8.8). Subfraction 11.19.8.8 was further purified by column chromatography (RP C_{18}, H_2O−MeOH, 50:50 to 0:100) to yield dihydrocubebin (8.8 mg). Subfraction 11.19.14 contained a mixture of menisdaurilide and aquilegiolide, which was further separated by column chromatography (2× , silica gel, *n*-hexane−EtOAc, 60:40 to 0:100, then CH_2Cl_2−MeOH, 100:0 to 99:1) to yield menisdaurilide (120.2 mg) and aquilegiolide (17.4 mg). Subfraction 11.22.11 was column chromatographed (silica gel, CH_2Cl_2−MeOH, 98:2 to 50:50) to yield 19 subfractions (11.22.11.1 to 11.22.11.19). Subfraction 11.22.11.10 was then purified (silica gel, CH_2Cl_2−MeOH, 98:2 to 80:20) to afford hederagenin (39.2 mg).

Fraction 12 was column chromatographed (silica gel, CH_2Cl_2−MeOH, 98:2 to 50:50) and was separated into 17 subfractions (12.1 to 12.17). Subfraction 12.9 was further chromatographed (silica gel, *n*-hexane−EtOAc, 70:30 to 0:100) to obtain 14 subfractions (12.9.1 to 12.9.14). Subfraction 12.9.8 was purified using reversed-phase column chromatography to yield 10 subfractions (12.9.8.1 to 12.9.8.10). Subfraction 12.9.8.3 was further purified (RP C_{18}, H_2O−MeOH, 100:0 to 30:70) to yield three subfractions. The first fraction of these after column chromatography (silica gel, *n*-hexane−EtOAc, 60:40 to 40:60) yielded boscialin (4.8 mg) and blumenol B (3.5 mg). Subfraction 12.11 after column chromatography (silica gel, *n*-hexane−EtOAc, 70:30 to 0:100, then EtOAc−MeOH, 100:0 to 65:35) gave 13 subfractions (12.11.1−12.11.13). Subfraction 12.11.10 gave six subfractions (12.11.10.1−12.11.10.6) after RP C_{18} column chromatography (MeOH−H_2O, 10:90 to 100:0). Further column chromatography of subfraction 12.11.10.6 (2× , silica gel, *n*-hexane−EtOAc, 60:40 to 50:50, then silica gel, *n*-hexane−EtOAc, 50:50) gave 2,3-dihydromenisdaurilide (163.8 mg) and 2,3-dihydroaquilegiolide (260.2 mg).

Fraction 13 was column chromatographed (silica gel, *n*-hexane−EtOAc, 50:50) to yield nine subfractions (13.1−13.9). Subfraction 13.4 was further chromatographed (silica gel, *n*-hexane−EtOAc, 65:35) to obtain 10 subfractions (13.4.1−13.4.10). Subfraction 13.4.6 contained 7-megastigmen-3,6,9-triol (63.9 mg). Subfraction 13.5 was further chromatographed (silica gel, CH_2Cl_2−MeOH, 96:4) to yield five subfractions (13.5.1−13.5.5). Subfraction 13.5.2 was purified using silica gel column chromatography (*n*-hexane−EtOAc, 20:80) to give compound **4** (26.3 mg). Subfraction 13.5.4 was column chromatographed (2× , silica gel, *n*-hexane−EtOAc, 40:60, then CH_2Cl_2−MeOH, 97:3) to give compound **5** (10.2 mg). Subfraction 13.4.8 was subjected to column chromatography (silica gel, CH_2Cl_2−MeOH, 94:4) to give five subfractions (13.4.8.1−13.4.8.5). Subfraction 13.4.8.2 after column chromatography (reversed-phase C_{18}, MeOH−H_2O, 80:20) gave three subfractions (13.4.8.2.1−13.4.8.2.3). Subfraction 13.4.8.2.1 was further chromatographed (silica gel, CH_2Cl_2−MeOH, 96:4) to yield three subfractions (13.4.8.2.1.1−13.4.8.2.1.3). The least polar subfraction, 13.4.8.2.1.1, contained compound **1** (20.6 mg). The most polar subfraction, 13.4.8.2.1.3, gave compound **2** (30.2 mg). The moderately polar subfraction, 13.4.8.2.1.2, after further purification (silica gel, *n*-hexane−EtOAc, 30:70), yielded compound **3** (5.5 mg). Compounds **4** and **5** were acetylated using Ac_2O in pyridine to obtain a triacetate (**4a**) and a tetraacetate (**5a**) derivative, respectively. ^1H and ^{13}C NMR chemical shifts of compounds **4a** and **5a** were assigned using the ^1H−^1H COSY, HMQC, and HMBC NMR correlations (Table S1, Supporting Information).

Bioassays. The cytotoxicity assay was performed using the colorimetric method described by Skehan and co-workers.[25] The reference compound ellipticine exhibited activities against the breast cancer (BC), nasopharyngeal carcinoma (KB), and NCI-H187 cell lines with IC_{50} values of 0.26 ± 0.08, 0.36 ± 0.07, and 0.32 ± 0.17 μg/mL, respectively.

3′-*O*-Acetyl-3-*O*-α-L-arabinopyranosyl hederagenin (1): mp 192−194 °C; $[\alpha]_D$ 32.4° (*c* 0.125, $CHCl_3$); IR (KBr) ν_{max} 3446, 2942, 1717, 1697, 1653, 1457, 1375, 1251, 1092, 757 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$), see Tables 1 and 2; HRFABMS *m/z* 645.4008 $[M + H]^+$ (calcd for $C_{37}H_{57}O_9$, 645.4003).

4′-*O*-Acetyl-3-*O*-α-L-arabinopyranosyl hederagenin (2): mp 272−274 °C; $[\alpha]_D$ 70.6° (*c* 0.190, MeOH); IR (KBr) ν_{max} 3436, 2943, 1729, 1696, 1456, 1376, 1252, 1092, 758, 647, 603, 454 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$), see Tables 1 and 2; HRFABMS *m/z* 645.4002 $[M + H]^+$ (calcd for $C_{37}H_{57}O_9$, 645.4003).

2′-*O*-Acetyl-3-*O*-α-L-arabinopyranosyl hederagenin (3): $[\alpha]_D$ 28.9° (*c* 0.045, $CHCl_3$); IR (KBr) ν_{max} 3418, 2926, 2856, 2651, 1731, 1693, 1455, 1365, 1305, 1269, 1239, 1168, 1137, 1053, 1006, 954, 932, 870, 824, 757, 646 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$), see Tables 1 and 2; HRFABMS *m/z* 645.4002 $[M + H]^+$ (calcd for $C_{37}H_{57}O_9$, 645.4003).

2′,3′,4′-Tri-*O*-acetyl-3-*O*-α-L-arabinopyranosyl oleanolic acid (4a): mp 227−230 °C; $[\alpha]_D$ 44.4° (*c* 0.150, $CHCl_3$); IR (KBr) ν_{max} 2944, 1752, 1698, 1462, 1370, 1325, 1222, 1179, 1194, 1056, 1023, 982, 911, 764, 601, 490 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$), see Table S1; HRESIMS *m/z* 737.4214 $[M + Na]^+$ (calcd for $C_{41}H_{62}O_{10}Na$, 737.4235).

24,2′,3′,4′-Tetra-*O*-acetyl-3-*O*-α-L-arabinopyranosyl hederagenin (5a): mp 177−179 °C; $[\alpha]_D$ 39.2° (*c* 0.155, $CHCl_3$); IR (KBr) ν_{max} 2945, 1746, 1697, 1464, 1370, 1224, 1179, 1104, 1055, 938, 884, 766, 643, 603, 490 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$), see Table S1; HRESIMS *m/z* 795.4274 $[M + Na]^+$ (calcd for $C_{43}H_{64}O_{12}Na$, 795.4290).

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Supporting Information Available: Structures of compounds **4**, **5**, **4a**, and **5a** and tabulated ^1H and ^{13}C NMR spectroscopic data of compounds **4a** and **5a** (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Vongvanich, N.; Kittakoop, P.; Kramyu, J.; Tanticharoen, M.; Thebtaranonth, Y. *J. Org. Chem*. **2000**, *65*, 5420−5423.
(2) Zhang, Y. J.; Tanaka, T.; Iwamoto, Y.; Yang, C. R.; Kouno, I. *J. Nat. Prod*. **2001**, *64*, 870−873.
(3) Sutthivaiyakit, S.; Na Nakorn, N.; Kraus, W.; Sutthivaiyakit, P. *Tetrahedron* **2003**, *59*, 9991−9995.
(4) Houghton, P. J.; Woldemariam, T. Z.; O'Shea, S.; Thyagarajan, S. P. *Phytochemistry* **1996**, *43*, 715−717.
(5) Bila, B. T.; Gedris, E.; Herz, W. *Phytochemistry* **1996**, *41*, 1441−1443.
(6) Lin, M. T.; Lee, S. S.; Liu, K. C. S. C. *J. Nat. Prod*. **1995**, *58*, 244−249.
(7) Somanabandhu, A.; Nitayanagkura, S.; Mahidol, C.; Ruchirawat, S.; Likitwitayawuid, K.; Shieh, H. L.; Chai, H.; Pezzuto, J. M.; Cordell, G. A. *J. Nat. Prod*. **1993**, *56*, 233−239.
(8) Gupta, D. R.; Ahmed, B. *J. Nat. Prod*. **1984**, *47*, 958−963.
(9) Miguel, O. G.; Calixto, J. B.; Sanntos, A. R. S.; Messana, I.; Ferrari, F.; Filho, V. C.; Pizzolatti, M. G.; Yunes, R. A. *Planta Med*. **1996**, *64*, 146−149.
(10) Anderson, G. E.; Goetz, C.; Suffness, M.; McLaughlin, J. L. *Phytochem. Anal*. **1991**, *2*, 107−111.
(11) Woo, W. S.; Choi, J. S.; Seligmann, O.; Wagner, H. *Phytochemistry* **1983**, *22*, 1045−1047.
(12) Andersson, R. T.; Popoff, P.; Theander, O. *Acta Chem. Scand. B* **1975**, *29*, 835−837.
(13) Razdan, T. K.; Qadri, B.; Harkar, S.; Waight, E. S. *Phytochemistry* **1987**, *26*, 2063−2069.
(14) Amaro-Luis, M.; Fronczek, F. R.; Massanet, G. M.; Pando, E.; Rodriguez-Luis, F.; Watkins, S. F.; Zubia, E. *Phytochemistry* **1988**, *27*, 3933−3935.
(15) Tillekeratne, L. M. V.; Jayamanne, D. T.; Weerasuria, K. D. V.; Gunatilaka, A. A. L. *Phytochemistry* **1982**, *21*, 476−478.
(16) Otsuka, H.; Ito, A.; Fujioka, N.; Kawamata, K. I.; Kasai, R.; Yamasaki, K.; Satoh, T. *Phytochemistry* **1993**, *33*, 389−392.
(17) Guerriero, A.; Pietra, F. *Phytochemistry* **1984**, *23*, 2394−2396.
(18) Galbraith, M. N.; Horn, D. H. S. *J. Chem. Soc., Chem. Commun*. **1972**, 113−114.
(19) Busch, J.; Grether, Y.; Ochs, D. O.; Sequin, U. *J. Nat. Prod*. **1988**, *61*, 591−597.
(20) Perez, C.; Trujillo, J.; Almonacid, L. N.; Trujillo, J.; Eduardo, N.; Alonso, S. J. *J. Nat. Prod*. **1996**, *59*, 69−72.
(21) Kizu, H.; Tomimori, T. *Chem. Pharm. Bull*. **1982**, *30*, 3340−3346.
(22) Aoki, T.; Suga, T. *Phytochemistry* **1978**, *17*, 771−773.
(23) Mahato, S. B.; Kundu, A. P. *Phytochemistry* **1994**, *37*, 1517−1575.
(24) Lavaud, C.; Voutquenne, L.; Massiot, G.; Men-Olivier, L. L.; Das, B. C.; Laprevote, O.; Serani, L.; Delaude, C.; Becchi, M. *Phytochemistry* **1998**, *47*, 441−449.
(25) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst*. **1990**, *82*, 1107−1112.

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