Two Antiproliferative Triterpene Saponins from *Nematostylis anthophylla* from the Highlands of Central Madagascar[1])

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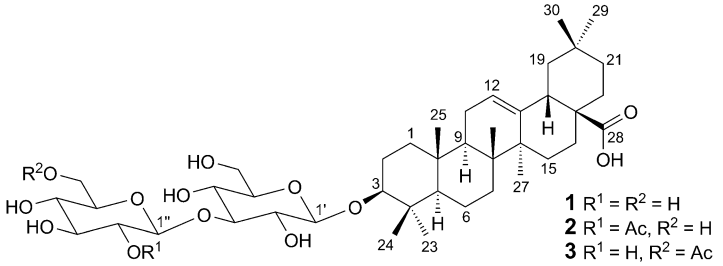
Investigation of the endemic Madagascan plant *Nematostylis anthophylla* (Rubiaceae) for antiproliferative activity against the A2780 ovarian cancer cell line led to the isolation of the known triterpene saponin randianin (**1**), and the two new bioactive triterpene saponins 2″-*O*-acetylrandianin (**2**) and 6″-*O*-acetylrandianin (**3**). The structures of the two new compounds were elucidated based on analysis of their 1D- and 2D-NMR spectra, and mass spectrometric data. The three isolated triterpene saponins displayed moderate but selective antiproliferative activities, with IC_{50} values of 1.2, 1.7, and 2.2 μM, respectively, against the A2780 ovarian cancer, but only weak inhibitions of the proliferation of A2058 melanoma and the H522 lung cancer cell lines.

Introduction. – As part of our engagement in an *International Cooperative Biodiversity Group* (*ICBG*) program, we are focusing on the search for antiproliferative natural products from a diversity of vegetation types in Madagascar [1–3]. The A2780 human ovarian cancer cell line is used as the primary screen, because it is a stable and yet relatively drug-sensitive cell line and gives reproducible results. As a part of this research, an EtOH extract from the roots of *Nematostylis anthophylla* (Rubiaceae) from the Highlands of Central Madagascar was investigated and found to exhibit antiproliferative activity against the A2780 cell line, with an IC_{50} value of 6.9 μg/ml. The Rubiaceae is a large family of 630 genera and *ca.* 13,000 species found worldwide [4]. This family is a rich source of indole alkaloids, terpenoids, and anthraquinones, all of which are well-known for their broad range of bioactivity, including antimicrobial, antimalarial, antidiabetic, vasorelaxant, cytotoxic, antioxidant, and anti-inflammatory activities among others [5–9]. Since *Nematostylis* is one of the many genera of the Rubiaceae that have not been systematically investigated for their phytochemical composition, the EtOH extract of *N. anthophylla* was selected for bioassay-guided fractionation to isolate its active components.

[1]) 'Biodiversity Conservation and Drug Discovery in Madagascar', Part 52. For Part 51, see [1].

Results and Discussion. – *Isolation of Bioactive Compounds.* An EtOH extract of the roots of *N. anthophylla* was subjected to liquid–liquid partitioning to give an active BuOH fraction with an IC_{50} value of 2.2 μg/ml. Bioassay-guided separation, including *LH-20* size-exclusion, *HP-20 Diaion*, and silica-gel normal-phase chromatography, was used to obtain three bioactive compounds comprising the known triterpene saponin randianin (**1**), and the two new related glycosides 2″-*O*-acetylrandianin (**2**) and 6″-*O*-acetylrandianin (**3**). All three compounds had moderate antiproliferative activities against A2780 ovarian cancer cells, with IC_{50} values of 2.2, 1.2, and 1.7 μM, respectively. Herein, we report the structure elucidation and antiproliferative properties of the two new isolates.



Identification of Compounds **1** *and* **2**. Compound **1** was identified as randianin (=oleanolic acid 3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranoside) by comparison of its chemical and spectroscopic data with those reported in the literature for the aglycone [10] and the glycoside [11].

Compound **2**, $[\alpha]_D^{21} = +12$ ($c = 1.2$, MeOH), was isolated as a light-yellow solid. Its positive-ion HR-ESI-MS exhibited cationized molecular-ion peaks at *m*/*z* 845.4692 ($[M+Na]^+$) and 861.4618 ($[M+K]^+$), corresponding to the molecular formula $C_{44}H_{70}O_{14}$. The observation of a C=O absorption at 1734 cm^{-1} in the IR spectrum, a ^{13}C-NMR resonance at δ(C) 170.7 ppm, and a *singlet* signal at δ(H) 1.98 ppm in the ^{1}H-NMR spectrum (*Table 1*) suggested the presence of an Ac group. Meanwhile, its glycosidic nature was corroborated by the presence of two anomeric H-atom signals at δ(H) 4.83 and 5.43 ppm. In addition to the Me and C=O C-atoms of the Ac group, there were 42 C-atom signals in the ^{13}C-NMR spectrum, among which 30 C-atom signals were assigned to a triterpenoid aglycone and the remaining 12 C-atoms to a disaccharide moiety. The ^{1}H-NMR spectrum of **2** indicated that the aglycone had seven Me groups corresponding to 3-H *singlet*s at δ(H) 0.80, 0.89, 0.97, 1.00, 1.03, 1.27 and 1.33, and one olefinic H-atom signal appeared at δ(H) 5.49. Correspondingly, signals for seven Me C-atoms at δ(C) 15.8, 17.2, 17.8, 24.1, 26.6, 28.5 and 33.7 ppm, and for two olefinic C-atoms at δ(C) 122.9 and 145.2 ppm were observed in the ^{13}C-NMR spectrum. The presence of a C=O absorption at 1689 and a broad OH absorption at 3453 cm^{-1} in its IR spectrum, together with a ^{13}C-NMR resonance at δ(C) 180.6 ppm, supported the presence of a carboxylic acid group.

Inspection of the ^{1}H- and ^{13}C-NMR spectra of compound **2** indicated that it had the same oleanolic acid aglycone as compound **1**. The HMB correlation between H–C(18) (*dd*, $J = 4.1$, 14.0) and C(28) confirmed that the carboxylic C-atom was connected to C(17) [12]. HMBCs between the anomeric H–C(1′) and C(3), as well as between

Table 1. *^{1}H- and ^{13}C-NMR Data for* **1**–**3**. Recorded in (D_5)pyridine at 500 and 125 MHz, resp.; δ in ppm, J in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	1.21–1.25 (*m*), 1.39–1.42 (*m*)	39.0	1.21–1.25 (*m*), 1.37–1.40 (*m*)	39.0	1.22–1.26 (*m*), 1.39–1.42 (*m*)	39.0
2	1.75–1.78 (*m*), 2.14–2.18 (*m*)	26.8	1.76–1.79 (*m*), 2.15–2.19 (*m*)	26.9	1.74–1.77 (*m*), 2.14–2.18 (*m*)	26.8
3	3.36 (*dd*, J=4.4, 11.9)	89.3	3.36 (*dd*, J=4.4, 11.9)	89.3	3.36 (*dd*, J=4.4, 11.7)	89.4
4	–	40.1	–	40.1	–	40.1
5	0.76–0.80 (*m*)	56.1	0.76–0.79 (*m*)	56.1	0.78–0.82 (*m*)	56.1
6	1.21–1.25 (*m*), 1.45–1.49 (*m*)	18.8	1.22–1.25 (*m*), 1.46–1.50 (*m*)	18.8	1.23–1.26 (*m*), 1.46–1.50 (*m*)	18.8
7	1.78–1.82 (*m*), 1.85–1.87 (*m*)	33.6	1.78–1.82 (*m*), 1.85–1.87 (*m*)	33.6	1.78–1.82 (*m*), 1.85–1.87 (*m*)	33.6
8	–	39.8	–	39.8	–	39.8
9	1.65 (br. *t*, J=8.9)	48.3	1.64 (br. *t*, J=8.9)	48.4	1.65 (br. *t*, J=8.9)	48.3
10	–	37.3	–	37.3	–	37.3
11	1.88–1.92 (*m*)	24.1	1.88–1.92 (*m*)	24.1	1.88–1.92 (*m*)	24.1
12	5.50 (*t*, J=3.3)	122.8	5.49 (*t*, J=3.3)	122.9	5.50 (*t*, J=3.3)	122.8
13	–	145.3	–	145.2	–	145.3
14	–	42.5	–	42.6	–	42.5
15	1.18–1.21 (*m*), 2.02–2.05 (*m*)	28.7	1.18–1.21 (*m*), 2.02–2.05 (*m*)	28.7	1.18–1.21 (*m*), 2.02–2.05 (*m*)	28.7
16	1.76–1.79 (*m*), 2.18–2.21 (*m*)	24.1	1.75–1.78 (*m*), 2.17–2.20 (*m*)	24.1	1.76–1.79 (*m*), 2.18–2.21 (*m*)	24.1
17	–	47.0	–	47.1	–	47.0
18	3.32 (*dd*, J=4.1, 14.0)	42.4	3.32 (*dd*, J=4.1, 14.0)	42.4	3.32 (*dd*, J=4.0, 13.9)	42.4
19	1.28–1.31 (*m*), 1.82–1.84 (*m*)	46.9	1.28–1.31 (*m*), 1.82–1.84 (*m*)	46.9	1.28–1.31 (*m*), 1.82–1.84 (*m*)	46.9
20	–	31.3	–	31.3	–	31.3
21	1.49–1.52 (*m*), 1.82–1.84 (*m*)	34.6	1.49–1.52 (*m*), 1.82–1.84 (*m*)	34.6	1.49–1.52 (*m*), 1.82–1.84 (*m*)	34.6
22	1.45–1.49 (*m*), 2.05–2.08 (*m*)	33.6	1.45–1.49 (*m*), 2.05–2.08 (*m*)	33.6	1.46–1.50 (*m*), 2.05–2.08 (*m*)	33.5
23	1.27 (*s*)	17.8	1.27 (*s*)	17.8	1.32 (*s*)	17.8
24	0.89 (*s*)	28.5	0.89 (*s*)	28.5	1.01 (*s*)	28.5
25	0.82 (*s*)	15.8	0.80 (*s*)	15.8	0.82 (*s*)	15.8
26	1.00 (*s*)	17.4	1.00 (*s*)	17.2	1.00 (*s*)	17.3
27	1.33 (*s*)	26.5	1.33 (*s*)	26.6	1.33 (*s*)	26.5
28	–	180.7	–	180.6	–	180.7
29	1.03 (*s*)	24.1	1.03 (*s*)	24.1	1.02 (*s*)	24.1
30	0.97 (*s*)	33.7	0.97 (*s*)	33.7	0.97 (*s*)	33.6
3-*O*-Glucosyl						
1′	4.91 (*d*, J=7.8)	106.7	4.83 (*d*, J=7.8)	107.1	4.91 (*d*, J=7.6)	106.7
2′	4.09–4.11 (*m*)	74.8	3.96–4.02 (*m*)	74.4	4.05–4.08 (*m*)	74.7
3′	4.23 (*t*, J=8.8)	89.3	4.15 (*t*, J=8.8)	89.3	4.18 (*t*, J=8.9)	89.7
4′	4.11–4.14 (*m*)	70.2	4.04–4.08 (*m*)	70.7	4.11 (*t*, J=9.3)	70.0
5′	3.92–3.98 (*m*)	78.3	3.89–3.93 (*m*)	78.1	3.92–3.98 (*m*)	78.3
6′	4.32 (*d*, J=11.3), 4.51 (*d*, J=11.0)	62.9	4.26 (*d*, J=11.4), 4.48 (*dd*, J=2.1, 11.5)	63.1	4.32 (*dd*, J=6.2, 11.8), 4.52 (*dd*, J=2.2, 11.8)	62.9

Table 1 (cont.)

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
3′-*O*-Glucosyl						
1″	5.32 (*d*, *J*=7.8)	106.3	5.43 (*d*, *J*=8.1)	103.7	5.25 (*d*, *J*=7.9)	106.3
2″	4.02–4.05 (*m*)	75.9	5.66 (*dd*, *J*=8.1, 9.1)	75.7	4.03–4.05 (*m*)	75.7
3″	4.26 (*t*, *J*=9.1)	75.8	4.31 (*t*, *J*=9.1)	76.6	4.22 (*t*, *J*=9.1)	75.7
4″	4.20 (*t*, *J*=9.2)	72.0	4.20 (*t*, *J*=9.2)	72.3	4.01 (*t*, *J*=9.1)	71.9
5″	4.07–4.09 (*m*)	79.1	4.07–4.10 (*m*)	79.1	4.23–4.26 (*m*)	78.3
6″	4.34 (*d*, *J*=11.1), 4.56 (*d*, *J*=10.7)	62.8	4.28 (*d*, *J*=11.1), 4.58 (*dd*, *J*=2.1, 11.5)	62.7	4.67 (*dd*, *J*=6.8, 11.7), 4.95 (*dd*, *J*=2.2, 11.8)	64.9
2″-AcO						
CO				170.7		
Me			1.98 (*s*)	21.5		
6″-AcO						
CO						171.2
Me					2.00 (*s*)	21.0

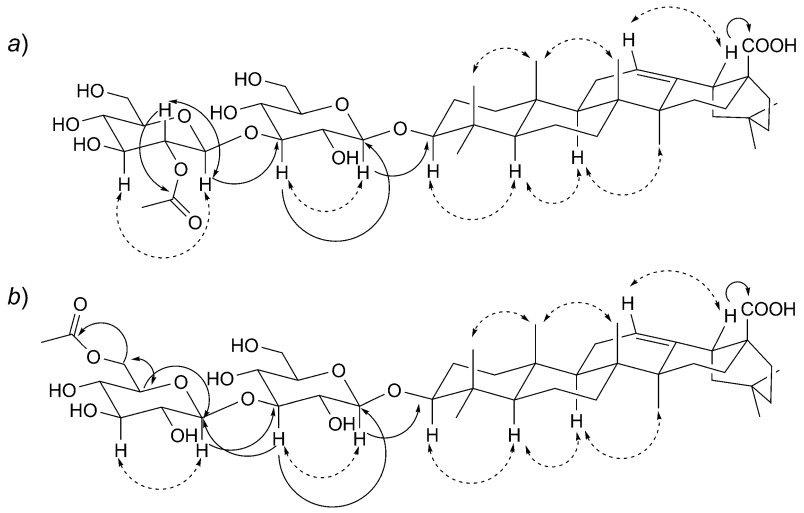


Figure. *HMBC* (H→C), *COSY* (H↔H), *and NOESY* (H← – →H) *correlations of* **2** (*a*) *and* **3** (*b*)

H–C(3) and the anomeric C(1′), confirmed that the disaccharide moiety was connected to C(3) (*Fig.*, *a*).

Both sugar molecules, which were represented by the of two sets of anomeric atom signals at δ(H) 4.83/δ(C) 107.1 ppm and δ(H) 5.43/δ(C) 103.7 ppm, respectively, were identified as glucose, based on the similarity of their ^{13}C-NMR chemical shifts with those of the sugar moiety of **1**. The linkage between the two glucopyranosyl units was determined as 1→3 on the basis of HMBCs between H–C(3′) and the two anomeric C-atoms C(1′) and C(1″), as well as the cross-peak between H–C(3′) and H–C(2′) in the

COSY spectrum (*Fig.*, *a*). The coupling constants between H–C(1′) and H–C(2′), and H–C(1″) and H–C(2″) ($J = 7.8$ and 8.1, resp.) indicated their axial–axial conformation, and thus the β-configuration of the two sugar units. The AcO group was deduced to be located at C(2″) of a glucopyranosyl residue, based on the comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** with those of **1**. Due to this acetylation, the chemical shift of H–C(2″) of **2** was δ(H) 5.66 as compared to δ(H) 4.02–4.05 for **1**, while other H-atoms in the distal glucose had chemical shifts similar to those of compound **1**. The position of the Ac group was confirmed by the COSY cross-peak between the downfield H–C(2″) and the corresponding anomeric H–C(1″), and a three-bond HMBC between H–C(2″) and the C=O C-atom of the Ac group (*Fig.*, *a*).

To determine the absolute configuration of the two glucose moieties and to confirm the overall structure assignment, compound **2** was hydrolyzed with 6M NH_4OH to yield a product identified as randianin (**1**) by its ^{1}H- and ^{13}C-NMR spectra. Further hydrolysis of **1** with 3M HCl yielded oleanolic acid, identified by its ^{1}H- and ^{13}C-NMR spectra, and a single monosaccharide, identified as D-glucose on the basis of a single TLC spot observed with the same R_f value as a D-glucose standard. Its absolute configuration was determined as D based on its positive optical rotation.

Based on these evidences, the structure of **2** was elucidated as oleanolic acid 3-*O*-β-D-glucopyranosyl-(1 → 3)-(2″-*O*-acetyl)-β-D-glucopyranoside, or 2″-*O*-acetylrandianin.

Identification of Compound **3**. Compound **3**, isolated as light-yellow solid, $[\alpha]_D^{21} = +17$ ($c = 1.2$, MeOH), had the same molecular formula as compound **2** as determined by HR-ESI-MS (*m*/*z* 845.4643 ($[M+Na]^+$) and 861.4569 ($[M+K]^+$)), corresponding to the molecular formula of $C_{44}H_{70}O_{14}$. Due to the similarity of its NMR data with those of compounds **1** and **2**, the aglycone portion of **3** was also assigned as oleanolic acid, with the disaccharide moiety connected to C(3) of the aglycone.

As in compound **2**, the presence of two sugar moieties was evidenced by the NMR spectra, which showed two sets of anomeric-atom signals at δ(H) 4.91/δ(C) 106.7 and δ(H) 5.25/δ(C) 106.3, respectively. The two sugar moieties were determined as glucosyls, as corroborated by the similarity of the ^{13}C-NMR chemical shifts of all C-atoms compared to those of compound **1**. The linkage between the two glucopyranosyl units was determined as 1 → 3 on the basis of the HMBCs between H–C(3′) and two anomeric C-atoms (C(1′) and C(1″)), as well as the cross-peak between H–C(3′) and H–C(2′) in the COSY spectrum (*Fig.*, *b*). The coupling constants between H–C(1′) and H–C(2′), and H–C(1″) and H–C(2″) ($J = 7.8$ and 7.8, resp.) indicated their axial–axial orientation and thus the β-configuration of the two sugar units. The presence of an Ac group was evidenced by a C=O absorption at 1727 cm^{-1} in its IR spectrum, ^{13}C-NMR resonances at δ(C) 171.2 ppm, and a *singlet* signal at 2.00 ppm in its ^{1}H-NMR spectrum. The 6″-OH group of the outer glucose moiety of **3** was acetylated, insted of the 2″-OH group of **2**. This was established by comparing the NMR data of the outer glucose moiety of **3** with those of **1**. The chemical shift of the two diastereotopic H-atoms, CH_2(6″), of **1** were shifted from δ(H) 4.34 and 4.56 ppm to δ(H) 4.67 and 4.95 ppm in **3**, while the resonances of the other H-atoms of the outer glucose moiety were similar to those of compound **1**. Furthermore, the location of the AcO group at C(6″) was confirmed by the COSY cross-peak between CH_2(6″) and H–C(5″), and a three-bond HMBCs between CH_2(6″) and the Ac C=O C-atom at 171.2 ppm, and between H–C(5″) and the anomeric C(1″) (*Fig.*, *b*).

As with compound **2**, the absolute configuration of the two glucose units and the overall structure assignment were confirmed by successive basic hydrolysis of **3** to randianin, followed by acidic hydrolysis to oleanolic acid and D-glucose. Based on this evidence, the structure of **3** was elucidated as oleanolic acid 3-*O*-*β*-D-glucopyranosyl-(1→3)-(6″-*O*-acetyl)-*β*-D-glucopyranoside, or 6″-*O*-acetylrandianin.

Biological Evaluation. Compounds **1**–**3** were tested for their antiproliferative activities against the A2780 ovarian cancer, the A2058 melanoma, and the H522 lung cancer cell lines. All three compounds showed modest inhibitions of the proliferation of A2780 ovarian cancer cells, with IC_{50} values in the low micromolar range. However, they showed only weak inhibition of the proliferation of A2058 melanoma and the H522 lung cancer cell lines (*Table 2*). Several hundred cytotoxic triterpene saponins have been identified from plants, but only a few of them showed selective antiproliferative activity [13]. 2″-*O*-Acetylrandianin (**2**) and 6″-*O*-acetylrandianin (**3**) are examples of compounds that selectively inhibit the proliferation of A2780 ovarian cancer cells. Furthermore, in the A2780 assay, the cytotoxicities of the two acetylated saponins are stronger than that of randianin (**1**), which has no Ac group in its structure. This suggests that the increase in activity on acetylation may be due to an increase in lipophilicity, facilitating cellular uptake [14].

Table 2. *Antiproliferative Activities* (IC_{50} [μM]) *of Compounds* **1**–**3**

Compound	A2780[a])	A2058	H522
1	2.2±0.2	7.63	7.32
2	1.7±0.1	>3.3, <10	>10
3	1.2±0.3	>3.3, <10	>10
Paclitaxel	0.028±0.003	ND[b])	ND
Vinblastine	ND	0.004	0.009

[a]) Mean of three replicates. [b]) ND, Not determined.

This project was supported by the *Fogarty International Center*, the *National Cancer Institute*, the *National Science Foundation*, the *National Heart, Lung and Blood Institute*, the *National Institute of Mental Health*, the *Office of Dietary Supplements*, and the *Office of the Director of NIH*, under Cooperative Agreement U01 TW000313 with the *International Cooperative Biodiversity Groups*. This project was also supported by the *National Research Initiative of the Cooperative State Research, Education and Extension Service, USDA*, Grant #2008-35621-04732. Funds for the plant collection and extraction were provided by *Dow AgroSciences* and *Eisai Inc*. This support is gratefully acknowledged. This work was also supported by the *National Science Foundation* under Grant No. CHE-0619382 for purchase of the *Bruker-Avance 500* NMR spectrometer and Grant No. CHE-0722638 for the purchase of the *Agilent 6220* mass spectrometer. We thank Mr. *Bill Bebout* for recording the mass spectra, Dr. *Hugo Azurmendi* for assistance with the NMR spectra, and Drs. *Donald Hahn* (*Dow AgroSciences*) and *Edward Suh* (*Eisai Inc.*) for their encouragement and support. Field work essential for this project was conducted under a collaborative agreement between the *Missouri Botanical Garden* and the *Parc Botanique et Zoologique de Tsimbazaza*, and a multilateral agreement between the ICBG partners, including the *Centre National d'Applications des Recherches Pharmaceutiques*. We gratefully acknowledge courtesies extended by the Government of Madagascar (Ministère des Eaux et Forêts).

Experimental Part

General. Optical rotations: *Jasco P-2000* polarimeter. IR Spectra: *MIDAC M-series* FT-IR spectrophotometer as a film; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: in (D_5)pyridine on a *Bruker Avance 500* spectrometer; chemical shifts δ in ppm, and coupling constants J in Hz. MS: *Agilent 6220 LC-TOF-MS* in the pos.-ion mode; m/z.

Antiproliferative Bioassays. Antiproliferative activities were evaluated at Virginia Polytechnic Institute and State University against the drug-sensitive A2780 human ovarian cancer cell line as described in [15]. The values reported are the mean of three replicates. Antiproliferative activities against the A2058 melanoma and the H522 lung cancer cell lines were determined at *Eisai Inc.* by similar procedures to those used for the H460 cell line [16].

Plant Materials. A sample of the roots of *Nematostylis anthophylla* (A.RICH.) BAILL. was collected in March 2011. The sample was a shrub of 60 cm with red flowers and succulent leaves, growing in rocky habitat on Ibity Massif in the Vakinakaratra region of the Antsirabe II district, Madagascar at an elevation of 1650 m, and coordinates 20°03′59″S 047°00′01″E (−20.0663889, 47.0002778). Duplicate voucher specimens (*Richard Randrianaivo et al. 1803)* have been deposited with the Parc Botanique et Zoologique de Tsimbazaza (TAN), the Centre National d'Application des Recherches Pharmaceutiques in Antananarivo, Madagascar (CNARP), the Missouri Botanical Garden in St. Louis, Missouri (MO), and the Muséum National d' Histoire Naturelle in Paris, France (P).

Extraction and Isolation. Dried root parts of *N. anthophylla* (273 g) were ground in a hammer mill, then extracted with EtOH by percolation for 24 h at r.t. to give the crude extract MG 4657 (12.4 g), of which 3.2 g was shipped to Virginia Tech for bioassay-guided isolation. A 1.1-g sample of MG 4657 (IC_{50} 6.9 μg/ml) was suspended in aq. MeOH (MeOH/H_2O 9:1; 100 ml), and extracted with hexane (3 × 100 ml). The aq. layer was then diluted to 60% MeOH (*v*/*v*) with H_2O and extracted with CH_2Cl_2 (3 × 150 ml). The remaining aq. layer was further extracted with BuOH (3 × 100 ml). The hexane fraction was evaporated *in vacuo* to leave 131.2 mg of material with $IC_{50} > 20$ μg/ml. The residue from the CH_2Cl_2 fraction (166.1 mg) had an IC_{50} value of 7.7 μg/ml, the residue from the BuOH fraction (248.6 mg) had an IC_{50} value of 2.5 μg/ml and the remaining aq. MeOH fraction had an IC_{50} value of 20 μg/ml. Chromatography of the CH_2Cl_2 fraction over a *Sephadex® LH-20* size-exclusion column with CH_2Cl_2/MeOH 1:1 was used to obtain six fractions, of which the most active fraction (40.3 mg) had an IC_{50} value of 2.0 μg/ml. This fraction was then applied to a silica-gel column with $CHCl_3$/MeOH 9:1 to give fourteen fractions, of which *Fr. 11* (4.8 mg) was the most active (IC_{50} 1.0 μg/ml) and yielded compound **3**. The BuOH fraction was applied to an open column of *Diaion HP-20* resin and eluted with a step MeOH/H_2O gradient of 40, 70, and 100% MeOH. The 100% MeOH fraction was the most active fraction (100 mg) with an IC_{50} value of 2.2 μg/ml. This fraction was applied to a silica-gel column and eluted with $CHCl_3$/MeOH 6:1 to give thirteen fractions, of which *Fr. 4* (1.8 mg) yielded compound **2**, with an IC_{50} value of 1.5 μg/ml, and *Fr. 7* (6.3 mg) yielded compound **1**, with an IC_{50} value of 1.9 μg/ml.

*2″-*O*-Acetylrandianin* (*=(3β)-3-{[3-*O*-(2-*O*-Acetyl-β-*D*-glucopyranosyl)-β-*D*-glucopyranosyl]oxy}-olean-12-en-28-oic Acid*; **2**). Light-yellow solid. $[\alpha]_D^{21} = +12$ ($c = 1.2$, MeOH). IR: 3453, 2935, 1734, 1689, 1027. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 845.4692 ($[M+Na]^+$, $C_{44}H_{70}NaO_{14}^+$; calc. 845.4663).

*6″-*O*-Acetylrandianin* (*=(3β)-3-{[3-*O*-(6-*O*-Acetyl-β-*D*-glucopyranosyl)-β-*D*-glucopyranosyl]oxy}-olean-12-en-28-oic Acid*; **3**). Light-yellow solid. $[\alpha]_D^{21} = +17$ ($c = 1.2$, MeOH). IR: 3439, 2935, 1727, 1689, 1027. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 845.4643 ($[M+Na]^+$, $C_{44}H_{70}NaO_{14}^+$; calc. 845.4663).

Hydrolysis of Compounds **2** *and* **3**. Compound **3** (3.0 mg) was hydrolyzed with 6M NH_4OH for 2 h at 110°. The soln. was evaporated to dryness under reduced pressure, and then the residue was dissolved in H_2O and extracted with BuOH (3 ×) [17][18]. The BuOH extract was evaporated to dryness and yielded a light-yellow powder (2.6 mg) identified as compound **1** by its ^{1}H- and ^{13}C-NMR spectra. The light-yellow powder was further hydrolyzed with 3M HCl for 4 h at 100°. The soln. was extracted with AcOEt (3 ×), and both the org. and the aq. layers were evaporated to dryness under reduced pressure. The structure of the white powder (1.4 mg) obtained from the org. layer was determined to be oleanolic acid by ^{1}H- and ^{13}C-NMR spectroscopy. The semisolid carbohydrate mixture from the aq. layer (0.9 mg) was

dissolved in 2 ml of H_2O and kept overnight before TLC analysis and determination of its optical rotation. The same procedure was also applied to compound **2**. The sugars from both **2** and **3** had R_f values identical to that of glucose by TLC on a silica-gel plate with $CHCl_3$/MeOH/H_2O 15 : 6 : 1, and had $[\alpha]_D^{21}$ values of +13.9 and +14.2, resp. ($c = 0.1$, H_2O).

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Received May 7, 2012