Antiproliferative Limonoids of a *Malleastrum* sp. from the Madagascar Rainforest[†,1]

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Bioassay-guided fractionation of an ethanol extract of a *Malleastrum* sp. afforded three new limonoids, malleastrones A−C (**1**−**3**), respectively. Each limonoid contained a rare tetranortriterpenoid skeleton. Structure elucidation of the isolates was carried out by analysis of one- and two-dimensional NMR and X-ray diffraction data. The novel isolates **1** and **2** were tested for antiproliferative activity against a panel of cancer cell lines and exhibited IC_{50} values ranging from 0.19 to 0.63 μM.

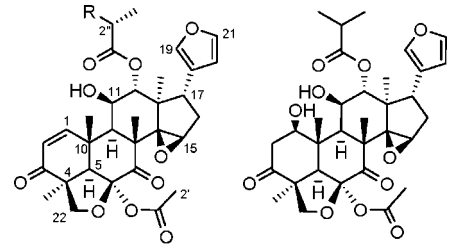
In our continuing search for biologically active natural products from tropical rainforests as part of an International Cooperative Biodiversity Group (ICBG) program,[2] we obtained an extract from *Malleastrum* sp. of the family Meliaceae. The taxonomy of the *Malleastrum* genus is complex, and it has not proved possible to identify this collection to the species level. The fragmented remaining forests of Madagascar's eastern escarpment are particularly diverse and continue to yield new species at an astonishing rate. The ICBG project has focused on inventory and sample collection in the remaining patches of forest that surround the Zahamena National Park.

The Meliaceae, or mahogany family, comprises approximately 550 species contained within 51 genera. Of the 51 genera, 22 are endemic to southern and eastern Africa and Madagascar.[3] The family is most widely known for its production of structurally unique, highly oxygenated, and biologically active limonoids.[4] To our knowledge this is the first reported chemical investigation of the genus *Malleastrum*.

Results and Discussion

Bioassay-guided fractionation of an ethanol extract of a *Malleastrum* sp. afforded three new limonoids (**1**−**3**), designated as malleastrones A−C. Each contained a hexacyclic tetranortriterpenoid skeleton, which has only rarely been reported in the literature.[5,6] Structure elucidation of compounds **1** and **2** was carried out by analysis of one- and two-dimensional NMR spectroscopy and X-ray diffraction data, while the structure of **3** was elucidated by analysis of g-COSY, g-HSQC, g-HMBC, and ROESY data and also by comparison of ^{13}C chemical shifts to those of malleastrone A (**1**).

Compound **1** was obtained as a colorless oil. Positive-ion HRFABMS analysis gave a pseudomolecular ion at *m*/*z* 583.2518 ($[M + H]^+$), which suggested a molecular formula of $C_{32}H_{38}O_{10}$. The ^{13}C and g-HSQC NMR spectra of **1** in $CDCl_3$ afforded evidence of two ester carbonyls (δ_C 179.7, C-1″; 170.7, C-1′), two ketone carbonyls (δ_C 201.3, C-3; 202.3, C-7), two sp^2-oxygenated carbons (δ_C 140.9, C-19; 142.0, C-21), and an additional four sp^2 carbons (δ_C 155.4, C-1; 128.4, C-2; 123.2, C-18; 112.5, C-20). There was also evidence of six sp^3-oxygenated carbons (δ_C 106.1, C-6; 75.6, C-11; 85.9, C-12; 74.6, C-14; 59.8, C-15; 77.8, C-22), one sp^3 methylene (δ_C 33.5, C-16), four sp^3 methines (δ_C 63.2, C-5; 43.0, C-9; 38.7, C-17; 33.9, C-2″), four quaternary carbons (δ_C 51.8, C-4; 49.1, C-8; 38.4, C-10; 46.3, C-13), and seven methyl groups (δ_C 28.0, C-23; 26.8, C-24; 25.6, C-25; 16.1, C-26; 21.0, C-2′; 18.1, C-3″; 19.1, C-4″). Select ^1H NMR resonances of **1** in $CDCl_3$ exhibited the characteristic pattern of a monosubstituted furan moiety (δ_H 7.16, br s, H-19; 6.17, br d, J = 1.0 Hz, H-20; 7.34, br t, J = 1.5 Hz, H-21). In addition signals for five hydrogens on oxygenated sp^3 carbons (δ_H 4.27, t, J = 3.0 Hz, H-11; 5.01, d, J = 3.5 Hz, H-12; 3.84, s, H-15; 4.34, d, J = 9.5 Hz, and 4.15, d, J = 9.5 Hz, H_2-22), and signals for two unsaturated methines (δ_H 7.51, d, J = 10 Hz, H-1; 6.03, d, J = 10 Hz, H-2) were observed.



1 R = CH_3
2 R = CH_2CH_3

3

The combination of g-COSY and g-HMBC spectra were critical in both assembling and linking together rings A−E. Ring A was assembled via a COSY correlation from H-1 to H-2 and subsequent HMBC correlations of H-1, H-2, and H_3-23 (δ_H 1.50, s) to the carbonyl (C-3). The linkage of ring A to B was evidenced by HMBC correlations from H_3-24 (δ_H 1.62, s) to C-1, C-10, and C-9, in addition to a correlation of H_3-25 (δ_H 1.73, s) to C-9 and a carbonyl at C-7. Also, the H-5 methine (δ_H 2.91, s) displayed long-range correlations with C-1 and a signal typical for a dioxygenated quaternary carbon (δ_C 106.1, C-6). The H_2-22 oxymethylene was found to be on a ring between rings A and B via HMBC correlations to C-3, C-4, C-5, and C-6. Fusion of ring B to the highly oxygenated C ring was determined primarily on the basis of COSY correlations from H-9 (δ_H 3.38, d, J = 3.5 Hz) to H-11, and H-11 to H-12, but was further supported by HMBC correlations from H_3-25 and H_3-26 (δ_H 1.33, s) to the quaternary epoxide carbon C-14. H_3-26 also exhibited long-range correlations with C-12, C-13, and C-17. The aforementioned data along with COSY connectivity from H-17 (δ_H 3.00, dd, J = 11, 6.0 Hz) to a methylene at position 16 (δ_H 2.12, dd, J = 13, 6.5 Hz, and 1.83, dd, J = 13, 11 Hz), and H_2-16 to an oxymethine (δ_H 3.84, s, H-15), thus suggested the fusion of ring C with a five-membered ring. The C ring epoxide moiety was partly deduced based on a characteristic ^{13}C signal for a shielded, cyclic

† Dedicated to Dr. G. Robert Pettit of Arizona State University for his pioneering work on bioactive natural products.

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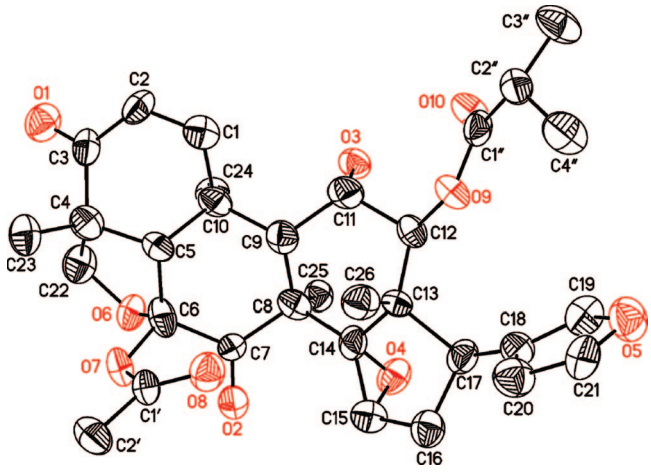

Figure 1. X-ray structure of malleastrone A (**1**).

epoxide carbon (δ_C 59.8, C-15). A long-range correlation from H-17 to C-18 and C-19 placed the monosubstituted furan moiety on C-17. The hydroxyl substituent was assigned to C-11 on the basis of the chemical shift of H-11 (δ_H 4.27) and C-11 (δ_C 75.6). Despite repeated efforts the relative positions of the remaining isobutyroxy and acetoxy substituents could not be assigned by NMR techniques due to a weak 2-D resonance of H-12 in conjunction with the absence of a hydrogen atom at C-6. Compound **1** was thus crystallized from a mixture of $CHCl_3$ and EtOH at room temperature and subjected to X-ray crystallography. The X-ray diffraction pattern (Figure 1) placed the isobutyroxy moiety at C-12 and the acetoxy substituent at C-6, completing the flat structure of **1**. The relative configuration of **1** was also established by its crystal structure, although the crystal quality did not allow assignment of absolute configuration.

Compound **2** was obtained as a colorless oil. Positive-ion HRFABMS analysis gave a pseudomolecular ion at *m/z* 597.2781 ($[M + H]^+$), which suggested a molecular formula of $C_{33}H_{40}O_{10}$. Compound **2** exhibited nearly identical ^1H and ^{13}C NMR resonances to those of **1**, and the g-COSY and g-HMBC spectra further confirmed that both compounds shared an identical basic skeleton. The distinction between the two sets of spectra was demonstrated by the presence of an additional methylene carbon signal at δ_C 25.8 (C-3″) in **2**, thus validating the additional 14 mass units found by mass spectrometry. In place of the two 3H doublets in the upfield region of the ^1H NMR spectrum of **1** (δ_H 1.07, d, J = 6.5 Hz, H_3-3″; 1.06, d, J = 7.5 Hz, H_3-4″), compound **2** displayed 3H doublet (δ_H 1.03, d, J = 7.5 Hz, H_3-5″) and triplet signals (δ_H 0.90, t, J = 7.0 Hz, H_3-4″), suggesting a modification to the C-12 substituent. The COSY spectrum of **2** indicated connectivity between H-2″ (δ_H 2.24, m, J = 7.0 Hz) and H_3-5″ and between H_2-3″ and H_3-4″, but no contour was present to indicate connectivity between H-2″ and H_2-3″. The flat structure of **2** was thus established by a 1-D TOCSY experiment with enhancement of the methyl triplet (H_3-4″). Five signals were observed, including signals for two nonequivalent methylene hydrogens (δ_H 1.60 m, H_a-3″; 1.36, m, H_b-3″).

The relative configuration of **2** was determined by analysis of its ROESY spectrum (Figure 2) and by comparison of ^1H and ^{13}C NMR chemical shifts to those of **1**, but the configuration at C-2″ could not be determined from these data. For this reason, we attempted to crystallize **2** and finally succeeded using a slow and controlled evaporation of a solution in $CHCl_3$/EtOH. The resulting X-ray diffraction data confirmed the previously assigned relative configuration of **2**, identified the configuration at C-2″, and also allowed determination of the absolute configuration of malleastrone B as (4*S*, 5*R*, 6*R*, 8*R*, 9*R*, 10*R*, 11*R*, 12*R*, 13*R*, 14*R*, 15*R*, 17*S*, 2″*S*).[7] Since compound **1** has an identical relative configuration

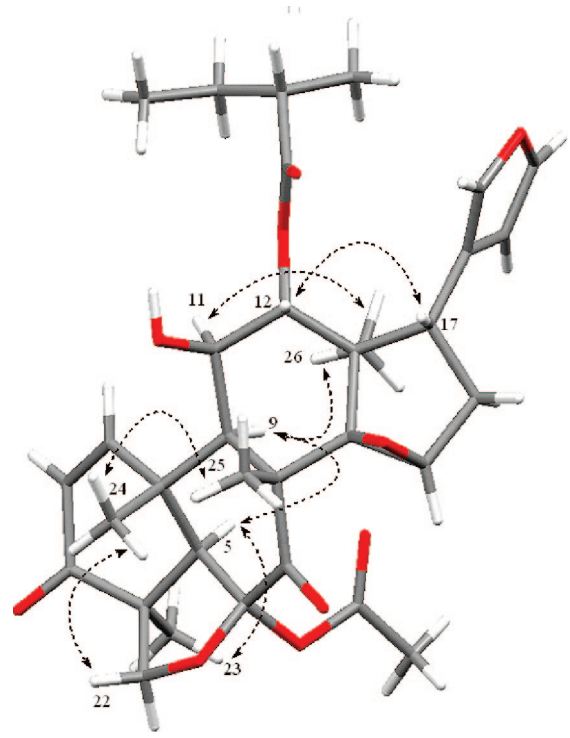

Figure 2. Key ROESY correlations of malleastrone B (**2**).

and a nearly superimposable CD spectrum with that of **2**, its absolute configuration could also be assigned as (4*S*, 5*R*, 6*R*, 8*R*, 9*R*, 10*R*, 11*R*, 12*R*, 13*R*, 14*R*, 15*R*, 17*S*).

Compound **3** was obtained as a greenish oil. Positive-ion HRFABMS analysis gave a pseudomolecular ion at *m/z* 583.2925, suggesting a composition [M − H_2O + H]. To confirm the molecular weight, low-resolution positive-ion ESIMS under direct infusion afforded a pseudomolecular ion at *m/z* 601.2 ($[M + H]^+$), which confirmed the molecular formula of $C_{32}H_{40}O_{11}$. The molecular ion peak proved to be unstable even under a low collision energy condition of 10 V. The dominant species was observed at *m/z* 541.2 [M − $C_2H_3O_2$], formed by loss of an acetoxy moiety. Compound **3** shared similar ^1H and ^{13}C spectral patterns with those of **1**, though additional signals and noticeable differences in chemical shift suggested the presence of a slightly different functionality. The molecular weight of **3** was 18 mass units greater than that of **1**, indicating the addition of a molecule of water. The absence of two olefinic hydrogen resonances in the ^1H NMR spectrum in addition to a downfield shift of the C-3 carbonyl (δ_C 212.4) signal signified a change to the A ring. Two α-methylene hydrogens (δ_H 3.04, dd, J = 17, 8.4 Hz, H_a-2 and 2.55, d, J = 17 Hz, H_b-2) showed a COSY correlation to one sp^3-oxygenated methine (δ_H 4.35, d, J = 8.4 Hz, H-1), and thus a hydroxyl moiety was placed at the 1-position. *J*-based analysis of the ^1H NMR signal of H-1 determined the 1-OH (δ_H 3.11, br s) to be equatorial. Further analysis of ROESY and CD spectra confirmed the relative and absolute configuration of the remainder of the molecule to be (1*R*, 4*S*, 5*R*, 6*R*, 8*R*, 9*R*, 10*R*, 11*R*, 12*R*, 13*R*, 14*R*, 15*R*, 17*S*), similar to that of **1** and **2**. Selected 2D NMR correlations of **3** appear in Figure 3.

All of the isolates were tested against the A2780 human ovarian cancer cell line, and compounds **1** and **2** were tested against four additional cell lines. The results are shown in Table 2. Of the three compounds, **1** and **2** displayed significant antiproliferative activity, while **3** was comparatively inactive (18 μM) against the A2780 human ovarian cancer cell line. It is apparent that saturation at the 1-position of this particular limonoid skeleton results in a complete loss of its antiproliferative properties and emphasizes the importance of A ring composition. Overall, compounds **1** and **2** appeared to

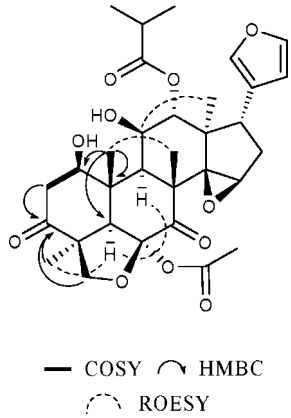

Figure 3. Key 2D NMR spectroscopic correlations of malleastrone C (**3**).

exhibit general antiproliferative activity toward tumor cells, and so no further exploration of their bioactive potential was carried out.

The hexacyclic tetranortriterpenoid skeleton of compounds **1**−**3** is of rare occurrence in nature.[5,6] Generally speaking the biosynthetic origin of limonoids is traced to the triterpene euphane, whose C_8 side chain undergoes cyclization and loss of four carbons to yield the C-17 substituted furan moiety.[5] Aside from the latter, reports of further biosynthetic modifications to the limonoid skeleton that describe mechanistic details have been limited. It is widely accepted and generally stated that after initial formation of the tetranortriterpenoid skeleton limonoids undergo a series of oxidations and skeletal rearrangements via ring cleavage reactions.[4,8–10] In the case of the malleastrone limonoids, esterification, D ring epoxidation, and cyclization to yield an A/B ring substituted tetrahydrofuran moiety are some of the modifications required to afford structures **1**−**3**. Thus, much work remains in order to reveal the specific biosynthetic mechanisms of such highly oxidized and bioactive chemical species.

Experimental Section

General Experimental Procedures. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. Melting points were obtained on a Buchi MP B-540 apparatus. NMR spectra were obtained on JEOL Eclipse 500, Varian Inova 400, and Varian Unity 400 spectrometers. Mass spectra were obtained on a JEOL JMS-HX-110 instrument and a Finnigan LTQ LC/MS. CD analysis was performed on a JASCO J-720 spectropolarimeter. Chemical shifts are given in δ (ppm), and coupling constants (J) are reported in Hz. HPLC was performed using Shimadzu LC-10AT pumps coupled with Varian Dynamax semipreparative diol and C-8 columns (250 × 10 mm). The HPLC system employed a Shimadzu SPD-M10A diode array detector.

Antiproliferative Bioassays. The A2780 ovarian cancer cell line antiproliferative assay was performed at Virginia Polytechnic Institute and State University as previously reported.[11]

Antiproliferative effects of compounds **1** and **2** were evaluated in four cultured human cancer cell lines: MDA-MB-435 breast cancer

Table 1. NMR Spectral Data of Limonoids **1**−**3** in $CDCl_3$[a,b]

	1		**2**		**3**	
pos	^{13}C	^{1}H[c] (J, Hz)	^{13}C	^{1}H[c] (J, Hz)	^{13}C	^{1}H[c] (J, Hz)
1	155.4	7.51 d (10)	155.3	7.50 d (10)	71.9	4.35 d (8.4)
2	128.4	6.03 d (10)	128.4	6.03 d (10)	46.4	3.04 dd (17, 8.4)
						2.55 d (17)
3	201.3		201.3		212.4	
4	51.8		51.8		52.8	
5	63.2	2.91 s	63.2	2.91 s	56.1	3.68 s
6	106.1		106.1		109.4	
7	202.3		202.3		199.6	
8	49.1		49.1		49.5	
9	43.0	3.38 d (3.5)	43.0	3.38 d (4.0)	41.0	3.26 s
10	38.4		38.4		40.4	
11	75.6	4.27 t (3.0)	75.6	4.27 t (3.0)	75.2	4.07 br s
12	85.9	5.01 d (3.5)	85.8	5.01 d (3.5)	85.3	4.90 br s
13	46.3		46.3		45.4	
14	74.6		74.6		70.9	
15	59.8	3.84 s	59.8	3.84 s	57.3	4.02 s
16	33.5	2.12 dd (13, 6.5)	33.6	2.12 dd (13, 6.5)	32.3	2.21 dd (14, 6.5)
		1.83 dd (13, 11)		1.83 dd (13, 11)		1.94 dd (14, 11)
17	38.7	3.00 dd (11, 6.0)	38.7	3.00 dd (11, 6.0)	41.2	2.89 dd (11, 6.8)
18	123.2		123.2		122.7	
19	140.9	7.16 br s	140.9	7.16 br s	140.4	7.12 d (1.0)
20	112.5	6.17 br d (1.0)	112.6	6.18 br s	111.9	6.12 br s
21	142.0	7.34 br t (1.5)	142.0	7.34 br t (1.6)	142.3	7.31 d (1.6)
22	77.8	4.34 d (9.5)	77.8	4.34 d (9.5)	78.9	4.48 d (9.0)
		4.15 d (9.5)		4.15 d (9.5)		4.08 d (9.0)
23	28.0	1.50 s	28.0	1.50 s	26.7	1.49 s
24	26.8	1.62 s	26.8	1.62 s	17.3	1.29 s
25	25.6	1.73 s	25.6	1.73 s	23.0	1.51 s
26	16.1	1.33 s	16.1	1.33 s	14.8	1.12 s
1′	170.7		170.7		170.8	
2′	21.0	2.10 s	21.0	2.10 s	21.4	2.13 s
1″	179.7		179.4		178.2	
2″	33.9	2.44 m	40.9	2.24 m	34.1	2.43 m
3″	18.1	1.07 d (6.5)	25.8	1.60 m, 1.36 m	18.4	1.03 d (7.0)[d]
4″	19.1	1.06 d (7.5)	11.8	0.90 t (7.0)	18.9	1.04 d (7.0)[d]
5″			16.9	1.03 d (7.5)		
11-OH		4.09 br s		4.14 s		3.49 s
1-OH						3.11 br s

[a] Assignments based on COSY, HMBC, and HSQC. [b] Chemical shifts (δ) in ppm. [c] br s, broad singlet; d, doublet; m, multiplet. [d] Values are interchangeable. [e] Signal overlapped with H-6′.

Table 2. Antiproliferative Data of Compounds **1**–**3**

	IC_{50} (μM)				
compound	A2780[a]	MDA-MB-435[b]	HT-29[b]	H522-T1[b]	U937[b]
1	0.49	0.41	0.24	0.24	0.20
2	0.63	0.34	0.22	0.23	0.19
3	18	ND	ND	ND	ND

[a] Concentration of each compound that inhibited 50% of the growth of the A2780 human ovarian cell line according to the procedure described,[11] with paclitaxel (IC_{50} 23.4 nM) as the positive control. [b] Concentration of a compound that inhibited cell growth by 50% compared to untreated cell populations, with vinblastine as the positive control (average IC_{50} 0.27 nM (MDA-MB-435), 0.53 nM (HT-29), 1.38 nM (H522-T1), and 0.49 nM (U937).

cells, HT-29 colon cancer cells, H522-T1 non-small-cell cancer cells, and U937 histiocytic lymphoma cells. The cells were placed into 96-well plates and grown in the absence or continuous presence of 0.3–10 000 nM compounds for 96 h. Cell growth was assessed using the CellTiter-Glo luminescent cell viability assay (Promega) according to manufacturer's recommendations. Luminescence was read on a Victor^{2}V 1420 MultiLabel HTS counter (Perkin-Elmer/Wallac). IC_{50} values were determined as the concentration of a compound that inhibits cell growth by 50% compared to untreated cell populations. Two separate replicate experiments were performed.

X-ray Diffraction Studies. Colorless needles of **1** were crystallized from $CHCl_3$/EtOH at room temperature. The chosen crystal was cut ($0.016 \times 0.067 \times 0.194$ mm^3) and mounted on the goniometer of an Oxford Diffraction Gemini diffractometer equipped with a Sapphire 3 CCD detector. The data collection routine, unit cell refinement, and data processing were carried out with the program CrysAlisPro.[12] The Laue symmetry and systematic absences were consistent with the orthorhombic space group $P2_12_12_1$. The structure was solved by direct methods and refined using SHELXTL NT.[13] The asymmetric unit of the structure comprises one crystallographically independent molecule. The final refinement model involved anisotropic displacement parameters for non-hydrogen atoms and a riding model for all hydrogen atoms. Due to insufficient crystal quality, the absolute configuration could not be determined from the Friedel pairs; the Friedel pairs were therefore merged for the final refinement. SHELXTL NT was used for molecular graphics generation.[13]

A colorless paralellepiped crystal of **2** (0.053 mm × 0.087 mm × 0.121 mm) was crystallized from chloroform/ethanol at room temperature. The crystal was centered on the goniometer of an Oxford Diffraction Nova diffractometer operating with Cu radiation. The data collection routine, unit cell refinement, and data processing were carried out with the program CrysAlis.[11] The Laue symmetry and systematic absences were consistent with the monoclinic space groups $P2_1$ and $P2_1/m$. As **2** was known to be enantiomerically pure, the chiral space group $P2_1$ was chosen. The structure was solved by direct methods and refined using SHELXTL NT.[12] The asymmetric unit of the structure comprises one crystallographically independent molecule. The final refinement model involved anisotropic displacement parameters for non-hydrogen atoms and a riding model for all hydrogen atoms. The absolute configuration was established from anomalous dispersion effects with the Flack parameter refining to 0.0(2). Using the Bijvoet pair method,[14,15] the correlation for the correct enantiomer, P2(true), was 1.000 and the incorrect enantiomer, P3(false), was 0.2×10^{-24}. SHELXTL NT was used for molecular graphics generation.[10]

Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Plant Material. The sample of *Malleastrum* sp. (Meliaceae) was collected in mid-elevation humid forest in the Zahamena region of Madagascar, in the province of Toamasina, 250 m from the hamlet of Antenina, 4 km from Ankosy (17°32′32″ S, 48°43′20″ E, 1250 m elevation) under the vernacular name Fanazava beravina, in December 2002. Duplicates of the voucher specimen (Randrianjanaka 766) were deposited at the Missouri Botanical Garden, St. Louis, Missouri (MO), the Muséum National d'Histoire Naturelle, Paris (P), the Département des Recherches Forestières et Pisicoles, Madagascar (TEF), and the Centre National d'Application des Recherches Pharmaceutique, Madagascar (CNARP). The tree had a height of 7 m and trunk diameter at chest height of 8 cm.

Extraction and Isolation. The dried plant sample described above (422 g) was extracted with EtOH to give 16.5 g of extract designated MG 1695, of which 3.6 g was made available to Virginia Polytechnic Institute and State University. Extract MG 1695 (1.2 g) was suspended in aqueous MeOH (MeOH/H_2O, 9:1, 500 mL) and extracted with hexanes (2 × 200 mL; > 20 μg/mL). The aqueous layer was then diluted to 40% water and extracted with DCM (2 × 200 mL). The DCM and aqueous MeOH fractions displayed antiproliferative activity (IC_{50} = 2.9 and 15 μg/mL, respectively). The DCM fraction was further chromatographed over a flash silica gel column to yield three fractions (A−C). Fraction A (497 mg, IC_{50} = 2.4 μg/mL) was chromatographed over a flash silica column to yield eight fractions (D−K). Malleastrones A (t_R 25 min, 1.9 mg, IC_{50} = 0.49 μM, A2780) and B (t_R 21.5 min, 1.8 mg, IC_{50} = 0.63 μM, A2780) were isolated from fraction H (304 mg, IC_{50} = 1.7 μg/mL) via semipreparative diol HPLC using an isocratic flow of hexanes/DCM (68:32). Compound **3** was extracted out of the aqueous MeOH fraction, which was partitioned between H_2O and *n*-BuOH. The *n*-BuOH fraction (425 mg, IC_{50} = 11 μg/mL) was subjected to a flash C18 column to yield three fractions (L−N). Fraction M (192 mg, IC_{50} = 9.5 μg/mL) was separated via preparative C18 HPLC over 25 min with a gradient of 50–100% MeOH to give five fractions (O−S). Fractions S (t_R 25–35 min, 45.7 mg, IC_{50} = 3.3 μg/mL) and the MeOH flush were subjected to semipreparative C8 HPLC employing an isocratic flow of MeOH/H_2O (62:38) to afford malleastrone C (t_R 23.5 min, 0.8 mg, IC_{50} = 18 μM, A2780).

Malleastrone A (1): colorless needles; mp 252–254 °C; UV ($CHCl_3$) λ_{max} (log ε) 243 (3.46) nm; IR ν_{max} 3473, 2918, 2850, 1721, 1685, 1458, 1375, 1259, 1162, 1004 cm^{-1}; CD (MeOH, *c* 0.0180) $[\theta]_{233}$ 53.7, $[\theta]_{337}$ −11.1; ^{1}H and ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 583.2518 [M + 1]$^+$ (calcd for $C_{32}H_{39}O_{10}$, 583.2543).

Malleastrone B (2): colorless needles; mp 239–241 °C; UV ($CHCl_3$) λ_{max} (log ε) 246 (3.47) nm; IR ν_{max} 3462, 2934, 1722, 1687, 1457, 1375, 1260, 1159, 1006 cm^{-1}; CD (MeOH, *c* 0.0012) $[\theta]_{235}$ 16.7, $[\theta]_{335}$ −1.65; ^{1}H and ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 597.2781 [M + 1]$^+$ (calcd for $C_{33}H_{41}O_{10}$, 597.2700).

Malleastrone C (3): light-green oil; UV ($CHCl_3$) λ_{max} (log ε) 245 (3.52), 275 (3.20) nm; IR ν_{max} 3423, 2919, 2850, 1719 (br), 1456, 1377, 1256 cm^{-1}; CD (MeOH, *c* 0.0090) $[\theta]_{249}$ 3.02, $[\theta]_{275}$ 5.05, $[\theta]_{319}$ −1.04; ^{1}H and ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 583.2925 [M − H_2O + 1]$^+$ (calcd for $C_{32}H_{39}O_{10}$, 583.2543); ESIMS *m*/*z* 601.2 ($C_{32}H_{40}O_{11}$).

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Supporting Information Available: X-ray crystallographic data for compounds **1** and **2** (crystal data, atom coordinates, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates, and isotropic displacement parameters) and ^{1}H NMR spectra for compounds **1**−**3** are available. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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