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## Antiproliferative Limonoids of a *Malleastrum* sp. from the Madagascar Rainforest,<sup>1,†</sup>

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### Abstract

Bioassay-guided fractionation of an ethanol extract of a *Malleastrum* sp. afforded three new limonoids (**1-3**), malleastrones A-C, respectively. Each limonoid contained the 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<sub>50</sub> values ranging from 0.19 – 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,<sup>2</sup> 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.

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

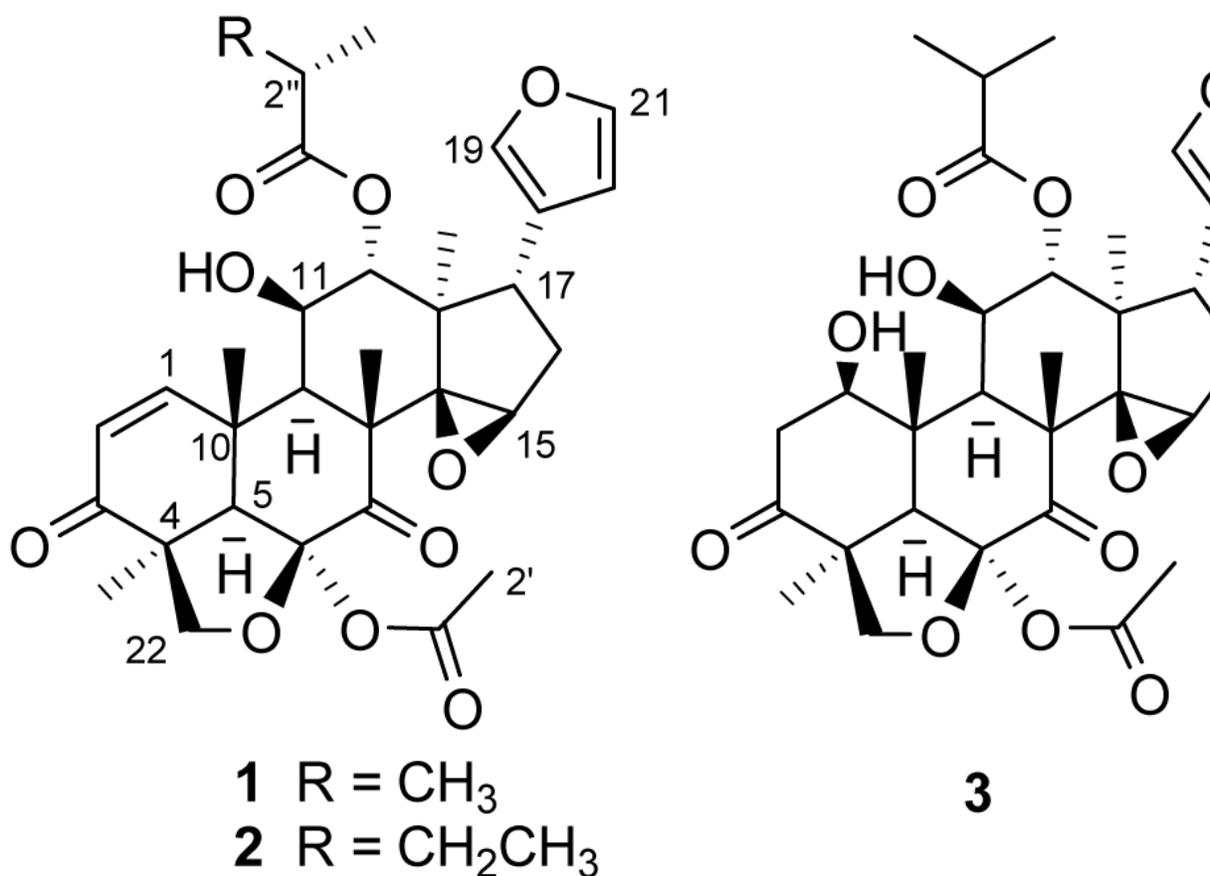
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**Supporting Information Available:** Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or deposit@ccdc.cam.ac.uk. 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 <sup>1</sup>H NMR spectra for compounds **1- 3** are available as Supporting Information. This material is available free of charge via the internet at <http://pubs.acs.org>

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.<sup>3</sup> The family is most widely known for its production of structurally unique, highly oxygenated, and biologically active limonoids.<sup>4</sup> To our knowledge this is the first reported chemical investigation of the genus *Malleastrum*.

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 that has only rarely been reported in the literature.<sup>5,6</sup> 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 <sup>13</sup>C chemical shifts to those of malleastrone A (**1**).

Compound **1** was obtained as colorless oil. Positive-ion HRFABMS analysis gave a pseudomolecular ion at  $m/z$  583.2518 ( $[M+H]^+$ ), which suggested a molecular formula of C<sub>32</sub>H<sub>38</sub>O<sub>10</sub>. The <sup>13</sup>C and g-HSQC NMR spectra of **1** in CDCl<sub>3</sub> afforded evidence of two ester carbonyls ( $\delta_C$  179.7, C-1''; 170.7, C-1'), two ketone carbonyls ( $\delta_C$  201.3, C-3; 202.3, C-7), two  $sp^2$ -oxygenated carbons ( $\delta_C$  140.9, C-19; 142.0, C-21), and an additional four  $sp^2$  carbons ( $\delta_C$  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 ( $\delta_C$  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 ( $\delta_C$  33.5, C-16), four  $sp^3$  methines ( $\delta_C$  63.2, C-5; 43.0, C-9; 38.7, C-17; 33.9, C-2''), four quaternary carbons ( $\delta_C$  51.8, C-4; 49.1, C-8; 38.4, C-10; 46.3, C-13), and seven methyl groups ( $\delta_C$  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 <sup>1</sup>H NMR resonances of **1** in CDCl<sub>3</sub> exhibited the characteristic pattern of a monosubstituted furan moiety ( $\delta_H$  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 ( $\delta_H$  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<sub>2</sub>-22), and signals for two unsaturated methines ( $\delta_H$  7.51, d,  $J$  = 10 Hz, H-1; 6.03, d,  $J$  = 10 Hz, H-2) were observed.



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<sub>3</sub>-23 ( $\delta_{\text{H}}$  1.50, s) to the carbonyl (C-3). The linkage of ring A to B was evidenced by HMBC correlations from H<sub>3</sub>-24 ( $\delta_{\text{H}}$  1.62, s) to C-1, C-10, and C-9, in addition to a correlation of H<sub>3</sub>-25 ( $\delta_{\text{H}}$  1.73, s) to C-9 and a carbonyl at C-7. Also, the H-5 methine ( $\delta_{\text{H}}$  2.91, s) displayed long range correlations with C-1 and a signal typical for a dioxygenated quaternary carbon ( $\delta_{\text{C}}$  106.1, C-6). The H<sub>2</sub>-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 ( $\delta_{\text{H}}$  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<sub>3</sub>-25 and H<sub>3</sub>-26 ( $\delta_{\text{H}}$  1.33, s) to the quaternary epoxide carbon C-14. H<sub>3</sub>-26 also exhibited long range correlations with C-12, C-13, and C-17. The aforementioned data along with COSY connectivity from H-17 ( $\delta_{\text{H}}$  3.00, dd,  $J = 11, 6.0$  Hz) to a methylene at position 16 ( $\delta_{\text{H}}$  2.12, dd,  $J = 13, 6.5$  Hz, and 1.83, dd,  $J = 13, 11$  Hz), and H<sub>2</sub>-16 to an oxymethine ( $\delta_{\text{H}}$  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 <sup>13</sup>C signal for a shielded, cyclic epoxide carbon ( $\delta_{\text{C}}$  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 based on the chemical shift of H-11 ( $\delta_{\text{H}}$  4.27) and C-11 ( $\delta_{\text{C}}$  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<sub>3</sub> 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  $\delta_C$  25.8 (C-3'') in **2**, thus validating the additional fourteen mass units found by mass spectrometry. In place of the two 3H doublets in the upfield region of the  $^1H$  NMR spectrum of **1** ( $\delta_H$  1.07, d,  $J = 6.5$  Hz, H<sub>3</sub>-3''; 1.06, d,  $J = 7.5$  Hz, H<sub>3</sub>-4''), compound **2** displayed 3H doublet ( $\delta_H$  1.03, d,  $J = 7.5$  Hz, H<sub>3</sub>-5'') and triplet signals ( $\delta_H$  0.90, t,  $J = 7.0$  Hz, H<sub>3</sub>-4''), suggesting a modification to the C-12 substituent. The COSY spectrum of **2** indicated connectivity between H-2'' ( $\delta_H$  2.24, m,  $J = 7.0$  Hz) and H<sub>3</sub>-5'', and between H<sub>2</sub>-3'' and H<sub>3</sub>-4'', but no contour was present to indicate connectivity between H-2'' and H<sub>2</sub>-3''. The flat structure of **2** was thus established by a 1-D TOCSY experiment with enhancement of the methyl triplet (H<sub>3</sub>-4''). Five signals were observed, including signals for two non-equivalent methylene hydrogens ( $\delta_H$  1.60 m, H<sub>a</sub>-3''; 1.36, m, H<sub>b</sub>-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*).<sup>7</sup> Since compound **1** has an identical relative configuration and a nearly super imposable 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 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 volts. 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 slightly different functionality. The molecular weight of **3** was eighteen 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 ( $\delta_C$  212.4) signal signified a change to the A-ring. Two  $\alpha$ -methylene hydrogens ( $\delta_H$  3.04, dd,  $J = 17.4, 8.4$  Hz, H<sub>a</sub>-2 and 2.55, d,  $J = 17$  Hz, H<sub>b</sub>-2) showed a COSY correlation to one  $sp^3$ -oxygenated methine ( $\delta_H$  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 ( $\delta_H$  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  $\mu$ M) 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 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.<sup>5,6</sup> Generally speaking the biosynthetic origin of limonoids is traced to the triterpene euphane, whose C<sub>8</sub> side chain undergoes cyclization and loss four carbons to yield the C-17 substituted furan moiety.<sup>5</sup> 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.<sup>4,8-10</sup> 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 point was 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  $\delta$  (ppm), and coupling constants ( $J$ ) are reported in Hz. HPLC was performed using Shimadzu LC-10AT pumps coupled with Varian Dynamax semi-preparative diol and C-8 columns (250  $\times$  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.<sup>11</sup>

Antiproliferative effects of compounds **1** and **2** were evaluated in four cultured human cancer cell lines: MDA-MB-435 breast cancer 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 – 10000 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<sup>2</sup>V 1420 MultiLabel HTS Counter (Perkin-Elmer/Wallac). IC<sub>50</sub> values were determined as the concentration of a compound which 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  $\text{CHCl}_3/\text{EtOH}$  at room temperature. The chosen crystal was cut ( $0.016 \times 0.067 \times 0.194 \text{ mm}^3$ ) and mounted on the goniometer of an Oxford Diffraction Gemini diffractometer equipped with a Sapphire 3<sup>TM</sup> CCD detector. The data collection routine, unit cell refinement, and data processing were carried out with the program CrysAlisPro.<sup>12</sup> 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.<sup>13</sup> 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.<sup>13</sup>

A colorless parallelepiped crystal of **2** ( $0.053 \text{ mm} \times 0.087 \text{ mm} \times 0.121 \text{ mm}$ ) 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.<sup>12</sup> 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.<sup>13</sup> 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,<sup>14,15</sup> the correlation for the correct enantiomer, P2(true), was 1.000 and the incorrect enantiomer, P3(false), was  $0.2 \times 10^{-24}$ . SHELXTL NT was used for molecular graphics generation.<sup>10</sup>

## 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 ( $17^\circ 32' 32'' \text{S}$ ,  $48^\circ 43' 20'' \text{E}$ , 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 Piscicoles, 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 breast 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 ( $\text{MeOH-H}_2\text{O}$ , 9:1, 500 mL) and extracted with hexanes ( $2 \times 200 \text{ mL}$ ;  $> 20 \mu\text{g/mL}$ ). The aqueous layer was then diluted to 40% water and extracted with DCM ( $2 \times 200 \text{ mL}$ ). The DCM and aqueous MeOH fractions displayed antiproliferative activity ( $\text{IC}_{50} = 2.9$  and  $15 \mu\text{g/mL}$ , respectively). The DCM fraction was further chromatographed over a flash silica gel column to yield three fractions (A-C). Fraction A (497 mg,  $\text{IC}_{50} = 2.4 \mu\text{g/mL}$ ) was chromatographed over a flash silica column to yield eight fractions (D-K). Malleastrone A ( $t_R$  25 min, 1.9 mg,  $\text{IC}_{50} = 0.49 \mu\text{M}$ , A2780) and B ( $t_R$  21.5 min, 1.8 mg,  $\text{IC}_{50} = 0.63 \mu\text{M}$ , A2780) were isolated from fraction H (304 mg,  $\text{IC}_{50} = 1.7 \mu\text{g/mL}$ ) via semi-preparative 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<sub>2</sub>O and *n*-BuOH. The *n*-BuOH fraction (425 mg, IC<sub>50</sub> = 11 µg/mL) was subjected to a flash C18 column to yield three fractions (L-N). Fraction M (192 mg, IC<sub>50</sub> = 9.5 µg/mL) was separated via preparative C18 HPLC over 25 minutes with a gradient of 50-100 % MeOH to give five fractions (O-S). Fractions S (*t*<sub>R</sub> 25-35 min, 45.7 mg, IC<sub>50</sub> = 3.3 µg/mL) and the MeOH flush were subjected to semi-preparative C8 HPLC employing an isocratic flow of MeOH-H<sub>2</sub>O (62:38) to afford malleastrone C (*t*<sub>R</sub> 23.5 min, 0.8 mg, IC<sub>50</sub> = 18 µM, A2780).

**Malleastrone A (1):** colorless needles; mp 252-254 °C; UV (CHCl<sub>3</sub>) λ<sub>max</sub> (log ε) 243 (3.46) nm; IR ν<sub>max</sub> 3473, 2918, 2850, 1721, 1685, 1458, 1375, 1259, 1162, 1004 cm<sup>-1</sup>; CD (MeOH, *c* 0.0180) [θ]<sub>233</sub> 53.7, [θ]<sub>337</sub> -11.1; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; HRFABMS *m/z* 583.2518 [M+1]<sup>+</sup> (calcd for C<sub>32</sub>H<sub>38</sub>O<sub>10</sub>, 583.2499).

**Malleastrone B (2):** colorless needles; mp 239-241 °C; UV (CHCl<sub>3</sub>) λ<sub>max</sub> (log ε) 246 (3.47) nm; IR ν<sub>max</sub> 3462, 2934, 1722, 1687, 1457, 1375, 1260, 1159, 1006 cm<sup>-1</sup>; CD (MeOH, *c* 0.0012) [θ]<sub>235</sub> 16.7, [θ]<sub>335</sub> -1.65; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; HRFABMS *m/z* 597.2781 [M+1]<sup>+</sup> (calcd for C<sub>33</sub>H<sub>40</sub>O<sub>10</sub>, 597.2655).

**Malleastrone C (3):** light-green oil; UV (CHCl<sub>3</sub>) λ<sub>max</sub> (log ε) 245 (3.52), 275 (3.20) nm; IR ν<sub>max</sub> 3423, 2919, 2850, 1719 (br), 1456, 1377, 1256 cm<sup>-1</sup>; CD (MeOH, *c* 0.0090) [θ]<sub>249</sub> 3.02, [θ]<sub>275</sub> 5.05, [θ]<sub>319</sub> -1.04; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; HRFABMS *m/z* 583.2925 [M-H<sub>2</sub>O+1]<sup>+</sup> (calcd for C<sub>32</sub>H<sub>39</sub>O<sub>10</sub>, 583.2543) and ESIMS *m/z* 601.2 (C<sub>32</sub>H<sub>40</sub>O<sub>11</sub>).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

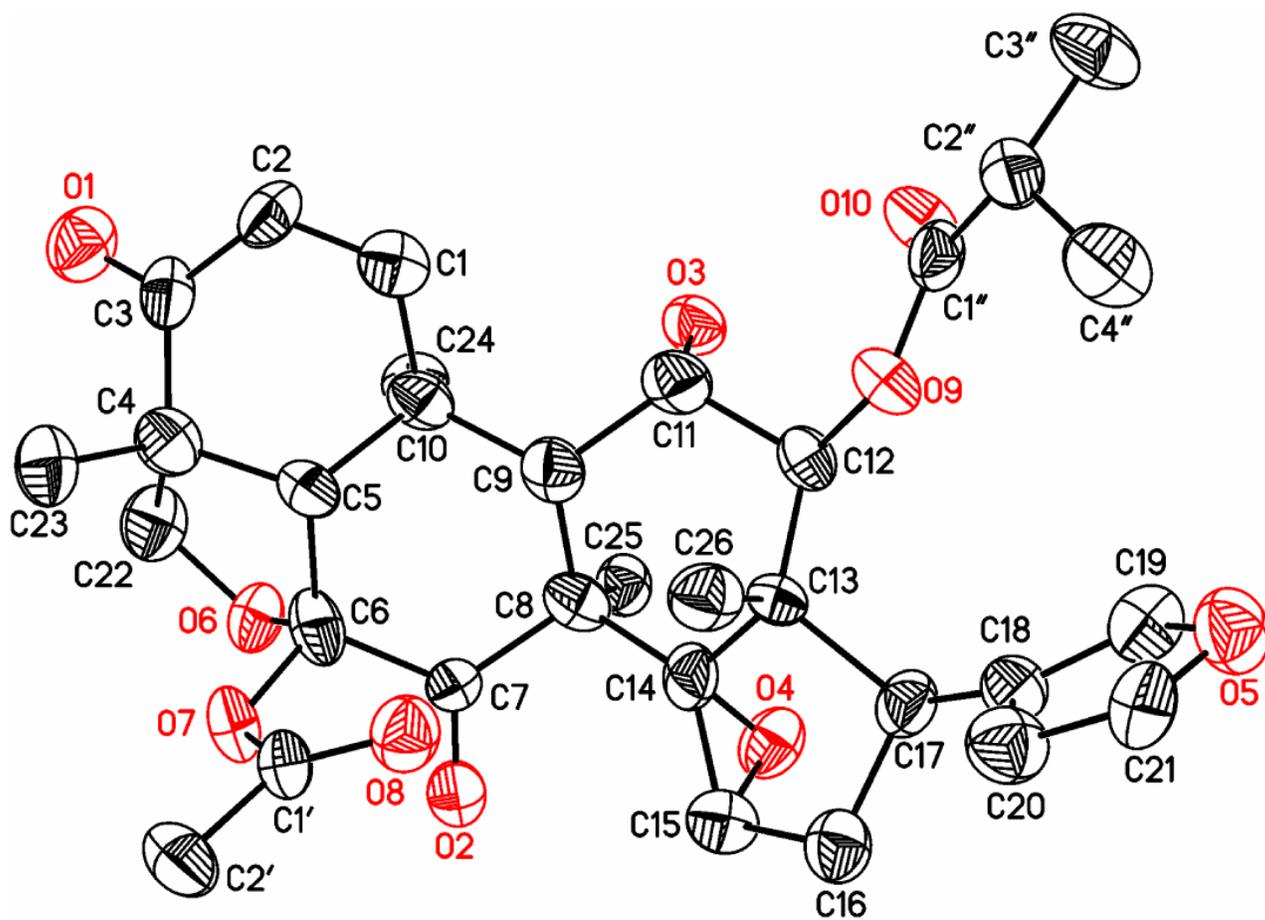
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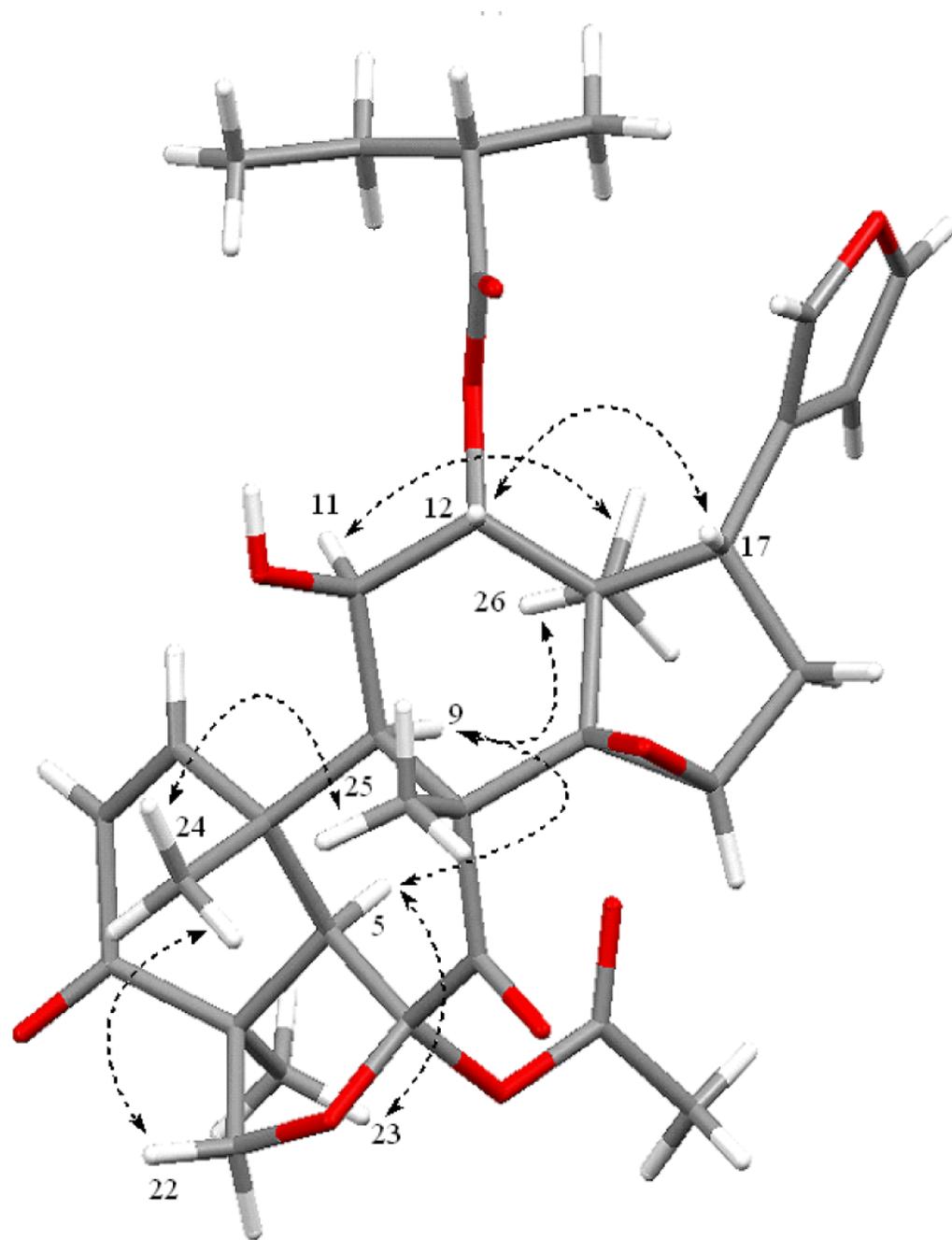
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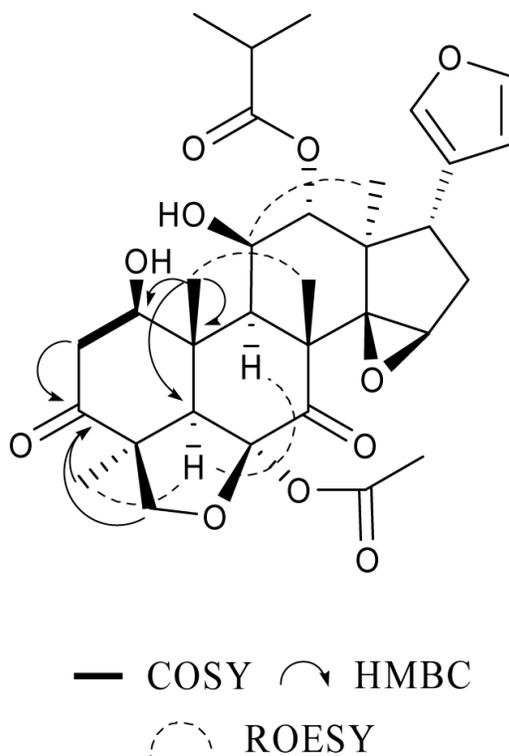
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**Figure 1.**  
X-ray Structure of Malleastrone A (1).



**Figure 2.**  
Key ROESY Correlations of Malleastrone B (2).



**Figure 3.**  
Key 2D NMR Spectroscopic Correlations of Malleastrone C (3).

Table 1

NMR Spectral Data of Limonoids **1-3** in CDCl<sub>3</sub> <sup>a,b</sup>

Pos	1		2		3	
	<sup>13</sup> C	<sup>1</sup> H <sup>c</sup> (J, Hz)	<sup>13</sup> C	<sup>1</sup> H <sup>c</sup> (J, Hz)	<sup>13</sup> C	<sup>1</sup> H <sup>c</sup> (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) 1.83 dd (13, 11)	33.6	2.12 dd (13, 6.5) 1.83 dd (13, 11)	32.3	2.21 dd (14, 6.5) 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) 4.15 d (9.5)	77.8	4.34 d (9.5) 4.15 d (9.5)	78.9	4.48 d (9.0) 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

Pos	1		2		3	
	$^{13}\text{C}$	$^1\text{H}^c$ (J, Hz)	$^{13}\text{C}$	$^1\text{H}^c$ (J, Hz)	$^{13}\text{C}$	$^1\text{H}^c$ (J, Hz)
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) <sup>d</sup>
4''	19.1	1.06 d (7.5)	11.8	0.90 t (7.0)	18.9	1.04 d (7.0) <sup>d</sup>
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

<sup>a</sup> Assignments based on COSY, HMBC, HSQC.

<sup>b</sup> Chemical shifts ( $\delta$ ) in ppm.

<sup>c</sup> br s: broad singlet; d: doublet; m: multiplet.

<sup>d</sup> Values are interchangeable.

<sup>e</sup> Signal overlapped with H-6'.

Table 2

## Antiproliferative Data of Compounds 1-3

compound	IC <sub>50</sub> (μM)				
	A2780 <sup>a</sup>	MDA-MB-435 <sup>b</sup>	HT-29 <sup>b</sup>	H522-T1 <sup>b</sup>	U937 <sup>b</sup>
<b>1</b>	0.49	0.41	0.24	0.24	0.20
<b>2</b>	0.63	0.34	0.22	0.23	0.19
<b>3</b>	18	ND	ND	ND	ND

<sup>a</sup>Concentration of each compound that inhibited 50% of the growth of the A2780 human ovarian cell line according to the procedure described,<sup>8</sup> with paclitaxel (IC<sub>50</sub> 23.4 nM) as the positive control.

<sup>b</sup>Concentration of a compound which inhibited cell growth by 50% compared to untreated cell populations, with vinblastine as the positive control (average IC<sub>50</sub> 0.27 nM (MDA-MB-435), 0.53 nM (HT-29), 1.38 nM (H522-T1) and 0.49 nM (U937).