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Euphane triterpenoids of *Cassipourea lanceolata* from the Madagascar rainforest*

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Abstract

Fractionation of an ethanol extract of a Madagascar collection of the leaves and fruit of *Cassipourea lanceolata* Tul. led to the isolation of three euphane triterpenoids **1–3**. The ¹H and ¹³C NMR spectra of all compounds were fully assigned using a combination of 2D NMR experiments, including COSY, TOCSY, HSQC (HMQC), HMBC and ROESY sequences. The three compounds showed weak antiproliferative activities against the A2780 human ovarian cancer cell line, with IC₅₀ values of 25, 25 and 32 μM, respectively.

Keywords

Cassipourea lanceolata; Rhizophoraceae; Euphanes; Triterpenoids; NMR; Antiproliferative activity

1. Introduction

In our continuing search for biologically active natural products from tropical rainforests as part of a Madagascar-based International Cooperative Biodiversity Group (ICBG) program, we obtained an extract from the leaves and fruit of a tree that was originally identified as a *Macarisia* sp., but was later re-identified as *Cassipourea lanceolata* Tul. (Rhizophoraceae). This extract showed weak antiproliferative activity against the A2780 human ovarian cancer cell line with an IC₅₀ value of 17 μg/mL. No previous work has been reported on the chemistry

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Appendix A. Supplementary data Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2009.12.009.

of any *Macarisia* sp., and so work was initiated on this novel genus on the basis of its antiproliferative activity and unknown phytochemistry. When the collection was later re-identified as *C. lanceolata*, it was noted that no phytochemical work had been carried out on this species, but studies of *Cassipourea guianensis* gave sulfur-containing alkaloids and amides (Kato et al., 1985, 1989; Ichimaru et al., 2000), and work on *Cassipourea gerrardii* yielded a flavonol glycoside (Drewes et al., 1992a) and proanthocyanidins (Drewes et al., 1992b), and an earlier study of ours yielded bioactive diterpenes from *Cassipourea madagascariensis* (Chaturvedula et al., 2006).

Bioassay-guided fractionation did not lead to any compounds with increased activity, but three new weakly active triterpenoids were obtained. Herein we report the structural elucidation of these triterpenoids as 1 β ,3 β ,11 α ,26-tetrahydroxy-7,24*E*-euphadiene (**1**), 3 β ,26-dihydroxy-8,24*E*-euphadien-11-one (**2**), and (24*S*)-1 β ,3 β ,24,25-tetrahydroxy-7,9(11)-euphadiene (**3**), and their bioactivities.

2. Results and discussion

1 β ,3 β ,11 α ,26-Tetrahydroxy-7,24*E*-euphadiene (**1**) was obtained as a white amorphous solid. Its molecular formula was established as C₃₀H₅₀O₄ on the basis of an [M+H]⁺ ion peak at *m/z* 475.3783 in its HRFAB mass spectrum. Its ¹H NMR spectrum showed signals for two olefinic protons, one oxymethylene, three oxymethines, and seven methyls (Table 1). Its ¹³C NMR and HSQC spectra indicated 30 carbons, consisting of four olefinic carbons, four quaternary carbons, four oxygenated carbons, four tertiary carbons, seven methylenes, and seven methyls (Table 1).

Based on this information and on the COSY, HMBC, and ROESY experiments described below, **1** was deduced to be a tetracyclic euphane triterpenoid. The complete ¹H and ¹³C NMR spectroscopic assignments were made by a combination of COSY, TOCSY, HSQC and HMBC data, and the key COSY and HMBC correlations are shown in Fig. 1.

The HMBC correlations allowed assignments of the seven methyl groups as indicated in Table 1. The three hydroxyl groups were determined to reside at C-1, C-3 and C-11 by analysis of the HMBC spectrum. HMBC correlations from H-1 to C-19 and from H₃-19 to C-1 indicated the presence of a hydroxyl group at C-1. The hydroxyl group at C-3 was assigned based on HMBC correlations from H-3 to C-28 and C-29 and from H₃-28 to C-3. The remaining group was assigned to C-11 by HMBC correlations from H₂-12 to C-18, and by COSY correlations from H-11 to H₂-12. The position of the internal double bond was assigned on the basis of an HMBC correlation from H₃-30 to C-8, in addition to the coupling system of H-5–H₂-6–H-7.

Analysis of ROESY correlations (Fig. 2) and coupling constants (Table 1) determined the relative configuration at C-1, C-3, C-5, C-9, C-10, C-11, C-13, C-14, C-17, C-20, and Δ^{24} , indicating that the compound has the normal euphane stereochemistry.

The configuration of the hydroxyl group at C-1 was indicated by the coupling constants of H-1 (*dd*, *J* = 11.6, 4.8 Hz), which showed that the hydroxyl group is in the β -equatorial position. The configuration of the Δ^{24} double bond was determined as *E* on the basis of ROESY correlations from H-24 to H₂-26 in addition to those from H₃-27 to H₂-23 and H-22a. The structure and relative stereochemistry of **1** was thus established as shown.

3 β ,26-Dihydroxy-8,24*E*-euphadien-11-one (**2**) was obtained as a white amorphous solid. Its molecular formula was established as C₃₀H₄₆O₃ on the basis of an [M+H]⁺ ion peak at *m/z* 457.3693 in its HRAPCI mass spectrum. Comparison of the ¹H NMR spectra of **1** and **2** showed that the two compounds were very similar. The major differences were that the olefinic methine (H-7) and the oxygenated methines (H-11 and H-1) that appeared in the ¹H NMR spectrum of **1** were absent in the ¹H NMR spectrum of **2** (Table 1). The ¹³C NMR spectrum of **2** also lacked signals for the C-7 olefinic methine and the two oxygenated methines of **1**, but contained a signal for a ketone carbonyl carbon (δ_C 201.7, C-11). The carbonyl group was shown to be part of an α,β -unsaturated ketone system by a UV absorption at 255 nm, consistent with the Woodward-Fieser rules. Complete ¹H and ¹³C NMR assignments and functional group locations were determined from a combination of COSY, TOCSY, HMQC and HMBC data. The α,β -unsaturated ketone unit from C-8 and C-9 to C-11 was indicated by HMBC correlations from H₃-30, H-6a and H₂-7 to C-8, and from H₃-19 and H₂-7 to C-9, and from H₂-12 to C-9.

The relative configuration of **2** was determined on the basis of its ROESY correlations and coupling constants (Table 1). The configurations of rings A and B were determined as the same manner as **1**. The C-3 hydroxyl group was determined to be β -equatorially oriented, and ring A/B adopted the chair/twisted chair conformation. The C-14 methyl group (C-30) was β -oriented based on a ROESY correlation from β -equatorial H-7a (*br dd*, *J* = 19.5, 5.6 Hz) to H₃-30, and H-7a was assigned a β -orientation due to its coupling constants and the ROESY correlation from α -axial H-5 to H-7b. Furthermore, 13-CH₃ had to be α -oriented from the biosynthetic point of view (Dewick, 2001). The ROESY correlation from H₃-30 to H-17 established that the side chain was in the α -orientation, and the configurations of ring C/D are consistent with those of the cooccurring **1**. The *E*-configuration of Δ^{24} was established in the same way as discussed in **1**. Thus, the structure and stereochemistry of **2** was established as shown.

(24*S*)-1 β ,3 β ,24,25-Tetrahydroxy-7,9(11)-euphadiene (**3**) was obtained as a white amorphous solid. Its molecular formula was established as C₃₀H₅₀O₄ on the basis of an [M+H–H₂O]⁺ ion peak at *m/z* 457.3693 in its HRAPCI mass spectrum and an [M+H]⁺ ion peak at *m/z* 475.3 in its ESIMS spectrum. Signals for two olefinic protons, were observed in its ¹H NMR spectrum (Table 1). Its ¹³C NMR and HSQC spectra indicated 30 carbons that were composed of four olefinic carbons, eight methyls, seven methylenes, three methines and four quaternary carbons (Table 1). On the basis of this information and comparison with the spectra of compounds **1** and **2**, compound **3** was deduced to be a third tetracyclic triterpenoid. The complete assignments of its ¹H and ¹³C NMR spectra were determined from a combination of COSY, TOCSY, HSQC and HMBC data. COSY correlations indicated the coupling systems H-1–H₂-2–H-3, H-5–H₂-6–H-7, H-11–H₂-12, H₂-15–H₂-16–H-17–H-20–H₃-21, and H-20–H₂-22–H₂-23–H-24. The positions of the eight methyls were assigned on the basis of the HMBC correlations that showed similar connectivities to those of **1** and **2**. The diene $\Delta^{7,9(11)}$ unit was established through HMBC correlations from H₂-12 to C-11 and C-9 in addition to those from H₂-12 to C-18, C-13 and C-14, the HMBC correlation from H₃-19 to C-9, and the HMBC correlation from H₃-30 to C-8, together with the related COSY correlations. The C-24–C-25–C-26/27 unit including hydroxyl groups at C-24 and C-25 was indicated on the basis of the HMBC correlations from both H₃-26 and H₃-27 to both C-24 and C-25, in addition to those from H₃-26 to C-27 and from H₃-27 to C-26. The C-1 and C-3 hydroxyl groups were located as described previously for **1** and **2**. The relative configuration of **3** was established as described for **1** and **2**, through analysis of its ROESY spectrum, measurement of coupling constants, and consideration of the biosynthetic pathway for tetracyclic triterpenoids (Dewick, 2001).

The absolute configuration of C-24 in **3** was determined by converting **3** to the two Mosher esters **4** and **5** with (*R*)- and (*S*)-methoxyphenylacetic acid (MPA), using the EDCI/DMAP coupling conditions (Scheme 1) (Seco et al., 2001). The structures of **4** and **5** were confirmed by analysis of both their HRESI mass and LC-ESIMS/MS spectra, and their 1D and 2D NMR spectroscopic data (Supporting information for NMR data and see Section 4 for MS data). The chemical shifts from H-20 to H₃-27 between the two esters were significantly different (Scheme 1), and that led to the conclusion that **3** had the *S*-configuration at C-24 (Seco et al., 2001).

The absolute configuration of C-20 in compounds **1–3** was determined primarily on the basis of ROESY correlations using a literature method (Wang et al., 2003). This method assigns the stereochemistry of euphane (C-20(*R*)) triterpenoids by virtue of a ROESY correlation from H₃-21 to H-16 α in addition to the absence of a ROESY correlation from H₃-21 to H₃-18, and that of tirucallane (C-20(*S*)) triterpenoids by the ROESY correlation from H₃-21 to H₃-18 in addition to that from H₃-21 to H-12. This argument was derived from careful analysis of the ROESY spectra of the two diastereomers, kansenone [C-20 (*R*)] and epi-kansenone [C-20 (*S*)] and by comparison with previously reported compounds. The configurations determined for the five compounds discussed in the literature, namely kansenone, epi-kansenone, kansenonol, 11-oxokansenonol and kansenol, were consistent with X-ray crystallographic studies of euphane and tirucallane triterpenoids (Nes et al., 1984; Wang et al., 2003). In the ROESY spectra of **1**, correlations of H₃-21 to H₂-16, H-17, and H₂-23, those of H₃-18 to H-16 α (δ_{H} 1.34, *m*) and H-20, and that of H-22a (δ_{H} 1.70, *m*) to H-12b (δ_{H} 1.58, *m*) were observed in addition to the absence of correlations between H₃-21 and H₃-18 and between H₃-21 and H₂-12 (Fig. 2). Those ROESY correlations showed a nearly identical pattern to those of kansenone and kansenonol (Wang et al., 2003) and indicated that **1** is a euphane

triterpenoid with the (*R*)-configuration at C-20, and not a triucallane triterpenoid with an (*S*)-configuration at C-20. Compound **2** was also determined as a euphane triterpenoid based on similar key ROESY correlations to those of **1**. Compound **3** had almost identical ROESY correlation patterns in ring C/D and H-20–H-22 to those of kansanol (Wang et al., 2003). In particular, a correlation from H₃-21 to H-16 α (δ_{H} 1.36, *m*) in addition to the absence of one from H₃-21 to H₃-18, indicated that **3** was a euphane triterpenoid with the (*R*)-configuration at C-20.

Based on these considerations, the structures of **1–3** were assigned as 1 β ,3 β ,11 α ,26-tetrahydroxy-7,24*E*-euphadiene, 3 β ,26-dihydroxy-8,24*E*-euphadien-11-one, and (24*S*)-1 β ,3 β ,24,25-tetrahydroxy-7,9(11)-euphadiene, respectively.

3. Conclusions

As described in the introduction, the work was initiated in part to investigate the phytochemistry of what was thought to be a previously uninvestigated genus. In the event, the plant of origin turned out to be a known genus, but this was only discovered after the chemical work was complete. The plant was, however, a previously uninvestigated species, and the discovery of three new compounds indicates that even well-investigated genera such as *Cassipourea* contain many new phytochemicals.

All the isolates were tested against the A2780 ovarian cancer cell line. Compounds **1–3** showed weak antiproliferative activities with IC₅₀ values of 25, 25, and 32 μ M, respectively. Compound **1** was also tested against the BT-549 and MCF-7 breast cancer, DU 145 prostate cancer, NCI-H460 and H522-T1 NSCLC, HCC-2998 and HT-29 colon cancer, OVCAR-5 ovarian cancer, SF-539 CNS cancer, SR and U937 lymphoma, and UACC-257 and MDA-MB-435 melanoma cell lines, but was found to have IC₅₀ values of >5 μ M in each of these assays. The weak antiproliferative activity of the initial extract is thus presumably due to other as yet unidentified compounds.

4. Experimental

4.1. General

Optical rotations were recorded on a JASCO P-2000 polarimeter. UV spectra were performed on a Shimadzu UV-1201 spectrophotometer. NMR spectra were obtained on Bruker Avance 600 equipped with a 1.7 mm CapProbe, JEOL Eclipse 500, Varian Inova 400, and Varian Unity 400 spectrometers. HRFAB mass spectra were obtained on a JEOL-JMS-HX-110 instrument. HRAPCI mass spectra were obtained on an Agilent 6220 TOF LC/MS. LC-ESIMS was performed on Agilent 1100 and Thermo TSQ Quantum instruments. Chemical shifts are given in δ (ppm), and coupling constants (*J*) are reported in Hz. HPLC was performed using Shimadzu LC-8A pumps coupled with a Varian Dynamax preparative phenyl column (250 $\times$ 21.4 mm) or a Varian Dynamax preparative C18 column (250 $\times$ 21.4 mm), and Shimadzu LC-10A pumps coupled with a Varian Dynamax semi-preparative C18 column (250 $\times$ 10 mm). Both HPLC instruments employed a Shimadzu SPD-M10A diode array detector and the Shimadzu LC-10A pumps were also coupled with a Sedex Model 75 evaporative light scattering detector.

4.2. Antiproliferative bioassay

The A2780 ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University as previously reported (Cao et al., 2007). The A2780 cell line is a drug-sensitive ovarian cancer cell line (Louie et al., 1985).

4.3. Plant material

Leaves and fruit of *C. lanceolata* Tul. were collected in October 2001 by N. M. Andrianjafy et al. and vouchered with the herbarium specimen NMA 213. The collection was made from a plant growing in dense humid forest adjacent to the Zahamena National Park, Analanjirofo Region, Madagascar (17°37'39"S; 048°56'48"E, elevation 600 m). The specimen accessed was a small tree 8 m in height with opposite toothed leaves, interpetiolar stipules, flowers with superior ovary and ten stamens and green fruits. The vernacular name for this plant is *Moara fotsy*, and it is used locally for house construction. Duplicate voucher specimens were deposited in herbaria at Centre National d'Application des Recherches Pharmaceutiques (CNARP), the Departement des Recherches Forestieres et Piscicoles in Antananarivo, Madagascar (TEF), the Missouri Botanical Garden, St. Louis, Missouri (MO), and the Museum National d'Histoire Naturelle in Paris, France (P).

4.4. Extraction and isolation

Dried leaves and fruit (317 g) were extracted for 24 h at room temperature with EtOH (1.2 L) and the ethanol evaporated to give an extract (12.9 g), which was assigned the number MG1002. An aliquot of this extract (2.8 g) was supplied to VPISU, and this had an IC₅₀ value of 17 µg/mL against A2780 cells. A portion of this extract (1.3 g) was suspended in aqueous MeOH (MeOH/H₂O, 9:1, 40 mL), and extracted with *n*-hexane (3 × 40 mL). The aqueous layer was then diluted to MeOH–H₂O (6:4, v/v) with H₂O and extracted with CH₂Cl₂ (3 × 60 mL). The CH₂Cl₂ extract (517.8 mg, IC₅₀ = 19 µg/mL) was separated via preparative HPLC over a phenyl column using MeOH–H₂O (85:15) to afford eight fractions (I–VIII), of which fractions II (54 mg), III (9.8 mg), IV (21 mg), VI (89 mg) and VIII (176 mg) were found to display the highest antiproliferative activity (IC₅₀ = 8.4, 14, 11, 9.5 and 9.2 µg/mL, respectively), and these fractions were subjected to the further separation. Fraction II was subjected to preparative HPLC on an RP-C18 column using MeOH–H₂O (75:25) to afford three fractions (A–C). Fraction III was further separated via semi-preparative HPLC on an RP-C18 column using MeOH–H₂O (80:20). Four fractions (D–G) were collected. Fraction E afforded **2** (1.0 mg, *t*_R 29.0 min). Fraction IV was subjected to semi-preparative HPLC on an RP-C18 column using MeOH–H₂O (75:25) to afford four fractions (H–K). Fraction I afforded **3** (9.0 mg, *t*_R 32.7 min). Fraction VI was separated using preparative RP-C18 HPLC using MeOH–H₂O (80:20). Five fractions (L–P) were collected. Fraction P afforded **1** (67 mg, *t*_R 51.1 min).

4.4.1. 1β,3β,11α,26-Tetrahydroxy-7,24E-euphadiene (1)—White amorphous solid; [α]_D²⁵ –4.8 (*c* 0.12, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 222 (3.49) nm; for ¹H and ¹³C NMR spectroscopic data, see Table 1; HRFABMS *m/z* 475.3782 [M+H]⁺ (calcd for C₃₀H₅₁O₄, 475.3787).

4.4.2. 3β,26-Dihydroxy-8,24E-euphadien-11-one (2)—White powder; [α]_D²⁵ +70.4 (*c* 0.12, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 207 (3.36), 255 (3.32), and 323 (2.88) nm; for ¹H and ¹³C NMR spectroscopic data, see Table 1; HRAPCIMS *m/z* 457.3693 [M+H]⁺ (calcd for C₃₀H₄₉O₃, 457.3682).

4.4.3. (24S)-1β,3β,24,25-tetrahydroxy-7,9(11)-euphadiene (3)—White powder; [α]_D²⁵ –30.2 (*c* 0.50, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 209 (3.38) and 232 (3.49) nm; for ¹H and ¹³C NMR spectroscopic data, see Table 1; HRAPCIMS *m/z* 457.3693 [M+H–H₂O]⁺ (calcd for C₃₀H₄₉O₃, 457.3682). ESIMS *m/z* (rel. int.): 475.3 (30) [M+H]⁺, 457.3 (100) [M+H–H₂O]⁺, 439.3 (60) [M+H–2H₂O]⁺, 421.3 (10) [M+H–3H₂O]⁺.

4.4.4. Mosher esters 4 and 5—(*R*)-MPA (6 mg) was added in a stirred solution of **3** (1 mg), DMAP (4 mg) and EDCI (18 mg) in 0.3 mL CH₂Cl₂. After 36 h, EtOAc was added and

then the solution was filtrated and evaporated under reduced pressure. The residue was subjected to RP-C18 HPLC using a gradient from MeOH/H₂O, 1:1, to 100% MeOH from 0 to 30 min followed by MeOH from 30 to 50 min). Analysis of LC/MS/MS spectra indicated a product of **4** that derived from esterification of all three secondary hydroxyl groups in **3**. Then the peak at $t_R = 39.0$ min was collected to generate **4** (0.5 mg). Compound **5** (0.5 mg, $t_R = 42.5$ min) was obtained from a reaction between **3** (1 mg) and (*S*)-MPA using the same procedure.

4.4.5. (R)-Methoxyphenylacetic acid Mosher ester 4—White powder; $[\alpha]_D^{25} -44.0$ (c 0.05, CHCl₃); UV (CHCl₃) λ_{max} (log ϵ) 245 (3.74) nm; ¹H NMR [CDCl₃, 150 MHz (CapProbe)]: δ 5.18 (1H, *br s*, H-7), 5.01 (1H, *dd*, $J = 12.1, 5.2$ Hz, H-1), 4.77 (1H, *dd*, $J = 7.2, 2.5$ Hz, H-24), 4.72 (1H, *dd*, $J = 12.4, 4.1$ Hz, H-3), 4.17 (1H, *br s*, H-11), 1.80 (1H, *m*, H-22a), 1.68 (1H, *m*, H-23a), 1.32 (1H, *m*, H-23b), 1.18 (1H, *m*, H-23b), 1.18 (1H, *m*, H-20), 1.15 (3H, *s*, H-26), 1.13 (3H, *s*, H-27), 0.91 (3H, *s*, H-29), 0.89 (3H, *s*, H-19), 0.74 (3H, *s*, H-28), 0.70 (3H, *d*, $J = 6.6$ Hz, H-21), 0.54 (3H, *s*, H-30), 0.32 (3H, *s*, H-18); ¹³C NMR (CDCl₃, 150 MHz (CapProbe), data extracted from its HSQC spectrum): δ 116.9 (C-11), 116.8 (C-7), 81.2 (C-24), 76.7 (C-3), 75.7 (C-1), 35.4 (C-20), 27.6 (C-22), 26.9 (C-28), 26.7 (C-23), 26.4 (C-27), 25.0 (C-26), 22.2 (C-30), 18.7 (C-21), 16.2 (C-18), 15.8 (C-29), 14.6 (C-19); HRESIMS m/z 941.5199 [M+Na]⁺ (calcd for, C₅₇H₇₄O₁₀Na, 941.5180). ESIMS/MS m/z (rel. int.): 941.5 (34) [M+Na]⁺, 775.4 (25) [M+Na-MPA]⁺, 609.3 (100) [M+Na-2MPA]⁺, 443.2 (4) [M+Na-3MPA]⁺, 189.0 (100) [MPA+Na]⁺.

4.4.6. (S)-Methoxyphenylacetic acid Mosher ester 5—White powder; $[\alpha]_D^{25} +10$ (c 0.05, CHCl₃); UV (CHCl₃) λ_{max} (log ϵ) 248 (3.78) nm; ¹H NMR [CDCl₃, 600 MHz (CapProbe)]: δ 5.16 (1H, *br s*, H-7), 5.02 (1H, *dd*, $J = 11.7, 4.2$ Hz, H-1), 4.76 (1H, *dd*, overlapping, H-24), 4.70 (1H, *dd*, $J =$ overlapping, H-3), 4.16 (1H, *br s*, H-11), 1.86 (1H, *m*, H-22a), 1.72 (1H, *m*, H-23a), 1.32 (1H, *m*, H-23b), 1.27 (1H, *m*, H-20), 1.23 (1H, *m*, H-23b), 0.95 (3H, *s*, H-26), 0.92 (3H, *s*, H-27), 0.90 (3H, *s*, H-19), 0.72 (3H, *s*, H-29), 0.82 (3H, *d*, $J = 6.6$ Hz, H-21), 0.56 (3H, *s*, H-30), 0.39 (3H, *s*, H-28), 0.39 (3H, *s*, H-18); ¹³C NMR (CDCl₃, 150 MHz (CapProbe), data extracted from its HSQC spectrum): δ 116.9 (C-7), 116.6 (C-11), 81.2 (C-24), 76.6 (C-3), 75.7 (C-1), 36.1 (C-20), 27.9 (C-22), 26.8 (C-23), 26.4 (C-28), 25.8 (C-27), 24.8 (C-26), 22.2 (C-30), 18.9 (C-21), 16.4 (C-18), 15.4 (C-29), 14.5 (C-19); HRESIMS m/z 941.5198 [M+Na]⁺ (calcd for, C₅₇H₇₄O₁₀Na, 941.5180). ESIMS/MS m/z (rel. int.): 941.5 (21) [M+Na]⁺, 775.4 (28) [M+Na-MPA]⁺, 609.3 (88) [M+Na-2MPA]⁺, 443.2 (3) [M+Na-3MPA]⁺, 189.0 (100) [MPA+Na]⁺.

Supplementary Material

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Fig. 1.
Key COSY (bold) and HMBC (arrows) correlations of compound **1**.

Scheme 1.

Table 1

¹H and ¹³C NMR data of compounds **1–3**.^a

Position	1^b		2^c		3^b	
	¹ H	¹³ C	¹ H	¹³ C	¹ H	¹³ C
1	3.64 <i>dd</i> (11.6, 4.8)	75.7	2.55 <i>m</i> 0.96 <i>m</i>	35.6	3.84 <i>dd</i> (11.6, 5.2)	74.7
2	1.85 <i>m</i> 1.80 <i>m</i>	37.0	1.71 <i>m</i> 1.63 <i>m</i>	28.4	1.86 <i>m</i> 1.80 <i>m</i>	38.2
3	3.25 <i>dd</i> (11.8, 4.2)	76.6	3.18 <i>dd</i> (11.3, 4.6)	79.7	3.23 <i>dd</i> (11.6, 4.0)	76.7
4		40.5		40.2		40.4
5	1.29 <i>dd</i> (11.6, 6.0)	50.0	1.05 <i>m</i>	53.3	1.21 <i>dd</i> (10.4, 5.6)	48.6
6	2.27 <i>m</i> , 2.10 <i>m</i>	25.6	1.81 <i>m</i> , 1.48 <i>m</i>	19.3	2.23 <i>m</i> , 2.22 <i>m</i>	25.0
7	5.34 <i>br d</i> (2.8)	121.1	2.42 <i>br dd</i> (19.5, 5.6) 2.23 <i>m</i>	31.1	5.33 <i>br s</i>	119.1
8		144.0		164.8		143.1
9	2.32 <i>m</i>	58.4		141.0		144.4
10		43.8		38.5		43.8
11	4.20 <i>ddd</i> (10.4, 10.0, 5.2)	66.9		201.7	5.81 <i>br s</i>	119.0
12	2.43 <i>dd</i> (10.4, 9.2) 1.58 <i>m</i>	47.6	2.55 <i>d</i> (18.8) 2.33 <i>d</i> (18.8)	52.4	2.26 <i>m</i> 2.26 <i>m</i>	39.9
13		45.2		45.9		45.3
14		52.5		52.5		50.9
15	1.58 <i>m</i> , 1.47 <i>m</i>	35.8	1.80 <i>m</i> , 1.43 <i>m</i>	36.9	1.70 <i>m</i> , 1.30 <i>m</i>	31.0
16	1.99 <i>m</i> , 1.34 <i>m</i>	29.4	2.07 <i>m</i> , 1.41 <i>m</i>	28.6	1.99 <i>m</i> , 1.36 <i>m</i>	29.4
17	1.54 <i>m</i>	54.8	1.77 <i>m</i>	51.8	1.66 <i>m</i>	52.2
18	0.89 <i>s</i>	22.1	0.93 <i>s</i>	18.0	0.67 <i>s</i>	17.1
19	0.84 <i>s</i>	9.6	1.20 <i>s</i>	20.4	0.94 <i>s</i>	14.3
20	1.47 <i>m</i>	37.3	1.44 <i>m</i>	37.3	1.47 <i>m</i>	38.0
21	0.90 ^d	19.1	0.92 ^d	18.8	0.90 <i>d</i> (6.8)	19.7
22	1.70 <i>m</i> , 1.07 <i>m</i>	36.3	1.52 <i>m</i> , 1.12 <i>m</i>	36.9	1.76 <i>m</i> , 1.33 <i>m</i>	29.2
23	2.13 <i>m</i> , 1.97 <i>m</i>	26.1	2.13 <i>m</i> , 1.98 <i>m</i>	25.5	2.00 <i>m</i> , 1.06 <i>m</i>	34.0

Position	1^b		2^c		3^d	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
24	5.42 <i>br t</i> (6.8)	127.2	5.39 <i>br t</i> (7.0)	127.2	3.18 <i>dd</i> (10.0, 1.6)	80.7
25		135.8		135.9		74.0
26	3.91 <i>s</i>	69.2	3.91 <i>s</i>	69.1	1.17 <i>s</i>	25.8
27	1.66 <i>s</i>	13.9	1.65 <i>s</i>	13.8	1.14 <i>s</i>	25.1
28	0.94 <i>s</i>	28.0	1.02 <i>s</i>	29.0	0.94 <i>s</i>	28.1
29	0.84 <i>s</i>	15.0	0.82 <i>s</i>	16.6	0.86 <i>s</i>	15.6
30	0.91 <i>s</i>	27.6	1.03 <i>s</i>	24.6	0.89 <i>s</i>	23.6

^aIn CD₃OD.

^b δ (ppm) 400 MHz (^1H), 100 MHz (^{13}C).

^c δ (ppm) 500 MHz (^1H), 125 MHz (^{13}C).

^dCoupling constants not measured due to overlapping signals.