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(9 β H)-Pimaranes and Derivatives from the Tuber of *Ipomoea trichantha*

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Abstract

New 17-nor-pimaranes (**1**, **2**), (9 β H)-pimaranes (**3**, **4**), and 17-nor-(9 β H)-pimarane (**5**) were isolated from the tuber of *Ipomoea trichantha*. The structures were elucidated based on spectroscopic and HRMS data. The absolute configurations of **3** and **5** were determined by single-crystal X-ray diffraction. Compound **5** possesses a unique 19,20- δ -lactone moiety. Compound **3** showed cytotoxicity against MDA-MB-435 (human melanoma cancer) cells with an IC₅₀ value of 7.04 μ M. A plausible biogenetic pathway for compounds **1** – **5** is proposed.

Ipomoea trichantha Oliv. (Ipomoeaceae) is a medicinal plant used in Nigeria and other regions of western Africa. The tuber is often used for the treatment of food poisoning, constipation, and malaria.^{1, 2} Our previous studies showed this plant is rich in (9 β H)-pimarane, 17-nor-(9 β H)-pimarane, and 17-nor-pimarane diterpenoids.^{3–5} Furthermore, a rearranged 17-nor-pimarane possessing an unprecedented carbon skeleton, icacintrichantholide, was identified.⁵ Several of these compounds were cytotoxic against MDA-MB-435 (human melanoma cancer), MDA-MB-231 (human breast cancer), and OVCAR3 (human ovarian cancer) cell lines.^{3, 4} During the course of continuing search for new chemical ingredients from *I. trichantha*, two 17-nor-pimaranes, two (9 β H)-pimaranes, and a 17-nor-(9 β H)-pimarane were obtained. Herein the isolation, structural elucidation, and cytotoxic properties of these diterpenoids are reported.

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Notes

The authors declare no competing financial interest.

Supporting Information

IR, 1D and 2D NMR spectroscopic data for compounds **1** – **5**. This material is available free of charge via Internet at <http://pubs.acs.org>.

RESULTS AND DISCUSSION

The tuber of *I. trichantha* was extracted with 80% aqueous MeOH by percolation. The extract was successively partitioned into petroleum ether-soluble, EtOAc-soluble, and *n*-BuOH-soluble fractions. Five new diterpenoid lactones, 12-hydroxyicacinlactone A (**1**), 7 β -hydroxyicacinlactone B (**2**), 14 α -methoxyhumirianthol (**3**), icacinlactone I (**4**) and icacinlactone J (**5**), were purified from the EtOAc-soluble fraction.

12-Hydroxyicacinlactone A (**1**) was obtained as an amorphous powder. A molecular formula of $C_{19}H_{18}O_6$ with 11 indices of hydrogen deficiency was deduced from HRESIMS (m/z [$M + H$] $^+$ 343.1182; calcd for $C_{19}H_{19}O_6^+$, 343.1176) and ^{13}C NMR spectroscopic data. The IR absorption at 1740 cm^{-1} indicated the presence of a γ -lactone moiety. A comparison of the NMR spectroscopic data with those of icacinlactone A revealed that **1** was a hydroxy derivative of the latter (Tables 1 and 2).⁴ A C-12 hydroxy group was assigned on the basis of the HMBC results (Figure 1). Indeed, in comparison with icacinlactone A, significant shielding of C-8 (-9.0 ppm), C-11 (-14.9 ppm), and C-13 (-10.9 ppm), with a concomitant deshielding of C-12 ($+30.6\text{ ppm}$), supported the assignment. The relative configuration of **1** was proposed based on the NOESY data (Figure 2), in which cross signals for H-5 (δ_H 2.34) / H-18 (δ_H 1.43), H-5 / H-6 (δ_H 5.16), H-6 / H-7 α (δ_H 4.09), and H-20a (δ_H 4.11) / H-7 β (δ_H 2.90) were observed. Consequently, the structure of **1** was elucidated as 3 β , 20:14,16-diepoxy-3 α ,12-dihydroxy-17-*nor*-pimar-8(9),11,13(14),15-tetraen-19,6 β -olide and given the trivial name, 12-hydroxyicacinlactone A.

7 β -Hydroxyicacinlactone B (**2**) was obtained as an amorphous powder. The HRESIMS (m/z 355.1204 [$M + H - H_2O$] $^+$; calcd for $C_{20}H_{19}O_6^+$, 355.1176) suggested a molecular formula of $C_{20}H_{20}O_7$ with eleven indices of hydrogen deficiency when the ^{13}C NMR spectroscopic data were taken into consideration. The presence of a γ -lactone moiety was evident by an IR absorption at 1740 cm^{-1} . A comparison of the NMR data (1H and ^{13}C) of **2** with those of **1** and icacinlactone B⁴ revealed that **2** possessed a similar structure to that of icacinlactone B. The 1H - 1H COSY results indicated the presence of three coupled spin systems, i.e., H-1 (δ_H 2.75, 1.87) / H-2 (δ_H 2.27, 2.06), H-5 (δ_H 2.49) / H-6 (δ_H 5.09) / H-7 (δ_H 5.91), and H-15

(δ_{H} 6.86) / H-16 (δ_{H} 7.68) (Figure 1). With the aid of HMBC experiment, the 2D structure of **2** was elucidated as the 7-hydroxy derivative of icacinlactone B. Thus, HMBC correlations between H-7 (δ_{H} 5.91) and C-5 (δ_{C} 53.4), C-8 (δ_{C} 114.7), C-9 (δ_{C} 138.3), and C-14 (δ_{C} 156.7) were observed. To determine the relative configuration of **2**, a NOESY experiment was performed. The α -orientations of H-5, H-6, H-7, and 4-CH₃ (δ_{H} 1.45), as well as the β -orientation of the C_{3,20}-epoxy bridge were proposed by the observation of the following NOESY correlations: from H-5 to H-1 α (δ_{H} 1.87), H-6, and CH₃-18 (δ_{H} 1.45); from H-6 to H-7 and CH₃-18; as well as between H-1 β (δ_{H} 2.75) and H-20 (δ_{H} 3.79) (Figure 2). The structure of **2** was thus elucidated as 3 β ,20:14,16-diepoxy-3 α ,7 β -dihydroxy-12-methoxy-17-*nor*-pimar-8(9),11,13(14),15-tetraen-19,6 β -olide and given the trivial name of 7 β -hydroxyicacinlactone B.

14 α -Methoxyhumirianthol (**3**) was obtained as an amorphous powder. A molecular formula of C₂₁H₂₈O₇ with eight indices of hydrogen deficiency was proposed based on its HRESIMS (m/z 393.1917 [M + H]⁺; calcd for C₂₁H₂₉O₇⁺, 393.1908) and ¹³C NMR spectroscopic data. An IR absorption at 1738 cm⁻¹ indicated the presence of a γ -lactone moiety. The NMR spectroscopic data (Tables 1 and 2) of **3** were similar to those of icacinol,³ when the solvent effects (between methanol-*d*₄ and DMSO-*d*₆) were taken into consideration. The 2D structure of **3** was derived from interpretation of the 2D NMR data (¹H-¹H COSY, HSQC, and HMBC) (Tables 1 and 2; Figure 1) as an 14-O-methyl derivative of humirianthol. Based on the NOESY spectrum, the presence of 5 α -H, 6 α -H, 9 β -H, 4 α -CH₃, 13 α -CH₃, 14 α -OCH₃, as well as a 3 β ,20-epoxy was proposed (Figure 2). Owing to the NMR signal overlap between H-15 and H-16 (δ_{H} 3.71), determination of H-15 orientation was not possible. Finally, the absolute configuration was resolved to be 3*S*, 4*R*, 5*R*, 6*S*, 9*S*, 10*S*, 13*S*, 14*S*, and 15*S* by a single-crystal X-ray diffraction experiment (Figure 3). The structure of the new (9 β H)-pimarane diterpenoid (**3**) was thus defined as (3*S*,4*R*,5*R*,6*S*,9*S*,10*S*,13*S*,14*S*,15*S*)-3 β ,20:14,16-diepoxy-3 α ,15 α -dihydroxy-14 α -methoxy-(9 β H)-pimar-7-en-19,6 β -olide, and given the trivial name of 14 α -methoxyhumirianthol.⁶

icacinlactone I (**4**) was obtained as an amorphous powder. A combination of HRESIMS (m/z 347.1871 [M + H]⁺; calcd for C₂₀H₂₇O₅⁺, 347.1853) and NMR spectroscopic data suggested a molecular formula of C₂₀H₂₆O₅ with eight indices of hydrogen deficiency. IR absorption at 1757 cm⁻¹ indicated the presence of a γ -lactone moiety. The ¹H NMR spectrum displayed signals for three methyls (δ_{H} 0.96, CH₃-17; 1.49, CH₃-18; and 0.90, CH₃-20) and an isolated olefinic proton (δ_{H} 5.96, H-7) (Table 1). Twenty carbons were observed in the ¹³C NMR spectrum (Table 2). The ¹H-¹H COSY spectrum displayed cross signals corresponding to H-1 (δ_{H} 2.02, 1.59) / H-2 (δ_{H} 2.55–2.62), H-5 (δ_{H} 2.56–2.57) / H-6 (δ_{H} 4.95) / H-7 (δ_{H} 5.96), H-9 (δ_{H} 2.27) / H-11 (δ_{H} 1.75) / H-12 (δ_{H} 1.42–1.47), as well as H-15 (δ_{H} 3.81) / H-16 (δ_{H} 4.41), allowing the establishment of the fragments of C-1 / C-2, C-5 / C-6 / C-7, C-9 / C-11 / C-12, and C-15 / C-16 (Figure 1). The connectivities between the last three fragments were established by the following HMBC correlations: between H-6 and C-8 (δ_{C} 149.0); between H-7 and C-9 (δ_{C} 45.0) and C-14 (87.4); between H-15 and C-12 (δ_{C} 33.6) and C-14; as well as between H-16 and C-14. The H-17 (δ_{H} 0.96) showed HMBC correlations to C-12, C-13, C-14, and C-15 (δ_{C} 79.8). The presence of a carbonyl carbon at C-3 (δ_{C} 208.2) was evident by its HMBC correlation with CH₃-18 (δ_{H} 1.49),

which also correlated to C-4 (δ_C 54.9), C-5 (δ_C 47.4), and C-19 (δ_C 177.0). The protons at both C-1 (δ_C 31.9) and C-20 (δ_C 21.6) correlated with C-5 and C-10 (δ_C 33.3) in the HMBC spectrum, supporting their assignments. The 2D structure of **4** was thus elucidated as shown. The relative configuration was established via a NOESY experiment was carried out (Figure 2), revealing the α -orientation of 4-CH₃, H-5, H-6, 13-CH₃, H-14, and H-15, as well as the β -orientation of H-9 and 10-CH₃. Consequently, the structure of compound **4** was defined as 3-oxo-14 β ,16-epoxy-15 β -hydroxy-(9 β H)-pimar-7-en-19,6 β -olide and given the trivial name of icacinlactone I.

Icacinlactone J (**5**) was obtained as an amorphous powder. The HRESIMS spectrum displayed a protonated-molecular ion at m/z 347.1461 [M + H]⁺ (calcd for C₁₉H₂₃O₆⁺, 347.1489), suggesting a molecular formula of C₁₉H₂₂O₆ with nine indices of hydrogen deficiency. Strong IR absorptions at 1713 and 1664 cm⁻¹ were consistent with the presence of δ -lactone and conjugated carbonyl functionalities, respectively. The ¹H NMR spectrum showed resonances for two olefinic protons at δ_H 6.63 (d, J = 2.0 Hz, H-15) and 7.51 (d, J = 2.0 Hz, H-16), and a methyl at δ_H 1.37 (s, CH₃-18). Nineteen carbon signals were observed in the ¹³C NMR spectrum, corresponding to a methyl, five methylenes, seven methines, two carbonyl carbons, an oxygenated sp² tertiary carbon, and three quaternary carbons. All proton signals were assignable via HSQC data (Tables 1 and 2). The ¹H-¹H COSY spectrum displayed three coupled spin systems of H-1 / H-2 / H-3, H-5 / H-6 / H-7 / H-8 / H-9 / H-11, and H-15 / H-16. Analysis of the ¹H-¹H COSY and HMBC data (Figure 1) led to the unambiguous assignment of the 2D structure for **5** as shown. Interestingly, the H-20 (δ_H 4.05, br d, J = 11.2 Hz) displayed HMBC correlation with C-19 (δ_C 176.6), suggesting the presence of a 19,20- δ -lactone moiety. The relative configuration of **5** was revealed by the observations of the following NOESY correlations (Figure 2): between H-3 (δ_H 3.52) and H-5 (δ_H 1.68) and CH₃-18; between H-5 and H-6 (δ_H 4.21) and CH₃-18; between H-20 (δ_H 4.76) and H-8 (δ_H 3.66) and H-9 (δ_H 2.10); as well as between H-8 and H-9. Finally, the absolute configuration of the compound was determined by a single crystal X-ray diffraction experiment (Figure 3). The structure of **5** was thus elucidated as (3*S*,4*R*,5*R*,6*R*,8*R*,9*R*,10*S*)-14,16-epoxy-3 β ,6 β -dihydroxy-12-oxo-17-*nor*-(9 β H)-pimar-13(14),15-dien-19 β ,20-olide, and given the trivial name of icacinlactone J. This is the first report on the isolation of a 17-*nor*-(9 β H)-pimarane possessing a unique 19,20- δ -lactone moiety from *Icacina* plants.

Compounds **1** – **5** were evaluated for cytotoxic activity against MDA-MB-435 (human melanoma cancer), MDA-MB-231 (human breast cancer), and OVCAR3 (human ovarian cancer) cell lines. Only 14 α -methoxyhumiranthol (**3**) showed a moderate activity on MDA-MB-435 cells with an IC₅₀ value of 7.04 μ M.

(9 β H)-Pimarane, 17-*nor*-pimarane, and 17-*nor*-(9 β H)-pimarane diterpenoids have been identified from the *Icacina* plants (*I. trichantha*, *I. claessensis*, *I. mannii*, and *I. guesfeldtii*), and several of them showed cytotoxicity against cancer cell lines.^{3–5, 7–9} Those diterpenoids bearing 9 β -H are generally more active.^{3, 4} Based on the fact that 9-H (if any) of all *Icacina* diterpenoids is β -oriented, these compounds are proposed to be biosynthesized through a (9 β H)-pimarane pathway rather than a pimarane pathway,⁵ as shown in Scheme 1.

In summary, new 17-*nor*-pimaranes (**1**, **2**), (9 β H)-pimaranes (**3**, **4**) and 17-*nor*-(9 β H)-pimarane (**5**) have been isolated from the tuber of *I. trichantha*. Unlike other diterpenoids obtained from *Ipacina* plants, icacinlactone J (**5**) possesses a unique 19,20- δ -lactone moiety. 14 α -Methoxyhumiranthol (**3**) showed moderate cytotoxicity on MDA-MB-435 cells with an IC₅₀ value of 7.04 μ M.

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotations at the sodium D line were measured with a Perkin-Elmer 241 digital polarimeter using a quartz cell with a path length of 100 mm at room temperature. Concentrations (*c*) are given in g/100mL. IR spectra were measured on a Jasco Fourier Transform IR Spectrometer (FT-IR model 410) loaded with an OMNIC software. NMR spectra were recorded on a Bruker DPX-400 spectrometer. All chemical shifts were quoted on the δ scale in ppm using residual solvent as the internal standard (methanol-*d*₄: 3.30 ppm for ¹H NMR, 49.90 ppm for ¹³C NMR). Coupling constants (*J*) are reported in Hz. For HPLC purification, a C₁₈ semi-preparative HPLC column (Phenomenex C₁₈ column, 250 $\times$ 10 mm, 5 μ m) and a Shimadzu UFLC system were used. HRESIMS were measured on a Waters SYNAPT hybrid quadrupole/time of flight spectrometer using positive electrospray ionization. Human melanoma cancer cells MDA-MB-435, human breast cancer cells MDA-MB-231, and human ovarian cancer cells OVCAR3 were purchased from the American Type Culture Collection (Manassas, VA). Molecular models in figure 2 were generated by Chem3D Pro 14.0 using MM2 force field calculation.

Plant Material

Fresh tubers of *Ipacina trichantha* Oliv. were collected in June, 2011 from the Orba village in Nsukka of the Enugu State, Nigeria, and authenticated by Prof. B.O. Olorede of the Botany Department, University of Abuja, Nigeria, and Mr. A. Ozioko, botanist at the BDCP laboratories, Nsukka, Nigeria. A voucher specimen (UNN/FVM 456) was deposited in the pharmacology laboratory at the University of Nigeria, Nsukka, Nigeria.

Extraction and Isolation

The powdered tuber of *I. trichantha* (1.5 kg) was extracted with 80% aqueous MeOH by percolation to yield 166 g of dry crude extract. The crude extract was partitioned into petroleum ether-soluble (11 g), EtOAc-soluble (17 g), *n*-BuOH-soluble (15 g), and H₂O-soluble (128 g) fractions. The EtOAc fraction (17 g) was separated into 88 sub-fractions on a silica gel column (5 $\times$ 60 cm) eluted with mixtures of petroleum ether and EtOAc (from 100:0 to 0:100 v/v; 600 mL each).

The combined subfractions 29 – 32 was separated by semipreparative HPLC eluted with MeOH–H₂O (48:52 v/v; 3.5 mL/min) to afford **1** (1.2 mg, *t*_R = 15.3 min, soluble in MeOH). Compound **2** (1.0 mg, *t*_R = 9.6 min, soluble in MeOH) was purified from sub-fractions 33 – 35 by HPLC (MeOH H₂O, 60:40 v/v; 3.5 mL/min). Subfractions 54 – 56 were further fractionated into 25 fractions using an MCI CHP20P resin column (2.5 $\times$ 20 cm) eluted by aqueous MeOH (from 20 to 80%). Compound **3** (20 mg, soluble in MeOH) was crystallized

from the 9th and 10th fractions. Compound **4** (0.9 mg, t_R = 10.1 min, soluble in MeOH) was purified from the 18th fraction using semipreparative HPLC, eluted with MeCN–H₂O (32:68 v/v; 3.5 mL/min). Compound **5** (2.2 mg, t_R = 10.7 min, soluble in MeOH) was purified from subfractions 60 – 61 by HPLC (MeCN–H₂O, 20:80 v/v; 3.5 mL/min).

12-Hydroxyicacinlactone A (1): colorless amorphous powder; $[\alpha]_D^{20} = +22$ (c 0.2, MeOH); IR (film) $\nu_{\max}$ 3375, 2915, 2848, 1740, 1597, 1345, 1235, 1181, 1127, 1110, 1038 cm⁻¹; ¹H NMR (methanol-*d*₄, 400 MHz), see Table 1; ¹³C NMR (methanol-*d*₄, 100 MHz), see Table 2; (+)-HRESIMS m/z [M + H]⁺ 343.1182; calcd for C₁₉H₁₉O₆⁺, 343.1176.

7β-Hydroxyicacinlactone B (2): colorless amorphous powder; $[\alpha]_D^{20} = +18$ (c 0.1, MeOH); IR (film) $\nu_{\max}$ 3499, 2916, 2848, 1740, 1604, 1499, 1463, 1375, 1337, 1324, 1295, 1239, 1180, 1131, 1103, 1044, 999 cm⁻¹; ¹H NMR (methanol-*d*₄, 400 MHz), see Table 1; ¹³C NMR (methanol-*d*₄, 100 MHz), see Table 2; (+)-HRESIMS m/z 355.1204 [M + H – H₂O]⁺; calcd for C₂₀H₁₉O₆⁺, 355.1176).

14α-Methoxyhumirianthol (3): colorless amorphous powder; $[\alpha]_D^{20} = -152$ (c 0.2, MeOH); IR (film) $\nu_{\max}$ 3512, 2928, 1738, 1430, 1350, 1300, 1214, 1122, 1091, 1067, 1050, 1032, 989, 931, 920 cm⁻¹; ¹H NMR (methanol-*d*₄, 400 MHz), see Table 3; ¹³C NMR (methanol-*d*₄, 100 MHz), see Table 2; (+)-HRESIMS m/z 393.1917 [M + H]⁺; calcd for C₂₁H₂₉O₇⁺, 393.1908).

Icacinlactone I (4): colorless amorphous powder; $[\alpha]_D^{20} = -129$ (c 0.1, MeOH); IR (film) $\nu_{\max}$ 3406, 2916, 2849, 1757, 1570, 1420, 1380, 1300, 1220, 1160, 1156, 1096, 1047, 986 cm⁻¹; ¹H NMR (methanol-*d*₄, 400 MHz), see Table 3; ¹³C NMR (methanol-*d*₄, 100 MHz), see Table 2. (+)-HRESIMS m/z 347.1871 [M + H]⁺; calcd for C₂₀H₂₇O₅⁺, 347.1853.

Icacinlactone J (5): colorless amorphous powder; $[\alpha]_D^{20} = -12$ (c 0.2, MeOH); IR (film) $\nu_{\max}$ 3399, 2921, 2848, 1713, 1664, 1594, 1454, 1421, 1289, 1264, 1206, 1153, 1123, 1073, 1051, 1022 cm⁻¹; ¹H NMR (methanol-*d*₄, 400 MHz), see Table 1; ¹³C NMR (methanol-*d*₄, 100 MHz), see Table 2; (+)-HRESIMS m/z 347.1461 [M + H]⁺ (calcd for C₁₉H₂₃O₆⁺, 347.1489).

Single Crystal X-Ray Structure Determination

X-Ray diffraction intensity data were collected at the Advanced Photon Source, Argonne National Laboratory, at LS-CAT (beamline 21-ID-D), with a 50 μm X-ray beam, at a distance of 100 mm and temperature of 100K, using a MAR CCD 300 mm area detector. For compound **3**, a needle-like crystal, roughly 10 × 10 × 50 μm, was used for data collection. The space group was determined to be P2₁ (No. 4) by identification of the systematic absences. The absolute configuration of the structure was inferred from an analysis of Bijvoet differences, with the Flack parameter of –0.2(2) (1.2 for the inverted structure), and a probability of P2(true) = 1.000. For compound **5**, a block-like crystal, roughly 10 × 10 × 10 μm, was used for data collection. The space group was determined to be P2₁2₁2₁ (No. 19) by systematic absences. The absolute configuration of the structure was

evident from an analysis of Bijvoet differences, with the Flack parameter of $-0.39(17)$ (1.2 for the inverted structure), and a probability of $P2(\text{true}) = 1.000$. Both structures were solved by SHELXS, and refined with SHELX-2014. The data for X-ray diffraction analyses of compounds **3** and **5** have been deposited at the Cambridge Crystallographic Data Centre. CCDC 1414791 (for **3**) and 1414790 (for **5**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via <http://www.ccdc.cam.ac.uk/conts/retrieving.html> (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44 1223 336033; E-mail: deposit@ccdc.cam.ac.uk).

Cytotoxicity Assays

The cell line was propagated at 37°C in 5% CO₂ in RPMI 1640 medium, supplemented with fetal bovine serum (10%), penicillin (100 units/mL), and streptomycin (100 µg/mL). Cells in log phase growth were harvested by trypsinization followed by two washings to remove all traces of enzyme. A total of 5,000 cells were seeded per well of a 96-well clear, flat-bottom plate (Microtest 96®, Falcon) and incubated overnight (37°C in 5% CO₂). Samples dissolved in DMSO were diluted and added to the appropriate wells (concentrations: 20, 4, 0.8, 0.16, and 0.032 µM; total volume: 100 µL; DMSO: 0.5%). The cells were incubated in the presence of test substance for 72 h at 37°C and evaluated for viability with a commercial absorbance assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega Corp, Madison, WI) that measured viable cells. IC₅₀ values are expressed in µM relative to the solvent (DMSO) control. Vinblastine was used as positive control, showing IC₅₀ values of 0.49, 8.78, and 1.82 nM against MDA-MB-435 (human melanoma cancer), MDA-MB-231 (human breast cancer), and OVCAR3 (human ovarian cancer) cell lines, respectively.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
 ^1H - ^1H COSY and selective HMBC correlations for **1** – **5**

Figure 2.
Key NOESY correlations for **1** – **5**

Figure 3.
ORTEP representations of **3** and **5**

Scheme 1.A plausible biogenetic pathway for compounds **1** – **5**

Table 1

¹H (400 MHz) NMR Spectroscopic Data for Compounds 1 – 5 (δ in ppm, J in Hz)^a

position	1	2	3	4	5
	δ_{H} (J in Hz)	δ_{H} (J in Hz)	δ_{H} (J in Hz)	δ_{H} (J in Hz)	δ_{H} (J in Hz)
1	2.63, ddd (4.5, 12.3, 12.3)	2.75, ddd (4.5, 12.4, 12.4)	1.62–1.79 ^d	2.02, ddd (9.2, 9.2, 12.5)	1.72–1.81 ^j
	1.87, dddd (4.0, 4.2, 12.0, 12.3)	1.87, dddd (4.0, 4.4, 12.0, 12.4)		1.59, ddd (2.0, 7.8, 12.5)	1.56, m
2	2.27, ddd (4.5, 12.0, 14.0)	2.27, ddd (4.5, 12.0, 13.9)	2.11, ddd (4.9, 12.0, 13.8)	2.55–2.62 ^h	2.00–2.06 ^k
	2.04, ddd (4.2, 12.3, 14.0)	2.06, ddd (4.4, 12.4, 13.9)	1.81–1.91 ^e		1.80–1.87 ^j
3					3.52, dd (4.7, 10.7)
5	2.34, dd (2.2, 8.1)	2.49, dd (2.2, 8.2)	2.39, dd (1.5, 7.0)	2.56–2.57 ^h	1.68, d (2.2)
6	5.16, ddd (5.8, 8.1, 9.6)	5.09, dd/br t-like (8.0, 8.2)	5.06, dd (5.0, 7.0)	4.95, dd/t-like (5.0, 5.0)	4.21, d (2.2)
7	4.09, dd (9.6, 16.4) ^b	5.91, d (8.0)	5.97, d (5.0)	5.96, dd (0.8, 5.0)	2.00–2.06 ^k
	2.90, dd (5.8, 16.4)				1.72–1.81 ^j
8					3.66, ddd/dt-like (4.2, 4.3, 13.2)
9			1.87–1.92 ^e	2.27, br dd (3.6, 12.9)	2.10, ddd/dt-like (4.2, 4.2, 14.0)
11	6.61, s	6.73, s	1.61–1.67 ^d	1.75, m	2.62, dd (14.0, 17.0)
			1.20–1.31 ^f	1.43–1.45 ⁱ	2.46, dd (4.2, 17.0)
12			1.41–1.47, m	1.42–1.47 ⁱ	
			1.24–1.36 ^f		
14				4.03, s	
15	6.85, d (2.2)	6.86, d (2.2)	3.71 ^g	3.81, br d (4.5)	6.63, d (2.0)
16	7.62, d (2.2)	7.68, d (2.2)	4.51, dd (6.5, 10.5)	4.41, dd (4.5, 10.0)	7.51, d (2.0)
			3.71 ^g	3.72, dd (0.9, 10.0)	
17			0.95, s	0.96, s	
18	1.43, s	1.45, s	1.39, s	1.49, s	1.37, s
20	4.11, dd (4.0, 9.5) ^b	4.87, dd (4.0, 9.3) ^c	3.96, dd (3.4, 9.1)	0.90, s	4.76, dd (2.1, 11.2)
	3.75, dd (2.2, 9.5)	3.79, dd (2.2, 9.3)	3.54, dd (1.5, 9.1)		4.05, br d (11.2)
OCH ₃		3.95, s	3.25, s		

$b-k$ Signal was partially obscured.

d Data measured in methanol

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Table 2

¹³C (100 MHz) NMR Spectroscopic Data for Compounds 1 – 5 (δ in ppm)^a

	1	2	3	4	5
position	δ_c , type	δ_c , type	δ_c , type	δ_c , type	δ_c , type
1	30.9, CH ₂	31.5, CH ₂	29.9, CH ₂	31.9, CH ₂	37.6, CH ₂
2	28.4, CH ₂	28.9, CH ₂	28.6, CH ₂	35.7, CH ₂	30.4, CH ₂
3	98.2, C	97.9, C	97.8, C	208.2, C	76.8, CH
4	50.8, C	51.5, C	51.8, C	54.9, C	48.3, C
5	52.2, CH	53.4, CH	45.4, CH	47.4, CH	46.6, CH
6	76.1, CH	76.1, CH	74.0, CH	74.4, CH	64.6, CH
7	26.6, CH ₂	60.1, CH	117.5, CH	117.8, CH	34.9, CH ₂
8	109.2, C	114.7, C	145.1, C	149.0, C	28.5, CH
9	135.9, C	138.3, C	40.2, CH	45.0, CH	40.2, CH
10	36.3, C	36.0, C	31.9, C	33.3, C	35.5, C
11	105.5, CH	101.8, CH	27.0, CH ₂	25.6, CH ₂	35.8, CH ₂
12	151.0, C	155.1, C	36.4, CH ₂	33.6, CH ₂	195.6, C
13	117.0, C	118.1, C	54.8, C	50.7, C	120.9, C
14	155.9, C	156.7, C	110.6, C	87.4, CH	173.6, C
15	105.0, CH	105.1, CH	79.3, CH	79.8, CH	106.9, CH
16	145.0, CH	145.4, CH	76.5, CH ₂	76.5, CH ₂	145.2, CH
17			13.0, CH ₃	15.5, CH ₃	
18	20.0, CH ₃	20.3, CH ₃	19.1, CH ₃	21.7 ^b , CH ₃	18.1, CH ₃
19	181.8, C	181.7, C	181.3, C	177.0, C	176.6, C
20	72.5, CH ₂	75.2, CH ₂	73.4, CH ₂	21.6 ^b , CH ₃	78.9, CH ₂
OCH ₃		56.2, CH ₃	49.9, CH ₃		

^aData measured in methanol-*d*₄.^bData were interchangeable.