Antiproliferative Trihydroxyalkylcyclohexenones from Pleiogynium timoriense

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ABSTRACT: Investigation of a DCM extract of the bark of Pleiogynium timoriense from the former Merck collection of natural product extracts for antiproliferative activity indicated that it was active with an IC$_{50}$ value of 1.3 μg/mL against the A2780 ovarian cancer cell line. Bioassay-directed fractionation of this extract yielded the three new bioactive trihydroxyalkylcyclohexenones 1−3. Their structures were determined by a combination of spectroscopic and chemical methods. Compounds 1−3 exhibited submicromolar antiproliferative activity against the A2780 human ovarian cancer cell line, with IC$_{50}$ values of 0.8, 0.7, and 0.8 μM, respectively.

As part of an investigation of the former Merck natural products extract library for antiproliferative constituents, now maintained by the Natural Products Discovery Institute,1 we identified a DCM fraction of the ethanol extract of the bark of Pleiogynium timoriense (Anacardiaceae) as a promising extract with an IC$_{50}$ value of 1.3 μg/mL against the A2780 ovarian cancer cell line. P. timoriense, also known as the Burdekin plum, is a tree found in northeast Australia and Malaysia as well as locations in the south-central Pacific and southwestern Pacific.2 Its fruit is used to make jam,3 and its leaves have been reported to be a source of antioxidants. Twelve compounds, including kaempferol, gallic acid, various kaempferol, quercitin, and myricetin glycosides, and three galloyl derivatives, have been identified from the ethanolic extract of the leaves.4 It has also been reported that cyanidin 3-glucoside can be found in the fruit of P. timoriense.5,6 This DCM fraction was selected for fractionation based on its antiproliferative activity and the lack of reported antiproliferative compounds from the species.

The DCM fraction (0.30 g) was fractionated using Sephadex LH-20 column chromatography and two rounds of C18 HPLC to yield the three active compounds 1−3. Compound 2 was obtained in the largest amount and was investigated first. 

13C NMR and HRESIMS data indicated that compound 2 had the molecular formula C$_{25}$H$_{44}$O$_{4}$ (M + H)$^+$ m/z 409.3291, calcd for C$_{25}$H$_{45}$O$_{4}$ 409.3312. Its 1H NMR spectrum indicated the presence of an α,β-unsaturated carbonyl group (H-2, δ 6.1, 1H, dd, J = 10.2, 0.7 Hz; H-3 δ 6.8, 1H, ddd, J = 10.1, 3.9, 1.3 Hz), which was confirmed by its 13C NMR spectrum (C-1, δ 200.2; C-2, δ 126.4; C-3, δ 145.9). A large peak for methylene protons in the 1H NMR spectrum (δ 1.22−1.34) as well as a triplet at δ 0.88 (H-19′, 3H, J = 6.9 Hz) indicated the presence of a long alkyl chain in 2. This was consistent with the 13C NMR data, which showed 10 signals at approximately δ 29 as well as signals at δ 23.0 (C-2′), 22.7 (C-18′), and 14.7 (C-19′). The NMR spectroscopic data are comparable to those of other known trihydroxyalkylcyclohexene derivatives.7−10 The presence of a double bond within the alkyl chain was indicated by a signal at δ 5.34 (2H, m) in the 1H NMR spectrum as well as by COSY correlations from δ 5.34 (H-10′, H-11′) to δ 2.00 (H-9′, H-12′).

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COSY correlations between H-3 and H-4 as well as H-4 and H-5 were used to establish the positions of C-4 and C-5 (Figure 1). The lack of other correlations in the COSY spectra indicated that C-5 must be attached to an oxygenated tertiary carbon (C-6). HMBC correlations of H-2 to C-6, H-4 to C-6, and H-5 to C-1 (Figure 1) indicated that the structure contained a cyclic moiety, which is consistent with the calculated hydrogen deficiency index of four.

The configuration of the double bond in the alkyl chain was assigned as Z based on the shifts of the adjacent carbon atoms (δ 27.2 C-9' and C-12'), which would have been more shielded in the case of an E-configuration (δ ∼27).11,12 The connectivity of the alkyl chain at C-6 was determined from the HMBC spectrum, which showed long-range correlations from H-1' to C-6. The remaining 13C NMR signals were assigned using HSQC and HMBC spectroscopy. Complete NMR assignments of all carbons and protons for 2 are reported in Table 1.

The location of the double bond in the alkenyl chain was determined through MS analysis of the products resulting from derivatization with dimethyl disulfide, following the method of Mansour13 and Roumy.9 The LC-MS of the dimethyl disulfide derivative of 2 contained a strong fragment ion at m/z 329.19 (calcld [C17H29O4S] + 329.18), indicating a Δ10,11 double bond.

The 1H NMR spectra of compounds 1 and 3 were similar to those of compound 2 (Table 1). The structures of 1 and 3 were assigned by comparison of NMR and MS data with those of 2. 1H and 13C NMR spectroscopic data indicated that the structure of the cyclic moiety was identical based on chemical shifts and coupling constants. The only differences in the structures were due to the length of the alkyl chain and the location of the double bond within the chain. HRESIMS and 13C NMR data were used to determine that 1 contained two fewer methylene groups than 2 ([M + H] + m/z 381.2972, calcd for C20H41O4S 381.2999) and that 3 contained two additional methylene groups ([(M + H)] + m/z 437.3604, calcd for C23H41O4S 437.3625).

LC-MS analysis of the dimethyl disulfide derivative of 1 showed a strong fragment ion at m/z 301.12 (calcd for [C15H25O4S] + 301.15), and that of the same derivative of 3 showed a strong fragment ion at 357.20 (calcld for

![Figure 1. Selected 2D NMR correlations of 2.](Image 147x638 to 214x705)

### Table 1. NMR Spectroscopic Data for Compounds 1–3

| Position | δ_C | δ_H (J in Hz) | δ_C | δ_H (J in Hz) | δ_C | δ_H (J in Hz) |
|----------|-----|---------------|-----|---------------|-----|---------------|
| 1        | 200.2, C | 6.10, dd (10.1, 0.8) | 200.2, C | 6.10, dd (10.2, 0.7) | 201.6, C | 6.10, dd (10.2, 0.8) |
| 2        | 126.4, CH | 6.80, ddd (10.2, 1.3, 0.7) | 145.9, CH | 6.80, ddd (10.1, 1.3, 1.0) | 154.5, CH | 6.80, ddd (10.1, 1.0, 1.3) |
| 3        | 78.1, C | 3.98, brs | 78.1, C | 3.98, brs | 75.3, CH | 3.98, ddd (10.3, 1.3, 1.3) |
| 4        | 78.1, C | 5.34, m | 78.1, C | 5.34, m | 77.8, C | 5.34, m |
| 5        | 36.1, CH3 | 1.83, s | 36.1, CH3 | 1.83, s | 36.1, CH3 | 1.83, s |
| 6        | 23.0, CH3 | 1.13, m | 23.0, CH3 | 1.13, m | 22.7, CH3 | 1.13, m |
| 7        | 29.8, CH3 | 1.26, brs | 29.8, CH3 | 1.26, brs | 29.4, CH3 | 1.25, brs |
| 8        | 27.2, CH3 | 2.00, m | 27.2, CH3 | 2.00, m | 27.2, CH3 | 2.00, m |
| 9        | 129.9, CH | 5.34, m | 129.9, CH | 5.34, m | 129.8, CH | 5.34, m |
| 10       | 27.2, CH3 | 2.00, m | 27.2, CH3 | 2.00, m | 27.1, CH3 | 2.00, m |
| 11       | 29.3, CH3 | 1.26, brs | 29.3, CH3 | 1.26, brs | 29.3, CH3 | 1.26, brs |
| 12       | 29.3, CH3 | 1.26, brs | 29.3, CH3 | 1.26, brs | 29.3, CH3 | 1.26, brs |
| 13       | 31.9, CH3 | 1.26, brs | 31.9, CH3 | 1.26, brs | 31.9, CH3 | 1.26, brs |
| 14       | 22.7, CH3 | 1.26, brs | 22.7, CH3 | 1.26, brs | 22.7, CH3 | 1.26, brs |
| 15       | 14.1, CH3 | 0.88, t (6.9) | 14.2, CH3 | 0.88, t (6.9) | 14.2, CH3 | 0.88, t (6.9) |
| 16       | 3.60 f | 3.59 f | 2.94 f | 2.90 f | 2.94 f | 2.87 f |
| 17       | 2.94 f | exchanges | 2.90 f | exchanges | 2.87 f | exchanges |

*CDCl3, 500 MHz, 150 MHz. 1HCDCl3, 500 MHz, 125 MHz. 1H 1H 1H 1H 1H 1H 1H 1H 1H

Interchangeable assignment within a column. Overlapping signals. Obtained from HMBC and HSQC spectra.
The 1H NMR spectrum of 4 (1H, dd, δ = 10.5, 2.2 Hz; H-3, dd, δ = 7.5, 2.3 Hz; H-5a, dd, δ = 7.5, 2.3 Hz; H-6a, δ = 2.7) showed a positive Cotton effect at 253 nm, as predicted by the Newman projections of the C-4/C-5 and C-5/C-6 bonds. The expected weaker negative second Cotton effect is presumably buried in the strong positive background ellipticity. Thus, the absolute configuration of 1 is assigned as 4S,5R,6R. Additionally, since 2 ([(α)2]D +21) and 3 ([(α)2]D +19) have similar values of specific rotation to 1 ([(α)2]D +23), their absolute configurations must also be 4S,5R,6R.

The three isolated compounds are similar in structure to other known hydroxyalkylcyclohexenones that are found from Tapirira obtusa, T. guianensis, and Lannea edulis in the family Anacardiaceae. Furthermore, they contain the same oxygenation pattern as the zeylenones, many of which have been isolated from various members of the Uvaria genus (Annonaceae).

ExPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a JASCO P-2000 polarimeter, and UV spectra were measured on a Shimadzu UV-1201 spectrophotometer. ECD analysis was performed on a JASCO J-810 spectropolarimeter with a 0.1 cm cell in MeOH at room temperature under the following conditions: speed 50 nm/min, time constant 1 s, bandwidth 2.0 nm. 1H and 13C NMR spectra were recorded using either Bruker Avance 500 or 600 spectrometers. Mass spectra were obtained on an Agilent 6220 LC-TOF-MS or a Thermo Electron TQS LC-ESI-MS. Semi-preparative HPLC was performed using a Shimadzu LC-10AT pumps coupled with a Shimadzu SPD M10A diode array detector, a SCL-10A system controller, and a Cogent Bidentate C18 column (250 × 10 mm) or a Varian Lichrosorb 5 Diol column (250 × 10 mm).

Antiproliferative Bioassay. The assay was performed at Virginia Tech according to specifications previously described. The A2780 ovarian cell line is a drug-sensitive ovarian cancer cell line.22

Plant Material. Bark of Pleiogynium timoriense (DC) Leenh. was collected by Dr. Paul Cox under the auspices of the New York Botanical Garden (NYBG) from a seaward-facing forest on the island of Eua, Tonga, in July 1987; a voucher specimen, PC01113 (ID number 40077), is on deposit at the NYBG.

Extraction and Isolation. An EtOH extract of the bark of P. timoriense was subjected to liquid–liquid partitioning to give active hexanes, DCM, and aqueous MeOH fractions with IC50 values against the A2780 ovarian cancer cell line of 3.0, 1.3, and 6.2 µg/mL, respectively. The active DCM fraction 23050-C6 (0.30 g) was further purified using Sephadex LH-20 column chromatography (1:1 DCM/MeOH) to generate an active fraction (222 mg, IC50 0.5 µg/mL). This fraction was further purified utilizing C18 HPLC (MeOH/H2O gradient) to yield three semipure active fractions. These fractions were further purified utilizing C18 HPLC (MeCN/H2O gradient) to yield the active compounds 1 (5.4 mg), 2 (6.9 mg), and 3 (1.4 mg).

Compound 1: [(α)2]D +23 (0.5 CHCl3); UV (MeOH) λmax (log ε) 215 (3.59) nm; ECD (MeOH) [Δε]381 nm = −0.17, [Δε]342 nm +1.72, [Δε]209 nm +1.07; 1H NMR (CDCl3, 500 MHz) see Table 1; 13C NMR (CDCl3, 500 MHz) see Table 1; HRESIMS [M + Na]+ m/z 381.2972 (calcd for C19H33O4S, 381.2999), [M + Na]+ m/z 403.2805 (calcd for C25H45O4S, 403.2819).

Compound 2: [(α)2]D +21 (0.7 CHCl3); UV (MeOH) λmax (log ε) 215 (3.44) nm; 1H NMR (CDCl3, 500 MHz) see Table 1; 13C NMR (CDCl3, 125 MHz) see Table 1; HRESIMS [M + H]+ m/z 409.3291 (calcd for C25H45O4S, 409.3291), [M + Na]+ m/z 431.3125 (calcd for C25H45O4SNa+, 431.3132).

Compound 3: [(α)2]D +19 (0.1 CHCl3); UV (MeOH) λmax (log ε) 215 (3.25) nm; 1H NMR (CDCl3, 500 MHz) see Table 1; 13C NMR...
(CDCl3, 150 MHz) see Table 1; HRESIMS [M + H]+ m/z 437.3604 (calcd for C24H34Br3O7 437.3625), [M + Na]+ m/z 459.3431 (calcd for C26H36NaO7 459.3445).

Reduction of Compound 2. Compound 2 (5.8 mg, 0.014 mmol) was dissolved in CHCl3 (3 mL), and Ph2SiH2 (5.3 μL, 53 μg, 0.028 mmol), ZnCl2 (0.5 mg, 0.0037 mmol), and Pd(PPh3)4 (0.3 mg, 0.0026 mmol) were added. The reaction mixture was stirred for 4 h at rt. The solvent was removed under reduced pressure, and the residue was purified utilizing silica gel column open column chromatography (7:3 hexanes/EtOAc) to yield 2.3 mg of residue was purified utilizing diol HPLC (hexanes/EtOAc gradient) to yield 0.8 mg (0.026 mmol, 39%).

Compound 4: 1H NMR (methanol-d4, 600 MHz) δ 5.38 (2H, m, H-10), 4.03 (1H, q, J = 4.3 Hz, H-4), 3.71 (1H, dd, J = 4.5, 1.1 Hz, H-5), 2.70 (1H, ddd, J = 13.4, 11.7, 5.6 Hz, H-2a), 2.37 (1H, dt, J = 13.4, 5.2 Hz, H-2b), 2.16 (2H, m, H-3a, H-1′b), 1.97 (4H, br, H-9′a), 1.84 (1H, m, H-3b), 1.70 (1H, ddd, J = 13.9, 12.4, 4.3 Hz, H-1′a), 1.29 (24H, brs, H-3′), 1.08 (2H, m, H-2′), 0.90 (3H, t, J = 7.0 Hz, H-9′b); HRESIMS [M + H]+ m/z 411.3479, [M + Na]+ m/z 433.3428 (calcd for C27H49O4 411.3479), [M + Na]+ m/z 433.3428.

p-Bromobenzoylation of Compound 1. Compound 1 (0.4 mg, 0.001 mmol) was dissolved in DCM (2 mL), and 44.9 mg (0.37 mmol) of p-bromobenzoyl chloride were added. The reaction mixture was stirred for 1.5 h at rt. The solution was diluted with DCM (5 mL) and washed with H2O (5 mL x 3), 3 M HCl (5 mL), and brine (5 mL). The organic layer was dried with anhydrous MgSO4, and the solvent was removed under vacuum. The resulting residue was purified utilizing diol HPLC (hexanes/EtOAc gradient) to yield compound 5 (0.8 mg, 82%).

Supporting Information

ASSOCIATED CONTENT

1H and 13C and 2D NMR spectra of compounds 1−3, 1H NMR spectra of 4 and 5, COSY, NOESY, selective TOCSY, and NOE spectra of 4, and ECD spectra of 1 and 5. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.SB00119.

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Notes

The authors declare no competing financial interest.