Four Clerodane Diterpenoids From *Ptychopetalum Olacoides*

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Abstract

A phytochemical component investigation of the bark of *Ptychopetalum olacoides* led to the isolation of 4 new clerodane-type diterpenoids, namely, psycholide V (1), 7α,20-dihydroxykolavelool (2), psycholide VI (3), and psycholide VII (4). Their structures were elucidated by extensive spectroscopic data and comparison of NMR data with that obtained for known compounds.

Keywords
clerodane, *Ptychopetalum olacoides*, Olacaceae, diterpenoids, psycholide

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The genus *Ptychopetalum* is a flowering plant of the Olacaceae family that is native to the Amazon rainforest. It is a shrub or small tree that reaches a height of about 4.3 m. The leaves are short-stalked, up to 7.6 cm in length and 5.1 cm in breadth, light green on the upper surface and dark brown on the lower surface. Indigenous names for the genus include marapuama, muirapuama, and mirantã, translating roughly to “potency wood.”¹² P. olacoides is known as Muirapuama or Marapuama and is used to treat chronic degenerative conditions of the nervous system.³ Previous pharmacological studies have shown that the EtOH extract of P. olacoides produces a series of beneficial effects on the central nervous system, consisting of neuroprotective, anti-stress, antidepressant, antioxidative, adaptogen-like activities, and inhibitory effects on acetylcholinesterase in mice.⁴ The IR spectrum showed absorption bands at 3407 and 1362 cm⁻¹, which are ascribed to a hydroxy group and a -lactone moiety, respectively. The NMR data for 1 was obtained as colorless oil, had the molecular formula C₂₀H₃₄O₃, as assigned from the high resolution-fast atom bombardment-MS (HR-FAB-MS) ion at m/z 362.0 (calcd for 362.2093). Its IR spectrum showed absorption bands at 3407 and 1762 cm⁻¹, which are ascribed to a hydroxy group and a γ-lactone moiety, respectively. The NMR data for 1 (Table 1) were found to be similar to those for psycholide II (5).¹³ Compound 5 was isolated as an acetal mixture, while compound 1 was isolated as a single compound. The structure of 1 was estimated to be a 15-hydroxy analogue. The methoxy group was shown to be connected to C-20, which was supported by the heteronuclear multiple bond coherence (HMBC) correlation of -OCH₃ to C-20 (105.7 ppm) (Figure 2). The relative configuration of 1 was deduced to be the same as that of 5 by a nuclear Overhauser and exchange spectroscopy (NOESY) experiment (Figure 2). The absolute configuration on C-15 in 1 was assigned by Gawronski’s method.¹⁴ Thus, the 15α configuration as the sole chromophore displays a positive n-π* Cotton effect at a wavelength of 244 nm and a negative π-π* Cotton effect at 215 nm (Figure 2). Thus, psycholide V was represented as 1.

Compound 2, obtained as a colorless oil, has the molecular formula C₁₂H₁₅O₅, as assigned from the high resolution-fast atom bombardment-MS (HR-FAB-MS) ion at m/z 345. The IR spectrum showed the presence of a hydroxy group at 3289 cm⁻¹. The ¹H NMR was very similar to that for 7α-hydroxykolavelool (6) except an oxymethylene group in 2 replaced the H₃-20 methyl in 6, implying that 2 is

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7α,20-dihydroxykolavelool. The partial structures (bold line in Figure 3) were deduced from the ¹H-¹H correlation spectroscopy and heteronuclear multiple quantum coherence spectra for 2 with 5 carbons at 36.9, 43.2, 73.4, 145.0, and 145.4, and the connectivity between these partial structures and the quaternary carbons was established by HMBC experiments (Figure 3). As a result, the ¹³C NMR chemical shift value at C-20 is shifted to the low field to 65.7 ppm, and the HMBC correlation of H-11 to C-20 and H₂-20 to C-9 (δC 43.2) indicate that the methyl group at the C-20 group is oxidized to a hydroxy group. The relative configuration of 2 was deduced by a NOESY experiment, as shown in Figure 3. The NOESY correlations of H-8 to H-6β, H-7 and H-10 and H₃-19 to H₂-20 and small vicinal J values of H-7 (3.4, 3.3, and 2.8 Hz) indicate that H-10 and the C-11~C-16 side chain are β-oriented, and the C-19, C-20, and hydroxy groups at C-7 are α-oriented (Figure 3). Accordingly, the structure of 2 is elucidated to be 7α,20-dihydroxykolavelool.
Compound 3 was obtained as a colorless oil and its molecular formula C_{21}H_{32}O_{5} was assigned by HR-FAB-MS at m/z 387 [M + Na]^{+}. The IR absorption bands at 1763 and at 3434 cm$^{-1}$ indicated the presence of a $\gamma$-lactone group and a hydroxy group. The 1H and 13C NMR spectra for 3 were very similar to those of 2 considering the decalin part, while the side chain looks like that of 5. Surprisingly, the presence of a 1:1 as in 5 could not be ascertained. The HMBC correlation of OCH$_3$ signal to C-15 implied an attachment of the methoxy group at C-15. The HMBC correlations of H-12 with the lactone carbonyl group as well as by a downfield shifted of H-14 ($\delta$C 141.4; $\delta$H 6.81) suggest that the position of the lactone carbonyl group is C-16. According to NOESY spectra of H-1$\sim$H-12, H-18$\sim$H-20 positions were the same as those of compound 2, indicating that the relative configuration of 3 is the same as that of 2, but stereochemistry of C-15 could not be determined. Thus, 3 was defined as Ptycholide VI.

Compound 4 had a molecular formula of C_{22}H_{36}O_{7}, as established by HR-FAB-MS. The IR spectrum showed the presence of a hydroxy group (3428 cm$^{-1}$) and carbonyl group (1699 cm$^{-1}$). The NMR data of 4 showed the presence of decalin parts as same as 3 and the 3 oxymethines ($\delta$C 80.4

![Figure 3. COSY and selected HMBC (a) and NOESY (b) correlations for 2.
Abbreviations: COSY, correlation spectroscopy; HMBC, heteronuclear multiple bond coherence; NOESY, nuclear Overhauser and exchange spectroscopy.](image1)

![Figure 4. COSY and selected HMBC (a) and NOESY (b) correlations for 4.
Abbreviations: COSY, correlation spectroscopy; HMBC, heteronuclear multiple bond coherence; NOESY, nuclear Overhauser and exchange spectroscopy.](image2)
Table 1. $^1$H (600 MHz) and $^{13}$C NMR (150 MHz) Data for 1-4 in CDCl$_3$.

| C   | $\delta$C | $\delta$H | $\delta$C | $\delta$H | $\delta$C | $\delta$H | $\delta$C | $\delta$H |
|-----|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 1   | 18.5      | 1.64 m    | 19.1      | 1.60 m    | 18.9      | 1.69 m    | 20.1      | 1.64 m    |
|     | 1.74 m    |           |           |           |           |           |           |           |
| 2   | 25.4      | 2.13 m    | 27.0      | 2.00 m    | 27.0      | 2.00 m    | 27.2      | 2.16 m    |
|     |           |           |           |           |           |           |           |           |
| 3   | 118.8     | 5.02 d (1.5) | 119.6    | 5.13 br s | 119.7    | 5.14 br s | 121.6     | 5.27 br s |
|     | 145.4     |           |           |           |           |           |           |           |
| 4   | 38.5      |           | 36.9      |           | 37.1      |           | 43.1      |           |
|     |           |           |           |           |           |           |           |           |
| 5   | 40.6      | 1.48 d (13.5) | 43.3      | 1.40 dd (14.1, 3.4) | 43.5      | 1.43 m    | 52.9      | 2.28 d (12.0) |
|     |           | 1.91 dd (13.5, 4.9) |           | 2.10 dd (14.1, 2.8) |           |           |           |           |
| 6   | 84.2      | 4.25 d (4.9) | 72.7      | 3.98 ddd (3.4, 3.3, 2.8) | 72.7      | 4.02 ddd (6.5, 3.1, 2.7) | 213.3     |           |
|     | 46.5      | 1.72 m    | 38.9      | 1.65 m    | 39.1      | 1.71 m    | 49.8      | 2.58 q (7.0) |
| 7   | 52.0      |           | 43.2      |           | 43.3      |           | 48.8      |           |
|     |           |           |           |           |           |           |           |           |
| 8   | 47.0      | 1.81 d (13.5, 2.5) | 46.5      | 1.49 d (12.0, 2.0) | 46.7      | 1.52 m    | 46.9      | 2.03 m    |
|     | 24.2      | 1.67 m    | 26.9      | 1.41 m    | 30.6      | 1.68 m    | 25.6      | 1.62 m    |
| 9   | 19.8      | 2.29 m    | 35.2      | 1.36 m    | 29.7      | 1.30 m    | 26.4      | 1.16 m    |
| 10  | 138.9     |           | 73.4      |           | 139.1     |           | 81.0      |           |
| 11  | 142.7     | 6.91 d (1.5) | 145.0    | 5.90 dd (17.4, 10.7) | 141.4     | 6.81 d (1.5) | 80.4      | 4.00 s    |
| 12  | 96.7      | 6.10 s    | 112.1     | 5.10 dd (10.7, 1.1) | 102.6     | 5.74 d (1.5) | 110.6     | 4.93 d (3.6) |
| 13  |           |           |           |           |           |           |           |           |
| 14  | 171.3     |           | 27.9      | 1.31 s    | 171.6     |           | 108.5     | 4.81 s    |
| 15  | 17.9      | 1.59 d (1.5) | 18.1      | 1.63 br s | 18.1      | 1.63 br s | 17.9      | 1.59 br s |
| 16  | 23.3      | 1.26 s    | 21.7      | 1.34 s    | 21.6      | 1.43 s    | 20.4      | 1.06 s    |
| 17  | 15.0      | 1.11 d (7.0) | 13.2      | 1.08 d (7.1) | 13.3      | 1.12 d (6.5) | 8.5       | 1.00 d (7.0) |
| 18  | 105.7     | 4.69 s    | 65.7      | 3.73 d (11.6) | 65.6      | 3.84 ddd (8.0, 5.1) | 65.8      | 3.53 d (11.5) |
| 19  |           |           |           |           |           |           |           |           |
| 20  |           |           |           |           |           |           |           |           |
|     |           |           |           |           |           |           |           |           |
| OCH$_3$ | 55.4     | 3.33 s    |           |           | 57.1      | 3.58 s    | 56.4, 55.2 | 3.48 s, 3.40 s |

Compounds 1-4 were isolated from the active fractions that promote neurite outgrowth. Thus, we evaluated their ability to induce neurite outgrowth in PC12 cells as previously our method. However, they had no effect on PC12 cells at concentrations ranging from 3 to 30 μM. This result suggests our previous conclusion that the furan ring in clerodane-type diterpenoids plays an important role for neurite outgrowth in PC12 cells.

**Experimental**

**General Experimental Procedures**

IR and UV spectra were recorded on JASCO FT-IR 410 infrared and Shimadzu UV-1650PC spectrophotometers, respectively. Optical rotations were measured with a JASCO P-1030 digital polarimeter. Circular dichroism spectra were recorded using a JASCO-J-725. $^1$H (600 MHz) and $^{13}$C NMR (150 MHz) spectra were measured with a Varian Unity 600 instrument. MS were recorded using JEOL JMS-HX 110 and JEOL AX-500 instruments. Silica gel (Merck, 70-230, 230-400 mesh, Wakogel C-300) was used for column chromatography (CC). Sephadex LH-20 was used for gel filtration chromatography. HPLC was performed using a JASCO PU-1580 HPLC equipped with a JASCO UV-1575 detector.
Plant Materials
The bark of Ptychopetalum olacoides was collected in Sao Paulo, Brazil in 2005. Dr G. Hashimoto (Centro de Pesquisas de Historia Natural) identified the plant, and a voucher specimen (1762BK) was deposited at the Institute of Pharmacognosy, Tokushima Bunri University, Japan.

Isolation and Purification
The extraction procedure has been reported previously. The extract was separated by CC on silica gel using a linear gradient solvent system (100% n-hexane to 100% EtOAc) to give 15 fractions (1-15). Fraction 12 (2.1 g) was separated by LH-20 (CH2Cl2:hexane = 1:1) to give 5 fractions. Fraction 12-3 (926.8 mg) was subjected to silica gel CC (hexane:EtOAc = 6:4) to give ten fractions. Fraction 12-3-5 (55.3 mg) was purified by HPLC (Cosmosil 5C18 MS-II, i.d. 10 × 250 mm; MeOH: H2O [7: 3; 2.0 mL/min]; det. 225 nm) to give 4 (1.44 g). Fraction 13 (1.44 g) was separated by LH-20 (MeOH) to give 7 fractions. The second fraction (1.0 g) was subjected to silica gel CC (hexane:EtOAc = 6:4) to give 3 fractions. The second fraction (1.0 g) was subjected to LH-20 (CH2Cl2:hexane = 1:1) to give 8 fractions. Fraction 13-5 (49.7 mg) was subjected to silica gel CC (hexane:EtOAc = 6:4) to give 3 fractions. Fraction 13-6-2 (7.8 mg) was purified by HPLC (Cosmosil 5C18 MS-II, i.d. 10 × 250 mm; MeOH: H2O [7: 3; 2.0 mL/min]; det. 210 nm) to give 5 fractions. Fraction 13-6-3-5 (55.3 mg) was purified by HPLC (Cosmosil 5C18 MS-II, i.d. 10 × 250 mm; MeOH: H2O [6: 4; 2.0 mL/min]; det. 210 nm) to give 6:4) to give ten fractions. Fraction 12-3-5 (55.3 mg) was purified by HPLC (Cosmosil 5C18 MS-II, i.d. 10 × 250 mm; MeOH: H2O [6: 4; 2.0 mL/min]; det. 210 nm) to give 2 (1.5 mg). Fraction 13-6-1 (24.7 mg) was purified by HPLC (Cosmosil π-NAP, i.d. 10 × 250 mm; MeOH: H2O: CH3CN [67: 28: 5; 2.0 mL/min]; det. 210 nm) to give 1 (5.7 mg).

Compound 1
α [α]D: -9.7 (c 0.0223, MeOH)
IR: νmax 3407 (OH), 2925, 1762 (lactone) cm⁻¹
UV: λmax (logε) 207 (3.5) nm
EI-MS: m/z 362 [M]+
HR-MS: m/z [M+] calecd for C21H30O5: 362.2093; found 362.2097.

Compound 2
α [α]D: -35.4 (c 0.0123, MeOH)
IR: νmax 3289 (OH), 2944 cm⁻¹
UV: λmax (logε) 206 (4.3) nm
FAB-MS: m/z 345 [M + Na]⁺, 361 [M + K]⁺
HR-FAB-MS: m/z [M + Na]⁺ calecd for C21H30O5Na: 345.2406; found 345.2419.

Compound 3
α [α]D: -28.5 (c 0.0036, MeOH)
IR: νmax 3434 (OH), 2918, 1763 (lactone) cm⁻¹
UV: λmax (logε) 206 (3.7) nm
FAB-MS: m/z 387 [M + Na]⁺, 403 [M + K]⁺
HR-FAB-MS: m/z [M + Na]⁺ calecd for C22H36O7Na: 387.2147; found 387.2142.

Compound 4
α [α]D: -28.0 (c 0.0248, MeOH)
IR: νmax 3428 (OH), 2925, 1699 (C = O) cm⁻¹
UV: λmax (logε) 206 (3.2) nm
FAB-MS: m/z 435 [M + Na]⁺
HR-FAB-MS: m/z [M + Na]⁺ calecd for C22H36O7Na: 435.2059; found 435.2365.

Screening for Neurite Outgrowth-Promoting Activity
PC12 (phenochromocytoma) cells were cultured in a 24-well plate at density of 8 × 10³ cells/mL in DMEM + 10% HS, 5% FBS, 100 IU/mL penicillin, and 100 µg/mL streptomycin at 37 °C under a humidified atmosphere of 95% air and 5% CO2 for 24 h. The culture medium was then changed to DMEM + 2% HS, 1% FBS, 100 IU/mL penicillin, and 100 µg/mL streptomycin. At the same time, different concentrations of test samples with or without 2 ng/mL NGF were added. One concentration experiment was repeated in 3 wells. After incubation with samples for 4 days, the cultures were fixed with 4% paraformaldehyde/PBS and stained with methylene blue. Cell morphology was observed under a phase-contrast microscope, and neurite length was quantified. Ten images were selected randomly under a microscope for each well, and 5 significantly differentiated cells were selected to measure the longest neurite length extending from a cell body for each picture. Statistical analyses were performed using Dunnett’s t test.

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