Pterocarpadiols A–D, Rare 6a,11b-Dihydroxypterocarpans from *Derris robusta*

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**Abstract**

Four hitherto unknown 6a,11b-dihydroxypterocarpans, namely pterocarpadiols A–D (1–4), were isolated from the ethanol extract of the twigs and leaves of *Derris robusta*. Their structures were elucidated on the basis of extensive spectroscopic analysis. Pterocarpadiols A–D are a kind of very rare 6a,11b-dihydroxypterocarpans, and their presence as markers may be helpful in chemotaxonomical classification.

**Graphical Abstract**

**Keywords** *Derris robusta* · 6a,11b-Dihydroxypterocarpan · Pterocarpadiol

**1 Introduction**

The genus *Derris* (Leguminosae) contains about 800 species, widely distributed in tropical and subtropical parts of the world [1]. The bark and leaves of *Derris* species are commonly utilized as folk medicine to treat human diseases such as arthritis and eczema [2]. The extracts and reported chemical constituents exhibit antioxidant, antibacterial and pesticidal activities [2]. Previous studies show the genus is a rich source of flavonoids, isoflavonoids, pterocarpans and rotenoids [2]. As part of a
BioBioPha [http://www.chemlib.cn] objective to assemble a large-scale natural product library very valuable in the discovery of new drug leads from nature [3–7], the phytochemical investigation on the twigs and leaves of Derris robusta led to the isolation of four new 6α,11β-dihydroxypterocarps, namely pterocarpadiols A–D (1–4), together with 10 known pterocarps: 1,11β-dihydro-11β-hydroxymaackiain (5) [8], 1,11β-dihydro-11β-hydroxymedicarpin (6) [8], pisin (7) [9], variabilin (8) [10], 6α-hydroxymaackiain (9) [11], 6α-hydroxymedicarpin (10) [12], maackiain (11) [13], medicarpin (12) [14], 3-hydroxy-8,9-methylene-dioxypertocarpene (13) [15], and anhydroglycinol (14) [16].

Table 1 1H NMR spectroscopic data for pterocarpadiols A–D (1–4) in CD3OD ($\delta_H 3.30$ ppm)

| No. | 1         | 2         | 3         | 4         |
|-----|-----------|-----------|-----------|-----------|
| 1   | 6.80 (d, 10.0) | 6.83 (d, 10.0) | 2.64 (td, 13.9, 4.5, Hβ) | 2.68 (td, 13.9, 4.0, Hβ) |
|     | 2.01 (ddd, 13.9, 4.5, 2.5, Hα) | 2.03 (ddd, 13.9, 5.0, 2.0, Hα) |
| 2   | 6.09 (dd, 10.0, 1.8) | 6.10 (dd, 10.0, 1.8) | 2.76 (ddd, 16.5, 13.9, 4.5, Hβ) | 2.77 (ddd, 16.2, 13.9, 5.0, Hβ) |
|     | 2.31 (ddd, 16.5, 4.5, 2.5, Hα) | 2.31 (ddd, 16.2, 4.0, 2.0, Hα) |
| 4   | 5.41 (d, 1.8) | 5.39 (d, 1.8) | 5.35 (s) | 5.34 (s) |
| 6   | 4.99 (d, 10.5, Hz) | 5.02 (d, 10.6, Hz) | 4.65 (d, 10.0, Hz) | 4.68 (d, 10.0, Hz) |
|     | 4.37 (d, 10.5, Hz) | 4.39 (d, 10.6, Hβ) | 4.30 (d, 10.0, Hβ) | 4.32 (d, 10.0, Hβ) |
| 7   | 6.81 (s) | 7.25 (d, 8.5) | 6.81 (s) | 7.25 (d, 8.0) |
| 8   | 6.55 (dd, 8.5, 2.4) | 6.59 (d, 2.4) | 6.34 (s) | 6.36 (d, 2.0) |
| 10  | 6.26 (s) | 6.29 (d, 2.4) | 6.34 (s) | 6.36 (d, 2.0) |
| 11a | 4.73 (s) | 4.75 (s) | 4.48 (s) | 4.51 (s) |
| OCH2O | 5.90 (d, 1.0) | 5.91 (s) | 5.89 (d, 1.0) |
| OCH3 | 3.72 (s) | 3.74 (s) |

Fig. 1 Pterocarps from Derris robusta (1–14) and hydroxycristacarpone (15)
Among the known pterocarps, compounds 5–10 and 14 were isolated from the genus for the first time. This paper reports the isolation and structure elucidation of pterocarpadiols A–D.

2 Results and Discussion

Compound 1, obtained as amorphous powder, had a molecular formula of C_{16}H_{12}O_{6} as deduced from its positive-ion HRESIMS at m/z 339.0462 [M+Na]+ (calcd for C_{16}H_{12}O_{6}Na, 339.0475), requiring 11 degrees of unsaturation. The 1H NMR spectrum (Table 1) showed three characteristic aliphatic protons of 6a-hydroxypterocarpan skeleton at δ_{H} 4.99, 4.37 (each 1H, d, J = 10.5 Hz, H-6), and 4.73 (s, H-11a) [17]. The 13C NMR spectrum (Table 3) displayed a total of 16 carbon resonances, including three typical oxygen-bearing carbons of 6a-hydroxypterocarpan at δ_{C} 70.2 (t, C-6), 78.7 (s, C-6a) and 91.4 (d, C-11a). Three olefinic protons at δ_{H} 6.80 (d, J = 10.0 Hz, H-1), 6.09 (dd, J = 10.0, 1.8 Hz, H-2), and 5.41 (d, J = 1.8 Hz, H-4) were assignable to A-ring by comparison with hydroxycristacarpine (15) (Fig. 1) [18], and their spectral difference was almost completely rooted in the D ring. Two aromatic singlets at δ_{H} 6.81 (s, H-7), 6.26 (s, H-10) and a methylenedioxy signal at δ_{H} 4.49, 4.28 (each 1H, d, J = 10.0 Hz) were newly detected, while the prenyl and methoxy signals disappeared, which suggested that the methylenedioxy group should be connected to C-8 and C-9. The inference was confirmed by the HMBC correlations from the proton at δ_{H} 6.81 (s, H-7) to the carbons at δ_{C} 78.7 (s, C-6a), 144.2 (s, C-8), and 151.6 (s, C-9), and from the methylenedioxy protons to the carbons at δ_{C} 144.2 (s, C-8), and 151.6 (s, C-9). Regrettably, it was inconclusive to establish relative configurations at C-6a, C-11a and C-11b by ROESY analysis, since the pivotal hydroxy signals were undetectable in CD_{3}OD. As we know, hydroxy proton signals were observable and often appeared as sharp peaks in DMSO-d_{6}, and their HMBC and ROESY correlations often played an important role in structure elucidation, especially the determination of relative configuration [19].

The clear ROESY correlation (DMSO-d_{6}, Fig. 2) of 6a-OH ⇔ H-11a revealed a cis fusion of the B/C ring junction, while the correlations of 11b-OH ⇔ H-11a and H-6a indicated z-orientation of the hydroxy group at C-11b. Accordingly, the structure of 1 was established and named as pterocarpadiol A.

Compound 2, white amorphous powder, had a molecular formula of C_{16}H_{14}O_{6} based on the positive-ion HRESIMS at m/z 325.0674 [M+Na]+ (calcd for C_{16}H_{14}O_{6}Na, 325.0683). The NMR spectroscopic data (Tables 1, 3) were similar to those of pterocarpadiol A (1), and the major difference was that its NMR spectra newly displayed a methoxy group (δ_{H} 3.72; δ_{C} 56.0) instead of the methylenedioxy. And then three aromatic protons at δ_{H} 7.25 (d, J = 8.5 Hz, H-7), 6.55 (dd, J = 8.5, 2.4 Hz, H-8), and 6.29 (d, J = 2.4 Hz, H-10) were assignable to an ABX-type aromatic D-ring. The methoxy group was linked to C-9 on the basis of the HMBC correlations from the proton at δ_{H} 7.25 (d, J = 8.5 Hz, H-7) to the carbons at δ_{C} 78.0 (s, C-6a), 164.2 (s, C-9), and 162.9 (s, C-10a), and from the methoxy protons at δ_{H} 3.72 (s) to the carbon at δ_{C} 164.2 (s, C-9). Therefore, the structure of 2 was established and named as pterocarpadiol B.

Compound 3, white amorphous powder, possessed a molecular formula of C_{16}H_{14}O_{6} according to its positive-ion HRESIMS at m/z 341.0620 [M+Na]+ (calcd for C_{16}H_{14}O_{6}Na, 341.0632), which was 2 m.u. higher than that of 1. Signals of an oxymethylene at δ_{H} 4.49, 4.28 (each 1H, d, J = 10.0 Hz, H-6), an oxymethine at δ_{H} 4.44 (s, H-11a), an olefinic proton at δ_{H} 5.22 (s, H-4), two aromatic singlets at δ_{H} 6.93 (s, H-7), 6.49 (s, H-10) and a methylenedioxy at δ_{H} 5.96, 5.94 (each 1H, s) were observed in the 1H NMR spectrum (Table 2). By comparison of the NMR spectra

![Fig. 2](image-url)  
**Fig. 2** Key HMBC (---) and ROESY (–) correlations of pterocarpadiol A (1)
Table 3 13C NMR spectroscopic data for pterocarpadiols A–D (1–4)

| No. | 1a  | 2a  | 3a  | 4a  | 1b  | 3b  |
|-----|-----|-----|-----|-----|-----|-----|
| 1   | 146.5 d | 146.5 d | 32.7 t | 32.8 t | 144.9 d | 31.2 t |
| 2   | 128.9 d | 128.9 d | 32.8 t | 32.8 t | 127.8 d | 31.8 t |
| 3   | 190.2 s | 190.2 s | 202.1 s | 202.1 s | 187.0 s | 197.7 s |
| 4   | 106.9 d | 106.9 d | 108.9 d | 108.9 d | 105.8 d | 101.6 t |
| 4a  | 172.9 s | 172.9 s | 174.2 s | 174.1 s | 169.8 s | 170.9 s |
| 6   | 70.2 t | 70.4 t | 70.5 t | 70.8 t | 68.7 t | 68.9 t |
| 6a  | 78.7 s | 78.0 s | 78.8 s | 78.1 s | 76.9 s | 76.9 s |
| 6b  | 119.9 s | 120.9 s | 121.0 s | 122.1 s | 119.7 s | 120.6 s |
| 7   | 104.1 d | 125.6 d | 104.1 d | 125.5 d | 103.9 d | 103.8 d |
| 8   | 144.2 s | 109.5 d | 144.2 s | 109.4 d | 142.1 s | 142.0 s |
| 9   | 151.6 s | 164.2 s | 151.4 s | 164.1 s | 149.4 s | 149.1 s |
| 9a  | 93.6 d | 96.4 d | 93.6 d | 96.6 d | 92.7 d | 92.6 d |
| 9b  | 156.5 s | 162.9 s | 155.8 s | 162.2 s | 154.5 s | 153.7 s |
| 10a | 91.4 d | 91.4 d | 91.8 d | 91.8 d | 89.6 d | 89.9 d |
| 11a | 68.8 s | 68.8 s | 69.7 s | 69.7 s | 67.1 s | 67.9 s |
| OCH2O | 103.0 t | 102.9 t | 101.6 t | 101.4 t |
| OCH3 | 56.0 q | 56.0 q |

a Measured in CD3OD (δ, c 49.0 ppm)
b Measured in DMSO-d6 (δ, c 39.5 ppm)

(Tables 2, 3) of 3 and 1, two ortho-coupled doublets and the corresponding olefinic carbons were absent whereas two sp3 carbons at δc 31.2 (t) and δc 31.8 (t) were newly detected, which hinted that 3 should be 1,2-dihydropterocarpidiolo A. The inference was confirmed by the HMBC correlations from the proton at δ6H 2.43 (td, 14.1, 4.5, H-1β) to the carbon at δc 89.9 (d, C-11a) and from the proton at δ6H 2.16 (dd, 16.0, 4.5, 2.8, H-2β) to the carbon at δc 107.5 (d, C-4). The clear ROESY correlations (DMSO-d6) of 6α-OH/11b-OH → H-11a indicated that 3 possessed the same stereochemistry as 1. Thus, the structure of 3 was established and named as pterocarpidiolo C.

Compound 4, white amorphous powder, had a molecular formula of C16H16O6 determined by the positive-ion HRESIMS at m/z 327.0819 [M+Na]+ (calcd for C16H16O6Na, 327.0839). The NMR spectroscopic data (Tables 1, 3) were similar to those of pterocarpidiolo C (3), and the obvious difference was that a methoxy signal (δH 3.74; δc 56.0) replaced the methylenedioxy in 3. According to the HMBC correlations from the proton at δ6H 7.25 (d, J = 8.0 Hz, H-7) to the carbons at δc 78.1 (s, C-6α), 164.1 (s, C-9), and 162.2 (s, C-10a), and from the methoxy protons at δ6H 3.74 (s) to the carbon at δc 164.1 (s, C-9), the methoxy group was positioned at C-9 as with the previous structure. Thus, the structure of 4 was established and named as pterocarpidiolo D.

Pterocarpsans isolated in our current research such as pisatin (7), variabilin (8), and maackiain (11) exhibited without exception negative optical rotation values (−286°, −304°, and −260°, respectively), and their absolute configurations had been established as 6αS,11aR (7), 6αS,11aS (8), and 6αR,11aR (11) [20]. As their related co-constituents, pterocarpsans A–D (1–4) also gave large negative optical rotation values (−484.0°, −397.0°, −507.0° and −476.0°, respectively), thereupon we assumed that the absolute configurations of 1–4 could be assigned as 6αS,11aR,11bS depicted in Fig. 1. Nonetheless, this issue deserved further studies in the future. Until now, only two 6a,11b-dihydroxypterocarpsans: hydroxytuberose [21] and hydroxyristcarpone [18], were reported and only from the family Leguminosae, therefore pterocarpsans A–D as chemical markers in Derris species may be helpful in chemotaxonomical classification.

3 Experimental Section

3.1 General Experimental Procedures

Optical rotations were measured on a Jasco P-1020 automatic digital polarimeter. UV data were obtained from HPLC online analysis. NMR spectra were carried out on a Bruker AV-400, Bruker DRX-500 or Bruker AV-800 spectrometer with deuterated solvent signals used as internal standards. ESI and HRESIMS were performed with a Shimadzu LC-IT-TOF mass spectrometer equipped with an ESI interface (Shimadzu, Kyoto, Japan). Silica gel 200–300 mesh (Qingdao Marine Chemical Inc., Qingdao, China), Chromatorex C-18 (40–75 μm, Fuji Silysia Chemical Ltd., Japan) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for normal pressure column chromatography (CC). Fractions were monitored and analyzed by TLC, in combination with Agilent 1200 series HPLC system equipped by Extend-C18 column (5 μm, 4.6 × 150 mm).

3.2 Plant Material

The twigs and leaves of D. robusta were collected from the Pu’er region of Yunnan Province, China, in May 2011, and identified by Mr. Yu Chen of Kunming Institute of Botany. A voucher specimen (BBP0350021DR) was deposited at BioBioPha Co., Ltd.

3.3 Extraction and Isolation

The air-dried and powdered twigs and leaves (12.0 kg) of D. robusta were extracted with 95 % EtOH at room temperature, and the solvent was removed under reduced pressure to give crude extract (ca. 870 g), which was fractionated by silica gel CC successively eluted with petroleum ether (PE)/acetone gradient and then MeOH to
yield nine fractions A–I. Fraction C (PE/acetone, 6:1) was subjected to silica gel CC (CHCl₃/MeOH, 100:0 → 100:2) and Sephadex LH-20 (CHCl₃/MeOH, 1:1; or MeOH) to give 5 (51 mg), 6 (185 mg), 7 (138 mg), 8 (262 mg), 11 (665 mg), and 12 (608 mg), and the remainder was separated by RP-18 (40 % MeOH/H₂O) and Sephadex LH-20 (MeOH) to yield 9 (661 mg) and 10 (349 mg). The fraction E (PE/acetone, 4:1) was repeatedly applied to silica gel CC (CHCl₃/MeOH, 30:1), Sephadex LH-20 (CHCl₃/MeOH, 1:1) and RP-18 (40 % MeOH/H₂O) to yield nine fractions A–I. Fraction C (PE/acetone, 6:1) was repeatedly isolated on silica gel CC (CHCl₃/MeOH, 10:1), Sephadex LH-20 (MeOH) to yield 13 (23 mg) and 14 (114 mg). The fraction F (PE/acetone, 3:1) was repeatedly isolated on silica gel CC (CHCl₃/MeOH, 10:1), Sephadex LH-20 (MeOH) and RP-18 (45 % MeOH/H₂O) to yield 3 (47 mg) and 4 (28 mg). The retention times (tR) of 1–4 on an analytical HPLC Extend-C18 column (20 % → 100 % MeOH in H₂O over 8.0 min followed by 100 % MeOH to 13.0 min, 1.0 ml/min, 25 °C) were 6.03, 6.16, 6.50, and 6.60 min, respectively.

3.4 Pterocarpadiol A (1)

White amorphous powder; UV (MeOH) λmax: 235, 306 nm; [α]D 23  484.0 (c 0.5, MeOH); 1H NMR data: see Tables 1 and 2; 13C NMR data: see Table 3; ESIMS (pos.): m/z 339 [M+Na]⁺; HRESIMS (pos.): m/z 339.0462 [M+Na]⁺ (calcd for C₁₆H₁₂O₇Na, 339.0475).

3.5 Pterocarpadiol B (2)

White amorphous powder; UV (MeOH) λmax: 230, 285, 305 nm; [α]D 22  397.0 (c 0.2, MeOH); 1H NMR data: see Table 1; 13C NMR data: see Table 3; ESIMS (pos.): m/z 325 [M+Na]⁺; HRESIMS (pos.): m/z 325.0674 [M+Na]⁺ (calcd for C₁₆H₁₂O₇Na, 325.0683).

3.6 Pterocarpadiol C (3)

White amorphous powder, UV (MeOH) λmax: 260, 308 nm; [α]D 23  507.0 (c 0.2, MeOH); 1H NMR data: see Tables 1 and 2; 13C NMR data: see Table 3; ESIMS (pos.): m/z 341 [M+Na]⁺; HRESIMS (pos.): m/z 341.0620 [M+Na]⁺ (calcd for C₁₆H₁₄O₇Na, 341.0632).

3.7 Pterocarpadiol D (4)

White amorphous powder, UV (MeOH) λmax: 232, 261 nm; [α]D 23  476.0 (c 0.2, MeOH); 1H NMR data: see Table 1; 13C NMR data: see Table 3; ESIMS (pos.): m/z 327 [M+Na]⁺; HRESIMS (pos.): m/z 327.0819 [M+Na]⁺ (calcd for C₁₆H₁₆O₆Na, 327.0839).

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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