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Pterocarpans from *Derris laxiflora*

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Six compounds were isolated from *Derris laxiflora* Benth., including two new pterocarpans, 7,6'-dihydroxy-3'-methoxypterocarpan (**1**) and derrispisatin (**2**), as well as four known ones, lespedezol D_1 (**3**), secundiflorol I (**4**), 6a-hydroxymaackiain (**5**) and pisatin (**6**). The structures of these compounds were determined by analysis of their spectroscopic data.

Keywords: *Derris laxiflora*, Leguminosae, Chinese Herb, Pterocarpans.

Derris laxiflora Benth., a member of the Leguminosae, is endemic in Taiwan, where it is distributed at altitudes of under 1000 meters. Its roots and stems were once used as an agricultural pesticide [1]. Flavonoids (3′-methoxylupinifonin, laxifolin, isolaxifolin, laxichalcone, derrichalcone, derriflavanone, and *epi*-derriflavanone) from roots [2a,b], and triterpenoids of oleanane and glutinane-types from whole plants of *D. laxiflora* [2c] have been characterized. In this study, we describe the isolation and structural elucidation of two new pterocarpans, 7,6'-dihydroxy-3'-methoxypterocarpan (**1**) and derrispisatin (**2**), together with four known compounds lespedezol D_1 (**3**) [3], secundiflorol I (**4**) [4a], 6a-hydroxymaackiain (**5**) [4b] and pisatin (**6**) [5] (Figure 1) from *D. laxiflora*.

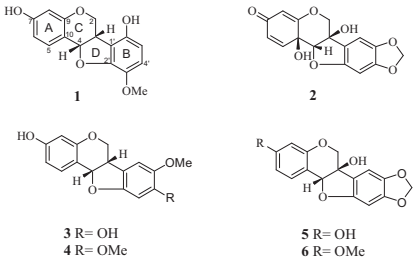

Figure 1: Structures of compounds **1**–**6**.

Compound **1**, a colorless solid, [M^+] at *m/z* 286.0842 ($C_{16}H_{14}O_5$), exhibited absorption bands at 230 and 279 nm in its UV spectrum. The ^{1}H NMR (Table 1) spectrum showed a set of mutually coupled four protons [δ 3.62 (t, *J* = 10.8 Hz), 4.21 (dd, *J* = 10.8, 4.8 Hz), 3.52 (m) and 5.53 (d, *J* = 6.8 Hz)] assignable to H-2, H-3 and H-4 in a pterocarpan skeleton. From the coupling constant values of the proton signals at δ 3.62 and 3.52, and 5.53 and 4.21, they are found to have axial and equatorial configurations, respectively. The coupling pattern and chemical shift data are similar to those of compound **3**. The *cis*-fusion of the C/D ring was discerned from NOESY correlation between H-3 and H-4. The presence of two hydroxyl (δ 5.25, 5.36) and a methoxyl (δ 3.85) group was also exhibited in the ^{1}H NMR spectrum, in addition to two aromatic proton doublets (δ 6.51 & 6.44) and three aromatic protons in an ABX spin system [δ 7.42 (d, *J* = 8.8 Hz), 6.60 (dd, *J* = 8.8, 2.0 Hz), 6.38 (d, *J* = 2.0 Hz)], characteristic of the pterocarpan skeleton. The HMBC spectrum shows that H-4 has correlation with C-2' confirming the type of connection between the D and C-rings. The other doublet aromatic protons [δ 6.44 (d, *J* = 8.4 Hz) and 6.51 (d, *J* = 8.4 Hz) confirmed the *ortho* relationship of the two protons. A comparison of ^{1}H and ^{13}C NMR spectra of **1** and **3** showed that the two compounds differ only in the position of the hydroxyl and methoxyl groups in ring B. NOESY showed a correlation between H-4' (δ 6.51) and OCH_3 (δ 3.85), but no NOESY correlation was observed between H-2 and OCH_3 showing that the OCH_3 was at C-3', adjacent to the H-4' proton at δ 6.51. The HMBC correlations of δ 6.51/C-2' and δ 6.44/C-1' also confirms the presence of the methoxyl group at C-3' and the hydroxyl group at C-6'. From these results, the structure of **1** was determined to be 7,6'-dihydroxy-3'-methoxypterocarpan.

Derrispisatin (**2**), a colorless oil, $C_{16}H_{12}O_7$ (HRMS), showed absorption bands at 233 nm and 304 nm in its UV spectrum. The IR spectrum of **2** showed absorption bands at 1595, 1460, 1162, and 1017 cm^{-1} ascribable to an aromatic ring and ether functionalities. The ^{1}H and ^{13}C NMR (Table 1) spectra of **2** showed signals assignable to two hydroxyl groups [δ 5.39 and 5.79 (1H each, br s, D_2O exchangeable)], a methylenedioxyl group [δ 5.94 (2H, s, -OCH_2O-)], two aromatic protons [δ 6.28 and 6.89 (1H each, both s, H-3', 6')], and three olefinic protons [δ 6.79 (1H, d, *J* = 10.0 Hz, H-5), 6.02 (1H, dd, *J* = 10.0, 1.7 Hz, H-6), and 5.31 (1H, d, *J* = 1.7 Hz, H-8)]. From the UV absorption band at 233 nm, as well as the

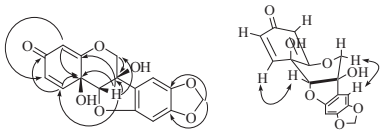

Figure 2: (a) Key HMBC (H→C) and (b) Key NOESY (H ◄► H) correlations of compound **2**.

Table 1: ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of compounds **1** and **2**.

No.	1	1	2	2
2	4.21 dd (10.8,4.8) 3.62 t (10.8)	66.4	4.41 d (10.0) 4.98 d (10.0)	69.5
3	3.52 m	40.2		78.4
4	5.53 d (6.8)	79.3	4.80 s	91.0
5	7.42 d (8.8)	130.5	6.79 d (10.0)	144.4
6	6.60 dd (8.8,2.0)	109.6	6.02 dd (10.0,1.7)	129.1
7		156.9		187.2
8	6.38 d (2.0)	103.4	5.31 d (1.7)	106.9
9		156.3		169.7
10		112.3		68.5
1'		121.5		102.2
2'		147.8		155.9
3'		140.5	6.28 s	93.2
4'	6.51 d (8.4)	114.7		143.4
5'	6.44 d (8.4)	103.7		150.8
6'		145.9	6.89 s	104.2
OC*H*$_3$	3.85 s	56.5		
OC*H*$_2$O			5.94 s	102.6
O*H*	5.25 s[a]		5.39 br. s[a]	
O*H*	5.36 s[a]		5.79 br. s[a]	

[a] D_2O exchangeable

^{13}C NMR signal at δ_c 187.2, it was found that there is a conjugated carbonyl system in **2**. The ^{1}H and ^{13}C NMR spectra of **2** resembled those of **5**. The planar structure of **2** was confirmed by an HMBC experiment, which showed long-range correlations (Figure 2a) between: H_2-2 and C-3, 4, 9, 1'; H-4 and C-2, 3, 5, 9, 10, 1'; H-5 and C-4, 6, 7, 9, 10; H-6 and C-5, 7, 8, 10; H-8 and C-6, 7, 9, 10; -OC*H*$_2$O- and C-4', 5'. The NOESY (Figure 2b) spectrum showed correlations of δ 6.89 (H-6') and 4.41 (H-2); δ 6.79 (H-5) and 4.80 (H-4), showing that H-4 (d 4.80) and H-2α (d 4.41) should have a pseudo-equatorial configuration (Figure 2b). The down field shift of H-2β (δ 4.98) was due to the presence of an OH group at C-10 showing that the C-ring should be in a boat-form configuration (Figure 2b). The C and D rings should be in a *cis*-fused form due to H-2α and H-6' having a NOESY correlation (Figure 2b). On the basis of this evidence, the structure of **2** was elucidated as shown. Compound **2** may be an oxidative product of compound **5**.

Experimental

General: Melting points, Yanagimoto micromelting point apparatus; IR, Perkin-Elmer 983G spectrophotometer; NMR, Varian Unity Plus 400 spectrometer; EIMS, UV, and specific rotations were determined using a JEOL JMS-HX 300, Hitachi S-3200 spectrometer, and JASCO DIP-180 digital polarimeter, respectively. Semi-preparative normal-phase HPLC column (250 x 10 mm, 7 m, LiChrosorb Si 60) on an LDC Analytical-III system.

Plant material: The whole plant of *D. laxiflora* was collected in Taitong County, Taiwan, in December 2001. The plant material was identified by Prof. Shang-Tzen Chang of National Taiwan University, and a voucher specimen was deposited at the herbarium of the School of Forestry and Resource Conservation, National Taiwan University, Taipei, Taiwan.

Extraction and isolation: Air-dried pieces of the whole plant of *D. laxiflora* (11.7 kg) were extracted with MeOH (140 L) twice at room temperature. The extract was evaporated under vacuum and concentrated in a rotary evaporator to a residue (400 g). This was suspended in H_2O and partitioned successively with EtOAc and *n*-BuOH to yield EtOAc (100 g), *n*-BuOH (83 g), and H_2O (217 g) soluble fractions. The EtOAc-soluble fraction was subjected to chromatography using a Geduran Si-60 (Merck, Darmstadt, Germany) column eluted with EtOAc/*n*-hexane (gradient elution by changing from 5/95 to 100/0) to give fractions A (8.7 g), B (10.1 g), C (11.2 g), D (9.3 g), E (8.7 g), F (9.3 g), G (7.5 g), H (4.5 g), and I (2.2 g). The fractions were further purified by repeated HPLC (normal phase on LiChrosorb Si 60), using a *n*-hexane-EtOAc solvent system and 7,6'-dihydroxy-3'-methoxypterocarpan (**1**) (8.2 mg), derrispisatin (**2**) (8.4 mg), lespedezol D_1 (**3**) (18.5 mg), secundiflorol I (**4**) (25.4 mg), 6a-hydroxymaackiain (**5**) (34.2 mg) and pisatin (**6**) (39.5 mg) were eluted from fraction F with 40% EtOAc in *n*-hexane.

7,6'-Dihydroxy-3'-methoxypterocarpan (**1**)
Colorless solid; mp 131-132 °C;
$[\alpha]_D^{25}$: -97.1 (*c* 0.46, CH_3OH).
UV λ_{max}^{MeOH} nm (log ε): 230 (4.05), 279 (3.63).
IR (film) ν_{max}: 3405, 2930, 2854, 1622, 1505, 1470, 1288, 1165, 1083 cm^{-1}.
^{1}H and ^{13}C NMR ($CDCl_3$): Table 1.
EIMS 70 eV, *m/z* (rel. int.): 286 $[M]^+$ (100), 271 $[M–Me]^+$ (29), 59 $[M–227]^+$ (22).
HREIMS *m/z*: 286.0842 (calcd. for $C_{16}H_{14}O_5$, 286.0837).

Derrispisatin (**2**)
Amorphous solid.
$[\alpha]_D^{22}$: +28.3° (*c* 0.33, CH_3OH).
UV λ_{max}^{MeOH} nm (log ε): 233 (4.09), 304 (3.99).
IR (film) ν_{max}: 3271, 1668, 1595, 1460, 1162, 1017 cm^{-1}.
^{1}H and ^{13}C NMR (CD_3COCD_3): Table 1.
EIMS 70 eV, *m/z* (rel. int.): 316 $[M]^+$ (97), 282 $[M–34]^+$ (42), 281 $[M–35]^+$ (46), 191 $[M–C_6H_5O_3]^+$ (100), 174 $[M–142]^+$ (44), 151 $[M–165]^+$ (30), 183 $[M–233]^+$ (24).
HREIMS: *m/z* 316.0575 (calcd. for $C_{16}H_{12}O_7$, 316.0579).

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