SHORT REPORT

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Pterocarpin and Isoflavan Derivatives from *Canavalia maritima* (Aubl.) Thou.

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Abstract: Pterocarpin and isoflavan derivatives were isolated from ethanol extract of *Canavalia maritima* (Aubl.) Thou on column chromatography. By analyzing spectral data, the structures were elucidated as 2-hydroxy-3, 9-dimethoxypterocarpin (**1**), 4-hydroxy-3-methoxy-8,9-methylenedioxypterocarpan (**2**), medicarpin (**3**), 7-hydroxy-2',4'-dimethoxy isoflavan (**4**), 7-hydroxy-4'-methoxyisofalvone (**5**) 5,7,4'-trihydroxyisoflavone (**6**), 3,7-dihydroxy-6-methoxylflavone (**7**), and quercetin (**8**). This paper firstly reports the compounds of pterocarpan and isoflavan from *C. maritima*, which would help understand the pharmaceutical mechanisms of these bioactive substances for wide medical applications. The ^{13}C-NMR spectral data of Compound **1** was reported for the first time.

Keywords: Pterocarpin; isoflavan; *Canavalia maritima* (Aubl.) Thou.

1. Plant Source

Canavalia maritima (Aubl.) Thou, belonging to Family *Leguminosae*, is widely distributed in southeastern China. *C. maritima* is common in mangrove habitats of coasts in southwestern India as wild legumes. Constituents of the seeds of *Canavalia maritima* were reported[1-3]. We report on the isolation of pterocarpin and isoflavan derivatives (Figure 1) from leaves and stems of *C. maritima* collected in China.

The plant of *C. maritima* was collected from coastal Guangxi, South China in November 2006. The samples were identified by Prof. WEI Fanan from Guangxi Institute of Botany. The voucher specimen is deposited in the Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China.

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2. Previous Studies

Studies on the chemical components of other parts from *Canavalia* are scarce. To my best knowledge, chemical investigation into *C. maritima* collected from China was reported for the first time. We firstly reported the ^{13}C-NMR spectral data of Compound **1**.

3. Present Study

Leaves and stems of *C. maritima* (0.65kg) without seeds were grounded and air-dried. The sample were marinated and extracted with 90% EtOH three times at room temperature for seven days. The total extract was concentrated in vacuum to afford a brown residue (12.0g). The residue was dissolved between water and petroleum ether. The petroleum ether extract (4.8g) was subject to silica gel column chromatography and eluted with petroleum ether-acetone in a gradient of 10:1 to 1:1. The eluents were combined into eight fractions upon TLC detection. Fraction 5 (70 mg) was further subject to silica gel column and eluted by petroleum ether-acetone (5:1) to produce compounds **1** (8.0 mg), **3** (15.0 mg), and **4** (5.6 mg). Fraction 7 (80.0 mg) was subject to silica gel column chromatography and eluted with petroleum ether-acetone (2:1), to obtain compounds **2** (11.0mg) and **6** (12.0 mg). Fraction 8 (85 mg) was separated on Sephadex LH-20 column with chloroform- MeOH (1:1) as the eluent and then further purified by ODS , to yield compounds **5** (18.0 mg), **7** (7.1 mg), and **8** (14.0 mg).

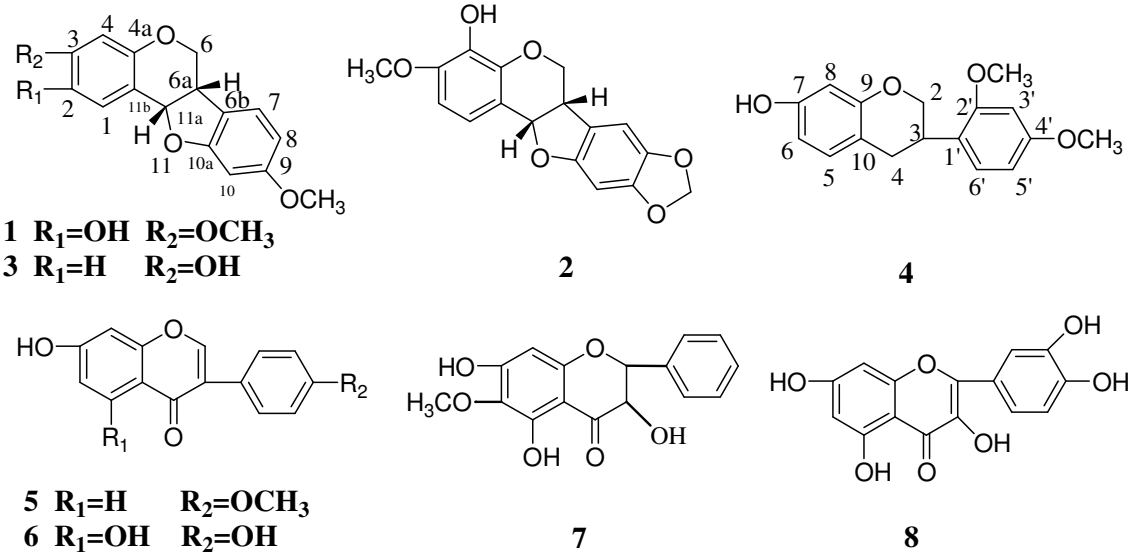

Figure 1: The chemical structures of compounds **1**–**8**

*Compound **1**: 2-hydroxy-4,9-dimethoxypterocarpin*; White amorphous powder, ESI-MS *m/z* 301.11[M+1]$^+$; ^{1}H-NMR (500MHz,CDCl$_3$) δ_H: 7.13 (1H, *d*, *J* = 9.0Hz, H-7), 7.05(1H, *s*, H-1), 6.47 (1H, *s*, H-4), 6.45 (1H, *d*, *J* = 3.0Hz, H-10), 6.44 (1H, *dd*, *J* = 9.0, 3.0Hz, H-8), 5.47 (1H, *d*, *J* = 7.0Hz, H-11a), 5.30 (br *s*, HO-2), 4.22 (1H, *dd*, *J* = 10.5, 5.0Hz, Hα-6), 3.87 (3H, *s*, -OC<u>H</u>$_3$-3), 3.77 (3H, *s*, -OC<u>H</u>$_3$-9), 3.62 (1H, *dd*, *J* = 10.5, 10.5Hz, Hβ-6), 3.55 (1H, *m*, *J* = 10.5, 7.0, 5.0Hz, H-6a). For the ^{13}C-NMR please see Table 1. The spectral data (^{1}H-NMR) are the same as in Yutaka[5].

*Compound **2**: 4-hydroxy-3-methoxy-8,9-methylenedioxypterocarpan*; White amorphous powder, ESI-MS *m/z* 315.09[M+1]$^+$; ^{1}H-NMR (500MHz, CDCl$_3$) δ_H: 7.05 (1H, *d*, *J* = 8.5Hz, H-1), 6.75 (1H, *s*, H-7), 6.69 (1H, *d*, *J* = 8.5Hz, H-2), 6.45 (1H, *s*, H-10), 5.94 (2H, each *d*, *J* = 15.0Hz, -OC<u>H</u>$_2$O-), 5.54 (1H, *d*, *J* = 7.0Hz, H-11a), 5.50 (br *s*, HO-4), 4.36 (1H, *dd*, *J* = 11.0, 5.0Hz, Hα-6), 3.93 (3H, *s*, -

OC<u>H</u>$_3$-3), 3.72 (1H, *dd*, *J* = 11.0, Hβ-6), 3.56 (1H, *ddd*, *J* = 11.0, 7.0, 5.0Hz, H-6a). For the ^{13}C-NMR please see Table 1. The spectral data are the same as in Chaudhuri et al[6].

*Compound **3**: medicarpin;* White amorphous powder, ESI-MS *m/z* 271.11[M+1]$^+$; ^{1}H-NMR (500MHz,$CDCl_3$) δ_H: 7.38(1H, *d*, *J* = 8.5Hz, H-1), 7.12 (1H, *d*, *J* = 9.0Hz, H-7), 6.55 (1H, *dd*, *J* = 8.5, 2.5Hz, H-2), 6.46 (1H, *dd*, *J* = 9.0, 2.5Hz, H-8), 6.45 (1H, *d*, *J* = 3.0Hz, H-10), 6.41 (1H, *d*, *J* = 2.5Hz, H-4), 5.49 (1H, *d*, *J* = 6.5Hz, H-11a), 5.12 (br *s*, HO-3), 4.23 (1H, *dd*, *J* = 11.0, 5.0Hz, Hα-6), 3.77 (3H, *s*, -OC<u>H</u>$_3$-9), 3.62 (1H, *dd*, *J* = 10.5Hz, Hβ-6), 3.54 (1H, *ddd*, *J* = 11.0, 6.5, 5.0Hz, H-6a). For the ^{13}C-NMR please see Table 1. The spectral data are the same as in Matos et al.[7] and Seo et al[8].

*Compound **4**: 7-hydroxy-2',4'-dimethoxyisoflavan*; White amorphous powder, EIMS *m/z* 286.6[M$^+$]; ^{1}H-NMR (500MHz,$CDCl_3$) δ_H: 7.04 (1H, *d*, *J* = 8.0Hz, H-6'), 6.51 (1H, *d*, *J* = 2.5Hz, H-3'), 6.49 (1H, *dd*, *J* = 8.0, 2.5Hz H-5'), 6.96 (1H, *d*, *J* = 8.0Hz, H-5), 6.38 (1H, *dd*, *J* = 8.0, 2.5Hz, H-6), 6.37 (1H, *d*, *J* = 2.5Hz, H-8), 4.80 (br *s*, HO-7), 4.32 (1H, *ddd*, *J* = 10.0, 3.5, 2.0Hz, Hα-2), 4.02 (1H, *dd*, *J* = 10.0Hz, Hβ-2), 3.58 (1H, *m*, H-3), 2.98 (1H, *dd*, *J* = 15.5, 10.0Hz, Hα-4), 2.88 (1H, *m*, *J* = 15.5, 5.0, 2.0Hz, Hβ-4), 3.84 (3H, *s*, OC<u>H</u>$_3$-2'), 3.83 (3H, *s*, OC<u>H</u>$_3$-4'); ^{13}C NMR (125MHz, $CDCl_3$) δ_C: 70.36 (C-2), 31.76 (C-3), 30.60 (C-4), 130.64 (C-5), 108.05 (C-6), 155.47 (C-7), 103.42 (C-8), 155.03 (C-9), 115.13 (C-10), 122.07 (C-1'), 158.12 (C-2'), 98.95 (C-3'), 159.92 (C-4'), 104.34 (C-5'), 127.78 (C-6'), 55.58 (O<u>C</u>H$_3$-2'), 55.61 (O<u>C</u>H$_3$-4') . The spectral data are the same as in Millerg et al[9].

*Compound **5**: 7-hydroxy-4'-methoxyisofalvone*; Colorless needles, mp. 262–264°C, EIMS *m/z* 268.2[M$^+$]; ^{1}H-NMR (500MHz,DMSO) δ_H: 8.25 (1H, *s*, H-2), 7.89 (1H, *d*, *J* = 8.5Hz, H-5), 7.50 (1H, *d*, *J* = 8.5Hz, H-2', 6'), 6.98 (1H, *d*, *J* = 8.5Hz, H-3', 5'), 6.82 (1H, *dd*, *J* = 8.5, 2.5Hz, H-6), 6.72 (1H, *d*, *J* = 2.5Hz, H-8), 3.78 (3H, *s*, -OC<u>H</u>$_3$-4'). The spectral data are the same as in [10,11].

*Compound **6**: 5,7,4'-trihydroxyisoflavone*; White amorphous powder, EIS-MS *m/z* 271.07 [M+1]$^+$; ^{1}H-NMR (500MHz, $CDCl_3$) δ_H: 12.29 (br *s*, HO-5), 8.33 (1H, *s*, H-2), 7.76 (br *s*, HO-7, 4'), 7.38 (2H, *d*, *J* = 8.5Hz, H-2', 6'), 6.85 (2H, *d*, *J* = 8.5Hz, H-3', 5'), 6.43 (1H, *s*, H-6), 6.29 (1H, *s*, H-8), The spectral data are the same as in Mahabusarakama et al[12].

*Compound **7**:3,7-dihydroxy-6-methoxylflavone;* Colorless needles, mp.204-206°C, ESI-MS *m/z* 287.09 [M+1]$^+$; ^{1}H-NMR (500MHz, DMSO) δ_H: 7.53(2H, *dd*, *J* = 8.5, 2.5Hz, H-2',6'), 7.41 (2H, *dd, J* = 8.5, 2.5Hz, H-3',5'), 7.39 (1H, *dd*, *J* = 8.5, 2.5Hz, H-4'), 7.17 (1H, *s*, H-5), 6.41 (1H, *s*, H-8), 5.12 (1H, *d*, *J* = 11.5Hz, H-2), 4.51 (1H, *d*, *J* = 11.5Hz, H-3), 3.78 (3H, *s*, -OC<u>H</u>$_3$-6); ^{13}C NMR (125MHz, DMSO) δ_C: 84.38 (C-2), 73.30 (C-3), 192.93 (C-4), 107.91 (C-5), 144.61 (C-6), 111.41 (C-7), 103.95 (C-8), 157.78 (C-9), 153.87 (C-10), 138.35 (C-1'), 128.83 (C-2'), 128.75 (C-3'), 129.19 (C-4'), 128.75 (C-5'), 128.83 (C-6'), 56.54 (O<u>C</u>H$_3$-6). The spectral data are same to those published[7].

*Compound **8**: quercetin (3, 5, 7, 3', 4'-pentahydroxyflavone)*;Yellow needles, mp.306-307°C, ESI-MS at *m/z*: 303.06[M+1]$^+$, ^{1}H-NMR (500MHz, DMSO) δ_H: 7.66 (1H, *d*, *J* = 2.5Hz, H-2'), 7.54 (1H, *dd*, *J* = 8.5, 2.5Hz, H-6'), 6.89 (1H, *d*, *J* = 8.5Hz, H-5'), 6.62 (1H, *d*, *J* = 2.5Hz, H-8), 6.42 (1H, *d*, *J* = 2.5Hz, H-6).The spectral data (^{1}H-NMR) are same to the data in Sun et al.[13].

Compound **1** was obtained as white amorphous powder, and its molecular formula is $C_{17}H_{16}O_5$ measured with ESI-MS data (*m/z* 301.11[M+1]$^+$). The ^{1}H-NMR spectrum shows a typical pterocarpin skeleton -OCH_2CHCHO- at δ_H: 4.22 (1H, *dd*, *J* = 10.5, 5.0Hz, Hα-6), 3.62 (1H, dd, J=10.5, 10.5Hz, Hβ-6), 3.55 (1H, ddd, J=10.5, 7.0, 5.0Hz, H-6a) and 5.47 (1H, d, J=7.0Hz, H-11a). A series of signals of aromatic protons suggests the presence of a substituted benzene ring, which is flavonoids derivatives.Three signals of aromatic protons at δ_H: 7.13 (1H, d, J=9.0Hz, H-7), 6.44 (1H, dd, J=9.0, 3.0Hz, H-8) and 6.45 (1H, d, J=3.0Hz, H-10) as an ABX type indicate the existence of a 1,2,4-trisubstituted aromatic nucleus. Two signals of aromatic protons at 7.05 (1H, s, H-1) and 6.47 (1H, s, H-4) suggest that compound **1** includes a 1,2,4,5-tetra-substituted benzene ring. The ^{13}C NMR and

DEPT spectra suggest that it contains 17 carbons, including two methyls, one methylene, seven methines, and seven quaternary carbons. The HMQC spectrum reveal two OCH_3 δ_H: 3.87 (3H, s, $OC\underline{H}_3$-3) and 3.77 (3H, s, $OC\underline{H}_3$-9), corresponding to δ_C: 55.98 (q, $O\underline{C}H_3$-3) and 55.51(q, $O\underline{C}H_3$-9), confirming the presence of two methyoxyl groups. Two protons δ_H: 4.22 (1H, dd, J=10.5, 5.0Hz) and 3.62 (1H, dd, J=10.5, 10.5Hz) are corresponded to δ 66.79(t, C-6), which confirms two protons (6-H)of pterocarpin skeleton. The main HMBC spectra (Fig. 2) show three protons δ_H: 4.22, 3.62, and 5.47, corresponding to δ_C: 149.26, reflecting that δ_C:149.26 is from 4a carbon signal; the relations between δ_H: 3.87 (3H, s, $OC\underline{H}_3$-3) and δ_C:147.89 (s, C-3), and between 3.77 (3H, s, $OC\underline{H}_3$-9) and δ_C:161.11 (s, C-9) show two -OCH3 that joined with C-3 and C-9; and the relations between δ_H:5.30 (br s, HO-) and δ_C: 114.98 (d, C-1), and between 140.57 (s, C-2) and 147.89 (s, C-3) indicate that hydroxyl group is at C-2. Therefore, the structure of compound **1** is 2-hydroxy-3,9-dimethoxypterocarpin**.** The spectra data of ^{1}H-NMR are the same to that of Yutaka[5]. We firstly reported the ^{13}C-NMR spectral data of Compound **1**, which was indicated by HMQC and HMBC NMR spectra.

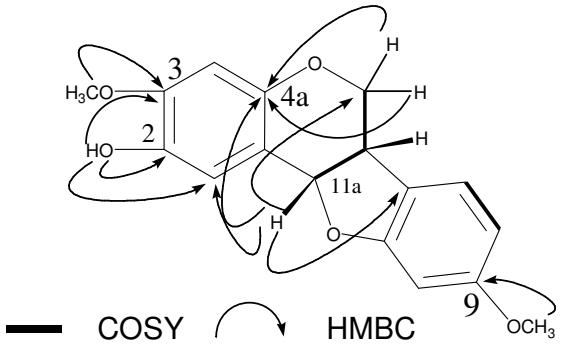

Figure 2. ^{1}H-^{1}H COSY correlations and the selected HMBC correlations of compound **1**

Compounds 2-8 were indentified to the NMR and MS as well as with values from the literature [6-13].

Table 1 ^{13}C-NMR data for Compounds **1-3** (at 125MHz in $CDCl_3$, δ in ppm)

Position (C)	1	2	3
1	114.98 d	120.91 d	132.22 d
2	140.57 s	105.24 d	109.78 d
3	147.89 s	147.25 s	156.68 s
4	100.06 d	133.84 s	103.68 d
4a	149.25 s	143.11 s	157.03 s
6	66.79 t	66.76 t	66.56 t
6a	39.76 d	40.15 d	39.59 d
6b	119.08 s	117.58 s	119.12 s
7	124.69 d	104.67 d	124.78 d
8	106.42 d	141.66 s	106.47 d
9	161.11 s	148.04 s	161.30 s
10	96.85 d	93.73 d	96.93 d
10a	160.11 s	154.29 s	160.66 s
11a	78.59 d	78.27 d	78.54 d
11b	111.89 s	113.82 s	112.64 s
OCH_3-3	55.98 q	56.23 q	-
OCH_3-9	55.51 q	-	55.53 q
OCH_2O	-	101.21 t	-

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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