Prenylated Flavonoids from *Pongamia pinnata*

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Z. Naturforsch. **60b,** 356 – 358 (2005);
received August 23, 2004

Two new prenylated flavonoids (**1, 2**), together with five known compounds, were isolated from the stem bark of *Pongamia pinnata*. Their structures were characterized on the basis of spectral data.

Key words: Pongamia pinnata, Flavonoid

Pongamia pinnata (Leguminosae) is widely distributed along southeastern Asia to the west Pacific and north Australia. All parts of the plant have been used as crude drug for the treatment of tumors, piles, skin diseases, wounds, ulcers, *etc.* [1]. Extracts of the plant possess significant anti-diarrhoeal, anti-fungal, anti-plasmodial, anti-ulcerogenic, anti-inflammatory, analgesic activities [2 – 6]. Previous phytochemical examination of this plant indicated the presence of abounding prenylated flavonoids such as furanoflavones, franoflavonols, chromenoflavones, furanochalcones, and pyranochalcones [7 – 9]. During our ongoing research on this plant, a new prenylated chalcone, 2'-hydroxy-3, 4-methylenedioxy-5'-prenyl-6", 6"-dimethylpyrano-[3', 4' : 2", 3"]- chalcone (**1**) and a new prenylated flavone, 3', 5'-dimethoxy-[2", 3" : 8, 7]-furano flavone (**2**), have been isolated from the stem bark of this plant, along with five known compounds, 2'-hydroxy-6'-methoxy-6", 6"-dimethyl-chromeno-[4', 3' : 2", 3"]-chalcone (3) [10], ovaliflavanone A (4) [11], 6,7,2,2-dimethylchromono-8,γ,γ-dimethylallylflavanone (5) [12] and isolonchocarpin (6) [13], millettocalyxin C (7) [14]. Their structural elucidations were based on the analyses of spectroscopic data.

Results and Discussion

Compound **1**, a yellow oil, exhibited a molecular ion $[M]^+$ peak at *m/z* 418.1777 in the HR-EIMS, indicating a molecular formula of $C_{26}H_{26}O_5$ (calcd. 418.1780). The ^{1}H, ^{13}C NMR of **1**, together with DEPT experiments, showed the presence of a chelated hydroxyl ($\delta_H = 13.6$, 1H, s), a conjugated ketone ($\delta_C = 191.9$, C-9), a double bond with a pair of *trans*-olefinic protons ($\delta_H = 7.77$, d, $J = 15.3$ Hz, H-7; $\delta_C = 143.8$, C-7; $\delta_H = 7.38$, d, $J = 15.3$ Hz, H-8, $\delta_C = 118.8$, C-8), a methylenedioxy group ($\delta_H = 6.03$, 2H, s; $\delta_C = 101.7$), an oxygenated quaternary carbon ($\delta_C = 77.7$, C-6"), an aliphatic methylene ($\delta_H = 3.24$, 2H, d, $J = 7.3$ Hz, H-1"'; $\delta_C = 28.2$, C-1"'), two *ortho*-coupled pyrano protons ($\delta_H = 6.75$, 1H, d, $J = 10.0$ Hz, H-4"; $\delta_H = 5.58$, 1H, d, $J = 10.0$ Hz, H-5"), three phenyl protons with an ABX system ($\delta_H = 7.17$, 1H, d, $J = 1.4$ Hz, H-2; $\delta_H = 6.86$, 1H, d, $J = 8.0$ Hz, H-5; $\delta_H = 7.13$, 1H, dd, $J = 8.0$, 1.4 Hz, H-6) and four tertiary methyls. All these revealed that **1** was a chalcone with two prenyl units, of which one was oxidized to form a conjugated pyrano ring.

In the HMBC spectra of **1**, the protons of two methyls ($\delta_H = 1.76$, s, 6H, H-4"', 5"'; $\delta_C = 25.8$, C-4"'; $\delta_C = 18.0$, C-5"') correlated to two olefinic carbons ($\delta_C = 132.4$, C-3"'; $\delta_C = 122.7$, C-2"'), one olefinic proton ($\delta_H = 5.26$, 1H, t, $J = 7.3$, H-2"') correlated to C-4"', C-5"' and two protons of a methylene ($\delta_H = 3.24$, 2H, d, $J = 7.3$ Hz, H-1"') correlated to C-2"', C-3"' and C-5' indicated the presence of a 2-isopentenyl group attached to the C-5' of the ring A. Moreover, the protons of another two methyls ($\delta_H = 1.46$, 6H, s, H-7", H-8"; $\delta_C = 25.8$, C-7", 8") correlated to C-5", C-6", and H-4", H-5" correlated to C-3' respectively, suggested the presence of a dimethylpyrano unit as shown in Fig. 1. Furthermore, the ^{1}H NMR ABX spin system ($\delta_H = 7.17$, 1H, d, $J = 1.4$ Hz, H-2; $\delta_H = 6.86$, 1H, d, $J = 8.0$ Hz, H-5; $\delta_H = 7.13$, 1H, dd, $J = 8.0$, 1.4 Hz, H-6), together with the HMBC correlations from two protons of methylenedioxy to C-3 and C-4 indicated the placement of the methylenedioxyl at C-3 and C-4. And a chelated hydroxyl signal ($\delta_H = 13.6$, 1H, s) correlated to C-2' disclosed its substituted position. Base on the above spectral evidence, compound 1 was identified as 2'-hydroxy-3,4-methylenedioxy-5'-prenyl-6", 6"-dimethylpyrano-[3', 4' : 2", 3"]-chalcone.

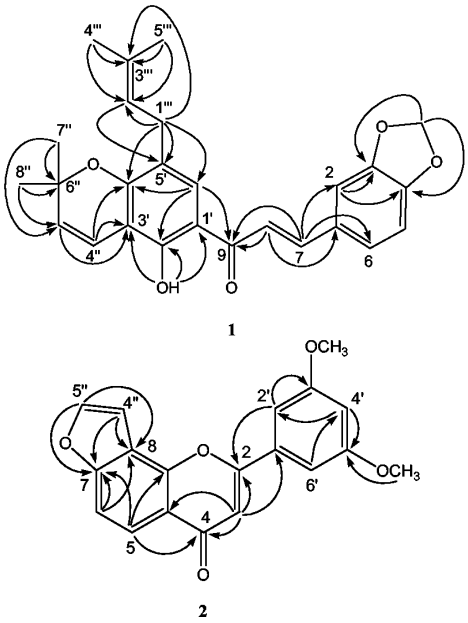

Fig. 1. Structures of compounds **1**, **2** and selected HMBC correlations.

Compound **2**, colorless needles, showed a molecular ion $[M]^+$ at *m/z* 322.0836 in the HR-EIMS, corresponding to the molecular formula $C_{19}H_{14}O_5$ (calcd. 322.0841). The IR band at 1653 cm^{-1} and the UV absorptions at 240 and 303 nm suggested that it was a furanoflavone [14]. Its NMR and EIMS spectral data were almost the same as those of millettocalyxin C, except for the additional signals of a methoxy group. In its EIMS, the fragment ions at *m/z* 160 and 162 resulting from retro-Diels-Alder cleavage of the $[M]^+$ were also observed. This indicated the placement of the furan ring on ring A and two methoxlys on ring B. The 1H NMR spectra showed a pair of *ortho*-coupled aromatic protons ($\delta_H = 8.16$, 1H, d, $J = 8.5$ Hz, H-5; $\delta_H = 7.57$, 1H, d, $J = 8.5$ Hz, H-6), two protons of furan ring ($\delta_H = 7.77$, 1H, d, $J = 2.0$ Hz, H-5"; $\delta_H = 7.20$, 1H, d, $J = 2.0$ Hz, H-4"), one olefinic proton ($\delta_H = 6.87$, 1H, *s*, H-3). With great similarity to those of millettocalyxin C, the above evidence suggested that compound **2** was a furanoflavone with a furan ring fused at C-7 (oxygenated) and C-8. Moreover, the remaining resonance for an A2B spin system including two symmetrical aromatic protons ($\delta_H = 7.08$, 2H, d, $J = 2.2$ Hz, H-2', 6'), *meta*-coupled to another aromatic proton ($\delta_H = 6.64$, 2H, t, $J = 2.2$ Hz, H-4'), and two symmetrical methoxy groups ($\delta_H = 3.90$, 6H, s, 3', 5'-OMe), indicated the presence of 3',5'-disubstituted methoxy groups on the B ring [9]. And this was confirmed by the strong HMBC correlation from H-2',6' to C-2, C-1', C-3',5' and C-4'. Thus, the structure of **2** was identified as 3',5'-dimethoxy-[2",3": 8,7]-furanoflavone.

Experimental Section

Apparatus

The NMR spectra were obtained on a Bruker AVANCE 500 spectrometer (500 MHz for ^1H NMR, 125 MHz for ^{13}C NMR). EI-MS and HR-EIMS spectra were recorded on a Finnigan MAT TSQ 700 mass spectrometer. UV spectra were obtained in a Beckman DU-640 UV spectrophotometer and IR spectra recorded on a Perkin-Elmer FT-IR 1760X spectrophotometer.

Plant material

The material investigated were stem bark of *Pongamia pinnata* collected in October 2002 from Hainan Province, southern China. The material was identified by Prof. Si Zhang, Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute Of Oceanology, Chinese Academy of Sciences. A voucher specimen is deposited at the herbarium of the South China Sea Institute of Oceanology (No. GKLMMM005).

Extraction

The dry powdered stem bark (6 kg) of *Pongamia pinnata* was extracted with 95% EtOH at 80 °C three times. After evaporation of the solvents under reduced pressure, the residue (300 g) was then extracted successively into four extracts: petroleum (80 g), ethyl acetate (60 g), *n*-butanol (60 g), and aqueous (80 g).

Isolation and characterization of **1** *and* **2**

The petroleum extract was subjected to silica gel CC, eluted with a gradient of petroleum-$CHCl_3$ (6:1 to 0:1) to afford 35 fractions. Fractions 21 – 23 were combined (3.3 g) and further separated on CC of silica gel (petroleum-ethyl acetate, 30:1) and Pharmacia-Sephadex LH-20 (MeOH-$CHCl_3$, 5:1) to yield compounds 1 (2 mg), 3 (7 mg), 4 (12 mg), 5 (21 mg), 6 (14 mg). The ethyl acetate extract was subjected to the CC of silica gel eluted with a gradient of $CHCl_3$-MeOH system (99:1 to 0:100) to afford 30 fractions. The fraction 3 (3 g) was further separated on the CC of silica gel with petroleum-ethyl acetate system (8:1) to afford 80 fractions. Then the fraction 36 and 52 recrystallized from acetone to give compounds 2 (30 mg) and 7 (20 mg), respectively.

Compound **1**: UV(MeOH): λ_{max} nm: 285, 356, 375. – MS (EI, 70 eV): *m/z* (%): 418$[M]^+$ (47), 403 (78), 255 (100). –

^{1}H NMR (500 MHz, $CDCl_3$): $\delta_H = 7.17$ (1H, d, $J = 1.4$ Hz, H-2), 6.86 (1H, d, $J = 8.0$ Hz, H-5), 7.13 (1H, dd, $J = 8.0$ Hz, 1.4, H-6), 7.77 (1H, d, $J = 15.3$ Hz, H-7), 7.38 (1H, d, $J = 15.3$ Hz, H-8), 7.49 (1H, s, H-6'), 6.75 (1H, d, $J = 10.0$ Hz, H-4"), 5.58 (1H, d, $J = 10.0$ Hz, H-5"), 1.46 (6H, s, H-7",8"), 3.24 (2H, d, $J = 7.3$ Hz, H-1"'), 5.26 (1H, t, $J = 7.3$ Hz, H-2"'), 1.76 (6H, s, H-4"',5"'), 13.6 (1H, s, OH-2'), 6.03 (2H, s, -OCH2O-). – ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 129.6 (C-1), 106.8 (C-2), 148.5 (C-3), 149.9 (C-4), 108.8 (C-5), 125.3 (C-6), 143.8 (C-7), 118.8 (C-8), 191.9 (C-9), 113.7 (C-1'), 159.6 (C-2'), 109.3 (C-3'), 157.8 (C-4'), 120.8 (C-5'), 129.9 (C-6'), 116.3 (C-4"), 127.9 (C-5"), 77.7 (C-6"), 28.4 (C-7",8"), 28.2 (C-1"'), 122.7 (C-2"'), 132.4 (C-3"'), 25.8 (C-4"'), 18.0 (C-5"').

Compound **2**: UV(MeOH) λ_{max} nm: 240, 303. – IR (KBr): ν = 3058, 2840, 1653, 1606, 1459, 1355, 1207, 1159, 1060, 879, 663 cm^{-1}. – MS (EI, 70 eV): *m/z* (%) = 322 $[M]^+$ (100), 162 (45), 161 (18), 160 (57). – ^{1}H NMR (500 MHz, $CDCl_3$): $\delta_H = 6.87$ (1H, s, H-3), 8.17 (1H, d, $J = 8.8$ Hz, H-5), 7.57 (1H, d, $J = 8.8$ Hz, H-6), 6.64 (1H, t, $J = 2.2$ Hz, H-4'), 7.08 (2H, d, $J = 2.3$ Hz, H-2',6'), 7.20 (1H, d, $J = 2.1$ Hz, H-4"), 7.77 (1H, d, $J = 2.2$ Hz, H-5"), 3.90 (6H, s, OMe-3',5'). – ^{13}C NMR (125 MHz, $CDCl_3$): $\delta_C = 162.5$ (C-2), 108.5 (C-3), 178.2 (C-4), 121.8 (C-5), 110.2 (C-6), 158.4 (C-7), 117.2 (C-8), 150.8 (C-9), 119.5 (C-10), 133.7 (C-1'), 104.6 (C-2',6'), 161.3 (C-3',5'), 103.3 (C-4'), 104.2 (C-4"), 145.8 (C-5"), 55.6 (OMe-3',5').

Acknowledgements

The authors are grateful to the financial support by the Knowledge Innovation Program of Chinese Academy of Sciences (grant: KZCX3-SW-216) and National Key Program for Base Research (grant: 2001CCA04700).

[1] T. Tanaka, M. Iinuma, Y. Fujii, K. Yuki, M. Mizuno, Chem. Pharm. Bull. **39(6)**, 1473 (1991).
[2] S. A. Dahanukar, R. A. Kulkarni, N. N. Rege, Ind. J. Pharmac. **32(4)**, 81 (2000).
[3] F. G. Shoba, M. Thomas, J. Ethnopharmacol. **76**, 73 (2001).
[4] H. T. Simonsen, J. B. Nordskjold, U. W. Smitt, U. Nyman, P. Palpu, P. Joshi, G. Varughese, J. Ethnopharmacol. **74**, 195 (2001).
[5] K. Srinivasan, S. Muruganandan, J. Lan, S. Chandra, S. K. Tandan, V. R. Prakash, J. Ethnopharmacol. **78**, 151 (2001).
[6] S. K. Misra, K. C. Sahu, Ind. J. Pharmac. **9**, 269 (1977).
[7] T. Tanaka, M. Iinuma, Y. Fujii, K. Yuki, M. Mizuno, Phytochemistry **31(3)**, 993 (1992).
[8] E. J. Carcache-Blanco, Y. H. Kang, E. J. Park, B.-N. Su, L. B. S. Kardono, R. Riswan, H. H. S. Fong, J. M. Pezzuto, A.D. Kinghorn, J. Nat. Prod. **66**, 1197 (2003).
[9] P. P. Yadav, G. Ahmad, R. Maurya, Phytochemistry **65(2)**, 439 (2004).
[10] V. P. Pathak, T. R. Saini, R. N. Khanna, Phytochemistry **22(5)**, 1303 (1983).
[11] R. K. Gupta, M. Krishnamurti, Phytochemistry **15(5)**, 832 (1976).
[12] S. Sultana, M. Ilyas, Phytochemistry **25(4)**, 963 (1986).
[13] M. C. D. Nascimento, W. B. Mores, Phytochemistry **11**, 3023 (1972).
[14] B. Stritularak B., L. Likhitwitayawuid, J. Conrad, B. Vogler, S. Reeb, I. Klaiber, W. Kraus, J. Nat. Prod. **65(4)**, 589 (2002).