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TWO ISOFLAVONES FROM THE BARK OF *PETALOSTEMON PURPUREUS*

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Key Word Index—*Petalostemon purpureus*; Leguminosae; bark; 6,7,8,3′,4′,5′-hexamethoxyisoflavone; 7,8,3′,4′,5′-pentamethoxyisoflavone; DNA-nicking assay.

Abstract—Two new isoflavones, 6,7,8,3′,4′,5′-hexamethoxyisoflavone and 7,8,3′,4′,5′-pentamethoxyisoflavone, have been isolated and characterized from the combined root bark and stem bark of *Petalostemon purpureus*.

INTRODUCTION

As part of our continuing search for antitumor agents from plants, we previously examined the flowers of *Petalostemon purpureus* Rydb. (Leguminosae), from which we reported the isolation of a series of pterocarpans. Among these pterocarpan analogues, (+)-3,4-dihydroxy-8,9-methylenedioxypterocarpan was found to be active in a mechanism-based DNA-nicking assay [1,2]. This observation prompted us to investigate the bark of the same plant. A literature survey revealed little scanty information is available on the genus *Petalostemon* [3–5]. A number of isoflavonoids were isolated from *P. candidum* [5]. However, no phytochemical study has been conducted on *P. purpureus* prior to our work. In this communication, we report the isolation and characterization of two new isoflavones (**1** and **2**) from a $CHCl_3$–MeOH (4:1)-soluble extract of the bark of *P. purpureus*. Both compounds were inactive in DNA-nicking assay when evaluated by a modification of the Hecht procedure [2].

RESULTS AND DISCUSSION

The molecular formula of **1** was determined as $C_{21}H_{22}O_8$, on the basis of its high-resolution EI mass spectrum (*m/z* 402.1317 $[M]^+$). The ^1H NMR spectrum of **1** (Table 1) was suggestive of an isoflavone nucleus (H-2, δ 8.07) [4]. A two-proton singlet at δ6.80 was attributable to both H-2′ and H-6′. The chemical shifts of 3′-OCH_3 and 5′-OCH_3 (δ_H 3.90 and δ_C 56.2) confirmed the symmetrical substitution pattern of ring B, which allowed the placement of a third methoxyl group at C-4′ (δ_H 3.88 and δ_C 60.8). The ^1H NMR resonances of **1** at δ3.96 (6-OCH_3), 4.04 (7-OCH_3) and 4.05 (8-OCH_3), and ^{13}C NMR peaks at δ56.2, 61.4 and 62.0 (6-OCH_3, 7-OCH_3 and 8-OCH_3, respectively) indicated that three further methoxyl substituents were positioned in ring A. The chemical shift of H-5 (δ7.45) is consistent with the substitution pattern of the methoxyl groups in ring A [6]. Complete ^1H and ^{13}C NMR spectral assignments of **1** were made with the aid of HMQC and HMBC experiments. Thus, in the HMBC spectrum of **1** each of the methoxyl proton signals showed three-bond long-range correlations with the respective carbon signals of the isoflavone nucleus. In a similar manner, unambiguous ^1H and ^{13}C NMR assignments were made for all carbons and protons of compound **1** (Table 1). The structure of **1** was therefore established as 6,7,8,3′,4′,5′-hexamethoxyisoflavone.

The elemental composition of **2** was deduced as $C_{20}H_{20}O_7$ from its high-resolution EI mass spectrum (*m/z* 372.1209). Comparison of the mass spectrum of **2** with that of **1** revealed that compound **2** has one less methoxyl unit. *Ortho*-coupled proton signals at δ7.08 and 8.04 were assigned to H-6 and H-5, respectively. Inspection of Table 1 indicates that the ^1H and ^{13}C NMR chemical shifts of ring B in **1** and **2** are almost identical, suggesting that this ring is intact in **2**. Comparison of ^1H and ^{13}C NMR chemical shifts of ring A in **2** with those reported for similarly substituted ring A in other isoflavones [7,8] permitted the placement of methoxyl groups at C-7 and C-8. These methoxyl signals occurred at δ_H 4.01 (7-OCH_3) and 4.02 (8-OCH_3), and δ_C 56.5 (7-OCH_3) and 61.7 (8-OCH_3). The structure of **2** was thus assigned as 7,8,3′,4′,5′-pentamethoxyisoflavone. Using a procedure described previously [1] compounds **1** and **2** were found to be inactive.

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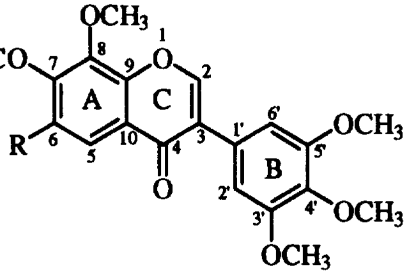
Table 1. ^{1}H and ^{13}C NMR data of **1** and **2** in $CDCl_3$

	1*		**2**†	
Position	δ_H	δ_C	δ_H	δ_C
2	8.07 *s*	152.5	8.04	152.7
3		124.5		124.8
4		175.4		175.9
5	7.45 *s*	100.3	8.04 *d* (9.1)	121.8
6		151.4	7.08 *d* (9.1)	110.2
7		147.7		156.5
8		141.7		136.6
9		145.8		150.5
10		120.3		119.2
1′		127.3		127.3
2′,6″	6.80 *s*	106.3	6.78 *s*	106.3
3′,5′		153.2		153.2
4′		138.2		138.2
6-OCH_3	3.96 *s*	56.2		
7-OCH_3	4.04 s	61.4	4.01 s	56.5
8-OCH_3	4.05 s	62.0	4.02	61.7
3′, 5′-OCH_3	3.90 s	56.2	3.90 s	56.2
4′-OCH_3	3.88 s	60.8	3.88 s	60.9

Coupling constants (in parentheses) in Hz.
*Measured at 500 MHz for ^{1}H and 125 MHz for ^{13}C.
†Measured at 250 MHz for ^{1}H and 62.5 MHz for ^{13}C.



1 R=OCH_3
2 R=H

EXPERIMENTAL

Mps: uncorr. UV: MeOH. IR: KBr disc. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 or 500 MHz instrument with TMS as internal standard. For MS measurements, VGZAB magnetic sector instrument with direct probe insertion was utilized. Chromatographic sepns were carried out on silica gel 60. TLC spots were detected under UV and heating the plates to about 100°. Prep. HPLC was performed with a Waters Model prep-3000 equipped with a Lambda Model 481 LC spectrophotometer and a Waters Data Module. A Dynamax Si column (2.15 × 25 cm) was used for prep. sepn. at a solvent flow rate of 20 ml min^{-1} with effluent detection at 254 nm.

Plant material. The combined root bark and stem bark of *Petalostemon purpureus* were cultivated at Maple, Texas, U.S.A., and harvested in November 1992. A voucher specimen (A1885) representing this collection has been deposited in the John G. Searle Herbarium, Field Museum of Natural History, Chicago, Illinois.

Extraction and isolation. The dried and ground barks (485 g) of *P. purpureus* were extracted with MeOH–$CHCl_3$ (1:1) (2 × 2 l) under reflux. The extract was concentrated *in vacuo* at 40° and partitioned with $CHCl_3$–MeOH (4:1) and H_2O. The organic portion was concd to give a residue which was suspended in MeOH–H_2O (9:1) and defatted with hexane. The 90% MeOH extract (7.34 g) was triturated with MeOH (3 × 100 ml) to yield a MeOH-soluble portion (5.4 g), which was subjected to silica gel CC, using chloroform as an eluting solvent and increasing the polarity by a gradual addition of MeOH. A fraction (0.42 g) containing isoflavones was eluted with $CHCl_3$–MeOH (98:2). A portion of this fraction (0.150 g) was further purified by prep. HPLC using the solvent isooctane–EtOH (95:5) to yield pure 6,7,8,3′,4′,5′-hexamethoxyisoflavone (**1**) (20.4 mg) and 7,8,3′,4′,5′-pentamethoxyisoflavone (**2**) (7.4 mg).

6,7,8,3′,4′,5′-Hexamethoxyisoflavone (**1**). Needles, mp 172°, UV $\lambda_{max}^{CHCl_3}$ nm (log ε) 320 (4.10) and 262 (3.93); IR ν_{max} KBr: 1637, 1600, 1505, 1463, 1426, 1317, 1250, 1183, 1128, 1064 and 997 cm^{-1}; HREIMS $[M]^+$ *m/z* 402.1317, calcd for $C_{21}H_{22}O_8$ $[M]^+$ 402.1314. MS (70 eV) *m/z* (rel. int.): 402 (100), 387 (70), 359 (10), 301 (12) and 186 (21); ^{1}H and ^{13}C NMR data, see Table 1.

7,8,3′,4′,5′-Pentamethoxyisoflavone (**2**). Needles, mp 144°, UV $\lambda_{max}^{CHCl_3}$ 250 nm (log ε 4.63); IR ν_{max} KBr: 1641, 1600, 1567, 1507, 1455, 1430, 1315, 1285, 1250, 1127 and 1098 cm^{-1}; HREIMS $[M]^+$*m/z* 372.1207, calcd for $C_{20}H_{20}O_7$ $[M]^+$ 372.1209. MS *m/z* (rel. int.): 372 (100), 357 (45), 329 (10), 271 (18) and 149 (14); ^{1}H and ^{13}C NMR data, see Table 1.

DNA-nicking assay. Compounds **1** and **2** were evaluated in DNA-nicking assay, an *in vitro* antitumor activity indicator based on mechanism of action [1, 2]. Bleomycin sulfate at 0.1 μg ml^{-1} was used as a positive control, with the concn of the test samples being 25 μg ml^{-1}.

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