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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 antitumour agents from plants, we have 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 has revealed that only 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 the present communication, we wish to report the isolation and characterization of two new isoflavones (**1** and **2**) from a chloroform–methanol (4:1) soluble extract of the bark of *P. purpureus*. Both compounds were inactive in the 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, $\delta$8.07) [4]. A two-proton singlet at $\delta$6.80 was attributable to both H-2′ and H-6′. The chemical shifts of 3′-$OCH_3$ and 5′-$OCH_3$ ($\delta_H$3.90 and $\delta_C$ 56.2) confirmed the symmetrical substitution pattern of ring B, which thus allowed the placement of a third methoxyl group at C-4′ ($\delta_H$ 3.88 and $\delta_C$ 60.8). The $^1H$ NMR resonances of **1** at $\delta$3.96 (6-$OCH_3$), 4.04 (7-$OCH_3$) and 4.05 (8-$OCH_3$), and $^{13}C$ NMR peaks at $\delta$56.2, 61.4 and 62.0 (6-$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 ($\delta$7.45) 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 **1** (Table 1). The structure of **1** was therefore established as 6,7,8,3′,4′,5′-hexamethoxyisoflavone.

The elemental compostion 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 **2** has one less methoxyl unit. *Ortho*-coupled proton signals at $\delta$7.08 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 $\delta_H$ 4.01 (7-$OCH_3$) and 4.02 (8-$OCH_3$), and $\delta_C$ 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.

## EXPERIMENTAL

Mps: uncorr. UV: MeOH. IR: KBr disc. $^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker 300 or 500 MHz instrument with TMS as int. standard.

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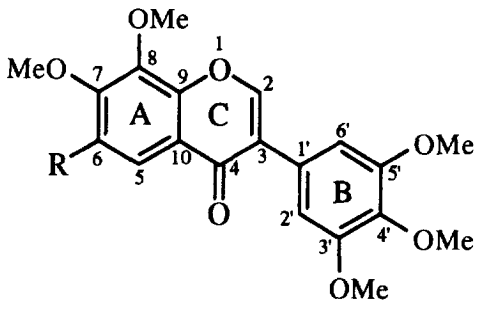


R
1 OMe
2 H

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

| | 1* | | 2† | |
|---|---|---|---|---|
| Position | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 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 | | 147.3 | | 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 | 3.888 *s* | 60.9 |

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

Chromatographic sepns were carried out on Silica gel 60. TLC spots were detected under UV and heating the plates to *ca* 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 *P. 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, U.S.A..

*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 concd *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 $CHCl_3$ as eluent and increasing the polarity by gradual addition of MeOH. A fr. (0.42 g) containing isoflavones was eluted with $CHCl_3$–MeOH (49:1). A portion of this fr. (0.150 g) was further purified by prep. HPLC using isooctane–EtOH (19:1) to afford pure **1** (20.4 mg) and **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), 262 (3.93); IR $\nu_{max}^{KBr}$: 1637, 1600, 1505, 1463, 1426, 1317, 1250, 1183, 1128, 1064, 997 $cm^{-1}$; HREIMS $[M]^+$ *m/z* 402.1317, calc. 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), 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 $\nu_{max}^{KBr}$: 1641, 1600, 1567, 1507, 1455, 1430, 1315, 1285, 1250, 1127, 1098 $cm^{-1}$; HREIMS $[M]^+$ *m/z* 372.1207, calc. for $C_{20}H_{20}O_7$ $[M]^+$ 372.1209. MS *m/z* (rel. int.): 372 (100), 357 (45), 329 (10), 271 (18), 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* antitumour activity indicator based on mechanism of action [1, 2]. Bleomycin sulphate at 0.1 μg $ml^{-1}$ was used as positive control, with the concn of test samples being 25 μg $ml^{-1}$. Using a procedure described previously [1], **1** and **2** were found to be inactive.

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