occurrence and it is significant because the oestrogenic activity of a synthetic sample was found to be one fourth of that of the isomeric 5,7,4′-trihydroxyisoflavone [4].

A comparison of the UV values of the aglucone with those of the glucoside suggested the presence of the sugar moiety at position 7. Methylation of the glucoside with CH_2N_2 for 30 hr and subsequent hydrolysis gave a compound, the UV (λ_{max}^{MeOH}: 255, 315, 330, 370 nm) and the spectral shifts (NaOAc, 260, 355, 365, 375 nm) of which indicated the presence of a free 7-hydroxyl. The glucoside could also be hydrolysed with emulsin indicating the presence of a β-linkage. Therefore the new isoflavone must be 5,7,2′-trihydroxyisoflavone 7-*O*-β-D-glucoside.

The second fraction from the column on repeated crystallization from MeOH–$CHCl_3$ yielded a terpene glycoside, mp 300°(d) which was identified as sitosterol β-D-glucoside by direct comparison with an authentic sample. Column chromatography of the C_6H_6-insoluble portion of the Me_2CO extract of the root bark yielded a major flavonoid, mp 290°. The IR, UV of the compound and PMR of its acetate [6] showed it to be 5,7,4′-trihydroxyisoflavone (genistein). The C_6H_6-soluble portion of the alcoholic extract was chromatographed on Si gel. Using petrol with increasing amounts of C_6H_6 as eluent sitosterol, lupeol, and α- and β-amyrin were isolated and identified by direct comparison with authentic samples.

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PETALOSTETIN, A NEW ISOFLAVONE FROM *PETALOSTEMON CANDIDUM*

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Key Word Index—*Petalostemon candidum*; Leguminosae; petalostetin; 6,7,8-trimethoxy-3′,4′-methylenedioxyisoflavone.

The genus *Petalostemon* has received relatively little attention. *P. gattingeri* has been reported to contain derivatives of 2-(4-hydroxybenzyl)-malic acid [1] and *P. villosum* contains unspecified flavonoid(s) [2]. *Petalostemon* belongs to the Leguminosae, in which isoflavones are common [3]. It was not surprising then, that the ethanol extract of *Petalostemon candidum* provided what we have called petalostetin, $C_{19}H_{16}O_7$, which appeared to be isoflavonoid in nature. Specifically, the IR (1635, 1600 and 1520 cm^{-1}), UV(λ_{max} 264 and 320 nm) and PMR (H-2 at 7.97 ppm; see Table 1) spectra of petalostetin (**1**) immediately suggested [4] that this compound was an isoflavone. Furthermore, the PMR spectrum (Table 1, compound **1**) showed the presence of one methylenedioxy and three methoxy groups. Preparation of dihydropetalostetin (**3**) and examination of its physical data allowed all of the methoxy groups to be placed on ring A and the methylenedioxy group to be located on ring C. That is, the MS of dihydropetalostetin exhibited as its major fragmentation pathway the retro-Diels–Alder process which is typical of such isoflavanones [5].

Table 1 shows a comparison of the PMR spectra of the closely-related isoflavonoids **1**–**7**. Comparison of the spectra of the known **2**, **5** and **7** with that of petalostetin (**1**) and the spectra of the known **4** and **6** with that of dihydropetalostetin (**3**) allowed all substituents to be located precisely. Specifically, the downfield position of one of the aromatic protons of petalostetin (7.42 ppm) and of dihydropetalostetin (7.15 ppm) indicates that this proton must be at C-5 (i.e. adjacent to the carbonyl group).

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Table 1. Assignment of chemical shifts (δ) of protons in the PMR spectra of isoflavonoids

	H-2	H-5	H-2′	H-5′	H-6′	H-3	6-OMe	7-OMe	8-OMe	3′,4′-O_2CH_2
Isoflavones										
1	7.97	7.42	0.07 (*d*,*J* = 2)	6.83 (*d*,*J* = 8)	6.99 (*dd*,*J* = 2,8)	—	4.02	4.02	3.96	5.96
2*	7.97	7.68	*m*	*m*	*m*	—	4.01	4.01	—	6.02
5†	7.93	7.56	7.12	6.85 (*d*,*J* = 8)	6.97	—	3.95	—	—	5.97
7‡	8.00	7.45	—	—	—	—	NA	NA	NA	—
Isoflavanones										
3	4.65 (*d*,*J* = 7)	7.15	6.74	6.74	6.74		4.00	3.90	3.83	5.92
4*	4.58 (*d*,*J* = 7)	7.32	6.75	6.75	6.75	3.78 (*t*,*J* = 7)	3.90	3.86	—	5.90
6§	4.66 (*d*,*J* = 7)	7.25	6.80	6.80	6.80	3.82 (*t*,*J* = 7)	3.87	—	—	5.95

Unless otherwise indicated, all signals are singlets and integrate for the appropriate number of protons. *J* in Hz. NA = not available.

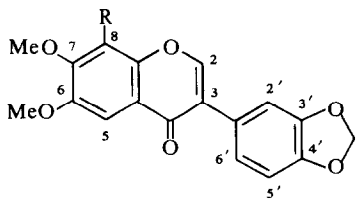
*Campbell, R. V. M., Harper, S. H. and Kemp, A. D. (1969) *J. Chem. Soc.* (*C*) 1787. †Highet, R. J. and Highet, R. F. (1967) *J. Org. Chem.* **32**, 1055. ‡Hayashi, T. and Thomson, R. H. (1974) *Phytochemistry* **13**, 1943. §Ollis, W. D., Rhodes, C. A. and Sutherland, I. O. (1967) *Tetrahedron* **23**, 4741.‖ Obscured by the methoxy signals.

Therefore, the three methoxy groups are located at C-6, C-7 and C-8, as in structure **1**. Furthermore, the methylenedioxy group must be placed C-3′,4′ due to the multiplicities of H-2′, 5′ and 6′ in the PMR spectrum of petalostetin and because of the similarities of the absorptions of these protons in the spectra of **1** and **5** and in the spectra of **3**, **4** and **6**. Therefore, petalostetin is correctly represented by structure **1**.

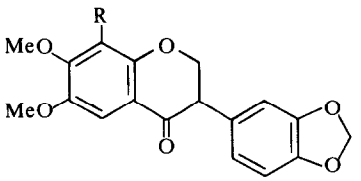
EXPERIMENTAL

Mps are uncorr. The plant was collected near Durango, Colorado in August, 1972, by Dr. E. R. Trumbull. Identification was confirmed by Dr. Charles Mason, Department of Botany, University of Arizona. A reference specimen has been deposited in The University of Arizona Herbarium.

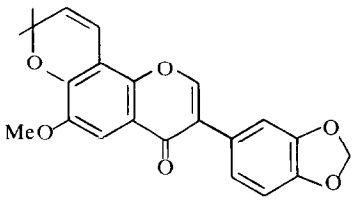
Isolation of petalostetin (**1**). Dry, whole plants of *Petalostemon candidum* were ground in a Wiley mill and exhaustively extracted in a Lloyd-type extractor. Extraction of plant material (16 kg) with petrol (bp 30–60°) gave an oily residue (485 g) after removal of solvent. The marc was then extracted with 95% EtOH and, after evaporation of the solvent, the extract was partitioned between $CHCl_3$ and H_2O (1:1). Upon removal of the solvent in air the $CHCl_3$ phase provided a sticky, green residue (176 g). A portion of the latter (50 g) was triturated with MeOH and filtered. The insoluble material (475 mg) was recrystallized from $CHCl_3$–MeOH to give petalostetin (**1**, 290 mg) as colorless needles, mp 169–170°. The IR ($CHCl_3$: 1635, 1600 and 1520 cm^{-1}), UV [$\lambda_{max}^{CHCl_3}$ 264 (log ε 4.38), 292 (inflection, 415) and 320 nm (3.86)], PMR ($CDCl_3$: see Table 1) and mass [*m/e* 356 (M^+, only significant peak)] spectra were in accord with structure **1**.



1 R = OMe
2 R = H



3 R = OMe
4 R = H



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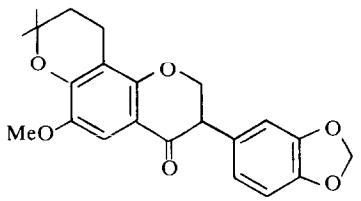
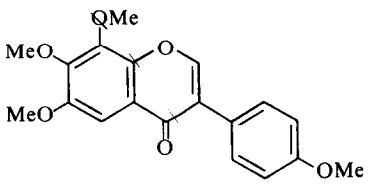


6



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Dihydropetalostetin (**3**). Petalostetin (**1**, 154 mg) was dissolved in HOAc (5 ml) and $CHCl_3$ (1 ml), the catalyst (5% Pd/C, 22 mg) was added and the mixture was subjected to hydrogenation at ambient temp. and atm. pres. for 0.5 hr. The crude product mixture (150 mg), after removal of catalyst and solvent, was separated by PLC in CH_2Cl_2–C_6H_6–EtOAc (3:6:1). The lower R_f spot (84 mg) was unreacted petalostetin and the higher R_f material (25 mg) was dihydropetalostetin (**3**). The latter was recrystallized from Et_2O–EtOH to colorless platelets, mp 119–20°. The IR ($CHCl_3$: 1680 and 1600 cm^{-1}), [UV $CHCl_3$: 238 (log ε 4.19), 280 (4.09) and 337 nm (3.60)], PMR ($CDCl_3$: see Table 1) and mass [*m/e* 358 (M^+, 31%), 210 (100%), 182 (25%), 157 (25%), 148 (55%) and 137 (27%)] spectra were in accord with structure **3**.

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