A New Prenylated Flavonol from the Root of *Petalostemon purpureus*

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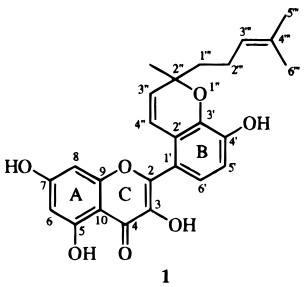
A new prenylated flavonol, petalopurpurenol (**1**), and a known dihydroflavonol, petalostemumol (**2**), have been isolated by DNA scission-guided fractionation of the organic portion of a 20% MeOH/$CHCl_3$/H_2O partition of a 50% MeOH/$CHCl_3$ extract of the roots of *Petalostemon purpureus*. Compound **2** displayed moderate activity in DNA-scission assay. Both compounds **1** and **2** were evaluated for cytotoxicity in a panel of human cancer cell lines. The structures of petalopurpurenol (**1**) and petalostemumol (**2**) were determined by spectroscopic analysis.

The genus *Petalostemon* (Leguminosae) appears to have received little phytochemical investigation. Unidentified flavonoids have been reported to occur in *Petalostemon villosum*.[1] The species *Petalostemon garttingeri* contains the compound 2-(4-hydroxybenzyl)malic acid,[2] and the isoflavone petalostein has been isolated from *Petalostemon candidum*.[3,4] In a recent study, Hufford et al.[5] have isolated a dihydroflavonol, petalostemumol (**2**), from the root of *Petalostemon purpureus*. As part of our continuing search for antitumor agents from plants, we have recently reported the isolation of pterocarpans[6] and isoflavones[7] from the flowers and bark of *P. purpureus*, respectively. The present paper describes the isolation of a new flavonol which we have designated petalopurpurenol (**1**) and the known petalostemumol (**2**) by DNA scission-guided fractionation of a 20% MeOH/$CHCl_3$ extract of the roots *P. purpureus* Rydb. (Leguminosae).



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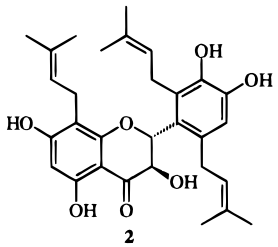

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Compound **1** was obtained as a yellow solid with $[a]^{21}_D$ −12° (c = 1.1, $CHCl_3$). Its molecular formula $C_{25}H_{24}O_7$ was deduced from high-resolution EIMS. The IR spectrum of **1** showed absorptions at 3565 (OH) and 1644 (C=O) cm^{-1}. The UV absorptions at 356 (band II), 255, 250 sh (band I) nm were suggestive of a flavonol skeletal type. The EIMS fragment at m/z 153 $(A_1 + H)^+$, arising from a retro Diels−Alder cleavage of **1** followed by a hydrogen transfer, indicated that two hydroxyl groups were present in ring A. The ^{1}H NMR spectrum of **1** showed two broad singlets at δ 6.20 (H-6) and 6.30 (H-8), which were consistent with a 5,7-dihydroxy substitution pattern in ring A.[8] This observation was confirmed by ^{13}C NMR chemical shift comparison of ring A carbons in **1** (Table 1) with those reported for flavonols with similarly substituted ring A.[9] The ortho-coupled signals at δ 6.80 and 7.01 (J = 8.1 Hz) suggested that the other three positions in ring B were substituted. Closer inspection of the ^{1}H, ^{13}C, and DEPT NMR spectra of **1** revealed the presence of three methyls (δ_H 1.44, 1.57, and 1.64; δ_C 17.9, 26.1 and 26.6 ppm), two methylenes (δ_C 41.8 and 24.0 ppm), one oxygenated quaternary carbon (δ_C 79.7 ppm), and two double bonds (δ_C 122.3, 131.6 and 125.4, 132.6 ppm). In the ^{1}H NMR spectrum

Table 1. ^{13}C-NMR Spectral Data in $CDCl_3$ (125 MHz) for Petalopurpurenol (**1**) and Observed HMBC Correlations

carbon	δC[a] (ppm)	HMBC
C-2	149.4	H-6′
C-3	138.1	
C-4	177.7	
C-5	162.7	H-6
C-6	99.5	H-8
C-7	165.6	H-6, H-8
C-8	94.7	H-6
C-9	158.8	H-8
C-10	105.2	H-6, H-8
C-1′	120.1	H-5′, H-4″
C-2′ (5″)	122.1	H-6′, H-4″
C-3′ (6″)	142.0	H-5′, H-4″
C-4′	148.3	H-5′, H-6′
C-5′	116.3	
C-6′	123.6	
C-2″	79.7	H-3″, H-4″
C-3″	131.6	
C-4″	122.3	H-3″
2″-CH_3	26.6*	
1‴	41.8	
2‴	24.0	
3‴	125.4	
4‴	132.6	
5‴	17.9	
6‴	26.1*	

[a] An asterisk indicates that chemical shifts are interchangeable.

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Table 2. Cytotoxic Activity of **1** and **2**

	cell line treated[a] (ED_{50}, μg/mL)											
compd	BC1	HT	Lu1	Mel2	Col2	KB	KB−V (+ VLB)	KB−V (− VLB)	A431	LNCa P	ZR-75-1	U373
1	9.9	7.3	14.8	17.1	>20	18.9	1.0	6.7	11.9	14.8	4.1	13.3
2	>20	10.3	>20	>20	>20	>20	1.2	>20	>20	>20	17.1	>20

[a] See ref 11 for full details of cell lines.

of **1**, vicinally-coupled protons at δ 5.68 and 6.36 (J = 10.1 Hz) indicated that an oxygen atom at C-3′ (6″) must have cyclized onto C-2″ of one of the prenyl groups (a part of a head−tail-connected geranyl group) to form a pyran ring as shown in the structure of **1**. This arrangement was supported by the observation of a fragment at m/z 353 (M − CH_2CH_2CH═$CH(Me)_2$) in the EIMS of **1**. The ^{13}C NMR signal at δ 148.3 indicated that C-4′ was hydroxylated. The ^1H and ^{13}C NMR assignments of **1** were confirmed by HMBC (Table 1). However, the stereochemistry at C-2″ was not determined. The structure of petalopurpurenol (**1**) was thus assigned as the novel 5,7,4′-trihydroxy-2″-methyl-2″-(4‴-methyl-3‴-pentenyl)pyrano[5″,6″:2′,3′]flavonol.

Petalostemumol (**2**) was isolated as an amorphous yellow solid. As noted earlier by Hufford et al.,[5] **2** showed a set of multiple peaks in its ^1H and ^{13}C NMR spectra. When **2** was measured in $CDCl_3$ or DMSO-d_6 solution, the prenyl groups appeared to rotate to give two rotamers which in turn gave rise to a set of multiple signals in certain regions of the ^1H and ^{13}C NMR spectra. However, in DMSO-d_6 one rotamer was found to be predominant as judged by the intensity of the signals in the ^{13}C NMR spectrum of **2**. A close agreement of the ^{13}C NMR chemical shift values was observed when the more intense ^{13}C NMR signals of **2** in DMSO-d_6 were compared with those reported for petalostemumol.[5]

Compound **2** showed moderate activity in the DNA strand-scission assay, while **1** was inactive. As noted previously in a pterocarpan series,[6] the presence of the catechol unit in ring B of **2** appears to enhance DNA-nicking activity.

Compounds **1** and **2** were evaluated for cytotoxic activity in a number of human cancer cell lines using established protocols.[11] As shown in Table 2, compound **1** exhibited modest cytotoxicity in a number of cell lines, whereas **2** was inactive in most of the cell lines. Both **1** and **2** were cytotoxic in the KB-V (+VLB) cell line and, thus, exhibit modest potential to reverse the multiple drug resistance phenotype.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. NMR experiments were performed on a Bruker AMX 500 spectrometer of a Bruker AMX 250 MHz operating at 500 or 250 MHz for proton and 125 or 62.5 MHz for carbon, respectively. $CDCl_3$ was used as solvent and TMS as internal standard. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. UV and IR spectra were recorded on a Varian 2290 UV−vis spectrometer and a Shimadzu IR-460 spectrometer, respectively. HREIMS was recorded on a VGZAB-E magnetic sector instrument. Column chromatography (CC) was carried out on Si gel 60 (70-230 mesh, Merck, Darmstadt, Germany). Fractions were monitored by TLC (silica 60 F254, 0.25 mm thick) with visualization under UV (254 and 365 nm) and by phosphomolybdate spray, 5% phosphomolybdic acid in EtOH. Preparative TLC was carried on Merck Si gel F254 plates (1 mm thickness).

Plant Material. The roots of *P. purpureus* were collected in July 1992 at Maple, TX. A voucher specimen (A1873) is on deposit in the John G. Searle Herbarium of the Field Museum of Natural History, Chicago, Illinois.

Extraction and Isolation. The ground roots (465 g) were extracted with 50% MeOH/$CHCl_3$ under reflux. The concentrated extract was partitioned between water and 20% MeOH/$CHCl_3$. The organic phase was concentrated, and the residue was suspended in 90% MeOH/H_2O and partitioned with hexane. The aqueous methanol portion (8.6 g) was chromatographed on a Si gel column (500 g), eluting with a $CHCl_3$/MeOH mixture of increasing polarity. Fractions were combined based on their TLC pattern to give 10 pooled fractions, F1−10. Fractions F6 and F8 were found to be active in DNA strand-scission assay. Fraction F6 (0.169 g) was further chromatographed on a Si gel column and finally purified by preparative TLC (toluene:EtOAc 1:1) to afford petalopurpunol (**1**) (22 mg). Fraction F8 (0.24 g) was subjected to a series of purification steps, including LH-20 CC, Si gel CC, and finally preparative TLC (MeOH:$CHCl_3$ 1:9) to obtain pure petalostemumol (**2**) (57 mg).

Petalopurpurenol (1): yellow solid; mp 92−95 °C; $[\alpha]^{21}_D$ −12° (c = 1.1, $CHCl_3$); IR ν max (KBr) 3565, 1644, 1594 cm^{-1}; UV λ max ($CHCl_3$) (log ϵ) 356 (4.01), 255 (4.18), 250 (4.18) nm; ^1H NMR (500 MHz, $CDCl_3$) δ 7.01 (1H, d, J = 8.1 Hz, H-6′), 6.80 (1H, d, J = 8.1 Hz, H-5′), 6.36 (1H, d, J = 10.1 Hz, H-4″), 6.30 (1H, s, H-8), 6.20 (1H, s, H-6), 5.68 (1H, d, J = 10.1 Hz, H-3″), 5.11 (1H, br s, H-3‴), 2.10−2.16 (2H, m, H_2−2‴), 1.68−1.81 (2H, m, H_2−1‴), 1.64, 1.57, 1.44 (each 3H, s, 2″-CH_3, H_3−5‴, H_3−6‴); ^{13}C NMR data see Table 1; EIMS m/z 436 (M^+, 43), 421 (M^+ − CH_3, 11), 367 (M^+ − CH_2CH═CMe_2, 10), 353 (M^+ − $(CH_2)_2CH$═CMe_2, 100), 227 (20), 153 (20), 69 (20); HREIMS m/z $[M]^+$ 436.1524 ($C_{25}H_{24}O_7$ requires 436.1522).

Petalostemumol (2): yellow solid; mp 124−127 °C; $[\alpha]^{21}_D$ +7° (c = 0.42, MeOH); IR ν max ($CHCl_3$) 3530, 3390, 2995, 1596, 1635, 1474, 1439 cm^{-1}; UV, MS, and ^1H and ^{13}C NMR data agree with those published.[5]

DNA Strand-Scission Assay. The DNA strand-scission assay was carried out according to a procedure described previously,[6] which is a modification of the Hecht procedure.[10] Compounds **1** and **2** were tested at a concentration of 25 μg/mL, and the activity was compared with that of a positive control, bleomycin sulfate at 0.1 μg/mL. Compound **2** was found to be equiactive (activity ratio = 1) with bleomycin sulfate at the concentration tested, while **1** was inactive.

Cytotoxicity Assay. Compounds **1** and **2** were evaluated for cytotoxic activity using previously described protocols.[11]

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