

New Flavonoids from Saudi collection of *Tephrosia purpurea* L. (Pers.)

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Abstract: Phytochemical investigation of the chloroform fraction obtained from Saudi collection of *Tephrosia purpurea* L. (Pers.) resulted in the isolation of four new and two known flavonoid derivatives. Three of the new compounds were 5-deoxyflavonoid derivatives identified as tephropurpugazanin (**1**), 4''-hydroxyapollinin (**4**), *epi*-tephroapollin E (**5**) as well as (-)-tephropurpulin A (**2**). The known compounds were identified as 3,7-dihydroxy-8-methoxy-2-(4-methoxyphenyl)-4H-1-benzopyran-4-one (**3**) and tephroapollin E (**6**). Structures were elucidated utilizing different spectroscopic tools including UV, optical rotation, 1D- and 2D-NMR as well as HRESIMS.

Keywords: *Tephrosia purpurea*; isolation; structure elucidation; 5-deoxyflavonoid. © 2021 ACG Publications. All rights reserved.

1. Introduction

The plant *Tephrosia purpurea* Family Fabaceae is traditionally used for the treatment of many ailments including malaria, wounds, gastro-duodenal, kidney, liver and heart disorders [1-2]. Extracts from different parts of *T. purpurea* showed antimicrobial, antidiabetic, antiurolithiatic, anticancer, wound healing and hepatoprotective effects [3-8]. Flavonoid glycoside fraction from *T. purpurea* exhibited insecticidal activity [9]. Prenylated flavones isolated from the aerial parts of the plant showed acetylcholine esterase inhibitory activity and neuroprotective effect [10]. Four prenylated flavones from the stems of *T. purpurea* reported to have antiplasmodial activity against *Plasmodium falciparum* with (*E*)-5-hydroxytephrostachin being the most active [11]. Terpurinflavone showed antiplasmodial activity against both chloroquine- sensitive and resistant *Plasmodium falciparum* [12]. Flavones from the plant stems also proved to possess diuretic effect [13].

Prenylated flavonoids are the most predominant secondary metabolites in the genus and can serve as biomarkers for species that exceed 350 in the genus [14-15]. In the current study we reported on the isolation and characterization of four new prenylated flavonoid derivatives from the entitled plant.

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2. Materials and Methods

2.1. General

Melting points were determined in open capillary tubes using a Thermosystem FP800 Mettler FP80 central processor supplied with an FP81 MBC cell apparatus and are uncorrected. Ultraviolet absorptions spectra were measured on a Unicam Heyios a UV–Visible spectrophotometer. Jasco P-2000 Polarimeter was used to measure the optical rotations and CD spectra were recorded using Jasco J-815 spectrometer (College of Pharmacy, King Saud University). ^1H , ^{13}C -NMR and 2D-NMR data were collected on a Bruker UltraShield Plus 500 MHz spectrometer at the NMR Unite, College of Pharmacy, Prince Sattam Bin Abdulaziz operated at 500 MHz for protons and 125 MHz for carbon atoms, respectively. The instrument is equipped with analytical probe using 1.7X103.5 mm tubes. Chemical shift values were reported in δ (ppm) relative to the residual solvents peaks. Coupling constants (J) were reported in Hertz (Hz). 2D-NMR experiments (COSY, HSQC, HMBC, H2BC, NOESY and/or ROESY) were performed utilizing the standard Bruker program. HRMS were determined by direct injection using Thermo Scientific UPLC RS Ultimate 3000 - Q Exactive hybrid quadrupole-Orbitrap mass spectrometer combines high performance quadrupole precursor selection with high resolution, accurate-mass (HR/AM) Orbitrap™ detection. Direct Infusion of isocratic elution Acetonitrile/Methanol (70:30) with 0.1 % formic acid was used to flush the samples. Runtime was 1 min using nitrogen as auxiliary gas with flow rate 5 $\mu\text{l}/\text{min}$. Scan range from 160- 1500 m/z was used. Resolving Power was adjusted to 70,000 @ m/z 200. Detection was in both positive and negative modes separately. Calibration was done using Thermo Scientific Pierce™ LTQ Velos ESI Positive Ion Calibration Solution including Caffeine, Met-Arg-Phe-Ala (MRFA), Ultramark 1621, n-Butyl-amine components and Pierce™ LTQ Velos ESI. Negative Ion Calibration Solution includes sodium dodecyl sulphate (SDS), sodium taurocholate, Ultramark 1621 components. Capillary temperature set at 320 °C and capillary voltage at 4.2 Kv. Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden), silica gel 60/230-400 mesh (EM Science), RP C-18 silica gel 40-63/230-400 mesh (Fluka) were used for column chromatography. The thin layer chromatography (TLC) analysis was performed on Kiesel gel 60 F254 and RP-18 F254 (Merck) plates. A UV lamp (entela Model UVGL-25) operated at 254 nm was used for detecting spots on the TLC plates.

2.2. Plant Materials

Plants of *Tephrosia purpurea* L. (Pers.) were collected in November, 2019 from Gazan province, Southern Saudi Arabia. The plants were identified by Dr. Mohammad Atiqur Rahman, taxonomist of the MAPPRC, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (# MAPPRC 10548) preserved at the herbarium of the Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC).

2.3. Extraction and Isolation

Air-dried powdered aerial parts (790 g) were repeatedly extracted with 95% ethanol by percolation at room temperature till exhaustion. The solvent was distilled off using rotary vacuum evaporator at 40 °C to give 53 g residue. Part of the obtained dried extract was dissolved in 800 mL of 40% aqueous ethanol and fractionated with light petroleum (500 mL \times 3) to yield 13.45 g petroleum ether soluble fraction, chloroform (500 mL \times 4) to yield 14.12 g of chloroform soluble fraction, ethyl acetate (400 mL \times 2) to yield 23.30 g of ethyl acetate soluble fraction and the left aqueous layer were freeze dried to yield 0.4 gm.

Part of the chloroform soluble fraction (12 g) was fractionated over silica gel column (150 \times 5 cm i.d., 300 g) eluting with chloroform followed by chloroform/methanol mixtures with gradual increase in methanol contents. Fractions of 200 mL each were collected, screened by TLC and similar fractions were pooled. Fractions eluted with chloroform (0.4 g) were purified over RP18 MPLC (45 cm X 1 cm id) eluting with 40% water in methanol with increasing methanol contents in a

gradient system till 100% methanol. Fractions 16-22 (49 mg) were subjected to PTLC using normal silica gel plates and chloroform/methanol (9.5:0.5) developing system to obtain 9 mg of **1**. Fractions 30-45 (65 mg) were subjected to PTLC using normal silica gel plates and chloroform/methanol (9.5:0.5) as mobile phase (double development) to obtain 11 mg of **2**. Fractions 50-55 (35 mg) were subjected to PTLC using normal silica gel plates and chloroform/methanol (9.5:0.5) as mobile phase (double development) to obtain 6 mg of **3**.

Fractions eluted with 5% methanol in chloroform (0.5 g) were purified over silica gel column (45 cm X 1 cm id, 40 gm) eluting with 25% ethyl acetate in petroleum ether with increasing the contents of ethyl acetate in a gradient system. Fractions eluted with 25% ethyl acetate (80 mg) afforded 35 mg of **4** on crystallization from methanol. Fractions eluted with 30 % ethyl acetate (100 mg) were purified over RP18 column (30 cm X 1 cm id, 15 gm) eluting with 35% water in methanol to afford 42 mg of **5** after crystallization from methanol. Fractions eluted with 40% of ethyl acetate (52 mg) were subjected to PTLC using silica gel plates and chloroform/methanol (9:1) to obtain 6 mg of **6**.

2.4. Compounds Characterization

Tephropurpugazanin (**1**): White powder; UV λ_{\max} MeOH: 242, 250, 315 nm; ^1H and ^{13}C NMR see Tables 1 and 2; HRESIMS $[\text{M}+1]^+$ m/z 337.1064 (calcd for $\text{C}_{20}\text{H}_{17}\text{O}_5+\text{H}$, 337.1076), $[\text{M}+\text{Na}]^+$ m/z 359.0882 (calcd for $\text{C}_{20}\text{H}_{17}\text{O}_5+\text{Na}$, 359.0895), $[\text{M}-1]^+$ m/z 335.0935 (calcd for $\text{C}_{20}\text{H}_{17}\text{O}_5-\text{H}$, 335.0919).

(-)-*Tephropurpulin A* (**2**): White powder; m.p 148.7 $^{\circ}\text{C}$; $[\alpha]_{\text{D}}^{25}$ -304; CD $[\theta]_{309} +599$, $[\theta]_{298} 0$, $[\theta]_{271} -3601$, $[\theta]_{246} 0$; UV λ_{\max} MeOH: 251, 285, 321 nm; ^1H and ^{13}C NMR see Tables 1 and 2; HRESIMS $[\text{M}+1]^+$ m/z 411.1436 (calcd for $\text{C}_{23}\text{H}_{22}\text{O}_7+\text{H}$, 411.1444).

4''-*Hydroxyapollinin* (**4**): White powder; m.p 280.4 $^{\circ}\text{C}$; UV λ_{\max} MeOH: 245, 252, 319 nm; ^1H and ^{13}C NMR see Tables 1 and 2; HRESIMS $[\text{M}+1]^+$ m/z 379.1172 (calcd for $\text{C}_{22}\text{H}_{18}\text{O}_6+\text{H}$, 379.1182), $[\text{M}+\text{Na}]^+$ m/z 401.0995 (calcd for $\text{C}_{22}\text{H}_{18}\text{O}_6+\text{Na}$, 401.1001), $[\text{M}-1]^+$ m/z 377.1025 (calcd for $\text{C}_{22}\text{H}_{18}\text{O}_6-\text{H}$, 377.1025), $[\text{M}+1-16]^+$ m/z 363.1220 (calcd for $\text{C}_{22}\text{H}_{18}\text{O}_5+\text{H}$, 363.1232), $[\text{M}+\text{Na}-16]^+$ m/z 385.1038 (calcd for $\text{C}_{22}\text{H}_{18}\text{O}_5+\text{Na}$, 385.1052).

epi-Tephroapollin E (**5**): White powder; m.p 165.7 $^{\circ}\text{C}$; $[\alpha]_{\text{D}}^{25} +94$; CD $[\theta]_{273} +2606$, $[\theta]_{258} +3408$; UV λ_{\max} MeOH: 251, 284, 318 nm; ^1H and ^{13}C NMR see Tables 1 and 2; HRESIMS $[\text{M}+1]^+$ m/z 353.1381 (calcd for $\text{C}_{21}\text{H}_{20}\text{O}_5+\text{H}$, 353.1389), $[\text{M}+\text{Na}]^+$ m/z 375.1198 (calcd for $\text{C}_{21}\text{H}_{20}\text{O}_5+\text{Na}$, 375.1208), $[\text{M}-1]^+$ m/z 351.1220 (calcd for $\text{C}_{21}\text{H}_{20}\text{O}_5-\text{H}$, 351.1232).

Tephroapollin E (**6**): CD $[\theta]_{331} +1201$, $[\theta]_{308} 0$, $[\theta]_{259} -3792$, $[\theta]_{237} 0$

Acetylation of Tephroapollin E (**6**): Two mg of **6** were dissolved in 200 μL of pyridine and 50 μL of acetic anhydride were added. The reaction mixture was kept at room temperature for 24 hr and then left to dry in hood to afford **6a**. ^1H and ^{13}C NMR see Tables 1 and 2.

3. Results and Discussion

3.1. Structure Elucidation

The NMR data (Tables 1, 2) indicated that compounds of **1-6** share 7, 8-disubstituted flavone skeleton. The ^1H -NMR indicated the presence of 5 aromatic overlapped protons in the range $\delta_{\text{H}} 7.27-7.93$ ppm assigned for unsubstituted ring B protons. H-3 proton in the three compounds appears as singlets in the range $\delta_{\text{H}} 6.40-6.74$ ppm. Protons at positions 5 and 6 appears as two *ortho* coupled doublets. H-5 appears at $\delta_{\text{H}} 8.09$ (d, $J=8.6$ Hz), 8.03 (d, $J=8.8$ Hz) and 7.69 (d, $J=8.5$ Hz) ppm, while H-6 appears at $\delta_{\text{H}} 6.88$ (d, $J=8.6$ Hz), 6.86 (d, $J=8.8$ Hz) and 6.84 (d, $J=8.5$ Hz) ppm in **1**, **4** and **5** respectively (Table 1). In the ^{13}C -NMR the chemical shift of C-7 ($\delta_{\text{C}} 165.84$, 163.17 and 166.44 ppm) and C-8 ($\delta_{\text{C}} 112.71$, 107.65 and 115.13 ppm) in **1**, **4** and **5** respectively (Table 2) were diagnostic for C-7 oxygenation and C-8 alkylation.

value of the =CH carbon as part of the 5-membered hetero cyclic ring in tephroglabrin at δ_C 175.8 ppm [17] is more than 20 ppm off the expected value. This structure needs careful revision.

The $C_{21}H_{20}O_5$ molecular formula was obtained for both **5** and **6**. The 1H -NMR and ^{13}C -NMR data of the two compounds were very similar to that reported for tephroapollin E [18].

Compound **2** compared to **5** and **6** bears extra hydroxyl group and one acetyl group. The HRESIMS showed an $[M+1]^+$ at m/z 411.1436 for the molecular formula $C_{23}H_{22}O_7$ were in support of the additional substituents. The disappearance of H-5 proton and presence of H-6 proton as singlet at δ_H 6.14 ppm in addition to the carbon signal at δ_C 163.76 ppm all pointed out to oxygenated C-5. The H-4'' proton appears at δ_H 3.83 (d, $J=8.5$ Hz) in **5** and at δ_H 5.55 (s) in **2** indicating that C-4'' is the site of acetylation. The data of **2** is closely similar to the literature data of tephropurpulin A [19].

Table 1. 1H -NMR data (δ ppm, J in parentheses in Hz)* of compounds **1**, **2**, **4-6a***

Pos	1		2	4	5	6a
	$CDCl_3$	C_6D_6	CD_3OD	$CDCl_3$	$CDCl_3$	$CDCl_3$
3	6.74 (s)	6.73 (s)	6.65 (s)	6.52 (s)	6.40 (s)	6.77 (s)
5	8.09 (d, 8.6)	8.33 (d, 8.8)	-	8.03 (d, 8.8)	7.69 (d, 8.5)	8.11 (d, 8.5)
6	6.88 (d, 8.6)	6.62 (d, 8.8)	6.14 (s)	6.86 (d, 8.8)	6.84 (d, 8.5)	6.87 (d, 8.5)
2',6'	7.89 (m, 2H)	7.68 (d, 7.8, 2H)	7.93 (d, 7.5, 2H)	7.54 (d, 6.9)	7.77 (d, 8.5)	7.95 (d, 6.7, 2H)
3',5'	7.52 (m, 3H)	7.15 m	7.53 (d, 7.3, 2H)	7.27 (m)	7.37 (t, 7.4)	7.55 m
4'	7.08 (t, 7.4, 2H)	7.08 (t, 7.4, 2H)	7.57 (t, 7.3)	-	7.45 (t, 7.4)	-
2''	4.69 (Overl)	4.16 (t, 9)	4.73 (t, 9.6)	-	4.69 (t, 8.4)	4.84 (t, 9.7)
	4.80 (t, 9.3)	4.22 (dd, 4.9, 9.2)	5.10 (q, 5)	-	4.79 (d, 8.7)	5.22 (q, 5.6)
3''	4.16 (Overl)	3.5 (m)	4.25 (q, 5)	-	4.10 (t, 7)	4.43 (q, 5.6)
4''	4.19 (Overl)	3.80 (bt, 10.7)	5.55 (s)	-	3.83 (d, 8.5)	5.74 (s)
	4.69 (Overl)	4.47 (dd, 4.3, 11)	-	-	-	-
Gem	-	-	1.24 (s)	1.45 (s, 6H)	1.41 (s)	1.35 (s)
2 CH ₃	-	-	1.39 (s)	-	1.42 (s)	1.49 (s)
COCH ₃	2.00 (s)	1.52 (s)	1.87 (s)	-	-	1.92 (s)
OCH ₃	-	-	-	3.73 (s)	-	-

*Assignments based on COSY, HSQC, HMBC, H2BC and comparison with literature data for known compounds.

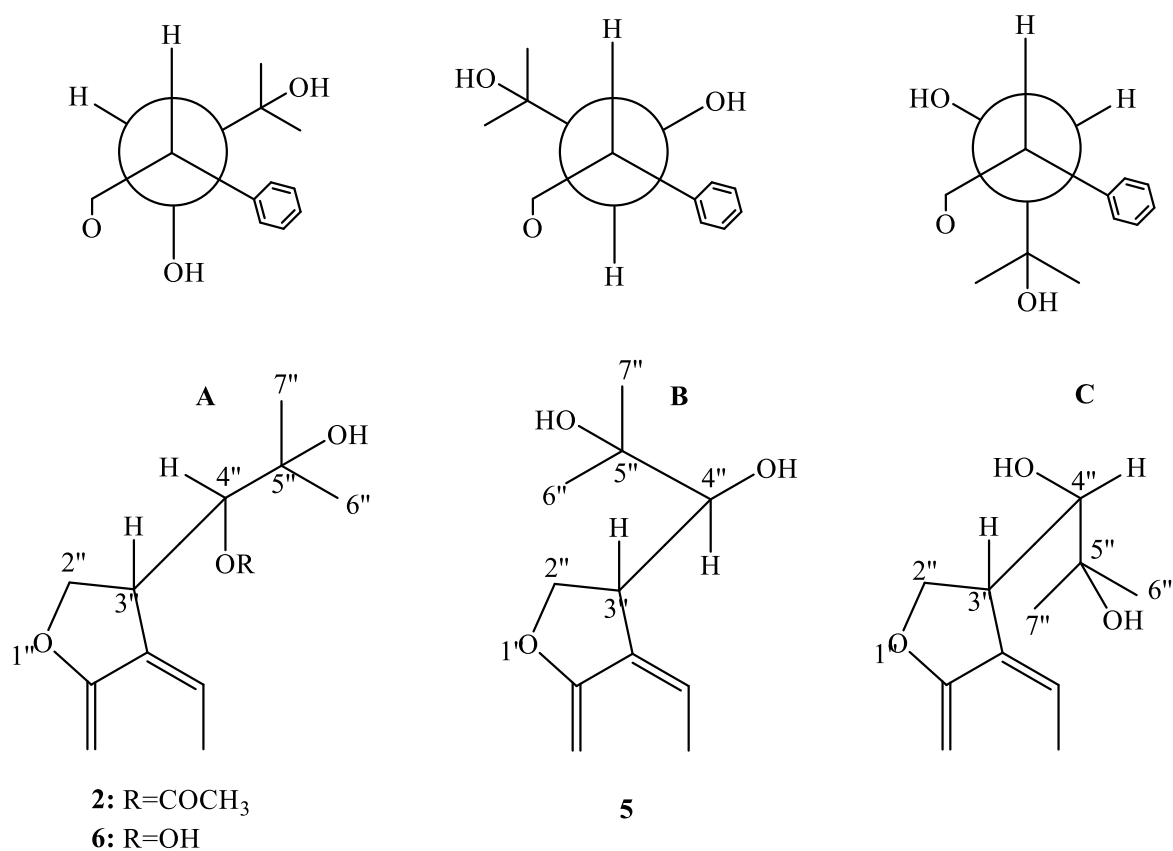
The relative stereochemistry of compounds **2**, **5** and **6** were accessed based of their spectral characters. The single bond between C-3'' and C-4'' allows free rotation and the existence of conformational isomers. The existence of **2**, **5** and **6** as pure rotamer indicating that the energy barrier for the interconversion of one conformer to another is high enough to restrict the free rotation. The possible conformers A-C are shown in figure 2. Conformer C is very unlikely due to the high steric hindrance with the aromatic system. In case of conformer A the dihedral angle between H-3'' and H-4'' is closer to 70° consequently H-4'' appears as singlet in **2** and **6** (3''R, 4''S). The dihedral angle in conformer B is about 168° leading to the appearance of H-4'' as doublet ($J=8.5$ Hz) in **5** (3''R, 4''R). 3D model of conformer A revealed a short distance in space between H-4'' and the two equivalents H-2' and H-6' aromatic protons. This fact was confirmed by the ROESY experiment results, where nOe correlation was observed between these protons (Supporting data). Conformer B model showed that H-4'' is directed in space away from the aromatic protons consequently no nOe correlation were observed. Other than The COSY correlations ROESY data did not provide additional evidences.

Structure similar to **2** was reported under the two names tephropurpulin A and 5-hydroxytephroapollin F [19-20]. Tephroapollin F was reported from *T. apollinea* with negative optical rotation and H-4'' signal as doublet (δ_H 5.08, $J=9$ Hz). Consequently, the name 5-hydroxytephroapollin F is inappropriate as the data of this reported compound showed positive optical rotation and H-4'' proton as singlet at δ_H 5.61 ppm [19]. Tephropurpulin A has very weak positive optical rotation and H-4'' signal appears as broad singlet and might be in fact racemic mixture. As compound **2** showed strong negative optical rotation (-304.4°) and H-4'' signal appears as singlet at δ_H 5.55 ppm it was described as the antipode of tephropurpulin A and given the name (-)-tephropurpulin A reported for the first time from natural source.

Table 2. ^{13}C -NMR data of compounds **1**, **2**, **4-6a***

Pos	1		2	3	4	5	6a
	CDCl_3	C_6D_6	CD_3OD	DMSO	CDCl_3	CDCl_3	CDCl_3
2	162.54	161.53	162.90	151.14	161.70	163.01	162.65
3	107.53	107.78	104.78	133.98	107.28	106.14	107.80
4	177.63	176.20	182.30	175.24	177.73	178.11	177.82
5	128.48	128.53	163.76	121.24	128.08	127.83	128.26
6	109.03	108.49	93.47	115.72	109.36	108.94	108.47
7	165.84	165.43	167.32	155.26	163.17	166.44	165.65
8	112.71	112.68	104.38	135.15	107.65	115.13	118.18
9	154.06	153.89	153.11	153.56	154.84	154.99	154.08
10	118.43	119.03	104.55	117.88	117.98	117.78	113.60
1'	131.67	131.98	130.94	123.41	131.86	131.58	132.27
2',6'	126.13	125.97	126.19	130.57	126.19	126.16	126.21
3',5'	129.19	128.79	128.87	114.09	129.00	128.89	129.05
4'	131.59	130.85	131.88	159.45	131.55	131.36	131.40
2''	76.17	75.62	73.79	-	170.70	77.84	73.65
3''	40.17	40.04	39.77	-	124.16	42.92	40.74
4''	64.39	63.91	77.13	-	160.04	79.08	76.75
5''	-	-	71.44	-	85.02	73.24	70.97
Gem 2	-	-	25.61	-	25.83	25.84	27.25
CH ₃	-	-	26.11	-	(2X)	26.64	27.39
COCH ₃	170.84, 20.84	169.62, 19.90	170.85, 19.06	8-OCH₃ 61.26 4'-OCH₃ 55.60	-	-	169.89 20.41
OCH ₃	-	-	-	-	56.61	-	-

*Assignments based on COSY, HSQC, HMBC, H2BC and comparison with literature data for known compounds.

**Figure 2.** Possible conformers of compounds **2**, **5** and **6**

Compound **5** expressed positive sign in optical rotation (+ 94.2⁰) and H-4'' signal appears as doublet at δ_H 3.83 ppm ($J=$ 8.5 Hz) indicating that the H-3'' and H-4'' have *anti* conformation. The similar structure known as tephroapollin E was reported to have negative optical rotation and H-4'' signal as broad singlet indicating the *syn* orientation of H-3'' and H-4'' [18]. Compound **5** has similar relative configuration to polystachin as they share the positive optical rotation and appearance of H-4'' as doublet [21]. Compound **5** was given the name *epi*-tephroapollin E reported for the first time from natural source.

The data of **6** is identical with those reported for tephroapollin E [18]. Comparison of the ¹³C-NMR data of **5** and **6** revealed a significant difference in the chemical shifts of C-2'' and C-4'' (Supporting data). In **5**, C-2'' and C-4'' chemical shifts were δ_C 77.84, 79.08, while in **6** the corresponding chemical shifts were δ_C 72.90 and 75.99 ppm, respectively. These significant differences in addition to splitting pattern of H-4'' can furnish effective tools for the differentiation between these epimers. CD data of **2**, **5** and **6** revealed similarities between **2** and **6** where negative cotton effects were observed at 271 and 259 nm respectively. On the other hand, **5** showed positive cotton effect at 258 nm. Acetylation of **6** resulted in the formation monoacetyl products **6a** clearly different from tephroapollin F (Supporting data) and could be described as the non-reported *epi*-tephroapollin F [18]. However, it was not isolated naturally from the plant.

Compound **3** was identified by comparison of its data with those reported for 3,7-dihydroxy-8-methoxy-2-(4-methoxyphenyl)-4H-1-benzopyran-4-one isolated from *Lolium multiflorum* ponce et al [22].

Tephrosia species are rich in flavonoid derivatives. Review of the secondary metabolites from 1911- 2014 indicated that 161 flavonoid derivatives were reported from the Genus. Among these thirty-one prenylated flavones including sixteen 5-dexoyflavones were identified [14]. Further investigation of the Genus indicated the presences of prenylated flavonos 5-deoxy derivatives in *T. egregia*, *T. tinctoria*, *T. apollinea* and *T. purpurea* [4, 10, 18, 20, 23, 24]. Due to the restricted distribution of prenylated flavonos 5-deoxy derivative they can be considered as taxonomic markers to the Genus *Tephrosia*.

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Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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