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PRENYLATED FLAVONOIDS FROM *TEPHROSIA APOLLINEA*

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Abstract – Re-investigation of the chloroform extract of the aerial part of *Tephrosia apollinea* afforded seven new 8-prenylated flavonoids namely tephroapollin A-G (1-7). The structures were established by spectroscopic methods, including HR-CI-MS, ^{1}H, ^{13}C, DEPT, HMQC, HMBC and ROESY experiments. The relative configuration analysis of stereocenters for **5-7** was carried out on the basis of the reported *J*-based method. General toxicity of the isolated compounds was determined by brine shrimp lethality bioassay.

INTRODUCTION

The genus *Tephrosia* (Leguminosae; subfamily Papilinoideae; tribe Tephrosieae) includes about 400 species.[1] Extracts of some species have been reported to possess antibacterial and antifungal,[2] insecticidal[3] and antiviral activities.[4] The juice of *Tephrosia villosa* is used to treat dropsy and diabetes.[5] The extract of the whole flowering and fruiting parts of *T. purpurea* Pers. induced quinone reductase (QR) activity on cultured Hepa 1c1c7 (mouse hepatoma) cells.[6,7] Phytochemical investigations on the genus gave prenylated flavonoids as the main constituent. *T. purpurea* have revealed the presence of glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, and flavones,[8] with chemotaxonomic importance in the genus.[9] Investigation of the aerial part of *T. apollinea* (Del.) link, taxon occurs through North-East Africa across to the Indian sub-continent, gave a total of eight complex 7-oxygenated-8-prenylflavones which were identified as (-)-semiglabrin, pseudosemiglabrin, (+)-glabratephrin,

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To the spirit of our late Prof. Dr. Ahmed A. Ahmed

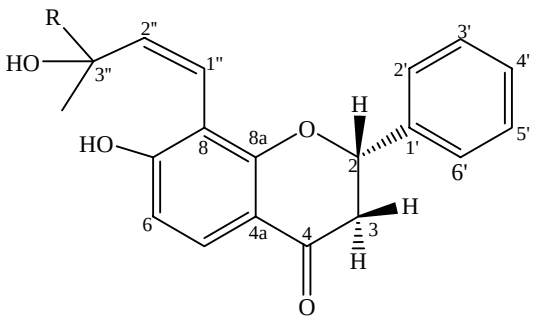
(+)-glabratephrinal, appollinine, lanceolatin-A, pseudosemiglabrinol, and semiglabrinol.[9] Re-investigation of the same species afforded seven new 8-prenylated flavonoids namely tephroapollin A-G (**1**-**7**).

RESULTS AND DISCUSSION

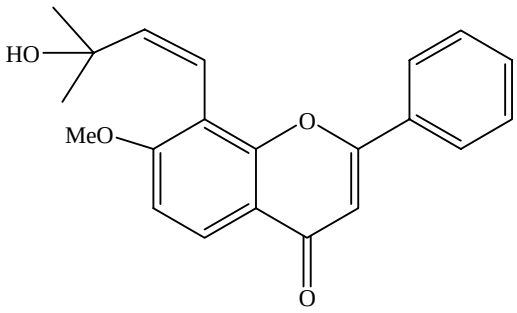
Tephroapollin A (**1**) was obtained as colorless powder, showed in the HRCIMS quasi-molecular ion peak $[M-H]^+$ at *m/z* 323.1294 (calc. 323.1283, $C_{20}H_{19}O_4$), suggesting the molecular formula $C_{20}H_{20}O_4$, which supported by ^{13}C and DEPT NMR analysis and comparison with the reported compound.[10] The presence of a flavanone structure was determined from ^1H-NMR spectrum which exhibited three typical ABX system signals as double doublets at δ_H 5.49 (dd, *J* = 13.5, 3.0 Hz, 1 H, H-2), 3.03 (dd, *J* = 17.0, 13.5 Hz, 1 H, H-3_{axi}), and 2.87 (dd, *J* = 17.0, 3.0 Hz, 1 H, H-3_{eq}). The large coupling constant of H-2 (*J* = 13.5 Hz) was indicative of an axial orientation of this proton. The aromatic protons could be assigned by ^1H-^1H COSY, HMQC and HMBC as following: H-5 at δ_H 7.75 (d, *J* = 8.5 Hz, 1 H), H-6 at δ_H 6.51 (d, *J* = 8.5 Hz, 1 H), H-2',6' at δ_H 7.47 (d, *J* = 8.5 Hz, 2 H), H-3', 5' at δ_H 7.44 (dd, *J* = 8.5, 8.5 Hz, 2 H) and H-4' at δ_H 7.38 (dd, *J* = 8.5, 8.5 Hz, 1 H). The prenyl moiety was identified as 3-methyl-3-hydroxy-1-butenyl on the basis of the ^1H and ^{13}C- NMR analysis: the proton signals at δ_H 6.66 (d, *J* = 10.0 Hz, 1 H, H-1''), 5.58 (d, *J* = 10 Hz, 1 H, H-2''), the two geminal methyl groups appeared at δ_H 1.48 (s, 3H) and 1.54 (s, 3H), and the carbon signals at δ_C 115.9 (d), 128.9 (d), 77.3 (s), 28.1 (q) and 28.4 (q). The coupling constant (*J* = 10.0 Hz) between H-1'' and H-2'' indicated the *cis*-orientation of the double bond. The placement of the prenyl moiety at C-8 was deduced from the HMBC measurements which showed correlation between H-2'' and C-8 at δ_C 109.4. Additional correlations were observed between H-1'' and C-3'' at δ_C 77.3 and C-7 at δ_C 159.7, as well as, between H-3 and C-1' at δ_C 139.0, C-2 at δ_C 79.8 and C-4 at δ_C 190.7, between H-5 and C-4 at δ_C 190.7 and C-8a at δ_C 157.7, also between H-6 and C-8 (δ_C 109.4) and C-4a (δ_C 114.7) and between H-2',6' and C-2 at δ_C 79.8. These results indicated the methyl-3-hydroxy-1-butenyl group was connected to C-8 of ring A. The NOESY spectral data also supported this substitution pattern by showing the correlations of H-1'' to H-2'' and one of the tertiary methyls. Thus, the ^1H and ^{13}C assignments of **1** were made by a combination of COSY, HSQC, HMBC, and NOESY experiments and by with the related synthetic 2,3-dihydro-8-(3-hydroxy-3-methylbut-1-enyl)-7-methoxy-2-phenyl-4H-1-benzopyran-4-one,[10] as it is the correct structure of the natural falciformin was previously reported from *Tephrosia falciformis*.[11] The specific optical rotation of **1** (-65.0°) together with the *trans* diaxial coupling constant of H-2 and H-3 ($J_{2,3ax}$ = 13.5 Hz) as well as the CD suggested the *S*-configuration at C-2 like those of known flavanones.[12] The absolute stereochemistry at C-2 was assumed to be *S* according to a positive CE at 331 (+2.99) nm for $n \rightarrow \pi^*$ absorption band and a negative CE at 286 (-3.67) for $\pi \rightarrow \pi^*$ absorption band in the CD spectrum.[13]

It is interesting to note totally 18 compounds represented prenylated flavanones which have been reported from the genus *Tephrosia* possess only chiral center at C-2 of the γ-pyrone ring have *S*-configuration like (-)-Isolonchocarpin,[8,14] 5-methoxy-8,8-dimethyl-2-phenyl-2,3-dihydro-8H-pyrano[2,3-*f*]chromen-4-one, 5-hydroxy-8,8-dimethyl-2-phenyl-2,3-dihydro-8H-pyrano[2,3-*f*]chromen-4-one,[15] 7-methylglabranin,[16] tephroleocarpin A, tephroleocarpin B, Quercetol C,[16] 8-prenylpinostrobin,[17] Spinoflavanone A,[18] Spinoflavanone B,[18] fulvinervin A,[19] 5,7-dimethoxy-8-(3-methyl-but-2-enyl)-2-phenyl-chroman-4-one,[20] 5-methyl obbovatin,[21] dehydroisoderricin,[22] (-)-dehydroisoderricin,[23] maxima flavanone A,[24] 5-hydroxy-7-methoxy-8-[(E)-3-oxo-1-butenyl]flavanone,[25] and 7-O-methylglabranin.[26] Up to now the 2*R*-configuration has not been reported from the genus *Tephrosia*. Although, 2*R*-flavanones represents a rare example of the preferential generation.

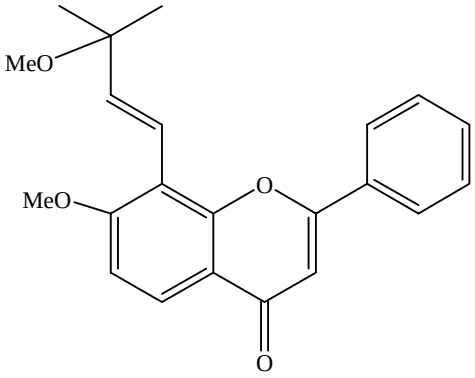
Tephroapollin B (**2**) was isolated as yellowish powder with molecular formula $C_{22}H_{22}O_6$ deduced from HRCIMS: $[M-H]^+$ at *m/z* 381.1345 (calc. 381.1338, $C_{22}H_{21}O_6$) and $[M-OH]^+$ at m/z 365.1387 (calcd. 365.1389, $C_{22}H_{21}O_5$). The ^{1}H- and ^{13}C-NMR data was quite similar to those of **1**, except the difference in the ^{1}H and ^{13}C signals of the prenyl moiety. It showed two sharp singlet signals at δ_H1.46 (3H) and 2.04 (3H), a pair of doublets at δ_H 4.13 (J = 11.5 Hz) and 4.26 (J = 11.5 Hz) correlated in HMQC with a carbon at δ_C 68.5 (C-4''). The olefinic protons H-1'' and H-2'' appeared as two doublets at δ_H 6.81 (J = 10.0 Hz) and 5.53 (J = 10.0 Hz), respectively, suggesting that the prenyl moiety as 3-acetoxymethyl-3-hydroxy-1-butenyl group. Again, the coupling constant (10.0 Hz) between H-1'' and H-2'' indicated the *cis*-orientation of both protons. The position of the prenyl moiety at C-8 was established by HMBC measurements. The main correlations were between H-2'' with C-8 (δ_C 109.6) and C-3'' (δ_C 78.4), between H-1'' with C-7 (δ_C 159.1) and C-3'' (δ_C 78.4), and between H-4'' with C-2'' (δ_C 124.1), C-3'' (δ_C 78.4) and the carbonyl of acetate group (δ_C 170.7). The absolute stereochemistry at C-2 was assumed to be *S* according to a positive CE at 331 (+3.22) nm for n → π* absorption band and a negative CE at 286 (-4.19) for π → π* absorption band in the CD spectrum.[13] The stereochemistry for 3-acetyl-3-hydroxy-1-butenyl group was quite assigned by the aid of NOE experiment. Irradiation of H-2'' (δ_H 5.53) enhancement the proton signal of H-1'' (δ_H 6.81, 18%), the two actylated protons (δ_H 4.13 and 4.26 12%), as well as the tertiary alcoholic methyl (δ_H 1.46, 6%). To be a good results for NOE experiments the tertiary alcoholic methyl should be α-position with the respect of the free sigma bond rotation of C-3''. In addition to comparison with the some synthetic compounds which contain only tertiary alcoholic methyl as chiral center, with the aid of the optical rotaion sign which was negative value C-3'' should possess *S*-cofiguration.[27] However, since this is only a working hypothesis, additional arguments are necessary to determine the stereochemistry of the chiral at C-3''.



1 R = Me
2 R = CH_2OAc



3



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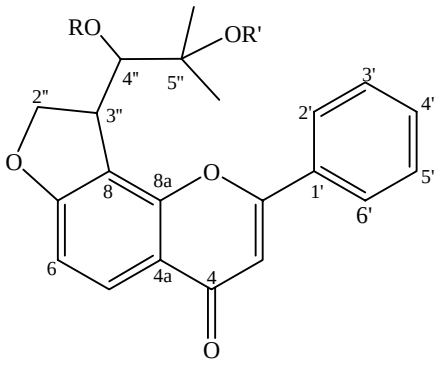

5 R = H, R' = H
6 R = Ac, R' = H
7 R = Ac, R' = Ac

Figure 1. Structures of Compounds (**1**-**7**)

Tephroapollin C (**3**) was obtained as a colorless solid with molecular formula $C_{21}H_{20}O_4$ deduced from HRCIMS ($[M+H]^+$ at *m/z* 337.1437 calcd. 337.1440, $C_{21}H_{21}O_4$). The ^{13}C NMR spectrum of **3** indicated the presence of one carbonyl carbon, six SP^2 quaternary carbons (three of them bearing oxygen atoms), ten SP^2 methines, one SP^3 quaternary carbon (bearing an oxygen atom) and three methyl carbons (one bearing an oxygen atom). The ^1H-NMR spectrum revealed the two olefinic protons are *cis* to each other, doublet at δ_H 6.27 (*J* = 12.5 Hz, H-1'') and a doublet at δ_H 6.15 (*J* = 12.5 Hz, H-2''). It showed also three singlet signals appeared at δ_H 1.26, 4.00 and 6.77 for the two geminal methyls of the prenyl moiety at C-3'', methoxyl group which at C-7 and for H-3, respectively. ^1H-^1H COSY indicated clear correlation between a doublet at δ_H 7.04 (*J* = 8.5 Hz, H-6) with a doublet at δ_H 8.15 (*J* = 8.5 Hz, H-5) and a correlation between a double doublets at δ_H 7.91 (*J* = 8, 2.5 Hz, H-2', 6') with a multiplet signal at δ_H 7.51 (H-3',4',5'). All protonated and quaternary carbons in **3** were assigned with the aid of HMQC and HMBC. Important correlations have been gained from HMBC measurements: H-1'' with C-3'' (δ_C 71.9), H-2'' and H-6 with C-8 (δ_C 115.5), H-3 with C-1' (δ_C 131.5), C-2 (δ_C 162.4), C-4 (δ_C 178.3) and C-4a (δ_C 118.2), H-5 with C-4 (δ_C 178.3), C-7 (δ_C 160.2) and C-8a (δ_C154.2), H-2',6' (δ_H 7.91) with C-4 (δ_C 131.4) and C-2 (δ_C 162.4) and OMe with C-7 (δ_C 160.2).

Table **1**: ^{1}H-NMR of compounds **1-7** (500 MHz, $CDCl_3$, δ-values)

Protons	1	2	3	4	5	6	7
$\mathbf{2_{ax}}$	5.49 dd (13.5, 3)	5.49 dd (13.5, 3)					
$\mathbf{3_{ax}}$	3.03 dd (17, 13.5)	3.03 dd (17, 13.5)	6.77 s	6.79 s	6.38 s	6.71 s	6.76 s
$\mathbf{3_{eq}}$	2.87 dd (17, 3)	2.86 dd (17, 3)					
5	7.75 d (8.5)	7.78 d (8.5)	8.15 d (8.5)	8.15 d (8.5)	7.67 d (8.5)	7.94 d (8.5)	8.10 d (8.5)
6	6.51 d (8.5)	6.54 d (8.5)	7.04 d (8.5)	6.92 d (8.5)	6.74 d (8.5)	6.80 d (8.5)	6.85 d (8.5)
2`,6`	7.47 d (8.5)	7.40 d (8.5)	7.91 dd (8, 2.5)	7.93 dd (8, 2.5)	7.65 dd (8, 2.5)	7.94 dd (8, 2.5)	7.90 dd (8, 2.5)
3`, 5`	7.44 dd (8.5, 8.5)	7.46 dd (8.5, 8.5)	7.51 t (8)	7.54 t (8)	7.44 t (8)	7.45 t (8)	7.44 t (8)
4`	7.38 dd (8.5, 8.5)	7.44 dd (8.5, 8.5)	7.51 t (8)	7.54 t (8)	7.52 t (8)	7.45 t (8)	7.45 t (8)
1``	6.66 d (10)	6.81 d (10)	6.27 d (12.5)	6.92 d (16.5)			
2``	5.58 d (10)	5.53 d (10)	6.15 d (12.5)	6.66 d (16.5)	4.76 t (9), 5.23 dd (9, 2.8)	4.69 dd (9, 8.3), 4.97 dd (9, 2.8)	4.80 t (9), 5.18 dd (9, 6)
3``					4.21 dd (9, 7.5)	4.21 ddd (9, 8.3, 2.8)	4.18 dd (9, 6)
4``		4.13 d (11.5), 4.26 d (11.5)			4.41 br. S	5.08 d (9)	5.78 d (0.6)
gem-Me_2	1.48 s 1.54 s	1.46 s	1.26 s	1.49 s	1.36 s 1.47 s	1.27 s 1.39 s	1.60 s 1.74 s
4``-OAc		2.04 s				1.97 s	2.10 s
5``-OAc							1.90 s
7-OMe			4.00 s	3.97 s			
3`-OMe				3.29 s			

The molecular formula of tephroapollin D (**4**) was determined as $C_{22}H_{22}O_4$ on the basis of HRCIMS and ^{13}C-NMR analysis. The CIMS exhibited an ion peak $[M+H]^+$ at *m/z* 351, followed by fragments at *m/z* 321 $[M\text{-}OCH_3]^+$ and 289 $[M\text{-}2xOCH_3]^+$. The HRCIMS showed a $[M+H]^+$ ion at *m/z* 351.1600 (calcd. 351.1596, $C_{22}H_{23}O_4$). The ^{1}H-NMR spectrum exhibited the aromatic protons with *ortho*-coupling at δ_H 8.15 (d, J = 8.5 Hz, 1H, H-5), 6.92 (d, J = 8.5 Hz, 1 H, H-6), 7.93 (dd, J = 8.0, 2.5 Hz, 2 H, H-2', 6') and 7.54 (t, J = 8.0 Hz, 3 H, H-3', 4', 5'). Additionally, it showed that the prenyl moiety as: sharp singlet signal at δ_H 1.49 (6H) and two doublets at δ_H 6.92 (J = 16.5 Hz, H-1'') and 6.66 (J = 16.5 Hz, H-2''). The coupling constant (J = 16.5 Hz) between H-1'' and H-2'' indicated the *trans*-orientation of both protons. Furthermore, two methoxyl groups appeared at δ_H 3.29 and 3.97. The ^{13}C-NMR spectrum displayed 22 carbon signals and classified by DEPT experiments as: four methyl carbon signals, ten methine carbon signals and eight quaternary carbon

signals. Of them, one carbonyl carbon at δ_C 178.3 (C-4), two olefinic carbons at δ_C 117.9 (C-1'') and 141.6 (C-2''), two methyl carbons at δ_C 26.0 (prenyl) and two methoxyl carbons at δ_C 50.6 and 56.3. All ^{1}H and ^{13}C signals were determined by ^{1}H-^{1}H COSY and HMQC and HMBC. The positions of the methoyxyl groups and the prenyl moiety were determined on the basis of HMBC: H-1'' with C-3'' (δ_C 75.7), C-7 (δ_C 161.3) and C-8a (δ_C 154.6), H-5 with C-4 (δ_C 178.3), C-7 (δ_C 161.3) and C-8a (δ_C 154.6), H-6 with C-8 (δ_C 114.3) and C-4a (δ_C 118.1), OMe (prenyl δ_H 3.29) with C-3'' (δ_C 75.7) and OMe which attached to C-7 (δ_H 3.97) with C-7 (δ_C 161.3) and H-2',6' and C-1' (δ_C 132.2). Comparison of the above data with those of the known compound lanceolatin A[9] resulted that **4** is 3'-methyl ether of lanceolatin A.

Tephroapollin E (**5**) was isolated as amorphous powder, and demonstrated an ion peak in the CIMS at *m/z* 353, followed by ion peak $[M+H-(C_4H_9O_2)]^+$ at *m/z* 265 (100%). The molecular formula was determined to be $C_{21}H_{20}O_5$ by HRCIMS (353.1391, (calc. 353.1389). The ^{1}H-NMR spectrum (Table 1) showed signals at δ_H 1.36 (s, 3 H) and δ_H 1.47 (s, 3 H) due to geminal dimethyl, δ_H 7.67 (d, J = 8.5 Hz, 1 H) and 6.74 (d, J = 8.5 Hz, 1 H) indicating the *ortho*-substitutions on the ring A, where as a sharp singlet at δ_H 6.38 was assigned to H-3, two triplets at δ_H 7.44 (J = 8.0 Hz, 2 H, H-3', 5') and 7.52 (J = 8.0 Hz, 1 H, H-4') and a double doublet at δ_H 7.65 (J = 8.0, 2.5 Hz, 2 H, H-2', 6'), indicating the lack of substitution in the ring B. ^{13}C-NMR and DEPT experiments (Table 2) revealed the presence of 21 carbon signals that included one primary alcoholic carbon at δ_C 72.9, one secondary alcoholic carbon at δ_C 75.3, tertiary alcoholic carbon at δ_C 72.9 and one aliphatic methine carbon at δ_C 42.5, in addition to five aromatic methines at δ_C 109.1, 125.9, 127.7, 129.0, 131.6. All protonated and quaternary carbons were assigned with the aid of HMQC and HMBC. In the HMQC spectrum, the broad singlet at δ_H 4.41 (H-4'') correlated with the carbon signal at δ_C 75.3, the methylene protons at δ_H 5.23 and 4.76 correlated with the carbon signal at δ_C 72.9 and the double doublet at δ_H 4.21 (H-3'') correlated to carbon signal at δ_C 42.5. HMBC allowed the assignment of the ^{1}H and ^{13}C-NMR spectra of **5**. In addition, the presence of a *gem*-dimethyl group at C-5'' in the terminal chain of the dihydrofuran ring was supported by HMBC. Thus, the resonance of the 5''-Me groups at δ_H 1.47 and 1.36 showed cross peaks with signals at δ_C 72.9 (C-5'') and 75.3 (C-4''). Furthermore, H-2'' (δ_H 4.76, 5.23) exhibited correlation with the carbon signals at δ_C 75.3 (C-4''), 166.4 (C-7) and 115.0 (C-8). An additional correlation was observed between H-3'' and the carbon signals at δ_C 72.9 (C-5''), 115.0 (C-8) and 166.4 (C-7), between H-4'' and the carbon signal at δ_C 115.0 (C-8) and between H-3 with the carbon signals at δ_C 162.6 (C-2), 177.6 (C-4), 117.5 (C-4a) and 131.6 (C-1').

The composition of tephroapollin F (**6**) was deduced to be $C_{23}H_{22}O_6$ from CIMS, ^{1}H-NMR and ^{13}C-NMR data. CI mass spectrum gave the molecular ion peak $[M+H]^+$ at *m/z* 395 followed by a peak at *m/z* 377, due to the loss of water molecule from the parent ion peak. HRCIMS gave a quasi-molecular ion peak at *m/z*

395.1494 (calcd. 395.1495). The ^{1}H- and ^{13}C-NMR data indicated that compound **6** was a flavone in which ring B was unsubstituted. Furthermore, the data revealed the presence of one hydroxyl group (IR,

Table **2** : ^{13}C-NMR of compounds **1-7** (125 MHz, $CDCl_3$, δ-values)

	1	**2**	**3**	**4**	**5**	**6**	**7**
2	79.8 (d)	79.9 (d)	162.4 (s)	163.4 (s)	162.6 (s)	162.0 (s)	162.8 (s)
3	44.3 (t)	44.3 (t)	101.7 (d)	107.0 (d)	106.9 (d)	106.7 (d)	107.8 (d)
4	190.7 (s)	190.5 (s)	178.3 (s)	178.3 (s)	177.6 (s)	177.8 (s)	177.6 (s)
4a	114.7 (s)	115.1 (s)	118.2 (s)	118.1 (s)	117.5 (s)	117.8 (s)	118.1 (s)
5	127.9 (d)	128.3 (d)	125.9 (d)	125.5 (d)	127.7 (d)	128.1 (d)	128.5 (d)
6	111.2 (d)	111.0 (d)	109.0 (d)	109.0 (d)	109.1 (d)	109.1 (d)	108.5 (d)
7	159.7 (s)	159.1 (s)	160.2 (s)	161.3 (s)	166.4 (s)	166.6 (s)	165.6 (s)
8	109.4 (s)	109.6 (s)	115.5 (s)	114.3 (s)	115.0 (s)	114.4 (s)	114.0 (s)
8a	157.7 (s)	157.6 (s)	154.2 (s)	154.6 (s)	153.8 (s)	154.2 (s)	154.1 (s)
1`	139.0 (s)	138.0 (s)	131.5 (s)	132.2 (s)	131.6 (s)	131.4 (s)	132.2 (s)
2`,6`	126.0 (d)	126.0 (d)	126.0 (d)	126.4 (d)	125.9 (d)	126.2 (d)	126.2 (d)
3`,5`	128.8 (d)	128.8 (d)	129.0 (d)	129.0 (d)	129.0 (d)	129.0 (d)	129.0 (d)
4`	126.8 (d)	128.7 (d)	131.4 (d)	131.5 (d)	131.6 (d)	131.7 (d)	131.5 (d)
1``	115.9 (d)	118.5 (d)	115.6 (d)	117.9 (d)			
2``	128.9 (d)	124.1 (d)	143.2 (d)	141.6 (d)	72.9 (t)	78.1 (t)	73.3 (t)
3``	77.3 (s)	78.4 (s)	71.9 (s)	75.7 (s)	42.5 (d)	40.7 (d)	40.8 (d)
4``		68.5 (t)			75.3 (d)	79.0 (d)	76.3 (d)
5``					72.9 (s)	72.2 (s)	82.6 (s)
gem-Me_2	28.1 (q) 28.4 (q)	23.7 (q)	29.7 (q)	26.0 (q)	27.7 (q) 25.6 (q)	27.5 (q) 25.2 (q)	23.6 (q) 22.3 (q)
4”-OAc		170.7 (s) 20.8 (q)				169.5 (s) 20.4 (q)	169.5 (s) 22.5 (q)
5”-OAc							169.7 (s) 20.3 (q)
7-OMe			56.3 (q)	56.3 (q)			
3`-OMe				50.6 (q)			

ν_{max}cm^{-1} 3450 cm^{-1}, δ_C 72.2), acetate (IR, ν_{max} cm^{-1} 1780 cm^{-1}, δ_C 169.5 and 20.4, δ_H 1.97), *gem*-dimethyl (IR, ν_{max} cm^{-1}, 1368-1236 cm^{-1}, δ_C 27.5 and 25.2, δ_H1.39 and 1.27), and a flavone carbonyl (IR, ν_{max} cm^{-1} 1690 cm^{-1}, δ_C 177.8). The ^{1}H-^{1}H COSY, HMQC and HMBC supported the proposed structure. ^{1}H-^{1}H COSY showed that the proton at δ_H 4.21(ddd, *J* = 9.0, 8.3, 2.8 Hz, 1 H, H-3''), coupled to the doublet at δ_H 5.08 (d, *J* = 9.0 Hz, 1 H, H-4''), double doublets at δ_H 4.69 (dd, *J* = 9.0, 8.3 Hz, 1 H, H-2a''), and double doublets at δ_H 4.97 (dd, *J* = 9.0, 2.8 Hz, 1 H, H-2b''). HMQC and HMBC established the connectivity of the carbons with protons and the location of quaternary carbons. Also, HMBC allowed the complete assignment, in which correlations were observed for the resonance at δ_H 5.08 (H-4''), with the signals of δ_C 27.5 (Me-5''), 40.7 (C-3''), 72.2 (C-5''), 78.1 (C-2'') and 169.5 (OAc-4''). Additional HMBC correlations were observed for the resonance at δ_H 4.21 (H-3''), with the signals of δ_C 72.2 (C-5''), 79.0 (C-4''), 114.4 (C-8) and 166.6 (C-7), H-5 with the signal of δ_C 154.2 (C-8a), H-6 with the signal of δ_C 117.8 (C-4a), 166.6 (C-7) and 114.4 (C-8) and between H-2',6' with the signal of δ_C 131.4 (C-1') and 162.0 (C-2).

Tephroapollin G (**7**) was isolated as colorless material with molecular formula $C_{25}H_{24}O_7$ deduced from HREIMS: $[M]^+$ at *m/z* 436.1529 (calc. 436.1524, $C_{25}H_{24}O_7$). The ^{1}H- and ^{13}C-NMR of compound **7** were quite similar to those of **6**, except the presence of a new acetyl signal at δ_H 1.90 (δ_C 169.7, 20.3), in addition to the former one which located at δ_H 2.10 (δ_C 169.5, 22.5). All protonated and quaternary carbons were assigned with the aid of HMQC and HMBC. Also, the position of the prenyl moiety was determined on the basis of HMBC: H-5 (δ_H 8.10), H-2'' (δ_H 4.80, 5.18), H-3'' (δ_H 4.18) with (C-7) (δ_C 165.6), H-3'' (δ_H 4.18) and H-5 (δ_H 8.10) with (C-8a) (δ_C 154.1), H-2',6' (δ_H 7.90), H-3 (δ_H 6.76) with (C-2) (δ_C 162.8).

The *J*-based configuration analysis, relying on the extensive use of $^3J_{H,H}$/$^{2,3}J_{C,H}$ couplings in combination with ROE/ NOE data, has been widely applied in the elucidation of relative configurations of natural compounds featuring acyclic chains bearing substituents such as hydroxyl, alkoxy, methyl groups.[28] This approach allows the determination of the predominant rotamer among the six main staggered conformers of each two-carbon fragment in which a molecule with consecutive and alternating stereogenic centers can be ideally divided. The large value of $^{3'',4''}J_{H-H}$ and the pattern of $^{3'',4''}J_{C-H}$ couplings, extracted from HETLOC spectra allowed to rule out all the 3D arrangements with H-3'' and H-4'' in a *gauche* relationship for compounds **5** and **7**, leaving out compound **6** in which H-3'' and H-4'' *anti*-conformer with opposite relative configurations. The ROESY spectrum (Fig. 2) contained a key dipolar coupling that permitted us to confirm unequivocally the correct relative configuration (3''*R*, 4''S) for compounds **5** and **7** (Table 3) and (3''*R*, 4''*R*) for compound **6** (Table 4). The ROESY spectrum showed between H-2'', H-3'' and H-4'' in addition to, irradiation of H-3'' (δ_H 4.21) enhancement the proton signals of H-2'' (δ_H 4.76, 12%) and H-4'' (δ_H 4.41, 6%) in NOE spectrum suggested, with a high confidence that, the configuration of the C-3''-C-4'' was a type of (3''*R*, 4''S) for compounds **5** and **7**. By the analysis of ROESY spectrum (Fig. 3) and the aid

of NOE spectrum which showed that irradiation of H-3'' (δ_H 4.21) enhancement the proton signal of H-2'' (δ_H 4.69, 11%) and a weak enhancement of the proton signal H-4'' (δ_H 5.08, 4%) resulted in the configuration of C-3''-C-4'' was a type of (3''*R*, 4''*R*) for compound **6**.

To the best of our knowledge, Polystachin was the only example for the flavanone possessed 2, 3-dihydofuran ring with the respect of the furan ring derivatives was previously isolated from T. polystachya.[29] Three compounds Pseudosemiglabrin, semiglabrinol, and (-)-semiglabrin,[9] have been reported from *T. apollinea*, *T. purpurea*, and *T.serniglabra*[8,9,30] revealed the teterahydrofurofuran moiety in their flavanone skeleton, which supported that compounds **5-7** resulted from the enzymes cleavage of mehine carbon and the oxygen of the furan ring.

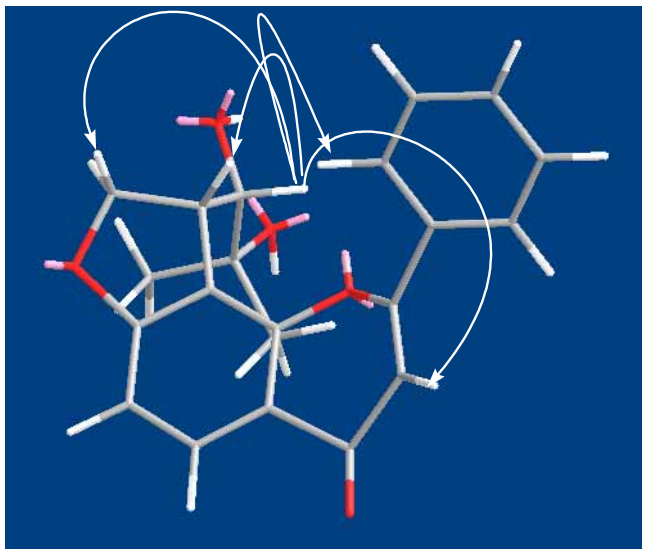

Figure 2. Important ROESY NMR correlations observed for **5**.

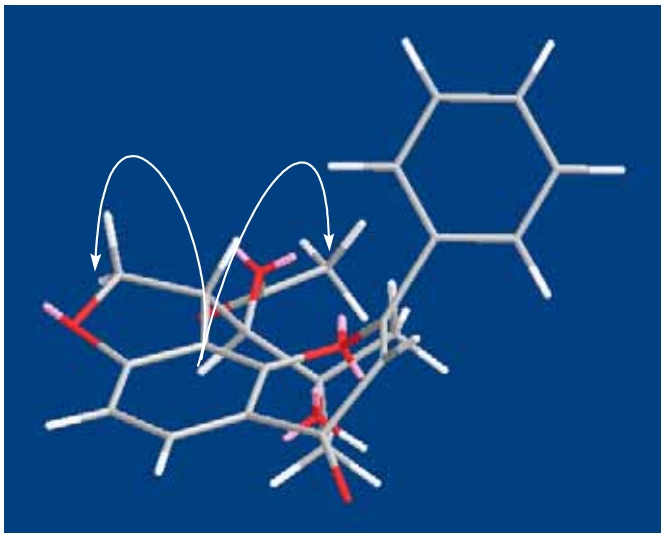

Figure 3. Important ROESY NMR correlations observed for **6**.

The compounds were examined for their cytotoxicity by brine shrimp lethality assay. All compounds possess weak activity (LC_{50} > 100 ppm). The toxicities for the pure compounds (**1-7**) were LC_{50} ppm 160, 222, 288, 299, 188, 215, and 252 respectively.

The present and earlier investigations have resulted in the interesting observation that the constituents of the various *Tephrosia* species differ fairly from one another. In particular *T. apollinea* biosynthesized prenylated falavanones similar to those characterized from the genus *Broussonetia*,[31] *Dorstenia*[32] (Moraceae), *Pseudowintera*[33] (Winteraceae), *Piper*[34] (Piperaceae), and *Macaranga*[35] (Euphorbiaceae), suggesting a chemotaxonmic relationship.

Table 3: $^{3}J_{HH}$, $^{2,3}J_{HC}$ and ROESY data of the C-3''-C-4'' fragment of compound **5**

syn-(3''*R*, 4''S)

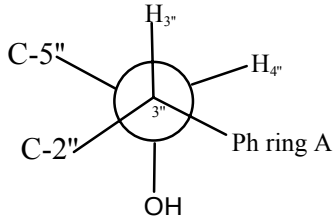

Table 3: $^{3}J_{HH}$, $^{2,3}J_{HC}$ and ROESY data of the C-3''-C-4'' fragment

Coupling constants (Hz)			ROESY
^{3}JH4''Me(5'')	^{3}JH4''H3''	^{3}JH4''C3''	
<1.1	<1.1	<1.1	H4''/H3'' (s)[a] H4''/H2''β (m) H4''/H2'(m) H4''/H2(w)

[a]The intensity of dipolar effects is expressed in terms of three categories s = strong, m = medium, w = weak

Table 4: $^{3}J_{HH}$, $^{2,3}J_{HC}$ and ROESY data of the C-3''-C-4'' fragment of compound **6**

syn-(3''*R*, 4''*R*)

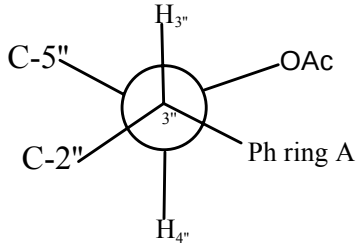

Table 4: $^{3}J_{HH}$, $^{2,3}J_{HC}$ and ROESY data of the C-3''-C-4'' fragment

Coupling constants (Hz)				
^{3}JH4''-3''	^{3}JH4''-Me(5'')	^{2}JH4''-C3''	^{2}JH3''-C3''	ROESY
9.0	2.1	-2.6	-2.2	H4''/H2''$_{\alpha}$ (m)

EXPERIMENTAL

General Experimental Procedures. Optical rotations were determined using a JASCO DIP-360 digital polarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1725×IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded using a JEOL-LA 500 Lambda (500 and 125 MHz respectively) spectrometer. Chemical shifts are given on δ (ppm) scale.^{2,3} $J_{C–H}$ values were obtained from phase-sensitive PFGPS- HMBC spectrum with a total of 32 scans/t_1, acquiring 4 K points in ω_2, and with a t_{1max} value of 4.7 ms. The delay for long-range coupling evolution (Δ) was set at 50 ms. Upon 2D-FT, zero-filling (4 K × 1 K)

was carried out in ω_2 and ω_1. Phase-sensitive PFG-HETLOC spectrum with a total of 64 scans/t_1, 4 K points in ω_2, a spin lock of 50 ms and a t_{1max} value of 43 ms. The data matrices were zero-filled to (4 K × 1 K) affording a digital resolution of 1.2 Hz in ω_2. ROESY spectra were executed with a number of scans/ t_1 ranging from 8 to 32, a t1max values in the range of 15–75 ms and a mixing time of 400 and 600 ms. CI and HR-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on kieslgel 60 (Merck; 230-400 mesh) and Sephadex LH-20 (Pharmacia Co. Tokyo, Japan). The CD spectrum was measured on a JASCO J-810 spectropolarimeter. TLC was performed on silica gel 60 F_{254} plated (0.25 mm, Merck Co.), and spots were detected under UV light and colored by spraying with 10% H_2SO_4 solution followed by heating.

Plant Material. The dried aerial parts of *Tephrosia apollinea* were collected in Spring of 1997, Elba mountain, Aswan, south of Egypt. A voucher specimen has been deposited in the Herbarium of the Department of Botany, Faculty of Science, Aswan, Egypt.

Extraction and isolation. Air-dried pieces of the aerial parts (2 Kg) were extracted successively with $CHCl_3$ at room temperature. After removal of solvent, the residue (83.0 g) was subjected to CC on silica gel and eluted with *n*-hexane, EtOAc, and MeOH in increasing order of polarity. The hexane fractions (11.7 g) were absorbed on silica gel column and eluted with hexane with increasing amounts of EtOAc (9:1). A total of 18 fractions were collected. Fractions 9-18 were combined and recomatographed on Sephadex LH-20 to afforded **1** (31.0 mg) and **2** (43.0 mg). Concentration of EtOAc fraction (29.3 g) was chromatographed on silica gel eluted with $CHCl_3$-MeOH (10:1) gave **3** (25.0 mg) and **4** (32.8 mg). The MeOH fraction (36.4 g) was applied to CC in a similar way, and then followed by Sephadex LH-20 eluted with EtOAc. Purification of the fractions by preparative TLC, $CHCl_3$-MeOH (15:1) provided **5** (23.1 mg), **6** (18.0 mg) and **7** (26.3 mg).

Tephroapollin A (1): colorless powder; $[\alpha]_D^{25}$ -65° (*c* 0.1, $CHCl_3$); CIMS $[M-H]^+$ *m/z* 323, 307, 291,279, 203; HRCIMS: $[M-H]^+$ at *m/z* 323.1294 (calcd. 323.1283, $C_{20}H_{19}O_4$); ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

Tephroapollin B (2): yellowish powder; $[\alpha]_D^{25}$ -57° (*c* 0.1, $CHCl_3$); CIMS $[M-H]^+$ *m/z* 381, 365, 351, 333, 321, 305, 291, 261, 214, 187; HRCIMS: $[M-H]^+$ at *m/z* 381.1345 (calc. 381.1338, $C_{22}H_{21}O_6$) and $[M-OH]^+$ at *m/z* 365.1387 (calcd. 365.1389, $C_{22}H_{21}O_5$); ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

Tephroapollin C (3): colorless solid; HRCIMS ($[M+H]^+$ at *m/z* 337.1437 calcd. 337.1440, $C_{21}H_{21}O_4$); ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

Tephroapollin D (4): colourless powder; CIMS $[M+H]^+$ *m/z* 351, $[M-OCH_3]^+$ *m/z* 321, $[M-2 \times OCH_3]^+$ *m/z* 289; HRCIMS $[M+H]^+$ m/z 351.1600 (calcd. 351.1596, $C_{22}H_{23}O_4$); ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

Tephroapollin E (5): amorphous powder; $[\alpha]_D^{25}$ -13° (*c* 0.1, $CHCl_3$); CIMS $[M+H]^+$ *m/z* 353, $[M+H-(C_4H_9O_2)]^+$ *m/z* 265 (100%); HRCIMS $[M+H]^+$ *m/z* 353.1391, (calc. 353.1389, $C_{21}H_{21}O_5$; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

Tephroapollin F (6): yellowish powder; $[\alpha]_D^{25}$ -110.9° (*c* 0.1, $CHCl_3$); CIMS $[M+H]^+$ *m/z* 395, $[M+H-H_2O]^+$ *m/z* 377, 363, 334, 291, 277, 263, 183. HRCIMS $[M+H]^+$ *m/z* 395.1494 (calcd. 395.1495, $C_{23}H_{23}O_6$); ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

Tephroapollin G (7): colorless material; $[\alpha]_D^{25}$ -45.6 (*c* 0.1, $CHCl_3$); EIMS $[M]^+$ *m/z* 436, $[M-H_2O]^+$ *m/z* 418, 392, 376, 316, 291, 239, 161, 133, 105; HREIMS $[M]^+$ m/z 436.1529 (calc. 436.1524, $C_{25}H_{24}O_7$); ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

Brine shrimp bioassay: A solution of sea water was made by dissolving 33.3 g [A natural blend of salts and trace element, for sea water fish (Sera Company, Germany, Aquaristik Gmbh, D5138 Henisberg)] in distilled water (1L). *ca* 1 mg of brine shrimp, *Artemia salina* (Leach), eggs was added in a hatching chamber (22 × 32cm). The hatching chamber was kept under an inflorescent bulb for 48 h for the eggs to hatch into shrimp larvae (nauplii). X mg of pure compounds, dissolved in X mL of solvent in which they were soluble and from this, 30, 75, 150 and 300 uL of each solution was transferred into vials corresponding to 10, 25, 50 and 100 ug/mL, respectively. Each dosage was tested in triplicate. The vials (5 per test compound) and one control containing 30, 75, 150 and 300 uL of solvent were allowed to evaporate. 10 larvae (nauplii) of *Artemia salina* were transferred into each vial and the volume completed into 3 mL with sea salt solution (± DMSO) immediately after adding the nauplii. 24 h later, the number of surviving shrimp at each dosage was counted and recorded. LC_{50} values were determined statistically.

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