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BIPHENYL ESTERS AND BIFLAVONOIDS FROM THE FRUITS OF *Schinus terebenthefolus*

M. E. S. Kassem, S. K. El-Desoky, and M. Sharaf

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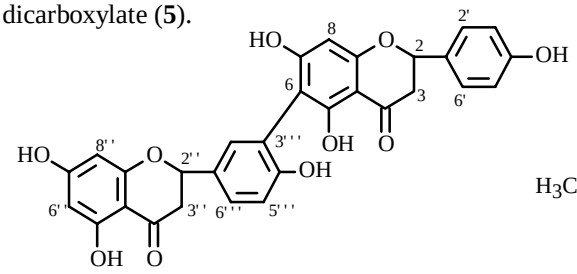
Two new biphenyl esters and three biflavonoids were isolated from the aqueous methanol extract of the fruits of Schinus terebenthefolus. *The spectral data of tetrahydrorobustaflavone have been recorded and assigned for the first time. Characterization of the structures was achieved by various spectroscopic methods.*

Key words: *Schinus terebenthefolus,* Anacardeaceae, biphenyl esters, biflavonoids.

Schinus terebenthefolus Radd. is a dioeciously small tree, native to Brazil, commonly cultivated in Egypt and known as Filfil aread [1] and belonging to the family Anacardeaceae, which is the main source of mono- and di-phenols substituted in the aromatic ring with aliphatic chain and biflavonoids [2]. The available reports are those dealing with the isolation of triterpene [3] and sitosterol from the berries [4]. The fruits have a paralyzing effect on birds upon ingestion and are reported to contain quercetin [5].

Many reports dealing with biflavonoid isolation mention the structure of agathisflavone, robustaflavone, and tetrahydrorobustaflavone [6–11]. These reports are based on chromatographic separation and/or synthesis of their methylether derivatives. Nothing has been reported on the spectral analysis of tetrahydrorobustaflavone as a naturally occurring isolate. The present communication deals with the structure elucidation of three biflavonoids and two new biphenyl esters from the fruits of *S. terebenthefolus* and identified as agasthisflavone (**1**), robustaflavone (**2**), tetrahydrorobustaflavone (**3**), 4′-ethyl-4-methyl-2,3′,5′,6-tetrahydroxy[1,1′-biphenyl]-4,4′-dicarboxylate (**4**), and 3-ethyl-3′-methyl-4,4′,5,5′,6,6′-hexahydroxy-[1,1′-biphenyl]3,3′-dicarboxylate (**5**).



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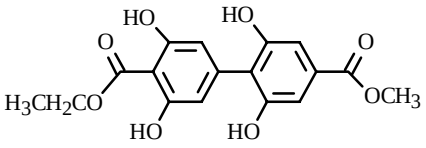

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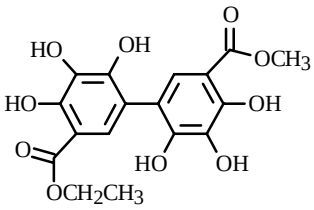

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Fruits of *S. terebenthefolus* were extracted with MeOH–H_2O (3:1). Compounds **1–5** were isolated. Purification was achieved by a combination of polyamide and sephadex LH-20 columns. Compounds **1** and **2** were identified as agasthisflavone and robustaflavone as they showed UV, MS, and NMR spectra similar to those previously reported [8, 9, 12].

Compound **3** was isolated as a yellowish white powder. It gave positive test with $NaBH_4$ indicating its flavanone nature. The UV spectral data with diagnostic shift reagents indicated a flavanone with 5,7,4′-trihydroxyl pattern and the absence of a free *ortho*-dihydroxy pattern at ring-B [12]. EI-MS gave a molecular ion peak at *m/z* 542, suggesting the tetrahydro-structure of **2**, and a fragment at 524 (M^+-H_2O) is observed, confirming the presence of hydroxyl groups *ortho* to the interflavonoid linkage [8]. The fragments at *m/z* 120, 107, 152, and 153 for B_3^+, B_4^+, A_1^+, and A_1^++1 were also detected. The fragment at *m/z* 422 is due to one of the component flavanones undergoing RDA reaction with the second still attached [8]. The ^{1}H-NMR spectrum of **3** is quite complicated due to the overlapping of the aromatic signals.

Phytochemistry and Plant Systematics Department, National Research Centre, Dokki-12311, Cairo, Egypt, e-mail: sharafali58@hotmail.com. Published in Khimiya Prirodnykh Soedinenii, No. 5, pp. 369-371, September-October, 2004. Original article submitted April 14, 2004.

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TABLE 1. ^{13}C-NMR Spectral Data of Compounds **3-5**

Carbon	Compounds		
	3	**4**	**5**
1	-	120.14	119.84
2	77.88	146.03	108.77
3	41.49	109.09	145.81
4	196.51	138.88	145.81
5	160.14	109.09	145.81
6	101.96	146.03	145.81
7	164.73	-	-
8	95.06	-	-
9	160.14	-	-
10	101.96	-	-
1′	129.02	119.84	119.54
2′	127.25	109.09	108.77
3′	115.60	146.03	145.81
4′	160.14	138.79	145.81
5′	115.60	146.03	145.81
6′	131.48	109.09	145.81
2″	78.74	-	-
3″	42.37	-	-
4″	196.90	-	-
5″	160.14	-	-
6″	95.60	-	-
7″	166.75	-	-
8″	95.88	-	-
9″	160.14	-	-
10″	101.81	-	-
1‴	129.02	-	-
2‴	128.03	-	-
3‴	121.00	-	-
4‴	157.70	-	-
5‴	115.06	-	-
6‴	128.03	-	-
-CH_2	-	60.75	60.37
-CH_3	-	14.61	14.50
-CH_3	-	52.04	51.92
-COO	-	166.90	166.69
-COO	-	166.39	166.19

However, a multiplet at δ 7.20 integrated for four protons was assigned to H-2′,6′,2‴,6‴; two doublets at δ 6.80 and 6.70 (J = 8.5 Hz) were assigned to H-5‴ and H-3′,5′. Two signals for three protons at δ 6.10 and 5.85 were assigned to H-8,8″ and H-6″ The flavanone nature was confirmed by the observance of a quartet at δ 5.40 (2H, J = 4.12 Hz) for H-2,2″. The *cis-trans* H-3,3″ were located at δ 2.80 and 3.10 as two unresolved multiplets. This was confirmed by the presence of four signals, two of which were located at δ 78.74 and 77.88 for C-2,2″ while the other two carbons were observed at δ 41.49 and 42.37 for C-3,3″, and the C-4,4″ were located at δ 196.51 and 196.90 in the ^{13}C-NMR spectrum of **3** (Table 1). The three signals observed at δ 95.88, 95.60, and 95.06 were assigned to C-8″,6″ and C-8. The two signals assigned for C-6 and C-3‴ were shifted downfield to 101.96 and 121.0 (ca ≈ 96.0, 115). These shifts are analogous to those reported for C-C linked flavonoids [13]. The H-H COSY spectrum confirms the suggested structure as shown by the clear connectivity between H-6″ and H-8″, H-6′ and H-5′, and no connectivity with H-8. From the above data compound **3** is identified as 6,3‴-biflavanone (tetrahydrorobustaflavone).

It was previously reported that the biflavonoid has pharmacological activity [14, 15]. Thus, the presence of biflavonoids in the plant motivates one to take into consideration their pharmacological activity and the possibility of their new application.

Compound **4** was isolated as a white powder. It gave a yellowish brown color with $FeCl_3$ solution and appeared as a mauve colored spot under UV light not changed on exposure to NH_3 vapor. The UV spectrum in MeOH showed two peaks at 366 and 255 nm. EI-MS gave a molecular ion peak at *m/z* 348 in accordance with the molecular formula $C_{17}H_{16}O_8$. Fragments at *m/z* 318 (M^+-31) and 303 (M^+-45) were also observed. The most significant fragment was observed as a base peak at *m/z* 302 for the lactone fragment, confirming the presence of an OH group in *ortho*-position to the ethyl ester group [16]. The corresponding fragment for methyl ester could not be detected. The ^{1}H-NMR spectrum of **4** showed a broad intense signal integrated for four protons at δ 7.00. The ethyl group appears as a triplet and a quartet at δ 1.10 and 4.10, while the esterified methyl group appears as a singlet at δ 3.60. The ^{13}C-NMR spectrum of **4** showed ten signals. The signals at δ 166.90 and 166.39 were assigned to two C=O groups. The signals at δ 60.75 and 14.61 were assigned to the ethyl group while that at δ 52.04 was assigned to the methyl group. The spectrum also shows two sharp symmetrical signals at 146.03 and 109.09 which were assigned to eight aromatic carbons (C-2,6,3′,5′ and C-3,5,2′,6′ (Table 1). From the above data compound **4** is identified as 4′-ethyl-4-methyl-2,3′,5′,6-tetrahydroxy[1,1′-biphenyl]-4,4′-dicarboxylate.

Compound **5** was isolated as a white powder; it gave a blue color with $FeCl_3$ solution and appeared as a dark colored spot under UV light, which changes to mauve on exposure to NH_3 vapor. The UV and ^{1}H-NMR spectra of **5** are quite similar to those of **4**, the only difference being the integration of the broad intense signal, for the aromatic protons, to two protons in the spectrum of **5**. EI-MS gave a molecular ion peak at *m/z* 380, suggesting structure **4** with two additional hydroxyl groups. The base peak was detected at *m/z* 302, indicating a dilactone structure and confirming the presence of an OH group *ortho* to both esterified carbon. A comparison of the ^{13}C-NMR spectrum of **5** with that of **4** showed similarity, the only difference being the absence of any symmetrical signals in the region from δ 105.00 to 150.00. Thus, the signal appearing at δ 108.77 was assigned to C-2,2′, while the other aromatic signals (eight carbons) appeared as a sharp signal at δ 145.81. This was confirmed by an off-resonance experiment which showed two doublets for the signals assigned to C-2,2′, confirming the protonation of both carbons. From the above data compound **5** is identified as 3-ethyl-3′-methyl-4,4′,5,5′,6,6′-hexahydroxy[1,1′-biphenyl]-3,3′-dicarboxylate.

EXPERIMENTAL

Equipments. NMR: Jeol EX-270 spectrometer, 270 MHz (^{1}H-NMR) and 67.5 MHz (^{13}C-NMR). ^{1}H resonance was measured relative to TMS and ^{13}C-NMR resonance to DMSO-d_6 and converted to the TMS scale by adding 39.5. Typical conditions: spectral width = 5000 Hz for ^{1}H and 20000 for ^{13}C, 32 K data points and a flip angle of 45°C. EI-MS: Finnigan Mat SSQ 7000 instrument, EI mode. UV: Shimadzu UV-visible 2410.

Plant Material. Fruits of *Schinus terebenthefolus* were collected in June 2001 from the Orman Garden, Giza, and authenticated by Dr. S. A. Kawashty. A voucher specimen is deposited at the herbarium of the NRC (CAIRC).

Isolation and Identification. The fruits (500 g) were extracted with MeOH–H_2O (3:1) over a hot water bath. The conc. extract was applied to a polyamide 6S column eluted with H_2O followed by a H_2O–MeOH mixture of decreasing polarities. Repeated fractionations over Sephadex LH-20 using MeOH as eluent afforded pure samples of **1–5**.

6,3‴-Biflavanone (tetrahydrorobustaflavone) (3). UV λ_{max} (nm) MeOH: 289, 330 sh; NaOMe: 268, 322; $AlCl_3$: 306, 373 sh; $AlCl_3$/HCl: 307, 373; NaOAc: 252, 273, 321; NaOAc/H_3BO_3: 264, 290, 328. EI-MS: 542 (47%), 524 (76%), 422 (8%), 153 (8%), 152 (7%), 120 (44%), 107 (16%). ^{1}H-NMR: δ ppm 7.20 (m, H-2′,6′,2‴,6‴), 6.80 (d, J = 8.5, H-5‴), 6.70 (d, J = 8.5, H-3′,5′), 6.10 (s, H-8), 5.85 (s, 2H, H-6″, H-8″), 5.40 (q, J = 4,12, H-2,2″), 2.80, 3.10 (2m, unres., H-3,3″). ^{13}C-NMR: see Table 1.

4′-Ethyl-4-methyl-2,3′,5′,6-tetrahydroxy[1,1′-biphenyl]-4,4′-dicarboxylate (4). UV λ_{max} (nm) MeOH: 366, 255; EI-MS: 348 (5%), 318 (4%), 303 (15%), 302 (100%); ^{1}H-NMR: δ ppm 7.00 (s, 4H, H-3,5,2′,6′), 4.10 (q, 2H, CH_2), 3.60 (s, 3H, CH_3COO), 1.10 (t, 3H, CH_3); ^{13}C-NMR: see Table 1.

3-Ethyl-3′-methyl-4,4′,5,5′,6,6′-hexahydroxy[1,1′-biphenyl]-3,3′-dicarboxylate (5). UV λ_{max} (nm) MeOH: 364, 255; EI-MS : 380 (3%), 303 (10%), 302 (100%); ^{1}H-NMR: δ ppm 7.00 (s, 2H, H-2,2′), 4.10 (q, 2H, CH_2), 3.65 (s, 3H, CH_3COO), 1.20 (t, 3H, CH_3); ^{13}C-NMR: see Table 1.

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