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Article

New Ester and Furocoumarins from the Roots of *Pituranthos tortuosus*

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Sete compostos foram isolados, a partir das frações solúveis em $CHCl_3$, de raízes de *Pituranthos tortuosos*; adicionalmente manitol foi cristalizado a partir do extrato alcoólico. Usando diferentes técnicas espectroscópicas os compostos isolados foram identificados como bergaptana, graveolana, xanthotoxinona, isopimpinela, dimetoxi aesculetina, 3-*O*-β-glicopiranosil estigmasterol e também o novo éster umbelato de 4-metoxifenila. A estrutura do novo éster foi confirmada pela síntese completa e se mostrou inativo a testes antimicrobiais e de citotoxicidade.

Seven compounds were isolated from the $CHCl_3$ soluble fraction of the roots of *Pituranthos tortuosus*; in addition, mannitol was crystallized out of the total alcoholic extract. Using different spectroscopic techniques, the isolated compounds were identified as bergapten, graveolone, xanthotoxin, isopimpinellin, aesculetin dimethyl ether, stigmasterol glucoside, in addition to the new ester *4*-methoxyphenylumbellate. The structure of the new ester was confirmed by total synthesis and it was found to be inactive in antimicrobial and cytotoxicity assays.

Keywords: *Pituranthos tortuosus*, Apiaceae, *4*-methoxyphenylumbellate, furanocoumarins, mannitol, stigmasterol glucoside, synthesis

Introduction

Members of the family Apiaceae (Umbelliferae) are well known producers of furanocoumarins. In this regard, Apiaceae is in the first place followed by Rutaceae and Moraceae.[1] Furanocoumarins have several interesting biological activities, such as analgesic, antiinflammatory, antibacterial, antiviral, anticoagulant, in addition to their well known photosensitizing effect.[2-8] In Egypt, the genus *Pituranthos (Deverra)* is represented by two species.[9] While five furanocoumarins were isolated from *P. triradiatus*, the aerial parts of *P. tortuosus* was found to be free from these compounds.[10] This result intiated the study of the plant roots for the presence of furanocoumarins since they are taxonomic markers for the family.

Results and Discussions

Spectral data indicated that compounds **1** and **3** are furanocoumarins with a methoxyl group at either C-5 or C-8. The position of the methoxyl was assigned based on comparison of the ^{13}C-NMR data with those in the literature[11]. The negative CIMS data of **1** (bergapten) showed a base peak at *m/z* 201 resulting from the fission of the aromatic ether bond indicating a 1, 3, 5 oxygenation of the aromatic ring which stabilizes the resulting phenoxide anion.[12] That ion in **3** (xanthotoxin) was of very low intensity indicating C-8 oxygenation.

Compounds **2**, **4** and **5** were identified as graveolone,[13-15] isopimpinellin[16] and aesculetin dimethyl ether,[17] respectively, by comparison of their data with those of the literature. However, the ^{13}C-NMR data for the linear dihydrobenzodipyrandione; graveolone (**2**) is reported here for the first time. Graveolone is a compound of very limited occurrence and has only been isolated from dill and parsley.[13, 16]

Compound **6** gives a positive reaction with $FeCl_3$ indicating the presence of free phenolic OH group(s). In the ^{1}H-NMR (Table 1), the ABX system (6.33, dd, *J* 2.2, 9.0 Hz; 6.31, d, *J* 2.2 Hz; 7.38, d, *J* 9.0 Hz) was assigned for a trisubstituted aromatic system. The chemical shifts of the protons and carbons supported a 1, 2, 4-trisubstitution with two oxygenations at 2, 4 rather than 1, 3, 4-trisubstitutions.[18-21] Two other doublets (*J* 16.0 Hz) each integrated for 1 proton at δ 6.58 and 8.03 with their correlated carbons (Table 1) were assigned to *trans* oriented conjugated vinyl protons. The substituted aromatic system along with the vinyl protons as well as the carbonyl signal at 167.91 ppm in the ^{13}C-NMR (Table 1) were assigned for a 2, 4-dioxygenated cinnamate moiety.

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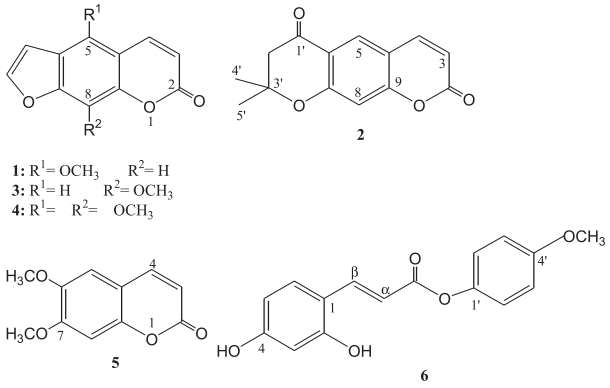

Both EIMS (M^+ at *m/z* 286) and CIMS (M^++1 at *m/z* 287) (see experimental) were consistent with the molecular formula $C_{16}H_{14}O_5$. Fourteen carbon signals were observed in the ^{13}C-NMR spectrum (Table 1). However, two of these signals (114.18 and 122.41 ppm) correlated by an HMQC experiment to two doublets at δ 6.92 (2H, *J* 8.8 Hz) and 7.03 (2H, d, *J* 8.8 Hz) were assigned for a *p*-dioxygenated benzene ring. The position of the OCH_3 (δ 3.77, 54.85 ppm in ^{1}H- and ^{13}C-NMR respectively) at C-4' was determined by a GOESY experiment where irradiation of the OCH_3 signal resulted in an enhancement in the doublet at δ 6.92 (2H, *J* 8.8 Hz).

The above discussion indicated that **6** is an ester of umbellic acid (2, 4-dihydroxy cinnamic acid) with 4-methoxyphenol.

As final proof, **6** was obtained by total synthesis (Figure 1). The commercially available umbelliferone (Aldrich) was treated with 5% alcoholic solution of NaOH to open the lactone ring. This reaction resulted in the formation of umbellic acid (2, 4-dihydroxy *trans*-cinnamic acid) **7** which was protected by *TBDMSCl* to give **7a**. Protected umbellic acid **7a** was then coupled with 4-methoxyphenol to produce **6a**. Deprotection of **6a** gave **6**. Umbellic acid is a possible precursor for umbelliferone which is the key compound in the biosynthesis of furanocoumarins.[22] The isolation of compounds **1- 6** from the roots of *P. tortuosus* indicated that its biosynthetic pathway is consistent with those in other members of the family Apiaceae.

Compound **6** was inactive when tested against A2780 (Human Ovarian cancer cell), *Escherichia coli, Staphylococcus albus* and *Candida albicans*.

Experimental

General procedure

Melting points were determined using Kofler's hot stage instrument and are uncorrected. UV spectra were determined using UV-1201 Shimadzu spectrometer. NMR spectra were recorded on a Varian Unity 400 NMR instrument at 399.951 MHz for ^{1}H and 100.578 MHz for ^{13}C. MS were taken on a VG 7070 E-HF. All chemicals used in the synthesis of **6** were obtained from Aldrich Chemicals Company.

Table 1. ^{1}H-[a] and ^{13}C-NMR data for compounds **6, 6a, 7** and **7a**

No.	6[b]		6a[b]		7[c]		7a[b]
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1		113.56		119.60		113.81	
2		157.51		156.41		158.70	
3	6.31 d *J* 2.2	102.34	6.34 d *J* 2.3	111.74	6.31 m	102.33	6.47 d *J* 2.5
4		159.30		157.28		160.90	
5	6.33 dd *J* 2.2,9.0	107.81	6.51 dd *J* 2.3, 8.7	114.53	6.31 m	107.59	6.48 dd *J* 2.5, 8.7
6	7.38 d *J* 9.0	130.85	7.49 d *J* 8.7	128.62	7.31 d *J* 9.0	130.22	7.44 d *J* 8.7
α	6.58 d *J* 16.0	143.22	6.45 d *J* 16.0	141.82	6.37 d *J* 15.9	141.27	6.28 d *J* 16.0
β	8.03 d *J* 16.0	112.23	8.20 d *J* 16.0	114.39	7.86 d *J* 15.9	114.30	8.10 d *J* 16.0
C=O		**167.91**		**166.61**		**171.83**	
1'		144.76		144.76			
2'	7.03 d *J* 8.8	122.41	6.91 d *J* 8.9	122.66			
3'	6.92 d *J* 8.8	114.18	7.07 d *J* 8.9	114.64			
4'		161.65		159.36			
5'	6.92 d *J* 8.8	114.18	7.07 d *J* 8.9	114.64			
6'	7.03 d *J* 8.8	122.41	6.91 d *J* 8.9	122.66			
OCH_3	3.77 s	54.85	3.81 s	55.83			
CH_3			0.23s, $2CH_3$	-4.11 $2CH_3$			0.22s, $2CH_3$
			0.25s, $2CH_3$	-4.03 $2CH_3$			0.24s, $2CH_3$
			0.99s, $3CH_3$	25.84 $3CH_3$			0.98s, $3CH_3$
			1.03s, $3CH_3$	25.97 $3CH_3$			1.04s, $3CH_3$

[a] *J* values in Hz; [b] Spectra were measured in $CDCl_3$; [c] Spectra were measured in CD_3OD.

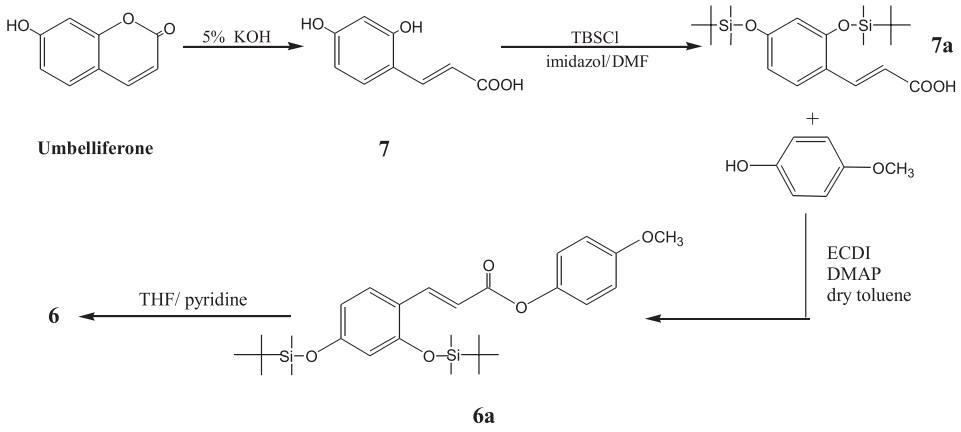

Figure 1. Scheme for synthesis of **6** from umbelliferone.

Plant material

Roots of *Pituranthos tortuosus* (Desf.) Benth. were collected on April 3, 2000 from Borg El-Arab, Alexandria, Egypt. The plant was identified by Dr Sanyia Kamal, Department of Botany, Faculty of Science, University of Alexandria. A voucher specimen MS-7 is deposited in the Pharmacognosy Department, Faculty of Pharmacy, University of Alexandria, Egypt.

Extraction and isolation

1.2 kg of the dried roots was extracted with MeOH (6L). The methanolic extract was concentrated to 200 mL. At that point 400 mg of colourless crystals of mannitol were separated out of the solution. The concentrated methanolic solution was diluted with water to 300 mL and extracted exhaustively with hexane (700 mL), $CHCl_3$ (700mL) and EtOAc (500 mL). The $CHCl_3$ soluble fraction (3 g) was fractionated over silica gel column (200 g, 3 cm) eluting with 1% MeOH in $CHCl_3$, with gradual increasing of the MeOH contents and 200 mL fractions were collected. Fractions 2- 4 (1% MeOH, 1.0 g) were rechromatographed over silica gel column (100 g, 2.5 cm) eluting with 25% hexane in $CHCl_3$, $CHCl_3$/ hexane and then $CHCl_3$/MeOH mixtures. Fraction 2 (50% hexane in $CHCl_3$, 150 mg) was subjected to repeated prep TLC using hexane/$CHCl_3$ (2: 1) as developing system (triple run) to afford **1** (8 mg), **2** (5 mg), **3** (14 mg), **4** (6 mg) and **5** (4 mg).

Fraction 5 (2% MeOH, 100 mg) was further purified over flash silica gel column (30 g, 2.5 cm) eluting with 1% MeOH in $CHCl_3$. Fraction 3 (7 mg) was subjected to prep TLC over RP18 and MeOH/ H_2O (7: 3) as developing system to afford 2 mg of **6**.

Fraction 9 afforded 50 mg of stigmasterol glucoside after crystallization from MeOH.

*Bergapten (5- Methoxypsoralen) (**1**).* $C_{12}H_8O_4$, mp 191-192 °C. EIMS *m/z* (rel. Int.): 216 (90, M^+). Negative CIMS *m/z* (rel. Int.): 217 (7, M^++1), 216 (10, M^+), 201 (100, M^+-CH_3).

*Graveolone (**2**).* $C_{14}H_{12}O_4$, mp 176- 178 ^{0}C. UV λ_{max}/ nm (MeOH) 253, 303, 308, 329, 344. IR (film): ν_{max}/cm^{-1}: 1733 (lactone C=O), 1688 (C=O), 1620, 1589, 1542, 821. ^{1}H-NMR (ppm,$CDCl_3$): δ 1.49(6H, s, 2XCH_3), 2.77 (2H, s, H-2'), 6.30 (1H, d, *J* 9.7 Hz, H-3), 6.83 (1H, s, H-8), 7.66 (1H, d, *J* 9.7 Hz, H-4), 8.03 (1H, s, H-5). ^{13}C-NMR (ppm, $CDCl_3$): δ 26.93 (C-4', C-5'), 48.86 (C-2'), 80.98 (C-3'), 105.83 (C-8), 113.53 (C-6), 114.82 (C-3), 117.85 (C-10), 127.60 (C-5), 143.56 (C-4), 159.49 (C-9), 160.15 (C-7), 162.61 (C-2), 191.07 (C-1'). EIMS *m/z* (rel. Int.): 244 (43, M^+), 229 (100, M^+- CH_3), 216 (8, M^+- CO), 201 (7, M^+-CH_3-CO), 189 (81, M^+- C_4H_7), 188 (40, M^+- C_4H_8), 160 (28), 132 (12), 104 (15), 76 (37).

*Xanthotoxin (8- Methoxypsoralen) (**3**).* $C_{12}H_8O_4$, mp 150- 151 °C. EIMS *m/z* (rel. Int.): 216 (100, M^+). Negative CIMS *m/z* (rel. Int.):217 (16, M^++1), 216 (100, M^+), 201 (6, M^+- CH_3).

*Isopimpinellin (**4**).* $C_{13}H_{10}O_5$, mp 115- 117 °C. EIMS *m/z* (rel. Int.): 246 (100, M^+), 231 (100, M^+- CH_3), 216 (15), 203 (17, M^+- CH_3-CO).

*Aesculetin dimethyl ether (**5**).* $C_{11}H_{10}O_4$, mp 145-146 °C. EIMS *m/z* (rel. Int.): 206 (100, M^+), 191 (36, M^+- CH_3), 178 (21, M^+-CO), 163 (44, M^+- CH_3-CO), 135 (42), 107 (43), 79 (52), 69 (74).

*4-Methoxyphenylumbellate (**6**).* $C_{16}H_{14}O_5$, mp 169 °C. UV λ_{max}/ nm (MeOH) 221, 244, 297, 337. ^{1}H- and ^{13}C-NMR (Table 1). EIMS *m/z* (rel. Int.): 286 (50, M^+), 255 (92, M^+-OCH_3), 223 (56), 211 (55), 195 (42), 194 (57), 179 (31, M^+-C_7H_7O), 177 (68), 171 (37), 165 (58), 163 (52, M^+-$C_7H_7O_2$), 161 (100). CIMS *m/z* (rel. Int.): 287 (47, M^++1), 286 (21, M^+), 269 (24), 255 (19), 213 (23), 179(27), 177

(45), 163 (100). HRCIMS *m/z*: 286.281 (M^+), calculated for $C_{16}H_{14}O_5$ 286.282.

*Synthesis of 4-Methoxyphenylumbellate (**6**)*

*Preparation of Umbellic acid (**7**).* 1 gm of umbelliferone (Aldrich) was dissolved in 100mL MeOH and stirred with an equal volume of 5% alcoholic KOH for 4 h. The reaction mixture was neutralized with diluted HCl and then extracted with EtOAc (500 mL). The residue left after evaporation of the solvent was purified over silica gel column (100 g, 3 cm) eluting with $CHCl_3$ and $CHCl_3$/MeOH mixtures. 455 mg of umbelliferone were recovered in the early fractions. Fractions 5- 11 eluted with 2% MeOH in $CHCl_3$ afforded 516 mg of umbellic acid: UV λ_{max}/nm (MeOH) 218, 239, 287, 323. ^{1}H- and ^{13}C-NMR Table 1. Found: C, 60.12; H, 4.61. Calc. for $C_9H_8O_4$ (180.1): C, 60.00; H, 4.48.

*Protection of umbellic acid to (**7a**).* To a solution of **7** (410 mg, 2.3 mmol) in 0.75 mL DMF, imidazol (938 mg, 13.8 mmol) and *tert*-butyldimethylsilylchloride (TBSCl) (810 mg, 5.4 mmol) were added. The reaction mixture was stirred for 15 min under argon, quenched with $NaHCO_3$, and stirring was continued for 5 min. The resulted solution was extracted with 300 mL EtOAc. The organic layer was washed twice with H_2O, brine solution, then H_2O again and finally dried over Na_2SO_4. Part of the residue left after evaporation of the EtOAc (**7a**) was checked by ^{1}H-NMR and the rest was dried for the next step. UV λ_{max}/nm ($CHCl_3$) 228, 241, 293, 325. ^{1}H-NMR Table 1. Found: C, 61.94; H, 8.79; Si, 13.91. Calc. for $C_{21}H_{36}O_4Si_2$ (408.6): C, 61.72; H, 8.88; Si, 13.74.

*Esterification of (**7a**) to (**6a**).* To a solution of **7a** (165 mg, 0.49 mmol) in dry toluene, ECDI (141 mg, 0.74 mmol) and DMAP (90 mg, 0.74 mmol) were added. After stirring for 10 min, a solution of 92 mg 4-methoxyphenol (0.74 mmol) in dry toluene was injected into the reaction solution and kept overnight at 55 °C with stirring. The product of the reaction was purified by silica gel column (30 g, 2 cm) eluted with $CHCl_3$ to afford 97 mg of (**6a**). UV λ_{max}/nm (MeOH) 241, 287, 296, 330. ^{1}H-^{13}C-NMR Table 1. Found: C, 65.40; H, 8.36; Si, 10.72. Calc. for $C_{28}H_{42}O_5Si_2$ (514.8): C, 65.32; H, 8.22; Si, 10.91.

*Deprotection of (**6a**) to 4-Methoxyphenylumbellate (**6**).* The protected ester (**6a**)(100 mg) was dissolved in dry THF, 2 mL of THF/pyridine were added and the solution was stirred at room temperature for 10 min. The reaction mixture was extracted with 5% $NaHCO_3$, then H_2O. The organic layer after evaporation and prep TLC on silica gel afforded 45 mg (**6**), which was identical with the isolated natural compound. HRCIMS *m/z*: 286.280 (M^+), calculated for $C_{16}H_{14}O_5$ 286.282.

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