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A new 3-arylcoumarin from the roots of an Egyptian collection of *Lotus polyphyllus*

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Further investigation of different fractions from *Lotus polyphyllus* Clarke roots resulted in the isolation of the new 3-arylcoumarin derivative; 4',6'-dihydroxy-7, 2'-dimethoxy-3-arylcoumarin (5). In addition, the known compounds β -sitosterol, 1-hexacosanol (1), *n*-tetracosyl *p*-coumarate (2), 4'-*O*-methylcherrone (4), and quercetin (6) were identified. The structures were determined from spectroscopic data including 1D- and 2D-NMR experiments.

Keywords: *Lotus polyphyllus*; Fabaceae; Flavonoids; *p*-Coumaric acid ester; 3-Arylcoumarin

1. Introduction

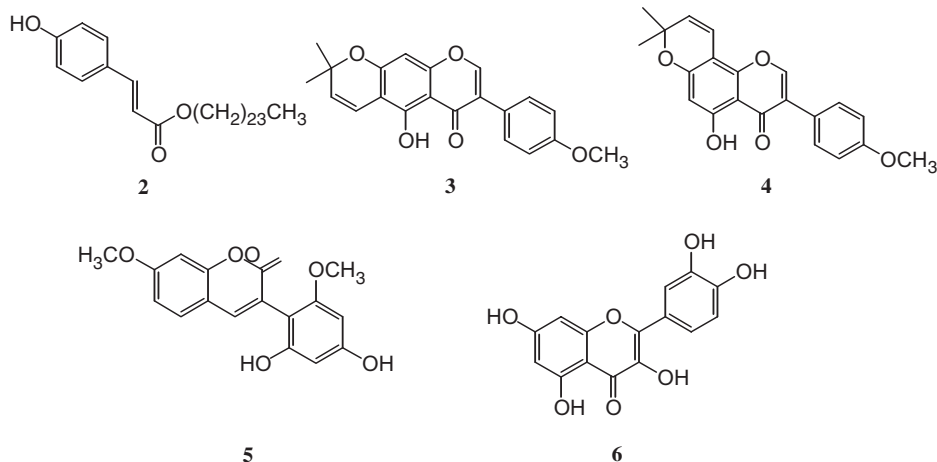
Phytochemical investigation of *Lotus* species revealed that the aerial parts are rich in flavone and flavonol derivatives [1–4]. The roots, as in many plants belonging to family Fabaceae, are rich in isoflavone derivatives [5–8]. We recently reported the isolation of two isoflavone derivatives from the roots of *Lotus polyphyllus* Clarke [7]. In the present study, further constituents including a new 3-arylcoumarin derivatives were obtained from different fractions of the same plant.

2. Results and discussion

Extraction of fresh roots of *L. polyphyllus* with 90% alcohol, followed by liquid–liquid partitioning and chromatography on silica gel, yielded five compounds 1, 2, 4–6 in addition to β -sitosterol. Compounds 1, 2, 4, and 6 are known, while 5 was found to be a new natural product. The known compounds 1-hexacosanol (1) and *n*-tetracosyl *p*-coumarate (2) [9] were isolated from the combined $\text{CHCl}_3/\text{EtOAc}$ fractions of the same plant [7]. From the same fractions the common flavonoid quercetin (6)

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was identified. Characterization of the known compounds was achieved by comparing their spectroscopic data with the literature.



35 The UV, MS, ^1H -, and ^{13}C -NMR data of **4** were diagnostic for 5-hydroxy, 4'-methoxyisoflavone with either C-6 or C-8 prenyl moiety. The prenyl group forms an additional six-membered ring with the C-7 hydroxyl group [10–12]. The data of **4** including COSY, HSQC, and HMBC were typical with those reported for 4'-O-methylderrone rather than 4'-O-methylalpinumisoflavone (**3**) [13–17].
40 Compound **4** was isolated only from three Fabaceae members; *Calopogonium mucunoides*, *Derris robusta*, and *Euchresta horsfieldii* [13,15,18]. The reported chemical shifts of C-8 and C-10 for **4** isolated from *E. horsfieldii* [15] must be revised. In the same publication the chemical shift assigned for C-6 is closer to that reported for C-8 in **3**. The differentiation between **3** and **4** based on the
45 chemical shift of C-5 OCH₃ group in **3** after derivatization is questionable. Extraction by reflux with CH₂Cl₂ raise the question whether the compound was natural or an artifact.

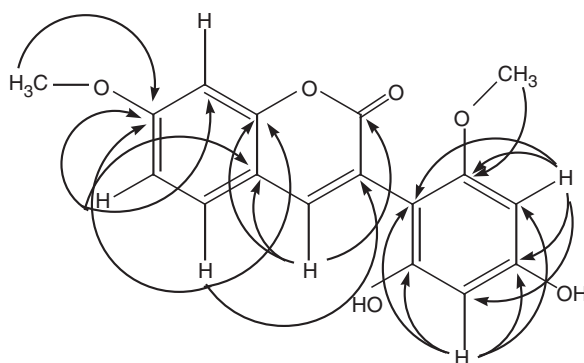
The UV spectrum of **5** (211, 260, 352 nm (experimental)) as well as the ^1H -NMR singlet at $\delta_{\text{H}}7.88$ correlated to carbon at $\delta_{\text{C}}136.3$ (table 1) in an HSQC experiment were diagnostic for 3-aryl coumarin skeleton [19–22]. ^1H -NMR showed a typical ABX system (δ 7.18, d, $J=8$ Hz; 6.43, dd, $J=8, 1.5$ Hz; 6.45, d, $J=1.5$ Hz) assigned for 7-oxygenated ring A protons. The two singlets at δ 6.35 and 6.37 were ascribed for H-3' and H-5', respectively, in a 2',4',6'-trioxygenated aryl moiety at C-3. Both ^1H - and ^{13}C -NMR indicated the presence of two methoxyls at $\delta_{\text{H}}3.73$, $\delta_{\text{C}}55.0$ and $\delta_{\text{H}}3.86$, $\delta_{\text{C}}56.0$, respectively. The failure of NaOAc to produce any shift in the UV spectrum of **5** indicated that C-7 hydroxyl is methoxylated [23]. The positions of the two methoxyls were assigned based on HMBC experiment (figure 1). The methoxyl singlet at $\delta_{\text{H}}3.86$ showed HMBC correlations (figure 1) to the carbon signal at $\delta_{\text{C}}160.0$ assigned for C-7. The second methoxyl at $\delta_{\text{H}}3.73$ showed HMBC correlations to carbon signal at $\delta_{\text{C}}156.8$ assigned for C-2'. In an NOESY experiment, NOE correlations between C-7 methoxyl at $\delta_{\text{H}}3.86$ and H-6 ($\delta_{\text{H}}6.43$, dd) and H-8 ($\delta_{\text{H}}6.45$, d); C-2' methoxyl at $\delta_{\text{H}}3.73$ and H-3' singlet at $\delta_{\text{H}}6.37$ further supported the positions of the methoxyl groups. The EIMS of **5** showed M^+ at m/z 314 consistent with the molecular formula C₁₇H₁₄O₆ fully supports the assigned structure. Based on the above discussion, **5** was identified as the

A new 3-arylcoumarin from *L. polyphyllus*

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Table 1. ^1H - and ^{13}C -NMR data of **4** and **5** (δ values, J in parenthesis in Hz)^a.

Position	4		5	
	^1H	^{13}C	^1H	^{13}C
2	7.87 (s)	152.3	–	160.1
3	–	123.6	–	118.6
4	–	180.9	7.88 (s)	136.3
5	–	162.3	7.18, d (8)	131.5
6	6.27 (S)	100.4	6.43, dd (8, 1.5)	104.4
7	–	159.6	–	160.0
8	–	101.1	6.45, d (1.5)	101.3
9	–	152.2	–	156.8
10	–	106.1	–	115.1
1'	–	122.9	–	102.3
2'	7.43, 2H, d (8.5)	130.1	–	155.4
3'	6.96, 2H, d (8.5)	114.1	6.35 (s)	94.5
4'	–	159.8	–	161.9
5'	6.96, 2H, d (8.5)	114.1	6.37 (s)	95.3
6'	7.43, 2H, d (8.5)	130.1	–	155.9
1''	6.66, d (10)	114.6	–	–
2''	5.56, d (10)	127.5	–	–
3''	–	78.1	–	–
4''	1.47 (s)	28.1	–	–
5''	1.47 (s)	28.1	–	–
5-OH	12.92 (s)	–	–	–
7-OCH ₃	3.83 (s)	55.4	3.73 (s)	55.0
4'-OCH ₃	–	–	3.86 (s)	56.0

^aAssignments made by combination of COSY, DEPT, HMQC, HMBC data, and comparison with the literature.Figure 1. HMBC Correlations of **5**.

65 new 4',6'-dihydroxy-7,2'-dimethoxy-3-arylcoumarin. This is the first report for the isolation of 3-arylcoumarin derivative from *Lotus* species.

3. Experimental

3.1. General experimental procedures

Melting points were determined using Kofler's hot stage instrument and are uncorrected. UV spectra were determined using Hewlett-Packard HP-845

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UV-Vis spectrometer. NMR spectra were recorded on a Bruker Avance DRX-500 instrument at 500 MHz for ^1H and 125 MHz for ^{13}C using the residual solvent signal as internal standard. Standard Bruker pulse programs were used for DEPT, 2D NMR COSY, HSQC, and HMBC spectra. EIMS were taken on an E.I. Finnigan model 4600 quadrupole system. FABMS were obtained on a Bruker Bioapex-FTMS. TLC silica gel 60 F254 (Merck) plates and silica gel 60/230–400 mesh (EM Science) were used for separation. Centrifugal preparative TLC (CPTLC) was performed using Chromatotrone, Harrison Research Inc. model 7924 equipped with 4 mm silica gel P₂₅₄ disc. β -Sitosterol, 1-hexacosanol, and quercetin were obtained from Aldrich chemical company.

3.2. Plant material

The plants of *L. polyphyllus* Clarke were described earlier [7].

3.3. Extraction and isolation

The fresh roots (2 kg) were sliced and exhaustively extracted with 90% ethyl alcohol (10 L). The concentrated ethanol extract (250 mL) was diluted with the same volume of distilled water and was successively extracted with petroleum ether (3 \times 300 mL), CH_2Cl_2 (3 \times 300 mL), EtOAc (3 \times 250 mL), and *n*-butanol (3 \times 200 mL).

The petroleum ether fraction (11.3 g) was subjected to a silica gel column (300 g, 100 \times 5 cm i.d.) eluting with light petroleum– CHCl_3 in different ratios. Fractions eluted with petroleum ether– CHCl_3 (3 : 1) (3.3 g) were rechromatographed on silica gel column (150 g, 45 \times 2 cm i.d.) eluting with light petroleum– CH_2Cl_2 with increasing amounts of CH_2Cl_2 . Fractions 5–7 eluted with 10% CH_2Cl_2 in petroleum ether and afforded **1** (8 mg) on crystallization from petroleum ether. Fractions 15–20 eluted with 10% CH_2Cl_2 in light petroleum and were crystallized from petroleum ether to afford **2** (32 mg). Fractions 29–30 eluted with 15% CH_2Cl_2 in petroleum ether and were crystallized from CHCl_3 to yield 6 mg of **4**.

Fractions eluted with petroleum ether– CH_2Cl_2 (1 : 1) (290 mg) afforded 75 mg of β -sitosterol.

Part of the combined CH_2Cl_2 and EtOAc extracts (15 g) was fractionated on silica gel column (300 g, 100 \times 5 cm i.d.) eluting with CHCl_3 then CHCl_3 –MeOH mixtures. Forty fractions 150 mL each were collected, screened by TLC, and similar fractions were pooled. Fractions 23–35 eluted with 3% MeOH in CHCl_3 (2.16 g) and were rechromatographed on a silica gel column (100 g, 45 \times 2 cm i.d.) eluting with petroleum ether–EtOAc mixtures. Fractions 23–36, eluted with 50% EtOAc, were subjected to CPTLC (4 mm silica gel GF₂₅₄ disk, solvent: 30% EtOAc in petroleum ether). Hundred fractions 150 mL each were collected, screened by TLC, and similar fractions were pooled. Crystallization of fractions 13–19 from CHCl_3 afforded **5** (7 mg). Fractions 27–62 were crystallized from MeOH to yield 24 mg of **6**.

3.3.1. 4'-O-Methyl derrone (4). $\text{C}_{21}\text{H}_{20}\text{O}_6$, Yellow crystals, m.p. 164°C, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 268, 349 (sh); NaOMe: 274, 375 (sh); AlCl_3 : 226, 283, 410 (sh); AlCl_3/HCl : 225, 282,

410 (sh); NaOAc: 269, 349 (sh). ^1H - and ^{13}C -NMR data in CDCl_3 (table 1). EIMS (rel. int., %): m/z 350 (M^+ , 43), 335 ($\text{M}^+ - \text{CH}_3$, 100), 221 (5), 203 (22), 146 (9), 135 (15), 117 (13). FAB-MS (rel. int., %): m/z 351 ($\text{M}^+ + 1$, 100), 350 (M^+ , 62), 335 ($\text{M}^+ - \text{CH}_3$, 62), 307 (65), 289 (48).

3.3.2. 4', 6'-Dihydroxy-7, 2'-dimethoxy-3-arylcoumarin (5). $\text{C}_{17}\text{H}_{14}\text{O}_6$, Yellow crystals, m.p. 159–160°C, UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 211, 260, 352. ^1H - and ^{13}C -NMR data in DMSO (table 1). EIMS (rel. int., %): m/z 314 (M^+ , 17), 310 (15), 296 ($\text{M}^+ - \text{HOH}$, 87), 286 ($\text{M}^+ - \text{CO}$, 7), 250 (9), 218 (7), 206(11), 176 (15), 119 (29), 107 (66), 91 (100).

Acknowledgment

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