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Two new isoflavone derivatives from the roots of an Egyptian collection of *Lotus polyphyllos*

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Investigation of the roots of *Lotus polyphyllos* Clarke resulted in the isolation of two new isoflavone derivatives; 4′-*O*-methylerythrinin C (**3**) and 4′-*O*-methyl-2′′-hydroxydihydroalpinumisoflavone (**4**). In addition, the three known isoflavone derivatives 4′-*O*-methylalpinumisoflavone (**1**) lupinalbin F (**5**), 4′,7-dimethoxy-5-hydroxyisoflavone (**6**), and the phenylpropanoid ester tetracosyl *p*-coumarate (**2**) were also identified. The structures were determined from spectroscopic data.

Keywords: *Lotus polyphyllos*; Fabaceae; Isoflavones; Phenylpropanoids

1. Introduction

About 18 members of the genus *Lotus* are present in the Egyptian flora [1]. Previous investigations of the aerial parts of *Lotus* species have resulted in the isolation of several flavone and flavonol derivatives [2–5]. On the other hand, investigation of the roots of *Lotus creticus* revealed that they are rich in isoflavonoid derivatives [6,7]. In the present study we report on the isolation of isoflavonoid and phenylpropanoid derivatives from the roots of *Lotus polyphyllos* Clarke.

2. Results and discussion

Extraction of fresh roots of *L. polyphyllos* with 90% alcohol, followed by liquid–liquid partitioning and chromatography on silica gel, yielded the six pure compounds **1**–**6**. Of these **1**, **2**, **5**, and **6** were identified as known compounds, while **3** and **4** were new.

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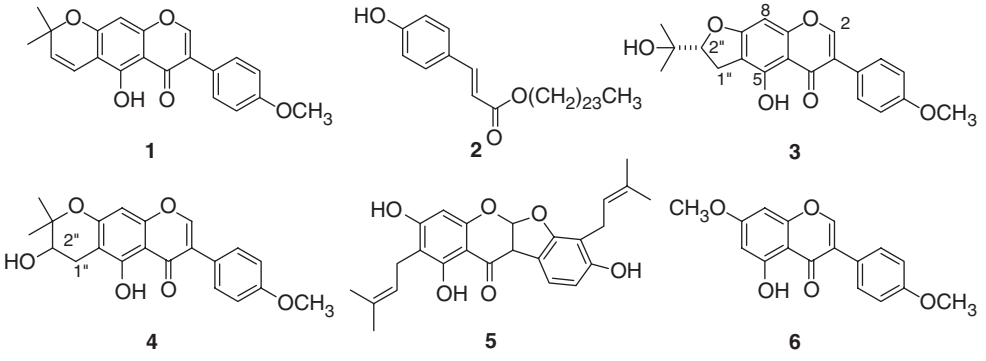

Table 1. ^{1}H- and ^{13}C-NMR data of **3** and **4** in $CDCl_3$ (δ values, J in parenthesis in Hz)[a].

	3		**4**	
Position	^{1}H	^{13}C	^{1}H	^{13}C
2	7.82 s	152.1	7.82 s	153.3
3	–	124.0	–	124.6
4	–	180.1	–	180.2
5	–	166.3	–	160.4
6	–	103.8	–	101.9
7	–	163.6	–	154.4
8	6.36 s	93.6	6.36 s	100.2
9	–	153.3	–	152.4
10	–	105.3	–	106.3
1′	–	123.2	–	123.5
2′	7.43, 2H, d (8)	130.1	7.45, 2H, d (8)	130.8
3′	6.99, 2H, d (8)	113.5	6.99, 2H, d (8)	114.2
4′	–	159.9	–	158.4
5′	6.99, 2H, d (8)	113.5	6.99, 2H, d (8)	114.2
6′	7.43, 2H, d (8)	130.1	7.45, 2H, d (8)	130.8
1″	3.23, 2H, d (9)	27.4	2.80 dd (17, 7) 3.02 dd (17, 6)	25.5
2″	4.82 t (9)	91.7	3.88 m	68.6
3″	–	70.9	–	78.2
4″	1.22, 3H, s	24.2	1.38, 3H, s	22.4
5″	1.38, 3H, s	26.0	1.52, 3H, s	25.2
5-OH	13.13		13.16	
OCH_3	3.83, 3H, s	54.4	3.82, 3H, s	56.8

[a]Assignments made by combination of COSY, DEPT, HMQC, HMBC data and comparison with the literature.

The known compounds 4′-*O*-methylalpinumisoflavone (**1**) [8], *n*-tetracosyl *p*-coumarate (**2**) [9], lupinalbin F (**5**) [10] and 4′,7-dimethoxy-5-hydroxyisoflavone (**6**) [11] were identified by comparison of their spectroscopic data with literature data.

Both the ^{1}H- and ^{13}C-NMR spectra (table 1) and HMQC data of compounds **3** and **4** showed characteristic signals for H-2 and C-2 of isoflavones at δ 7.82, 152.1 and 7.82, 153.3 ppm, respectively [12,13]. In the ^{1}H-NMR spectrum of **3** the two doublets at δ 6.99, 7.43 (2H each, $J = 8$ Hz) were correlated with carbons at 113.5 and 130.1 ppm in its HMQC spectrum. In the spectra of **4**, proton signals at δ 6.99, 7.45 (2H each, $J = 8$ Hz) were correlated with carbons at 114.2, 130.8 ppm in an HMQC experiment;

these shifts and correlations were diagnostic for a 4′-monooxygenated B ring. Both spectra indicated the presence of an OCH_3 group (δ 3.83, 54.4 ppm in **3** and δ 3.82, 56.8 ppm in **4**). In NOE experiments, irradiation of the OCH_3 singlet resulted in significant enhancement of the doublets at δ 6.99 assigned to H-3′ and H-5′ in **3** and **4**. These results confirmed unambiguously the position of the OCH_3 at C-4′.

The ^{1}H-NMR singlets at δ 6.36 in the spectra of **3** and **4** indicated a trisubstituted A ring. The fact that both **3** and **4** gave positive reactions with Gibbs' reagent [14] enabled the assignment of these singlets to H-8, and this assignment was supported by characteristic ^{1}H-NMR singlets for the C-5 hydroxyl protons at δ 13.13 (**3**) and δ 13.16 (**4**).

The main differences between **3** and **4** were observed in the signals assigned for the C-6 prenyl substituents. In **3** the ^{1}H-, ^{13}C-NMR COSY, HMQC and HMBC spectra indicated the presence of CH_2 (δ 3.23, d, $J = 9$ Hz; 27.4 ppm), CH–O (δ 4.82, d, $J = 9$ Hz; 91.7 ppm), C–O (70.9 ppm), CH_3 (δ 1.22, s; 24.2 ppm) and CH_3 (δ 1.38, s; 26.0 ppm) groups, which were assigned to a 3″-hydroxyprenyl residue forming a furan ring with a hydroxyl group [15,16]. The signal at δ 13.13 for the C-5 hydroxyl proton noted above indicates that cyclization utilized the C-7 hydroxyl rather than the C-5 group. HMBC correlations were in full agreement with this proposed structure. Thus HMBC correlations were observed from the C-1″ CH_2 protons of the isoprenyl group at δ 3.23 to the C-5 and C-7 carbons at 166.3 and 163.6 ppm, confirming that the isoprenyl substituent was cyclized onto C-6 instead of C-8. The molecular formula was confirmed to be $C_{21}H_{20}O_6$ by HREIMS, with M^+ m/z 368.127 (calculated value for $C_{21}H_{20}O_6$ is 368.126). The unreported compound **3** was thus assigned as 4′-*O*-methylerythrinin C [15]. Lack of material prevented the assignment of absolute stereochemistry, but it is most probably the same as that of erythrinin C.

The HREIMS of **4** (M^+ m/z 368.126 for $C_{21}H_{20}O_6$) indicated that it is a structural isomer of **3**. The ^{1}H-NMR spectrum of **4** showed a singlet at δ 13.14 characteristic for a free C-5 hydroxyl group. The prenyl signals in the ^{1}H and ^{13}C-NMR spectra of **4** showed the same number and type of protons and carbons as in **3**, but with significant differences in their chemical shifts. The signals in **4** were CH_2 (δ_H 2.80, 3.02, dd, $J = 6.17$ Hz; δ_C 25.5 ppm), CH–O (δ_H 3.88, m; δ_C 68.6 ppm), C–O (δ_C 78.2 ppm), CH_3 (δ_H 1.38, s; δ_C 22.4 ppm) and CH_3 (δ_H 1.52, s; δ_C 25.2 ppm). These data supported a structure in which a hydroxy prenyl group forms a pyran ring with the C-7 hydroxyl group [10,17]. Comparison of ^{1}H and ^{13}C-NMR data with those of 2″-hydroxydihydroalpinumisoflavone [10] indicated that the compounds were identical except for the presence of an additional *O*-methyl group in **4**. Compound **4** is thus assigned the structure 4′-*O*-methyl-2″-hydroxydihydroalpinumisoflavone; this is the first report of its isolation from a natural source.

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 UV-1201 Shimadzu spectrometer.

NMR spectra were recorded on a JEOL 500 NMR instrument at 500 MHz for ^{1}H and 125 MHz for ^{13}C. MS were taken on a VG 7070 E-HF.

3.2. *Plant material*

The plants of *Lotus polyphyllos* Clarke were collected from the Northern Coastal area 21 Km west of Alexandria in April 2002. The plants were identified by Dr Sanyia Kamal, Department of Botany, Faculty of Science, University of Alexandria. A voucher specimen L-2 is deposited in the Pharmacognosy Department, Faculty of Pharmacy, University of Alexandria, Egypt.

3.3. *Extraction and isolation*

The fresh roots (2 kg) were sliced and extracted with 90% alcohol (10 L) to exhaustion. The concentrated alcohol extract (250 mL) was diluted with same volume of distilled water and was successively extracted with light petroleum (3×300 mL) CH_2Cl_2 (3×300 mL), EtOAc (3×250 mL), and *n*-butanol (3×200 mL). The CH_2Cl_2 and EtOAc extracts were combined to give 19.8 g of combined extract. A 15 g portion of this extract was fractionated using silica gel column chromatography (300 g, 5 cm diameter) eluting with $CHCl_3$ then $CHCl_3$–MeOH mixtures. Forty fractions of 150 mL were collected and screened by TLC, and similar fractions were combined.

Fractions 3 and 4 (120 mg) from this original column, eluted with 0.5% MeOH in $CHCl_3$, were purified by column chromatography, PTLC using $CHCl_3$–MeOH (19 : 1), and by HPLC on a C-18 column with MeOH–H_2O (9 : 1) as mobile phase to afford **5** (14 mg).

Fractions 5–16 (3.5 g) of the original column, eluted with 1% MeOH in $CHCl_3$, were rechromatographed on a silica gel column (100 g, 2 cm diameter) eluting with light petroleum-$CHCl_3$ mixtures. Thirty fractions of 50 mL were collected, and similar fractions were combined. Fractions eluted with light petroleum–$CHCl_3$ (1 : 2) (200 mg) were further purified on a silica gel column (30 g, 1 cm diameter). Elution with $CHCl_3$ followed by crystallization from $CHCl_3$ afforded 13 mg of **1**. Fractions eluted with light petroleum–$CHCl_3$ (1 : 3) (175 mg) were crystallized from MeOH to afford 40 mg of **2**. Fractions eluted with $CHCl_3$ (368 mg) afforded 25 mg of **3** and 8 mg of **4** after repeated column chromatography, PTLC using 5% MeOH in $CHCl_3$, and HPLC on a C-18 column with MeOH–H_2O (19 : 1) as mobile phase. Fractions eluted with 1% MeOH in $CHCl_3$ (300 mg) afforded **6** (50 mg) on crystallization from MeOH.

4′-*O*-Methylerythrinin C (3): UV $C_{21}H_{20}O_6$, Yellowish crystals, m.p. 178–179°C, λ_{max}^{MeOH} nm: 208, 266, 345 (sh); NaOMe: 209, 265; $AlCl_3$: 209, 280, 389; $AlCl_3$/HCl: 208, 280, 386. ^{1}H- and ^{13}C-NMR data (table 1). EIMS (rel. int., %): *m*/*z* 368 (M^+, 18), 335 (3), 297 (M^+–C_4H_8O, 21), 268 (5), 165 (12), 132 (19), 117 (11), 89 (22), 71 (18), 57 (38), 43 (100). HREI-MS: *m*/*z* 368.127 (calculated for $C_{21}H_{20}O_6$, 368.126).

4′-*O*-methyl-2″-hydroxydihydroalpinumisoflavone (4): $C_{21}H_{20}O_6$, Yellowish crystals, m.p. 107–108°C, UV λ_{max}^{MeOH} nm: 207, 267; NaOMe: 208, 268; $AlCl_3$: 207, 220, 380, 325 (sh), 375 (sh); $AlCl_3$/HCl: 207, 220, 380, 325 (sh), 375 (sh). ^{1}H- and ^{13}C-NMR data (table 1). EIMS (rel. int., %): *m*/*z* 368 (M^+, 6), 335 (2), 297 (M^+- C_4H_8O, 10), 268 (3), 165 (8), 132 (9), 107 (13), 89 (18), 77 (32), 57 (34), 43 (100). HREI-MS: *m*/*z* 368.126 (calculated for $C_{21}H_{20}O_6$, 368.126).

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