TWO ISOFLAVANS AND A 3-ARYLCOUMARIN FROM THE ROOTS OF *LOTUS LALAMBENSIS* GROWING IN SAUDI ARABIA

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ABSTRACT

Phytochemical investigation of *Lotus lalambensis* roots resulted in the isolation and identification of two isoflavan derivatives; vestitol (**1**) and lotisoflavan (**3**). Lotisoflavan (**3**) is isolated for the first time from plants under normal conditions. In addition a 3-arylcoumarin derivative; 2',7-dihydroxy-4',5-dimethoxy-3-arylcoumarin (**2**) was also isolated. Structures of the compounds were elucidated utilizing UV, [α], 1D- 2D-NMR spectroscopy as well as MS. The positions of the methyl groups were unequivocally assigned based on combination of HMBC and NOESY experiments. *www.relaquim.com*

Keywords: *Lotus lalambensis*; Fabaceae; 3-arylcoumarin; isoflavans

RESUMEN

La investigación fitoquímica de las raíces de *Lotus lalambensis* dió como resultado el aislamiento y la identificación de dos isoflavanos: vestitiol (**1**) y lotisoflavano (**3**). El lotisoflavano (**3**) es aislado por vez primera de plantas en condiciones normales. También se aisló una 3-arilcumarina: 2',7-dihidroxi-4',5-dimetoxi-3-arilcumarina (**2**). Las estructuras de de los compuestos se elucidaron utilizando espectroscopía UV, [α], 1D-, 2D-RMN así como también EM. La posición de los grupos metilo se asignaron inequívocamente por la combinación de los experimentos HMBC y NOESY. *www.relaquim.com*

Palabras clave: *Lotus lalambensis*; Fabaceae; 3-arilcumarina; isoflavanos

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INTRODUCTION

The genus *Lotus*, belonging to family Fabaceae, contains approximately 100 species distributed throughout the world, especially around the Mediterranean region (Evans, 2002). About twelve *Lotus* species are present in Saudi Arabia (Migahid, 1978; Chaudhary, 2001). In folk medicine, plants of genus *Lotus* are used as contraceptives, prophylactics and treatment of sexually transmitted disorders and peptic ulcers (El-Mousallami *et al.* 2002). *Lotus halophilus* has a good antimicrobial activity against Gram-positive, Gram–negative bacteria and fungi (Mahasneh, 2002). Flavones and flavonols are the most common constituents of the aerial parts of *Lotus* species (El-Mousallami *et al.* 2002; Abdel-Ghani *et al.* 2001; Abdel-Kader *et al.* 2007). Previously we reported on the isolation and identification of the aerial parts constituents of *Lotus lalambensis* Schweinf (El-Youssef *et al.* 2008a; El-Youssef *et al.* 2008b). The roots of such plants are usually rich in isoflavone derivatives (Yang, Gao *et al.* 1989; Mahmoud *et al.* 1990; Abdel-Kader *et al.* 2006). Previous investigation of the roots of *Lotus polyphyllos* resulted in the isolation of 3- arylcoumarin derivative (Abdel-Kader *et al.* 2008). In the present study, isoflavans and 3-arylcoumarin were identified from the roots of *Lotus lalambensis*.

MATERIALS AND METHODS

General

Melting points were determined in open capillary tubes using Thermosystem FP800 Mettler FP80 central processor supplied with FP81 MBC cell apparatus, and were uncorrected. Ultraviolet absorption spectra were obtained in methanol and with different shift reagents on a Jasco UV-Visible V-630 spectrophotometer. Specific rotations were measured on a Jasco P-2000 polarimeter, using a one-decimeter tube. ^{1}H and ^{13}C NMR spectra were recorded on a UltraShield Plus 500MHz (Bruker) (NMR Unite, College of Pharmacy, Salman Bin Abdulaziz University) spectrometer operating at 500 MHz for proton and 125 MHz for carbon, respectively. The chemical shift values were reported in δ (ppm) relative to the residual solvent peak, the coupling constants (*J*) were reported in Hertz (Hz). 2D-NMR experiments (COSY, NOESY, HSQC and HMBC) were obtained using standard Bruker programs. ESIMS were measured using an Agilent Technologies model 6410 Triple quadrupole LC/MS system. Centrifugal preparative TLC (CPTLC) was preformed using Chromatotron (Harrison Research Inc. model 7924): 2 mm silica gel P254 disc. Silica gel 60/230–400 mesh (EM Science) and RP C-18 silica gel 40-63/230-400 mesh (Fluka) were used for column chromatography, while silica gel 60 F254 (Merck) was used for TLC.

Plant material

The roots of *Lotus lalambensis* Schweinf were collected in March, 2010 from Aqubat Al-Abnaa Baljorashi, southern region of Saudi Arabia. The plant was identified by Dr. M. Atiqur Rahman, Prof. of Taxonomy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (#11180) was deposited at the herbarium of the Research Center for Medicinal, Aromatic and Poisonous Plants of the same college.

Extraction and Isolation

Air-dried powdered roots of *L. lalambensis* (150 g) were exhaustedly extracted with 90% ethanol (2 L) at room temperature. The ethanol extract was evaporated under vacuum to yield 12 g of dark brown residue. The residue was dissolved in 0.3 L H_2O/MeOH (4:6) and successively extracted with hexane (3 X 0.2 L), $CHCl_3$ (3 X 0.25 L) and EtOAc (3 X 0.2 L).

Evaporation of the hexane soluble fraction under vacuum left 0.8 g residue. Part of the hexane soluble fraction (0.38 g) was

subjected to CPTLC (2 mm silica gel disc) using 15% EtOAc in hexane with gradual increase of the EtOAc contents. 100 Fractions 5 ml each were collected, screened by TLC and similar fractions were combined. Fractions eluted with 35% EtOAc in hexane yielded 50 mg of β-sitosterol.

The residue left after evaporation of $CHCl_3$ (2 g) were subjected to silica gel column chromatography (80 g, 2.5 cm). Elution started with 5% EtOAc in $CHCl_3$ and polarity was increased by increasing the EtOAc contents in a gradient elution technique. Twenty fractions, 150 ml each were collected, screened by TLC and similar fractions were pooled. Fractions 6- 14 (150 mg) were further purified over C_{18} silica gel column (20 g, 2.5 cm) eluted with 25% H_2O in MeOH. Eighteen fractions 30 ml each were collected, screened by TLC and similar fractions were pooled. Fractions 12- 15 provided 26 mg of **1**.

The EtOAc soluble fraction (1.4 g) was subjected to column chromatography on Sephadex LH-20® (30 g, 2.5 cm) eluting with MeOH. Fraction 3 (120 mg) was further purified using by CPTLC (2 mm silica gel disc) using 25% EtOAc in hexane to give 10 mg of **2**. Fractions 8- 10 (170 mg) were subjected to C_{18} column chromatography eluting with 10% H_2O in MeOH to yield 17 mg of **3**.

(-) Vestitol (1). $C_{16}H_{16}O_4$, white crystals, m.p. 145- 146 ˚C, $[\alpha]_D$ – 74.25° (*c* =0.4, MeOH). UV λ_{max} nm: (MeOH) 225, (NaOMe) 290. ^{1}H- and ^{13}C-NMR (CD_3OD): Table 1. ESIMS (rel. abund. %): 295 (M^++Na, 34), 273 (M^++H, 100), 272 (M^+, 23). ESIMS (rel. abund. %) negative mood: 271 (M^+-1, 100).

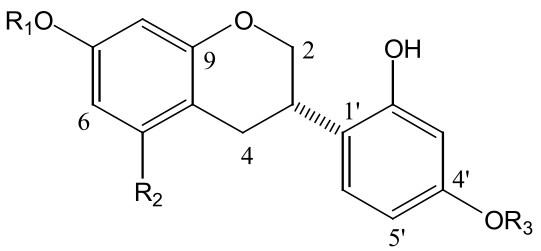
2',7-Dihydroxy,4',5-dimethoxy-3-arylcoumarin (2). $C_{17}H_{14}O_6$, yellow crystals, m.p. 258- 260 ˚C, UV λ_{max} nm: (MeOH) 210, 259, 351, (NaOMe) 275, 393, (NaOAc) 213, 262, 271, 373. ^{1}H- and ^{13}C-NMR (DMSO d_6 and Pyridine d_5): Table 1. ESIMS (rel. abund. %): 315 (M^++H, 28), 314 (M^+, 100). ESIMS (rel. abund. %) negative mood: 313 (M^+-1, 100).

(-) Lotisoflavan (3). $C_{17}H_{18}O_5$, semisolid, $[\alpha]_D$ – 13.5° (*c* =0.3, MeOH). UV λ_{max} nm: (MeOH) 279, (NaOMe) 291. ^{1}H- and ^{13}C-NMR (CD_3OD): Table 1. ESIMS (rel. abund. %): 325 (M^++Na, 36), 303 (M^++H, 100), 302 (M^+, 17). ESIMS (rel. abund. %) negative mood: 301 (M^+-1, 100).

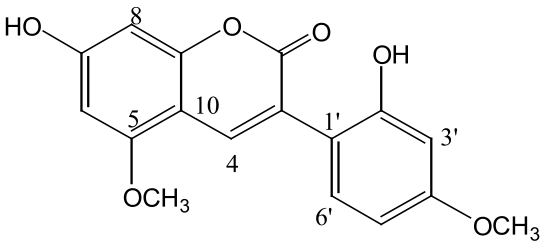
RESULTS AND DISCUSSION

L. lalambensis roots were extracted with ethanol followed by liquid-liquid fractionation. Chromatographic purification of the hexane fraction afforded β-sitosterol. While the $CHCl_3$ and EtOAc fractions afforded two isoflavans (**1**), (**3**) and an arylcoumarin (**2**). Structures were elucidated based on 1D- and 2D-NMR as well as MS data.

The UV spectrum of **2** (210, 259, 351 nm)(experimental) as well as the ^{1}H-NMR (DMSO *d6*) singlet at δ_H 7.88 correlated to carbon at δ_C 136.3 (table 1) in an HSQC experiment were diagnostic for 3-arylcoumarin skeleton (Murray *et al.* 1982; Hatano *et al.* 1989; Macías *et al.* 1999; Hatano *et al.* 2000).The shift in the UV spectrum produced after addition of NaOAc (213, 262, 271, 373 nm) indicated a C-7 free hydroxyl group (Jurd, 1959). ^{1}H-NMR showed a typical ABX system (δ_H 7.19, d, *J*= 8.5 Hz; 6.43, dd, *J*= 8.5, 1.5 Hz; 6.46, d, *J*= 1.5 Hz) and two coupled broad singlets at δ_H 6.35 and 6.37 ppm. The ^{1}H- and ^{13}C-NMR also showed two methyl singlets at δ_H 3.70; δ_C 55.0 and δ_H 3.86; δ_C 56.0 ppm. The ^{1}H- and ^{13}C-NMR data of **2** are closely related to the previously reported 4',6'-dihydroxy,7,2'-dimethoxy-3-arylcoumarin (Abdel-Kader *et al.* 2008). However, UV shift with NaOAc represents a major significant difference. ^{1}H-, ^{13}C-NMR, COSY, HSQC data of **2** can be assigned to either 5,7,2',4'- or 7,2',4',6'-tetra oxygenated skeleton (Abdel-Kader *et al.* 2008; Agrawal, 1989). In the second possibility, taking in consideration the presence of free C-7 hydroxyl group, the two methoxyl must be present in the 3-aryl ring.



1 R_1=R_2= H R_3= CH_3
3 R_1= CH_3 R_2= OCH_3 R_3=H



2

Figure 1. The structures of compounds **1-3**.

The results of NOESY experiment in both DMSO *d6* and Pyridine *d5* were crucial for the determination of the structure of **2** (Fig. 1). The methoxy singlet at δ_H 3.86 showed NOE correlation with the broad singlet at δ_H 6.35 while the other methoxy singlet at δ_H 3.70 showed NOE correlations with the two protons of the ABX system at δ_H 6.43 (dd, *J*= 8.5, 1.5 Hz) and δ_H 6.46 (d, *J*= 1.5 Hz). Due to the crowding of the aromatic protons in very narrow area of the spectrum data for **2** were collected in Pyridine *d5* for better resolution. Similarly the methoxy singlet at δ_H 3.73 showed NOE correlation with the doublet at δ_H 6.62 while the other methoxy singlet at δ_H 3.70 showed NOE correlations with the two protons of the ABX system at δ_H 6.73 (dd, *J*= 2.4, 8.5 Hz) and δ_H 6.95 (d, *J*= 2.4 Hz). These results indicated that the two mthoxyl groups are located on two different rings. The δ_H 3.70 methoxyl must be located at C-4' position of the aryl moiety, while the second methoxyl (δ_H 3.86 in DMSO *d6* and δ_H3.73 in Pyridine *d5*) was assigned to C-5 position. From the above data **2** was identified as 2',7-dihydroxy, 4',5-dimethoxy-3-arylcoumarin.

Compound (**1**) was identified as (-) vestitol a common phytoalexin with Anti-*Helicobacter pylori* activity (Hamburger *et al.* 1987; Russell *et al.* 1978; Fukai *et al.* 2002).

The ^{1}H- and ^{13}C-NMR data of **3** (Table 1) were typical for an isoflavan derivative with 5,7,2',4'-tetra oxygenation (Agrawal, 1989). In comparison with **1** compound **3** posses an additional methoxyl group. In an NOESY experiments both methoxyl groups at δ_H 3.74 and δ_H 3.79 showed NOE correlation with the proton doublet at δ_H 6.10 (*J*=2.5 Hz) while the methoxyl at δ_H 3.74 showed another NOE correlation with the doublet at δ_H 6.02 (*J*=2.5). Consequently, the methoxyl groups at δ_H 3.79 and δ_H 3.74 were assigned to positions 5 and 7 respectively. Literature search revealed that **3** is the phytoalexin lotisoflavan isolated from two lotus species infected with *Helminthosporium carbonurn*, however, identification was accomplished by UV, MS as well as TLC comparison with synthesized sample (Ingham, & Dewick, 1980). The concept of phytoalexins was introduced over 70 years ago (Müller, & Börger, 1940). Phytoalexins are low molecular mass secondary metabolites with antimicrobial activity produced under stress conditions including attack by potential pathogens. They represent an important part of the plant defense repertoire (Hammerschmidt, 1999; Pedras *et al.* 2011). The field of phytoalexins has evolved also due to their health-promoting effects (Pedras *et al.* 2011; Yang *et al.* 2009; Boue *et al.* 2009; Ng *et al.* 2011). Although **3** was isolated as phytoalexin produced under infection, this is the first time to report **3** as a secondary metabolite under normal conditions as well as recording its full NMR data.

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

	1[b]		2[c]		2[d]		3[b]	
Position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2	3.94 (1H, t, J=10.2) 4.22 (1H, dt, J= 2.3, 10.2)	71.1	-	162.0	-	163.7	3.92 (1H, t, J=10) 4.20 (1H, dt, J= 2.3, 10)	71.2
3	3.45 (1H,m)	33.2	-	118.6	-	120.7	3.37 (1H,m)	32.6
4	2.78 (1H, dd, J=10.5, 16.5) 2.93 (1H, dd, J= 4.3, 15.2)	31.4	7.88 (1H, s)	136.3	8.40 (1H, s)	138.0	2.64 (1H, dd, J=11, 16.5) 2.80 (1H, dd, J= 5.4, 16.9)	26.1
5	6.88 (1H, d, J=8)	131.2	-	160.1	-	162.0	-	160.1
6	6.33 (1H, dd, J=2.3, 8)	109.1	6.35 (1H, bs)	95.3	6.62 (1H, d, J=1.7)	96.4	6.10 (1H, d, J=2.5)	94.6
7	-	157.5	-	156.8	-	158.3	-	160.8
8	6.25 (1H, d, J=2.3)	103.9	6.37 (1H, bs)	94.6	6.71 (1H, d, J=1.7)	95.9	6.02 (1H, d, J=2.5)	91.9
9	-	156.4	-	155.5	-	157.2	-	157.2
10	-	115.0	-	102.3	-	104.1	-	105.3
1'	-	121.5	-	115.2	-	117.7	-	120.4
2'	-	157.2	-	156.0	-	158.2	-	157.1
3'	6.34 (1H, d, J=2.5)	102.6	6.46 (1H, d, J=1.5)	101.4	6.95 (1H, d, J=2.4)	103.3	6.34 (1H, d, J=2.5)	103.7
4'	-	160.9	-	160.0	-	161.7	-	158.0
5'	6.41 (1H, dd, J=2.5, 7.5)	105.9	6.43 (1H, dd, J=1.5, 8.5)	104.5	6.73 (1H, dd, J=2.4, 8.5)	105.4	6.28 (1H, dd, J=2.5, 7.5)	107.7
6'	6.98 (1H, d, J=7.5)	128.8	7.19 (1H, d, J=8.5)	131.5	7.67 (1H, d, J=8.5)	133.0	6.87 (1H, d, J=7.5)	128.8
5-OCH$_3$	-	-	3.86 (3H,s)	56.0	3.73 (3H,s)	56.2	3.79 (3H,s)	55.9
7-OCH$_3$	-	-	-	-	-	-	3.74 (3H,s)	55.7
4'-OCH$_3$	3.74 (3H,s)	55.6	3.70 (3H,s)	55.0	3.70 (3H,s)	55.5	-	

[a] Assignments made by combination of COSY, DEPT, HSQC, HMBC, NOESY data, and comparison with the literature.
[b] Data were collected in CD_3OD.
[c] Data were collected in DMSO-*d6*.
[d] Data were collected in Pyridine-*d5*.

CONCLUSION

The results of the present study are in complete agreement with the previous investigations of the roots of members of the family Fabaceae. Isoflavone derivatives are the dominant secondary metabolites of such plant roots. On searching for isoflavones as natural xenoestrogens the roots of such plants must be considered as the most reliable plant source. Further pharmacological and toxicological evaluation of the root extract of *L. lalambensis* is required.

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