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Flavone C-glycosides from *Lupinus hartwegii*

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Abstract

From the aerial parts of *Lupinus hartwegii*, two new flavone C-glycosides apigenin-7-*O*-β-apiofuranosyl-6, 8-di-*C*-β-glucopyranoside (**1**) and apigenin-7-*O*-β-apiofuranosyl-6-*C*-β-glucopyranosyl-8-*C*-(6‴-*O*-*E*-feruloyl)- β-glucopyranoside (**2**) have been isolated together with two known isoflavonoid glucosides genistein-7-*O*-β-glucopyranoside (**3**) and genistein-7, 4′-di-*O*-β-glucopyranoside (**4**) as well as two known compounds ferulic acid 4-*O*-β-glucopyranoside (**5**) and sparteine (**6**). The structures of the isolated compounds were verified by means of MS and NMR spectral analyses.

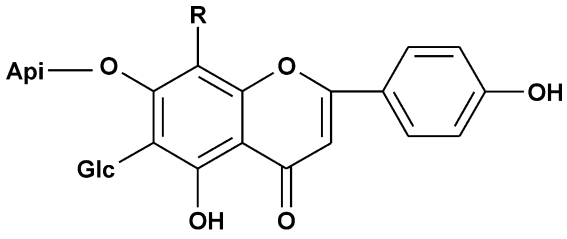
Keywords: *Lupinus hartwegii*; Leguminosae; Flavone C-glycosides; Isoflavonoid glucosides

1. Introduction

Lupinus hartwegii Lindl (*L. maxicanus*) is a cultivated plant in Egypt and known as Termis Al-zuhoor. Fifteen alkaloids were detected in the different organs of the plant by capillary GC and GC–MS techniques (El-Shazly et al., 2001). Moreover, two triterpenes (dimethyl serratagenate and acetyl dimethyl serratagenate) together with three coumaranochromones (lupinalbins A, C and E) and three common isoflavones (genistein, luteone and 2′-hydroxy genistein) have been already detected in the aerial parts of *L. hartwegii* (Hassanean, 1998). This work describes the isolation and structural elucidation of two new flavone C-glycosides together with two known isoflavonoid glucosides, one known phenolic glucoside and one known alkaloid from the aerial parts of *L. hartwegii*.

2. Results and discussion

The ethanolic extract of the aerial parts of *L. hartwegii* was defatted with diethylether and the aqueous layer was subjected to column chromatography on Diaion HP-20. The methanol–H_2O (1:1) and methanol eluates were repeatedly chromatographed on columns of silica gel and then by MPLC and HPLC to afford four compounds (**1**–**4**) from the 50% methanol eluate and two compounds (**5**, **6**) from the methanol eluate.



Compd	R
(1)	Glc
(2)	Glc (6‴– feruloyl)

The molecular formula of compound **1** was deduced as $C_{32}H_{38}O_{19}$ from HR FAB-MS spectrometry (see experimental section). Inspection of the ^{13}C NMR (Table 1) and DEPT spectra of **1** displayed the presence of apigenin as aglycone together with three sugar residues. The identity of apigenin was verified from the two signals at δ_C 129.0 and 115.9 (each one for two methine carbons) revealing the A_2B_2 system of ring-B of the aglycone (C-2′, 6′ and C-3′, 5′ respectively) and the methine carbon signal at δ_C 102.5 was assigned to C-3

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Table 1
^{13}C NMR spectral data of compounds **1** (DMSO) and **2** (CD_3OD) (100 MHz)

C	1	2	C	1	2
2	164.1	166.6	Glc		
3	102.5	103.7	**1‴,1⁗**	74.7, 74.0	76.9, 76.1
4	182.3	184.1	**2‴,2⁗**	72.2, 71.6	74.0, 73.4
5	158.6	160.3	**3‴,3⁗**	78.9, 78.9	79.8, 79.1
6	107.2	107.6	**4‴,4⁗**	70.6, 69.0	72.2, 71.1
7	161.2	162.0	**5‴,5⁗**	81.8, 80.9	82.7, 80.7
8	104.8	106.0	**6‴,6⁗**	61.1, 59.7	62.9, 64.0
9	155.5	157.8			
10	103.7	105.4	Fer		
1′	121.6	123.4	**1⁗′**		127.6
2′	129.0	130.0	**2⁗′**		111.4
3′	115.9	117.0	**3⁗′**		150.5
4′	160.4	162.5	**4⁗′**		149.2
5′	115.9	117.0	**5⁗′**		116.5
6′	129.0	130.0	**6⁗′**		124.5
Api			**7⁗′**		114.8
1″	109.3	110.9	**8⁗′**		147.4
2″	77.8	78.3	**9⁗′**		168.9
3″	79.1	80.7	Ome		56.5
4″	72.9	74.3			
5″	64.0	65.2			

Api: β-apiofuranose, Glc: β-glucopyranose, Fer: feruloyl.

(Markham and Chari, 1982). This identity was supported by the ^1H NMR spectrum of **1** (Table 2) that showed two *ortho*-coupled doublet signals at δ_H 8.2 and 6.8 (each 2H, *d*, $J=8.8$ Hz) assignable to H-2′, 6′ and H-3′, 5′ respectively together with a singlet at δ_H 6.7 for H-3. The absence of the two meta coupled protons of H-6 and H-8 in the ^1H NMR spectrum of **1** together with the downfield shifts of their carbon signals in the ^{13}C NMR spectrum to δ_C 107.2 and 104.8 respectively indicated the glycosylation of the aglycone at these positions (Agrawal, 1989). On the other hand, the three sugar residues have been identified as β-apiofuranose and two β-glucopyranose units from the ^{13}C NMR spectrum (Table 1) (Bradbury and Jenkins, 1984; Markham et al., 1982). The upfield shifts of the anomeric carbon signals of the glucosyl residues (δ_C 74.7, 74.0) indicates that these glucosyl units are C-linked to C-6 and C-8 of the aglycone (Agrawal, 1989). The attachment of the apiosyl unit to the hydroxyl group at C-7 was established from the chemical shift of C-4 of the aglycone at δ_C 182.3. If this glycosylation had occurred at the 5-hydroxyl group, the hydrogen bonding between the 5-hydroxyl and the carbonyl would be broken leading to a significant upfield shift of C-4 and downfield shift of C-3 (Markham et al., 1978). From the ^1H NMR spectral data of **1** (Table 2), the coupling constant of the doublet at δ_H 5.2 ($J=4.4$ Hz) representing the anomeric proton of the apiofuranosyl unit, and the two doublets at δ_H 4.6 ($J=9.5$ Hz) and 4.1 ($J=9.0$ Hz) of the two glucopyranosyl units, proved their β-configuration. The negative ion FAB-MS spectrum of **1** exhibited M^+ at *m*/*z* 725 $[M-H]^-$ as well as a significant peak at *m*/*z* 593 $[M-H-apiose]^-$. Consequently, the structure of compound **1** was determined as apigenin-7-*O*-β-apiofuranosyl-6, 8-di-*C*-β-glucopyranoside.

Table 2
^1H NMR spectral data of compounds **1** (DMSO) and **2** (CD_3OD) (400 MHz)

H	1	2
3	6.7, *s*	7.1, *s*
2′,6′	8.2, *d* (8.8)	7.8, *d* (8.5)
3′,5′	6.8, *d* (8.8)	6.8, *d* (8.5)
Api-1″	5.2, *d* (4.4)	5.2, *d* (4.1)
Glc-1‴, 1⁗	4.6, *d* (9.5), 4.1, *d* (9)	5.0, *d* (9.5), 4.9, *d* (10)
C-5 OH	13.7, *s*	
Fer-2⁗′		6.7, *d* (2)
Fer-5⁗′		6.5, *d* (8.1)
Fer-6⁗′		7.0, *dd* (2, 8.1)
Fer-7⁗′		6.3, *d* (15.9)
Fer-8⁗′		7.6, *d* (15.9)
Ome		3.7, *s*

Chemical shifts in ppm, *J* values in parentheses in Hz.

The molecular formula of compound **2** was deduced as $C_{42}H_{46}O_{22}$ from HR FAB-MS spectrometry (see Experimental section). ^{13}C, ^1H NMR (Tables 1 and 2 respectively) and DEPT experiments of **2** revealed a similar pattern as those of **1**. However, 10 additional signals were present in the spectrum of **2** (see Table 1) and these are typical for a feruloyl moiety (Kamel et al., 2001). The identity of the feruloyl moiety was substantiated from the ^1H NMR spectrum of **2** (Table 2). The two doublets at δ_H 6.7 ($J=2.0$ Hz) and 6.5 ($J=8.1$ Hz) were assigned to H-2⁗′ and H-5⁗′ of the feruloyl moiety respectively while the doublet doublet at δ 7.0 ($J=2.0$, 8.1 Hz) represented H-6⁗′. Moreover the coupling constant (15.9 Hz) of the two doublets at δ_H 7.6 and 6.3 (H-8⁗′ and 7⁗′ respectively) indicated the *trans* configuration of the feruloyl moiety (Silverstein et al., 1996). The downfield resonance (δ_C 64.0, Table 1) for one C-6 of the two glucosyl units (C-6‴ or C-6⁗) indicated its substitution with this feruloyl moiety (Miyase et al., 1992). Because of the similarity of the chemical shifts of the two glucosyl units attached to C-6 and C-8 of the aglycone and due to the difficulty in determining which one is substituted with the feruloyl moiety at its C-6, 2D NMR experiments were performed. The HMQC spectral analysis of **2** revealed correlations between each carbon and its directly attached protons while the proton–proton couplings were established by measurements of H–H COSY. The HMBC spectral analysis (Fig. 1) revealed correlation peaks between H-1 of a glucopyranosyl unit (δ_H 4.9, H-1⁗) with C-8 of the aglycone (δ_C 106.0), H-1 of the second glucosyl unit (δ_H 5.0, H-1‴) and C-6 of the aglycone (δ_C 107.6) and H-1″ of the apiofuranosyl residue (δ_H 5.2) and C-7 (δ_C 162.0) of apigenin. Additionally, the 2D NOESY spectrum of **2** revealed a correlation peak between H-1⁗ of the glucopyranosyl

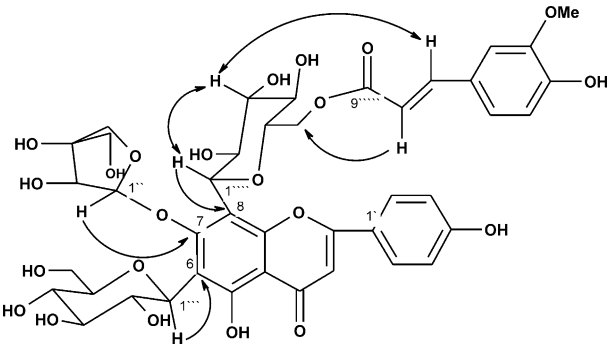

Fig. 1. Significant HMBC (→) and NOE (↔) correlations of compound **2**.

unit at C-8 (δ_H 4.9) and H-3‴″ of the same residue (δ_H 4.8, dd, $J=6.2$ and 7.3 Hz). H-3‴″ also showed a NOESY correlation peak with H-7⁗″ (δ_H 6.3) of the feruloyl moiety. Therefore, it is concluded that the feruloyl moiety is attached to C-6‴″ of the glucopyranosyl unit at C-8 of the aglycone that was confirmed from the correlation peak between H-8⁗″ (δ_H 7.6) of the feruloyl moiety and C-6‴″ (δ_C 64.0) of this glucosyl unit in the HMBC spectrum. The β configuration of the anomeric protons of the sugar residues was deduced from the ^{1}H NMR spectrum of **2** which showed three doublets at δ_H 5.2 ($J=4.1$ Hz), 5.0 ($J=9.5$ Hz) and 4.9 ($J=10.0$ Hz) for β-apiofuranosyl and two β-glucopyranosyl units respectively. The negative ion FAB-MS spectrum of **2** exhibited M^+ at m/z 901 $[M-H]^-$ as well as a significant peak at m/z 769 $[M-H-apiose]^-$. Consequently, the structure of compound **2** was determined as apigenin-7-*O*-β-apiofuranosyl-6-*C*-β-glucopyranosyl-8-*C*-(6‴″-*O*-*E*-feruloyl)- β-glucopyranoside.

After determination of the structures of compounds **3**–**6** by ^{1}H and ^{13}C NMR analyses, their structures were found to be known as follows: genistein-7-*O*-β-glucopyranoside (**3**), genistein-7, 4′-di-*O*-β-glucopyranoside (**4**) (Yasufumi et al., 2000; Agrawal, 1989), ferulic acid 4-*O*-β-glucopyranoside (**5**) (Baderschneider and Winterhalter, 2001) and sparteine (**6**) (Boczon and Skolik, 1989; Mohamed, 1991).

3. Experimental

^{1}H and ^{13}C NMR (TMS as int. standard): 400 MHz and 100 MHz respectively were recorded on a Jeol JNM α-400 spectrometer. MS spectra were recorded on a Jeol JMS-SX 102 spectrometer by direct inlet method at an ionizing voltage of 70 eV. MPLC: RP-18 column (20 mm i.d.×40 cm); flow rate of mobile phase 3 ml/min. HPLC: polyamine column (20 mm i.d.×25 cm, YMC) with a Toyo Soda high speed chromatograph HLC-803 D pump and a Tosoh refraction index (RI-8) detector; flow rate of mobile phase 6 ml/min, injection vol. 0.8–1.0 ml. CC: Kieselgel 60 (70–230 mesh, Merck) and Diaion HP 20 (Mitsubishi). TLC: silica gel 60 precoated plates, F-254 and HPTLC using RP-18 precoated plates, F-254 s (Merck).

3.1. Plant material

Aerial parts of *L. hartwegii* Lindl were collected from the Experimental Station of Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt in March 1996. The plant was identified by Professor A. Fayed, Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt. A voucher specimen is deposited at the Herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

3.2. Extraction and isolation of compounds (1–6)

The air dried powdered aerial parts of *L. hartwegii* (1.5 kg) were extracted with EtOH–H_2O (7:3). The dried ethanolic extract (130 g) was suspended in H_2O and defatted with diethylether. The aq. fr. was applied to a column of Diaion HP-20 and eluted with H_2O, MeOH–H_2O (1:1), MeOH and acetone successively. The MeOH–H_2O (1:1) eluate (25 g) was chromatographed on silica gel CC using EtOAc–MeOH–H_2O (85:15:1 and 75:25:2) to give five fractions. Fraction 4 eluted with EtOAc–MeOH–H_2O (75:25:2) was subjected to MPLC on ODS column and MeOH–H_2O (5.5:4.5) as a solvent system followed by HPLC using polyamine column and MeCN–H_2O (93:7) to afford compounds **1** (yellow powder, 80 mg), **2** (yellow powder, 25 mg), **3** (yellow powder, 200 mg) and **4** (yellow powder, 55 mg). The MeOH eluate (10 g) was chromatographed on silica gel CC using CH_2Cl_2–MeOH–H_2O (80:20:1) to afford 3 fractions. Fraction 2 was subjected to HPLC on polyamine column using MeCN–H_2O (95:5) as a solvent system to give compounds **5** (white powder, 30 mg) and **6** (white powder, 12 mg).

3.3. Compound (1)

Apigenin-7-*O*-β-apiofuranosyl-6, 8-di-*C*-β-glucopyranoside. R_t 18 min (polyamine, MeCN–H_2O, 93 : 7). UV λ_{max} MeOH nm: 274, 328. HR FAB-MS (negative) m/z: 725.6291 $[M-H]^-$ $C_{32}H_{37}O_{19}$ (req. 725.6250). ^{13}C NMR (DMSO, Table 1). ^{1}H NMR (DMSO, Table 2).

3.4. Compound (2)

Apigenin-7-*O*-β-apiofuranosyl-6-*C*-β-glucopyranosyl-8-*C*-(6‴″-*O*-*E*-feruloyl)- β-glucopyranoside. R_t 23 min (polyamine, MeCN- H_2O, 93:7). UV λ_{max} MeOH nm: 272, 325 and 291 (sh). HR FAB-MS (negative) m/z: 901.7873 $[M-H]^-$ $C_{42}H_{45}O_{22}$ (req. 901.7930). ^{13}C NMR (CD_3OD, Table 1). ^{1}H NMR (CD_3OD, Table 2).

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