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Flavone *C*-Glycosides from Seeds of Fenugreek, *Trigonella foenum-graecum* L.

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Fenugreek (*Trigonella foenum-graecum* L.) is particularly used in Asia, Africa, and Mediterranean countries for its nutritional and medicinal value. The flavone *C*-glycosides, apigenin 6-*C*-β-chinovopyranosyl-8-*C*-β-galactopyranoside (**6**) and apigenin 6-*C*-β-xylopyranosyl-8-*C*-(6‴-*O*-(3-hydroxy-3-methylglutaroyl)-β-glucopyranoside) (**7**), in addition to the known flavone *C*-glycosides, apigenin 6,8-*C*-di-β-galactopyranoside (**1**), apigenin 6-*C*-β-xylopyranosyl-8-*C*-β-galactopyranoside (**2**), apigenin 6-*C*-β-arabinopyranosyl-8-*C*-β-galactopyranoside (**3**), luteolin 8-*C*-β-glucopyranoside (**4**), luteolin 6-*C*-β-glucopyranoside (**5**), apigenin 8-*C*-β-glucopyranoside (**8**), apigenin 6-*C*-β-glucopyranoside (**9**), luteolin 8-*C*-(2″-*O*-(*E*)-*p*-coumaroyl-β-glucopyranoside) (**10**), and apigenin 8-*C*-(2″-*O*-(*E*)-*p*-coumaroyl-β-glucopyranoside) (**11**) were isolated from fenugreek seeds. Compounds **1**, **5**, and **10** were reported for the first time in this species. Signal duplication in the NMR spectra, with exception of spectra of the mono-6-*C*-substituted compounds, revealed the presence of rotameric conformers, created by rotational hindrance at the *C* (sp^3)-*C* (sp^2) glycosyl-flavone linkage in these flavone *C*-glycosides.

KEYWORDS: Fenugreek; *Trigonella*; seeds; flavone *C*-glycosides; rotameric conformers; chinovopyranosyl; 3-hydroxy-3-methylglutaroyl; 2D NMR

INTRODUCTION

Fenugreek is particularly used in Asia, Africa, and Mediterranean countries for the nutritional and medicinal values of its leaves (herbs) and seeds (spice) (*1*). It has a long history as a traditional medicinal plant used for treatment of diabetes (*2*). The aerial part of the plant has been used to treat renal diseases while the seeds have been used as a tonic and for stomach disorders (*3*). The seeds are rich in polyphenolic compounds including flavonoids (*4*), which have been correlated to the beneficial health effects of fenugreek (*5*,*6*). Almost four decades ago, Adamska and Lutomski (*7*) isolated the *C*-glycosylflavones vitexin and vitexin-7-glucoside from fenugreek seeds in addition to two compounds, which were tentatively identified as an arabinoside of either orientin or iso-orientin, and an unknown diglycoside. Seshadri et al. (*8*) reported acacetin 6,8-di-*C*-glucoside and its monoacetate from seeds of *Trigonella corniculata*, and later they found the 8-*C*-glucoside, the 6,8-di-*C*-glucoside, and the 6,8-di-*C*-glucoside monoacetate of apiginin in the seeds of *Trigonella corniculata* L., in addition to apigenin 6-*C*-glucoside and apigenin 8-*C*-glucoside in seeds of fenugreek (*9*). At the same time, Wagner et al. (*10*) independently reported apigenin 6-*C*-xyloside-8-*C*-glucoside (vicenin 1) and apigenin 6,8-di-*C*-glucoside (vicenin 2) in addition to apigenin 8-*C*-glucoside to occur in seeds of fenugreek. In 1976, Sood et al. isolated vitexin 2″-*O*-*p*-coumarate from the same species (*11*). Kawashty et al. (*12*) found kaempferol 3-glucoside, kaempferol 7-glucoside, kaempferol 3-galactosylglucoside, kaempferol 3,7-diglucoside, kaempferol 7-diglucoside-3-*p*-coumaryl-glucoside, quercetin 3-glucoside, quercetin 7-glucoside, quercetin 3-galactosyl-glucoside, quercetin 7-diglucoside-3-*p*-coumarylglucoside, 7,4′-dihydroxyflavone, 7,3′,4′-trihydroxyflavone, formononetin, kaempferol and quercetin from the aerial part of eight different *Trigonella* species. Han et al. (*3*) has reported kaempferol 3-*O*-β-D-glucosyl(1→2)-β-D-galactoside, kaempferol 3-*O*-β-D-glucosyl(1→2)-β-D-galactoside 7-*O*-β-D-glucoside, kaempferol 3-*O*-β-D-glucosyl(1→2)-(6″-*O*-acetyl)-β-D-galactoside 7-*O*-β-D-glucoside and quercetin 3-*O*-β-D-glucosyl(1→2)-β-D-galactoside 7-*O*-β-D-glucoside from the stems of fenugreek, while Yuldashev et al. (*13*) reported the presence of biochanin A, luteolin, quercetin, and the 7-*O*-β-D-glucopyranosides of quercetin and luteolin in *Trigonella grandiflora* (Fabaceae).

C-Glycosylflavones are important bioactive constituents of some medicinal plants (*14*), and they demonstrate a range of biological effects including antioxidant, antifungal, and antimicrobial activities ((*15*); references therein). They contain at least one nonhydrolyzable glycosidic unit attached to the flavone aglycone, and the recently reported transformation of these compounds to *C*-glycosylanthocyanidins (*16*) has the potential of overcoming problems normally related to hydrolytic instability of the naturally occurring anthocyanin-*O*-glycosides used as food colorants and nutraceuticals. Fenugreek appears to be a rich source of some *C*-glycosylflavones with restricted availability from other sources. In this paper, we report on the isolation and identification of two new and nine known *C*-glycosylflavones isolated from seeds of fenugreek.

MATERIALS AND METHODS

Voucher specimen of fenugreek has been deposited at the ARBOHA at the University of Bergen (accession number H507).

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Isolation of Flavones. Fenugreek was cultivated in Beit Noba (Palestine). Seeds of fenugreek (500 g) were crushed and extracted overnight 7 times with portions of 2 L of MeOH at 4 °C. The filtered extract was concentrated under reduced pressure, purified by partition against diethyl ether, and subjected to Amberlite XAD-7 column chromatography. Seven grams of XAD-7 purified extract was subjected to an 100 × 10 cm Sephadex LH-20 column using MeOH−H_2O/trifluoracetic acid (0.2%) containing increasing proportions of MeOH; 10% (2000 mL), 20% (1450 mL), 30% (2420 mL), 40% (4140 mL), 50% (1862 mL), 60% (3846 mL) (**S6**), 70% (3253 mL), 80% (2529 mL) (**S8**), and 85% (1616 mL) (**S9**). The flow rate was 18.5 mL min^{-1}. Compounds **7**, **1**, and (**2**, **3**, and **6** in mixture) were obtained after the elution of 303, 2341, 2841 mL of **S6**, respectively. Compounds **2**, **3**, and **6** were separated by preparative HPLC. Compounds **8** and **9** (in mixture) were eluted directly after changing the eluent to **S8**. Compounds **4** and **5** (in mixture) were eluted with **S9**.

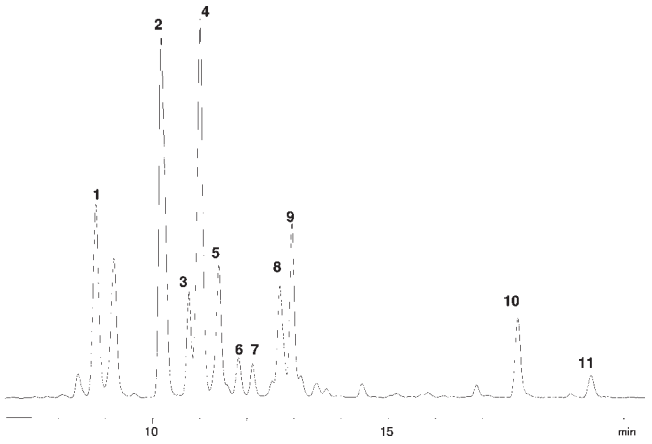


Figure 1. HPLC profile detected at 338 ± 20 nm of fenugreek seeds extract after subjecting it to Amberlite XAD-7 column chromatography purification.

Compounds **10** and **11** were isolated using a Toyopearl HW-40F column, in which 1.6 g of the XAD-7 purified material was used. The solvents used were water and acetonitrile (MeCN). The MeCN content in the elution profile was as follows: 10% (v/v) for the first 15 fractions, 20% in fractions 16−27, 30% in fractions 28−36, 40% in fractions 37−47, followed by 60% for the last five fractions, respectively. The volume of each fraction was 28−30 mL. Compounds **10** and **11** were isolated from fractions 47 and 41−44, respectively. Individual compounds (3−5 mg) were further purified by preparative HPLC.

Preparative HPLC. Preparative HPLC (Gilson 305/306 pump equipped with an HP-1040A photodiode array detector) was performed using an Econosil C18 column (250 mm × 22 mm; length × I.D., 10.0 μm). The elution profile consisted of pure water for the first 5 min, MeCN/H_2O (10:90; v/v) for the next 48 min, followed by MeCN/H_2O (20:80; v/v) for additional 15 min. The flow rate was 12 mL min^{-1}. Samples (about 10−15 mg) were dissolved in 50 mL of water and transported to and applicated on the column using the HPLC pump.

Analytical HPLC. Analytical HPLC was performed with an ODS-Hypersil column (20 × 0.5 cm, length × i.d., 5 μm) using the solvents A, H_2O containing 0.5% TFA (v/v) and B, acetonitrile containing 0.5% TFA (v/v). The following gradient was used: 10−15% B in 0−5 min, 15−40% B (linear gradient) from 5 to 21 min, 40% B from 21 to 23 min, 40−60% from 23 to 29 min. The flow rate was 1.0 mL min^{-1}.

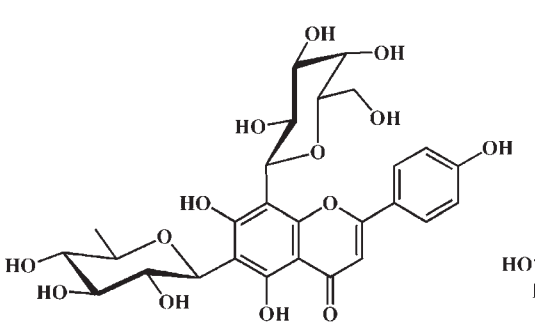
Spectroscopy. UV−vis absorption spectra were recorded online during HPLC analysis using a photodiode array detector (HP 1050, Hewlett-Packard). Spectral measurements were made over the wavelength range 240−400 nm in steps of 2 nm.

The NMR experiments (1D ^1H, 2D $^1H-^{13}C$ HMBC, $^1H-^{13}C$ HSQC, $^1H-^1H$ COSY, $^1H-^1H$ TOCSY, $^1H-^1H$ ROESY, $^1H-^1H$ NOESY, and

Table 1. Chromatographic (HPLC) and Spectral (UV and MS) Data Recorded for Compounds **1**−**11**

peak	t_R (min)	UV (λ_{max}) (nm)	[M^+] (*m*/*z*) observed	[M^+] (*m*/*z*) calculated	relative flavonoid content (%)[b]
1	8.8	336, 271	595.1612	595.1663	11
2	10.2	336, 271	565.1546	565.1557	20
3	10.8	336, 271	565.1533	565.1557	4
4	11.0	349, 270, 258	449.1108	449.1084	20
5	11.4	349, 268, 257	449.1108	449.1084	7
6	11.8	337, 272	579.1703	579.1714	2
7	12.1	336, 271	709.1955	709.1980	2
8	12.7	337, 269	433.1176	433.1135	6
9	12.9	337, 271	433.1176	433.1135	8
10	17.7	271, 302[a], 317, 360[a]	595.1031	595.1442	4
11	19.3	271, 301[a], 318	579.1090	579.1503	1

[a] Shoulder. [b] Recorded at 338 ± 20 nm without taken into account the different molar absorptivity values of individual flavonoids.



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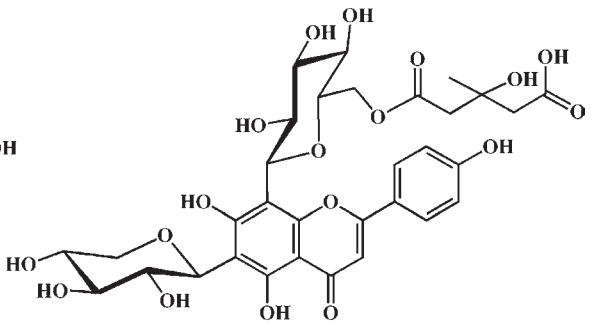

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Figure 2. Structures of apigenin 6-*C*-β-chinovopyranosyl-8-*C*-β-galactopyranoside (**6**) and apigenin 6-*C*-β-xylopyranosyl-8-*C*-(6‴-*O*-(3-hydroxy-3-methylglutaroyl)-β-glucopyranoside) (**7**) isolated from fenugreek seeds.

Table 2. ^{1}H Spectral Data (δ in ppm and J in Hz) for **1**−**11** Dissolved in DMSO-d_6 at 25 °C[a]

	1 Ma, 77% Mi, 23%	**2** Ma, 77% Mi, 23%	**3** Ma, 63% Mi, 37%	**4** Ma, 77% Mi, 23%	**5** Ma, 100%	**6** Ma, 63% Mi, 37%	**7** Ma, 59% Mi, 41%	**8** Ma, 77% Mi, 23%	**9** Ma, 100%	**10** Ma, 83% Mi, 17%	**11** Ma, 83% Mi, 17%
3	6.84 *s*	6.79 *s*	6.84 *s*	6.64 *s*	6.66 *s*	6.78 *s*	6.81 *s*	6.77 *s*	6.78 *s*	6.66	6.79
	6.80 *s*	6.80 *s*	6.79 *s*			6.82 *s*	6.79 *s*			6.58	6.67
6				6.27 *s*				6.27 *s*		6.14	6.14
										6.18	
8					6.49 *s*				6.51 *s*		
2′	8.30 *'d'* 8.9	7.96 *'d'* 8.9	8.30 *'d'* 8.9	7.49 *d* 2.2	7.40 *d* 2.3	7.95 *'d'* 8.8	7.96 *'d'* 8.9	8.02 *'d'* 8.8	7.92 *'d* 8.8	7.53 *'d'* 2.3	8.09 *'d'* 8.8
	7.96 *'d'* 8.9	8.30 *'d'* 8.9	7.95 *'d'* 8.9	7.40 (*b*)		8.30 *'d'* 8.9	7.94 *'d'* 8.9	7.91 *'d'* 8.8		7.47 *dd* 2.4, 0.6	7.98 *'d'* 8.9
3′	6.88 *'d'* 9.0	6.92 *'d'* 8.9	6.87 *'d'* 8.9			6.92 *'d'* 8.8	6.92 *'d'* 8.9	6.89 *'d'* 8.8	6.92 *'d'* 8.8		6.91 *'d'* 8.8
	6.93 *'d'* 8.9	6.89 *'d'* 8.9	6.93 *'d'* 8.9			6.87 *'d'* 8.9	6.92 *'d'* 8.9	6.90 *'d'* 8.8			7.02 *'d'* 8.9
5′	6.88 *'d'* 9.0	6.92 *'d'* 8.9	6.87 *'d'* 8.9	6.87 *d* 8.5	6.89 *d* 8.2	6.92 *'d'* 8.8	6.92 *'d'* 8.9	6.89 *'d'* 8.8	6.92 *'d'* 8.8	6.89 *d* 8.3	6.91 *'d'* 8.8
	6.93 *'d'* 8.9	6.89 *'d'* 8.9	6.93 *'d'* 8.9			6.87 *'d'* 8.9	6.92 *'d'* 8.9	6.90 *'d'* 8.8		7.47 *dd* 8.0, 0.6	7.02 *'d'* 8.9
6′	8.30 *'d'* 8.9	7.96 *'d'* 8.9	8.30 *'d'* 8.9	7.53 *dd* 8.5, 2.2	7.41 *dd* 8.2, 2.3	7.95 *'d'* 8.8	7.96 *'d'* 8.9	8.02 *'d'* 8.8	7.92 *'d'* 8.8	7.61 *dd* 8.3, 2.3	8.09 *'d'* 8.8
	7.96 *'d'* 8.9	8.30 *'d'* 8.9	7.95 *'d'* 8.9			8.30 *'d'* 8.9	7.94 *'d'* 8.9	7.91 *'d'* 8.8		7.45 *m*	7.98 *'d'* 8.9
	6-*C*-gal	6-*C*-xyl	6-*C*-ara		6-*C*-glc	6-*C*-chin	6-*C*-xyl		6-*C*-glc	8-*C*-glc	8-*C*-glc
1″	4.78 *d* 9.6	4.54 *d* 9.8	4.71 *d* 9.6		4.61 *d* 9.8	4.61 *d* 9.9	4.65 *d* 9.8		4.59 *d* 9.8	4.90 *d* 10.2	4.91 *d* 10.1
		4.66 *d* 9.8	4.53 *d*(*b*) 9.6			4.74 *d*(*b*) 9.6	4.57 *d* 9.8			5.13 *d* 10.0	4.90 *d* 10.2
2″	3.82 *dd* 9.9, 9.1	4.10 *s*(*b*)	3.82 *m*		4.05 *dd* 9.8, 8.6	4.13 *m*(*b*)	3.67 *m*		4.03 *t* 9.8 (*b*)	5.41 *dd* 10.2, 9.2	5.42 *dd* 10.1, 9.1
		3.67 *s*(*b*)	4.26 *t* 9.6			3.86 *m*	3.98 *m*			5.38 *dd* 10.0, 9.3	5.36 *dd* 10.2, 9.2
3″	3.47 *m*	3.14 *d* 8.7	3.46 *dd* 9.2, 2.7		3.22 *t*(*b*) 8.6	3.15 *m*	3.21 *d* 8.8		3.20 *t* 8.6	3.57 *m*	3.57 *t* 9.1
		3.49 *m*	3.49 *m*			3.24 *m*	3.49 *m*			3.74 *m*	3.70 *t* 9.2
4″	3.80 *d*(*b*) 2.7	3.40 *m*	3.8 *m*		3.16 *dd* 9.4, 8.6	2.93 *t* 9.1	3.50 *m*		3.12 *t* 9.2	3.47 *m*	3.50 *m*
						3.06 *m*	3.43 *m*			3.41 *m*	3.40 *m*
5(A)″	3.56 *m*	3.76 *m*	3.82 *m*		3.19 *m*	3.18 *dd* 9.1, 6.3	3.86 *m*		3.16 *m*	3.36 *m*	3.34 *m*
		3.86 *m*				3.37 *m*	3.79 *m*			3.52 *m*	3.54 *m*
5B″		3.07 *d* 10.7	3.64 *d* 11.7		3.70 *dd* 11.9, 2.1		3.18 *d* 10.8				
		3.19 *m*					3.09 *m*				
6(A)″	3.56 *m*				3.43 *dd* 11.9, 6.0	1.13 *d* 6.3			3.68 *dd* 11.9, 2.0	3.84 *dd* 11.2, 5.6	3.80 *dd* 10.1, 5.8
						1.22 *d* 6.1				3.75 *m*	3.83 *m*
6B″									3.41 *dd* 11.9, 6.1	3.61 *m*	3.59 *m*
										3.57 *m*	3.61*m*
	8-*C*-gal	8-*C*-gal	8-*C*-gal	8-*C*-glc		8-*C*-gal	8-*C*-glc	8-*C*-glc			
1‴	4.71 *d* 9.6	4.98 *d* 9.4	4.70 *d* 9.9	4.70 *d* 9.9		4.97 *d* 9.6	4.77 *d* 9.9	4.68 *d* 9.9			
	4.99 *d* 9.5	4.69 *d* 8.7	4.93 *d* 9.7	4.85 *d* 9.7		4.68 *d* 9.6	5.01 *d* 9.8	4.83 *d* 9.6			
2‴	4.26 *dd* 9.6, 9.1	3.80 *t* 9.4	4.24 *t* 9.7	3.85 *dd* 9.9, 8.8		3.81 *m*	3.93 *dd* 9.9, 8.7	3.83 *dd* 9.9, 8.8			
		4.22 *t* 9.2	3.94 *ddd* 7.2, 3.8, 1.4	3.88 *m*		4.24 *t* 9.6		3.86 *dd* 9.6, 8.8			
	3.84 *m*						3.58 *m*				
3‴	3.44 *m*	3.55 *m*	3.43 *dd* 9.1, 3.4	3.27 *t*(*b*) 8.8		3.55 *m*	3.29 *m*	3.26 *t* 8.8			
	3.57 *m*	3.41 *m*	3.50 *m*	3.40 *m*		3.41 *m*	3.40 *m*	3.33 *t* 8.8			
4‴	3.87 *dd* 3.5, 1.3	3. 84 *d*(*b*) 2.5	3. 87 *dd* 3.4, 0.8	3.39 *dd* 9.7, 8.8		3. 84 *d*(*b*) 2.6	3.38 *m*	3.38 *t* 9.2			
	3.82 *dd* 3.0, 0.6		3.90 *dd* 3.4, 1.3	3.28 *m*		3.87 *m*	3.39 *m*	3.24 *m*			
5‴	3.52 *m*	3.73 *m*	3.51 *m*	3.27 *ddd* 9.7, 6.5, 1.8		3.73 *t* 6.2	3.49 *m*	3.23 *m*			
	3.55 *m*	3.57 *m*	3.69 *m*	3.38 *m*		3.51 *m*	3.72 *m*	3.33 *m*			
6A‴	3.58 *m*	4.55 *m*	3.57 *m*	3.81 *dd* 12.1, 1.8		3.55 *m*	4.39 *m*	3.76 *dd* 12.0, 2.1			
	3.57 *m*		3.55 *m*	3.71 *m*		3.52 *m*	4.41 *m*	3.68 *dd* 12.0, 2.2			
6B‴				3.58 *dd* 12.1, 6.5			3.99 *dd* 11.8, 6.5	3.52 *dd* 12.0, 5.9			

Table 2. Continued

	1	2	3	4	5	6	7	8	9	10	11
	Ma, 77% Mi, 23%	Ma, 77% Mi, 23%	Ma, 63% Mi, 37%	Ma, 77% Mi, 23%	Ma, 100%	Ma, 63% Mi, 37%	Ma, 59% Mi, 41%	Ma, 77% Mi, 23%	Ma, 100%	Ma, 83% Mi, 17%	Ma, 83% Mi, 17%
				3.53 *m*			4.17 *dd* 12.1, 6.1	3.50 *m*			
acyl							6‴-*O*-3-Me-glutaroyl			2″-*O*-*p*-coumaroyl	
						2A‴′	2.61 *d* 14.5		2‴,6‴	7.43 *d*′ 8.8	7.43 *d*′ 8.6
										6.91 *d*′ 8.8	
						2B‴′	2.57 *d* 14.5		3‴,5‴	6.73 *d*′ 8.8	6.73 *d*′ 8.6
										6.59 *d*′ 8.8	6.90 *d*′ 8.6
						4A‴′	2.43 *d* 14.8		7‴ (*β*)	7.28 *d* 15.9	7.28 *d* 15.9
										7.08 *d* 16.0	7.07 *d* 15.9
						4B‴′	2.36 *d* 14.8		8‴ (*α*)	6.09 *d* 15.9	6.09 *d* 15.9
										5.88 *d* 16.0	5.87 *d* 15.9
						CH_3	1.14 *s*				
							1.24 *s*				

[a] Ma and Mi denote the major and minor rotamer of each flavone, respectively. gal = galactoside; xyl = xyloside; ara = arabioside; glc = glucoside; chin = chinovoside.

1D ^{13}C CAPT) were obtained at 600.13 and 150.90 MHz for ^{1}H and ^{13}C, respectively, on a Bruker Biospin AV-600 MHz instrument equipped with a TCI ^{1}H−^{13}C/^{15}N CryoProbe. All experiments were recorded at 298K and the chemical shift values were set relative to the deuterio-methyl ^{13}C signal and the residual ^{1}H signal of the solvent ($(CD_3)_2SO$) at δ 39.6 and δ 2.49, respectively. Crosspeaks in the ^{1}H−^{13}C HMBC spectra of particular importance for structure elucidation of **6** and **7** were observed at δ 6.76/163.8 (H-3/C-2), δ 6.76/182.5 (H-3/C-4), δ 6.76/102.9 (H-3/C-10), δ 6.76/121.2 (H-3/C-1′), δ 4.61/159.8 (H-1″/C-5), δ 4.61/161.0 (H-1″/C-7), δ 1.13/75.5 (H-6″/C-4″), δ 1.13/76.1 (H-6″/C-5″), δ 4.97/161.0 (H-1‴/C-7), and δ 4.97/153.5 (H-1‴/C-9) for **6**, and at δ 6.81/163.9 (H-3/C-2), δ 6.81/182.4 (H-3/C-4), δ 6.81/104.1 (H-3/C-10), δ 6.81/121.6 (H-3/C-1′), δ 4.65/159.3 (H-1″/C-5), δ 4.65/160.8 (H-1″/C-7), δ 4.77/160.8 (H-1‴/C-7), δ 4.77/155.2 (H-1‴/C-9), δ 4.39/170.7 (H-6A‴/C-1⁗), δ 3.99/170.7 (H-6B‴/C-1⁗), δ 2.61/170.7 (H-2A⁗/C-1⁗), δ 2.61/68.9 (H-2A⁗/C-3⁗), δ 2.61/45.2 (H-2A⁗/C-4⁗), δ 2.43/45.0 (H-4A⁗/C-2⁗), δ 2.43/172.4 (H-4A⁗/C-5⁗) and δ 1.14/68.9 (CH_3⁗/C-3⁗) for **7**, respectively.

High-resolution LC-electrospray mass spectrometry (ESI^+/TOF) spectra were recorded using a JEOL AccuTOF JMS-T100LC in combination with an Agilent Technologies 1200 Series HPLC system. A Zorbax SB-C18 (50 mm × 2.1 mm, length × i.d., 1.8 μm) column was utilized for separation using the same two solvents, A and B, as in Analytical HPLC: 0−1 min 5% B (isocratic), 1−3 min 5 to 13% B (linear gradient), 3−6 min 13% B (isocratic), 6−8 min 13 to 30% B (linear gradient), 8−14 min 30 to 40% B (linear gradient). The flow rate was 0.4 mL min^{-1}.

RESULTS AND DISCUSSION

Identification of Flavones. The HPLC profile of the methanolic crude extract of fenugreek showed two major and several minor flavonoids (**Figure 1**). The methanolic extract of the seeds of fenugreek was purified by partition against diethyl ether followed by Amberlite XAD-7 column chromatography. The flavonoids in the purified extract were fractionated by Sephadex LH-20 and Toyopearl HW-40F column chromatography. Compounds **2** and **6**−**7** were obtained after further purifications using preparative HPLC.

Compounds **1, 3**−**5**, and **8**−**11** were identified as the known compounds apigenin 6,8-di-*C*-*β*-galactopyranoside (**1**), apigenin 6-*C*-*β*-arabinopyranosyl-8-*C*-*β*-galactopyranoside (**3**), luteolin 8-*C*-*β*-glucopyranoside (**4**), luteolin 6-*C*-*β*-glucopyranoside (**5**), apigenin 8-*C*-*β*-glucopyranoside (**8**), apigenin 6-*C*-*β*-glucopyranoside (**9**), luteolin 8-*C*-(2″-*O*-(*E*)-*p*-coumaroyl)-*β*-glucopyranoside (**10**), and apigenin 8-*C*-(2″-*O*-(*E*)-*p*-coumaroyl)-*β*-glucopyranoside (**11**), respectively, by NMR, UV−vis spectroscopy and high-resolution electrospray MS (**Tables 1**−**3**).

The 1D ^{1}H NMR spectrum of **7** showed a 4H AA'XX' system at δ 7.96 (semidoublet, J = 8.9 Hz; H-2′/6′) and δ 6.92 (H-3′/5′), and a 1H singlet at δ 6.84 (H-3) in the aromatic region in accordance with a 6,8-di-*C*-substituted apigenin derivative. The 13 ^{13}C resonances belonging to the aglycone in the 1D ^{13}C CAPT spectrum of **7** were assigned by the observed crosspeaks in the HMBC spectrum. The sugar regions of the 1D ^{1}H and 1D ^{13}C CAPT spectra of **7** showed the presence of one glucose unit and one xylose unit (**Tables 2** and **3**). All the ^{1}H sugar resonances were assigned by COSY and TOCSY experiments, and the corresponding ^{13}C resonances were then assigned by the ^{1}H−^{13}C HSQC experiment. The anomeric coupling constants (9.9 and 9.8 Hz, respectively) and the 11 ^{13}C resonances in the sugar region of the ^{13}C CAPT spectrum of **7** were in accordance with one *C*-*β*-glucopyranosyl unit and one *C*-*β*-xylopyranosyl unit (*17*). The downfield shifts of H-6A‴ (δ 4.39) and H-6B‴ (δ 3.99) belonging to the glucose unit indicated the presence of acyl substitution. The acyl moiety was identified as 3-hydroxy-3-methylglutaroyl by the two 2H doublets at δ 2.61 and δ 2.57 (J = 14.5 Hz; H-2A⁗, H-2B⁗) and δ 2.43 and δ 2.36 (J = 14.8 Hz; H-4A⁗, H-4B⁗), respectively, in the 1D ^{1}H NMR spectrum (assigned by the 2D COSY spectrum), a 3H singlet (3-methyl) at δ 1.14 correlated to a

Table 3. ^{13}C Spectral Data (ppm) for **1**−**11** Dissolved in DMSO-d_6 at 25 °C[a]

	1 Ma, 77% Mi, 23%	**2** Ma, 77% Mi, 23%	**3** Ma, 63% Mi, 37%	**4** Ma, 77% Mi, 23%	**5** Ma, 100%	**6** Ma, 63% Mi, 37%	**7** Ma, 59% Mi, 41%	**8** Ma, 77% Mi, 23%	**9** Ma, 100%	**10** Ma, 83% Mi, 17%	**11** Ma, 83% Mi, 17%
2	164.3	163.7	164.3	164.2	163.8	163.8	163.91	164.0	163.5	164.2	164.1
	163.5	164.1	163.6			163.7	163.8	163.7		163.5	
3	101.9	102. 4	101. 9	102.4	102.9	102.4	102.74	102. 4	102.5	102.3	102.3
	102.5	101.9	102.5			102.5	102.7			102.1	
4	182.3	182.5	182.3	182.1	181.9	182.5	182.4	182.1	182.0	182.0	182.1
	182.2	182.2	182.4			182.2	182.3	182.9		181.7	182.0
5	158.0	159.9	158.4	160.5	160.8	159.8	159.33	160.4	160.9	160.7	160.7
		159.3				158.8	160.2	160.7		161.0	
6	108.0	109.3	107.8	98.3	108.9	109.1	108.13	98.0	108.9	97.5	97.5
		108.0				107.3	109.2	99.3		99.3	
7	160.9	162.2	161.0	162.6	163.3	161.0	160.82	162.6	163.3	162.1	162.1
		160.8	161.0			160.4	161.1	163.0		163.3	
8	105.1	103.8	105.4	104.6	93.6	103.7	104.80	104.6	93.5	102.4	102.5
	103.6	105.2	104.5			105.0	102.7	104.1		102.1	
9	155.0	153.5	155.2	156.1	156.3	153.5	155.16	156.1	156.2	156.5	156.5
	153.3	155.4	155.2			154.9	153.9	155.5		154.5	
10	103.5	103.0	103.8	104.1	103.5	102.9	104.14	104.0	103.5	104.0	104.0
	103.1	104.2	103.1			103.4	103.3	103.7		103.1	
1′	120.9	121.4	121.1	121.1	121.5	121.2	121.56	121.7	121.2	122.1	121.7
	121.2	121.5	121.3			121.3	121.42	121.4		121.5	
2′	129.7	128.6	129.8	114.0	113.3	128.6	128.74	129.1	128.6	113.9	128.8
	128.7	129.6	128.8			129.6	128.82	129.0		113.1	
3′	115.9	115.9	116.0	146.0	145.8	115.8	116.00	115.7	115.9	145.9	115.7
	116.0	115.8					116.06			146.0	
4′	161.1	161.3	161.3	149.7	149.7	161.0	161.34	161.2	161.3	149.7	161.3
	161.2	161.2	161.2				161.3	161.4		150.2	
5′	115.9	115.9	116.0	115.6	115.9	115.8	116.00	115.7	115.9	115.5	115.7
	116.0	115.8					116.06			116.0	
6′	129.7	128.6	129.8	119.3	118.9	128.6	128.74	129.1	128.6	119.3	128.8
	128.7	129.6	128.8			129.6	128.82	129.0		119.0	
	6-*C*-gal	6-*C*-xyl	6-*C*-ara		6-*C*-glc	6-*C*-chin	6-*C*-xyl		6-*C*-glc	8-*C*-glc	8-*C*-glc
1″	73.7	73.6	73.9		73.2	72.8	74.96		72.9	70.9	70.9
		74.8	73.7		74.2	74.0	74.12			71.8	
2″	69.7	69.7	68.4		70.2	69.9	71.43		70.1	72.0	72.0
		71.2	67.7		70.9	70.6	71.32			72.5	72.5
3″	74.4	79.2	73.7		78.9	78.9	78.56		78.8	75.6	75.7
		80.9	74.5		78.4	77.7	78.56			75.3	75.3
4″	68.4	69.9	68.3		70.5	75.5	69.61		70.5	70.6	70.4
					70.0	74.9	69.86			69.9	69.8
5″	79.9	70.2	70.0		81.5	76.1	70.40		81.5	82.0	81.9
		70.2				76.6	70.42			81.4	81.3
6″	60.8				61.4	18.4			61.3	61.2	60.8
					60.9	17.9				60.6	61.2
	8-*C*-gal	8-*C*-gal	8-*C*-gal	8-*C*-glc		8-*C*-gal	8-*C*-glc	8-*C*-glc			
1‴	73.7	74.8	73.9	73.5		74.8	73.79	73.2			
	74.9	74.1	74.7	74.2		73.9	75.19	74.2			
2‴	68.1	69.9	69.9	70.7		69.9	70.81	70.7			
	68.5	68.2	68.2	70.9		67.9	71.27	70.8			
3‴	75.7	74.1	75.6	78.7		74.1	78.60	78.5			
	74. 3	77.6	74.9	78.4		75.5	78.73				
4‴	69.2	68.3	69.1	70.6		68.3	70.84	70.4			
	69. 7		69.1	70.0		69.0	69.87	69.9			
5‴	80.6	79.3	80.5	81.9		79.3	78.48	81.7			
	79.1	79.2	79.5	81.5		80.5	78.7	81.5			
6‴	61.3	60.7	61.1	61.6		60.7	64.25	61.2			
	60.9		60.8	60.9		60.9	63.60	61.3			
acyl							6‴-*O*-3-Me-glutaroyl		2″-*O*-*p*-coumaroyl		
						1‴′ (C═O)	170.7		1‴	125.1	125.1
							170.5			124.7	
						2‴′	45.25		2‴,6‴	130.1	130.0
							44.96			130.4	
						3‴′	68.93		3‴,5‴	115.5	115.5
							69.03			115.4	
						4‴′	45.15		4‴	159.8	159.8
							45.48			159.7	
						5‴′ C═O)	172.4		7‴ (β)	144.1	144.1
							172.1			143.5	
						CH_3	27.12		8‴ (α)	113.8	113.7
							27.20			113.7	
									9‴ (C═O)	165.5	165.5
										164.8	

[a] Ma and Mi denote the major and minor rotamer of each flavone, respectively. gal = galactoside; xyl = xyloside; ara = arabioside; glc = glucoside; chin = chinovoside.

^{13}C signal at δ 27.12 (3-methyl) in the 2D HSQC spectrum, the five ^{13}C signals at δ 170.7 (C-1‴), δ 45.25 (C-2‴), δ 68.93 (C-3‴), δ 45.15 (C-4‴), and δ 172.4 (C-5‴) in the 1D ^{13}C CAPT spectrum, and the crosspeaks in the 2D HMBC spectrum. The linkages between the aglycone, sugar units and 3-hydroxy-3-methylglutaroyl were determined by the long-range correlations in the 2D HMBC spectrum. A molecular ion at *m*/*z* 709.1955 in the high resolution ESI-MS spectrum, confirmed the identity of **7** to be the novel compound apigenin 6-*C*-β-xylopyranosyl-8-*C*-(6‴-*O*-(3-hydroxy-3-methylglutaroyl)-β-glucopyranoside) (**7**) (**Figure 2**).

The ^{1}H and ^{13}C resonances of pigment **2** shared many similarities with the corresponding resonances of **7** (**Tables 2** and **3**), in accordance with a 6,8-di-*C*-substituted apigenin derivative. The coupling constants and the chemical shift values for the 8-*C*-glycosyl unit were in agreement with a nonacylated galactopyranosyl. A molecular ion at *m*/*z* 565.1546 in the high resolution ESI-MS spectrum, confirmed the identity of **2** to be apigenin 6-*C*-β-xylopyranosyl-8-*C*-β-galactopyranoside) (**2**). This compound has previously been reported in two patents to occur in tubers of *Pinellia ternata* and fenugreek seeds, respectively (*18*, *19*). Similarly, the NMR spectra of **6** partly resembled those of **2** (**Tables 2** and **3**), in accordance with a 6,8-di-*C*-substituted apigenin derivative. The linkages between the *C*-glycosyls and the aglycone were determined by the 2D ^{1}H−^{13}C HMBC spectrum. On the basis of the ^{13}C and ^{1}H chemical shift values, in addition to the observed ^{1}H coupling constants (**Tables 2** and **3**) the glycosyl unit attached to the 8-position of the aglycone was identified as β-galactopyranose. The ^{1}H and ^{13}C chemical shift values of the glycosyl unit attached to the 6-position of the aglycone was in accordance to a deoxyhexose (**Tables 2** and **3**). This sugar unit was identified as β-chinovopyranose by the large axial−axial coupling constants observed for J_{H1-H2} (9.8 Hz), J_{H3-H4} (9.1 Hz), and J_{H4-H5} (9.1 Hz) which revealed that all the ring protons of this unit were in axial positions, in agreement with earlier reported values for this glycosyl moiety (*20*). A molecular ion at *m*/*z* 579.17025 in the high resolution ESI-MS spectrum confirmed the identity of **6** to be the novel compound, apigenin 6-*C*-β-chinovopyranosyl-8-*C*-β-galactopyranoside (**Figure 2**). Signal duplication in the NMR spectra of **6** revealed the presence of rotameric conformers, created by rotational hindrance at the *C* (sp^3)-*C* (sp^2) glycosyl-flavone linkage in these flavone *C*-glycosides. On the basis of integration of signals in the 1D ^{1}H NMR spectrum the relative proportions of the major and minor rotamers of **6** were determined to be 70:30. Only a very limited number of natural products containing a chinovosyl unit have hitherto been reported, including three flavone *C*-glycosides (*20*−*23*).

Relative Proportions. The relative proportions of the flavones **1**−**11** in the acidified methanolic extract of seeds of fenugreek are presented in **Table 1**, without taking into account the different molar absorptivity values of individual flavonoids. The three major flavones apigenin 6,8-di-*C*-β-galactopyranoside (**1**), apigenin 6-*C*-β-xylopyranosyl-8-*C*-β-galactopyranoside (**2**), and luteolin 8-*C*-β-glucopyranoside (**4**) constitute together 51% of the total flavonoid content.

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