Half of this residue was subjected to CC on silica gel with CH_2Cl_2 to give two products which were purified by silica gel TLC (CH_2Cl_2–MeOH 97:3), to yield 50 mg of lichenxantone and 20 mg of 5-hydroxy-7-methoxy-2-pentyl chromone (**1**).

171 g of *Z. microcarpum* bark were extracted in a Soxhlet apparatus with 3 l of petrol which yielded 1.6 g of residue after concentration, which then was submitted to the same procedure as above to yield 10 mg of lichenxanthone and 6 mg of **1**.

5-Hydroxy-7-methoxy-2-pentylchromone (**1**). Crystallized from EtOH as white crystals, mp 55–57°. UV λ_{max} (MeOH) nm (ε): 240, 246, 280, 328 (32 280, 33 634, 4912, 4561); λ_{max} (MeOH + NaOH) nm (ε): 208, 236, 272, 350 (23 333, 32 280, 7543, 5263); IR ν_{max}^{KBr} cm^{-1}: 1675, 1640, 1570, 1340, 1200, 1160; ^1H NMR ($CDCl_3$, 250 Mz): δ0.91 (3H, *t*, J = 7.0 Hz, Me-5'), 1.34 (4H, *m*, H-3' and H-4'), 1.67 (2H, *m*, H-2'), 2.48 (2H, *t*, J = 7.5 Hz, H-1'), 3.86 (3H, *s*, OMe-7), 6.17 (1H, *s*, H-3), 6.31 (1H, *d*, J = 2.2 Hz, H-8), 6.45 (1H, *d*, J = 2.2 Hz, H-6), 11.13 (1H, *s*, OH-5); ^{13}C NMR (62.83 MHz, $CDCl_3$): δ13.83 (C-5'), 22.27 (C-4'), 26.43 (C-3'), 31.07 (C-2'), 33.21 (C-1'), 55.60 (OMe-7), 100.19 (C-6), 101.04 (C-8), 103.91 (C-3), 139.53 (C-2), 158.16 (C-8a), 163.75 (C-5),166.56 (C-7), 166.87 (C-4); HRMS *m/z* (rel. int.): 262.1209 $[M]^+$ (68) (Cal. 262.1205), 244 $[M-H_2O]^+$ (10), 229 $[M-H_2O-Me]^+$ (12), 206 $[M-C_4H_8]^+$ (58), 191 $[M-C_4H_8-Me]^+$ (11), 177 (17), 164 (100), 135 (21).

Lichenxanthone. Crystallized from Me_2CO as white crystals, mp 195°. (Ref. [4], mp 187°). UV λ_{max}^{MeOH} nm: 245, 310, 340; ^1H NMR ($CDCl_3$, 250 Mz): δ2.83 (3H, *s*, Me-8), 3.87 (3H, *s*, OMe-3), 3.89 (3H, *s*, OMe-6), 6.30 (1H, *d*, J = 2.3 Hz, H-2), 6.34 (1H, *d*, J = 2.3 Hz, H-4), 6.66 (1H, *m*, H-7), 6.69 (1H, *d*, J = 2.4 Hz, H-5), 13.25 (1H, *s*, OH-1); ^{13}C NMR (62.83 MHz, $CDCl_3$): δ182.51 (*s*, C-9), 165.94 (*s*, C-3), 163.88 (*s*, C-1), 163.89 (*s*, C-6), 159.52 (*s*, C-4b), 157.06 (*s*, C-4a), 143.60 (*s*, C-8), 115.45 (*d*, C-7), 113.05 (*s*, C-8a), 104.2 (*s*, C-9a), 98.53 (*d*, C-5), 96.81 (*d*, C-2), 92.09 (*d*, C-4), 55.65 (*c*, OMe-3), 55.60 (*c*, OMe-6), 23.31 (*c*, Me-8); MS *m/z* (rel. int.): 286 $[M]^+$ (100), 257 (98), 243 (37), 228 (16), 199 (25), 129 (38).

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ACETYLATED FLAVONOL GLYCOSIDES FROM *VICIA FABA* LEAVES

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Key Word Index—*Vicia faba*; Leguminosae; broad bean; acetylated flavonol glycosides; kaempferol 3-*O*-(2″-*O*-α-L-rhamnopyranosyl-6″-acetyl-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside; ^1H NMR.

Abstract—From the leaves of *Vicia faba*, one known and five new flavonol glycosides have been identified: kaempferol 3-*O*-(2″-α-L-rhamnopyranosyl-6″-acetyl-β-D-galactopyranoside)-7-*O*-α-L-rhamnopyranoside, kaempferol 3-*O*-(6″-acetyl-β-D-galactopyranoside)-7-*O*-α-L-rhamnopyranoside, quercetin 3-*O*-(6″-acetyl-β-D-galactopyranoside)-7-*O*-α-L-rhamnopyranoside and their deacylated derivatives. The structures have been established by UV, IR, ^1H NMR and COSY experiments and by identification of controlled acid hydrolysis intermediates.

INTRODUCTION

Vicia faba is cultivated for its pods and seeds. As part of a study for the use of agricultural waste as a source of biologically active compounds, we have found that broad bean leaves are very rich in highly glycosylated flavonoids. This prompted us to study their structures. Previously, only kaempferol 3-galactoside-7-rhamnoside [1] and kaempferol 3-glucoside-7-rhamnoside (2] have been identified from *Vicia faba* leaves. However, an antibacterial flavonol glycoside, quercetin 3-galactosyl-(1→6)-glucoside has been characterised from leaves of *Vicia angustifolia* [3]. The aim of the present work is the identification of the flavonoid glycosides in broad bean leaves.

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RESULTS AND DISCUSSION

From the leaves of *Vicia faba* six flavonoids (**1**–**6**) have been isolated by a combination of PC, low pressure liquid chromatography and open column liquid chromatography (see Experimental). Three of these compounds (**2**, **4** and **6**) were slowly transformed into compounds **1**, **3** and **5** respectively. This transformation was enhanced in alkaline media, suggesting that the former compounds were acylated derivatives. This was confirmed by reversed-phase HPLC analyses, in which the former compounds, eluting with higher retention times (**2** = 3.36 min, **4** = 7.37 min, **6** = 4.24 min), were partly transformed to the corresponding counterparts (retention times: **1** = 1.90 min, **3** = 4.31 min, **5** = 3.64 min) during the HPLC analysis.

Acid hydrolysis of **1**, **2**, **3** and **4** yielded kaempferol, rhamnose and galactose, which were identified by TLC and PC comparisons with authentic samples. Their UV spectra in methanol and after addition of the usual shift reagents indicated that the hydroxyls at the 5- and 4′-positions were free and that the hydroxyls at 3- and 7-positions were substituted. The IR spectra of **2** and **4** indicated the presence of additional acyl-carbonyls in these compounds (1727 and 1738 cm^{-1} respectively), confirming their acylated nature.

Controlled acid hydrolysis of **1** and **2** showed, after PC separation with 15% HOAc, three intermediates identified as kaempferol 3-galactoside-7-rhamnoside, kaempferol 3-rhamnosylgalactoside and kaempferol 7-rhamnoside by UV study and total acid hydrolysis and analysis of the products. The ^{1}H NMR spectra of the intermediates confirmed that they were kaempferol 3-rhamnosylgalactoside-7-rhamnosides (Table 1). The δ values of the protons of galactose and the rhamnose residue linked to galactose suggested that the interglycosidic linkage was (1 → 2) [4]. This was confirmed by comparison of the chemical shifts of the protons of galactose in **1** and **2** with the unsubstituted galactoside **4** (Table 1). This ^{1}H NMR study also confirmed that the sugar linked directly to the 3- position is galactose. It is well known that flavonoid rutinosides (rhamnosyl(1 → 6)glucosides) and neohesperidosides (rhamnosyl(1 → 2)glucosides) can be easily differentiated by ^{1}H NMR [5]. Thus, rutinosides are characterized by a C-1‴ proton signal near 4.2–4.4 ppm and a methyl group signal at 0.7–1.0 ppm, whereas in neohesperidosides, the rhamnose C-1‴ proton absorbs at 4.9–5.0 ppm and the signal for the methyl appears at 1.1–1.3 ppm. From a reviewed of ^{1}H NMR data for rhamnosylgalactosides [6–10], it seems clear that ^{1}H NMR differentiation of isomers is also possible, since in rhamnosyl (1 → 6)galactosides, the rhamnose is characterized by a C-1‴ proton signal near 4.5 ppm (similar to rutinosides) and a methyl group signal at 1.05–1.26 ppm (different from rutinosides), whereas in rhamnosyl (1 → 2)galactosides, the rhamnose C-1‴ proton absorbs at 5.0 ppm (similar to neohesperidosides) and the signal for the methyl group appears at 0.7–0.85 ppm (different from neohesperidosides). This confirms that the interglycosidic linkage in compound **1** and **2** is (1 → 2). In addition, the ^{1}H NMR spectrum of compound 2 shows the presence of an acetyl group (singlet at 1.64 ppm). which is absent in the spectrum of **1**. The analysis of the COSY spectra of both compounds allowed the identification of the acetyl attachment point. The δ values of protons of the two rhamnoses are nearly identical in both flavonoids, while the resonances corresponding to 6A and 6B protons of galactose are significantly shifted downfield (*ca* 0.7 ppm), thus supporting the 6-position as the linkage point. Thus, compound **2** is kaempferol 3-*O*-(2″-α-L-rhamnosyl-6″-acetyl-β-D-galactoside)7-*O*-α-L-rhamnoside, and compound **1** the corresponding deacylated derivative.

Table 1. ^{1}H NMR chemical shifts of compounds **1**, **2** and **4** (360 MHz, d_6-DMSO, δ values are downfield from TMS)

Hydrogens	Multiplicity	Compounds		
		1	**2**	**4**
H-6	(1H, *d*)	6.43	6.43	6.45
H-8	(1H, *d*)	6.83	6.82	6.84
H-2′, 6′	(2H, *m*)	8.12	8.07	8.08
H-3′, 5′	(2H, *m*)	6.86	6.86	6.87
CH_3–CO	(3H, *s*)	—	1.64	1.69
7-*O*-α-L-rhamnose				
H-1‴	(1H, *d*, *J* = 1 Hz)	5.54	5.54	5.55
H-2‴		3.84	3.84	3.84
H-3‴		3.63	3.63	3.63
H-4‴		3.28	3.28	3.29
H-5‴		3.40	3.40	3.41
CH_3–	(3H, *d*, *J* = 6 Hz)	1.10	1.10	1.10
3-*O*-β-D-galactose				
H-1′	(1H, *d*, *J* = 8 Hz)	5.65	5.57	5.34
H-2″		3.78	3.77	3.54
H-3″		3.57	3.59	3.40
H-4″		3.64	—	3.40
H-5″		3.29	3.62	3.61
H-6″A		3.33*	4.07	4.08
H-6″B		3.41*	3.87	3.89
3-*O*-α-L-rhamnose				
H-1‴	(1H, *d*, *J* = 1 Hz)	5.04	5.04	—
H-2‴		3.72	3.73	—
H-3‴		3.45	3.46	—
H-4‴		3.10	3.12	—
H-5‴		3.72	3.73	—
CH_3–	(3H, *d*, *J* = 6 Hz)	0.72	0.77	—

*Assignments may be interchanged.

$J_{2''',3'''}$ and $J_{2''',3'''}$ = 4 Hz; $J_{3''',4'''}$ and $J_{3''',4'''}$ = 9 Hz; $J_{4''',5'''}$ and $J_{4''',5'''}$ = 8 Hz; $J_{2'',3''}$ and $J_{3'',4''}$ = 9 Hz.

Controlled acid hydrolysis of compounds **3** and **4** yielded as an intermediate product kaempferol 7-rhamnoside, suggesting that these were diglycosides. The ^{1}H NMR analysis of **4** confirmed the presence of an acetyl (singlet at 1.69 ppm). Again, protons 6A and 6B of galactose appear at the lowest field (apart from the anomeric ones) (Table 1), a fact that was not observed in unsubstituted galactose protons [4, 11], indicating that the acetyl residue is linked to position 6 of galactose. Thus compound **4** is kaempferol 3-*O*-(6″-acetyl)-β-D-galactoside-7-*O*-α-L-rhamnoside and compound **3** is the corresponding deacylated derivative.

Compounds **5** and **6** were only present in trace amounts. After total acid hydrolysis, quercetin, rhamnose and galactose were identified by TLC and PC comparisons with authentic markers. Their UV spectra in methanol and after addition of the classical shift reagents showed that hydroxyls at the 5,3′ and 4′ positions were

free and hydroxyls at the 3 and 7 positions were blocked. After controlled acid hydrolysis quercetin 7-rhamnoside was detected as an intermediate, identified by UV study and total acid hydrolysis and identification of the hydrolysis products. All these data suggest that compound **6** is quercetin 3-*O*-(6″-acetyl)-β-D-galactoside-7-*O*-α-L-rhamnoside and compound **5** is the corresponding deacylated derivatives.

Only compound **3** has been previously reported in nature in *Vicia faba* [1], the remaining compounds being new naturally occurring flavonoids.

EXPERIMENTAL

Extraction and isolation. Fresh leaves of *Vicia faba* (*ca* 600 g) were extracted with MeOH–H_2O (7:3) overnight at room temp. The concd extract was dissolved in H_2O and successively extracted with EtOAc and *n*-BuOH. The concd *n*-BuOH extract was fractionated by PC in *n*-BuOH–HOAc–H_2O (4:1:5, upper phase). The flavonoid fractions were visualized under UV-light (366 nm) and 6 (**1**–**6**) compounds were isolated by a combination of PC on Whatman no 3 with H_2O and 2% HOAc, and low pressure liquid chromatography (Lobar C-8 with MeOH–H_2O 7:13, solvent flow 3 ml/min). The isolated compounds were purified on a Sephadex LH-20 column with MeOH as eluant.

Acid hydrolysis. Total hydrolysis was performed with 2M HCl at 90° for 1 hr mild acid hydrolysis was achieved by streaking a MeOH soln of the flavonoid on Whatman No 3 paper, depositing 2 MHCl onto the dried flavonoid strip and heating for 1 min with a hair-drier. This was repeated two or three times and then developed with 15% HOAc. The different intermediates are clearly visualized between the naturally occurring glycoside and the aglycone.

Spectral data. IR spectra were measured in nujol cm^{-1}. ^1H NMR (360 MHz) were recorded at 22° on a Bruker VW-360 spectrometer. Samples were dissolved in d_6-DMSO, and a trace of *d*-trifluoroacetic acid added to increase the exchange rate of OH protons. Two dimensional COSY spectra were obtained in the phase sensitive mode with saturation of the HDO solvent signal. In combination with some conventional double resonance experiments, the COSY spectra allowed the assignation of nearly all the flavonoid sugar resonances. Approximate *J* values were obtained from the COSY correlation peaks.

HPLC analysis. HPLC was performed isocratically with MeOH–H_2O (2:3), on a Lichrospher 100 RP-18 Column (5 μm) (flow 1 ml/min) and detection at 250 nm.

1. UV λ_{max}^{MeOH} 350, 266; +NaOMe, 388, 270; +$AlCl_3$, 401, 353, 274; +$AlCl_3$+HCl, 399, 349, 275; +NaOAc, 353, 265; +NaOAc+H_3BO_3, 351, 265.

2. UV λ_{max}^{MeOH} 349, 266; induced shifts identical to those of compound **1**. IR: 3364, 1727, 1559, 1625, 1591.

3. UV λ_{max}^{MeOH} 351, 266; induced shifts as for **1**.

4. UV λ_{max}^{MeOH} 348, 266; induced shifts as for **1**. IR: 3341, 1738, 1659, 1629, 1596.

5 and **6.** UV λ_{max}^{MeOH} 359, 265sh, 257; +NaOAc, 369, 261; +NaOAc+H_3BO_3, 381, 261.

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