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2″-*p*-COUMAROYLVITEXIN 7-GLUCOSIDE FROM *MOLLUGO OPPOSITIFOLIA*

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Key Word Index—*Mollugo oppositifolia*; Aizoaceae; *C*-glycosylflavones; vitexin 7-*O*-β-D-glucopyranoside; EIMS; 2″-*p*-coumaroylvitexin 7-*O*-β-D-glucopyranoside; FAB-MS; ^{13}C NMR.

Abstract—Besides vitexin, two compounds have been isolated from *Mollugo oppositifolia* and identified as vitexin 7-glucoside and 2″-*p*-coumaroylvitexin 7-glucoside. The latter is a new natural compound. Some features common to the electron-impact mass spectra of permethyl vitexin 7-glucoside and permethyl isovitexin 7-glucoside are discussed.

In continuation of our work on the anthocyanin-producing genus *Mollugo* [1–3], we report here the isolation and characterization of three *C*-glycosylflavonoids from *Mollugo oppositifolia*, including one new compound. From the methylethylketone-soluble fraction of the ethanolic extract of fresh aerial parts, a yellow solid was isolated and identified as vitexin by direct comparison with an authentic specimen [4]. From the *n*-butanol-soluble fraction of the same extract, two homogeneous compounds **1** and **2** were obtained by column chromatography on silica gel in a chloroform–methanol gradient followed by repeated paper and thin-layer chromatography. Both compounds appeared as dark spots on paper under UV light, and their UV spectra and chromatographic properties indicated an apigenin glycoside structure.

Whereas the UV spectrum of **1** showed two bands at λ_{max} 271 (II) and 327 (I) nm of nearly equal intensity, band I (321 nm) was much higher than band II (272 nm) in the UV spectrum of **2**, suggesting the presence of an additional chromophore. However, the usual diagnostic reagents [5] disclosed in both compounds a 7-*O*-substituted apigenin structure with free 5- and 4′-hydroxyl groups. Both compounds gave, on acid hydrolysis, vitexin and traces of isovitexin (identified by HPLC under conditions where vitexin and 8-*C*-galactosylapigenin are separated [6]), but, surprisingly, only traces of sugars could be detected (TLC) in the hydrolysis mixture. On alkaline hydrolysis, **1** was recovered unchanged (UV and TLC) whereas **2** gave **1** together with *p*-coumaric acid. Permethylation of **1** followed by TLC led to one main product which gave a mass spectrum showing an intense molecular ion at *m/z* 734 corresponding to a permethyl *O*-hexosylvitexin. Also consistent with this formulation was the presence of important ions at *m/z* 516 (corresponding to the loss of the *O*-hexosyl residue with hydrogen transfer to the oxygen atom) and *m/z* 218, 187 and 155 (characteristic of the hexosyl residue). It follows that **1** is a 7-*O*-hexosylvitexin. For compound **2**, the MW was determined by fast atom bombardment mass spectrometry. The spectrum showed a protonated peak $[MH]^+$ at 741, which was confirmed by the presence of an ion $[M+Na]^+$ at 763. Thus only one *p*-coumaroyl group is present in **2**. This group is not attached to a phenolic hydroxyl group (UV data), but to a hydroxyl group of either the 7-*O*-hexosyl residue or the 8-*C*-glucosyl residue. In order to identify the 7-linked hexose and to locate the *p*-coumaroyl group, **1** and **2** were studied by ^{13}C NMR spectrometry.

The ^{13}C NMR resonances for the carbon atoms of the flavonoid nuclei and the *p*-coumaroyl group of **1** and **2** are consistent with the partial formulations outlined above (for data see Experimental). Sugar carbon chemical shifts are presented in Table 1 together with the chemical shifts for an 8-*C*-β-glucopyranosyl moiety (from vitexin [7]) and a 7-*O*-β-glucopyranosyl moiety (from saponarin [7]). It is clear from the level of agreement between the spectra of **1** and a combination of the two models that both the *C*-

Table 1. Chemical shift data for sugar carbons in the ^{13}C NMR spectra of **1**, **2** and models vitexin and saponarin*

1	2	*C*-Glucopyranosyl†	*O*-Glucopyranosyl‡
101.2	101.2	—	101.7 (C-1′)
81.8	81.7	81.4 (C-5)	—
78.6	—	78.7 (C-3)	—
77.1	77.1	—	77.3 (C-5′)
75.8	76.2	—	76.2 (C-3′)
—	74.8 (C-3)	—	—
73.3	—	73.9 (C-1)	—
73.3	73.1	—	73.0 (C-2′)
71.6	72.0	71.4 (C-2)	—
70.6	70.7	—	70.5 (C-4′)
—	70.7 (C-1)	—	—
69.4	69.5	69.9 (C-4)	—
61.2	61.0	61.5 (C-6)	—
60.6	60.5	—	61.1 (C-6′)

*Chemical shifts in ppm; solvent DMSO-d_6; temperature 30°.
†From vitexin (assignments from ref. [7])
‡From saponarin (assignments from ref. [7]).

and *O*-linked glucopyranosyl moieties are present in **1**, thus defining **1** as vitexin 7-*O*-*β*-D-glucopyranoside. In the spectrum of its mono-*p*-coumaroyl derivative, **2**, the resonances assigned to the *O*-glucosyl moiety in **1** are largely unchanged. Two of the *C*-glucosyl signals, C-1 and C-3, however, have shifted upfield by δ3.8 and 2.6, respectively. This behaviour is the expected result of cinnamoylation of the C-2 hydroxyl of the *C*-glucosyl residue [8, 9], the signal for C-2 itself shifting only slightly (δ0.4) downfield. The structure of **2** is therefore, defined as 2″-*O*-*p*-coumaroylvitexin 7-*O*-glucoside, a new natural compound.

The comparison of the mass spectrum (electron impact) of permethyl **1** with that of permethyl saponarin (7-*O*-glucosylisovitexin) [10] disclosed striking similarities in the fragmentation pattern of the *C*-glucosyl residue in spite of its different position in the flavone. As both spectra are superimposable between *m/z* 510 and 297 (flavone ion), it follows that after loss of the 7-*O*-hexosyl residue, the fragmentation of the *C*-glycosyl residue is the same for the 6-*C*-glucosylflavone as for the 8-*C*-glucosylflavone. However, characteristic differences between the two spectra permit the determination of the position of the *C*-glucosyl residue unambiguously. In the spectrum of permethyl saponarin, the molecular peak is followed by an ion $[M-15]^+$ of similar importance and by a more intense peak $[M-31]^+$ (base peak for *m/z* values higher than 250), a feature common to the mass spectra of all permethyl 6-*C*-glycosylflavones [11], whereas the molecular peak itself is very intense (base peak for *m/z* values higher than 250) in the spectrum of permethyl **1**, a feature common to the mass spectra of all permethyl 8-*C*-glycosylflavones [11]. Moreover, the rupture of the 7-*O*-glycosidic bond takes place *without* hydrogen transfer to the oxygen atom in the case of permethyl 7-*O*-glycosyl-6-*C*-glycosylflavones [10], giving rise to an important ion at *m/z* 515 $[M-219]^+$ in the mass spectrum of permethyl saponarin, whereas this hydrogen transfer (always observed with permethyl flavone *O*-glycosides) takes place in the case of permethyl **1** and gives rise to an important ion at *m/z* 516 $[M-218]^+$.

Whereas isovitexin 7-glucoside is a widely distributed and well-characterized natural compound, vitexin 7-glucoside is a rare compound which until now had been characterized only by its acid hydrolysis products: glucose and vitexin. Owing to our repeated observation that glucose is easily found in the acid-hydrolysis products of isovitexin 7-glucoside whereas it is not from vitexin 7-glucoside under the same conditions, some doubt may be expressed about the exact nature of the previously reported vitexin 7-glucoside. This anomalous behaviour towards acid hydrolysis seems to be restricted to vitexin 7-glucoside since we could find glucose in the acid-hydrolysis products of 4′-*O*-methylvitexin 7-glucoside.

EXPERIMENTAL

Plant material. Mollugo oppositifolia L. syn. *M. spergula* L. Aizoaceae (voucher specimen No. 4/76 deposited at Jawaharlal Institute), a diffuse, prostrate or ascending annual with numerous branched stems up to 2 feet long and a stout tap root occurring over the greater parts of India [12], was collected from Pondicherry.

Isolation. Fresh aerial parts of *M. oppositifolia* (2 kg) were extracted with boiling 80% EtOH (3 × 4 l.). After concn under red. pres. and filtration, the aq. residue was extracted with C_6H_6, EtOAc, MeCOEt and *n*-BuOH. Recrystallization of the MeCOEt extract in MeOH–Me_2CO yielded vitexin. The *n*-BuOH concentrate was chromatographed over a column of silica gel and eluted with a gradient of $CHCl_3$–MeOH. Fractions from 25% to 100% MeOH contained two flavone *C*-glycosides together with some triterpene saponin impurities. The flavonoids were further separated by PPC (15% HOAc) followed by TLC (silica gel; $CHCl_3$–MeOH, 3:1) and finally PC (BAW) to yield two homogeneous compounds, **1** and **2**.

Vitexin 7-glucoside (**1**). TLC (cellulose) R_f 0.34 (15% HOAc), 0.32 (BAW). UV λ_{max}^{MeOH} nm: 271, 327; + $AlCl_3$ 278, 303, 345, 386, + $AlCl_3$ + HCl 277, 302, 342, 385, + NaOH 252, 269, 300 sh, 393↗; + NaOAc 250 sh, 270, 300 sh, 390↗; + NaOAc + H_3BO_3 270, 335. ^{13}C NMR (DMSO-d_6): δ182.3 (C-4), 164.5 (C-2), 162.6 (C-7), 161.1 (C-4′), 160.7 (C-5), 155 (C-9), 128.8 (C-2′,6′), 121.2 (C-1′), 115.8 (C-3′,5′), 107.3 (C-8), 102.4 (C-3), 98.5 (C-6), for sugar carbon resonances see Table 1. Permethyl ether: EIMS 70 eV, *m/z* (rel. int.): 734 $[M]^+$ (86), 720 (8), 703 (12), 671 (5), 559 $[M-175]^+$ (6), 516 $[AH]^+$ (26), 515 $[A]^+$ (8), 501 $[AH-15]^+$ (26), 485 $[AH-31]^+$ (75), 483 (33), 469 $[AH-47]^+$ (22), 453 $[AH-63]^+$ (60), 439 (12), 381 (13), 367 (15), 355 $[AH-161]^+$ (11), 353 $[AH-163]^+$ (11), 341 $[AH-175]^+$ (27), 327 $[AH-189]^+$ (18), 323 (12), 311 $[AH-205]^+$ (7), 218 (26), 187 (36), 155 (19), 101 (100).

2″-p-Coumaroylvitexin 7-glucoside (**2**). TLC (cellulose) R_f 0.63 (15% HOAc), 0.64 (BAW). UV λ_{max}^{MeOH} nm: 272, 321; + $AlCl_3$ 279, 304, 332, 387; + $AlCl_3$ + HCl 280, 304, 330, 385; + NaOH 248, 268 sh, 299, 311, 380↗; + NaOAc 256 sh, 270, 300, 314 sh, 387, + NaOAc + H_3BO_3 271, 321. ^{13}C NMR (DMSO-d_6): 182.2 (C-4), 165.5 (C-9, *p*-cou), 164.5 (C-2), 161.5/161.0 (C-7,4′), 159.6 (C-4 *p*-cou), 155.6 (C-9), 144 (C-7 *p*-cou), 130.0 (C-2,6 *p*-cou), 129.1 (C-2′,6′), 125.2 (C-1 *p*-cou), 121.2 (C-1′), 115.9/115.7 (C-3′,5′), 115/114.6/114.2 (C-3,5,8 *p*-cou), 104.7/105.3 (C-8,10), 102.4 (C-3), 98.0 (C-6), for sugar carbons resonances see Table 1.

Acid hydrolysis. The samples were dissolved in MeOH–4 M HCl (1:1) and heated at 100° for 1 hr in a sealed tube. After repeated evapns of the solvent, the residue was taken up in H_2O and extracted with *n*-BuOH. The aglycones were identified in the *n*-BuOH extract by TLC (silica gel) in EtOAc–pyridine–H_2O–MeOH (16:4:2:1); (cellulose) in 15% HOAc and BAW (4:1:5), the sugars in the aq. phase by TLC on Na_2HPO_4 (0.2 M) impregnated silica gel in Me_2CO–H_2O (9.1) against standard markers. The flavones and sugars were detected with bis-diazotized benzidine and aniline malonate, respectively.

Alkaline hydrolysis. **2** was dissolved in MeOH–4 M NaOH (1:1) and left under N_2 in a sealed tube for 2 hr at room temp. After acidification with 4 M HCl and evapn to dryness under red. pres., the residue was extracted with Et_2O, EtOAc and *n*-BuOH. Et_2O and EtOAc extracts showed the same UV spectrum (λ_{max}^{MeOH} 294 sh, 301 and 311 nm) and chromatographic behaviour as *p*-coumaric acid; the *n*-BuOH extract the same UV spectra and chromatographic behaviour as **1**.

Permethylation. See ref. [11]. EIMS and FAB-MS were run at the Centre de Spectrométrie de Masse de Lyon

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FLAVONOID GLUCOSIDES FROM LICORICE

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Key Word Index—*Glycyrrhiza uralensis*; Leguminosae; licorice; flavonoid glycosides; liquiritigenin 4′-*O*-*β*-apiofuranosyl(1 → 2)-*β*-glucopyranoside; liquiritigenin 7,4′-diglucoside; apigenin 6,8-di-*C*-glucoside.

Abstract—Two new flavanone glycosides, liquiritigenin 4′-apiosyl(1 → 2)-glucoside and liquiritigenin 7,4′-diglucoside together with a known flavone, apigenin 6,8-di-*C*-glucoside, have been isolated from licorice.

Licorice, the dried root of *Glycyrrhiza uralensis* (Leguminosae), is prescribed in many Chinese traditional medicines as a flavouring, a diluting agent and an anti-inflammatory agent. It has been reported to contain triterpenoids (glycyrrhizin, etc.) and a variety of flavonoids [1–4] (isoflavonoids, chalcones and flavones). As part of our chemical examination of phenolic constituents in Chinese crude drugs, we have undertaken a further analysis of licorice. This has resulted in the isolation and structural characterization of two new flavanone glycosides (**1** and **2**), together with apigenin 6,8-di-*C*-glucoside (**3**).

Compound **1** showed UV absorptions at 274 and 312 nm, characteristic of flavanones. The ^{1}H NMR spectrum of **1** exhibited two anomeric proton signals at δ 5.38 (*br s*) and 4.95 (*d*, J = 7 Hz). Enzymatic hydrolysis of **1** with crude hesperidinase [5] yielded liquiritigenin (7,4′-dihydroxy flavanone) (**1a**), glucose and apiose. The sugar sequence and the configuration in **1** were determined by the analogy of the chemical shifts (Table 1) with those of the corresponding chalcone glycoside licurazid [6], which contains a *β*-apiofuranosyl(1 → 2)-*β*-glucopyranosyl moiety. The location of the sugar moiety in **1** was confirmed by comparison of the ^{13}C NMR spectrum of **1** with that of **1a**. On going from **1a** to **1**, the carbon resonances of C-1′, C-3′ and C-5′ in the flavanoid B-ring were displaced downfield by 3.0, 0.9 and 0.9 ppm, respectively, while the carbon resonances arising from the A- and C-rings remained unchanged. From these observations, the structure of **1** was assigned as liquiritigenin 4′-*O*-*β*-apiofuranosyl(1 → 2)-*β*-glucopyranoside.

Compound **2** showed UV absorption similar to **1** (270 and 313 nm). Enzymatic hydrolysis of **2** yielded **1a** and glucose. The occurrence of two *β*-glucosyl moieties in **2** was deduced from the fact that its ^{1}H NMR spectrum showed two anomeric proton signals at δ4.99 and 4.90 (each *d*, J = 7 Hz). Furthermore, these two glucose residues were shown to be attached to the A- and B-rings in the flavanoid skeleton by comparison of the ^{13}C NMR resonances in **2** with those of **1a**; the carbon resonances of C-6, C-8, C-4a, C-1′, C-3′ and C-5′ in **2** were shifted downfield by 0.4, 0 9, 1.7, 2.7, 1.0 and 1.0 ppm, respectively. Accordingly, the structure of **2** was determined as liquiritigenin 7,4′-di-*O*-*β*-glucopyranoside.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR and ^{13}C NMR spectra were recorded at 100 and 25 05 MHz, respectively, and chemical shifts are given in the δ (ppm) scale with TMS as internal standard. TLC was performed on silica gel and compounds were detected by

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