(43.3%, SO), 456 (33.2%, S + H), 455 (100%, S), 341 (83.9%, Fl—CH=OH$^+$), 325 (12.3%, Fl—CH$_2^+$), etc.

Conditions for co-chromatography. (a) *Permethyl ethers*, TLC, Si gel using Me_2CO–$CHCl_3$ (1:4), $CHCl_3$–EtOAc–Me_2CO (5:4:1) and EtOAc-pyridine–H_2O–MeOH (16:4:2:1). (b) *Flavone C-arabinosides*, PC using BAW, HOAc, H_2O and $CHCl_3$–HOAc–H_2O (30:15:2).

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ACYLATED FLAVONE-*C*-GLYCOSIDES FROM THE SEEDS OF *ZIZYPHUS JUJUBA**

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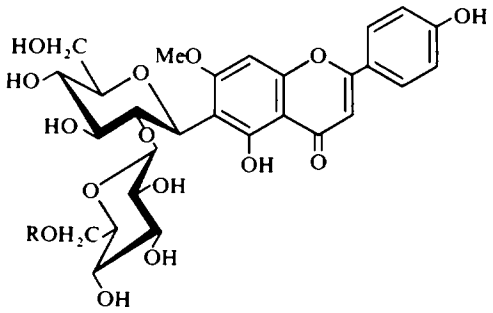
Key Word Index—*Zizyphus jujuba*; Rhamnaceae; acylated flavone-*C*-glycoside; 6‴-sinapoylspinosin; 6‴-feruloylspinosin; 6‴-*p*-coumaroylspinosin.

In a previous paper [1], we reported the isolation and structure elucidation of a new flavone-*C*-glycoside, spinosin, from the seeds of *Zizyphus jujuba* (*Z. vulgaris* var. *spinosus*) which have long been used in traditional medicine for treating insomnia and nervous debility. This communication deals with the chemistry of new acylated spinosins. Repeated column chromatography of the ethyl acetate-soluble fraction of the MeOH extract of the seeds on silica gel yielded an acylated spinosin mixture. This was further separated using another solvent system into three components **1**–**3** in order of decreasing polarity.

1. $C_{39}H_{42}O_{19}\cdot 2H_2O$, mp 198–204, $[\alpha]_D^{20} - 40.5°$ (MeOH), gave characteristic flavonoid colour reactions and a positive Molisch test. IR showed OH, α,β-unsaturated ester and carbonyl absorptions at 3350, 1690 and 1650 cm^{-1} respectively and a broad C—O stretching band in the region 1100–1000 cm^{-1}, suggesting its glycosidic nature. Acid hydrolysis of **1** gave swertisin, mp 242–244° (mmp, co-TLC), glucose (TLC and GLC as TMSi ether) and sinapic acid (GLC as TMSi ether), while mild alkaline hydrolysis yielded spinosin (**4**), mp 255–256° (mmp and co-TLC). The UV absorption of **1** and the bathochromic shifts with diagnostic reagents [2] suggested that the acyl residue must be attached to one of sugar OH groups.

Acetylation gave a nonaacetate, mp 124–128°, showing six sugar acetate methyl signals, three phenolic acetate methyl signals and three oxymethyl signals in its ^{1}H NMR spectrum. This observation indicated that **1** was composed of 1 mol of **4** and 1 mol of sinapic acid. Acetone treatment yielded a monoisopropylidene derivative. Permethylated **1** and permethylated monoisopropylidene derivative showed in the mass spectra intense peaks at *m/e* 499 and 515, and 511 and 527, respectively, corresponding to the loss of

* Part 18 in the series "Structure of Flavone-*C*-glycosides". For Part 17 see [1].



1 R = sinapoyl
2 R = feruloyl
3 R = *p*-coumaroyl
4 R = H

permethylated sinapoylglucosyl moiety with and without oxygen from respective molecular ions. These results demonstrated the presence of acyl residue on the 4‴ or 6‴-position. Methanolysis of permethylated **1** gave 2″-OH free permethylated isovitexin (mmp, MS, co-TLC) and methyl-2,3,4-tri-*O*-methylglucoside (GLC). Therefore the point of the attachment of acyl residue proved to be the 6‴-position of **4**.

2, $C_{38}H_{40}O_{18}\cdot 2H_2O$, mp 194–197°, $[\alpha]_D^{20}$ − 45.2° (MeOH), showed, as described in the Experimental, the same physico-chemical properties and spectral data (UV, IR, MS, NMR) as those of **1** with an exception that the acyl moiety was ferulic acid. Therefore, in the case of **2**, feruloyl residue was also linked to the 6‴-position of **4**. ^{13}C NMR chemical shifts of **2** were also in agreement with the formulation of **2** as 6‴-feruloylspinosin.

The signals for the sugar carbon atoms which appeared in the region from 81.4 to 61.9 ppm were compared with the corresponding carbon signals in the spectrum of **4** [1]; the C-6‴ signal in **2** was 2.1 ppm downfield and the C-5‴ signal was 2.3 ppm upfield. Such changes in the chemical shifts of C-6‴ and C-5‴ can only be explained if the primary hydroxyl group at the 6‴-position of **4** is esterified with ferulic acid [3].

3 was a minor component and was not obtained in a pure state. Crude **3** (*ca* 70%) gave *p*-coumaric acid (GLC as TMSi ether) on acid hydrolysis and physico-chemical properties were very similar to those of **1** and **2**. Therefore **3** was suggested to be 6‴-*p*-coumaroylspinosin. These acylspinosins showed mild sedative activity, data of which will appear elsewhere [4].

EXPERIMENTAL

Isolation of acylspinosins. The commercially available seeds of *Zizyphus jujuba* were defatted by repeated extraction with petrol (bp 60–80°). They were then extracted with MeOH and the extract was concd. The residue was partitioned between Et_2O and H_2O. The aq. layer was extracted with EtOAc. The EtOAc extract was concd to a dark brown residue which was subjected to column chromatography on Si gel, using $CHCl_3$–MeOH–H_2O (13:7:2) as an eluent to yield an acylflavone-*C*-glycoside fraction.

The fraction showed 3 peaks on HPLC (reverse phase column material: COPELL ODS, 2 × 250 mm; eluent: THF–H_2O, 4:53; pump pressure, 32 bar; flow rate: 1.7 ml/mm) which was designated as compounds A, B and C in order of decreasing polarity. The mixture was rechromatographed on Si gel and eluted with EtOAc–MeOH–H_2O (6:1:0.7).

6‴-*Sinapoylspinosin* (**1**) was crystallized from MeOH as a pale yellow amorphous powder, mp 198–204°, $[\alpha]_D^{20}$ − 40.5° (*c* = 0.2, MeOH), Zn–HCl, pink; Mg–HCl, yellowish orange; Molisch test, +. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1690 (ester), 1650 (CO), 1605 (C=C), 1100–1000 (glycoside); UV λ_{max}^{EtOH} nm (log ε): 275 (4.08), 333 (4.39); with NaOEt, 232 (4.24), 268 (4.14), 318 (3.61), 395 (4.50); with NaOAc, 273 (4.10), 335 (4.29), 404 (3.75); with NaOAc + H_3BO_3, 274 (4.04), 333 (4.35); with $AlCl_3$ + HCl, 238 (4.30), 287 (4.09), 308 (4.19), 344 (4.31); (Found: C, 54.76; H, 5.56. $C_{39}H_{42}O_{19}\cdot 2H_2O$ requires: C, 55.06; H, 5.45%).

6‴-*Feruloylspinosin* (**2**) was crystallized from MeOH as a pale yellow amorphous powder, mp 194–197°, $[\alpha]_D^{20}$ − 45.2° (*c* = 0.383, MeOH); Zn–HCl, pink; Mg–HCl, yellowish orange; Molisch test, +. IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1695 (ester), 1650 (CO), 1600 (C=C), 1100–1000 (glycoside); UV λ_{max}^{EtOH} nm (log ε): 276 (4.21), 332 (4.41); with NaOEt: 270 (4.14), 311 (3.78), 396 (4.52); with NaOAc: 275 (4.16), 334 (4.35), 404 (3.55); with NaOAc + H_3BO_3: 276 (4.18), 333 (4.39); with $AlCl_3$ + HCl: 234 (4.27), 287 (4.23), 307 (4.28), 340 (4.34); (Found: C, 55.41; H, 5.82. $C_{38}H_{40}O_{18}\cdot 2H_2O$ requires: C, 55.61; H, 5.40%). ^{13}C NMR (DMSO-d_6): Aglycone: δ 182.1 ppm (C-4), 164.2 (C-2, C-7), 161.1 (C-5, C-4′), 157 (C-9), 128.4 (C-2′, C-6′), 121.5 (C-1′), 116.0 (C-3′, C-5′), 109.4 (C-6), 105.1 (C-10), 103.3 (C-3), 90.6 (C-8), 56.8 (OMe), 56.1 (OMe); *C*-glucosyl δ 81.4 (C-2″, C-5″), 78.8 (C-3″), 71.2 (C-1″), 70.7 (C-4″), 61.9 (C-6″); *O*-glucosyl δ 105.1 (C-1‴), 76.5 (C-3‴), 74.6 (C-2‴), 73.6 (C-5‴), 69.4 (C-4‴), 62.6 (C-6‴); feruloyl δ 166.2 (C-γ), 149.6 (C-4‴′), 148.1 (C-3‴′), 144.6 (C-α), 125.8 (C-1‴′), 122.8 (C-6‴′), 115.8 (C-5‴′), 114.4 (C-β), 111.8 (C-2‴′).

6‴-p-*Coumaroylspinosin* (**3**) was not obtained in a pure state.

Acid hydrolysis of **1**, **2** *and* **3**. Each of the samples (50 mg) in 5% HCl (10 ml) was heated on the water bath for 2 hr. After cooling the resulting mixture was extracted with Et_2O. The Et_2O layer was dried, filtered and concd. The residues from the samples of **1** and **2** and the crude sample of **3** were found to be sinapic acid (R_t, 10.5 min), ferulic acid (5.5 min) and *p*-coumaric acid (3.0 min) (mixture with ferulic acid), respectively by GLC of their TMSi derivatives (column, 3%, SE 30, 60–80 mesh, 1.5 m × 4 mm; column temp., 175°; injector temp., 185°; FID temp., 200°; N_2, 45 ml/min). The water layer was extracted with BuOH. The BuOH layer was concd and crystallized from MeOH to yield swertisin, mp 242–244°, from all samples, which was identical with an authentic sample (mmp, UV, IR, co-TLC). The aglycone-freed water layer was neutralized with Ag_2CO_3 and evapd under red. pres. All residues from the three samples were found to be glucose by TLC (Si gel G, MeOH–$CHCl_3$–Me_2CO–NH_4OH, 5:2:3:2, R_f, 0.20) and GLC of their TMSi derivatives (column, 3%, OV-1, 60–80 mesh, 1.5 m × 4 mm; column temp., 170°; injector temp., 180°; FID temp., 200°; N_2, 45 ml/min; R_t, 3.55, 4.75 min).

Alkali hydrolysis of **1**, **2** *and* **3**. Each of the samples (50 mg) in 0.1 N KOH (10 ml) were heated at 70° for 5 min. After cooling and acidification, the reaction mixture was extracted with Et_2O. The Et_2O layer was treated and applied to GLC under similar conditions as above to detect sinapic acid, ferulic acid and *p*-coumaric acid in the samples from **1**, **2** and **3** respectively. The water layer was extracted with BuOH. The BuOH soln was concd and purified by prep. TLC ($CHCl_3$–MeOH–H_2O, 13:7:2, R_f, 0.27) and crystallized from MeOH to give **4**, mp 255–256°, which was identical with an authentic sample (mmp, IR, UV).

Acetylation of **1** *and* **2**. Acetylation of each of the samples (80 mg) with Ac_2O and pyridine at room temp. gave two products which were separated into a nonaacetate (R_f, 0.32) and an octaacetate (R_f, 0.44) by prep. TLC (C_6H_6–Et_2O–MeOH, 8:2:1). Sinapoylspinosin nonaacetate: mp 124–128°; ^{1}H NMR ($CDCl_3$) δ 1.90–2.13 (6 × sugar acetyl), 2.31 (3H, phenolic acetyl), 2.37 (3H, phenolic acetyl), 2.43 (3H, C-5 acetyl) 3.89 (6H, MeO) and 3.90

(3H, MeO). Sinapoylspinosin octaacetate: mp 149–152°, ^{1}H NMR ($CDCl_3$) δ 1.92–2.13 (6 × sugar acetyl), 2.30 (3H, phenolic acetyl) and 2.37 (3H, phenolic acetyl), 3.83 (6H, MeO), 3.95 (3H, MeO). Feruloylspinosin nonaacetate: mp 153–156°; ^{1}H NMR ($CDCl_3$) δ 1.90–2.13 (6 × sugar acetyl), 2.32 (6H, phenolic acetyl), 2.44 (3H, C-5 × acetyl), 3.87 (3H, MeO), 3.98 (3H, MeO). Feruloylspinosin octaacetate: mp 142–145°; ^{1}H NMR ($CDCl_3$) δ 1.92–2.13 (6 × sugar acetyl), 2.33 (6H, phenolic acetyl), 3.86 (3H, MeO), 3.96 (3H, MeO).

Permethylation of **1** *and* **2**. Each of the samples (100 mg) was permethylated according to the method of Hakomori [5] and purified by prep. TLC (C_6H_6–Me_2CO–MeOH, 40:20:1). Permethylated sinapoylspinosin: mp 105–112°; R_f, 0.35; MS *m/e*: 940 (M^+), 719 (M – PM-sinapoyl), 515 (M – PM-Glu-sinapoyl), 499 (M – O-PM-Glu-sinapoyl), 221 (PM-sinapoyl). Permethylated feruloylspinosin: mp 102–110°; R_f, 0.35; MS *m/e*: 910 (M^+), 719 (M – PM-feruloyl), 515 (M – PM-Glu-feruloyl), 499 (M – O-PM-Glu-feruloyl), 191 (PM-feruloyl).

Methanolysis of permethylated compound A and permethylated compound B. Each of the samples (100 mg) in 3% HCl–MeOH was heated under reflux for 2 hr and concd under red. pres. to remove MeOH. After addition of water the resulting ppt. was filtered and purified by prep. TLC (C_6H_6–Me_2CO–MeOH, 40:20:1) to yield 2″-OH free permethylated isovitexin, mp 128–134°, R_f, 0.24, which was identical with an authentic sample obtained by hydrolysis of permethylated spinosin (mmp and MS). The filtrate was extracted with $CHCl_3$. The $CHCl_3$ soln was evapd. The presence of methyl-2,3,4-tri-*O*-methylglucoside in each of residues was proved by TLC (C_6H_6–Me_2CO–MeOH, 40:20:1, R_f, 0.33 and R_f, 0.23) and GLC (column, 5% NPGS, 100–120 mesh, 1.5 m × 4 mm, column temp., 175°; injector temp., 185°; FID temp., 200°; N_2, 45 ml/min, R_t, 4.3, 5.9 min).

Acetonide formation of **1** *and* **2**. Each of the samples (200 mg) in dry Me_2CO (300 ml) was stirred overnight in the presence of dry $CuSO_4$ (300 mg) and filtered. After removal of the solvent under red. pres., the residue was subjected to prep. TLC ($CHCl_3$–MeOH–H_2O, 13:7:2). Monoisopropylidene sinapoylspinosin: mp 250–256°: R_f, 0.57; MS of permethylated derivative: *m/e*; 952 (M^+), 731 (M – PM-sinapoyl), 527 (M – PM-Glu-sinapoyl), 511 (M – O-PM-Glu-sinapoyl), 221 (PM-sinapoyl). Monoisopropylidene feruloylspinosin: mp 236–242°; R_f, 0.57; MS of permethylated derivative: *m/e*; 922 (M^+), 731 (M – PM-feruloyl), 527 (M – PM-Glu-feruloyl), 511 (M – O-PM-Glu-feruloyl), 191 (PM-feruloyl).

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