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THE STRUCTURE OF SPINOSIN (2″-*O*-β-GLUCOSYLSWERTISIN) FROM *ZIZYPHUS VULGARIS* VAR. *SPINOSUS**

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Key Word Index—*Zizyphus vulgaris* var. *spinosus*; Rhamnaceae; flavone-*C*-glycoside; spinosin; 2″-*O*-β-D-glucopyranosylswertisin.

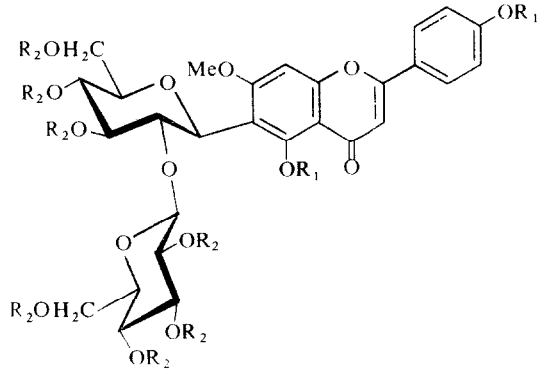
The seeds of *Zizyphus vulgaris* var. *spinosus* have been used in traditional medicine for treating insomnia. Chemical investigation so far on the seeds has only been reported to yield triterpenoids [1–3]. This communication records the isolation and structure elucidation of a new flavone *C*-glycoside, spinosin (**1**) which exhibited a mild sedative action [4]. Repeated column chromatography of the BuOH soluble part of the MeOH extract of the seeds on silica gel and crystallization yielded a pure compound as a yellow microcrystalline substance, $C_{28}H_{32}O_{15}\cdot H_2O$, mp 255–6°, $[\alpha]_D^{24}$ +16.5° (MeOH). It gave characteristic flavonoid colour reactions, purplish brown with $FeCl_3$, yellow with NaOH, yellowish orange with Mg–HCl, pink with Zn–HCl, and a yellow precipitate with basic lead acetate. IR showed OH and α,β-unsaturated carbonyl absorptions at 3300 and 1645 cm^{-1}, respectively and a broad C—O stretching band in the region 1000–1100 cm^{-1}, suggesting its glycosidic nature. The UV spectrum of the compound (**1**), exhibiting maxima at 273 and 336 nm, was very similar to those reported for a number of flavonoids of the apigenin type [5]. The bathochromic shifts of the UV absorption band I of spinosin with NaOEt and with $AlCl_3$ suggested the presence of free 4′- and 5-hydroxyl groups in the flavonoid aglycone. Acid hydrolysis gave an aglycone, mp 242–4°, $[\alpha]_D^{24}$ +40.7° (MeOH) which was identical with an authentic sample of swertisin (mmp, UV, NMR, co-TLC). In the hydrolysate, freed of the aglycone, only D-glucose was detected by TLC and GLC (TMS ether). The PMR spectrum of the compound in DMSO-d_6 showed a three proton singlet at δ 3.93 ppm (MeO), two doublets of one proton each at δ 4.23 ppm (J = 7 Hz, H-1‴) and δ 4.75 ppm (J = 10 Hz, H-1″) and a two proton singlet at δ 6.8 ppm (H-3 and H-8), two pairs of *ortho* coupled doublets of two protons each at 6.97 ppm (J = 9 Hz, H-3′ and H-5′) and δ 7.97 ppm (J = 9 Hz, H-2′ and H-6′) and a broad signal at δ 12.4 ppm (5-OH) for one proton. Treatment of spinosin with excess diazomethane followed by hydrolysis of the resulting di-*O*-methyl ether (**2**) with 5% H_2SO_4 for 4 hr afforded fine colourless needles, mp 300–2°, identical with tri-*O*-methylisovitexin and exhibited no change in the UV spectrum in the presence of alkaline reagents. These experiments established that compound **1** was a swertisin-X″-*O*-glucoside. That the interglycosidic linkage was β-oriented was evident from the PMR data of **1** and from the molecular rotation differences [6]. The molecular rotation difference (−81°) between spinosin and swertisin is very similar to $[M]_D$ (−62°) of methyl-β-D-glucopyranoside. Treatment of spinosin with acetone in the presence of $CuSO_4$ afforded mono- and di-isopropylidene derivatives indicating that the second glucose moiety was not linked to 4″-*O*- or 6″-*O*-positions. When **1** was acetylated with pyridine and acetic anhydride a nona-acetate (**3**) was obtained. One acetyl signal, in the PMR spectrum of the nona-acetate, appeared at an unusually high field position (δ 1.81 ppm). This information coupled with the fact that di-*O*-methylspinosin consumed 1.9 mol of periodate, yielding swertisin after hydrolysis of the oxidation product would lead to the postulation of (1‴ → 3″) linkage of the *O*-β-D-glucosyl unit. The mass spectrum of permethylated spinosin (M^+ *m/e* 734) however, did not exhibit the fragmentation characteristic of such a linkage. The absence of M-15 and M-31 peaks (*ca* 0.3% when measured with an AEI MS-30 instrument) in the mass spectrum of permethylated spinosin is indicative of a 2″-*O*-glycosylated compound [8]. In order to resolve this ambiguity permethylated spinosin was subjected to hydrolysis and subsequent acetylation. The resulting hexa-*O*-methylisovitexin monoacetate was subjected to spin decoupling experiments. The PMR spectrum of this compound showed the presence of a doublet at δ 4.88 ppm (J = 10 Hz) for H-1″ and a multiplet at δ 5.82 ppm for the proton on the acetylated oxymethine carbon atom. Irradiation of H-1″ resulted in a broad doublet for the signal at δ 5.82 ppm. Decoupling of the proton at δ 5.82 ppm resulted in a sharp singlet for H-1″ indicating that it must be H-2″. This thus fixes the site of acetylation and thereby the position of the second glucose unit at 2″-O of the swertisin moiety in spinosin. The ^{13}C NMR spectrum of the compound in DMSO-d_6 was also in agreement with this structure. The glycosidation effect on C-2″ was *ca* +10 ppm, this signal appearing at δ 80.3 ppm. On the basis of these observations the structure of spinosin was established as 2″-*O*-β-D-glucopyranosylswertisin (**1**).

Implicit in the above results is the restricted applicability of the high field acetyl signal as being diagnostic for the 2″-*O*-acetyl in X″-*O*-glycosylated flavonoid-6-*C*-glycoside acetates [7]. In this connection it is interesting to note that in flavonoid 6-*C*-glycoside acetates methylation or glycosidation of 7-OH leads to an upfield shift (0.07 ppm) of the highest field acetyl signal. Thus in 6-*C*-glycosyltricin acetate the signal at δ 1.83 ppm moves to δ 1.75 ppm on 7-*O*-glycosidation. A similar effect is seen on comparing the upfield acetate signal in the acetates of isovitexin (δ = 1.82 ppm) and its 7-*O*-methyl ether (swertisin) (δ = 1.75 ppm). This is probably due to conformational effects brought about by substitution of

*Part XVII in the series "Structure of Flavone-*C*-glykosides."

the 7-OH. Further glycosidation of the existing *C*-glycosyl unit as in the case of spinosin would also lead to the existence of preferred conformations in solution. The results of periodate titration of such compounds should therefore be treated with caution.

An *O*-glucosylswertisin – flavoayamenin – has been isolated from the petals of *Iris nertshinskia* [9], the linkage position of the *O*-glycosyl moiety being however undetermined. Though its mp 258–9° is very near to that of spinosin, no comparison could be made due to unavailability of an authentic sample of flavoayamenin.



1 Spinosin $R_1, R_2 = H$
2 Di-*O*-Methylspinosin $R_1 = Me$; $R_2 = H$
3 Spinosin nona-acetate $R_1, R_2 = Ac$
4 Permethylspinosin $R_1, R_2 = Me$

EXPERIMENTAL

Isolation and purification of spinosin (**1**). The commercially available seeds (5 kg) of *Zizyphus vulgaris* var. *spinosus* were defatted by repeated extraction with petrol (60–80°). They were then extracted with MeOH and the extract was concd to a dark brown viscous residue which was partitioned between Et_2O and H_2O. The aq. layer was extracted with EtOAc followed by BuOH. The BuOH extract was concd to a dark brown residue which was subjected to column chromatography on Si gel, using MeOH–$CHCl_3$ as eluent, to yield spinosin (**1**). The compound was crystallized from MeOH as yellowish needles, mp 255–256°, $[\alpha]_D^{24}$ + 16.5 (*c*, 0.21 in MeOH); IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1645 (CO), 1605, (C=C), 1100–1000 (C—O); λ_{max}^{EtOH} nm (log ε): 273 (4.34), 336 (4.43); with NaOEt 235 (4.39), 273 (4.37), 392 (4.61); with NaOAc 273 (4.24), 338 (4.27), 399 (4.10), with NaOAc + H_3BO_3 273 (4.29), 337 (4.38), 403 (3.51); with $AlCl_3$ 284 (4.26), 304 (4.24), 355 (4.39); (Found: C, 53.24; H, 5.76. $C_{28}H_{32}O_{15}\cdot H_2O$ requires: C, 53.67; H, 5.47%). ^{13}C NMR (DMSO-d_6): Aglycone: δ = 181.6 ppm (C-4), 164.3 (C-7), 163.7 (C-2), 161.1 (C-4'), 159.9 (C-5), 156.9 (C-9), 127.9 (C-2', C-6'), 120.9 (C-1'), 115.6 (C-3', C-5'), 108.7 (C-6), 104.1 (C-10), 102.8 (C-3), 90.2 (C-8), 55.9 (OMe); *C*-glucosyl δ = 81.0 ppm (C-5''), 80.3 (C-2''), 78.2 (C-3''), 70.3 (C-4''), 70.7 (C-1''), 61.3 (C-6''), *O*-glucosyl δ = 104.6 ppm (C-1'''), 76.1, 75.9 (C-3''', C-5'''), 74.3 (C-2'''), 69.6 (C-4'''), 60.5 (C-6''').

Hydrolysis of **1**. A solution of **1** in 5% HCl was heated under reflux for 3 hr. The solid, separating on cooling, was crystallized from H_2O to give a yellowish powder, mp 242–4°, $[\alpha]_D^{24}$ +40.7° (*c*, 0.17 in MeOH), which was identified as swertisin by mmp, UV, IR NMR and co-TLC with an authentic sample. The aq. layer was neutralized with Ag_2CO_3, filtered and the filtrate was evapd under red. pres. The residue was found to contain only D-glucose by TLC and GLC (TMS ether).

Di-O-methylspinosin (**2**). A sample of **1** (200 mg) was etherified with ethereal CH_2N_2 in the usual way and the product, purified by preparative TLC (MeOH–$CHCl_3$–H_2O 28:52:8, R_f 0.42), separated from MeOH–H_2O as a brownish powder, mp 183–5°, $[\alpha]_D^{24}$ +15.4° (*c*, 0.23 in MeOH). λ_{max}^{EtOH} nm (log ε): 265 (4.36), 324 (4.53); (Found C, 57.04; H, 5.78. $C_{30}H_{36}O_{15}$ calcd: C, 56.6; H, 5.7%). Acid hydrolysis of the above compound by refluxing for 3 hr with 5% HCl in MeOH yielded di-*O*-methylswertisin, mp 300–2°, identified by mmp and TLC comparison with an authentic sample.

Isopropylidene derivatives of spinosin. A soln of spinosin (100 mg) in dry Me_2CO (200 ml) was refluxed for 5 hr in presence of dry $CuSO_4$ (200 mg) and filtered. After removal of the solvent under red. pres., the residue was subjected to preparative TLC (MeOH–$CHCl_3$–H_2O 28:52:8) to yield the di-isopropylidene derivative, R_f 0.7, mp 233–6°, MS (*m/e*): 688 (M^+), and the mono-isopropylidene derivative, R_f 0.59, mp 218–22°, MS (*m/e*): 648 (M^+). Further treatment with Me_2CO and $CuSO_4$ of the latter yielded the di-isopropylidene derivative.

Spinosin nona-acetate (**3**). Spinosin (100 mg) was heated with Ac_2O (4 ml) and Py (3 ml) for 5 hr and worked up as usual. The acetate was purified by preparative TLC ($CHCl_3$–EtOAc 7:3, R_f 0.21), mp 150–3°. PMR ($CDCl_3$) δ = 1.82 ppm, 1.93, 2.02, 2.04, 2.07, 2.13 (singlets, 21 H, sugar acetyls), 2.34 (*s*, 3 H, 4'-*O*-acetyl), 2.47 (*s*, 3 H, 5-*O*-acetyl); (Found C, 54.82; H, 5.5. $C_{46}H_{50}O_{24}\cdot H_2O$ calcd: C, 54.98; H, 5.22%).

Permethylation of spinosin. Spinosin (120 mg) was permethylated according to the method of Hakomori [10]. The product (**4**), after the usual workup, was separated as a brownish powder mp 93–95°, from MeOH–H_2O $[\alpha]_D^{24}$ +3.1° (*c*, 0.56 in MeOH). The IR spectrum showed the absence of OH band indicating permethylation. MS (*m/e*) AEI-MS 90, MS-30: 734 (M^+, 1.4%), 687 (1.0), 573 (0.9), 559 (3.2), 545 (5.9), 515 (45.1), 499 (100), 467 (13.5), 397 (8.2), 355 (11.8), 341 (89.2), 325 (27.9), 311 (10.8). (Found: C, 60.58; H, 6.81. $C_{37}H_{50}O_{15}$ calcd: C, 60.48; H, 6.86%).

Hexa-O-methylisovitexin 2-O-monoacetate. Per-*O*-methylspinosin was hydrolysed in a sealed tube with 2 N HCl–MeOH for 3.5 hr at 120°. The product was purified by preparative TLC (C_6H_6–Me_2CO–MeOH 200:100:5, R_f 0.25) and acetylated in the cold with Ac_2O and Py for 15 hr to yield the monoacetate, R_f 0.65. PMR ($CDCl_3$, 200 MHz) δ = 7.78 ppm (*d*, *J* = 8.5 Hz, 2 H, H-2' and H-6'), 6.96 (*d*, *J* = 8.5 Hz, 2 H, H-3' and H-5'), 6.70 (*s*, 1 H, H-8), 6.54 (*s*, 1 H, H-3), 5.82 (*m*, 1 H, H-2''), 4.88 (*d*, *J* = 10 Hz, 1 H, H-1''), 3.93–3.37 (23 H, MeO and sugar protons), 1.81 (*s*, 3 H, OAc-2'').

Periodate oxidation of di-O-methylspinosin. A solution of di-*O*-methylspinosin (63.6 mg) in MeOH (10 ml) was added to 10 ml of aq. 0.05 M HIO_4 and was allowed to react in the dark for 5 days till TLC showed the absence of starting material. The residual HIO_4 in soln was estimated by titration with 0.01 M Na_3AsO_3. Periodate consumption was 1.9 mol per mol of di-*O*-methyl spinosin. The reaction mixture after removal of excess periodate, was hydrolysed with 5% HCl–MeOH for 4 hr. Di-*O*-methyl swertisin, mp 300–2°, was identified (mmp and co-TLC) as the degradation product.

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FLAVONOIDS OF *PROSOPIS SPICIGERA* FLOWERS

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Prosopis spicigera, a moderate-sized thorny tree, is reported to possess medicinal properties [1]. During the course of our work on plants of medicinal interest, *Prosopis spicigera* flowers which had not been systematically studied before, were investigated. Earlier, the isolations of patulitrin [2], sitosterol [3] and spicigerin [4] were reported from this plant. This communication reports the isolations and characterizations of two phenolic compounds, prosogerin-A and B.

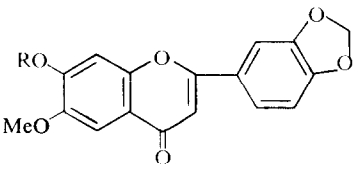
Air-dried flowers (5 kg) of *P. spicigera* were extracted with petrol and then benzene. The benzene extract on preparative TLC (silica gel) using C_6H_6–MeOH (9:1) yielded prosogerin-A and B which were characterized as 6-methoxy-7-hydroxy-3′,4′-methylenedioxyflavone (**1**) and 2′,4′-dihydroxy-5′-methoxy-3,4-methylenedioxychalkone (**2**), respectively.

Prosogerin-A

$C_{17}H_{12}O_6$ (M^+ 312). Colour reactions and spectral data indicated it to be a flavonoid. Prosogerin-A (**1**) formed a monoacetate (**1a**) and a monoethyl ether (**1b**) showing a free OH. **1** developed a bluish-green colouration with gallic acid–H_2SO_4 characteristic for the methylenedioxy group at δ 6.05 besides those for the other trum of its acetate (**1a**) which had a signal for the methylenedioxy group at δ 6.05 besides those for the other substituents: an acetoxyl (3H, δ 2.34) and a methoxyl (3H, δ 3.90). On alkali fission, **1** gave piperonylic acid fixing methylenedioxy group at C-3′ and C-4′ positions in the B-ring. Further, the signal at δ 6.64 (1H) was considered due to an aromatic proton at C-3 as observed for flavonoids having an unsubstituted C-3 position [5,6]. The signal at δ 7.66 (1H) due to the aromatic proton at C-5 was a singlet thereby showing a substituent at C-6. As the signal due to the aromatic proton at C-8 was shifted downfield to δ 7.22, the acetoxyl function was considered at C-7. Consequently the methoxyl function was placed at C-6 and prosogerin-A must be 6-methoxy-7-hydroxy-3′,4′-methylenedioxyflavone (**1**). This structure was fully supported by its mass spectrum and by the synthesis of its 7-*O*-ethyl ether (**2b**) [7].

Prosogerin-B

$C_{17}H_{14}O_6$ (M^+ 314). Colour reactions and spectral data showed it to be a chalkone derivative having a methylenedioxy, a methoxyl and hydroxyl (s) as the substituents. Prosogerin B (**2**) yielded a monoethyl ether (**2a**) which dissolved in 10% aq. NaOH and also gave positive ferric reaction indicating that **2** contained a chelated OH at C-2′ besides one more OH function. On alkali fission, **2** yielded piperonylic acid showing the methylenedioxy function at C-3 and C-4. UV spectral shifts with $AlCl_3$ and the colour with alc. $FeCl_3$ confirmed a chelated OH at C-2′, **2** did not give a Gibbs



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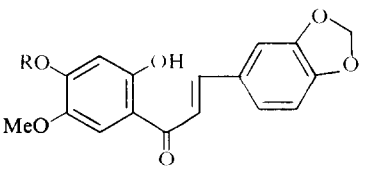
1a R = COMe
1b R = Et



2 R = H
2a R = Et