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A NEOLIGNAN AND STEROLS IN FRUITS OF *SOLANUM SISYMBRIFOLIUM**

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Key Word Index—*Solanum sisymbrifolium*; Solanaceae; fruits; neolignan; sisymbrifolin; sterols; carpesterol.

Abstract—A new neolignan, designated as sisymbrifolin, and carpesterol were isolated from the berries of *Solanum sisymbrifolium*. Their structures were established mainly on the basis of 2D NMR analyses.

INTRODUCTION

Solanum sisymbrifolium [2] is a densely prickly and stellate-pubescent, erect, perennial undershrub, with oblong or oblong-lanceolate leaves. Its berries are globose–obovoid, shiny red when ripe, 0.5–1.5 cm in diameter. A native to South America, this species is distributed mainly in the hotter parts of India.

The family Solanaceae is well known [3] to elaborate steroidal alkaloids and spirostane sapogenins through common biogenetic precursors. It is also reported [4–8] that in some *Solanum* species, steroidal alkaloids are the only constituents present in roots, while their aerial part produce exclusively the spirostane derivatives. Though *S. sisymbrifolium* is reported to yield the steroidal alkaloid, solasodine [9], as well as spirostane derivatives [10], *viz.* nuatigenin and isonuatigenin, no alkaloid or spirostane derivatives could be detected in any part of plants collected in June–July around Calcutta. On the other hand, a new neolignan, designated as sisymbrifolin (**1**), and carpesterol (**2**), a rare C_{30} sterol, together with β-sitosterol and its β-D-glucoside, were isolated from the berries of this species. To the best of our knowledge, this is the first report of the isolation of a lignan from a *Solanum* species and as such it may have chemotaxonomic significance. The present paper deals with the structural elucidation of **1** and the characterization of **2**, mainly on the basis of 2D-NMR spectral analysis.

RESULTS AND DISCUSSION

A methanol extract of defatted barries was fractionated by successive extractions with methylene di-chloride, ethylacetate and *n*-butanol. The ethylacetate extract on repeated column chromatography over silica gel yielded a fraction containing compound **1**. Further purification was effected *via* the corresponding acetate. The crude acetate on chromatography over neutral alumina furnished two acetates (**3** and **4**). The latter could, however, be converted into the former by treatment with acetic anhydride-pyridine. Compound **2**, on the other hand, was obtained from the methylene dichloride extract.

The electron impact mass spectra of **3** and **4** showed a $[M]^+$ at *m/z* 602 and 560, corresponding to the molecular formulae $C_{30}H_{34}O_{13}$ and $C_{28}H_{32}O_{12}$, respectively. The 500 MHz ^{1}H NMR spectrum of **3** showed the presence of five acetoxyl groups, including one phenolic acetoxyl (δ2.30), which was absent in the spectrum of **4**. It was, therefore, evident that **1** contains four alcoholic hydroxyl and one phenolic hydroxyl groups. Moreover, the spectrum of **3** displayed two three-proton singlets at δ3.812 and 3.905 for two methoxyl groups, two multiplets for two protons each at δ3.78 and 4.26, a one-proton double doublet at δ4.460 (J = 11 and 4.5 Hz), a one-proton doublet of a double doublet at δ5.405 (J = 7, 7 and 4 Hz) and two doublets at δ5.531 (J = 7 Hz) and 5.885 (J = 7 Hz), besides signals for aromatic protons, viz. three singlets at δ6.82, 6.83 and 7.0, and two one-proton double doublets at δ6.94 (J = 8.5 and 2.5 Hz) and δ7.0 (J = 8.5 and 2.0 Hz). The ^{13}C NMR spectrum of **3** displayed signals for 18 skeletal carbons, besides, two methoxyl and five acetoxyl carbons. Of these, 12 carbons (δ109.6–150.9), comprising five hydroxyl and seven quaternary carbons, belong to aromatic moieties; the remaining six carbons (four methine and two methylene) are aliphatic in nature. The chemical shifts of these aliphatic carbons indicated that three methine (δ72.1–87.6) and two methylene (δ61.9 and 64.8) carbons are attached to the oxygen functions and one methine (δ50.2) is linked to other carbons.

*Part 96 in the series on 'Indian Medicinal Plants'. For Part 95, see ref. [1].

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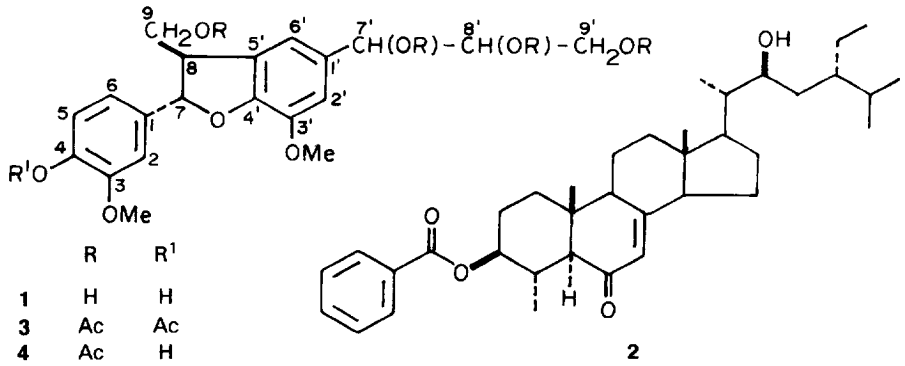
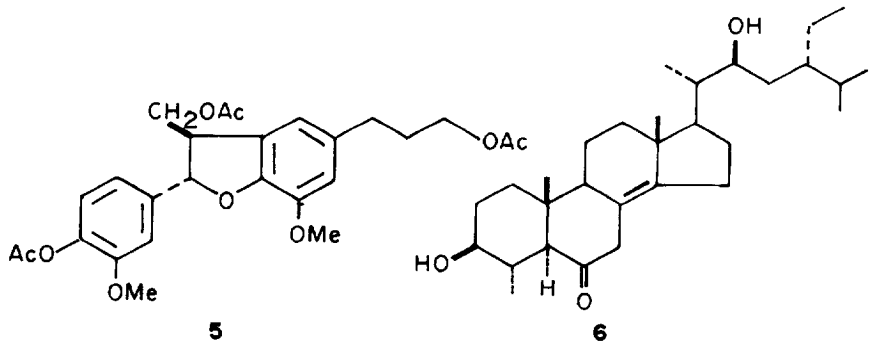


	R	R^1
1	H	H
3	Ac	Ac
4	Ac	H

2



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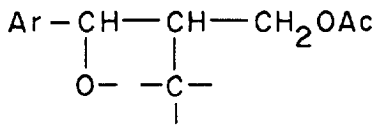
Ar–CH(OAc)–CH(OAc)–CH_2OAc

A



B

The ^{1}H–^{1}H COSY spectrum of **3** showed that one methine (δ_C72.1; δ_H5.41) proton was coupled to another methine (δ_C73.4; δ_H5.89) proton, as well as two non-equivalent protons of a CH_2OAc (δ_H3.80 and 4.25) group indicating the presence of part structure **A** in the molecule. Again, the methine (δ_C50.2) proton resonating at δ3.78, was found to be correlated with a methine (δ_C87.6, δ_H5.53) proton, as well as with the methylene protons (δ_H4.29 and 4.46) of a CH_2OAc group, demonstrating the presence of the second part structure **B** in the molecule.

Based on the above evidence, a neolignan structure having a dihydrobenzofuran ring system was assigned for **1**. That the aromatic part of the C_6–C_3 unit of **1** must be 3-methoxy-4-hydroxyphenyl, became apparent on the following considerations. (i) Two one-proton double doublets at δ7.0 and δ6.94, assigned respectively to H-5 and H-6, showed mutual correlation in the ^{1}H–^{1}H COSY spectrum of **3**. Also, the H-5 and H-6 signals suffer unequal splitting (2.0 and 2.5 Hz) due to their interaction with H-2. (ii) ^{13}C chemical shifts of C-1 to C-6 of **3** were in excellent agreement with those reported [11] for dihydrodehydrodiconiferyl alcohol triacetate (**5**).

The observed very close ^{13}C chemical shifts of C-7 to C-9 of **3** with those reported [11] for **5** indicated a *trans*-relationship between aryl and CH_2OAc groups attached respectively to C-7 and C-8, as in **5**. The α-orientation of the aryl group at C-7 was evident from the negative Cotton effect [12] at 248 nm in the circular dichroic spectrum of **3**. The precise stereochemistry at C-7′ and C-8′, however, could not be established from the available data.

Though **2** was eventually characterized as the rare C_{30} sterol benzoate, carpesterol, initially we had difficulty in identifying the skeleton to which the compound belongs due to the complex nature of the molecule. This sterol was previously isolated [13] from *S. xanthocarpum* and its structure established [14] by X-ray crystallography only; no detailed spectroscopic data, particularly ^{1}H and ^{13}C NMR were available for comparison. We would, therefore, like to include a brief discussion on its characterization based on 2D-NMR studies, particularly to strengthen the potential of modern pulse NMR techniques in solving structural problems of complex natural products.

The molecular formula of **2** was determined to be $C_{37}H_{54}O_4$ ([M]$^+$ *m/z* 562.4052) by high resolution mass

Table 1. ^1H–^{13}C Multiple-bond correlation data for compound **2**

δ_H (ppm)			δ_C (ppm)		
4.693 (H-3)	17.48 (C-30)	26.19 (C-2)	31.80 (C-4)	166.45 (PhCOO)	
1.096 (H_3-30)	31.80 (C-4)	60.04 (C-5)	78.97 (C-3)		
2.27 (H-5)	14.72 (C-19)	17.48 (C-30)	31.80 (C-4)	39.34 (C-10)	51.06 (C-9)
	200.21 (C-6)				
0.929 (H_3-19)	36.27 (C-1)	39.34 (C-10)	51.06 (C-9)	60.04 (C-5)	
2.23 (H-9)	14.72 (C-19)	39.34 (C-10)	60.04 (C-5)	161.02 (C-8)	
5.702 (H-7)	51.06 (C-9)	54.96 (C-14)	60.04 (C-5)		
0.622 (H_3-18)	38.84 (C-12)	45.11 (C-13)	53.13 (C-17)	54.96 (C-14)	
1.45, 2.14 (H_2-12)	21.74 (C-11)	45.11 (C-13)	51.06 (C-9)	54.96 (C-14)	
2.06 (H-14)	12.35 (C-18)	22.57 (C-15)	45.11 (C-13)	123.68 (C-7)	161.02 (C-8)
1.32 (H-17)	12.35 (C-18)	12.52 (C-21)	38.84 (C-12)	42.64 (C-20)	45.11 (C-13)
0.956 (H_3-21)	42.64 (C-20)	53.13 (C-17)	71.08 (C-22)		
1.72 (H-20)	12.52 (C-21)	30.02 (C-23)	53.13 (C-17)		
3.735 (H-22)	12.52 (C-21)	41.43 (C-24)			
1.29 (H-24)	11.83 (C-29)	23.63 (C-28)	28.76 (C-25)		
0.898 (H_3-29)	23.63 (C-28)	41.43 (C-24)			
0.813 (H_3-26)	20.54 (C-27)	28.76 (C-25)	41.43 (C-24)		
0.907 (H_3-27)	17.64 (C-26)	28.76 (C-25)	41.43 (C-24)		

spectrometry. Its IR and 500 MHz ^1H NMR spectra showed the presence of a benzoyloxyl, an α,β-unsaturated C=O and a secondary hydroxyl groups. In addition, the ^1H NMR spectrum displayed signals for one primary, four secondary and two tertiary methyl groups. The compound on treatment with 1% methanolic KOH yielded the debenzoylated product (**6**) as an oil as the major compound, along with some other minor inseparable isomers. The ^1H NMR spectrum of **6** showed that hydrolysis of the benzoyl group of **2** was accompanied by the migration of the conjugated trisubstituted double bond to an unconjugated tetrasubstituted position. The ^{13}C NMR spectra of **2** and **6** were equally inconclusive in determining the nature and skeleton of the compounds.

The unambiguous assignment of all the ^1H and ^{13}C NMR signals of **2** could be accomplished by analyses of its ^1H–^1H COSY, ^1H–^{13}C COSY and HMBC spectra. The one-bond and multiple-bond (Table 1) ^1H–^{13}C correlation data clearly demonstrated the presence of a carbon skeleton as indicated by the heavy lines in structure **7**. It can be seen that no correlation was obtained for C-16 (δ27.03). However, the chemical shift could be assigned for it unambiguously by the method of elimination since all other carbon signals were already assigned.

Having established the structure of the benzoate as **2**, the relative stereochemistry of the ring chiral centres could be determined from the NOE interactions observed in its NOESY spectrum (Fig. 1). However, the sterochemistry of the side-chain chiral centres could not be established from the available data.

The structure of the KOH hydrolysis product of **2** (see above) could now be identified as the $\Delta^{8(14)}$ isomer **6** on

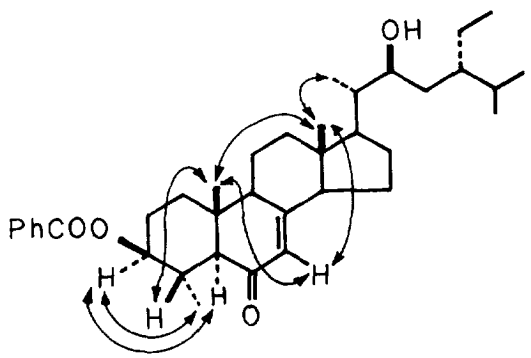
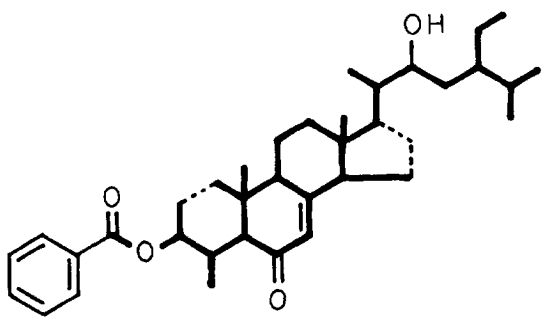


Fig. 1. NOE interactions observed in NOESY spectrum of **2**.



7

the basis of the deshielding of the C-15 and C-18 carbon signals (see Experimental) in its ^{13}C NMR spectrum by 3.5 and 4.4 ppm, respectively, when compared with those of **2**.

EXPERIMENTAL

General. Mps: uncorr. NMR: TMS as int. standard.

Plant material. Berries of *S. sisymbrifolium* Lam. were collected in the vicinity of Calcutta during June–July, 1994. A voucher specimen is deposited in the herbarium of the Indian Institute of Chemical Biology, Calcutta.

Extraction and isolation. Powdered and milled berries (0.9 kg) were extracted with MeOH at room temp. for 72 hr. The extract was concd (50 ml) *in vacuo*. The concentrate was then diluted with H_2O (300 ml) and successively extracted with CH_2Cl_2, EtOAc and *n*-BuOH. The CH_2Cl_2-soluble fr. (3.5 g) on chromatography over silica gel yielded **2** (70 mg), besides β-sitosterol (0.1 g) and its β-D-glucoside (0.12 g). The EtOAc-soluble fr. (3.0 g) was chromatographed over silica gel. Elution with $CHCl_3$–MeOH (9:1) yielded a viscous oil (0.2 g), which, on further chromatography over the same adsorbent, gave a fr. containing **1** as the major compound. Since no further purfication was possible, the fr. was acetylated with Ac_2O–pyridine at room temp. The product on chromatography over neutral alumina furnished sisymbrifolin pentacetate (**3**, 80 mg) and sisymbrifolin tetraacetate (**4**, 10 mg).

Sisymbrifolin pentaacetate (**3**). Viscous oil. $[\alpha]_D$ + 10.7° ($CHCl_3$; *c* 1.96). UV λ_{max}^{MeOH} nm (log ε): 258 (2.82), 279 (3.17). IR ν_{max}^{KBr} cm^{-1}: 1744, 1730, 1606, 1240. ^1H NMR ($CDCl_3$) δ 2.05 and 2.06 (3H each, *s*, OAc × 2), 2.06 (6H, *s*, OAc × 2), 2.30 (3H, *s*, phenolic OAc), 3.81 and 3.91 (3H each, *s*, OMe × 2), 3.78 (1H, *m*, H-8), 3.80 and 4.25 (1H each *m*, H_2-9'), 4.29 (1H, *d*, *J* = 11.0 Hz, H_a-9), 4.46 (1H, *dd*, *J* = 11.0, 4.5 Hz, H_b-9), 5.41 (1H, *ddd*, *J* = 7.0, 7.0, 4.0 Hz, H-8'), 5.53 (1H, *d*, *J* = 7.0 Hz, H-7), 5.89 (1H, *d*, *J* = 7.0 Hz, H-7'), 6.82, 6.83 and 7.0 (1H each, *s*, H-2, H-2' and H-6'), 6.94 (1H, *dd*, *J* = 8.5, 2.5 Hz, H-6), 7.0 (1H, *dd*, *J* = 8.5, 2.0 Hz, H-5). ^{13}C NMR ($CDCl_3$): δ 139.4 (C-1), 109.6 (C-2), 150.9 (C-3), 139.0 (C-4), 122.5 (C-5), 117.8 (C-6), 87.6 (C-7), 50.2 (C-8), 64.8 (C-9), 127.2 (C-1'), 111.6 (C-2'), 144.1 (C-3'), 148.1 (C-4'), 129.4 (C-5'), 115.4 (C-6'), 73.4 (C-7'), 72.1 (C-8'), 61.9 (C-9'), 55.5 and 55.8 (OMe × 2), 20.3 and 20.5 ($OCOCH_3$ × 5), 168.4, 169.3, 169.6, 169.9 and 170.2 ($OCOCH_3$ × 5). EIMS *m/z* (rel. int.): 602 $[M]^+$ (10), 542 (6), 500 (15), 440 (3), 415 (5), 331 (28), 313 (17), 292 (7), 250 (16), 189 (25), 169 (100).

Sisymbrifolin tetraacetate (**4**). Viscous oil. $[\alpha]_D$ + 6.9°. ($CHCl_3$: *c* 4.87). UV λ_{max}^{MeOH} nm (log ε): 257 (2.61), 280 (2.96). IR ν_{max}^{KBr} cm^{-1}: 3460, 1751, 1727, 1604, 1248. ^1H NMR ($CDCl_3$) δ 2.02 and 2.07 (3H each, *s*, OAc × 2), 2.09 (6H, *s*, OAc × 2), 3.78 and 3.90 (3H each, *s*, OMe × 2), 3.82 (1H, *m*, H-8), 3.91 (1H, *m*, H_a-9), 4.25 (1H, *dd*, *J* = 12.0, 4.0 Hz, H_b-9'), 4.30 (1H, *dd*, *J* = 11.0, 7.5 Hz, H_a-9), 4.45 (1H, *dd*, *J* = 11.0, 5.0 Hz, H_b-9), 5.42 (1H, *m*, H-8'), 5.46 (1H, *d*, *J* = 7.5 Hz, H-7), 5.90 (1H, *d*, *J* = 7.5 Hz, H-7), 6.80–6.90 (5H, *m*, Ar-H × 5). EIMS *m/z* (rel. int.): 560 $[M]^+$ (12).

Carpesterol (**2**). Recrystallized $CHCl_3$–MeOH, needles, mp 248°–249° (lit. mp 248°) [13]. $[\alpha]_D$ + 68.3° ($CHCl_3$; *c* 0.98) (lit. + 67°) [15]. IR ν_{max}^{KBr} cm^{-1}: 3548, 1702, 1674, 1272. ^1H NMR ($CDCl_3$): δ 0.62 (3H, *s*, H_3-18), 0.81 (3H, *d*, *J* = 6.8 Hz, H_3-26), 0.90 (3H, *t*, *J* = 7.3 Hz, H_3-29), 0.91 (3H, *d*, *J* = 6.8 Hz, H_3-27), 0.93 (3H, *s*, H_3-19), 0.96 (3H, *d*, *J* = 6.7 Hz, H_3-21), 1.10 (3H, *d*, *J* = 5.5 Hz, H_3-30), 3.74 (1H, *dd*, *J* = 10.7, 1.5 Hz, H-22), 4.69 (1H, *ddd*, *J* = 10.6, 10.6, 4.5 Hz, H-3), 5.70 (1H, *dd*, *J* = 2.1, 2.1 Hz, H-7), 7.57 (1H, *ddd*, *J* = 7.5, 7.5, 1.5 Hz, H-4'), 7.45 (2H, *dd*, *J* = 7.6, 7.6 Hz, H-3' and H-5'), 8.06 (2H, *dd*, *J* = 8.5, 1.5 Hz, H-2' and H-6'). ^{13}C NMR ($CDCl_3$) δ 11.8 (C-29), 12.4 (C-18), 12.5 (C-21), 14.7 (C-19), 17.5 (C-30), 17.6 (C-26), 20.5 (C-27), 21.7 (C-11), 22.6 (C-15), 23.6 (C-28), 26.2 (C-2), 27.0 (C-16), 28.8 (C-25), 30.0 (C-23), 31.8 (C-4), 36.3 (C-1), 38.8 (C-12), 39.3 (C-10), 41.4 (C-24), 42.6 (C-20), 45.1 (C-13), 51.1 (C-9), 53.1 (C-17), 55.0 (C-14), 60.0 (C-5), 71.0 (C-22), 79.0 (C-3), 123.7 (C-7), 128.4 (C-3' & C-5'), 129.6 (C-2' & C-6'), 130.5 (C-1'), 132.9 (C-4'), 161.0 (C-8), 166.5 (CO), 200.2 (C-6). EIMS *m/z* (rel. int.): 562.4052 $[M]^+$ (4), 544 (3), 440 (85), 434 (12), 426 (15), 312 (100), 297 (22), 257 (69), 244 (16), 129 (6), 111 (9), 109 (9), 105 (7).

Hydrolysis of **2**. Compound **2** (50 mg) was hydrolysed with 1% KOH in MeOH under reflux for 2 hr. After removing most of the MeOH from the reaction mixt. *in vacuo*, the concentrate was diluted with H_2O (15 ml) and extracted with CH_2Cl_2. The product on chromatography over silica gel yielded **6** (30 mg) as an oil. ^1H NMR ($CDCl_3$): δ 3.16 (1H, *m*, H-3), 3.78 (1H, *m*, H-22). ^{13}C NMR ($CDCl_3$): δ 36.0 (C-1), 29.9 (C-2), 76.0 (C-3), 34.5 (C-4), 63.4 (C-5), 208.4 (C-6), 47.9 (C-7), 123.6 (C-8), 49.7 (C-9), 41.1 (C-10), 20.4 (C-11), 37.0 (C-12), 43.6 (C-13), 145.0 (C-14), 26.0 (C-15), 26.6 (C-16), 53.9 (C-17), 16.7 (C-18), 14.0 (C-19), 41.5 (C-20), 12.3 (C-21), 71.0 (C-22), 30.3 (C-23), 41.3 (C-24), 28.7 (C-25), 17.6 and 20.4 (C-26 and C-27), 23.5 (C-28), 11.8 (C-29), 18.1 (C-30). EIMS *m/z* (rel. int.) 458 $[M]^+$ (7).

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