Phytochemistry, Vol. 28, No. 9, pp. 2419–2422, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00
Maxwell Pergamon Macmillan plc.

A FURANOID DITERPENE GLUCOSIDE FROM *TINOSPORA CORDIFOLIA*

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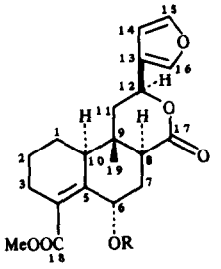
(*Received in revised form* 16 *January* 1989)

Key Word Index—*Tinospora cordifolia*; Menispermaceae; novel furanoid; diterpene glucoside.

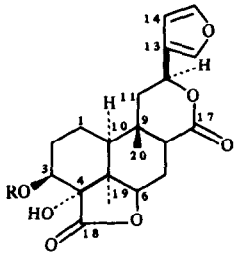
Abstract—A novel furanoid diterpene glucoside with the molecular formula $C_{26}H_{34}O_{11}$ was isolated from the stems of *Tinospora cordifolia*. Its complete structure was determined by spectroscopic and chemical studies along with the comparison of the spectral data with the related furanoid diterpene glucosides.

INTRODUCTION

Chemical investigation of *Tinospora cordifolia* has led to the isolation of a phenolic lignan and five diterpenic furano lactones which were reported earlier [1–7]. In the present paper the isolation and structure of a novel furanoid diterpene glucoside, without an angular methyl group at C-5 is reported. The structure (**1**) was established with the help of high resolution ^{1}H NMR, spin-decoupling, ^{13}C NMR and mass spectral studies of the parent compound, the tetra-acetate and acid hydrolysed product. The structure was further supported by comparing the spectral data with those of related furanoid diterpene glucosides such as borapetoside A (**4**) [8] borapetoside B (**5**) [9] and palmatoside A (**6**) [10].



1 R = β-D-glucopyranosyl
2 R = tetra-*O*-acetyl-β-D-glucopyranosyl
3 R = H



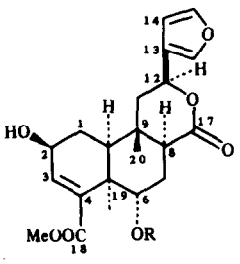
4 R = β-D-glucopyranosyl

RESULTS AND DISCUSSION

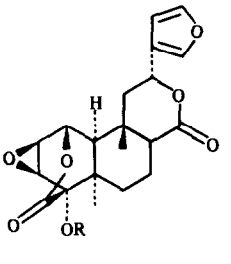
The hot chloroform extract of the stems of *Tinospora cordifolia* was subjected to column chromatography over silica gel in petrol–ethylacetate (1:1) and (3:7) to yield the two diterpenic furano lactones described in the preceding papers [4, 6]. The column was further eluted with ethyl acetate to provide a sticky mass, which showed a single spot on TLC (R_f 0.29). This was repeatedly crystallized from acetone–ethanol (1:5) to give compound **1** as colourless flakes, mp 199–201°.

The elemental analysis and mass spectral data *m/z* 343, $[M-(O\text{-}\beta\text{-D-glucopyranose})]^+$, established the molecular formula as $C_{26}H_{34}O_{11}$ for the furanoid diterpene glucoside. The IR spectrum showed characteristic absorptions for hydroxyl groups (3560–3430 cm^{-1}), a lactone carbonyl (1715 cm^{-1}), an α,β-unsaturated ester (1705 cm^{-1}), an tetra-substituted olefinic band (1675 cm^{-1}) and a furan ring (3140, 1510, 1130, 880 cm^{-1}, also positive Ehrlich test). The UV spectrum showed the presence of an α,β-unsaturated carbonyl (λ_{max} 272 nm, ε_{max} 2640).

The ^{1}H NMR assignments are given in Table 1. The signals at δ7.71 (*br s*, 1H), 7.68 (*br s*, 1H) and 6.65 (*br s*, 1H) were assigned to two α- and one β-protons of a β-substituted furan moiety. An angular methyl group was observed as a singlet at δ0.92 (3H). A very broad, D_2O exchangeable, singlet at δ4.48 (4H) was observed and assigned to four hydroxyl protons of a glucose moiety. The signals at δ5.57 (*dd*, $J_1=12.34$, $J_2=3.32$, 1H), 2.17 (*dd*, $J_1=14.51$, $J_2=3.84$, overlapped signals) and 1.94 (*dd*, appears to be *t*, 1H; from the irradiation experiment, as given below, $J_1=14.45$, $J_2=12.30$ Hz), were assigned to an 'ABX' system (C-11, C-12) as given in the part structure A. Two very broad singlets at δ4.49 (1H) and 2.58 (1H) were assigned to protons at C-6 and C-8, respectively. A broad doublet at δ2.64 ($J=14.59$, 1H) and one of the protons at 2.17 (*dd*, $J_1=14.51$, $J_2=3.84$, 2H,



5 R = β-D-glucopyranosyl

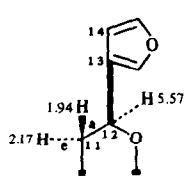


6 R = β-D-glucopyranosyl

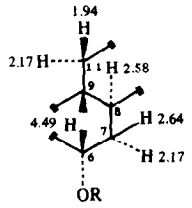
Table 1. ^{1}H NMR spectral data of diterpene glucoside **1** and related compounds (δ)

H	1*	2†	3	4	5	6
1	1.45 (*m*, 1H)	1.55 (*m*, 2H)				
	1.54 (*m*, 1H)					
2	1.56 (*m*, 1H)	1.63 (*m*, 1H)				
	1.72 (*m*, 1H)	1.75 (*m*, 1H)				
3	1.82 (*m*, 2H)	1.86 (*m*, 2H)		4.04	6.34	
6	4.49 (*br s*, 1H)	4.58 (*br s*, 1H)	3.68–3.81 (*m*, 1H)	5.02		
7	2.64 (*br d*, J = 14.59, 1H)	2.85 (*br d*, J = 15.19, 1H)				
	2.17‡ (*dd*, J = 14.51, 3.84, 1H)	2.16 (*m*, 1H)				
8	2.58 (*br s*, 1H)	2.44 (*m*, 1H)		2.78		2.78
10	2.39 (*m*, 1H)	2.38 (*m*, 1H)				1.62
H_e-11	2.17‡ (*dd*, J = 14.51, 3.84, 1H)	2.27 (*dd*, J = 14.68, 3.26, 1H)				
H_a-11	1.94‡ (*dd*, J = 14.45, 12.30, 1H)	2.44 (*m*, 1H)				
H_a-12	5.57 (*dd*, J = 12.34, 3.32, 1H)	5.45 (*dd*, J = 12.26, 2.74, 1H)	5.45 (*dd*, J = 12.26, 2.74, 1H)	5.90	5.49	5.48
14	6.65 (*br s*, 1H)	6.45 (*br s*, 1H)	6.57 (*br s*, 1H)	6.55	6.56	6.65
15	7.68 (*br s*, 1H)	7.43 (*br s*, 1H)	7.42 (*br s*, 1H)	7.51	7.65	7.68
16	7.71 (*br s*, 1H)	7.48 (*br s*, 1H)	7.48 (*br s*, 1H)	7.64	7.71	7.75
–Me	0.92 (*s*, 3H)	1.03 (*s*, 3H)	0.91 (*s*, 3H)	1.23, 1.12	1.45, 0.85	1.25, 1.05
–COOMe	3.65 (*s*, 3H)	3.74 (*s*, 3H)	3.78 (*s*, 3H)		3.69	
Sugar part						
–OH	4.48 (*br s*, 4H)					
1′	4.12 (*d*, J = 7.89, 1H)	4.60 (*d*, J = 7.99, 1H)		4.33	4.27	4.59
2′	4.23 (*t*, J = 5.30, 1H)	5.14 (*t*, J = 9.50, 1H)				
3′	3.00 (*m*, 1H)	4.90 (*t*, J = 8.77, 1H)				
4′	3.45 (*m*, 1H)	5.07 (*t*, J = 9.69, 1H)				
5′	2.83 (*m*, 1H)	3.66 (*m*, 1H)				
6′	3.13 (*m*, 2H)	4.28 (*dd*, J = 12.24, 4.34, 1H)				
		4.14 (*br d*, J = 12.20, 1H)				
Me CO		2.10 (*s*, 3H)				
		2.02 (*s*, 6H)				
		2.00 (*s*, 3H)				

*Measured in DMSO-d_6 at 500 MHz.
†Measured in $CDCl_3$ at 500 MHz, TMS as internal standard coupling constant (J) in Hz.
‡Overlapped signals.



A



B

overlapped signals) were assigned to protons at C-7. Signal at δ3.65 (*s*, 3H) was assigned to the ester methyl group. The signals at δ4.23 (*t*, J = 5.30, 1H) and 4.12 (*d*, J = 7.89, 1H) were assigned to sugar protons at C-2′ and C-1′, respectively. A multiplet at δ3.45 (1H) was assigned to a proton at C-4′. The signal at δ3.06 (*m*, 3H) was assigned to protons at C-3′, C-6′α and C-6′β. The signal for C-5′H was observed as a multiplet at δ2.83 (1H).

The above assignments were confirmed on the basis of 500 MHz ^{1}H NMR spin-decoupling experiments. On irradiating the signal at δ5.57 (H-12, X of ABX) the signals at 2.17 and 1.94 (H_e-11 and H_a-11, AB of ABX) collapsed into two doublets (J_{AB} = 14.45 Hz). Irradiation of the signal at δ2.17 (H_e-11, B of ABX) caused signals at δ5.57 and 1.94 (H_a-12 and H_a-11, AX of ABX system) to collapse into two doublets (J_{AX} = 12.30 Hz). These observations suggested a partial structure **A** having an axial proton at C-12 and vicinal methylene protons at C-11. It is also interesting to note that the methylene protons at C-11 have long range coupling with protons at C-8, C-7 and C-6. On irradiating the signals at δ2.17 (H_e-11), the broad singlet at δ2.58 (H-8) became a broad doublet. When the signal at δ1.94 (H_a-11) was irradiated the broad signal at δ4.49 (H-6) changed to a broad doublet and one of the peaks of the broad doublet at δ2.64 (H-7) changed to a broad doublet. These observations suggested a partial structure **B**.

The noise decoupled and single frequency off-resonance ^{13}C NMR spectrum contained signals arising from two methyl carbons (*q*, δ23.32 and 51.89), six methylene carbons (*t*, δ18.52, 22.74, 26.98, 28.70, 40.04 and 63.45), nine methine carbons (*d*, δ40.00, 49.78, 71.15, 72.14, 72.36, 75.21, 78.56, 78.43 and 102.40), four furan carbons (δ109.69 *d*, 140.83 *d*, 144.57 *d* and 126.49 *s*), two tetrasubstituted olefinic carbons (*s*, δ131.17 and 144.49), one quaternary carbon (*s*, δ38.68), and two carbonyl carbons of an ester and a lactone (*s*, δ169.45 and 171.94). These values compared well with the values reported for furanoid diterpene glucosides (Table 2).

Acetylation of compound **1** with acetic anhydride in pyridine at room temperature gave a tetraacetate (**2**) as colourless needles, mp 171–173°, which indicated the presence of four hydroxyl groups in **1**. Acid hydrolysis of **1** with dilute hydrochloric acid in methanol gave D-glucose and an aglycone (**3**) as a colourless gum which could not be crystallized (TLC single spot). The spectral assignments of these compounds are shown in Tables 1 and 2.

Compound **1** on refluxing with acetic anhydride and dry sodium acetate (under similar conditions in which the inversion of C-8 columbin to isocolumbin acetate was achieved) [11] gave as the only product compound **2**. This indicated the B/C ring juncture to be *trans* [9]. The high field chemical shift (δ0.92) observed for the methyl group at C-9, was due to the influence of the furan ring which is on the same side as 9-Me [10]. The β-configuration of the glycosidic linkage of glucopyranose was indicated by the anomeric proton in the ^{1}H NMR spectrum of **1**, at δ4.12 (*d*) with the coupling constant 7.89 Hz [9]. The stereochemistry of the C-10 proton was established by irradiation studies. When the signal at δ2.39 (H-10) was irradiated the splitting pattern of the signal at δ2.17 (*br dd*, H_e-11) was changed (could be due to the spacial coupling of H-10 and H_e-11) [2]. This is possible when H-10 is on the same side as H_e-11. Hence the proton at C-10 could be α-oriented as H_e-11 is also α.

The proposed structure **1** for the furanoid diterpene glucoside clearly satisfied the above spectral data. The mass fragmentation pattern also supported the structure **1**. The highest peak observed in the mass spectrum was *m/z* 343 [M − *O*-β-D-glucopyranose]$^+$ indicating the immediate loss of a glucose moiety, peaks at *m/z* 95, 94 and 81 were derived from the furan moiety [2, 4].

EXPERIMENTAL

Mps: uncorr. UV spectra were taken in MeOH. IR spectra were recorded either as nujol mulls or in $CHCl_3$. ^{1}H NMR and ^{13}C NMR spectra were obtained at 500 MHz with $CDCl_3$ and $(CD_3)_2SO$ as solvents and TMS as int. standard. Silica gel (100–200 mesh) used for CC was activated by heating at 120° for 4 hr.

Stems of *Tinospora cordifolia* Miers (26 kg) were collected from IIT Campus, Bombay and identified by Mr R. D. Shinde (Blatter Herbarium St. Xavier's College, Bombay). The stems were dried, finely powdered and extracted with $CHCl_3$ (60 l) in a Soxhlet for 48 hr. CC over silica gel with EtOAc yielded **1**, as colourless flakes, mp 199–201°. $[\alpha]_D^{20}$ = +23.8° (MeOH; *c* 3.14); IR ν_{max}^{nujol} cm^{-1}: 3560–3430, 3140, 1715, 1705, 1675, 1510, 1380, 1200, 1130, 880; UV λ_{max}^{MeOH} nm (ε): 220 (12896) and 272 (2640); ^{1}H NMR (500 MHz, DMSO-d_6) (Table 1); ^{13}C NMR (125 MHz, DMSO-d_6) (Table 2); MS *m/z* (rel. int.): 343 [M−(*O*-β-D-glucopyranose)]$^+$ (19), 316 (13), 270 (12), 248 (14), 216 (37), 171 (61), 143 (39), 95 (100), 94 (35), 81 (31); Anal. Calcd. for $C_{26}H_{34}O_{11}$: C, 59.77; H, 6.56. Found C, 59.96; H, 6.39%.

Tetraacetate of compound (**1**). Acetylation of **1** (350 mg) with Ac_2O (3 ml) in pyridine (2 ml) at room temp. for 12 hr gave a tetraacetate (**2**, 240 mg) as colourless needles (from MeOH), mp 171–173°, $[\alpha]_D^{20}$ = +47.5° ($CHCl_3$; *c* 2.52). IR ν_{max}^{nujol} cm^{-1}: 3140, 1760–1725, 1650, 1510, 1450, 1380, 1230, 1210, 1080, 980, 880. UV $\lambda_{max}^{CHCl_3}$ nm (ε): 231.3 (3071). ^{1}H NMR (500 MHz, $CDCl_3$): Table 1. ^{13}C NMR (125 MHz, $CDCl_3$): Table 2. MS *m/z* (rel. int.): 343 (4), 316 (3), 248 (5), 216 (15), 171 (18), 169 (15), 157 (15), 143 (11), 98 (21), 95 (46), 81 (10), 43 (100); Anal. Calcd. for $C_{34}H_{42}O_{15}$: C, 59.13; H, 6.13. Found: C, 59.27; H, 5.98%.

Acid hydrolysis of compound **1**. Compound **1** (100 mg) was stirred with HCl (2 ml) in 50% aq. MeOH (10 ml) for 48 hr, H_2O was added, the ppt. formed was collected and the filtrate concd under red. pres. The ppt. showed several spots on TLC, which on repeated CC and prep. TLC gave a colourless gum (**3**), which could not be crystallized (TLC single spot). Paper chromatography of the aq. concentrate showed a single spot which was identified as D-glucose on comparison with the authentic sample. For the aglycone (**3**), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1720, 1710, 1510, 930, 880. UV $\lambda_{max}^{CHCl_3}$ nm: 243.7 and 275. ^{1}H NMR (500 MHz, $CDCl_3$): Table 1. MS *m/z* (rel. int.): 360 [M]$^+$ (2), 342 [M − H_2O]$^+$ (3), 164 (34), 143 (35), 95 (88), 94 (100); Anal. Calcd. for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.58; H, 6.80%.

Table 2. ^{13}C NMR spectral data (δ values) of diterpene glucoside **1** and related compounds

C	1*	2†	4	5	6
1	18.52 *t*	17.45 *t*	17.4 *t*	28.0 *t*	69.0 *d*
2	22.74 *t*	21.98 *t*	24.6 *t*	62.2 *d*	48.7 *d*
3	26.98 *t*	26.25 *t*	75.6 *d*	140.2 *d*	49.6 *d*
4	144.49 *s*	143.47 *s*	79.5 *s*	137.4 *s*	85.9 *s*
5	131.17 *s*	128.55 *s*	45.2 *s*	40.9 *s*	41.8 *s*
6	75.21 *d*	72.85 *d*	69.8 *d*	79.0 *d*	25.5 *t*
7	28.70 *t*	27.84 *t*	27.2 *t*	26.4 *t*	16.6 *t*
8	49.78 *d*	49.28 *d*	46.3 *d*	49.2 *d*	42.0 *d*
9	38.68 *s*	38.19 *s*	34.3 *s*	36.5 *s*	34.8 *s*
10	40.00 *d*‡	39.13 *d*	46.1 *d*	40.4 *d*	53.5 *d*
11	40.04 *t*‡	39.65 *t*	42.9 *t*	44.0 *t*	43.3 *t*
12	71.15 *d*	71.28 *d*‡	69.4 *d*	69.9 *d*	71.1 *d*
13	126.49 *s*	124.78 *s*	124.2 *s*	124.5 *s*	125.3 *s*
14	109.69 *d*	108.23 *d*	108.7 *d*	109.2 *d*	109.0 *d*
15	140.83 *d*	139.38 *d*	140.3 *d*	140.2 *d*	140.2 *d*
16	144.57 *d*	144.99 *d*	143.5 *d*	143.7 *d*	143.7 *d*
17	171.94 *s*	171.13 *s*	172.6 *s*	174.6 *s*	172.2 *s*
18	169.45 *s*	169.12 *s*	177.6 *s*	167.5 *s*	168.4 *s*
19	23.32 *q*	23.38 *q*	32.6 *q*	28.4 *q*	23.7 *q*
20	—	—	17.9 *q*	22.8 *q*	17.4 *q*
-COOMe	51.89 *q*	51.16 *q*	—	51.6 *q*	—
Sugar part					
1′	102.40 *d*	98.99 *d*	102.4 *d*	104.6 *d*	100.0 *d*
2′	72.36 *d*[a]	70.36 *d*[a]	74.1 *d*	73.9 *d*	73.8 *d*
3′	78.56 *d*[b]	72.66 *d*[a]	76.7 *d*	77.4 *d*	76.7 *d*
4′	72.14 *d*[a]	68.12 *d*[a]	73.1 *d*	69.4 *d*	69.9 *d*
5′	78.43 *d*[b]	71.29 *d*‡[a]	75.8 *d*	76.6 *d*	76.9 *d*
6′	63.45 *t*	61.77 *t*	60.7 *t*	61.0 *t*	61.1 *t*
-COMe	—	20.30 *q* × 4	—	—	—
-OCO Me	—	170.31 *s*, 169.94 *s*, 168.73 *s*, 167.77 *s*			

*Measured in DMSO-d_6 at 125 MHz.
†Measured in $CDCl_3$ at 125 MHz.
‡Overlapping signals in each column.
[a,b]These assignments may be reversed in each column.

Acknowledgement—The use of facilities provided by the 500 MHz FT-NMR National Facility at T.I.F.R., Bombay are gratefully acknowledged.

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