$-RCO_2H]^+$ (88) (calc. for $C_{13}H_8O$: 180.058), 152 [180 $-C_2H_4]^+$ (26), 71 $[RCO]^+$ (100); UV $\lambda_{max}^{Et_2O}$ nm: 373, 347, 324, 304, 285, 272, 258, 239; ^1H NMR ($CDCl_3$): δ 3.74 and 3.71 (*dd*, H-1), 5.39 (*ddd*, H-2), 6.35 (*dd*, H-3), 5.83 (*dd*, H-4), 2.01 (*s*, H-13), OCOR: 2.61 (*qq*, H-2'), 1.20 (*d*, H-3', H-4'); (*J* [Hz]: 1,2 = 3.5; 1',2 = 2,3 = 6; 2,4 = 1; 2',3' = 2',4' = 7].

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TINOSPORASIDE, AN 18-NORCLERODANE GLUCOSIDE FROM *TINOSPORA CORDIFOLIA*

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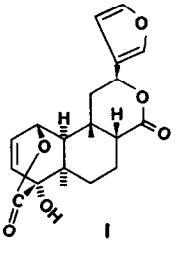
Key Word Index—*Tinospora cordifolia*; Menispermaceae; diterpene; 18-norclerodane glucoside; tinosporaside.

Abstract—The stem wood of *Tinospora cordifolia* has yielded a novel 18-norclerodane diterpene *O*-glucoside which has been assigned the trivial name tinosporaside. On the basis of extensive NMR studies this has been assigned the structure (relative stereochemistry) 1,17-dioxo-8β,10α,12α,19α,20β-18-norclerod-2,13(16),14-trien-4α-β-D-glucopyranoside-12,17;15,16-dioxide.

INTRODUCTION

Tinospora cordifolia Miers has long been used in Ayurvedic medicine for the treatment of jaundice, rheumatism and urinary diseases [1]. Previous studies on this and other species of *Tinospora* [1–4] have revealed the presence of a range of clerodane-derived sesquiterpenes related to columbin (**1**) [5]. In this paper we report the isolation of an 18-norclerodane glucoside from the stem bark of *T. cordifolia*.



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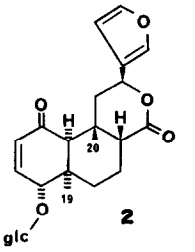

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RESULTS AND DISCUSSION

Column chromatography of the ethyl acetate soluble part of an ethanolic extract of the stem bark gave, on repeated recrystallization from methanol, tinosporaside (**2**). High resolution EIMS indicated an empirical formula $C_{25}H_{32}O_{10}$ with major fragments at *m/z* 330 $[C_{19}H_{22}O_5]^+$ and 314 $[C_{19}H_{22}O_4]^+$ for loss of a hexose sugar moiety (as an *O*-glycoside) with, respectively, retention and loss of the ether oxygen. The IR spectrum revealed the presence of two carbonyl bands at 1708 cm^{-1} (γ-lactone) and 1670 cm^{-1} (α,β-unsaturated carbonyl).

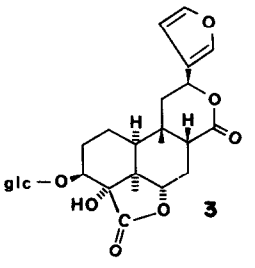

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Table 1. ^1H and ^{13}C NMR chemical shift data for **2**-Ac_4 and ^{13}C data for **1** [6] and **3**-Ac_5 [2]

C	^1H spectrum (*J* values: Hz) **2**-Ac_4	^{13}C spectrum **1**	**2**-Ac_4	**3**-Ac_5
1	—	74.1	201.8	16.9
2	5.99 *d* (10.3)	136.7	129.4	25.4
3	6.70 *ddd* (10.3, 4.9, 1.3)	128.9	141.8	78.5
4	4.22*d* (4.9)	80.4	72.0	87.9
5	—	37.1	43.3	46.9
6_{eq}	2.25 *ddd* (14.3, 4.0, 4.0)	25.6	29.2	70.6
6_{ax}	1.02 *ddd* (14.3, 14.0, 3.8)			
7_{eq}	2.19 *m*	17.3	18.9	28.4
7_{ax}	1.63 *dddd* (14.3, 14.1, 8.3, 1.5)			
8	2.30 *br s*	47.6	48.7	46.3
9	—	35.2	35.5	36.3
10	2.17 *s*	44.4	48.5	46.1
11_{eq}	2.25 *dd* (15.0, 3.6)	41.9	40.6	44.0
11_{ax}	1.82 *dd* (15.0, 12.2)			
12	5.47 *dd* (12.2, 3.6)	70.6	69.8	70.6
13	—	124.7	124.6	124.4
14	6.41 *dd* (1.4, 0.9)	108.3	108.0	108.3
15	7.43 *t* (1.4)	143.8	144.7	139.8
16	7.52 *t* (0.9)	139.5	139.5	143.8
17	—	173.1	171.0*	172.1
18	—	175.3	—	171.4
19	1.18 *s*	28.2	31.1	33.4
20	0.80 *s*	24.3	25.7	18.5
1′	4.65 *d* (7.7)	—	100.3	101.9
2′	4.94 *dd* (9.5, 7.8)	—	71.4	71.8
3′	5.18 *t* (9.4)	—	72.2	74.5†
4′	5.04 *t* (9.6)	—	68.0	68.6
5′	3.65 *ddd* (10.0, 4.6, 2.9)	—	72.0	72.3†
6′	4.14 *dd* (12.4, 4.8)	—	61.6	61.8
	4.11 *dd* (12.4, 2.8)			
Ac	2.01/1.99/1.97/1.97	—	170.2 */20.5	170.4†/21.7
			170.0*/20.4	169.9†/20.9
			169.9*/20.3	169.7†/20.6
			168.7*/20.3	169.4†/20.5
				168.7†

All spectra run in $CDCl_3$. ^1H NMR at 360 MHz for **2**; ^{13}C NMR at 62.5 MHz for **1**, 90.56 MHz for **2**-Ac_4 and 22.5 MHz for **3**-Ac_5.
*†Assignments are interchangeable.

Peracetylation yielded a tetra-acetate (**2**-Ac_4) the ^1H NMR spectrum of which allowed resolution of all 40 protons (Table 1). Coupling constants for the methine protons H-1′ to H-5′ of the hexose showed an all *trans*-axial relationship and together with the methylene (H-6′) resonances confirmed the identity of the sugar as β-glucose. Hydrolysis of **2** gave a hexose ($[\alpha]_D + 49°$), thus establishing the occurrence of β-D-glucose.

Olefinic resonances in the ^1H NMR spectrum at δ 6.41, 7.43 and 7.52 were typical for a 3-substituted furan system and an isolated ABX system at δ 5.47, 1.82 and 2.25 could be assigned to H-12 and H-11 protons with H-12 axial. Both patterns are closely comparable to the spectrum of compound **1** [6].

Two further olefinic protons resonated δ 5.99 and 6.70, typical of the α- and β-protons of an α,β-unsaturated ketone. The β-proton showed an additional coupling to a proton resonating at δ 4.22, which can be assigned to the carbinolic position of glycosidic attachment. These data require the system –CO–CH=CH–CH(O–glucose)–CR^3 which can be attributed to the A-ring of a columbin-like clerodane diterpene in which the 18-methyl group has been lost. A sharp singlet at δ 2.17 could be assigned to H-10. A second, broadened singlet at δ 2.30 must be attributed to H-8. This is unusual; in compound **1** H-8 appears as a double-doublet with a large coupling to one of the H-7 protons [6]. In **2**-Ac_4 the axial H-6 and H-7 protons are clearly visible as shielded resonances, that for H-7_{ax} showing two large (J_{gem} and J_{6ax}) and two small (J_{6eq} and J_8) couplings. Thus the stereochemistry of the B-ring of **2** appears to be a normal chair in which H-8 is equatorial. This contrast with **1** where it is considered to occur in the boat conformation [6].

The ^{13}C NMR spectrum of **2**-Ac_4 was obtained and correlated with the proton spectrum. A comparison with that obtained for compound **1** (Table 1) showed the anticipated differences related to changes in A-ring substituents. One striking feature was the deshielded nature of C-19 (δ 31.1) which is similar to that recorded for boreptoside-A (**3**) (Table 1) isolated from *Tinospora tuber-*

Table 2. NOE enhancements for 2-Ac_4

Irradiation	Enhancement (%)
H-4	H-3 (3), H-1′ (1), H-6_{eq} (1)
H-7_{ax}	H-7_{eq} (3.5), H-6_{eq} (1)
H-11_{ax}	H-11_{eq} (2.5), H-8 (2.5)
H-12	H-10 (5), H-16 (3)
Me-19	H-10 (3.5)
Me-20	H-2 (2), H-4 (4), H-7_{ax} (8), H-8 (3), H-11_{ax} (7)

culata [2]. The C-20 methyl resonance is also somewhat deshielded in comparison with many similar clerodanes. This appears to be attributable to its relative proximity to the C-2/C-3 double-bond.

The stereochemistry of compound **2** was further explored through a series of NOE experiments (Table 2). The key observations were as follows: (i) a strong interaction between H-12 and H-10; (ii) H-11_{ax} (on the opposite face of the molecule to H-12) interacts with H-8. Together these findings indicate H-8 and H-10 are on opposite faces and that H-8 and H-11_{ax} are 1,3-diaxial. (iii) Irradiation of Me-20 caused strong enhancement of H-11_{ax}, H-8, H-7_{ax} and H-4. This requires the anticipated *cis* B/C ring junction and places H-4 on the same face as Me-20. (iv) Irradiation of Me-19 causes a strong enhancement of H-10 confirming that the A/B ring junction is also *cis*.

On this basis the glycoside was assigned structure **2** or its enantiomer and it has been given the trivial name tinosporaside. The dextrorotatory nature of **2** suggests the stereochemistry is as depicted. Compound **2** is obviously related to **3** and shows comparable stereochemistry for both A/B and B/C ring junctions. Surprisingly, a similar diterpene reported from *T. cordifolia* [1] has been assigned a *trans* B/C ring junction although the reasoning for this is not clear and the ^{13}C NMR resonance for C-11 (δ 41.9) is similar to that found in compounds **1**–**3**. By contrast in floribundic acid, where the B/C ring fusion is *trans*, C-11 resonates at (δ 45.8).

EXPERIMENTAL

Plant material. The stem bark of *T. cordifolia* was collected from the outskirts of Hyderabad. A voucher specimen (No. 63) has been deposited at the Herbarium of the Department of Botany, Osmania University, Hyderabad, India.

Extraction and isolation of compound **2**. Air-dried stem bark (5 kg) was defatted with petrol (bp 60–80°) and then extracted to exhaustion (Soxhlet) with EtOH. The extract was concd and EtOAc added. After cooling a brown solid was formed which was collected and refluxed several times with C_6H_6. The C_6H_6 insoluble material was then subjected to CC eluting with 5% MeOH in $CHCl_3$ to give **2** which was recrystallized from aq. MeOH as needles (2 g), mp 228–229°, $[\alpha]_D$ +65° (MeOH; *c* 1.00). UV λ_{max} nm: 218. IR ν_{max} cm^{-1}: 3480–3400, 1708, 1670, 1075, 1022. EIMS *m/z* (rel. int.): 492.2017 $[M]^+$ (41) ($C_{25}H_{32}O_{10}$ requires 492.1892); 330 $[C_{19}H_{22}O_5]^+$ (58), 314 $[C_{19}H_{22}O_4]^+$ (32), 312 (61), 205 (72), 204 (52), 121 $[C_8H_9O]^+$ (100).

Acetylation of **2** (200 mg) was achieved by refluxing with Ac_2O (2 ml) in pyridine (2 ml) for 7 hr. Normal work-up and recrystallization from MeOH gave 2-Ac_4, mp 218–219°. IR ν_{max} cm^{-1}: 1750, 1730, 1680, 1230, 1210, 1040. ^1H and ^{13}C NMR, see Table 1.

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