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A SESQUITERPENE GLUCOSIDE FROM *TINOSPORA CORDIFOLIA**

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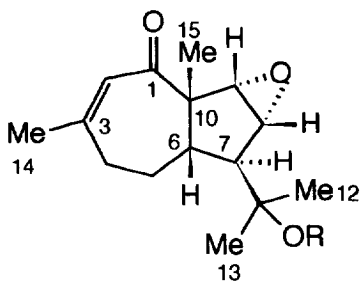
Key Word Index—*Tinospora cordifolia*; Menispermaceae; tinocordifolioside; sesquiterpene glucoside.

Abstract—A new daucane-type sesquiterpene glucoside, tinocordifolioside, has been isolated from the stem of *Tinospora cordifolia* and the structure was established by detailed spectroscopic studies. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Tinospora cordifolia Miers occurs throughout the plains of India. It has been used for several centuries in the Indian system of medicine for the treatment of jaundice, diabetes, skin diseases and anaemia [1]. Recently, we reported on the isolation and characterization of several new furanoditerpene glucosides [2, 3] and two phenylpropene disaccharides [4] from *T. cordifolia*. In the present paper, we describe the structural elucidation of a new sesquiterpene glucoside (**1**) isolated as its tetraacetate (**2**). We have named it tinocordifolioside.



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

RESULTS AND DISCUSSION

The *n*-butanol soluble fraction on chromatography over silica gel, eluted with methanol–ethylacetate (1:49), gave a fraction which was found to be a mixture. Repeated efforts to obtain pure compounds from this mixture were unsuccessful. Acetylation, followed by repeated chromatography led to the isolation of tinocordifolioside tetraacetate (**2**). The IR spectrum of compound **2** showed strong absorptions at 1750, 1670 and 1230 cm^{-1}, indicating the presence of acetyl carbon, an α,β-unsaturated carbonyl and an epoxide ring, respectively. Further evidence for an α,β-unsaturated carbonyl in compound **2** was provided by absorption at 254 nm in the UV spectrum.

The FAB-mass spectrum contained a peak at *m/z* 603 $[M+Na]^+$ corresponding to the molecular formula $C_{29}H_{40}O_{12}$. This conclusion was supported by the ^{13}C NMR and DEPT spectra. Elimination of a tetraacetylglucose moiety was indicated in the mass spectrum by an ion peak at *m/z* 331. The ^1H NMR (Table 1) coupling constants for methine protons H-1′ to H-5′ of the hexose showed all *trans* axial relationships which, together with a methylene H-6′ resonance, suggested that the sugar was β-D-glucose. This was further confirmed by hydrolysis of the parent mixture to give glucose which was identified by comparison of its optical rotation and R_f values with those of an authentic sample.

In addition to the signals of the tetra-*O*-acetyl-β-D-glucopyranosyl moiety, the ^{13}C NMR data (Table 1) of compound **2** revealed 15 carbon signals that were assigned by DEPT analysis to four methyls (δ 20.1, 22.4, 22.8 and 23.8), two methylenes (δ 36.4 and 20.1), five methines (two CH at δ 49.1 and 57.0; two HC-O- at δ 53.1 and 54.5 and one olefinic carbon at δ 121.4), four quaternary carbons (one at δ 56.9, one olefinic carbon at δ 169.8, one oxygenated at δ 81.1, one carbonyl at δ 203.3). The anomeric proton of the sugar moiety appeared at δ 4.63 as a doublet with a large coupling constant ($J = 7.9$ Hz) indicating a β linkage. The location of the sugar moiety at C-11 was shown by a three-bond HMBC correlation between H-1′ and C-11. The ^1H NMR spectrum of compound

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Table 1. ^1H and ^{13}C NMR data of compound **2** ($CDCl_3$)*

Position	δ_C	δ_H(*J* in Hz)
1	203.4	—
2	121.4	5.75(*q*, *J* = 1.50)
3	169.8	—
4a	20.1	1.76 (*m*)
4b		1.50 (*m*)
5a	36.4	1.88 (*m*)
5b		1.72 (*m*)
6	57.0	1.92 (*m*)
7	49.1	1.94 (*m*)
8	54.5	2.68 (*dd*, *J* = 1.60, 7.70)
9	53.1	2.76 (*brs*)
10	56.9	—
11	81.1	—
12	22.4†	1.13 (*s*)
13	22.8†	1.05 (*s*)
14	23.8	2.02 (*d*, *J* = 1.50)
15	20.1	0.94 (*s*)
1′	94.8	4.64 (*d*, *J* = 7.93)
2′	71.5	4.93 (*dd*, *J* = 7.93, 9.46)
3′	73.0	5.20 (*t*, *J* = 9.46)
4′	68.8	5.02 (*t*, *J* = 9.77)
5′	71.5	3.65 (*m*)
6′a	62.3	4.19 (*dd*, *J* = 5.95, 12.06)
6′b		4.07 (*dd*, *J* = 2.45, 12.06)
OCO*Me*	20.7	2.06 (*s*)
	20.7	2.02 (*s*)
	20.6	2.01 (*s*)
	20.6	1.99 (*s*)
OC*O*Me	170.6	
	170.3	
	169.4	
	169.1	

* Chemical shifts are in ppm from internal TMS, *J* values are in Hz.

† Values may be interchanged.

2 showed three methyl singlets at δ 0.94, 1.05 and 1.13, a vinyl methyl at δ 2.02 (*d*, *J* = 1.5 Hz) coupled to an olefinic proton at δ 5.75 (*q*, 1.5 Hz), and two methine groups bearing oxygen at δ 2.76 (*s*, 1H) and δ 2.68 (*dd*, *J* = 1.6, 7.7 Hz, 1H).

The signals at δ 2.76 (*br s*, 1H), δ_C 53.1, 2.68 (*dd*, *J* = 1.6, 7.7 Hz, 1H), δ_C 54.5 and 1.92 (*m*, 1H), δ_C 49.1 could be explained by an epoxide ring attached to a five-membered ring system. Taking into account the above data, compound **2** was deduced to be a daucane-type of sesquiterpene [5, 6]. The structure of compound **2**, with a β-oxygenated isopropyl group fits better with the spectral data than its C-7 epimer and is supported by biogenetic considerations. The C-7α proton coupled with the proton at C-8 with coupling constant 7.7 Hz indicates that they are *trans* to each other and show a typical long-range M-coupling (*J* = 1.6 Hz) with the proton at C-6. A broad singlet was observed for H-9, showing clearly the *cis* relationship of the vicinal epoxide protons. Hence, the epoxide group present in the compound appeared to be α. Further, the relative configuration of the chiral centres were supported by 2D NMR, ^1H–^1H COSY, ^{13}C–^1H HMQC, HMBC and NOE analysis. Thus tinocordifolioside tetraacetate can be represented by structure **2** and the corresponding parent glucoside structure by **1**, which has not been previously reported from natural sources.

EXPERIMENTAL

General. Mps: uncorr.; IR: KBr pellets; 1H and ^{13}C NMR: 400 Mhz and 100.57 MHz, respectively. Flash chromatography: silica gel (230–400 mesh); TLC: precoated silica gel plates (Merck).

Extraction and isolation. The plant material was collected from Palampur (H.P.) and was confirmed as *T. cordifolia* by comparison with the specimen kept in the herbarium of our institute. The powdered stem (5 kg) was extracted with 70% aq. EtOH at room temp. After removal of the EtOH by evaporation the remaining extract was washed with petrol and then extracted with *n*-BuOH. The *n*-BuOH extract was freed from solvent and on repeated flash chromatography over silica gel (230–400 mesh, Merck with EtOAc–MeOH (49:1) yielded a fr. (fr. 1) found to be mixt. This fr. was collected, conc., dried and stirred with Ac_2O and pyridine at room temp. for 16 hr. The solvent was then removed *in vacuo*. Careful flash chromatography using hexane–EtOAc (7:3) allowed the isolation of compound **2** (35 mg).

Tinocordifolioside tetraacetate (**2**). Recrystallized from MeOH, mp 166–167°, $[\alpha]_D^{22} - 13.01°$($CHCl_3$; *c* 1.13); HR-MS, CI, *m/z* $[MH]^+$581.2598, calcd for $C_{29}H_{41}O_{12}$ $[MH]^+$581.2598; FAB-MS *m/z*: 603[M+Na]$^+$, 565, 331, 217, 189, 59; IR ν_{max}^{KBr} cm^{-1}: 2965, 2940, 2920, 1750, 1670, 1375, 1230, 1030; UV$\lambda_{max}^{CHCl_3}$ nm: 254; ^1H and ^{13}C NMR: Table 1.

Acid hydrolysis of Fr. 1. A soln of fr. 1 (50 mg) in 1 M methanolic HCl (5 ml) was refluxed for 30 min. The reaction mixt. was worked up in the usual manner and the sugar fraction isolated on an activated carbon column to give D-glucose identified by comparison with an authentic sample (TLC) and by optical rotation.

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