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NORDITERPENE FURAN GLYCOSIDES FROM *TINOSPORA CORDIFOLIA*

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Key Word Index—*Tinospora cordifolia*; Menispermaceae; norditerpene furan glycosides.

Abstract—Two new norditerpene furan glycosides (cordifoliside D and cordifoliside E) were isolated, as their tetraacetates, from the polar butanol extract of *Tinospora cordifolia* stems. The structural elucidations and relative configurations are based on high-resolution 1D and 2D NMR spectroscopy.

INTRODUCTION

Tinospora cordifolia Miers is a traditional medicinal plant of Indian subcontinent which forms part of various Ayurvedic formulations. The aqueous extract of the stems has been shown to possess immunostimulant activity [1]. Our earlier studies, with the polar butanol extract of the plant, resulted in the isolation and characterization of phenylpropane glycosides [2] and norditerpene furan glycosides [3]. Diterpene furan glycosides, phytoecdysones and an acid amide have also been obtained (unpublished results). This communication describes the isolation and characterization of two more norditerpene furan glycosides, cordifoliside D (**1**) and cordifoliside E (**2**), in the tetraacetate forms **1a** and **2a**, respectively.

RESULTS AND DISCUSSION

The tetraacetates of cordifoliside D (**1a**) and cordifoliside E (**2a**) are solids showing UV absorptions at 203, 226 (sh), 278 nm (sh). The IR absorption bands at 3140, 1507 and 874 cm^{-1} indicated the presence of furan rings. IR of both **1a** and **2a** also showed the presence of a hydroxyl group. Resistance to normal acetylation suggested the tertiary nature of the hydroxyl group. Acid hydrolysis of both **1** and **2** resulted in the isolation of a monosaccharide that was identified a glucose on the basis of its TLC and GLC. In both compounds, the sugar was found to have the β-conformation on the basis of the chemical shifts and coupling constant [4] of the anomeric proton (H-1′, δ_H4.6, *d*, J = 8.0 Hz). Comparison of the spectral data of the two acetates with those of cordifolisides A, B and C tetraacetates [3] revealed that both **1a** and **2a** were norditerpene furan glucosides. The FAB mass spectrum of both the acetates showed the same molecular ion at *m/z* 729 $[M + Na]^+$, indicating a molecular weight of 706 $[M]^+$ for **1a** and **2a**. Thus, compared with the molecular weight of 690 for the three stereoisomers, cordifolisides A, B, C [3], compounds **1a** and **2a** possessed an additional hydroxyl group. Accordingly, the molecular formula for both **1a** and **2a** is $C_{34}H_{42}O_{16}$ and for the parent glucosides (**1** and **2**) $C_{26}H_{34}O_{12}$.

DEPT, in combination with other NMR data, indicated the presence of six methyls (one $-COOCH_3$, four $-O-CO-CH_3$, one CH_3), six methylenes (one $-OCH_2-$, five $-CH_2-$), eleven methines (three =CH–, seven –O–CH <, one > CH–) and eleven quaternary 'C' signals. Of the 11 methines, only one could be identified as a tertiary methine (δ_H2.45; δ_H40.2) whereas the NMR spectra of cordifolisides A–C showed the presence of two angular methines (C-8, C-10). Therefore the tertiary hydroxyl group could be assigned one of these positions. Assignment of the detailed structure and stereochemistry was decided on the basis of various 2D NMR experiments: $^{13}C-^1H$ COLOC, $^{13}C-^1H$ HETCOR, and $^1H-^1H$ COSY. The relative configurations were decided on the basis of $^1H-^1H$ NOESY experiments and ^{13}C chemical shifts of the neighbouring carbons.

The resonances at δ_H2.3 and δ_C41.7 for cordifoliside D tetraacetate (**1a**) were assigned to a tertiary methine at C-10 on the basis of its COLOC interaction with two quaternary carbons: C-4 (δ_C151.3), C-5 (δ_C127.1) and C-20 methyl (δ_C15.8). Therefore, the tertiary hydroxyl could be placed at C-8. The downfield oxygen bonded quaternary C-8 (δ_C74.9) showed COLOC cross peaks with H-20 methyl (δ_H0.94), thus confirming its placement at C-8. The positions of two methylenes at C-1, C-2 and methine at C-3 were fixed on the basis of COSY as spin–spin couplings were observed between H-10 (δ_H2.3) $\leftrightarrow$ $H_{a,b}$-1 (δ_{Ha}1.85, δ_{Hb}1.64; δ_C16.9), H_a-1 (δ_H1.85) $\leftrightarrow$ H_a-2 (δ_H1.53; δ_C28.3) and $H_{a,b}$-2 (δ_{Ha}1.53, δ_{Hb}2.15; δ_C28.3) $\leftrightarrow$ H-3 (δ_H4.68; δ_C72.5). The comparative downfield shift of the latter suggested that it could be attached to oxygen bonded carbon. This signified that the glycosidic linkage could be at C-3. The same was confirmed by COLOC, as cross interactions were observed between quaternary C-5 (δ_C127.1) $\leftrightarrow$ H-3 and C-1′

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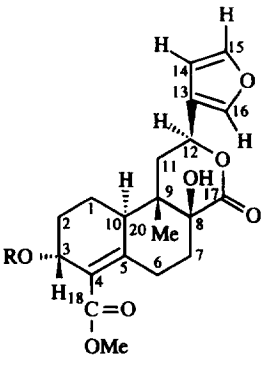
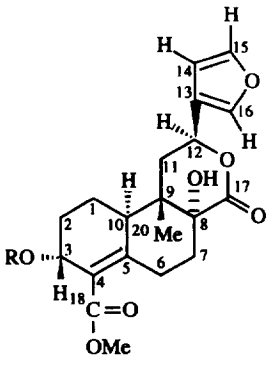


1 R



2 R

1 β - D - Gluco-pyranosyl, Cordifoliside D

1a Tetra - *O* - aceyyl - β - D - Gluco-pyranosyl, Cordifoliside D tetraacetate

2 β - D - Gluco-pyranosyl, Cordifoliside E

2a Tetra - *O* - aceyyl - β - D - Gluco-pyranosyl, Cordifoliside E tetraacetate

Table 1. 1D and 2D NMR data of Cordifoliside D tetraacetate (**1a**) in $CDCl_3$

Pos.	δ_C	δ_H*	COSY	NOESY	COLOC
1	16.9	1.85 (*m*, H_a)	H_b-1, H-10, H_a-2(w)	H-3	
		1.64 (*m*, H_b)	H_a-1, H-10	H-10	
2	28.3	1.53 (*m*, H_a)	H_b-2, H-3, H_a-1 (w)	H-3	H_a-1
		2.15 (*m*, H_b)	H_a-2, H-3		
3	72.5	4.68 (*br s*)	$H_{a,b}$-2	H_a-2, H_a-1	
4	151.3				H_a-6, H-10
5	127.1				H-3
6	28.3	3.29 (*d*, 11, H_a)	H_b-6, H_a-7	H_a-7 (w)	H_a-7
		1.90† (*m*, H_b)	H_a-6		
7	30.6	2.51 (*m*, H_a)	H_a-6		
		1.80 (*m*, H_b)		H_a-6 (w)	
8	74.9				H_a-6, H-20
9	42.1				H-20
10	41.7	2.30 (*m*)	$H_{a,b}$-1	H-12, H_b-1	H_b-2, H-20
11	33.9	1.93† (*m*, H_a)	H_b-11, H-12	H_b-11	H-20
		2.58 (*m*, H_b)	H_a-11, H-12	H-14, H-20, H_a-11	
12	71.5	5.43 (*dd*, 13.75, 5.5)	$H_{a,b}$-11	H-10	
13	124.7				H-15
14	108.8	6.50 (*br s*)	H-15	H_b-11, H-15	H-16
15	139.7	7.47 (*br s*)	H-14	H-14	
16	143.9	7.40 (*br s*)			H-14
17	172.0				H_b-7
18	168.3				3.77 (–OCH_3)
19					
20	15.8	0.94 (*s*)		H_b-11	H-10
COOMe	51.6	3.77 (*s*)			
1′	100.9	4.66 (*d*, 7.5)	H-2′		H-3
2′	71.3	4.95 (*t*, 8.6)	H-1′, H-3′		
3′	71.7	3.72 (*m*)	H-2′, H-4′		
4′	68.0	5.05 (*t*, 9.5)	H-3′, H-5′		
5′	72.9	5.21 (*t*, 9.0)	H-4′, $H_{a,b}$-6′		
6′	62.0	4.12 (*dd*, 12, 2, H_a)	H-5′, H_b-6′		
		4.24 (*dd*, 12.1, 4.75, H_b)	H-5′, H_b-6′		
OCOMe	169.0, 169.3				
	170.2, 170.5				
MeCO	20.5 × 4	1.96, 1.98, 2.01			
		2.07 (all *s*)			

*The proton assignments are based on ^{13}C–^1H HETCOR and ^1H–^1H COSY experiments.
†Signals overlapping with other protons.

Table 2. 1D and 2D NMR data of Cordifoliside E tetraacetate (**2a**) in $CDCl_3$

Pos.	δ_C	δ_H*	COSY	NOESY	COLOC
1	18.8	1.64 (*m*, H_a)	$H_{a,b}$-2, H_b-1, H-10,		
		1.90 (*m*, H_b)	H_a-1, H_b-2, H-10	H-10	
			H-3 (1*r*)		
2	26.0	2.68 (*m*, H_a)	H_a-1		
		2.80 (*m*, H_b)	$H_{a,b}$-1		
3	73.2	4.68 (*br s*)	H_b-1 (*lr*), H_b-6 (*lr*)		
4	150.5				H-3, H-10
5	126.4				H-3, H-10
6	26.7	1.59 (*m*, H_a)	H_b-6, H_a-7		
		1.72 (*m*, H_b)	H_a-6, H_a-7, H-3 (*lr*)		
7	29.6	1.96 (*m*, H_a)†	H_b-7, $H_{a,b}$-6		
		2.21 (*m*, H_b)	H_a-7		
8	88.8				H_b-7, H-10, H-20
9	50.1				H-10, H-20
10	38.8	2.6 (*m*)	H_b-11, $H_{a,b}$-1	H-12, H_b-1	
				H_b-11	
11	44.4	2.04 (*m*, H_a)	H_b-11, H-12	H-20, H-14	H-20
		2.30 (*m*, H_b)	H-10, H_a-11, H-12	H-10, H-12	
12	73.0	5.22 (*m*, 1H)	$H_{a,b}$-11	H-10, H_a-11	
13	124.4				H-15
14	109.5	6.54 (*br s*)	H-15, H-16 (1*r*)	H_a-11, H-15	H-16
15	140.9	7.47 (*br s*)	H-14, H-16	H-14	H-14
16	143.4	7.35 (*br s*)	H-14 (1*r*), H-15		H-14
17	177.9				$H_{a,b}$-7
18	167.9				3.74 ($-OCH_3$)
19					
20	19.8	0.98 (*s*)		H_a-11	
COOMe	51.3	3.74 (*s*)			
1′	99.8	4.60 (*d*, 8.0)	H-2′		H-3
2′	71.5	4.90 (*t*, 8.9)	H-1′, H-3′		
3′	71.6	3.63 (*m*)	H-2′, H-4′		
4′	68.4	5.05 (*t*, 9.5)	H-3′, H-5′		
5′	72.9	5.17 (*t*, 9.3)	H-4′, $H_{a,b}$-6′		
6′	62.1	4.11 (*dd*, 12, 2, H_a)			
		4.23 (*dd*, 12.1,4.75, H_b)	H-5′, H_b-6′		
OCOMe	169.0, 169.3				
	170.3, 170.6				
MeCO	20.6 × 4	1.98, 2.00, 2.01			
		and 2.07 (all *s*)			

*The proton assignments are based on ^{13}C–^1H HETCOR and ^1H–^1H COSY experiments.
†Signals overlapping with other protons.

(δ_C100.9) ↔ H-3. The H-3 did not show further COSY cross peaks and was therefore adjacent to quaternary C-4 (δ_C151.3). The two methylenes at C-6 and C-7 were assigned on the basis of COLOC, as cross peaks were observed between C-17 (δ_C172.0) ↔ H_b-7 (δ_H1.8; δ_C30.6), C-8 (δ_C74.9) ↔ H_a-6 (δ_H3.29; δ_C28.3), C-4 (δ_C151.3) ↔ H_b-6 (δ_H1.9) and C-6 (δ_C28.3) ↔ H_b-7 (δ_H1.8). These assignments were further supported by COSY on the basis of spin–spin couplings observed between H_a-6 (δ_H3.29) ↔ H_a-7 (δ_H2.51). The position of the remaining methylene at C-11 (δ_{Ha}1.93, δ_{Hb}2.58; δ_C33.9) was fixed on the basis of its COLOC interaction with H-20 methyl protons. This in turn, was involved in COSY interaction with a methine proton (δ_H5.43), confirming the position of that methine at C-12 (δ_H71.5). The other important COSY and COLOC interactions are as noted in Table 1.

The relative configuration of **1a** was fixed on the basis of the ^1H–^1H NOESY spectrum. The important NOE, observed between H-10 (δ_H2.3) and H-12 (δ_H5.43) suggested that these protons were on the same side of the molecule. No interaction was observed between H-10/H-12 and angular methyl at C-20, indicating that the C-20 methyl was on the other side of the molecule. The other NOEs observed were between H-10 (δ_H2.30) ↔ H_b-1 (δ_H1.64) and H_a-1 (δ_H1.85) ↔ H-3 (δ_H4.68). This indicated that the glycosidic 'H' was on the side opposite to H-10. The relative configuration of the C-8 tertiary hydroxyl could not be ascertained on the basis of NOESY. It will be discussed later in conjunction with the relative configuration of cordifoliside E tetraacetate (**2a**).

The structural elucidation of cordifoliside E tetraacetate (**2a**) was also based on the interpretations of

2D NMR experiments. The resonance at δ_H2.6 was assigned to H-10 on the basis of its COLOC interactions with three quaternary carbons, C-4 (δ_C150.5), C-5 (δ_C126.4) and C-9 (δ_C50.1). Therefore, the tertiary hydroxyl could be placed at C-8. Its downfield chemical shift (δ_C88.8) supports this assignment. This assignment gave COLOC interactions with H-20 methyl (δ_H0.98; δ_C19.8). The positions of various methylenes were fixed on the basis of respective COSY and COLOC interactions (Table 2). The placement of the glycosidic linkage at C-3 (δ_H4.68; δ_C73.2) was on the basis of COLOC interactions of H-3 with the two quaternary carbons C-4 and C-5. It is thus concluded that **2a** is an epimer of **1a**, both having the tertiary hydroxyl at C-8, but having opposite relative configurations.

The NOESY spectrum showed the cross peaks between H-10 (δ_H2.6) and H-12 (δ_H5.22), indicating that the two protons were on the same side of the molecule, as in compound **1a**. Also, no interaction was observed between H-10/H-12 and angular methyl at C-20, indicating that C-20 methyl was on the other side of the molecule.

It is evident from the above studies that compounds **1a** and **2a** differed only in the disposition of the tertiary hydroxyl group at the C-8 position. Their relative stereochemistries were derived on the basis of the ^{13}C NMR chemical shifts of the neighbouring carbons. According to Roberts *et al.* [5], the ^{13}C NMR shifts are very sensitive to steric effects and conformational changes in the molecule. Any carbon that exists in *gauche*-orientation with respect to another carbon or heteroatom shows an upfield shift compared with its *anti*-isomer. The shifts are generally more pronounced in the case of γ-carbons. The relative shifts of the γ-carbons C-20 and C-10 in **1a** and **2a** suggested the possible orientation of the hydroxyl group. As listed in Tables 1 and 2, C-20 in **1a**, appeared $\approx$ 4.0 ppm upfield (δ_C15.8) of that of **2a** (δ_C19.8). Thus, the methyl group at C-9 and the hydroxyl groups at C-8 are *cis-gauche*-disposed in **1a** and *anti*-disposed in **2a**. Molecular models of **1a** and **2a**, constructed using DTMM software [6], confirmed this and further revealed that the C-10 carbon is *anti*-disposed to the hydroxyl group at C-8 in **1a** and *cis-gauche*-disposed in **2a**. Therefore, δ_C values of C-10 are expected to be downfield in **1a** compared with that of **2a**. The observed chemical shifts of C-10 in **1a** (δ_C41.7) and **2a** (δ_C38.8) were in complete agreement with this. Therefore, cordifoliside D and E tetraacetates were assigned the structures **1a** and **2a**, respectively, with the corresponding parent glucosides structures **1** and **2**, respectively.

EXPERIMENTAL

Mps are uncorr. ^{1}H and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively. All the 1D and 2D experiments were carried out with 0.01 M solutions in $CDCl_3$.

Isolation of Cordifolisides D and E tetraacetates. The plant material (fresh wt. 5.8 kg) was collected from Trombay campus, Bhabha Atomic Research Centre, Bombay and identified by Dr V. Abraham of the Nuclear Agriculture Division, BARC. Fresh stems were subjected to cold extraction, with MeOH, through percolation. Isolation of compounds was carried out following the experimental and chromatographic procedure reported earlier for cordifolisides A, B and C [3]. Thus, a combination of exhaustive radial and prep. TLC resulted in the isolation of cordifolsides D and E as well as palmatosides C and F, ecdysterone, makisterone A and N-*trans*-feruloyl tyramine as their respective acetates (unpublished results).

Cordifoliside D tetraacetate (**1a**). Solid (16 mg); $C_{34}H_{42}O_{16}$; mp 151°; $[\alpha]_D^{28}$ − 87.5° ($CHCl_3$, *c* 0.160). IR ν_{max}^{KBr} cm^{-1}: 3453, 3250, 3150, 2954, 2855, 1756–1714, 1650, 1559, 1541, 1509, 1437, 1377, 1229, 1163, 1127, 1048, 999, 914, 897, 874, 803, 768, 685, 602. UV λ_{max}^{MeOH} nm: 209.5, 225 (sh), 276 (sh). FAB-MS *m/z*: 729 $[M + Na]^+$, 717, 707 $[M + H]^+$, 690, 689, 496, 495, 460, 408, 359, 331, 328, 307, 289, 273, 247, 229, 215, 187, 169, 154, 136, 127, 107, 95, 89, 81, 77, 63, 56, 52.

Cordifoliside E tetraacetate (**2a**). Solid (15 mg); $C_{34}H_{42}O_{16}$; mp 134°; $[\alpha]_D^{28}$ − 17.39° ($CHCl_3$, *c* 0.230), IR ν_{max}^{KBr} cm^{-1}: 3475, 2954, 2876, 1751–1701, 1663, 1507, 1449, 1437, 1372, 1318, 1244, 1146, 1048, 949, 922, 886, 876, 847, 801, 602. UV λ_{max}^{MeOH} nm^{-1}: 203, 226 (sh), 278 (sh). FAB-MS *m/z*: 729 $[M + Na]^+$, 713, 706 $[M]^+$, 460, 414, 359, 329, 307, 154, 120, 77, 52.

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REFERENCES

1. Thatte, U. M. and Dahanukar, S. A. (1989) *Phytochemistry Res.* **3**, 43.
2. Sipahimalani, A. T., Nörr, and Wagner, H. (1994) *Planta Medica.* **60**, 596.
3. Gangan, V. D., Pradhan, P., Sipahimalani, A. T. and Banerji, A. (1994) *Phytochemistry* **37**, 781.
4. Roberts, J. D., Weigert, F. J., Kroschwitz, J. I. and Reich, H. J. (1970) *J. Am. Chem. Soc.* **92**, 1338.
5. Desktop Molecular Modeller (version 1.0), Oxford Electronic Publishing, OUP, Oxford.