

Amritosides A, B, C and D: clerodane furano diterpene glucosides from *Tinospora cordifolia* [☆]

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Abstract

Four new clerodane furano diterpene glucosides (amritosides A, B, C and D) were isolated as their acetates from *Tinospora cordifolia* stems. The structures of these compounds were established on the basis of spectroscopic studies.

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1. Introduction

Tinospora cordifolia Miers. (Menispermaceae) popularly known as Amrita in Sanskrit, has been used for several centuries in Ayurvedic medicine for the treatment of various ailments (Chadha, 1976). This species is rich in clerodane derived diterpenes (Pachaly and Adnan, 1992; Rahman et al., 1992; Fukuda et al., 1993; Gangan et al., 1995; Maurya, 1996; Martin et al., 1996). Previously, we reported on the isolation and characterization of several new furanoditerpene glucosides (Wazir et al., 1995; Maurya et al., 1995) from *T. cordifolia*. In the present paper, we describe the structure elucidation of four more new clerodane furano diterpene glucosides, designated as amritosides A, B, C and D (**1–4**), in the acetate forms (**1a–4a**), respectively.

2. Results and discussion

The *n*-BuOH soluble fraction of the EtOH extract of *T. cordifolia* stems on chromatography over silica gel,

eluted with CHCl₃–MeOH mixture, gave two fractions which were found to be a complex mixture. Repeated efforts to obtain pure compounds from these mixtures were unsuccessful. IR spectra showed the presence of strong OH and C=O absorptions although acetyl signals were absent in ¹H NMR spectrum of mixture. Acetylation followed by repeated flash chromatography led to the isolation of four compounds as polyacetates. However, in nature these exist as non-acetylated compounds. We report on the structural elucidation of four new clerodane furano diterpene glucosides (**1–4**) isolated as its acetate (**1a–4a**). We have named them amritoside A, B, C and D.

The IR spectra of (**1a–4a**) showed strong absorptions in the carbonyl region (1705–1754 cm^{−1}) indicating the presence of acetyl carbonyl groups and the possibility of δ -lactone and/or ester carbonyl. The presence of a furan ring was deduced from the IR absorption at 1505, 880 cm^{−1}. IR of **1a–4a** also showed the presence of hydroxyl group, resistance to normal acetylation suggested the tertiary nature of the hydroxyl group. The fragment at *m/z* 81, 94 and 95 in the FAB mass, of compounds **1a–4a** resulted from the cleavage of C-11/C-12 bond, and cleavage of the δ -lactone ring along the C-9/C-11 and C-12 bond, these observations clearly indicated that the furan ring occupied the C-12 position. The ¹³C NMR

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Table 1

¹H NMR spectral data of **1a**, **2a**, **3a** and **4a** in CDCl₃

H	1a δ (J in Hz)	2a δ (J in Hz)	3a δ (J in Hz)	4a δ (J in Hz)
1a	1.52 (m)		1.55 (m)	2.28 (m)
b	1.52 (m)		1.85 (m)	2.52 (m)
2a	1.70–1.91 (m)		2.32 (m)	6.65 (m)
b	1.70–1.91 (m)			–
3a	2.85 (m)			5.91 (m)
b	2.41 (m)			
4	–		–	4.61 (d, 7.6)
5	–		–	
6	4.57 (d, J = 5.0)	4.56 (d, J = 4.8)	4.55 (d, J = 4.6)	1.15 (m)
7a	1.70–1.91 (m)		1.61 (m)	2.20 (m)
b	1.70–1.91 (m)		2.63 (m)	
10	2.27 (m)		1.90 (m)	
11a	2.54 (m)		1.31 (m)	2.41 (dd, 11.9, 3.5)
b	1.70–1.91 (m)		2.51 (m)	1.88 (m)
12	6.03 (t, 8.7)	6.04 (t, 8.6)	5.42 (dd, 3.9, 12.5)	5.45 (dd, 11.9, 3.5)
14	6.40 (s)	6.42 (s)	6.48 (s)	6.45 (s)
15	7.42 (s)	7.43 (s)	7.42 (s)	7.43 (s)
16	7.44 (s)	7.46 (s)	7.49 (s)	7.48 (s)
17	9.10 (brs)	9.50 (brs)	–	–
18	–	–	–	–
19	–	1.26 (s)	1.25 (s)	1.27 (s)
20	0.93 (s)	0.95 (s)	0.96 (s)	1.10 (s)
CO ₂ Me	3.74 (s)	3.75 (s)	3.79 (s)	–
1'	4.60 (d, 7.8)	4.60 (d, 7.8)	4.57 (d, 7.7)	4.61 (d, 7.6)
2'	5.17 (t, 9.3)	5.19 (t, 9.2)	5.19 (t, 9.3)	5.10 (t, 9.1)
3'	4.89 (t, 8.2)	4.90 (t, 8.2)	4.95 (t, 8.0)	4.81 (t, 8.1)
4'	5.06 (t, 9.4)	5.07 (t, 9.4)	5.05 (t, 9.6)	5.05 (t, 9.5)
5'	3.66 (m)	3.70 (m)	3.79 (m)	3.66 (m)
6'a	4.27 (dd, 4.5, 12.3)	4.43 (dd, 4.4, 2.2)	4.26 (dd, 4.8, 12.1)	4.33 (dd, 4.6, 12.1)
b	4.10 (dd, 2.1, 12.3)	4.10 (dd, 2.3, 12.2)	4.14 (dd, 3.7, 12.1)	4.12 (dd, 2.9, 12.1)
OCOMe	2.09 (s)	2.10 (s)	2.19 (s)	2.16 (s)
	2.02 (s)	2.03 (s)	2.09 (s)	2.02 (s)
	2.01 (s)	2.02 (s)	2.03 (s)	2.00 (s)
	51.99 (s)	2.00 (s)	1.99 (s)	
	–	–	1.48 (s)	

chemical shifts of the angular methyls are useful to distinguish between *cis* and *trans* A/B ring junctions in clerodane diterpenes (Manabe and Nishino, 1986). In the case of the *cis* clerodanes the C-19 methyl resonates in a region lower than δ 20, and in the corresponding *trans* compounds it resonates in the region δ 11–19. The C-19 methyl signals of **2a–4a** were found at 23.0–29.7, thus their A/B ring junctions are *cis*. The relative stereochemistries of tertiary hydroxyl group at C-8 position were determined on the basis of the ¹³C NMR chemical shift of C-20 (Gangan et al., 1995). Steric effects and conformational changes in the molecule render some characteristic shifts in ¹³C values. As summarized in Table 2, the ¹³C NMR chemical shifts of C-20 in **2a** and **3a** appeared upfield at δ 14.5 and δ 15.9 whereas that of **1a** and **4a**, appeared downfield at δ 23.5 and δ 21.8, respectively. Thus, the methyl group at C-9 and hydroxyl group at C-8 is *cis-gauche*-disposed in **2a** and **3a** and *anti*-disposed in **1a** and **4a**. Further the FAB mass and the NMR data of compounds **1a–4a** suggested certain common structural features attributable to one

glucose moiety. The elimination of tetraacetylglucose moiety was indicated in the FAB mass spectra of **1a–4a** by an ion peak at *m/z* 331. The NMR data for compounds **1a–4a** with the anomeric protons at δ 4.60 (*d*, *J* = 7.8), 4.60 (*d*, *J* = 7.8), 4.57 (*d*, *J* = 7.7) and 4.61 (*d*, *J* = 7.6) with corresponding carbon signals at δ 99.7, 99.3, 101.4 and 99.8, the coupling constant of the signal resulting from the anomeric proton of the glucopyranoside indicated the glucosidic linkage to have β -configuration. 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 view of the spectral evidences, it was reasonable to infer that the above compounds could be diterpene furan glucoside.

Compound **1a** afforded [M + H]⁺ and [M + Na]⁺ ions at *m/z* 767 and 789, respectively, in the FAB mass spectrum, suggesting the molecular formula C₃₆H₄₆O₁₈, supported by NMR spectra. The ¹H and ¹³C NMR spectra (Tables 1 and 2) together with a DEPT and ¹H–¹³C COSY experiments indicated the presence of

Table 2
¹³C NMR spectral data of **1a**, **2a**, **3a** and **4a** in CDCl₃

C	1a δ	2a δ	3a δ	4a δ
1	18.1	17.7	17.0	26.6
2	25.7	25.3	28.4	130.0
3	27.4	27.0	151.4	129.3
4	145.6	77.6	127.3	73.6
5	129.1	35.2	42.2	37.8
6	73.8	75.0	72.8	27.8
7	29.3	29.7	28.4	29.6
8	75.5	77.6	74.9	74.8
9	41.9	41.4	42.8	39.0
10	43.8	40.4	39.9	43.8
11	40.6	40.2	33.8	40.0
12	71.2	71.7	71.3	71.2
13	126.3	125.9	124.8	125.0
14	108.8	108.4	108.6	108.8
15	139.9	139.5	139.9	141.8
16	144.2	143.9	143.8	139.9
17	172.9	172.0	172.2	171.4
18	168.5	170.5	168.4	–
19	–	23.0	29.7	23.6
20	23.5	14.5	15.9	21.8
CO ₂ Me	51.9	51.6	51.7	–
1'	99.7	99.3	101.4	99.8
2'	72.2	73.5	71.3	69.9
3'	72.1	70.8	72.5	68.8
4'	68.8	68.8	68.7	68.2
5'	72.2	73.0	71.6	72.4
6'	62.5	62.1	62.1	61.8
OCOMe	171.1	170.7	174.8	170.2
	170.7		170.3	169.8
	169.8		169.5	168.4
	169.4		169.2	168.2
OCOMe	21.1	21.1	20.8	21.2
	21.0	21.0	20.7	20.8
			20.6	
			20.5	

carboxylic acid (δ 9.1 very *brs*; δ 172.9), ester carbonyl (δ 168.5), a furan ring (δ 6.40 *s*, 7.42 *s*, 7.44 *s*; δ 108.8, 139.9, 144.2, 126.3), an ester methyl (δ 3.74 *s*; δ 51.9) and tetra-substituted olefin (δ 145.6 and 129.1). One proton triplet at δ 6.03 ($J = 8.7$); δ 71.2 was assigned to H-12, the downfield shift of this proton signal indicated that the OH group attached to C-12 was acetylated. One proton doublet at δ 4.57 ($d, J = 5.0$); δ 73.8 assigned to proton at C-6, the down-field shift suggested that it could be attached to oxygen bonded carbon. This signified that the glycosidic linkage could be at C-6. One proton multiplet at δ 2.27; δ 43.8 was assigned to methine at C-10, the free carboxylic acid and tertiary hydroxyl could be placed at C-8 (δ 75.5). The relative configuration of **1a** was determined by NOE-difference spectral measurements (Fig. 1). The important correlations were observed between H-6, CH₃-20 and H-10, H-

12 and anomeric-H, H-6, suggested that H-6 and CH₃-20 are on the same side and H-10, H-12 are on the other side. Thus, amritoside A pentaacetate can be represented by structure **1a** and the corresponding parent glucoside structure by **1**, which has not been previously reported.

Compound **2a** has the molecular formula C₃₇H₅₀O₁₉ as determined by FAB mass at m/z 799 [M + H]⁺ and 821 [M + Na]⁺ supported by NMR spectra. The spectra of **2a** are summarized in Tables 1 and 2, suggested the presence of all the common structural features of isolated compound mentioned above. Moreover, in its NMR spectrum, an additional angular methyl group at (δ 1.26 *s*; δ 23.0) assigned at C-5 (δ 35.2) and one tertiary hydroxyl group at C-4 exists at δ 77.6. The methoxy-carbonyl group (δ 3.75 *s*; δ 51.6) and carboxylic acid group (δ 9.50 very *brs*; δ 172.0) are attached to C-4 and C-8, respectively. The linkage position of the glucose moiety at C-6 (δ 4.56 *d, J = 4.8*; δ 75.0), and acetoxy group at C-12 (δ 6.04 *t, J = 8.6*; δ 71.7) as determined by ¹H–¹H COSY and ¹H–¹³C COSY experiments. The two angular methyl groups (δ 1.26 *s*; δ 23.0) and (δ 0.95 *s*; δ 14.5) are assigned at C-5 (δ 35.2) and C-9 (δ 41.4), respectively. The C-19 methyl signal of **2a** was found at δ 23.0, thus their A/B ring junctions are *cis* (Manabe and Nishino, 1986). On the basis of these results the amritoside B pentaacetate was determined as shown in the formula **2a** and the corresponding parent glucoside structure by **2**, which has not been previously reported.

Compound **3a**, C₃₇H₄₆O₁₈, showed the molecular ions at m/z 779 [M + H]⁺ and 801 [M + Na]⁺ in the FAB mass spectrum. ¹³C NMR signals of compound **3a** revealed that it contains a δ -lactone (δ 172.2), a methoxycarbonyl group at (δ 168.4; 51.7), and tetra-substituted olefin (δ 151.4; δ 127.3). The signal at δ 151.4 showed presence of acetylated OH group attached to C-3. An isolated ABX system at δ 5.42 (*dd, J = 3.9, 12.5*), 1.31 (*m*) and 2.51 (*m*) could be assigned to H-12 and H-11, respectively. Since C-12 is an oxygen-bearing carbon, it was deduced that the ester carbonyl (δ 172.2) is in fact a δ -lactone ring existing between C-8 and C-12. Further the downfield shift of quaternary C-8 suggesting tertiary hydroxyl could be placed at this position. A doublet at δ 4.55 (*d, J = 4.6*) was assigned to one proton at C-6, whereas multiplets appeared at δ 1.61 and 2.63 for the two protons at C-7. The down field shift of proton at C-6 indicated that the glucopyranosyl residue is attached at this position. The two angular methyl groups (δ 1.25 *s*; δ 29.7) and (δ 0.96 *s*; δ 15.9) are assigned at C-5 (δ 42.2) and C-9 (δ 42.8), respectively. The C-19 methyl signal of **3a** was found at δ 29.7, thus their A/B ring junctions are *cis* (Manabe and Nishino, 1986). Thus, amritoside C pentaacetate was assigned the structure **3a**, with corresponding parent glucoside structure **3**.

Compound **4a** has the molecular formula C₃₃H₄₂O₁₄ as determined by FAB mass at m/z 663 [M + H]⁺ and

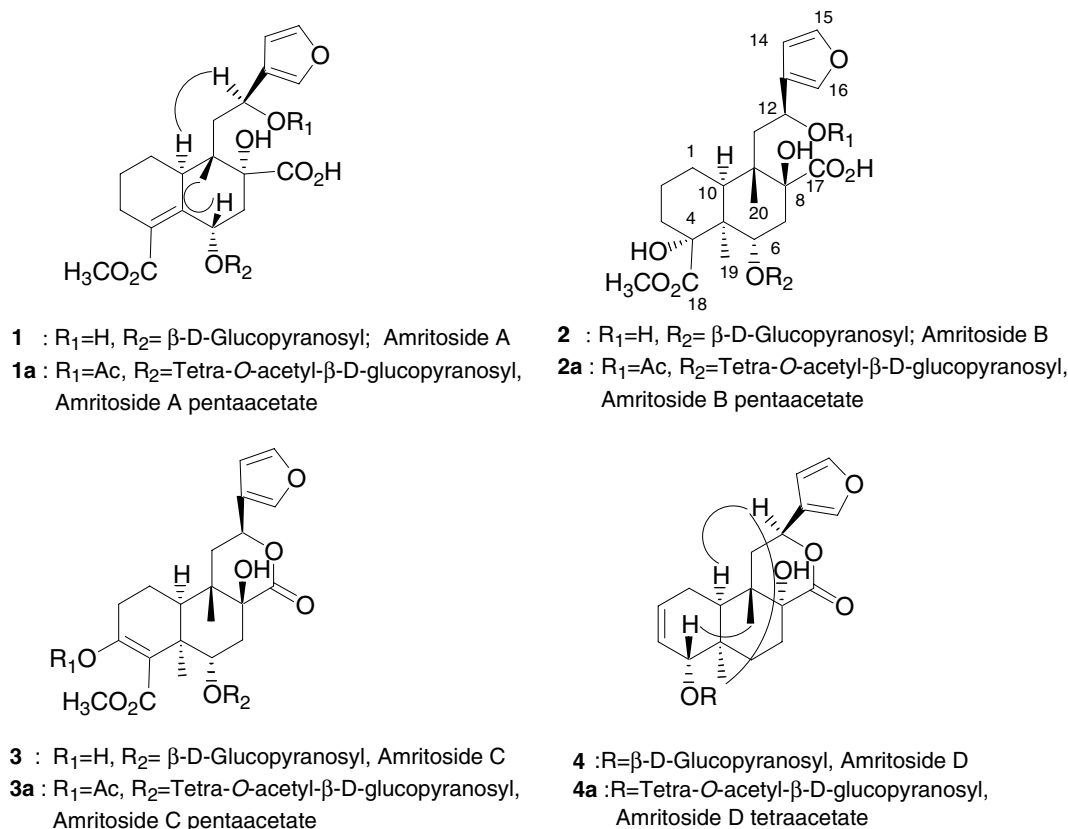


Fig. 1. Structure of isolated compounds and selected NOE correlations for **1a** and **4a**.

685 $[M + Na]^+$ supported by NMR spectra. The NMR spectra of **4a** are summarized in Tables 1 and 2, suggested the presence of all the common structural features of isolated compound mentioned above. The presence of two olefinic protons in ring A was resonated as multiplets at δ 6.65 and 5.91, and δ 130.0 and 129.3 and was assigned to C-2 and C-3, respectively, in the NMR spectra. The C-3 proton showed an additional coupling to a proton resonating at δ 4.61 ($d, J = 7.6$) assigned to H-4. The attachment of the glucose moiety was determined to be at C-4, based on the downfield shift of the signal attributed to this carbon δ 73.6. The NMR spectra of **4a** showed a close similarity with tinocordioside previously reported from the same plant (Maurya et al., 1995) but with a remarkable downfield shift of carbon C-8 (δ 74.8), indicating the presence of an additional hydroxyl group. Furthermore, irradiation of the C-20 methyl signal gave rise to a NOE for the signal corresponding to H-4, while irradiation of the H-12 signal showed NOE for the signals of H-10 and Me-19 (Fig. 1). These showed that H-10, H-12 and Me-19 are located on the same side while Me-20 and H-4 are located on the other side. Based on all these data, we deduced that it had the new furanoditerpene glucoside structure depicted in the formula **4a** for amritoside D tetraacetate, with corresponding parent glucoside structure **4**, which has not been reported.

3. Experimental

3.1. General

Mps: uncorr., on a Complab melting point apparatus. IR spectra (KBr) were recorded on a Hitachi 270-30 spectrophotometer. UV spectra were obtained on a Perkin–Elmer λ -15 UV spectrophotometer, optical rotations on Perkin–Elmer Model 241 digital polarimeter. NMR spectra were run on an Bruker DPX-200 MHz spectrometer; FAB MS were carried out on JEOL SX 102/DA-6000 mass spectrometer. Elemental analyses were obtained in a Carlo–Erba-1106 CHN elemental analyzer. Column chromatography was performed using flash silica gel (230–400 mesh); TLC: pre-coated silica gel plates (Merck).

3.2. Plant material

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.

3.3. Extraction and isolation

The powdered stem (5 kg) was extracted with 70% aqueous EtOH at room temperature. After removal of

the EtOH by evaporation the remaining extract was washed with petrol and CHCl_3 and then extracted with *n*-BuOH. The *n*-BuOH extract was freed from solvent and on MPLC (silica gel 230–400 mesh) with CHCl_3 –MeOH (9:1) yielded fraction (Fr. 1) and with CHCl_3 –MeOH (8:2) afforded fraction (Fr. 2), found to be mixt. These frs. were collected, conc., dried and stirred separately with Ac_2O and pyridine at room temperature for 16 h. The solvent was then removed in vacuo afforded Fr. 1Ac and Fr. 2Ac, respectively. Careful flash chromatography of Fr. 1Ac using hexane–EtOAc (7:3) allowed the isolation of compound **3a** (15 mg) and **4a** (11 mg). Careful flash chromatography of Fr. 2Ac using hexane–EtOAc (1:4) allowed the isolation of compound **1a** (19 mg) and **2a** (10 mg).

3.4. Acid hydrolysis of Fr. 1 and Fr. 2

The solution of Fr. 1 and Fr. 2 separately (50 mg) in 1 M methanolic HCl (5 ml) was refluxed for 30 min. The reaction mixture was worked up in the usual manner and the sugar fraction isolated on an activated carbon column to give D-glucopyranose identified by comparison with an authentic sample (TLC) and by optical rotation.

3.5. Amritoside A pentaacetate (**1a**)

White crystals from MeOH; mp 138–139 °C; $[\alpha]_{\text{D}}^{22}$ –53.6° (CHCl_3 , *c* 0.110). UV (MeOH) λ_{max} nm: 231; IR ν_{max} (KBr) cm^{-1} : 3450, 3140, 1735–1715, 1674, 1510, 1240, 1130, 880. ^1H NMR (CDCl_3 , 200 MHz) and ^{13}C NMR (CDCl_3 , 50 MHz) see Tables 1 and 2; FAB MS (pos.): *m/z* 767 $[\text{M} + \text{H}]^+$, 789 $[\text{M} + \text{Na}]^+$, 331, 121, 95, 94, 81, 42. Elemental analysis: (Found: C, 56.48, H, 5.95; $\text{C}_{36}\text{H}_{46}\text{O}_{18}$ requires: C, 56.39, H, 6.05%).

3.6. Amritoside B pentaacetate (**2a**)

White solid; mp 157–158 °C; $[\alpha]_{\text{D}}^{22}$ –37.9° (CHCl_3 , *c* 0.131). UV (MeOH) λ_{max} nm: 213; IR ν_{max} (KBr) cm^{-1} : 3470, 1740–1710, 1674, 1510, 880. ^1H NMR (CDCl_3 , 200 MHz) and ^{13}C NMR (CDCl_3 , 50 MHz) see Tables 1 and 2; FAB MS (pos.): *m/z* 799 $[\text{M} + \text{H}]^+$, 821 $[\text{M} + \text{Na}]^+$, 331, 121, 95, 94, 81, 42. Elemental analysis: (Found: C, 55.70, H, 6.25; $\text{C}_{37}\text{H}_{50}\text{O}_{19}$ requires: C, 55.63, H, 6.31%).

3.7. Amritoside C pentaacetate (**3a**)

Powder, $[\alpha]_{\text{D}}^{22}$ –77.9° (CHCl_3 , *c* 0.101). UV (MeOH) λ_{max} nm: 212; IR ν_{max} (KBr) cm^{-1} : 3440, 1750–1705, 1664, 1515, 875. ^1H NMR (CDCl_3 , 200 MHz) and ^{13}C NMR (CDCl_3 , 50 MHz) see Tables 1 and 2; FAB MS (pos.): *m/z* 779 $[\text{M} + \text{H}]^+$, 801 $[\text{M} + \text{Na}]^+$, 331, 121, 95, 94, 81, 42. Elemental analysis: (Found: C, 57.16, H, 5.83; $\text{C}_{37}\text{H}_{46}\text{O}_{18}$ requires: C, 57.07, H, 5.95%).

3.8. Amritoside D tetraacetate (**4a**)

Powder, $[\alpha]_{\text{D}}^{22}$ –17.2° (CHCl_3 , *c* 0.120). UV (MeOH) λ_{max} nm: 218; IR ν_{max} (KBr) cm^{-1} : 3545, 1750–1705, 1670, 1515, 1240, 885. ^1H NMR (CDCl_3 , 200 MHz) and ^{13}C NMR (CDCl_3 , 50 MHz) see Tables 1 and 2; FAB MS (pos.): *m/z* 663 $[\text{M} + \text{H}]^+$, 685 $[\text{M} + \text{Na}]^+$, 331, 121, 95, 94, 81, 42. Elemental analysis: (Found: C, 59.91, H, 6.45; $\text{C}_{37}\text{H}_{42}\text{O}_{14}$ requires: C, 59.81, H, 6.39%).

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