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CORDIOSIDE, A CLERODANE FURANO DITERPENE GLUCOSIDE FROM *TINOSPORA CORDIFOLIA*

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

Abstract—The structure of cordioside isolated from the stem of *Tinospora cordifolia* was characterized on the basis of NMR spectroscopy.

INTRODUCTION

Tinospora cordifolia Miers. is distributed throughout India. It has been used in Ayurvedic medicine for the treatment of various diseases [1]. Previous studies on this and other species of *Tinospora* [2–7] have revealed the presence of a range of clerodane-derived diterpenes. The present work deals with the isolation and structure determination of a new diterpene glucoside named cordioside (**1**) together with four known compounds **2**, **7**–**9** from stems of the plant.

RESULTS AND DISCUSSION

Cordioside showed UV absorption at 243 nm (α,β-unsaturated carbonyl group). The IR spectrum (nujol) showed absorptions at 3560–3400 (OH), 1715 (δ-lactone) and 1505, 880 cm^{-1} (furan ring). The presence of a furan ring was also indicated by a positive Ehrlich colour test [8]. Compound **1** afforded $[M + Na]^+$ and $[M + H]^+$ ions at *m/z* 561 and 539, respectively, in the FAB mass spectrum, suggesting the molecular formula $C_{26}H_{34}O_{12}$. The presence of hydroxyls in the compound did not allow the resolution of all the portons in the NMR spectrum. Therefore, **1** was acetylated to yield tetraacetate **3**, mp 120°, $C_{34}H_{42}O_{16}$, (FAB mass spectrum *m/z* 730 $[M + Na + H]^+$). The mass spectrum of **3** showed two major ion peaks at *m/z* 347 and 331 for the loss of hexose tetraacetate (as an *O*-glycoside with, respectively, retention and loss of ether oxygen). ^1H NMR coupling constants for methine protons H-1′ to H-5′ of the hexose showed an all *trans*-axial relationship which, together with a methylene H-6′ resonance, confirmed the identity of the sugar as β-D-glucose. This was confirmed by the hydrolysis of **1**. The ^1H and ^{13}C NMR spectra together with a DEPT experiment indicated the presence of δ-lactone and ester carbonyls (δ171.1, 170.3), a furan ring (δ 6.48 *s*, 7.43 *s*, 7.50 *s*; δ 108.6, 139.9, 143.4, 125.0), an ester methyl (δ3.74 *s*; δ51.5) and tetra-substituted olefin (δ143.4 and 128.9). An isolated ABX system at δ5.42 (*dd*, J = 12.6, 2.9 Hz), 2.25 (*dd*, J = 16.6, 3.3 Hz) and a multiplet at δ2.40 could be assigned to H-12 and H-11, respectively, with H-12 being axial. A broad singlet at δ4.60 was assigned to one proton at C-6, whereas multiplets appeared at δ2.16 and 2.97 for the two protons at C-7. The ^{13}C NMR spectra of **3** and **4** showed a close similarity, but with a remarkable downfield shift at the carbon atom bearing an additional hydroxyl group in **3**, a doublet at δ49.5 in **4** and a singlet at δ72.0 in **3** in relative ^1H coupled spectra. By comparison of the ^1H NMR spectra of **3** and **4**, we observed the absence of a multiplet at δ2.44 in **4** assigned to the C-8 proton in **4** and the appearance of a D_2O exchangeable singlet at δ5.03 for a tertiary hydroxy group, thus confirming its position. Cordioside (**1**) was, thus presumed to have the same stereochemistry as that of **4** except for the hydroxyl group at the C-8 position by detailed comparison of their ^1H and ^{13}C NMR spectra.

The closely related diterpenoids **5** [3, 9] and **6** [10] with a 8β-hydroxyl group have earlier been reported from *T. cordifolia* and *Chasmanthera dependens*, respectively. The absolute stereochemistry and β-orientation of the hydroxyl group of **5** was established by X-ray studies and that of **6** by detailed study of the deshielding β-effects on carbons in the ^{13}C NMR spectrum. The spectral properties of cordioside are practically identical to those of **5** and 8β-hydroxycolumbin (**6**). Furthermore, the NOE difference measurements were carried out at different centres to determine the relative stereochemistry. The key observations were that irradiation of the C-19 proton resulted in 5% NOE of C-8OH, 2% NOE of C-11H and 4% NOE of C-6H, while irradiation of C-6H resulted in 3% NOE of the C-19 proton, 4% NOE of C-7e, 2% NOE of C-8OH and 1% NOE of C-1′H, thereby establishing that C-6H, C-8OH, C-19 proton and C-11Ha were β-oriented, which confirmed the stereochemistry of cordioside.

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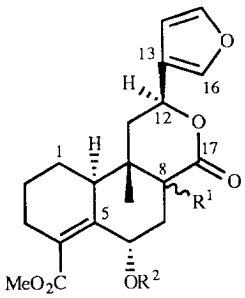
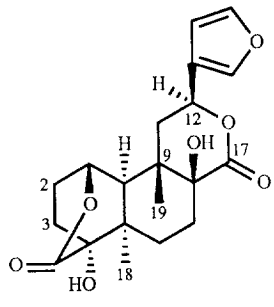
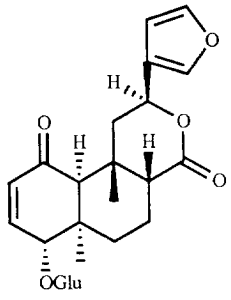


	R^1	R^2
1	β - OH	β - D - glucopyranosyl
2	α - H	β - D - glucopyranosyl
3	β - OH	tetra - O - acetyl - β - D - glucopyranosyl
4	α - H	tetra - O - acetyl - β - D - glucopyranosyl



5 2, 3 - epoxide
6 2, 3 - dehydro



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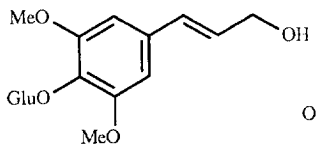

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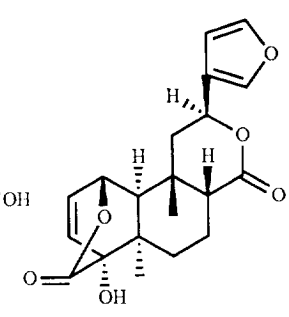

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The four known compounds, tinosporaside (**7**) [6], syringin (**8**) [11], columbin (**9**) [12] and **2** were also isolated. The occurrence of **8** is reported for the first time. Compound **2** has recently been isolated from *T. cordifolia* [13] but in our hands showed the opposite sign of rotation.

EXPERIMENTAL

Mps were determined in open capillary tubes and are uncorr. Flash chromatography was carried out over silica gel (230–400 mesh) and TLC over E. Merck precoated silica gel plates. ^{1}H NMR (400 and 300 MHz) and ^{13}C NMR (100 and 75 MHz) spectra were taken using TMS as int. standard.

Extraction and isolation. Plant material (5 kg) was extracted with 70% EtOH at room temp., the extract evapd to dryness *in vacuo* and the resulting residue was suspended in H_2O and extracted successively with $CHCl_3$ and *n*-BuOH. The *n*-BuOH extract was freed from solvent and partialy purified by MPLC using mixts of $CHCl_3$ and MeOH as eluting solvent. Careful flash chromatography of the $CHCl_3$–MeOH (9:1) eluate allowed the isolation of **1** (100 mg), **2** (200 mg), **7** (200 mg), **8** (100 mg) and **9** (300 mg).

Cordioside (**1**). Hygroscopic amorphous solid, UV λ_{max}^{MeOH} nm: 243. IR ν_{max}^{nujol} cm^{-1}: 3560–3400, 3140, 1715, 1705 and 880. ^{1}H NMR (400 MHz, DMSO-d_6): δ0.93 (3H, *s*, Me), 1.45–2.30 (11H, *m*, aliphatic protons), 3.68 (3H, *s*, CO_2Me), 4.99–5.07 (5H, broad hump, hydroxyls), 6.65 (1H, *br s*, H-14), 7.68 (1H, *br s*, H-15) and 7.74 (1H, *br s*, H-16). ^{13}C NMR (100 MHz, DMSO-d_6): δ17.7 (C-1), 23.1 (C-18), 26.7 (C-3), 28.4 (C-2), 31.6 (C-7), 33.8 (C-11), 33.2 (C-10), 34.6 (C-9), 51.5 (C-20), 63.5 (C-6′), 71.2 (C-12), 72.0 (C-8), 72.1 (C-4′), 72.4 (C-2′), 75.7 (C-6), 78.4 (C-5′), 78.6 (C-3′), 102.4 (C-1′), 108.6 (C-14), 125.0 (C-13), 128.3 (C-4), 139.0 (C-15), 143.2 (C-5), 169.2 (C-19) and 171.1 (C-17). FAB-MS *m/z* (rel. int.): 577 [M + K]$^+$ (40), 561 [M + Na]$^+$ (86), 539 (80), 376 (38), 359 (40), 279 (82) and 81 (10).

Cordioside tetra-acetate (**3**). A mixt. of **1** (20 mg) and Ac_2O (0.5 ml) in pyridine (0.7 ml) was left at room temp. for 16 hr; solvent was removed *in vacuo*, and the mixt. filtered through a short column of silica gel to yield **3** (15 mg), mp 120°, $[\alpha]_D^{22}$ −46.3° (0.139 $CHCl_3$; *c*) UV $\lambda_{max}^{CHCl_3}$ nm: 231. IR ν_{max}^{KBr} cm^{-1}: 3540, 3140, 2900, 1725–1715, 1674, 1510, 1240, 1130 and 880. ^{1}H NMR (400 MHz, $CDCl_3$); δ0.97 (3H, *s*, Me), 1.45–1.95 (6H, *m*, H-1, 2, 3), 2.0 (3H, *s*), 2.01 (6H, *s*), 2.10 (3H, *s*) for OCOMe, 2.16 (1H, *m*, H-7), 2.25 (1H, *dd*, *J* = 16.6, 3.3 Hz, He-11), 2.37 (1H, *m*, H-10), 2.40 (1H, *m*, Ha-11), 2.97 (1H, *d*, *J* = 19.1 Hz, H-7), 3.68 (1H, *m*, H-5), 3.74 (3H, *s*, CO_2Me), 4.15 (1H, *dd*, *J* = 12.22, 1.2 Hz, H-6′a), 4.27 (1H, *dd*, *J* = 12.22, 4.3 Hz, H-6′b), 4.60 (1H, *br s*, H-6), 4.61 (1H, *d*, *J* = 8 Hz, H-1′), 4.90 (1H, *t*, *J* = 9.8 Hz, H-3′), 5.03 (1H, *br s*, OH), 5.07 (1H, *t*, *J* = 9.72 Hz, H-4′), 5.18 (1H, *t*, *J* = 9.6 Hz, H-2′), 5.42 (1H, *dd*, *J* = 12.6, 2.9 Hz, H-12), 6.48 (1H, *s* H-14), 7.43 (1H, *s*, H-15), 7.50 (1H, *s*, H-16). ^{13}C NMR (100 MHz, $CDCl_3$): δ17.7 (C-1), 20.5 (4× OCOMe), 23.1 (C-18), 26.7 (C-3), 28.3 (C-2), 30.6 (C-7), 33.9 (C-11), 34.6 (C-9), 39.1 (C-10), 51.5 (C-20), 62.4 (C-6′), 68.4 (C-4′), 71.1 (C-2′), 71.7 (C-5′, C-12), 72.0 (C-8), 72.9 (C-3′), 99.4 (C-1′), 108.6 (C-14), 125.0 (C-13), 128.9 (C-5), 139.9 (C-15), 143.4 (C-16, C-4), 168.7, 169.9, 170.0, 170.2 (OCOMe), 170.3 (C-19) and 171.1 (C-17). FAB-MS *m/z* (rel. int.): 730 [M + Na + H]$^+$ (30), 705 (20), 376 (20), 359 (40), 347 (60), 331 (80), 327 (100), 309 (50), 281 (40), 95 (12) and 81 (12).

Acid hydrolysis of **1**. A soln of **1** (50 mg) in 1 N methanolic HCl (5 ml) was refluxed for 30 min. The reaction mixt. was worked up in the usual manner and the sugar fr. isolated on an activated carbon column to give D-glucose, identified by comparison with an authentic sample (TLC) and $[\alpha]_D^{22}$ + 51° (H_2O; *c* 0.61).

Compound **2**. A powder, mp 200°, $[\alpha]_D^{22}$ − 20° (MeOH; *c* 3.0).

Syringin (**8**). As amorphous powder, mp 190°, $[\alpha]_D^{20}$ − 17.3° (H_2O; *c* 0.4), UV λ_{max}^{MeOH} nm: 224 and 264. IR ν_{max}^{KBr} cm^{-1}: 3540–3430, 3140 and 1610–1510. ^1H NMR (300 MHz, MeOH-d_4): δ3.83 (6H, *s*, 2 × OMe), 3.00–5.00 (7H, broad hump, H-1″, 2″, 3″, 4″, 5″, 6″), 4.13 (2H, *d*, *J* = 6.5 Hz, H-1), 5.00–5.50 (4H, hump, hydroxyls), 6.30, (1H, *dt*, *J* = 16.49, 6.5 Hz, H-3), 6.63 (1H, *d*, *J* = 16.49 Hz, H-2) and 6.73 (2H, *s*, H-2′, 6′). ^{13}C NMR (75 MHz, MeOH-d_4): δ 56.3 (2 × OMe), 64.2 (C-6″), 66.3 (C-1), 70.3 (C-4″), 72.3 (C-3″), 73.3 (C-2″), 75.3 (C-5″), 101. 2 (C-1″), 104.3 (C-2′, C-6′), 123.2 (C-3), 133.0 (C-1′), 133.1 (C-4′), 134.0 (C-2) and 153.8 (C-3′, C-5′). FAB-MS *m/z* (rel. int.): 395 $[M + Na]^+$ (100), 373 (90), 210 (50), 193 (40), 179 (20) and 163 (41).

Syringin penta-acetate. A mixt. of syringin (15 mg), Ac_2O (1.5 ml) and pyridine (2 ml) was kept at room temp. overnight. The usual work up gave syringin penta-acetate (14 mg) as amorphous powder crystallized from MeOH, mp 125°, $[\alpha]_D^{22}$ − 5.90° ($CHCl_3$; *c* 0.065) UV $\lambda_{max}^{CHCl_3}$ nm: 224 and 261. IR λ_{max}^{KBr} cm^{-1}: 3140, 1725, 1610–1510 and 1240. ^1H NMR (300 MHz, $CDCl_3$): δ2.01 (6H, *s*, 2 × OCOMe), 2.02 (6H, *s*, 2 × OCOMe), 2.10 (3H, *s*, OCOMe), 3.70 (1H, *m*, H-5″), 3.83 (6H, *s*, 2 × OMe), 4.11 (1H, *dd*, *J* = 12.22, 4.30 Hz, H-6a″), 4.24 (1H, *dd*, *J* = 12.22, 1.2 Hz, H-6″b), 4.71 (2H, *d*, *J* = 6.56 Hz, H-1), 5.05 (1H, *d*, *J* = 7.0 Hz, H-1″), 5.26–5.35 (3H, *m*, H-2″, 3″, 4″), 6.21 (1H, *dt*, *J* = 16.49, 6.5 Hz, H-3), 6.54 (1H, *d*, *J* = 16.49, H-2) and 6.58 (2H, *br s*, H-2′, 6′). ^{13}C NMR (75 MHz, $CDCl_3$): δ20.5–21.1 (5 × OCOMe), 56.3 (2 × OMe), 62.5 (C-6″), 64.9 (C-1), 68.5 (C-4″), 71.7 (C-2″), 73.1 (C-5″), 72.1 (C-3″), 101.2 (C-1″), 104.1 (C-2′, C-6′), 123.3 (C-3), 133.1 (C-1′), 133.9 (C-4′), 134.0 (C-2), 153.1 (C-3′, C-5′) and 169–172 (5 × OCOMe), FAB-MS *m/z* (rel. int.): 621 $[M + K]^+$ (80), 606 (20), 605 (40), 331 (100), 252 (20) and 209 (10).

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