Pergamon

0031-9422(94)00686-5

Phytochemistry, Vol. 38, No. 3, pp. 659–661, 1995

0031-9422/95 $9.50 + 0.00

CLERODANE DITERPENOIDS FROM *TINOSPORA CORDIFOLIA*

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(*Received in revised form* 2 *August* 1994)

Key Word Index—*Tinospora cordifolia*; Menispermaceae; stem; tinosponone, tinocordioside.

Abstract—Tinosponone and tinocordioside have been isolated from the stem of *Tinospora cordifolia*. The structures were established by spectroscopic studies and chemical correlation.

INTRODUCTION

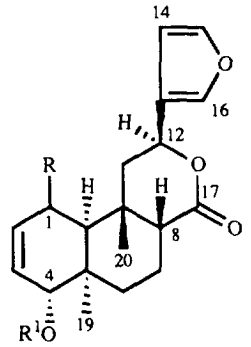
Tinospora cordifolia Miers. is distributed throughout the plains of India. It has been used for several centuries in Ayurvedic medicine for the treatment of liver and intestinal disorders [1]. This species [2–7] is rich in clerodane derived diterpenes. Recently we reported on the isolation and biological evaluation of several new furano diterpene glucosides [8, 9] and phenyl propene glycosides [10] from *T. cordifolia*. In the present communication, the structure elucidation of two new clerodane diterpenoids tinosponone (**1**) and tinocordioside (**4**) is described.

RESULTS AND DISCUSSION

The clerodane diterpene tinosponone (**1**) and the clerodane diterpene glucoside tinocordioside (**4**) were isolated from the polar fraction of *T. cordifolia* stem.

The molecular formulae of compounds **1** and **2** were determined to be $C_{19}H_{22}O_5$ and $C_{25}H_{34}O_9$, respectively, by mass measurements [FAB-MS: *m/z* 353 $[M + Na]^+$ and 369 $[M + K]^+$, and 501 $[M + Na]^+$ and 517 $[M + K]^+$, respectively] and from the ^{13}CNMR spectra. The mass spectra of both compounds exhibited fragment peaks at *m/z* 81, 94 and 121 owing to the furan moiety. The fragment at *m/z* 121 suggests that these compounds are furanoid diterpenes possessing an oxygen function at C-12 and a methyl group at C-9 [5, 11]. The fragments at *m/z* 179 in 4 and 331 in **5** indicate that each compound contains a hexose moiety.

The IR spectra of **1** indicate the presence of hydroxyl (3430 cm^{-1}), δ lactone (1710 cm^{-1}), α,β-unsaturated ketone (1678 cm^{-1}) and furan (874 cm^{-1}) functions. The UV absorption at 241 nm supported the presence of an α,β-unsaturated ketone group. Furthermore, the presence of an α,β-unsaturated ketone in ring A was confirmed by a doublet ascribed to H-2 at δ5.97 (J = 10.8 Hz) and a double doublet ascribed to H-3 at δ6.65 (J = 10.85, 5 Hz) in the ^1H NMR spectrum and resonances at δ200.0, 129.0



	R	R^1
1	O	H
2	O	β - D - glucopyranosyl
3	O	tetra - O - acetyl - β - D - glucopyranosyl
4	H_2	β - D - glucopyranosyl
5	H_2	tetra - O - acetyl - β - D - glucopyranosyl

and 143.4 ascribed to C-1, C-2 and C-3, respectively, in the ^{13}CNMR spectrum. The β-proton showed an additional coupling to a proton resonating at δ4.35 assigned to H-4. The presence of a carbinolic carbon was also evident from ^{13}CNMR signal at δ70.6. The characteristic ^1H and ^{13}CNMR signals revealed the existence of a β-substituted furan ring (δ6.38 *s*, 7.36 *s* and 7.42 *s*; δ108.4, 143.8, 139.7, 125.1). The one proton double doublet at δ5.57 (J = 12, 4 Hz) was assigned to the C-12 proton and two one-proton double doublets at δ2.35 (J = 15, 4 Hz) and 1.71 (J = 15, 12 Hz) and 1.71 (J = 15, 12 Hz) were assigned to the C-11eq and C-11ax protons, respectively. The two methyl groups at C-9 and C-5 were observed as three proton singlets at δ1.30 and 0.78, respectively. The signals at δ2.30 and 2.25 were assigned to the protons at C-8 and C-10; the C-6 and C-7 methylene protons resonating at δ2.25 (*m*), 1.08 (*dt*, J = 14, 4 Hz) and 2.19 (*m*), 1.65 (*dddd*, J = 14.2, 14, 8.2, 1.5 Hz), respectively. The ^{13}CNMR chemical shifts were also consistent with the

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structure **1**. These patterns are very similar to those of tinosporaside (**2**) previously reported by Waterman *et al.* [12] from the same plant. Its structure, however, was determined with the help of UV, IR and MS spectral data of **2** and the NMR data for its tetraacetate derivative (**3**). The ^{1}H and ^{13}C NMR spectral data of **2** have not yet been published.

Additional support for structure **1** for tinosponone was provided by its transformation to tinosporaside tetraacetate (**3**). Condensation of **1** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of mercuric cyanide [13], gave the expected tinosporaside tetraacetate (**3**). The spectral properties of the synthetic product were identical with that of natural compound [12]. Thus tinosponone (**1**) is an aglycone of **2** and is presumed to have the same absolute stereochemistry.

The presence of four hydroxyls in **4** did not allow the resolution of all the protons in the ^{1}H NMR spectrum. Therefore, **4** was acetylated to yield the tetraacetate derivative **5** which had comparable ^{1}H and ^{13}C NMR spectra to those of tinosporaside tetraacetate (**3**) [12]. The most striking differences in the spectra were the lack of a keto function at C-1 and the appearance of methylene protons as multiplets at δ1.59 and 2.38 (δ_C26.1).

The two olefinic protons of ring A resonated as multiplets at δ6.48 and 6.40, and δ_C132.4 and 130.7 and were assigned to C-2 and C-3, respectively. The proton at C-10 appeared as multiplet at δ1.3. The signals of the other protons differed slightly from those of **3**. Based on all these data, we deduced that it had the new furano diterpene glucoside structure depicted in the formula (**4**) and gave it the trivial name tinocordioside.

EXPERIMENTAL

Plant material (5 kg) was extracted with 70% aq. EtOH at room temp. The EtOH was evapd and the remaining extract washed with petrol and then extracted with *n*-BuOH. The *n*-BuOH extract was freed from solvent and subjected to repeated flash CC over silica gel (230–400 mesh. Merck) to yield tinosponone (**1**, 0.120 g) and tinocordioside (**4**, 0.064 g).

Tinosponone (**1**). Mp 172°; $[\alpha]_D$ + 46.3° ($CHCl_3$; *c* 0.3); FAB-MS *m/z* (rel. int.): 369 $[M + K]^+$ (12), 353 $[M + Na]^+$ (15), 313 (45), 219 (80), 121 (25), 95 (8), 94 (10), 81 (40); IR ν_{max} cm^{-1}: 3430, 1710, 1678, 1450, 1210, 874; UV λ_{max} nm: 241; ^{1}H NMR (400 MHz, $CDCl_3$): δ7.42 (*s*, H-16), 7.36 (*s*, H-15), 6.65 (*dd*, *J* = 10.8, 5 Hz, H-3), 6.38 (*s*, H-14), 5.97 (*d*, *J* = 10.8 Hz, H-2), 5.57 (*dd*, *J* = 12, 4 Hz, H-12), 4.35 (*d*, *J* = 5 Hz, H-4), 2.35 (*dd*, *J* = 15, 4 Hz, H-11eq), 2.30 (*br s*, H-8), 2.25 (*m*, H-6eq), 2.24 (*br s*, H-10), 2.19 (*m*, H-7eq), 1.71 (*dd*, *J* = 15, 12 Hz, H-11ax), 1.65 (*dddd*, *J* = 14.2, 14, 8.2, 1.5 Hz, H-7ax), 1.30 (*s*, H-19), 1.08 (*dt*, *J* = 14, 4 Hz, H-6ax), 0.78 (*s*, H-20); ^{13}C NMR: δ202.5 (C-1), 171.7 (C-17), 143.8 (C-15), 143.4 (C-3), 139.7 (C-16), 129.0 (C-2), 125.1 (C-13), 108.4 (C-14), 70.6 (C-4), 64.5 (C-12), 50.6 (C-10), 49.2 (C-8), 43.6 (C-5), 40.8 (C-11), 36.1 (C-9), 31.9 (C-19), 29.7 (C-6), 25.6 (C-20), 19.1 (C-7).

Tinosporaside (**2**). ^{1}H NMR (400 MHz, DMSO-d_6): δ7.72 (*s*, H-16), 7.68 (*s*, H-15), 6.94 (*dd*, *J* = 10.8, 5.2 Hz, H-3), 6.65 (*s*, H-14), 5.91 (*d*, *J* = 10.8 Hz, H-2), 5.75 (*dd*, *J* = 12.2, 3.2 Hz, H-12), 5.25 (*d*, *J* = 6 Hz, OH), 5.02 (*d*, *J* = 6 Hz, OH), 4.97 (*d*, *J* = 6 Hz, OH), 4.58 (*t*, *J* = 6 Hz, H-2′), 4.38 (*d*, *J* = 5.2 Hz, H-4), 4.33 (*d*, *J* = 7.8 Hz, H-1′), 3.65 (*m*, H-4′), 3.45 (*br s*, OH), 3.12 (*m*, H-6′), 3.02 (*m*, H-3′), 2.96 (*m*, H-8, H-5′), 2.45 (*s*, H-10), 2.38 (*dd*, *J* = 15.0, 3.2 Hz, H-11eq), 2.11 (*br d*, *J* = 14 Hz, H-7eq), 1.99 (*br d*, *J* = 14 Hz, H-6eq), 1.85 (*dd*, *J* = 15.0, 12.2 Hz, H-11ax), 1.50 (*br t*, *J* = 14.0 Hz, H-7ax), 1.22 (*s*, Me), 0.88 (*br t*, *J* = 15 Hz, H-6ax), 0.70 (*s*, Me); ^{13}C NMR: δ202.7 (C-1), 170.0 (C-17), 145.4 (C-15), 143.9 (C-3), 140.3 (C-16), 127.2 (C-13), 125.4 (C-2), 109.0 (C-14), 104.6 (C-1′), 77.0 (C-3′), 76.5 (C-5′), 73.5 (C-2′), 72.4 (C-12), 70.0 (C-4), 69.8 (C-4′), 61.2 (C-6′), 47.9 (C-8), 47.3 (C-10), 42.7 (C-5), 40.1 (C-11), 35.4 (C-9), 31.1 (Me), 28.2 (C-6), 24.3 (Me), 18.8 (C-7).

Tinosporaside tetraacetate (**3**). Semi-synthetic, ^{1}H NMR (300 MHz, $CDCl_3$): δ7.57 (*s*, C-16), 7.48 (*s*, H-15), 6.75 (*dd*, *J* = 10.2, 5 Hz, H-3), 6.46 (*s*, H-14), 6.04 (*d*, *J* = 10.2 Hz, H-2), 5.52 (*dd*, *J* = 12.2, 3.6 Hz, H-12), 5.21 (*t*, *J* = 9.4 Hz, H-3′), 5.08 (*t*, *J* = 9.7 Hz, H-4′), 4.99 (*dd*, *J* = 9.4, 7.8 Hz, H-2′), 4.67 (*d*, *J* = 7.7 Hz, H-1′), 4.25 (*dd*, *J* = 12.4, 4.8 Hz, H-6′), 4.22 (*d*, *J* = 5.2 Hz, H-4), 4.18 (*dd*, *J* = 12.4, 2.8 Hz, H-6′), 3.67 (*m*, H-5′), 2.35 (*dd*, *J* = 15.0, 3.6 Hz, H-11eq), 2.30 (*m*, H-8), 2.25 (*m*, H-6eq), 2.22 (*br s*, H-10), 2.19 (*m*, H-7eq), 2.07, 2.04, 2.03, 2.02 (*s*, 4 × OCOMe), 1.88 (*dd*, *J* = 15.0, 12.2 Hz, H-11ax), 1.23 (*s*, Me), 1.08 (*dt*, *J* = 14, 4 Hz, H-6ax), 0.86 (*s*, Me).

Tinocordioside (**4**). Hygroscopic, $[\alpha]_D$ + 8.0° (MeOH; *c* 0.4); FAB-MS *m/z* (rel. int.): 517 $[M + K]^+$ (90), 501 $[M + Na]^+$ (90), 478 $[M]^+$ (50), 179 (30), 95 (20), 94 (22), 81 (30); IR ν_{max} cm^{-1}: 3560, 3140, 1705, 1675, 1510, 880; UV λ_{max} nm: 212.

Tinocordioside tetraacetate (**5**). Semisolid; $[\alpha]_D$ + 20.0° ($CHCl_3$; *c* 0.1); FAB-MS *m/z* (rel. int.): 685 $[M + K]^+$ (20), 669 $[M + Na]^+$ (80), 646 $[M]^+$ (20), 331 (100), 121 (60), 95 (60), 94 (30), 81 (20); IR ν_{max} cm^{-1}: 3140, 1725–1705, 1670, 1240, 880; UV λ_{max} nm: 218; ^{1}H NMR (400 MHz $CDCl_3$: δ7.49 (*s*, H-16), 7.44 (*s*, H-15), 6.60 (*m*, H-3), 6.48 (*m*, H-2), 6.44 (*s*, H-14), 5.42 (*dd*, *J* = 12.2, 3.6 Hz, H-12), 5.05–5.31 (*m*, H-2′, H-3′, H-4′), 4.95 (*d*, *J* = 7.7 Hz, H-1′), 4.30 (*dd*, *J* = 12.4, 4.8 Hz, H-6′a), 4.22 (*d*, *J* = 5.2 Hz, H-4), 4.16 (*dd*, *J* = 12.4, 2.8 Hz, H-6′b), 3.75 (*m*, H-5′), 2.60 (*m*, H-8), 2.38 (*m*, H-1a), 2.28 (*dd*, *J* = 15, 3.6 Hz, H-11eq), 2.20 (*m*, H-5, H-7eq), 2.15, 2.10, 2.01, 2.00 (*s*, 4 × OCOMe), 1.88 (*dd*, *J* = 15, 2.2 Hz, H-11ax), 1.59 (*m*, H-1, H-7ax), 1.30 (*m*, H-10), 1.15 (*m*, H-6ax), 1.26 (*s*, Me), 0.96 (*s*, Me); ^{13}C NMR: δ173.3 (C-17), 170.8, 170.3, 169.4, 168.7 (4 × OCOMe), 143.9 (C-15), 139.6 (C-16), 132.4 (C-2), 130.7 (C-3), 125.0 (C-13), 108.3 (C-14), 98.0 (C-1′), 72.9 (C-5′), 72.6 (C-3′), 72.3 (C-4), 71.8 (C-2′), 70.6 (C-12), 68.5 (C-4′), 62.0 (C-6′), 47.3 (C-8), 44.3 (C-10), 41.8 (C-11), 38.4 (C-5), 35.2 (C-9), 29.6 (C-6), 28.2 (Me), 26.1 (C-1), 24.0 (Me), 20.7, 20.7, 20.6, 20.6 (4 × OCOMe), 17.3 (C-7).

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