Tinocordiside, a New Rearranged Cadinane Sesquiterpene Glycoside from *Tinospora cordifolia*

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A rearranged cadinane sesquiterpene glycoside named tinocordiside (**1**), consisting of a tricyclic skeleton with a cyclobutane ring, has been isolated from the immunomodulatory aqueous fraction of the Indian medicinal plant *Tinospora cordifolia*. The structure has been established by spectroscopic and chemical methods.

The Indian medicinal plant *Tinospora cordifolia* Miers (Menispermaceae) is one of the most widely used plants in various traditional medicinal systems, including the Ayurvedic medicine, for the treatment of jaundice, rheumatism, urinary and skin diseases, diabetes, and anemia and for its antiallergic and antiinflammatory properties.[1,2] The aqueous extract of *T. cordifolia* has shown interesting immunomodulatory effects against diverse experimentally induced infections.[3] The earlier chemical investigations of the plant led to isolation of a number of structurally interesting clerodane furanoditerpenoids,[4] clerodane norditerpenoids and their glycosides,[5] phenolic lignans,[6] phenolic propane glycosides,[7] and steroids.[8] Keeping in view the high reputation and wide application of this plant in the indigenous system of medicine for treatment of various ailments, we have initiated an evaluation of natural products from *Tinospora cordifolia* for immunomodulatory activity. In this paper, we wish to report the isolation and structure elucidation of a novel and unusual tricyclic sesquiterpenoid glycoside named tinocordiside (**1**).

The aerial parts of *T. cordifolia* were sequentially extracted with CH_2Cl_2−MeOH (1:1) followed by H_2O, and the aqueous extract was subfractionated into *n*-BuOH- and H_2O-soluble fractions, respectively. The *n*-BuOH fraction on flash column chromatography and preparative reversed-phase HPLC afforded tinocordiside (**1**) and two other compounds. The UV spectrum of **1** indicated presence of extended carbonyl conjugation (208.0 and 260.0 nm). The IR spectrum showed absorption bands at 1730, 1705 (C=O, ketone), 1625 (C=C, olefin), 1310, and 1285 cm^{-1}, confirming an α,β unsaturated carbonyl group in the molecule. The electrospray mass spectrum (ESMS) showed the molecular ion peak for tinocordiside (**1**) at *m/z* 397.0 $(M + H)^+$ corresponding to the molecular formula $C_{21}H_{32}O_7$. Acetylation (Ac_2O/Py) of **1** led to a tetraacetyl derivative **2**, which indicated four hydroxyl groups in **1**. Under mild acidic conditions **1** was readily transformed into a H_2O-soluble white solid, indicating its labile nature and tendency towards acid-catalyzed rearrangement; however, the tetraacetyl derivative (**2**) was fairly stable. We have used various NMR (^1H, ^{13}C, DEPT, DQF−COSY, HMQC, HMBC, and NOESY), FTIR, UV, and ESMS techniques for structural assignments of **1** and **2**. The ^1H- and ^{13}C-NMR spectral data showed that **1** had one carbonyl group (δ_c 203) and four methyl groups (δ_H 0.88, 1.08, 1.15, 1.93 and δ_c 19.5, 22.0, 22.8, and 23.5 ppm, respectively). The most downfield methyl signal at δ_H 1.93 was assigned to the methyl attached to an olefinic double bond. The signals at δ_H 5.58, ddq, and δ_C 122.0 and 169.0 clearly showed the presence of a β-trisubstituted olefinic double bond attached to a carbonyl group that shifted the β-carbon atom further down-field at δ_c 169.0 due to extended conjugation and the diamagnetic anisotropic effect of the carbonyl group. The ^1H- and ^{13}C-NMR spectra indicated the presence of a β-glucopyranose unit by the characteristic anomeric proton appearing at δ_H 3.70 (d, $J = 10$ Hz) and δ_c 97.0 for **1** and δ_H 4.58 (d, $J = 8.2$ Hz) and δ_c 95.0 for **2**, respectively. All other protons of the sugar appeared in the normal positions of a β-D-glucopyranoside ring (Table 1). The ^1H-NMR spectra of both **1** and **2** showed no primary or secondary carbinolic protons, indicating that the sugar was linked to a tertiary hydroxyl of the aglycon. It is worth mentioning here that during our studies we found soft ionization positive-ion ESMS extremely useful for detection of molecular ions of these and other glycosides.

Acid hydrolysis of **2** led to the isolation of aglycon (**3**) as oily material, and a monosaccharide identified as glucose on the basis of TLC correlation and ^1H-NMR data. The β-D-glucopyranose conformation of the sugar was confirmed by ^1H chemical shifts and coupling constant of the anomeric proton and NOE difference measurements. ESMS of the aglycon **3** gave the molecular ion at *m/z* 235 for $[C_{15}H_{22}O_2 + H]^+$ and ^1H- and ^{13}C-NMR spectra clearly showed the presence of only one double bond in **3**. This combined MS and NMR indicated the presence of a tricyclic sesquiterpene skeleton in tinocordiside (**1**) and its aglycon **3**.

The ^1H-NMR assignments were confirmed by 2D DQF−COSY, and connectivities of protons with carbons were determined by inverse HMQC and long-range HMBC experiments. The DEPT experiment showed five methine, two methylene, four methyl- and three quaternary carbons for the aglycon of **1**. The two methine protons of **1** at δ_H 1.93 and 2.68 showed large $^4J_{HH}$ W coupling ($J = 6.6$ Hz) characteristics of *cis* protons located on opposite vertices of a cyclobutane ring,[9] and they were identified as H-5 and H-1. The H-5 also showed small $^4J_{H,H}$ coupling (0.9 Hz) and a cross peak with Me-14 at δ_H 2.01 in the $^1H-^1H$ correlation spectrum confirming the position and assignment for H-5, and H−14. The singlet at δ_H 2.77 did not show

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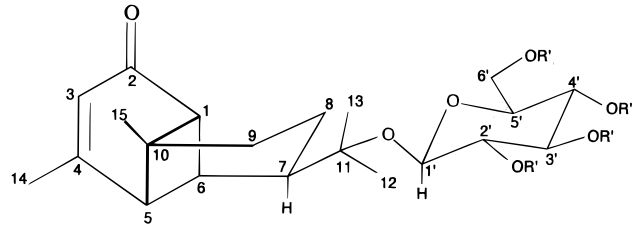
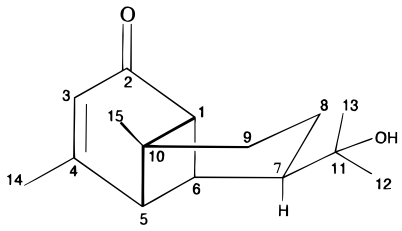
Table 1. ^{1}H- and ^{13}C-NMR Data (300 and 75 MHz) of Compounds **1**, **2,** and **3**

	1		2		3
position	δ_H[a] (J)	δ_C[a]	δ_H[b] (J)	δ_C[b]	δ_H[b] (J)
1	2.61 (dd, 6.7, 1.3)	57.0	2.68 (dd, 6.6, 1.3)	57.0	2.67 (dd,6.7, 1.3)
2		203.0		203.0	
3	5.58 (ddq, 0.9, 1.3, 1.4)	122.0	5.74, (ddq, 0.9, 1.2,1.4)	121.0	5.6 (ddq 0.9,1.3,1)
4		169.0		169.0	
5	1.84 (dd, 6.7, 0.9)	54.8	1.93 (dd, 6.6 , 0.9)	55.8	1.90 (dd, 6.7, 0.9)
6	2.95, s	53.5	2.77, s	53.5	2.85, s
7	1.73, m	48.5	1.68, m	48.5	1.70, m
8	1.23, m	21.0	1.20, m	21.3	1.20, m
9.	2.03, m	36.0	1.95, m	36.0	1.95,m
10		55.0		58.0	
11		80.0		81.3	3.40 (OH)
12	1.08,[c] s	19.5[c]	1.06,[c] s	20.0[c]	1.05,[c] s
13	0.88,[c] s	22.0[c]	0.95,[c] s	21.5[c]	0.91,[c] s
14	1.93, s	22.8[c]	2.01, s	22.0[c]	2.10, s
15	1.15,[c] s	23.5[c]	1.14,[c] s	24.5[c]	1.09,[c] s
1′	3.7 (d, 10)	97.0	4.58 (d, 8.2)	95.0	
2′	4.4 5(t, 5.3)	71.5	5.18 (t, 9.4)	72.0	
3′	3.03,[d] m	77.5	4.90 (t, 7.2)	73.5	
4′	3.53, m	74.5	5.08 (t, 10)	69.0	
5′	3.10,[d] m	76.5	3.64, m	72.0	
6′	3.20,[d] m	62.5	4.06 (dd, 12, 2.4)	62.5	
			4.18 (dd, 12.3, 5.9)		
Ac × 4			2.05−1.97		

[a] Measured in acetone-d_6 at 300 MHz. [b] Measured in $CDCl_3$ at 300 MHz. [c] These values are interchangeable in each column. [d] Overlapped signals.



1, R'= H
2, R'= Ac



3

significant coupling with H-1 or H-5, and this was assigned to H-6, placed at the third ring junction but on the opposite side of the cyclobutane ring. In an attempt to correlate the structure of **1** and its aglycon **3** with known tricyclic sesquiterpenoids, significant similarities were observed for **3** with the reported NMR and other spectral data for the sesquiterpene mustakone,[10−12] except that instead of the isopropyl at C-7 of mustakone, tinocordiside (**1**) contained an isopropyl alcohol moiety at the same position, and tertiary C_{11}−OH was a part of the glycosidic linkage. Absence of the carbon signal at δ_c 31.73 (assigned for C-11 of mustakone) in **1** and the appearance[13] of a quaternary carbon at δ_c 80.0 (HMBC and DEPT experiments) indicated presence of C_{11}−OH in aglycon **3** and C_{11}−O-glucopyranose in tinocordiside (**1**). The linkage of β-glucopyranose at tertiary C_{11}−OH position was also confirmed by HMBC spectrum of **2**, which showed that H-1′ (δ_H 4.58) was correlated to C-11 (δ_C 81.3) of the aglycon part of the molecule. Thus, tinocordiside, its tetraacetate, and its aglycon were assigned as **1, 2,** and **3**, respectively. These compounds represent the second example of this unusual class of rearranged cadinane having a cyclobutane ring; the first example of this class, mustakone, was reported[11,12] in 1963 from the essential oil of *Cyperus rodundus* and later[10] in 1988 from *Cyperus articulatus.*

Experimental Section

General Experimental Procedures. Flash chromatography was carried out over Si gel (200−300 mesh) and TLC over precoated Si gel plates (E. Merck). Preparative HPLC was done with Waters Maxima 820 systems and Rainin's Dynamax HPLC systems using C-18 bondapack reversed-phase columns (2.1 × 300 nm) and a linear solvent gradient of H_2O and CH_3CN. The UV and IR spectra were recorded on Shimadzu and Nicolet FT-IR 400D instruments, respectively. The ESMS were obtained from a Fisons VG Platform-II using CH_3CN-H_2O (50:50) as the mobile phase and nitrogen as nebulizer and drying gas, the probe temperature was set at 70 °C. The ^{1}H-, ^{13}C-, and 2D-NMR spectral data were recorded on a Bruker (DRX) 300 MHz instrument using TMS as internal standard, the HMQC and HMBC experiments were carried out using a high-sensitivity inverse-gradient NMR probe.

Extraction and Isolation of Tinocordiside (1). Plant Material. The *T. cordifolia* plant material was procured from the New Delhi market and authenticated by the pharmacognosy division of the Hamdard University, New Delhi, where a voucher specimen has been deposited.

The dried powdered stems of the plant (5 kg) were extracted with $MeOH-CH_2Cl_2$ (1:1, 5 × 10 L). The residue was extracted with H_2O (5 × 10 L). This aqueous extract was concentrated and partitioned into

n-BuOH- and H_2O-soluble fractions. Then the *n*-BuOH-soluble fraction was divided into six fractions by flash chromatography on Si gel (solvent gradient of 0% to 50% MeOH−$CHCl_3$). The fraction obtained with 20% MeOH−$CHCl_3$ as eluent afforded tinocordiside (**1**) as a mixture. Further purification of this compound was carried out by preparative HPLC on a Waters semiprep C-18 bondapack (2.1 × 300 mm) column with a solvent gradient of 100% H_2O to 65% H_2O−CH_3CN (with 0.1% TFA) by a run of 60 min at the 9 mL/min flow rate. Compound **1** was eluted from the column at 41 min. Pure **1** was obtained as a semisolid mass after removing the solvent by repeated vacuum distillation and freeze drying. Tinocordiside (**1**), (20 mg, 0.0004% on the basis of plant dry wt): UV (MeOH) λ_{max} (log ϵ), 208.0 (4.29), 260.0 (3.50) nm; IR (KBr, liquid film) ν_{max} 1730, 1705, 1625, 1310, 1285 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESMS (positive ion) *m/z* 397 $[M + H]^+$, 235 $[(M - C_6H_{11}O_5) + H]^+$, 217 $[(C_{15}H_{20}O + H)]^+$.

Preparation of Tinocordiside Tetraacetate (2). Tinocordiside (**1**) (15 mg) and Ac_2O (5 mL) in pyridine (0.7 mL) were left at room temperature for 16 h. The tetraacetate was collected as a semisolid mass and was further purified by passing through a Si gel column by elution with hexane−EtOAc (1:1). UV ($CHCl_3$) λ_{max} (log ϵ), 226 (3.25) nm; IR ($CHCl_3$ film) ν_{max} 1795, 1680, 1610, 1395 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESMS (positive ion) *m/z* 565 $[M + H]^+$, 587, $[M + Na]^+$ and 217 $[(C_{15}H_{20}O) + H]^+$.

Preparation of 3. A solution of tinocordiside tetraacetate (**2**) in $CHCl_3$ and 5% glacial HOAc was heated at 100 °C for 6 h, the usual workup by extraction with $CHCl_3$ led to isolation of the aglycon **3**. ^1H NMR, see Table 1; ESMS (positive ion) *m/z* 235.1 $[M + H]^+$, 216 $[C_{15}H_{20}O + H]^+$. The aqueous fraction was concentrated under reduced pressure, and TLC profile and ^1H-NMR data of the monosaccharide were compared with those of standard glucose.

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References and Notes

(1) *The Wealth of India, Raw Materials*; Publication & Information Directorate, Council of Scientific & Industrial Research: New Delhi, 1982; Vol. 10, pp 252−257.
(2) Rastogi, R. P.; Mehrotra, B. *The Compendium of Indian Medicinal Plants*; Publication & Information Directorate, Council of Scientific & Industrial Research: New Delhi, 1993; Vol. 2, pp 679−680.
(3) Thatte, U.; Chhabria, S.; Karandikar, S. M.; Dahanukar, S. *Indian Drugs*, **1987**, *25*, 95−97.
(4) Bhatt, R. K.; Sabata, B. K. *Phytochemistry* **1989**, *28*, 2419−2422.
(5) Gagan, V. D.; Pradhan, P.; Siphamalani, A. T.; Banerji, A. *Phytochemistry* **1994**, *37*, 781−786.
(6) Hanuman, J. B.; Mishra, A. K.; Sabata, B. K. *J. Chem. Soc., Perkin Trans I* **1986**, 1181−1185.
(7) Sipahimalani, A. T.; Norr, H.; Wagner, H. *Planta Med.* **1994**, *60*, 596−597.
(8) Pathak, A. K.; Agarwal, P. K.; Jain, D. C.; Sharma, R. P.; Howarth, O. W. *Indian. J. Chem.* **1995**, *34B*, 674−676.
(9) Silverstein, R. M.; Bassler, G. C.; Morril, T. C. *Spectrometric Identification of Organic Compounds*, 5th ed.; John Wiley & Sons: New York, 1991; p 198.
(10) Nyasse, B.; Ghogomu, T.; Sondengam, M. T.; Martin, M. T.; Bodo, B. *Phytochemistry* **1988**, *27*, 3319−3321.
(11) Kapadia, V. H.; Nagasampagi, B. A.; Naik, V. G.; Dev, S. *Tetrahedron* **1965**, *21*, 607−618.
(12) Kapadia, V. H.; Nagasampagi, B. A.; Naik. V. G.; Dev, S. *Tetrahedron Lett.* **1963**, *28*, 1933−1939.
(13) Yahara, S.; Higahasi, T.; Iwaki, K.; Nohara, T.; Marubayashi, N.; Ueda, I.; Khoda, H.; Goto, K.; Izumi, I.; Nuno, M.; Katsuki, S.; Isoda, S.; Satake, M. *Chem. Pharm. Bull.* **1989**, *37* , 2995−3000.

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