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Isolation and Characterisation of clerodane diterpenoids from the traditional medicinal plant -*Tinospora glabra (*Burm. f.*)* Merrill.

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ABSTRACT

Four clerodane diterpenoids , namely, tinosporide, 8-hydroxy tinosporide, columbin and columbin acetate have been isolated from the chloroform extract of the aerial parts of the traditional medicinal plant Tinospora glabra. furthertinosporide was acylated to form acetyl and diacetyltinosporide. The structures of the compounds were characterized by using Mass, Proton and 13C-NMR respectively.

Keywords: *Tinospora glabra*, Menispermaceae, clerodane diterpenoids , Medicinal plants

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INTRODUCTION

The family Menispermaceae is another rich source of *neo*-clerodane diterpenoids. *Tinospora* genus (Menispermaceae) consists of deciduous woody climbers, distributed throughout the tropics of Asia, Africa and Australia. *Tinospora glabra* widely occurs in India[1]. The aqueous extract of this plant is widely used in the traditional system for the treatment of jaundice, rheumatism, urinary diseases, intermittent fever, eye and liver ailments. It is known in Ayurveda for its adaptogenic and immuno-modulatory activity in fighting infections[2]. The aqueous and alcoholic extract of the plant significantly reduces the blood glucose; it also showed protective effects on CCl_4 induced hepatotoxicity[3]. Chronic diarrhoea and dysentery were reported to be controlled by the use of the starch obtained from the plant[4].

The bitterness of the stem of *Tinospora genus* is largely due to the *neo*-clerodane glycosides present in the plant[6]. As part of our work to obtain information on the phytochemistry of medicinal plants[5-9], examination of *Tinospora glabra* was taken up

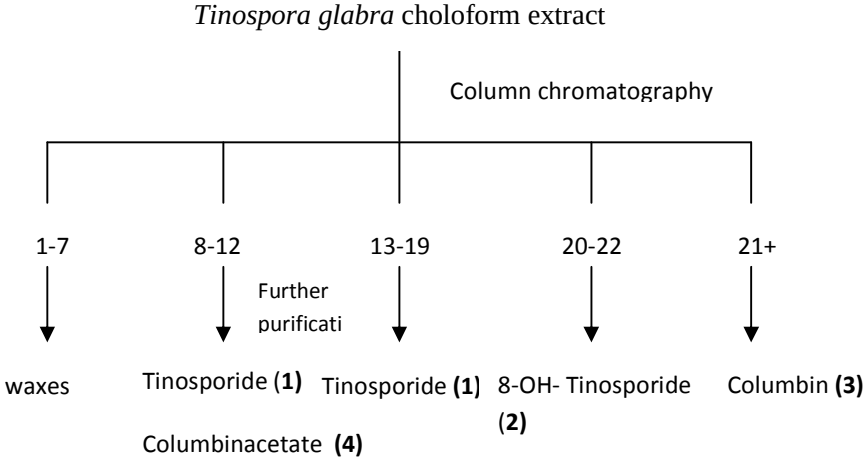
MATERIALS AND METHODS

General experimental procedure: All reagents were purchased from Sigma-Aldrich. TLC was monitored with silica gel-precoated aluminum sheets (Type 60 F254, Merck, Darmstadt, Germany) and the spots were visualized in the ultraviolet light chamber, Iodine chamber, 5% MeOH-H_2SO_4 mixture. Elemental analyses were carried out on an automatic Flash EA 1112 Series, CHN Analyzer (Perkin elmer, series II 2400). ^{1}H NMR and ^{13}C NMR spectra

were determined on a Bruker-300 NMR spectrometer and chemical shifts were expressed as part per million against TMS as internal reference. Mass spectra were recorded on Agilent 1200 (Liquid Chromatography), Agilent 6320 (Quadrupole Mass Analyzer) spectrophotometer.

Plant material:3kg of aerial part of *Tinospora glabra* was collected from tropical evergreen forests of Western Ghats, India. The plant was authenticated by Dr. Jayendran, Department of Bo\tany, Government Arts College, Ootacamund, India. A voucher specimen (JTG 1808) was deposited in Government Arts College, Ootacamund, India. The collected plant material was shade dried and coarsely powdered for extraction. Extracts were prepared by soaking plant material in Methanol successively at room temperature for 24 h and repeated thrice with the residue. The extract was filtered through Whatman No.1 filter paper and concentrated by usign Rotary evaporator (Buchi® Rotavap R-210).

Extraction and isolation:
Finely powdered *Tinospora glabra* aerial parts (3kg) were percolated with chloroform (10 L, three times) for 24 hrs. The resulting extract was then concentrated under vacuum to obtain a residue (100 g). The residue was chromatographed over a silica column (70-325 mesh) and eluted with $CHCl_3$/MeOH combinations [1-100%].The column elution was monitored by TLC and fractions were pooled based on similar TLC profiles. Initial Fractions eluted in chloroform alone yielded sterols and waxes. Fractions 8-12 (eluted with 2% MeOH in $CHCl_3$) afforded tinosporide**1** and columb in acetate**4**. Fractions 13-19 yielded **1** alone. Further elution with 5% MeOH in $CHCl_3$ yielded **2**. The Fractions 20-22 were chromatographed using BIOTAGE-FLASH MPLC and was eluted with toluene- ethyl acetate [4:1], to yield 8-OH tinosporide**2**and columbin**3**respectively.The compounds were carefully separated, evaporated to dryness and characterized for structural elucidation.



RESULTS AND DISCUSSION

Figure 1 clearly represents the structures of the molecules isolated from the aerial part of *T.glabra.* Those isolated compounds have been characterized by using ^{1}H and ^{13}C NMR which is discussed below.

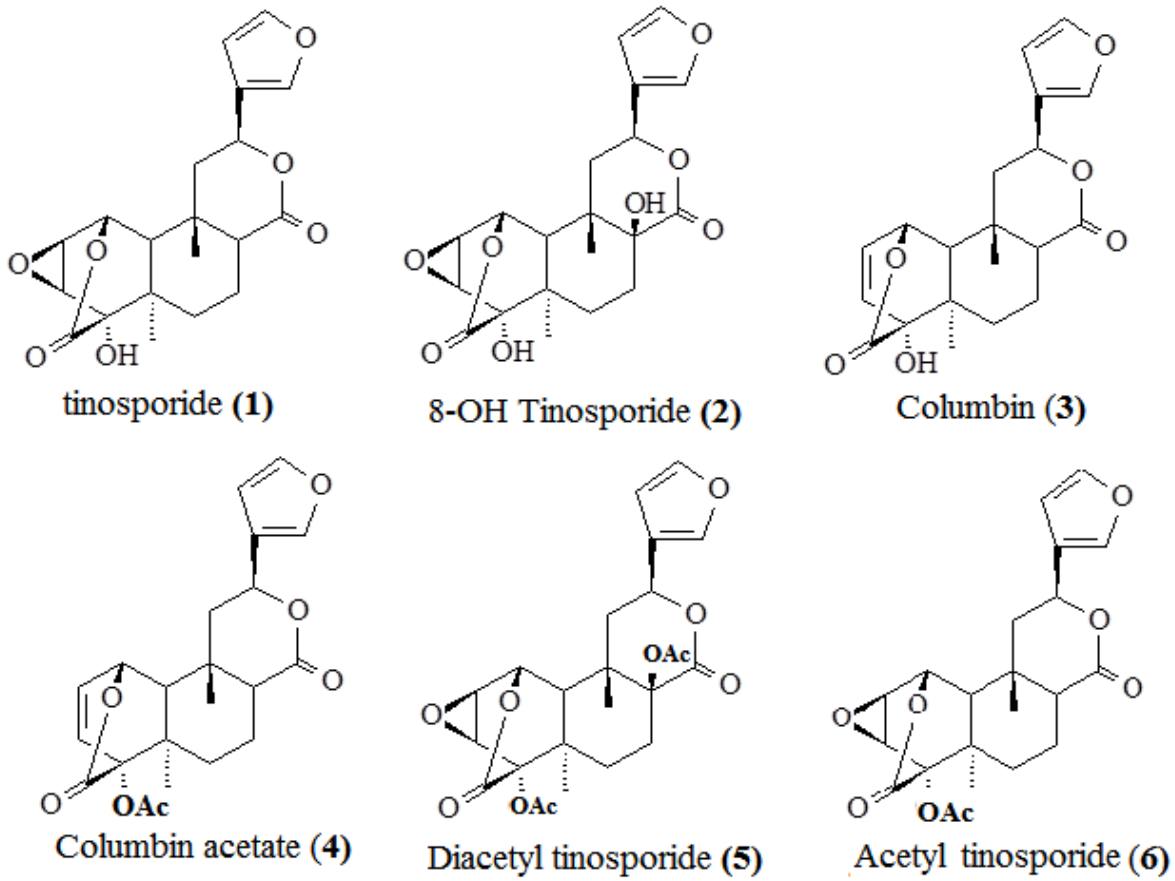


Figure 1. Structure of the compounds isolated from *Tinospora glabra*

Tinosporide (1):
Molecular formula: $C_{20}H_{22}O_7$ **M.p:** 240° C **Mass (m/z):** 374[M^+]; **IR (γ_{max}^{KBr} cm^{-1}):** 3500, 3045, 1745, 1500,870 **[α_D]** = -17° (DMSO, c-0.50); **^{1}H- NMR**-(δ,DMSO-d_6)**:** 4.99, d (J-2.8Hz;H1), 3.93, dd (J-2.8,4.2Hz;H2), 3.63,d (J-4.2Hz;H3), 2.40, m (H8), 1.98 br s (H10), 2.54,dd (J-14.5,3.8Hz;H11b), 5.67,dd (J-12.1,4Hz;H12), 6.45,br s (H14), 7.69,br s (H15), 7.74,br s (H16), 1.11,s (3H,H19), 1.04,s (3H,H20).**^{13}C- NMR**-(δ,DMSO-d_6)**:** 71.7(C1),50.2(C2), 51.8(C3), 81.6(C4), 34.9(C5), 26.3(C6), 17.2(C7), 43.7 (C8), 41.5 (C9), 45.8 (C10), 41.5(C11), 71.4(C12), 126.9(C13), 110.0(C14), 141.2(C15), 144.8(C16), 175.8(C17), 173.2(C18), 28.1(C19), 20.3(C20),

8-hydroxy tinosporide (III.2):
Molecular formula: $C_{20}H_{22}O_8$ **M.p:** 231° C **Mass (m/z):** 390[M^+]; **IR (γ_{max}^{KBr} cm^{-1}):** 3470,1771,1700,1504,881, 3150. **[α_D]** = +29.6° (DMSO, c=0.62) **^{1}H- NMR**-(δ,DMSO-d_6)**:** 5.16, d (J-1.9Hz;H1), 4.05, dd (J-4.1,1.9Hz;H2), 3.72,d (J-4.1Hz;H3), 1.95 br s (H10), 2.25 dd (J-14.5,3.8Hz;H11b), 5.77,dd (J-11.9,5.2Hz;H12), 6.55,br s (H14), 7.67,br s (H15), 7.71,br s (H16), 1.11,s (3H,H19), 1.06,s (3H,H20).**^{13}C- NMR**-(δ,DMSO-d_6)**:** 71.8(C1),51.1(C2), 53.0(C3), 82.2(C4), 40.5(C5), 28.5(C6), 28.0 (C7), 73.5 (C8), 40.5 (C9), 47.1 (C10), 36.5(C11), 72.4(C12), 127.3(C13), 110.9(C14), 141.9(C15), 145.8(C16), 174.8(C17), 173.4(C18), 24.0(C19), 22.0(C20),

Columbin(3):
Molecular formula: $C_{20}H_{22}O_6$ **M.p:** 235° C **Mass (m/z):** 358[M^+] **IR (γ_{max}^{KBr} cm^{-1}):** 3500, 3480, 1750, 1720, 3180, 1510, 1020, 880, 800, 3160, 1210. **[α_D]** = -5° (DMSO, c=0.60) **^{1}H- NMR**-(δ, DMSO-d_6)**:** 5.45, d (J-2.9Hz;H1), 6.55, dd (J-2.9, 8Hz;H2), 6.20,d (J-8Hz;H3), 5.70,dd (J-14.5,3.8Hz;H12), 6.64,br s (H14), 7.70,br s (H15), 7.74,br s (H16), 1.09, s (3H,H19), 0.85,s (3H,H20). **^{13}C- NMR**-(δ, DMSO-d_6)**:** 71.4(C1),51.1(C2), 51.6(C3), 86.4(C4),

35.4(C5), 26.6(C6), 17.2(C7), 44.2 (C8), 41.5 (C9), 46.6(C10), 41.5(C11), 71.9(C12), 126.9(C13), 109.6(C14), 140.7(C15), 144.5(C16), 174.5(C17), 171.0(C18), 28.2(C19), 23.4(C20).

Columbin Acetate (4)
Molecular formula: $C_{22}H_{24}O_7$**Mass (m/z):** 400[M^+] **IR (γ_{max}^{KBr} cm^{-1}):** 1750,1735,1504, 881. **^{1}H- NMR-**(δ, DMSO-d_6)**:** 5.45, d (J-2.9Hz;H1), 6.55, dd (J-2.9, 8Hz;H2), 6.20,d (J-8Hz;H3), 5.70,dd (J-14.5,3.8Hz;H12), 6.64,br s (H14), 7.70,br s (H15), 7.74,br s (H16), 1.09, s (3H,H19), 0.85,s (3H,H20), 2.11,s (3H,OCO$\underline{CH_3}$). **^{13}C- NMR-**(δ, DMSO-d_6)**:** 71.4(C1),51.1(C2), 51.6(C3), 86.4(C4), 35.4(C5), 26.6(C6), 17.2(C7), 44.2 (C8), 41.5 (C9), 46.6(C10), 41.5(C11), 71.9(C12), 126.9(C13), 109.6(C14), 140.7(C15), 144.5(C16), 174.5(C17), 171.0(C18), 28.2(C19), 23.4(C20),169.1 (O$\underline{C}$OMe),21.2 (OCO$\underline{C}H_3$)

Diacetyl tinosporide (5):
Molecular formula: $C_{24}H_{26}O_{10}$**Mass (m/z):** 474[M^+] **IR (γ_{max}^{KBr} cm^{-1}):** 3160, 1785, 1720, 1610, 1510, 1210, 1020, 950, 860 **^{1}H- NMR**(δ, DMSO-d_6)**:** 5.15, d (J-2.8Hz;H1), 5.21, dd (J-2.8,4.2Hz;H2), 5.15,d (J-4.2Hz;H3), 2.11 br s (H10), 2.33,dd (J-14.5,3.8Hz;H11b), 5.70,dd (J-14.5,3.8Hz;H12), 6.64,br s (H14), 7.70,br s (H15), 7.74,br s (H16), 1.11,s (3H,H19), 1.10,s (3H,H20), 2.14 and 2.11, each s (3H,OCO$\underline{Me}$). **^{13}C- NMR**(δ, DMSO-d_6)**:** 71.4(C1), 51.1(C2), 51.6(C3), 86.4(C4), 35.4(C5), 26.6(C6), 17.2(C7), 72.2 (C8), 41.5 (C9), 46.6(C10), 41.5(C11), 71.9(C12), 126.9(C13), 109.6(C14), 140.7(C15), 144.5(C16), 174.5(C17), 171.0(C18), 28.2(C19), 23.4(C20),169.1, 168.2 (O$\underline{C}$OMe**),** 21.1,20.9(OCO$\underline{Me}$).

Acetyl Tinosporide (6):
Molecular formula: $C_{22}H_{24}O_8$ **Mass (m/z):** 416[M^+] **IR (γ_{max}^{KBr} cm^{-1}):** 3160, 1785, 1720, 1610, 1510, 1210, 1020, 950, 860 **^{1}H- NMR-**(δ,DMSO-d_6)**:** 5.12, d (J-2.8Hz;H1), 5.12, dd (J-2.8,4.2Hz;H2), 5.12,d (J-4.2Hz;H3), 4.01, m (H8), 2.00 br s (H10), 2.33,dd (J-14.5,3.8Hz;H11b), 5.70,dd (J-14.5,3.8Hz;H12), 6.64,br s (H14), 7.70,br s (H15), 7.74,br s (H16), 1.11,s (3H,H19), 1.10,s (3H,H20), 2.14,s (3H,OCO$\underline{CH_3}$). **^{13}C- NMR-**(δ,DMSO-d_6)**:** 71.4(C1), 51.1(C2), 51.6(C3), 86.4(C4), 35.4(C5), 26.6(C6), 17.2(C7), 44.2 (C8), 41.5 (C9), 46.6(C10), 41.5(C11), 71.9(C12), 126.9(C13), 109.6(C14), 140.7(C15), 144.5(C16), 174.5(C17), 171.0(C18), 28.2(C19), 23.4(C20),168.2 (O$\underline{C}$OMe**),** 21.1(OCO$\underline{Me}$)

Compound **1**, was a white crystalline solid and had a molecular formula of $C_{20}H_{22}O_7$. The IR bands at 1745cm^{-1} showed the presence of a δ-lactone. Also bands for hydroxyl (3500 cm^{-1}) and epoxide (3045 cm^{-1}) were detected. The PMR spectrum of **1**displayed signals at δ 7.74 (1H, br s), δ 7.69 (1H, br s) and δ6.45(1H, br s) assignable to the protons of the β-substituted furan moiety. Two angular methyls were observed as singlets at δ1.04 and δ1.10. A D_2O exchangeable signal at δ6.29 was assigned to a tertiary hydroxyl group. Formation of a monoacetyl derivative **6**also supported this. The signals at δ5.67 (1H,dd, J=12.1,4Hz), was assigned to the C12 proton, bearing the β-substituted furan moiety. The signals at the aliphatic region δ2.52(1H,dd, J=14.3,12.2Hz), δ2.29(1H,dd, J=14.3,4Hz) were attributed to the C-11 methylene protons. By HOMOCOSY correlation profile, two multiplets observed at δ2.39 and 1.91 were assigned to protons at C-7. The downfield signal at δ2.39 was allocated to an axial proton on C-7, which experienced an anisotropic effect of the lactone carbonyl. The signals at δ4.99(1H,d, 4.8Hz), δ3.93(1H,dd, 4.3, 2.9) and δ3.63 (1H,dd, 4.3Hz) were given to protons on C-1, C-2 and C3 of ring A. On the basis of the coupling constants, the oxirane ring is assumed to adopt a β-orientation. From the ^{13}C-NMR and DEPT-135 experiments of **1**, six methyls, three methylenes, six methines, four furanoid carbons, three quarternary carbons and two lactone carbonyls were detected. It is interesting to note that the stereochemistry of A/B ring junction was deduced to be *cis* on the basis of the ^{13}C chemical shift values of the angular methyl at C-5 and literature reports. In case of *cis* clerodanes the C-19 methyl carbons[20] atom resonates above δ20 and in corresponding *trans* compounds, it resonated in the region δ11-19. Based on the above data **1** was found to be TINOSPORIDE. The physical and spectroscopic were in agreement with the literature.

Compound **2** also exhibited a similar proton and carbon NMR profile. On careful comparison of the NMR data **1** and**2**, it is clear that **2**was similar in all respects to **1**, with an exception that there was an additional tertiary hydroxyl group in **III.2**. The carbon bearing the tertiary hydroxyl group resonated at δ71.7 in the ^{13}C-NMR spectrum. From literature reports we could find that 6-OH and 8-OH substituted tinosporide have been isolated from *T.*cordifolia[10]. To confirm the substitution pattern, DEPT-135 experiments of **2** was performed and by comparison

with the carbon multiplicity, the compound **2** was found to be 8-HYDROXY TINOSPORIDE. Also HOMOCOSY and HETCOR experiments indisputably confirmed the above structure for **2**.

Compound **3** obtained from a chromatography using Biotage-Flash MPLC had a molecular formula $C_{20}H_{22}O_7$. It displayed bands pertinent to lactone (1710cm^{-1}), hydroxyl (3510cm^{-1}) moieties in the IR spectra. In the PMR of **3**, signals at δ7.68, 7.75 and 6.66 obtained were allocated to the β-substituted furan moiety. Also singlets accounting for three protons each at δ1.09 and 0.85 were duly assigned to the tertiary methyls. The resonance at δ5.71 was ascribed to the proton at C12 bearing the furan moiety. The C1 proton was obtained at δ5.30 as in the case of **1** and **2**. The above signals clearly established that **3** had a clerodane skeleton. The structural distinction between **1** and **3** was that an oxirane ring between C2 and C3 was absent. Absence of peaks at δ4.05 and δ3.72 as in the case of **1** augments the above argument.

Instead C2 and C3 protons were obtained at δ6.50 (dd,J-2.9,8Hz) and δ6.18(d,J-8Hz) respectively. Also in the ^{13}C-NMR resonances at 134.2 and 136.2 ppm were obtained. This established that an olefinic bond was present between C2 and C3. Thus **3**was found to be Columbin based on complete spectral and physical data.

Compounds 1 to 3 were subjected to acylation using pyridine and acetic anhydride. Afterthe usual work-up, the acetyl derivatives of each compound (**4- 6**) were obtained and they further corroborated the structures of the clerodane diterpenoids isolated from *Tinospora glabra*.

CONCLUSION

In conclude the present work, Four clerodane diterpenoids , namely, tinosporide, 8-hydroxy tinosporide, columbin and columbin acetate have been isolated from the chloroform extract of the aerial parts of the traditional medicinal plant *Tinospora glabra*. This kind of studies is useful in the future for the investigation of bioactive secondary metabolites present in the rare medicinal plants.

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