Crispene A, B, C and D, Four New Clerodane Type Furanoid Diterpenes from *Tinospora crispa* (L.)

Farhad Hossen, Rubaida Ahasan¹, Mohammad Rashedul Haque², Bilkis Begum, Choudhury Mahmood Hasan²

Departments of Clinical Pharmacy and Pharmacology, ¹Pharmaceutical Technology and ²Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

**ABSTRACT**

**Background:** *Tinospora crispa* (L.) is used to alleviate the symptoms of diabetes mellitus in folk medicine. It is also used for hypertension and to treat malaria, remedy for diarrhea, and as vermifuge.

**Materials and Methods:** Stems of *T. crispa* were collected, sun dried for several days followed by oven dried for 24 h at a considerably low temperature and then ground into coarse powder. The powdered stems were soaked in methanol at room temperature for 14 days with occasional shaking. The extract was collected by filtration, and the solvent was evaporated under reduced pressure in a rotary evaporator to obtain a solid residue which was then subjected to fractionation using the modified Kupchan partitioning method into n-hexane, CC1₅, CHCl₃ and aqueous soluble fractions. The n-hexane soluble fraction was chromatographed over sephadex (LH-20) and the column was eluted with n-hexane: CH₂Cl₂:MeOH (2:5:1) followed by CH₂Cl₂:MeOH (9:1) and MeOH (100%) in order to increase the polarities. The column fractions were then concentrated and subjected to thin layer chromatography screening and the fractions with a satisfactory resolution of compounds were rechromatographed over silica gel to isolate the pure compounds.

**Results:** Four new furanoid diterpenes of clerodane types, Crispene A, B, C, and D (1–4), including one known furanoid diterpene glucoside, borapetoside E (6), were isolated from the stems of *T. crispa*. The structures of these compounds were elucidated by means of extensive spectroscopic analysis and by comparison of their spectral data with closely related compounds.

**Conclusion:** We have reported four new furanoid diterpenes of clerodane types, including one known furanoid diterpene glucoside. This is the first report of any clerodane diterpene having olefinic bond between C-6 and C-7.

**Key words:** Clerodane, Crispene, furanoid diterpene, *Menispermaceae*, *Tinospora crispa*

**SUMMARY**

- Crispene A, B, C, and D, four new furanoid diterpenes of clerodane types from *Tinospora crispa*
- Crispene C, an unusual furanoid diterpene with olefinic bond between C-6 and C-7

**INTRODUCTION**

*Tinospora crispa* (L.) (Synonym: *Tinospora rumphii* Boerl., *Menispermum crispa* L.; Local name: Gulancha; family - *Menispermaceae*) is a woody climber with numerous protrusions on the stem and is native to Malesia, Indochina, Indian subcontinent, and China.¹,² In traditional medicine, it is used for hypertension, diabetes mellitus, to treat malaria, remedy for diarrhea, and as vermifuge.¹ Pharmacological studies on this plant also demonstrated its anti-inflammatory, antioxidant, antimalarial, antiprotozoal, and hypoglycemic activities.¹ Previous chemical investigation on *T. crispa* have reported the isolation of borapetol A and B, borapetoside A, B, C, E, and F, tinocrisposide, *N*-formylanondine, *N*-formynornuciferine, *N*-acetylnornuciferine, g-sitosterol, picrocitrin, tinotrubide, jatrohorzine, magnoflorine, palmatine, protoberberine, tembolarine, diasminet, cycloecualenol, cycloecualenone and other clerodane type furanoid diterpenes and their glycosides.³–⁴

This paper describes the phytochemical investigation of the stems of *T. crispa*, which resulted in the isolation and structural elucidation of four new furanoid diterpenes of clerodane types, Crispene A, B, C, and D (1–4), along with a previously isolated and reported furanoid diterpene glucoside, borapetoside E (5) [Figure 1].⁵,⁶

**Abbreviation used:** TLC: Thin layer chromatography, NMR: Nuclear magnetic resonance, COSY: Correlation spectroscopy, NOE: Nuclear overhauser effect, HPLC: High-performance liquid chromatography, ESI-MS: Electrospray ionization mass spectroscopy.

**Correspondence:**

Dr. Choudhury Mahmood Hasan,
Department of Pharmaceutical Chemistry,
Faculty of Pharmacy, University of Dhaka,
Dhaka-1000, Bangladesh.
E-mail: cmhasan@gmail.com
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MATERIALS AND METHODS

General experimental procedure

Column chromatography was performed on sephadex (LH-20). Optical rotations were measured on an ADP 220 polarimeter (Bellingham Stanley Ltd.,) and concentrations (c) are given in g/100 ml. 1H nuclear magnetic resonance (NMR) and 13C NMR spectra were acquired at 300 K using Bruker Advance NMR spectrometer at 400 MHz and 100 MHz respectively. Chemical shifts are reported relative to TMS using Bruker Advance NMR spectrometer at 400 MHz and 100 MHz respectively. Chemical shifts (δ H) are quoted in ppm referenced to CDCl3 residual chloroform signal (δ H).

Evaporation of solvent was performed using a Waters Micromass ZQ instrument coupled to a Waters 2695 Electrospray ionization mass spectroscopy (ESI-MS) data were collected using a Waters Micromass ZQ instrument coupled to a Waters 2695 high-performance liquid chromatography with a Waters 2996 photodiode array. Waters Micromass ZQ parameters used were capillary (kV), 3.38; cone (V), 35; extractor (V), 3.0; source temperature (°C), 100; desolvation temperature (°C), 200; cone flow rate (L/h), 50; and desolvation flow rate (L/h), 250.

Plant material

Stems of T. crispa were collected from the Tangail district of Bangladesh, in the month of March 2009. The plant was identified by Mr. Sardar Nasir Uddin, Senior Scientific officer, Bangladesh National Herbarium, Dhaka, where a voucher specimen has been deposited (DACB accession number: 35291). The stems were sun dried for several days followed by oven dried for 24 h at a considerably low temperature. The dried stems were then ground into coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Faculty of Pharmacy, University of Dhaka.

Extraction and isolation

The powdered stems (600 mg) were soaked in methanol (3 L) at room temperature for 14 days with occasional shaking and the extract was collected by filtration. The solvent was evaporated under reduced pressure in a rotary evaporator to obtain a solid residue (5 g) which was then subjected to fractionation using the modified Kupchan partitioning method into n-hexane, CHCl3, CH2Cl2 and aqueous soluble fractions. Evaporation of solvent afforded n-hexane (400 mg), CHCl3 (1.56 g), CH2Cl2 (140 mg), and aqueous soluble fractions. The n-hexane soluble fraction was chromatographed over sephadex (LH-20), and the column was eluted with n-hexane: CHCl3:MeOH (2:5:1) followed by CHCl3:MeOH (9:1) and MeOH (100%) in order to increase the polarities. The column fractions were then concentrated and subjected to thin layer chromatography (TLC) screening. The fractions with a satisfactory resolution of compounds were rechromatographed over silica gel separately to obtain the pure compounds 1 (1.4 mg), 2 (1.3 mg), 3 (1.7 mg), 4 (1.6 mg), and 5 (3.8 mg).

RESULTS AND DISCUSSION

The methanol extract of the dried stems of T. crispa was successively partitioned with n-hexane, carbon tetrachloride, and chloroform. The n-hexane fraction was then subjected to column chromatography using sephadex (LH-20) followed by TLC screening and preparative TLC to isolate five pure compounds (1–5) [Figure 1]. The NMR spectra of these compounds suggested that four of them were furanoid diterpenes of clerodane series and another was furanoid diterpene glucoside of the same series.

The 1H NMR spectra of 1–4, which are summarized in Tables 1 and 2, suggested some common structural features: The presence of an olefinic bond (C-3 and C-4), a furan ring (C-13–C-16), a carbonyl group (C-18), and two angular methyl groups (C-19 and C-20). The spectra also suggested the presence of two sp3-hybridized quaternary carbons (C-5 and C-9), two sp3-hybridized methines (C-8 and C-10) and an olefinic proton (C-3), which confirmed the absence of proton at C-4. The one proton broad signals at δ 6.72–7.01 and no cross connection between C-3 proton and any olefinic protons in correlation spectroscopy (COSY) experiment exclude the possibility of the double bond at C-1 and C-2.

Figure 1: Structures of compounds 1–5

Figure 2: Structure of Crispene C showing nuclear overhauser effect
The spectra of 1, 2, and 4 showed signals attributed to four sp3-hybridized methylenes (C-1, C-2, C-7, and C-11) and two oxygenated methine groups (C-6 and C-12). Whereas, the spectrum of 3 indicated three sp3-hybridized methylenes (C-1, C-2, and C-11) and one oxygenated methine group (C-12).

The ESI-MS of compound 1 showed a pseudo molecular ion [M + Na]+ peak at m/z 365.0 corresponding to the molecular formula C_{20}H_{22}O_{5} and compound 2 showed a pseudo molecular ion [M + H]+ peak at m/z 361.10 relevant to C_{19}H_{20}O_{5}. The 1H NMR spectra of 1 and 2 [Table 1] displayed one proton double doublet at δ 5.51 (J = 11.6, 4.8 Hz) and δ 5.33 (J = 12.0, 4.0 Hz), which were assigned to C-12. The difference in chemical shifts of C-12 (δ 5.51 and δ 5.33) may be due to the dissimilarity in the fusion of B/C rings (cis-fused in 1 and trans-fused in 2). [46] The downfield chemical shifts of the protons at C-12 were also indicative of the presence of a lactone ring at C-12 and C-17. Strong cross peaks were observed between C-12 proton with the C-11β and C-11α protons in the COSY spectra. The other proton doublet of doublets (dd) at δ 4.24 (J = 4.0, 3.6 Hz) in 1 and δ 4.28 (J = 12.6, 2.2 Hz) in 2 at C-8 were indicative of the presence of neighboring carbonyl group at C-17. The proton at C-6 of compound 1 showed a downfield dd at δ 4.62 (1H, J = 10.6, 6.6 Hz) which revealed the presence of another lactone ring between C-6 and C-18. The large coupling (10.6 Hz) is indicative of the presence of a free hydroxyl group (C-6). Again, the large coupling (10.6 Hz) confirmed that the oxymethine proton at C-6 is axial. Based on these results and from a

### Table 1: 1H NMR spectral data of compounds 1-5 (400 MHz, CDCl3) [46]

| Position | 1     | 2     | 3     | 4     | 5     |
|----------|-------|-------|-------|-------|-------|
| 1a       | 2.01 m* | 1.98-2.10 m* | 1.98-2.03 m* | 1.86-1.96 m* | 1.90 m* |
| 1b       | 2.01 m* | 1.98-2.10 m* | 1.98-2.03 m* | 1.86-1.96 m* | 1.90 m* |
| 2a       | 1.89 m* | 2.34-2.46 m* | 1.98-2.03 m* | 2.34 m | 2.36 m (15.2, 3.6) |
| 2b       | 1.89 m* | 2.34-2.46 m* | 1.98-2.03 m* | 2.34 m | 2.36 m (15.2, 3.6) |
| 3        | 6.89 dd (3.6, 3.6) | 6.88 dd (3.6, 3.6) | 6.72 | 6.72 | 6.72 |
| 4        | 4.62 dd (10.6, 6.6) | 4.46 dd (10.6, 7.4) | 6.38 dd (10.4) | 5.46 dd (6.0) | 5.45 dd (6.0) |
| 7a       | 1.52 m | 1.52 m | 6.56 dd (10.4) | 1.98 bd (12.4) | 1.95 bd (12.4) |
| 7b       | 2.80 add (14.2, 7.0, 3.6) | 2.58 add (14.2, 7.4, 2.4) | 2.09 br s | 2.17 dd (12.0, 6.0) | 2.13 dd (12.0, 6.0) |
| 8        | 2.42 dd (4.0, 3.6) | 2.48 dd (12.6, 2.2) | 2.09 br s | 2.63 dd (5.6) | 2.69 dd (5.6) |
| 9        | 2.00 bd (6.0) | 1.94 bd (6.8) | 2.54 dd (6.0, 2.0) | 1.86-1.96 m* | 1.90 m* |
| 10       | 4.61 br s | 4.62 br s | 6.41 br s | 6.51 t (0.8) | 6.46 d (1.2) |
| 11       | 7.43 br s | 7.42 br s | 7.43 br s | 7.36 t (1.6) | 7.38 dd (1.2, 1.6) |
| 12       | 7.46 br s | 7.47 br s | 7.46 br s | 7.41 d (0.8) | 7.48 br s |
| 13       | -     | -     | -     | -     | -     |
| 14       | -     | -     | -     | -     | -     |
| 15       | 1.25 s | 1.30 s | 1.25 s | 1.33 s | 1.31 s |
| 16       | 1.10 s | 0.98 s | 0.83 s | 1.27 s | 1.25 s |
| 17       | -     | -     | -     | -     | -     |
| CH3OCO−  | -     | -     | -     | -     | -     |
| AcO−     | -     | -     | -     | -     | -     |
| Glc 1′   | -     | -     | -     | -     | 4.29 d (8.0) |

*Signals overlapped in each column. NMR: Nuclear magnetic resonance

### Table 2: Comparison of 1H NMR spectral data of compounds 4 and 5 with the published data [9]

| Position | 4 (CDCl3 at 400 MHz) | Aglycone of borapetoside E (CDCl3 at 90 MHz)[9] | 5 (CDCl3 at 400 MHz) | Borapetoside E (CDCl3 at 90 MHz)[9] |
|----------|----------------------|-----------------------------------------------|----------------------|-----------------------------------|
| 1a       | 1.86-1.96 m          | -                                             | 1.90 m               | -                                 |
| 1b       | 1.86-1.96 m          | -                                             | 1.90 m               | -                                 |
| 2a       | 2.39 m               | -                                             | 2.36 m (15.2, 3.6)   | -                                 |
| 2b       | 2.29 dq (16.4, 4.4)  | -                                             | 2.29 dq (15.2, 5.0)  | -                                 |
| 3        | 7.01 dd (4.0, 4.0)   | 7.02 dd (3.6, 4.1)                            | 6.99 dd (4.0, 3.6)   | 7.01 dd (3.6, 4.1)               |
| 6        | 5.46 d (6.0)         | 5.46 d (6.0, 5.1)                             | 5.45 d (6.0)         | 5.46 d (5.1)                     |
| 7a       | 1.98 bd (12.4)       | -                                             | 1.95 bd (12.4)       | -                                 |
| 7b       | 2.17 dd (12.0, 6.0)  | -                                             | 2.13 dd (12.0, 6.0)  | -                                 |
| 8        | 2.63 d (5.6)         | 2.63 br.d (5.1)                               | 2.69 d (5.6)         | 2.71 br d (5.1)                  |
| 10       | 1.86-1.96 m          | 2.03 m                                        | 1.90 m               | 2.00 m                            |
| 11a      | 2.04 dd 15.2, 9.2    | -                                             | 2.19 dd (15.2, 3.6, 3.6) | -                 |
| 11b      | 1.78 bd (14.4)       | -                                             | 1.67 dd (15.2, 2.8)  | -                                 |
| 12       | 5.13 bd (8.4)        | 5.08 dd (3.1, 8.2)                            | 5.27 dd (9.6, 2.8)   | 5.24 dd (3.6, 8.2)               |
| 14       | 6.51 t (0.8)         | 6.51 m                                        | 6.46 d (1.2)         | 6.54 m                           |
| 15       | 7.36 t (1.6)         | 7.44-7.32 (2H, m)                             | 7.38 dd (1.2, 1.6)   | 7.36 m                           |
| 16       | 7.41 d (0.8)         | -                                             | 7.48 br s            | 7.52 m                           |
| 19       | 1.33 s               | 1.33 s                                        | 1.31 s               | 1.32 s                           |
| 20       | 1.27 s               | 1.28 s                                        | 1.25 s               | 1.27 s                           |
| CH3OCO−  | 3.73 s               | 3.72 s                                        | 3.73 s               | 3.73 s                           |
| Glc 1′   | -                    | -                                             | 4.29 d (8.0)         | 4.25 d (7.2)                     |

NMR: Nuclear magnetic resonance
The relative configuration at various centers in 1 and 2 were derived from extensive NMR model studies and comparing the published 1H NMR data of similar compounds. In the literatures, the structure of 1 and 2 were characterized as shown. Compound 1 and 2 are found to be new diterpenes and given the trivial names Crispene A and Crispene B, respectively.

In compound 1 and 2, the presence of two protons at C-8, 12, respectively, showed that these protons only couple with each other, hence this double bond must be at β orientation. Since both H-8 and C-20 methyl are in β orientation, B/C rings are cis-fused. The oxymethylene proton at C-12 appears as dd at δ 5.51 (J = 11.6, 4.8 Hz) and the large coupling (11.6 Hz) confirmed it to be axial (α). In compound 2, the H-8 proton appears as dd at δ 5.33 (J = 12.0, 4.0 Hz), the large coupling of 12.0 Hz indicative of axial orientation and α as the C ring in half-chair conformation.[14] In both 1 and 2, the relative deshielding of C-7 equatorial proton at δ 2.80 and 2.58, respectively, support earlier finding[14] that C (8)-C (17)-O-C (12) are on the same plane.

The 1H NMR spectrum of Crispene A (1) and 2 was elucidated as shown. To our knowledge, there is no record of a compound 5 except the data for glucose moiety at C-12. Compound 5 was characterized as borapetoside E by comparing the spectral data [Tables 1 and 2] with the published data of this compound.[9,10] This diterpene glucoside was first reported from *Tinospora tuberculata* and later on isolated and the structure was revised as 5.[10]

**Crispene A (1)**

Amorphous powder. 1H-NMR (CDCl3, 400 MHz): Table 1. ESI-MS (positive-ion mode) m/z: 365.0 [M + Na]+ (Calculated for C20H22O5Na: 365.40), 350, 329, 288, 255, 155, 151, 102, 100, 91, 89.

**Crispene B (2)**

Amorphous powder. 1H-NMR (CDCl3, 400 MHz): Table 1. ESI-MS (positive-ion mode) m/z: 361.10 [M + H]+ (Calculated for C19H21O5: 361.41), 289, 238, 214, 158, 141, 116, 101, 100, 90, 89.

**Crispene C (3)**

Amorphous powder. 1H-NMR (CDCl3, 400 MHz): Table 1. ESI-MS (positive-ion mode) m/z: 417.20 [M + H]+ (Calculated for C21H22O6: 417.48), 379, 358, 347, 288, 255, 198, 142, 123, 100, 91, 89, 84.

**Crispene D (4)**

Colorless gum. 1H-NMR (CDCl3, 400 MHz): Tables 1 and 2. ESI-MS (positive-ion mode) m/z: 375.20 [M + H]+ (Calculated for C21H22O6: 375.44), 357, 339, 289, 214, 158, 141, 130, 119, 102, 89.

**Borapetoside E (5)**

Amorphous powder. 1H-NMR (CDCl3, 400 MHz): Tables 1 and 2. 13C-NMR (CDCl3, 100 MHz) δ:16.60 (C-1, CH3), 24.17 (C-2, CH3), 142.37 (C-3, CH3), 134.30 (C-4, C), 39.30 (C-5, C), 82.87 (C-6, CH), 29.45 (C-7, CH), 46.55 (C-8, CH), 39.26 (C-9, C), 45.57 (C-10, CH), 46.62 (C-11, CH3), 69.0 (C-12, CH2), 125.86 (C-13, C), 108.93 (C-14, CH), 143.66 (C-15, CH), 140.20 (C-16, CH), 178.39 (C-17, C), 166.8 (C-18, CH), 27.15 (C-19, CH), 21.69 (C-20, CH3), 51.69 (CH2OOC-), 98.99 (C-1, CH), 73.86 (C-2, CH), 76.40 (C-3, CH), 70.73 (C-4, CH), 75.08 (C-5, CH), 62.55 (C-6, CH2).

**CONCLUSION**

We have isolated and characterized four new furanoid diterpenes of clerodane types, Crispene A, B, C and D (1–4), including one known furanoid diterpene glucoside, borapetoside E (5), from the stems of *T. crispa* (L.). To the best of our knowledge, there is no record of any other diterpene, like Crispene C (3), having olefinic bond between C-6 and C-7.

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Conflicts of interest
There are no conflicts of interest.

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ABOUT AUTHOR

Dr. Choudhury Mahmood Hasan, is a Professor of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Bangladesh. His research focuses on the chemical and biological studies of local medicinal plants with emphasis on structure elucidation of the secondary metabolites by spectroscopic techniques (UV, IR, NMR, MS etc.). He is the main/co-author of 232 peer-reviewed papers published in different international and local scientific journals. Dr. Hasan is a member of American Chemical Society (ACS), American Society of Pharmacognosy (ASP), Royal Society of Chemistry (RCS) and a fellow of Bangladesh Academy of Sciences (BAS).