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FULL PAPER CHEMISTRY & BIODIVERSITY

Three Undescribed Furanoditerpenoids from the *Tinospora crispa* that Inhibit NO Production

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Phytochemical study on the methanol extract of the stems of *Tinospora crispa* (L.) Hook.f. & Thomson led to the isolation of thirteen compounds including three undescribed *cis*-clerodane-type furanoditerpenoids (**1**–**3**) and ten known ones (**4**–**13**). Their chemical structures were determined by IR, HR-ESI-MS, 1D-, and 2D-NMR spectra. Compounds **2**–**4**, **6** and **8** inhibited moderately NO production in LPS activated RAW 264.7 cells with the IC_{50} values of 83.5, 57.6, 75.3, 78.1, and 74.7 μM, respectively.

Introduction

Tinospora crispa (L.) Hook.f. & Thomson (Menispermaceae) grows abundantly in the Northwest, growing wild everywhere in the mountains and plains of Vietnam.[1] According to the traditional medicine using, *T. crispa* has the effect of curing jaundice, rheumatism, urinary disorders, fever, malaria, diabetes, fracture, scabies and hypertension.[2-4] Modern scientific researches suggested that this plant has anti-inflammatory,[5-9] antidiabetic,[10-16] anticancer,[17] and antioxidant activities.[18-20] In addition, phytochemical analyses of *T. crispa* revealed the presence of diterpenes, diterpene glycosides, triterpenes, alkaloids, flavonoids, flavone glycosides, clerodane-type furanoditerpenoids, sterols, and lignans.[2-4] However, there have been no papers reported on the chemical constituents and bioactivities of *T. crispa* growing in Vietnam. In our screening NO inhibitory activity, the MeOH extract of the stems of this plant exhibited a significant inhibition and was selected for further study. This paper reported three undescribed *cis*-clerodane-type furanoditerpenoids and ten known compounds isolated from *T. crispa* and their NO production inhibition activity in LPS activated RAW 264.7 cells.

Results and Discussion

The MeOH extract of of *T. crispa* stems was isolated by using various chromatography methods to get three new and ten known compounds. The known compounds were identified to be borapetoside B (**4**),[21, 22] (5*R*,6S,9*S*,10*S*,12*S*)-15,16-epoxy-2-oxo-6-*O*-(*β*-D-glucopyranosyl)-cleroda-3,7,13(16),14-tetraen-17,12-olid-18-oic acid methyl ester (**5**),[23] tinosinenoside A (**6**),[24] rumphioside I (**7**),[23, 25] borapetoside H (**8**),[26] manglieside E (**9**),[27] (7*S*,8*R*,8'*R*)-4,4',9-trihydroxy-3,3',5,5'-tetramethoxy-7,9'-epoxylignan-7'-one-4-*O*-*β*-D-glucopuranoside (**10**),[28] (-)-*seco*isolariciresinol 4-*O*-*β*-D-glucopyranoside (**11**),[29] 8,8'-bisdihydrosiringenin glucoside (**12**),[30] acanthoside **B** (**13**).[31]

Compound **1** was isolated as a white amorphous powder. The IR spectrum of **1** revealed the presence of hydroxy (3416 cm^{-1}), carbonyl (1720 cm^{-1}), and ether (1080 and 1016 cm^{-1}) functionalities. The molecular formula of **1** was ditermined to be $C_{27}H_{34}O_{12}$ by the HR-ESI-MS [found *m/z* 551.2128 $[M+H]^+$, calcd. for $[C_{27}H_{35}O_{12}]^+$: 551.2123

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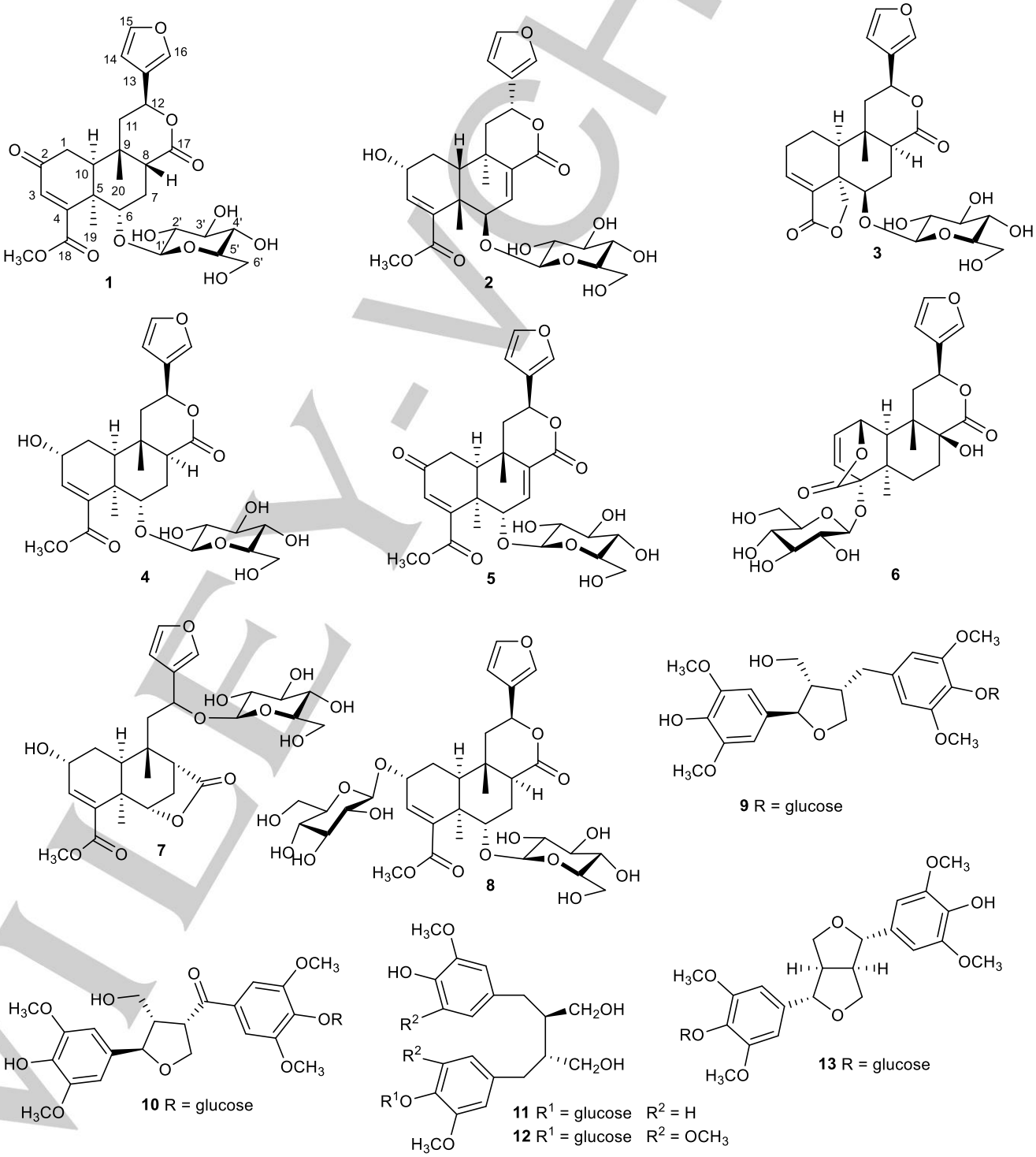

Figure 1. Chemical structures of compounds **1** – **13**

(Δ= -0.9 ppm)], and $[M+Na]^+$ at *m/z* 573.1949 (calcd. for $[C_{27}H_{34}O_{12}Na]^+$: 573.1942, Δ=+1.2 ppm), indicating eleven degrees of unsaturation. The ^{1}H-NMR spectrum of **1** showed the signals of two angular methyl groups [δ_H 1.69 (3H, s, H-19) and 1.01 (3H, s, H-20)], four olefinic protons [δ_H 6.45 (s, H-3), 7.63 (t, *J* = 0.6 Hz, H-16), 7.52 (dd, *J* = 1.8, 0.6 Hz, H-15), and 6.57 (dd, *J* = 1.8, 0.6 Hz, H-14), two methine carbinol groups at δ_H 5.81 (dd, *J* = 12.0, 2.4 Hz, H-12), and 4.46 (d, *J* = 4.8 Hz, H-6)], a methoxy group (δ_H 3.88, s), and one anomeric proton (δ_H 4.46, d, *J* = 7.8 Hz). The ^{13}C NMR and HSQC spectra of **1** indicated 27 carbons including 6 of one glucose, one methoxy, and 20 of a clerodane-type furanoditerpenoid aglycone, a main substance class of *T. crispa*.[2-4, 23, 25] One ketone (δ_C 200.9, C-2), two carboxylate carbons (δ_C 176.4 (C-17) and 168.1 (C-18), one glucose (δ_C 105.9, 75.4, 77.9, 70.7, 77.4, and 62.3), three double bonds

(134.2/155.8, 109.6/144.8, and 126.8/141.3) were identified. The NMR assignments of **1** were revealed with the aid of COSY, HSQC, and HMBC spectra in comparison with the published data (Table 1),[23, 25] which were similar to those of 15,16-epoxy-2-oxo-6-*O*-(*β*-D-glucopyranosyl)-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester. However, the carbon chemical shifts at C-6, C-8, C-9, C-20 were changed from 79.6, 49.8, 38.5, and 23.3 to 78.0, 45.5, 36.4, and 27.9 (in **1**), respectively, as well as H-6 proton was changed from 4.39 (br d, 2.6 Hz) to 4.46 (d, 4.8 Hz) (in **1**) (measured in the same solvent, CD_3OD). The above evident suggested *β/equatorial* for H-6 and *β/equatorial* for H-8.[23, 25] These were further confirmed by NOESY cross peaks of H-20/H-8 and H-10/H-1′ (glc) as shown in Figure 3. In addition, NOESY crosspeak of H-10 and H-19 was clearly observed indicating *cis*-A/B ring.[23] Furthermore, The HMBC correlations from H_3-19 to C-4/C-5/C-6/C-10, from H-10 to C-1/C-4/C-5, from H_3-20 to C-8/C-9/C-10/C-11, from H-12 to C-11/C-14/C-16, and from H-7 and H-8 to C-17 further indicated the planar structure of **1** as shown in Figure 1. In addition, HMBC correlations from methoxy protons (δ_H 3.88) to C-18 (δ_C 168.1), and from anomeric proton (δ_H 4.46) to C-6 (δ_C 78.0) confirmed methoxy was at C-18 and glucose moiety linked to C-6 by an ether linkage. The above analyses established the conformation of the A−B−C ring to be in a twisted boat−chair−chair conformation as shown in Figure 3.[23, 25] Absolute configuration of **1** was proposed to be 5*R*, 6*S*, 8*R*, 9*S*, 10*S*, and 12*S* by comparison its ECD spectrum with that of tinopanoid S, which shared the same clerodane-diterpene backbone (Supporting information). The large $^3J_{1',2'}$ value of the anomeric proton at δ_H 4.46 suggested *β*-form of the glycosidic linkage. Compound **1** gave D-glucose with the acid hydrolysis test, identified by comparison with authentic samples via TLC, and from the positive sign of the optical rotations.[32, 33] From the above evidence, the chemical structure of compound **1** was determined as shown in Figure 1, an undescribed compound and named as tinocrioside A.

Table 1. ^{13}C NMR spectral data for compounds **1** - **3** in CD_3OD

No.	**1**		**2**		**3**	
	$^a\delta_C$	$^b\delta_H$ (**mult.**, ***J* in Hz**)	$^a\delta_C$	$^b\delta_H$ (**mult.**, ***J* in Hz**)	$^a\delta_C$	$^b\delta_H$ (**mult.**, ***J* in Hz**)
1	36.4	2.65 (brd, 16.8) 2.94 (dd, 16.8, 6.6)	32.3	1.47-1.52 (m) 2.21-2.25 (m)	20.0	1.72-1.77 (m) 1.96-1.99 (m)
2	200.9	-	67.4	4.33 (m)	26.7	2.38-2.43* 2.60 (ddd, 14.4, 5.4, 5.4)
3	134.2	6.45 (s)	141.0	6.36 (d, 0.6)	138.4	6.95 (t, 3.0)
4	155.8	-	139.8	-	133.4	-
5	43.4	-	44.0	-	47.5	-
6	78.0	4.46 (d, 4.8)	81.4	4.70 (d, 2.4)	83.1	4.09 (dd, 12.0, 4.8)
7	29.9	2.84 (ddd, 13.8, 4.8, 1.8) 1.83 (dd, 13.8, 6.0)	140.9	6.98 (d, 2.4)	29.9	2.40* 2.20-2.27 (m)
8	45.5	2.43 (dd, 6.0, 1.8)	136.9	-	46.5	2.80 (dd, 13.2, 3.6)
9	36.4	-	38.1	-	36.2	-
10	40.6	2.96*	48.7	2.04 (dd, 12.0, 3.0)	48.9	1.78*
11	42.5	2.24 (dd, 15.0, 2.4) 1.71 (dd, 15.0, 12.0)	46.2	2.09 (dd, 14.4, 11.4) 2.37 (dd, 14.4, 3.6)	37.3	2.10 (dd, 14.4, 8.4) 1.87 (dd, 14.4, 7.8)
12	71.3	5.81 (dd, 12.0, 2.4)	72.5	5.29 (dd, 11.4, 3.6)	72.2	5.83 (dd, 8.4, 7.8)
13	126.8	-	125.3	-	127.8	-
14	109.6	6.57 (dd, 1.8, 0.6)	109.8	6.56 (d, 1.8)	109.6	6.54 (s)
15	144.8	7.52 (dd, 1.8, 0.6)	145.0	7.53 (t, 1.8)	145.3	7.54 (s)
16	141.3	7.63 (t, 0.6)	141.6	7.64 (t, 1.8)	141.1	7.61 (s)
17	176.4	-	171.1	-	175.3	-
18	168.1	-	172.3	-	173.8	-
19	27.5	1.69 (s)	23.2	1.36 (s)	75.7	5.07 (d, 9.0) 4.29 (d, 9.0)
20	27.9	1.01 (s)	28.4	1.18 (s)	28.9	1.11 (s)
6-*O*-Glucose			**6-*O*-Glucose**		**6-*O*-Glucose**	
1′	105.9	4.46 (d, 7.8)	105.7	4.44 (d, 7.8)	105.4	4.29 (d, 7.8)
2′	75.4	3.22 (dd, 9.0, 7.8)	75.5	3.24 (dd, 9.0, 7.8)	75.4	3.16 (dd, 9.0, 7.8)
3′	77.9	3.35 (t, 9.0)	77.7	3.40 (t, 9.0)	78.0	3.27 (t, 9.0)
4′	70.7	3.42 (t, 9.0)	71.3	3.38 (t, 9.0)	71.4	3.26 (t, 9.0)
5′	77.4	3.25-3.27 (m)	78.2	3.30-3.32 (m)	77.9	3.30-3.32 (m)
6′	62.3	3.75 (dd, 12.0, 4.2) 3.85 (dd, 12.0, 2.4)	62.5	3.74 (dd, 12.0, 5.4) 3.89 (dd, 12.0, 1.8)	62.8	3.65 (dd, 12.0, 6.0) 3.87 (dd, 12.0, 1.8)
OCH_3	53.2	3.88 (s)	52.8	3.87 (s)		

[*] Overlapped signals, [a] Recorded in 150 MHz, [b] Recorded in 600 MHz

Compound **2** was isolated as a white amorphous powder. Its IR spectrum revealed the presence of hydroxy (3420 cm^{-1}), carbonyl (1722 cm^{-1}), and ether (1074, 1024 cm^{-1}) functionalities. The molecular formula of **2** was $C_{27}H_{34}O_{12}$ as determined from the HR-ESI-MS [found m/z 551.2152 $[M+H]^+$, calcd. for $[C_{27}H_{35}O_{12}]^+$: 551.2123 (Δ= +5,0 ppm)] and $[M+Na]^+$ at m/z 573.1955 (calcd. for $[C_{27}H_{34}O_{12}Na]^+$: 573.1943, Δ=+2,0 ppm), indicating eleven degrees of unsaturation. The ^1H and ^{13}C NMR spectra (Table 1) displayed singlets for the two methyl groups (C-19 and C-20), a methoxycarbonyl, four double bonds (two of the furan ring), and a glucose moiety, protoncharacteristic of a furanoditerpene bearing a sugar unit.[23, 25] The NMR spectrum data of compound **2** was relatively similar to compound **1** (Table 1), except the ketone goup in 1 (δ_C 200.9) was replaced by a hydroxy group at C-2 (δ_C 67.4), and the additional signals of one double bond (δ_C140.9/δ_H 6.98 and δ_C 136.9). The NMR data of **2** were assigned based on HSQC, COSY, and HMBC spectra (Figure 2). In the HMBC spectrum, H_3-19 (δ_H 1.36) correlated to C-4 (δ_C 139.8)/C-5 (δ_C 44.0)/C-6 (δ_C 81.7)/C-10 (δ_C 48.6), H_3-20 (1.18) correlated to C-4/C-9 (δ_C 38.1)/C-8 (δ_C 136.9), H-3 (δ_H 6.36) correlated to C-1 (δ_C 32.3)/C-4 (δ_C 139.8)/C-5 (δ_C 44.0)/C-18 (172.3), and methoxy protons (δ_H 3.78) to C-18, and H-1′ (δ_H 4.44) correlated to C-6 (δ_C 81.4). In addition, COSY cross peaks of H-1/H-2/H-3 and H-6/H-7 were observed. These evidence indicated a hydroxy at C-2, the double bonds C-3/C-4 and C-7/C-8, methoxy at C-18, and glucose unit attached to C-6 (Figure 2). The NMR data of **2** were closely similar to those of (2*R*,5*R*,6*S*,9*S*,10*S*,12*S*)-15,16-epoxy-2-hydroxy-6-*O*-(*β*-D-glucopyranosyl)-cleroda-3,7,13(16),14-tetraen-17,12-olid-18-oic acid methyl ester, a furanoditerpenoids isolated from *T. crispa*.[23] However, the carbon chemical shifts at C-2 was shifted downfield from δ_C 64.0 to 67.4 (measured in the same solvent, CD_3OD) suggesting *β*-oriented for the hydroxyl group at C-2.[23] The relative configuration of **2** was revealed on the basis of NOESY interactions. In the NOESY spectrum, the cross peaks between H-2 and H-10, H-10 and H_3-19, and H-6 and H_3-20 (Figure 3) suggesting *cis*-A/B ring, and H-2 and H-6 were in *α*- and *β*-orintations, respectively (Figure 3).[23, 25] Absolute configuration of **2** was proposed to be 2*R*, 5*S*, 6*R*, 9*R*, 10*R*, and 12*R* by comparison its ECD spectrum with that of tinopanoid R, which shared the same clerodane-diterpene backbone (Supporting information). Furthermore, the coupling constants ($^3J_{1',2'}$ = 7.8 Hz) observed for the anomeric protons at δ_H 4.44 in the ^1H NMR spectrum suggested the *β*-glycoside linkage. Acid hydrolysis of **2** gave D-glucose, identified by comparison with authentic samples via TLC, and from the positive sign of the optical rotations.[32, 33] Thus, chemical structure of compound **2** was determined as shown in Figure 1, an undescribed compound and named as tinocrioside B.

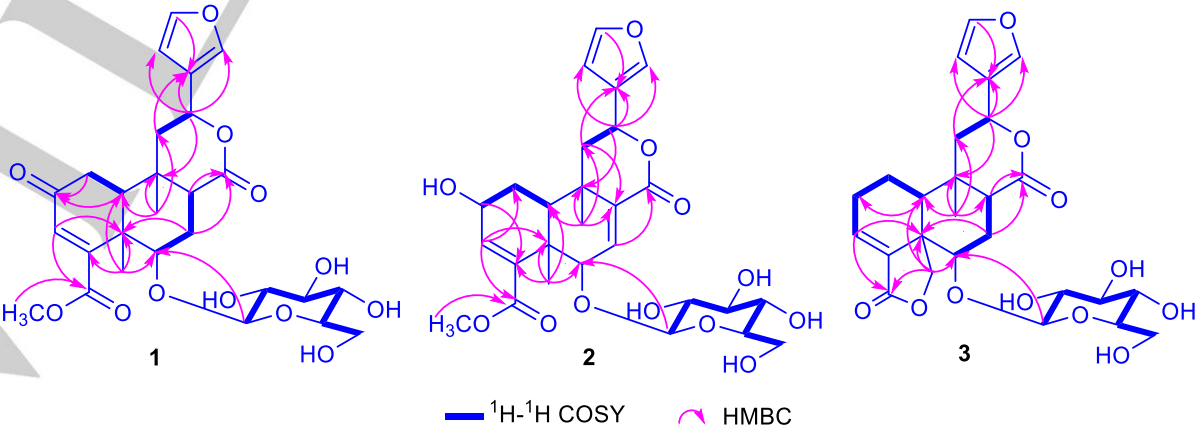

Figure 2. The key HMBC and ^1H-^1H COSY correlations of compounds **1** – **3**

Compound **3** was isolated as a white amorphous powder. The IR spectrum of **3** suggested the presence of hydroxy (3416 cm^{-1}), carbonyl (1737 cm^{-1}), and ether (1075, 1024 cm^{-1}) functionalities. The HR-ESI-MS of **3** exhibited the quasi molecular ion peaks at *m/z* 521.2026 $[M+H]^+$ (calcd. for $[C_{26}H_{33}O_{11}]^+$: 521.2017) and *m/z* 538.2268 $[M+NH_4]^+$, calcd. For $[C_{26}H_{36}NO_{11}]^+$: 538.2283 determining the molecular formula of $C_{26}H_{32}O_{11}$ with ten degree of unsaturation. The NMR data of **3** were closely resembling those of **1** except for the absence of the ketone and methoxy groups at C-2 and C-18, respectively, and the C-19 methyl group was replaced by an oxygenated methylene group (δ_C 75.7/ δ δ_H 5.07 and 4.29, each 1H, d, *J* = 9.0 Hz). The two carboxylate groups (δ_C 173.8 and 175.3), one furan

ring (δ_C 127.8, 109.6, 145.3, 141.1), and one glucose sugar (δ_C 105.4, 75.4, 78.0, 71.4, 77.9, and 62.8) were identified (Table 1). The HMBC correlations from H_2-19 (δ_H 5.07 and 4.29) to C-4 (δ_C 133.4)/C-5 (δ_C 47.5)/C-6 (δ_C 83.1)/C-18 (δ_C 173.8), and from H-3 (δ_H 6.95) to C-4/C-5/C-18 suggested $\Delta^{3,4}$-double bond and an ester linkage was fromed between C-18 and C-19. In addition, the glucose moiety linked to C-6 by an ether linkage as determined by HMBC correlation from H-1′ (δ_H 4.29) to C-6 (δ_C 83.1). Proton H-8 appeared at δ_H 2.80 with a large coupling constant ($^3J_{7,8}$ = 13.2 Hz) indicating *anpha/axial* orientation for H-8. The NOESY cross peaks of C-10 and H-19 was observed suggesting *cis*-A/B ring.[23, 25] In addition, the large $^3J_{6,7}$ value (12.0 Hz) of the H-6 proton at δ_H 4.09 suggested *anpha/axial* orientation for H-6. These were further confirmed by NOESY spectrum as shown in Figure 3. The ECD spectrum of **3** showed a negative Cotton effect at 250 nm and a positive Cotton effect at 216 nm, corresponding to the n→π and π→π electronic transitions of the α,β-unsaturated γ-lactone ring and hence indicated for *R* absolute configuration at C-5 (Supporting information). Thus, absolute configuration of **3** was proposed to be 5*R*, 6*R*, 8*S*, 9*S*, 10*S*, and 12*S*. The large $^3J_{1',2'}$ value of the anomeric proton at δ_H 4.29 suggested β-from of the glycosidic linkage. Acid hydrolysis of **3** gave D-glucose, identified by comparison with authentic samples via TLC, and from the positive sign of the optical rotations.[32, 33] Thus, chemical structure of compound **3** was determined as shown in Figure 1, an undescribed compound and named as tinocrioside C.

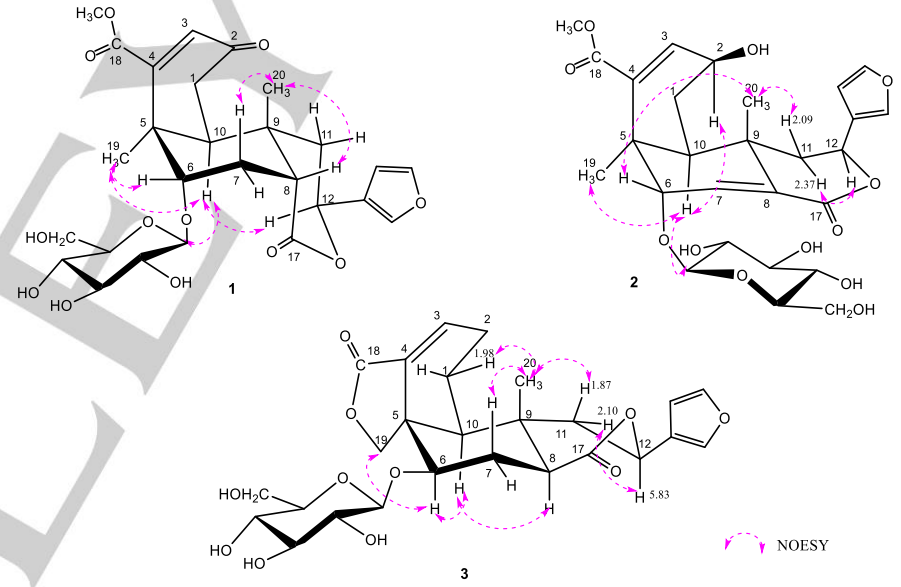

Figure 3. The key NOESY correlations of compounds **1** - **3**

Table 2. NO inhibitory effects in LPS-activated RAW 264.7 cells of the isolated compounds

Compounds	NO inhibition (IC_{50}, μM)
2	83.5±1.5
3	57.6±1.4
4	75.3±2.4
6	78.1±1.3
7	>100
8	74.7±1.8
9	>100
10	>100
11	>100
12	>100
Dexamethasone*	13.1±1.3

[*]positive control compound

Compounds **1**-**12** were selected to evaluate NO production inhibitory activity in LPS stimulated RAW 264.7 cells. Except compounds **1** and **5** (which showed cytotocxic activity, Table S1), the remainning compounds were further screened for their NO production effects in LPS stimulated RAW 264.7 cells. As shown in Table 2, compounds **2**-**6** and **8** showed moderately effects with IC_{50} value ranging from 57.6 to 83.5 μM, compared to that of the positive control compound, dexamethasone, which showed IC_{50} value of 13.1 μM. Compounds **7**, **8**-**12** were inactive with IC_{50} value over 100 μM. There is no relationship between chemical structure and biological activity in these results.

Conclusions

Three undescribed *cis*-clerodane-type furanoditerpenoids (**1**-**3**) and ten known compounds (**4**-**13**) were isolated from the methanol extract

of the stems of *Tinospora crispa*. Their chemical structures were elucidated by IR, HR-ESI-MS, 1D- and 2D NMR spectra in comparison with the reported data. Compounds **2**-**4**, **6** and **8** inhibited moderately NO production in LPS activated RAW 264.7 cell with the IC50 values of 83.5, 57.6, 75.3, 78.1, and 74.7 μM, respectively, while the remainning compounds were inactive IC_{50} value over 100 μM.

Experimental Section

General

The optical rotations were measured on a Jasco P2000 polarimeter. The infrared spectra (IR) were recorded on a Spectrum Two FT-IR spectrometer. The high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was acquired on an Agilent 6530 Accurate Mass Q-TOF LC/MS. The NMR spectra were recorded on a Bruker 600 MHz spectrometer. Semi-preparative high-performance liquid chromatography (HPLC) were run on an Agilent 1260 system including binary pump, autosampler, DAD detector, and semi-preparative HPLC column YMC J'sphere ODS-H80 (4 μm, 20 × 250 mm). Isocratic mobile phase with the flow rate of 2.5 mL/min was used in Semi-prep-HPLC. The compound was monitored at wavelengths of 205, 230, 254, and 280 nm. Flash column chromatography was performed using silica gel, reversed phase C-18, and diaion HP-20 resins as stationary phase. Thin layer chromatography was carried out on pre-coated silica gel 60 F_{254} and RP-18 F_{254S} plates. The spots were detected by spraying with aqueous solution of H_2SO_4 5%, followed by heating with a heat gun. The RAW 264.7 cells were kindly received from Perugia University, Italy.

Plant material

The stems of *Tinospora crispa* were collected in Me Linh, Vinh Phuc, Vietnam, in September 2022 and identified by Dr Nguyen The Cuong, Institute of Institute of Ecology and Biological Resources. A voucher specimen (NCCT-P107) was deposited at the Institute of Marine Biochemistry, VAST.

Extraction and isolation

The dried stems of *T. crispa* (3.5 kg) were minced and ultrasonic extracted with MeOH to obtain the MeOH extract (TC1, 154 g). This was suspended in water and then partitioned with EtOAc to get EtOAc extract (TC2, 43 g) and water layer (TC3). The water layer was isolated on a Diaion HP20 eluting with MeOH/H_2O (25%, 50%, 75%, and 100% MeOH) to get four fractions, TCWA-TCWD. Fractions TCWB and TCWC were combined (TCW3, 36 g) and isolated on a silica gel column eluting CH_2Cl_2/MeOH/H_2O (3/1/0.1) to get two fractions, TCW3A-TCW2B. Fraction TCW3A (7.5 g) was chromatographed on an YMC R18 column eluting with MeOH/H_2O (1/3) to get three fractions, TCW3A1-TCW3A3. Fraction TCW3A1 (850 mg) was isolated on the HPLC eluting with 45% MeOH to give compounds **9** (5.2 mg, t_R 28.39 min). Fraction TCW3A2 (428 mg) was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (10/1) to get two fractions, TCW3A2A and TCW3A2B. Fraction TCW3A2A (125 mg) was isolated on the HPLC eluting with 25% ACN to give compound **4** (10.6 mg, t_R 32.35 min). Fraction TCW3A2B (79 mg) was purified on the HPLC eluting with 28% ACN to give compound **3** (9.5 mg, t_R 38.0 min). Fraction TCW3A3 was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (10/1) to get two fraction TCW3A3A and TCW3A3B. Fraction TCW3A3A (52.0 mg) was purified on the HPLC eluting with 20% ACN to give compounds **11** (5.5 mg, t_R 32.10 min) and **10** (6.5 mg, t_R 38.10 min). Fraction TCW3A3B (120.3 mg) was purified on the HPLC eluting with 25% ACN to give compound **2** (10.5 mg, t_R 85.15 min). Fraction TCW3B (8.8 g) was isolated on an YMC R18 column eluting with methanol/water (1/3) to get four fractions, TCW3B1-TCW3B4. Fraction TCW3B1 (850 mg) was purified on the HPLC eluting with 30% ACN to give compounds **1** (5.1 mg, t_R 40.08 min) and **5** (5.0 mg, t_R 45.10 min). Fraction TCW3B2 (482 mg) was isolated on the HPLC eluting with 27% MeOH to give compound **12** (5.2 mg, t_R 43.95 min). Fraction TCW3B3 (615 mg) was isolated on the HPLC eluting with 25% ACN to give compound **13** (5.2 mg, t_R 24.70 min). Fraction TCW3B4 (256 mg) was chromatographed on a silica gel column eluting with CH_2Cl_2/CH_3OH/H_2O (5/1/0.1) to get three fractions, TCW3B4A, TCW3B4B and TCW3B4C. Fraction TCW3B4A (65 mg) was isolated on the HPLC eluting with 25% ACN to give compound **7** (5.2 mg, t_R 50.58 min). Fraction TCW3B4B (112 mg) was isolated on the HPLC eluting with 18% ACN to give compound **8** (5.5 mg, t_R 35.05 min). Fraction TCW3B4C (85 mg) was isolated on the HPLC eluting with 25% ACN to give compound **6** (6.5 mg, t_R 38.93 min).

Tinocrioside A (**1**)

A white amorphous powder; $[\alpha]_D^{25}$: +56.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (nm) 210. IR (KBr) ν_{max} (cm^{-1}): 3416, 2918, 2850, 1720, 1674, 1080, 1016; ECD (MeOH) $\theta_{(\lambda\ nm)}$: $-14.3_{(251)}$, $-1.1_{(373)}$ mdeg; HR-ESI-MS *m/z* 551.2128 $[M+H]^+$, calcd. for $[C_{27}H_{35}O_{12}]^+$: 551.2123 (Δ=+0.9 ppm); *m/z* 573.1949 $[M+Na]^+$, calcd. for $[C_{27}H_{34}O_{12}Na]^+$: 573.1942 (Δ=+1.2 ppm); *m/z* 585.1743 $[M+^{35}Cl]^-$, calcd. for $[C_{27}H_{34}O_{12}{}^{35}Cl]^-$: 585.1744 (Δ=-0.2 ppm), *m/z* 587.1738 $[M+^{37}Cl]^-$, calcd. for $[C_{27}H_{34}O_{12}{}^{37}Cl]^-$: 587.1714 (Δ=+4.1 ppm), ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are shown in the Table 1 (Figures S1-S8).

Tinocrioside B (**2**)

A white amorphous powder; $[\alpha]_D^{25}$: +48.0 (*c* 0.1, MeOH); UV (MeOH)

λ_{max} (nm) 210. IR (KBr) ν_{max} (cm^{-1}): 3420, 2918, 2850, 1722, 1074, 1024; ECD (MeOH) $\theta_{(\lambda\ nm)}$: −8.0$_{(226)}$ mdeg; HR-ESI-MS *m/z* 551.2152 $[M+H]^+$, calcd. for $[C_{27}H_{35}O_{12}]^+$: 551.2123 (Δ=+5.0 ppm); *m/z* 573.1955 $[M+Na]^+$, calcd. for $[C_{27}H_{34}O_{12}Na]^+$: 573.1942 (Δ=+2.0 ppm); *m/z* 568.2394 $[M+NH_4]^+$, calcd. for $[C_{27}H_{38}O_{12}N]^+$: 568.2389 (Δ=+1.0 ppm); *m/z* 585.1720 $[M+^{35}Cl]^-$, calcd. for $[C_{27}H_{34}O_{12}{}^{35}Cl]^-$: 585.1744 (Δ=-0.9 ppm), *m/z* 587.1733 $[M+^{37}Cl]^-$, calcd. for $[C_{27}H_{34}O_{12}{}^{37}Cl]^-$: 587.1714 (Δ=+2.4 ppm), ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are shown in the Table 1 (Figures S9-S16).

Tinocrioside C (**3**)

A white amorphous powder; $[\alpha]_D^{25}$: +24.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (nm) 210. IR (KBr) ν_{max} (cm^{-1}): 3416, 2918, 2851, 1722, 1075, 1024; ECD (MeOH) $\theta_{(\lambda\ nm)}$: +55.4$_{(216)}$, −10.0$_{(250)}$ mdeg; HR-ESI-MS *m/z* 521.2026 $[M+H]^+$, calcd. for $[C_{26}H_{33}O_{11}]^+$: 521.2017 (Δ=+1.7 ppm); *m/z* 538.2268 $[M+NH_4]^+$, calcd. for $[C_{26}H_{36}NO_{11}]^+$: 538.2283 (Δ=-2.8 ppm); *m/z* 543.1854 $[M+Na]^+$, calcd. for $[C_{26}H_{32}O_{11}Na]^+$: 543.1837 (Δ=-2.8 ppm); *m/z* 555.1622 $[M+^{35}Cl]^-$, calcd. for $[C_{26}H_{32}O_{11}{}^{35}Cl]^-$: 555.1639 (Δ=-3.4 ppm), *m/z* 519.1867 $[M-H]^-$, calcd. for $[C_{26}H_{31}O_{11}]^-$: 519.1872 (Δ=-1.0 ppm), ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are shown in the Table 1 (Figures S17-S25).

*Acid hydrolysis of compounds **1**-**3***

Acid hydrolysis of compounds **1**-**3** were the same as described in previous work[32, 33] referred to Supplementary information.

Nitric oxide assay

The NO assay protocol is the same as described in previous papers [34-36] referred to Supplementary information.

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/....

Additional references cited within the Supporting Information.

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Author Contribution Statement

Kiem PV, Tai BH, Yen PH, Giang LD designed experiments, elucidated chemical structures and wrote the paper. Quoc NV, Hoang NH, Hang DTT, Huong PTT, Bang NA, Dung DT, Trang DT extracted and isolated compounds and prepared sample for bioassay.

Keywords

Tinospora crispa, Menispermaceae, furanoditerpenoid, tinocrioside, NO inhibitory activity.

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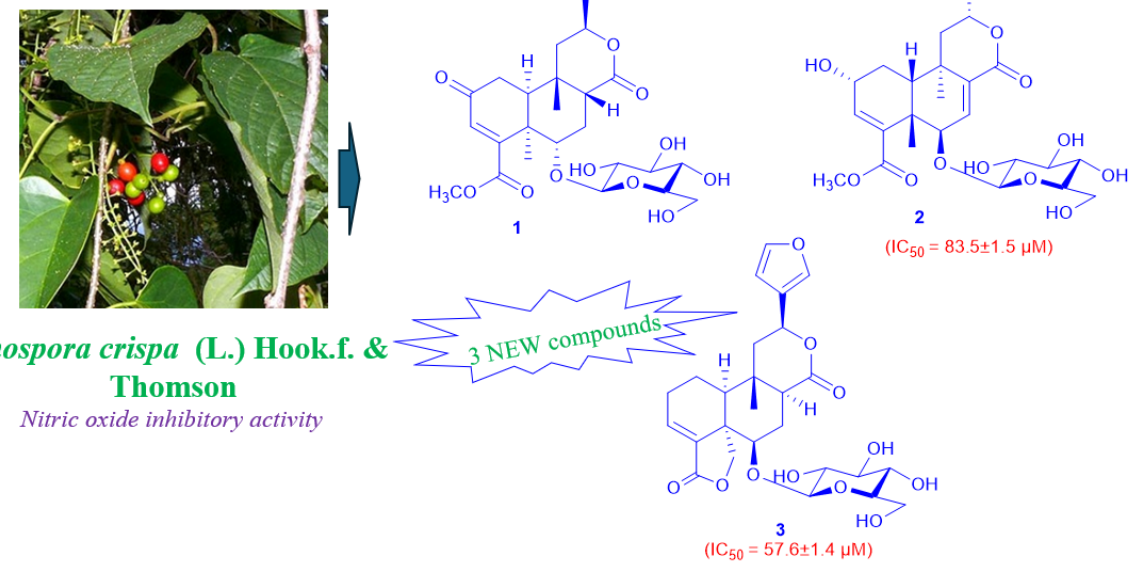

Twitter Text

- Isolation and determination of three undescribed *cis*-clerodane-type furanoditerpenoids and ten known compounds from the stems of *Tinospora crispa* and evaluation their NO production inhibitory activity in LPS-stimulated RAW264.7 macrophages.
- *Tinospora crispa*, Menispermaceae, furanoditerpenoid, tinocrioside, NO inhibitory activity.
- Corresponding author's name: Phan Van Kiem, and Twitter account: PVKiem_IMBC.