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RESEARCH ARTICLE

A new *cis*-clerodane-type furanoditerpenoid from the leaves of *Tinospora crispa* with inhibitory effect on LPS induced NO production in RAW 264.7 cells

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Abstract

Three *cis*-clerodane-type furanoditerpenoids (**1**-**3**) including one new compound named tinocrioside D (**1**) together with four known compounds (**4**-**7**) were isolated from the leaves of *Tinospora crispa*. Their chemical structures were determined based on the 1D, 2D-NMR, HR-ESI-MS, and ECD data analyses in comparison with the reported data. Compounds **1**–**3** showed weak inhibition of LPS-induced NO production in mouse mononuclear macrophages.

KEYWORDS
furanoditerpenoid, Menispermaceae, NO inhibitory activity, tinocrioside D, *Tinospora crispa*

1 | INTRODUCTION

Tinospora crispa belongs to family Menispermaceae, which is distributed in the mountainous areas of Vietnam.[1] The leaves and stems of this plant were used in folk medicine to treat rheumatism, jaundice, fever, urinary disorders, malaria, fracture, diabetes, scabies and hypertension.[2–4] The previous studies shown that *T. crispa* has anti-inflammatory,[5–9] antidiabetic,[10–14,5,15] anticancer,[16] and antioxidant activities.[17–19] Phytochemical studies resulted diterpenes, diterpene glycosides, triterpenes, flavonoids, alkaloids, flavone glycosides, clerodane-type furanoditerpenoids, sterols, and lignans have been isolated from the aerial parts of this plant.[2–4] This paper reports one new and six known compounds isolated from the leaves of *T. crispa* and their inhibition of LPS-induced NO production in mouse mononuclear macrophages.

2 | MATERIALS AND METHODS

2.1 | Plant materials

The leaves of *T. crispa* (L.) Hook.f. and Thomson (collected in Me Linh, Vinh Phuc, Vietnam, in September 2022) were identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources. A voucher specimen (NCCT-P107L) was deposited at the Institute of Marine Biochemistry, VAST.

2.2 | General experimental procedures

The used equipment for this study are described in the supporting information.

TABLE 1 NMR data for compounds **1**-**3** in CD_3OD.

No.	1		2		3	
	δ_C[a]	δ_H[b,c]	δ_C[a]	δ_H[b,c]	δ_C[a]	δ_H[b,c]
1	29.0	2.28 (dd, 15.0, 7.8) 2.03 (m)	30.9	1.95 (m) 2.07 (m)	26.6	2.28 (m)
2	64.8	4.53 (m)	64.0	4.39 (m)	73.0	4.70 (m)
3	141.7	6.63 (d, 3.6)	138.5	6.44 (d, 3.6)	137.8	6.45 (d, 3.6)
4	139.6	–	141.5	–	143.0	–
5	42.0	–	43.5	–	36.7	–
6	78.3	4.57 (d, 4.8)	81.1	4.56 (brd 1.8)	30.3	1.78 2.29
7	29.7	1.75 (dd, 13.8, 6.0) 2.71 (ddd, 13.8, 4.8, 1.8)	140.9	6.98 (d, 2.4)	27.1	1.65 1.80
8	46.5	2.35 (dd, 6.0, 1.8)	137.0	–	75.8	–
9	35.8	–	38.2	–	41.2	–
10	42.0	2.58 (d, 6.0)	46.5	2.10 (m)	49.6	1.05 (s)
11	43.1	1.67 (dd, 15.2, 12.0) 2.22 (dd, 15.2, 1.2)	45.1	2.43 (dd, 14.4, 3.0) 2.10 (dd, 14.4, 11.4)	42.8	2.12 (dd, 13.2, 8.4) 2.00 (dd, 13.2, 8.4)
12	71.3	5.80 (dd, 12.0, 1.8)	72.4	5.28 (dd, 11.4, 3.0)	72.4	6.08 (t, 8.4)
13	126.8	–	125.4	–	127.9	–
14	109.7	6.56 (d, 1.8)	109.7	6.57 (s)	109.8	6.51 (1.2, 0.6)
15	144.7	7.52 (dd, 1.8, 1.2)	145.0	7.54 (s)	145.1	7.52 (t, 1.2)
16	141.2	7.63 (d, 1.2)	141.6	7.64 (s)	141.1	7.60 (t, 0.6)
17	177.1	–	171.2	–	175.4	–
18	169.1	–	172.0	–	170.2	–
19	28.7	1.58 (s)	23.9	1.48 (s)	34.1	1.43 (s)
20	29.5	1.11 (s)	27.5	1.15 (s)	24.8	1.05 (s)
6-*O*-Glucose			**6-*O*-Glucose**		**2-*O*-Glucose**	
1′	105.7	4.44 (d, 7.8)	105.7	4.45 (d, 7.8)	103.6	4.48 (d, 7.8)
2′	75.3	3.20 (dd, 9.0, 7.8)	75.6	3.27 (dd, 9.0, 7.8)	75.1	3.18 (dd, 9.0, 7.8)
3′	77.9	3.34 (t, 9.0)	77.8	3.40 (t, 9.0)	78.0	3.37 (t, 9.0)
4′	70.5	3.43 (t, 9.0)	71.3	3.39 (t, 9.0)	71.6	3.30 (t, 9.0)
5′	77.1	3.24 (m)	78.1	3.30 (m)	77.9	3.30 (m)
6′	62.2	3.82 (dd, 12.0, 1.8) 3.73 (dd, 12.0, 5.4)	62.5	3.89 (dd, 12.0, 1.8) 3.74 (dd, 12.0, 5.4)	62.8	3.90 (dd, 12.0, 1.8) 3.68 (dd, 12.0, 5.4)
OCH_3	52.4	3.37 (s)	52.9	3.80 (s)	52.2	3.77 (s)

[a]150 MHz.
[b]600 MHz.
[c](mult., *J* in Hz).

2.3 | Extraction and isolation

The detailed extraction and isolation of the isolated compounds were given in the Supporting Information.

2.3.1 | Tinocrioside D (1)

A white amorphous powder; mp 236–238 C; $[\alpha]_D^{25}$: +67.4 (*c* = 0.1, MeOH); UV (MeOH) λ_{max} (nm) 260. IR (KBr) ν_{max} (cm^{-1}): 3415, 2948, 1716, 1506, 1073, 1024; ECD (MeOH) $\theta_{(\lambda\ nm)}$: $+27.0_{(220)}$, $-15.0_{(250)}$ mdeg; HR-ESI-MS *m/z* 553.2286 $[M+H]^+$, calcd. for $[C_{27}H_{37}O_{12}]^+$: 553.2280 (Δ = +1.1 ppm); *m/z* 575.2096 $[M + Na]^+$, calcd. for $[C_{27}H_{36}O_{12}Na]^+$: 575.2099 (Δ = −0.4 ppm); *m/z* 570.2561 $[M+NH_4]^+$, calcd. for $[C_{27}H_{40}O_{12}N]^+$: 570.2545 (Δ = +2.8 ppm). ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are shown in Table 1 (Figures S1–S10).

2.3.2 | (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 (2)

A white amorphous powder; mp 232–234 °C; $[\alpha]_D^{25}$: +15.2 (*c* = 0.1, MeOH). ECD (MeOH) $\theta_{(\lambda\ nm)}$: $+8.1_{(220)}$, $-5.9_{(252)}$

mdeg; HR-ESI-MS *m/z* 585.1728 $[M+^{35}Cl]^-$, calcd. for $[C_{27}H_{34}O_{12}{}^{35}Cl]^-$: 585.1744, Δ = −2.7 ppm. ^1H NMR and ^{13}C NMR data are shown in Table 1.

2.3.3 | (2*R*,5*R*,6*R*,8*R*,9*S*,10*S*,12*S*)-Tinospinoside B (3)

A white amorphous powder; mp 231–233 °C; $[\alpha]_D^{25}$: +26.4 (*c* = 0.1, MeOH. ECD (MeOH) $\theta_{(\lambda\ nm)}$: $+53.0_{(218)}$, $-11.0_{(250)}$ mdeg; HR-ESI-MS *m/z* 575.2105 $[M+Na]^+$, calcd. for $[C_{27}H_{36}O_{12}Na]^+$: 575.2099 (Δ = +1.0 ppm); *m/z* 570.2544 $[M+NH_4]^+$, calcd. for $[C_{27}H_{40}O_{12}N]^+$: 570.2545 (Δ = −0.2 ppm), ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are shown in Table 1.

2.3.4 | Citrusin A (4)

A colorless powder; mp 224–226 °C; $[\alpha]_D^{25}$: +57.8 (*c* = 0.07, MeOH). HR-ESI-MS *m/z* 561.1956 $[M+Na]^+$, calcd. for $[C_{27}H_{34}O_{12}Na]^+$: 561.1942 (Δ = +1.1 ppm); *m/z* 556.2396 $[M+NH_4]^+$, calcd. for $[C_{27}H_{40}O_{12}N]^+$: 556.2389 (Δ = +1.2 ppm), ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are shown in Table 2.

2.3.5 | 4,7,9,3′,9′-Pentahydroxy-3,5′-dimethoxy-8-4′-oxyneolign-7′-ene-3′-*O*-β-D-glucopyranoside (5)

A colorless powder; mp 224–226 °C; $[\alpha]_D^{25}$: −11.8 (*c* = 0.09, MeOH). ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data are shown in Table 2.

2.3.6 | *N-trans*-Caffeoyltyramine (*trans-N*-caffeoyltyramine) (6)

A gray solid; mp 217–219 °C; HR-ESI-MS *m/z* 300.1230 $[M+H]^+$, calcd. for $[C_{17}H_{18}NO_4]^+$: 300.1230 (Δ = 0 ppm); *m/z* 322.1040 $[M+Na]^+$, calcd. for $[C_{17}H_{17}NO_4Na]^+$: 322.1050 (Δ = −0.3 ppm); *m/z* 298.1081 $[M-H]^-$, calcd. for $[C_{17}H_{16}NO_4]^-$: 298.1085, Δ = −1.3 ppm. *m/z* 334.0846 $[M+^{35}Cl]^-$, calcd. for $[C_{17}H_{17}NO_4{}^{35}Cl]^-$: 334.0852, Δ = −1.8 ppm. ^1H NMR (CD_3OD, 600 MHz) *J* in Hz, *δ*: 7.02 (1H, d, br s, H-2), 6.78 (1H, d, 7.8, H-5), 6.91 (1H, br d, 7.8, H-6), 7.40 (1H, d, 15.6, H-7), 6.35 (1H, d, 15.6, H-8), 7.06 (2H, d, 8.4, H-2′, H-6′), 6,74 (2H, d, 8.4, H-3′, H-5′), 2.76 (2H, t, 7.2, H-7′), 3.47 (2H, t, 7.2, H-8′).

2.3.7 | (3*S*,9*S*)-Megastigman-5-en-3,9,12-triol (7)

A colorless solid; mp 242–245 °C; $[\alpha]_D^{25}$: -85.4 (*c* = 0.1, MeOH), HR-ESI-MS *m/z* 229.1797 $[M+H]^+$, calcd. for $[C_{13}H_{25}O_3]^+$: 229.1797 (Δ = −0.4 ppm); *m/z* 251.1620 $[M+Na]^+$, calcd. for $[C_{13}H_{24}O_3Na]^+$: 251.1620 (Δ = +0.7 ppm);. ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are shown in Table 2.

The HR-ESI-MS and NMR spectra of **2–7** were shown in Supplementary information (Figures S11–S20).

2.4 | Acid hydrolysis of compound 1

Acid hydrolysis of compound **1** was described in Supplementary information.

2.5 | Nitric oxide assay

The NO assay protocol was described in Supplementary information.

3 | RESULTS AND DISCUSSION

Compound **1** (Figure 1) was isolated as a white amorphous powder. The molecular formula of **1** was determined to be $C_{27}H_{36}O_{12}$ by the HR-ESI-MS (found *m/z* 553.2286 $[M+H]^+$, calcd. for $[C_{27}H_{37}O_{12}]^+$: 553.2280 (Δ = +1.1 ppm); *m/z* 575.2096 $[M+Na]^+$, calcd. for $[C_{27}H_{36}O_{12}Na]^+$: 575.2099 (Δ = −0.4 ppm); *m/z* 570.2561 $[M+NH_4]^+$, calcd. for $[C_{27}H_{40}O_{12}N]^+$: 570.2545 (Δ = +2.8 ppm). The IR spectrum of **1** suggested the presence of OH (3415 cm^{-1}), C═O (1716 cm^{-1}), C═C (1506 cm^{-1}), and C—*O*—C (1073, 1024 cm^{-1}) functional groups. The ^1H NMR spectrum of **1** exhibited two methyl groups [δ_H 1.58 (3H, s, C-19) and 1.11 (3H, s, H-20)], four sp^2 olefinic methine protons [δ_H 6.63 (d, *J* = 3.6 Hz, H-3), 6.56 (d, *J* = 1.8 Hz, H-14), 7.52 (dd, *J* = 1.8, 1.2 Hz, H-15), and 7.63 (d, *J* = 1.2 Hz, H-16)], two methine carbinol groups [δ_H 4.53 (m, H-2) and 4.57 (d, *J* = 4.8 Hz, H-6)], one anomeric proton [δ_H 4.44 (d, *J* = 7.8 Hz, H-1′), and methoxy protons [δ_H 3.37 (3H, s). In addition, two protons of one oxygenated methylene group were identified at δ_H 3.82 (dd, *J* = 12.0, 1.8 Hz) and 3.73 (dd, *J* = 12.0, 5.4 Hz), which were assigned to the glucose moiety. The ^{13}C NMR and HSQC spectra revealed 27 carbons, including 20 of a diterpene aglycone, 1 of methoxy, and 6 of a glucose unit. Of these, two carboxy carbons [δ_C 177.1 (C-17) and 169.1 (C-18)], two methyl's [δ_C 28.7 (C-19) and 29.5 (C-20)], three double bonds [δ_C 141.7 (CH, C-3), 139.6 (C, C-4), 126.8 (C, C-13), 109.7 (CH, C-14), 144.7 (CH, C-15, and 141.2 (CH, C-16)], two oxygenated methine groups [δ_C 64.8 (C-2) and 78.3 (C-6), and anomeric carbon (δ_C 105.7) were identified (Table 1). The above data suggested that compound **1** was a clerodane-type furanoditerpenoids bearing one glucose moiety, the main class of compounds from *T. crispa*.[5,20,21] The assigned NMR data of **1** were revealed using ^1H-^1H COSY, HSQC, and HMBC spectra in comparison with the published data (Table 1).[20,21] The HMBC correlations from H-3 to

TABLE 2 NMR data for compounds **4**, **5**, and **7** in CD_3OD.

	4		**5**			**7**	
No.	δ_C[a]	δ_H[b,f]	δ_C[c]	δ_H[d,f]	**No.**	δ_C[a]	δ_H[f]
1	133.1	–	134.9	–	1	44.2	–
2	112.6	7.14 (d, 1.8)	111.2	6.98 (d, 1.8)	2	43.6	2.09 (dt, 12.6, 1.8) 1.80 (m)
3	151.7	–	148.8	–	3	65.1	3.99 (m)
4	149.1	–	146.8	–	4	42.9	2.23 (m) 1.95 (m)
5	118.6	6.97 (d, 8.0)	115.9	6.77 (d, 8.0)	5	128.9	–
6	120.8	6.91 (dd, 8.0, 1.8)	120.2	6.78 (dd, 8.0, 1.8)	6	135.0	–
7	73.6	4.96 (d, 5.4)	74.0	5.00 (d, 4.0)	7	25.5	1.95 (m) 2.24 (m)
8	86.6	4.36 (m)	88.7	4.28 (m)	8	40.6	1.49 (m) 1.50 (m)
9	61.9	3.50[e] 3.78 (dd, 11.4, 5.4)	61.4	3.60 (dd, 12.0, 3.0) 3.92[e]	9	69.1	3.72 (m)
1′	137.4	–	133.5	–	10	23.2	1.19 (d, 7.0)
2′	111.3	7.06 (d, 1.8)	109.5	7.05 (d, 2.4)	11	24.7	1.05 (s)
3′	150.6	–	152.8	–	12	69.0	3.33 (d, 10.8) 3.41 (d, 10.8)
4′	147.4	–	137.4	–	13	20.2	1.69 (s)
5′	117.7	7.13 (d, 8.0)	154.8	–			
6′	120.7	6.99 (dd, 8.0, 1.8)	106.4	6.82 (d, 2.4)			
7′	131.4	6.55 (d, 16.0)	130.1	5.57 (br d, 15.6)			
8′	128.7	6.28 (ddd, 16.0, 6.6, 6.6)	131.2	6.36 (dt, 15.6, 6.6)			
9′	63.7	4.23 (d, 6.6)	63.6	4.24 (dd, 6.6, 1.5)			
3-OCH_3	56.6	3.86 (s)	56.4	3.82 (s)			
3′-OCH_3	56.7	3.89 (s)	56.6	3.82 (s)			
4-*O*-Glucose			**3′-*O*-Glucose**				
1′	103.0	4.88 (d, 7.8)	103.5	4.92 (d, 7.8)			
2′	74.9	3.49 (dd, 9.0, 7.8)	75.0	3.55 (m)			
3′	78.2	3.42 (t, 9.0)	77.8	3.49 (m)			
4′	71.4	3.42 (t, 9.0)	71.5	3.40 (t, 9.0)			
5′	77.8	3.50 (m)	77.3	3.47 (m)			
6′	62.5	3.88 (dd, 12.0, 1.8) 3.72 (dd, 12.0, 5.4)	62.6	3.93[e] 3.72 (dd, 12.0, 5.4)			

[a]150 MHz.
[b]600 MHz.
[c]125 MHz.
[d]500 MHz.
[e]Overlapped signals.
[f](mult., *J* in Hz).

C-1/C-4/C-5/C-18, from H-19 to C-4/C-5/C-6/C-10, from H-20 to C-10/C-9/C-8/C-11, from H-8 to C-17, H-7 to C-17, from H-12 to C-13/C-14/C-16, from H-6 to C-1′, and from methoxy protons to C-18 were observed (Figure 2). In addition, the COSY cross peaks of H-1/H-2/H-3 and H-1′/H-2′/H-3′/H-4′/H-5′/H-6′ were observed. These indicated three double bonds at C-3/C-4, C-14/C-15 and C-13/C-16, two carboxy groups at C-17 and C-18, two methine carbinol groups at C-2 and C-6, methoxy group at C-18, and the glucose linked to C-6 by an ether linkage (Figure 2). The NMR data of **1** were closely related to those of 6′-*O*-lactoylborapetoside B (**1a**) except that some slight differences of the proton and carbon chemical shifts as well as the coupling constant of proton and carbon at C-8 position.[21] The values $\delta_{C\text{-}8}$ 42.1/δ_H 3.36 (t, J = 9.1 Hz) in 6′-*O*-lactoylborapetoside B were changed to be as $\delta_{C\text{-}8}$ 46.5/δ_H 2.35 (dd, J = 6.0, 1.8 Hz) in **1** suggesting 8β-H with *equatorial* orientation.[5,20,21] this was indicated by the small $^3J_{7,8}$ (6.0 Hz) of proton H-8 as

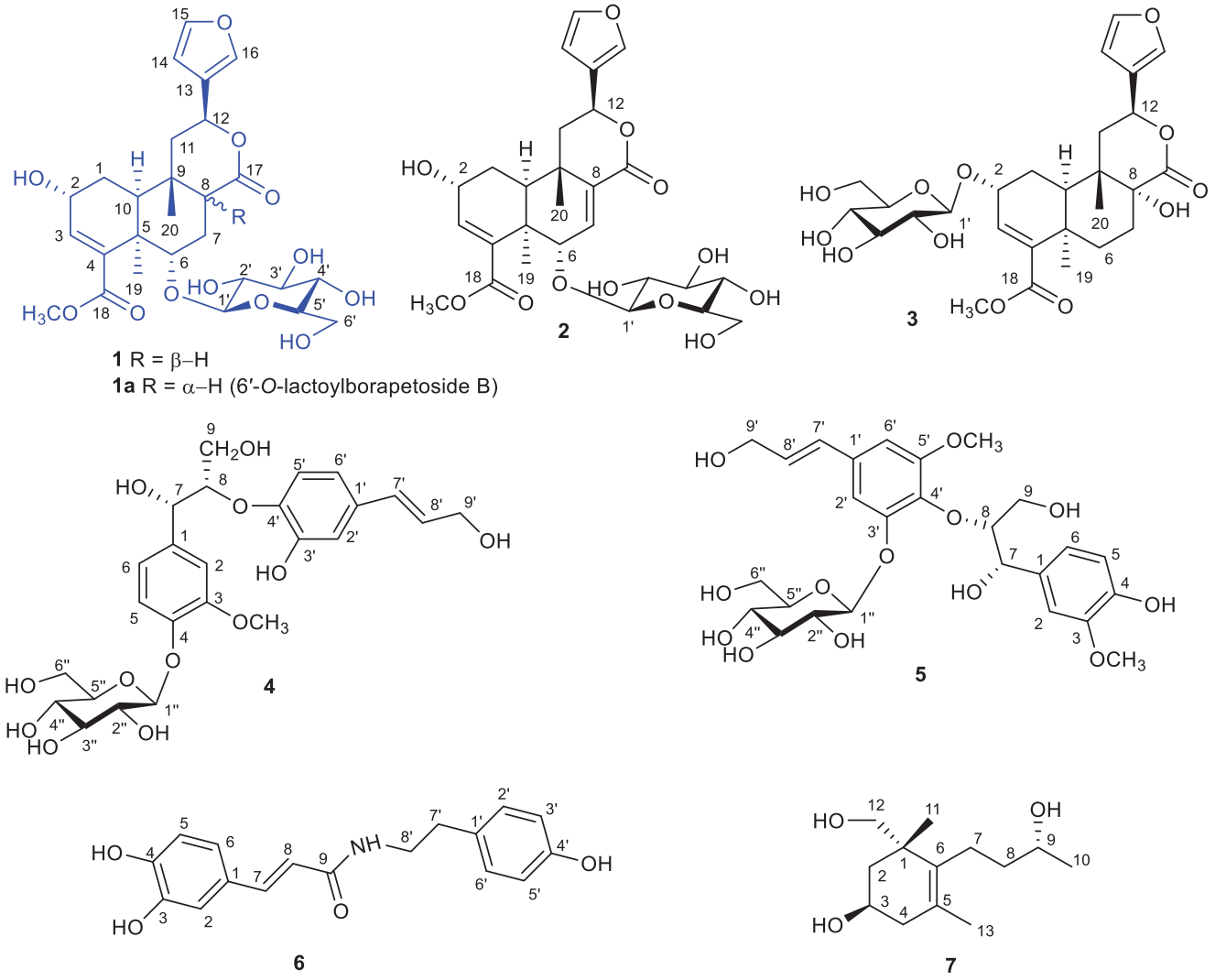

FIGURE 1 Chemical structures of compounds **1**–**7**.

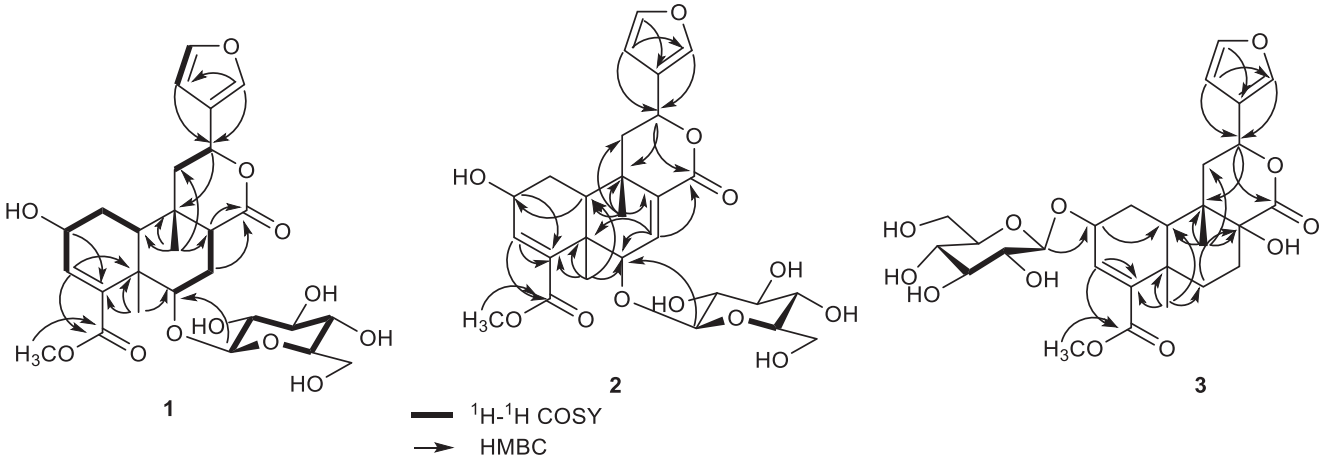

FIGURE 2 The key ^{1}H-^{1}H COSY and HMBC correlations of compounds **1**–**3**.

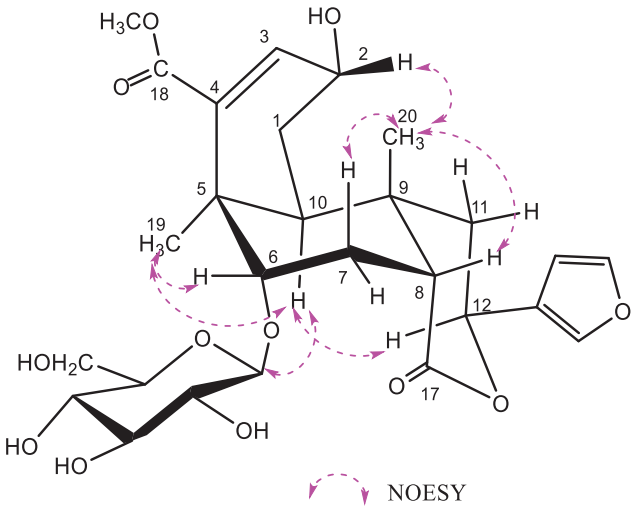

FIGURE 3 The key NOESY correlations of compound **1**.

well as by NOESY cross peak from H-8 to H-20 (Figure 3).[21] The NOESY correlation of H-10 and H-19 supported *cis*-A/B ring configuration, similar to that of *cis*-clerodane-type furanoditerpenoids isolated from *T. crispa*.[5,20,21] Proton H-6 appeared at δ_H 4.57 with small $^3J_{6,7}$ (4.8 Hz) suggested *β*/*equatorial* for H-6. This was further confirmed by NOESY cross peak of H-10 (δ_H 2.58 and H-1′ (δ_H 4.44). The hydroxy group at C-2 was *α*-oriented as suggested by NOESY cross peak of H-2 (δ_H 4.53) and H-20 (δ_H 1.11) (Figure 3).[21] The above data suggested the conformation of the A/B/C ring to be twisted boat/chair/chair conformation, as shown in Figure 3, with H-8 in ring B being *equatorially* oriented. The large *J* value (7.8 Hz) of an anomeric proton at δ_H 4.44 indicated the *β*-form of the glycosidic linkage. Acid hydrolysis of **1** gave D-glucose which was identified by comparison with authentic samples via TLC, and from the positive sign of the optical rotations.[22] Naturally, clerodane diterpenoids exist enantiomers commonly,[23] Therefore, the absolute configuration of **1** was determined by ECD spectrum. The ECD spectrum of **1** exhibited positive Cotton effect at 220 nm, similar to that of tinobakisin (isolated from *T. bakis*),[24] and tinocrisposide A (isolated from *T. crispa*),[25] which shared the same clerodane-diterpene backbone and showed positive Cotton effects at ≈217–220 nm, proposing 2*R*, 5*R*, 6*S*, 8*R*, 9*S*, 10*S*, 12*S* configurations for **1**. Thus, the chemical structure of compound **1** was determined as shown in Figure 1, a new compound named as tinocrioside D.

The HR-ESI-MS of compound **2** showed a quasi molecular ion peak at *m/z* 585.1728 $[M+^{35}Cl]^-$ (calcd. for $[C_{27}H_{34}O_{12}{}^{35}Cl]^-$: 585.1744, Δ = −2.7 ppm) determining the molecular formula of $C_{27}H_{34}O_{12}$ with 11 degrees of unsaturation. The NMR spectra of **2** were very similar to those of **1** except for the additional signals due to one double bond [(δ_C 140.9 (CH, C-7)/δ_H 6.89 (*d*, *J* = 2.4 Hz, H-7) and δ_C 137.0 (C, C-8)] and the downfield shift of carbon chemical shift at C-6 (from δ_C 78.3 in **1** to 81.1 in **2**). This suggested **2** was a *cis*-clerodane-type furanoditerpenoid compound.[20,21] Two carboxyl groups [δ_C 172.0 (C-18) and 171.2 (C-17)], two methyls [δ_C 23.9/δ_H 1.48 (s) and δ_C 27.5/δ_H 1.15 (s)], the furano ring [δ_C/δ_H: 125.4, 109.7/6.57, 145.0/7.54, 141.6/7.64], $\Delta^{3,4}$ double bond [δ_C/δ_H: 138.5 (C-3)/6.44 (*d*, *J* = 3.6 Hz) and 141.5 (C-4)], together with one glucose sugar [δ_C/δ_H: 105.7/4.45 (*d*, *J* = 7.8 Hz), 75.6/3.27, 77.8/3.40, 71.3/3.39, 78.1/3.30, 62.5/3.89 and 3.74] were identified. The sugar unit was linked to C-6 as confirmed by HMBC correlation from H-1′ (δ_H 4.45) to C-6 (δ_C 81.1) and from H_3-19 (δ_H 1.48) to C-4 (δ_C 141.5)/C-5 (δ_C 43.5)/C-6 (δ_C 81.1). The methoxy group at C-18 and the additional $\Delta^{7,8}$ double bond were indicated by HMBC correlations from methoxy protons (δ_H 3.80) to C-18 (172.0) and from H_3-20 (δ_H 1.15) to C-10 (δ_C 46.5)/C-9 (δ_C 38.2)/C-8 (δ_C 137.0). The hydroxy group at C-2 is evident from HMBC correlations from H-2 to C-3/C-4 and from H-3 to C-2, as well as by HSQC correlation from H-2 (δ_H 4.39) to C-2 (δ_C 64.0). All the NMR data of **2** including the multiplicity and proton-proton coupling constants were coincident with 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 (Table 1), which is a known compound from the aerial parts of *T. crispa*.[20] However, up to now, the absolute configuration of this compound has not been determined. Therefore, the ECD spectrum of compound **2** was carried out. The

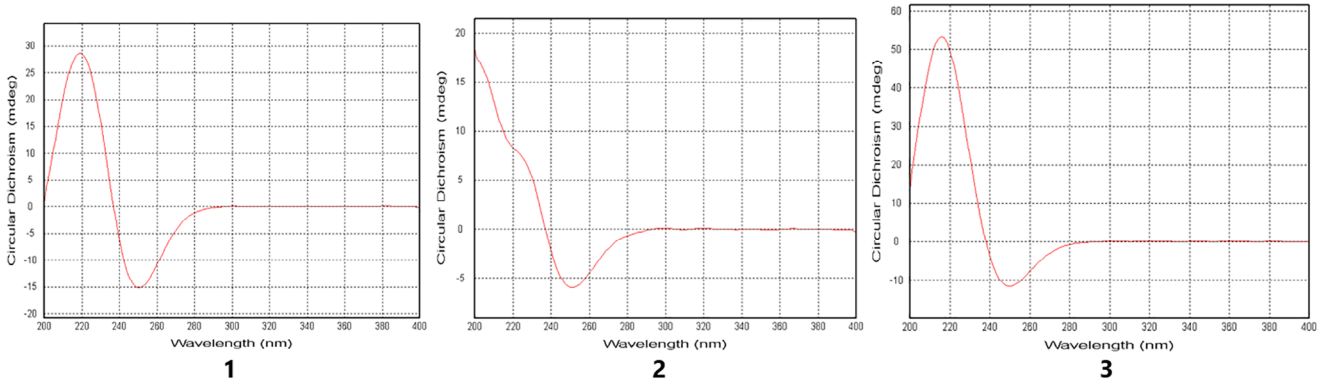

FIGURE 4 The ECD spectra of compounds **1**–**3**.

TABLE 3 The NO inhibitory activity of compounds **1–3**.

Compounds	NO inhibition (IC_{50}, μM)
1	67.3 ± 3.5
2	89.1 ± 1.7
3	78.4 ± 2.3
Dexamethasone[a]	13.1 ± 1.3

[a]Positive control compound.

ECD spectrum of **2** was similar to that of **1** (Figure 4) indicating that the absolute configuration of **2** was (2*R*,5*R*,6*S*, 9*S*,10*S*,12*S*).[25]

The molecular formula of compound **3** was determined to be $C_{27}H_{36}O_{12}$ by HR-ESI-MS (found *m/z* 575.2105 $[M+Na]^+$, calcd. for $[C_{27}H_{36}O_{12}Na]^+$: 575.2099 (Δ = +1.0 ppm); *m/z* 570.2544 $[M+NH_4]^+$, calcd. for $[C_{27}H_{40}O_{12}N]^+$: 570.2545 (Δ = −0.2 ppm). The NMR spectra of **3** were similar to those of compounds **1** and **2** suggesting a *cis*-clerodane-type furanoditerpenoid glycoside (Table 1).[21,22] The NMR data of **3** were assigned based on the HSQC and HMBC analysis in comparison with the corresponding data of compounds **1** and **2** (Table 1). The glucose moiety was linked to C-2 as confirmed by HMBC correlation from H-1′ (δ_H 4.48) to C-2 (δ_C 73.0). The additional oxygenated quaternary hydroxy group at C-8 was confirmed by HMBC correlation from H_3-20 (δ_H 1.05) to C-10 (δ_C 49.6)/C-9 (δ_C 41.2)/C-8 (δ_C 75.8)/C-11 (δ_C 42.8). The HMBC correlations from methoxy protons (δ_H 3.77) to C-18 (δ_C 170.2) and from H_3-19 (δ_H 1.43) to C-4 (δ_C 143.0)/C-5 (δ_C 36.7)/C-6 (δ_C 30.8)/C-10 (δ_C 49.6) indicated the position of methoxy carboxylate group and $\Delta^{3,4}$ double bond (Figure 1). All the NMR data of **3** were perfectly match those of tinospinoside B, a known compound isolated from *T. sagittata*.[26] The ECD spectrum of **3** (Figure 4) was similar to that of **1** suggesting the absolute configuration of **3** was (2*R*,5*R*,6*R*,8*R*,9*S*,10*S*,12*S*).[26]

Compounds **4**–**7** were identified to be citrusin A (**4**),[27] 4,7,9,3′,9′-pentahydroxy-3,5′-dimethoxy-8-4′-oxyneolign-7′-ene-3′-*O*-*β*-D-glucopyranoside (**5**),[28] *trans*-N-caffeoyltyramine (**6**),[29] and (3*S*,9*S*)-megastigman-5-en-3,9,12-triol (**7**).[30] The NMR data of these compounds were coincident with the published data in the literature. This is the first report of these compounds from *T. crispa* growing in Vietnam.

Three *cis*-clerodane-type furanoditerpenoid glycosides (**1**-**3**) were selected for evaluation of their NO production inhibitory activity in LPS stimulated RAW 264.7 cells. At a concentration of 100 μM, these compounds did not show significant cytotoxic activity and therefore were further evaluated for NO production inhibition.[31] As shown in Table 3, compounds **1**–**3** showed weak activity with IC_{50} values of 67.3, 89.1, and 78.4 μM, respectively, compared to that of the positive control compound, dexamethasone, which showed IC_{50} value of 13.1 μM.

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