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Clerodane Diterpenoid Glucosides from the Stems of *Tinospora sinensis*

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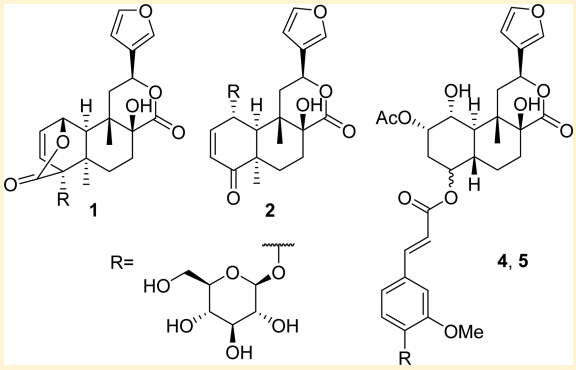
Supporting Information

ABSTRACT: Ten new clerodane diterpenoid glucosides (**1**−**10**) and three known analogues (**11**−**13**) were isolated from an EtOAc extract of the stems of *Tinospora sinensis*. Spectroscopic analyses and chemical methods were used to elucidate the structures of these isolates. The absolute configurations of tinosinenosides A−C (**1**−**3**) were established by using experimental and calculated ECD data. Their cytotoxicity against the human epithelioid cervical carcinoma (HeLa) cell line and the nitric oxide production inhibitory activity of lipopolysaccharide-activated N9 microglial cells were tested. 1-Deacetyltinosposide A (**12**) exhibited mild cytotoxicity against HeLa cells, with an IC_{50} value of 8.35 ± 0.60 μM.



Clerodane diterpenoids are commonly found in the genus *Tinospora*,[1−4] and a number of those have been reported to possess diverse biological activities such as cytotoxic,[5,6] immunomodulatory,[7] antifeedant,[8] and anti-inflammatory effects.[9] The biological activities and chemical structures of clerodanes have led to extensive efforts toward their synthesis.[10−12]

Tinospora sinensis (Lour.) Merr. is widely distributed and cultivated in the South of China.[13] The stems of this species, commonly known as "Kuan-Jin-Teng" in Chinese, have been used as a traditional Yao medicine to treat rheumatism, lumbar muscle strain, and sciatica.[14] The crude extracts of *T. sinensis* exhibited potent immunomodulatory and anti-inflammatory activities.[15,16] A wide array of clerodane diterpenoids have been reported to be the main components of *T. sinensis*.[1,2] Owing to its fascinating physiological properties and in an ongoing search for the bioactive agents of traditional Chinese medicines,[17] the clerodane diterpenoids of *T. sinensis* have been investigated. Ten new diterpenoid glucosides (**1**−**10**) and three known compounds (**11**−**13**) were obtained from the stems of *T. sinensis*. The rare presence of a C-4 carbonyl group was demonstrated in tinosinenoside B (**2**).[1,18−21] Seven new compounds (**4**−**10**) exhibited a dinorclerodane diterpenoid scaffold attached to a *cis*- or *trans*-feruloyl group. Herein, the isolation and structural elucidation of **1**−**10** are discussed. The cytotoxicity against the human epithelioid cervical carcinoma (HeLa) cell line and the nitric oxide (NO) production inhibitory activity of lipopolysaccharide (LPS)-activated N9 microglial cells were also tested. Cytotoxicity evaluations revealed that 1-deacetyltinosposide A (**12**) exhibited mild cytotoxicity against HeLa cells.

RESULTS AND DISCUSSION

The HRESIMS data of tinosinenoside A (**1**) showed a sodium adduct ion at *m*/*z* 559.1780 $[M + Na]^+$, consistent with the molecular formula $C_{26}H_{32}O_{12}$. The presence of hydroxy groups (3416 cm^{-1}) and a δ-lactone moiety (1738 cm^{-1}) was evident from their characteristic IR absorptions. The presence of a furan moiety was indicated by typical signals at δ_H 6.54 (dd, J = 1.5, 0.5 Hz, H-14), 7.49 (t, J = 1.5 Hz, H-15), and 7.58 (br s, H-16) in the ^{1}H NMR spectrum (Table 1). There were 20 carbon signals aside from those of the sugar moiety (δ_C 101.5, 75.2, 78.3, 71.3, 78.1, and 62.6) in the ^{13}C NMR spectrum (Table 1), which were classified by the HSQC data as two methyls (δ_C 20.7 and 25.1), three methylenes (δ_C 28.0, 28.2, and 37.2), three methines (δ_C 48.5, 73.0, and 74.7), two oxygenated tertiary carbons (δ_C 74.0 and 87.9), two sp^3 quaternary carbons (δ_C 40.1 and 41.1), one double bond (δ_C 131.8 and 133.1), a furan moiety (δ_C 109.7, 127.0, 141.4, and 145.0), and two lactone carbonyls (δ_C 175.2 and 175.9). The lactone moieties were distinguished via the HMBC cross-peaks from H-1 (δ_H 5.36) to C-18 (δ_C 175.2) and from H-12 (δ_H 5.58) to C-17 (δ_C 175.9), respectively. According to the literature, the NMR signals of tinosinenoside A (**1**) and palmatoside

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Chart 1

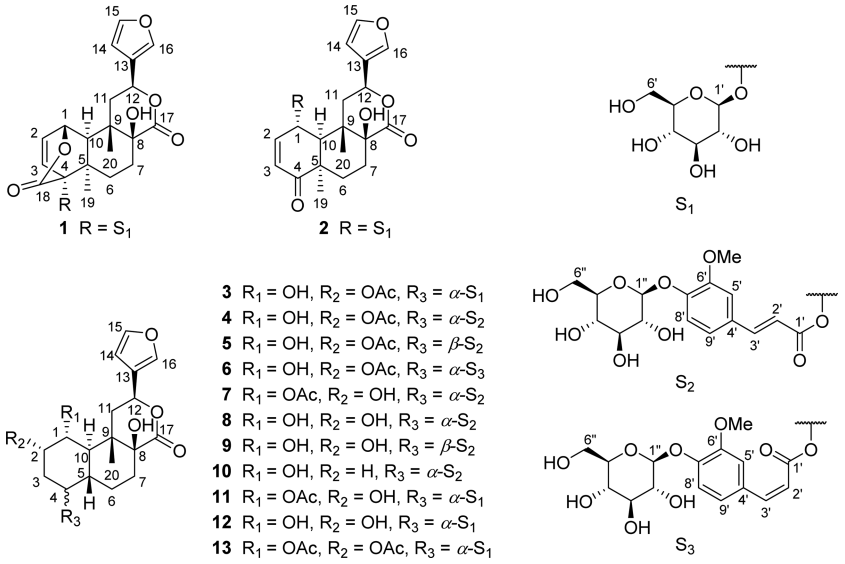

Table 1. ^{1}H and ^{13}C NMR Data of Compounds 1−3

	1[a]		2[b]		3[c]	
no.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	74.7	5.36, br d (5.0)	73.1	4.38, d (4.4)	71.8	3.88, dd (8.4, 3.2)
2	131.8	6.63, dd (8.0, 5.0)	145.9	6.97, dd (10.0, 4.4)	74.4	4.99, m
3a	133.1	6.83, dd (8.0, 1.0)	127.1	5.91, d (10.0)	30.82	2.28, ddd (15.6, 4.4, 1.6)
3b						1.77, dt (15.6, 4.4)
4	87.9		202.5		74.6	3.99, m
5	40.1		42.3		38.6	1.69, m
6a	28.0	1.92, dd (14.0, 8.0)	29.2	2.20, dt (14.0, 3.6)	26.8	1.67, overlapped
6b		1.66, m		0.92, td (14.0, 3.6)		1.57, m
7a	28.2	3.03, m	26.9	2.03, dt (14.0, 3.6)	30.78	2.33, m
7b		1.72, m		1.38, td (14.0, 3.6)		1.67, overlapped
8	74.0		74.3		76.7	
9	41.1		40.0		41.1	
10	48.5	1.81, br s	47.9	2.44, br s	40.1	2.19, dd (10.4, 8.4)
11a	37.2	2.43, dd (14.6, 11.5)	33.2	2.27, dd (14.4, 12.8)	36.5	2.55, dd (14.0, 4.4)
11b		2.12, dd (14.6, 5.5)		2.07, dd (14.4, 3.6)		2.46, dd (14.0, 12.2)
12	73.0	5.58, dd (11.5, 5.5)	70.2	5.71, dd (12.8, 3.6)	73.7	5.71, dd (12.2, 4.4)
13	127.0		125.3		127.3	
14	109.7	6.54, dd (1.5, 0.5)	108.9	6.56, d (1.4)	109.7	6.50, dd (2.0, 0.8)
15	145.0	7.49, t (1.5)	144.0	7.68, t (1.4)	144.8	7.47, dd (2.0, 1.6)
16	141.4	7.58, br s	140.3	7.72, br s	141.1	7.55, m
17	175.9		171.6		175.2	
18	175.2					
19	25.1	1.10, s	31.0	1.20, s		
20	20.7	1.18, s	17.9	0.61, s	15.3	1.14, s
2-Ac					173.7	
					21.6	2.10, s
Glc-1′	101.5	4.70, d (7.5)	105.1	4.35, d (7.6)	101.3	4.24, d (7.6)
2′	75.2	3.38, overlapped	73.5	2.95, m	75.0	3.16, dd (9.2, 7.6)
3′	78.3	3.38, overlapped	76.6	3.12, m	78.3	3.32, m
4′	71.3	3.38, overlapped	70.0	3.03, m	72.0	3.24, m
5′	78.1	3.29, m	77.0	3.15, m	78.1	3.20, m
6′a	62.6	3.81, dd (12.0, 2.0)	61.2	3.66, m	63.1	3.84, dd (11.8, 2.2)
6′b		3.66, dd (12.0, 5.0)		3.43, dd (12.0, 5.6)		3.62, dd (11.8, 5.8)

[a]In MeOH-d_4, ^{1}H NMR at 500 MHz, ^{13}C NMR at 125 MHz. [b]In DMSO-d_6, ^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz. [c]In MeOH-d_4, ^{1}H NMR at 400 MHz, ^{13}C NMR at 125 MHz.

C are similar, except for the presence of a hydroxy group at C-8 in **1** replacing the proton in the known analogue.[22] The key HMBC cross-peaks from H_2-6 (δ_H 1.66, 1.92) and H_3-20 (δ_H 1.18) to C-8 (δ_C 74.0) verified this relation. Following acid

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hydrolysis, the sugar moiety was identified as D-glucose by using an HPLC equipped with an optical rotation detector.[23,24] The β-configuration was based on the large $^3J_{1,2}$ value (7.5 Hz) and the chemical shift of its anomeric carbon at δ_C 101.5.

In the NOESY spectrum, the cross-peaks of H-1 (δ_H 5.36)/H-10 (δ_H 1.81), H-10/H_3-19 (δ_H 1.10), H-1′/H_3-19, and H-10/H-12 (δ_H 5.58) indicated that H-1, H-10, H-12, H_3-19, and the β-D-glucopyranosyl moiety were on the same side, and they were assigned randomly as α-oriented, while the furan moiety was assigned as β-oriented (Figure 1). The NOESY

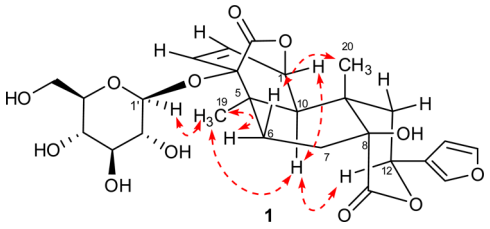

Figure 1. Selected NOESY correlations of **1**.

cross-peaks of H_3-19/H-6α (δ_H 1.92) and H-6β (δ_H 1.66)/H_3-20 suggested that H_3-20 was β-oriented. These data suggested that **1** shared the same 2D structure and relative configuration as palmatoside C, except for the oxygenated tertiary C-8. The configuration of C-8 was determined based on the structure elucidated via computer-aided simulation and critical NOESY information obtained from H-10.[25] The strong cross-peak of H-10/H-12 and the absence of a cross-peak of H-10/H_2-11 (δ_H 2.12, 2.43) in **1** suggested that OH-8 was β-oriented. This was further confirmed by the γ-gauche effect induced by OH-8,[26] which caused the decreased chemical shifts of C-11 (δ_C 37.2) and C-20 (δ_C 20.7) in **1** relative to palmatoside C (δ_C 40.2, C-11; δ_C 23.89, C-20).[22] By comparing the experimental and calculated ECD spectra predicted by time-dependent density functional theory (TDDFT) at the B3LYP/6-311G** level, the absolute configuration of **1** was established. The experimental electronic circular dichroism (ECD) spectrum of **1** (Figure 2) showed a negative Cotton effect (CE) at 203 nm

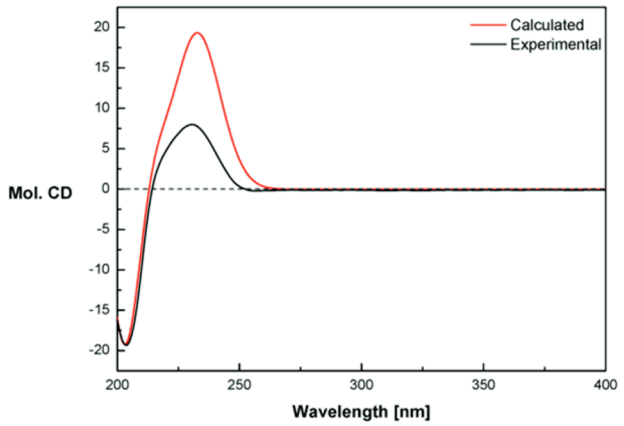

Figure 2. Comparison of experimental and calculated ECD spectra of **1**.

($\Delta\varepsilon$ = −19.3) and a positive CE at 230 nm ($\Delta\varepsilon$ = +8.0), fitting well with the calculated ECD spectrum for (1*R*,4*R*,5*R*,8*S*,9*S*,10*S*,12*S*)-**1**. Hence, the structure of **1** was established.

The HRESIMS data of tinosinenoside B (**2**) produced a chloride adduct ion at *m*/*z* 543.1643 $[M + Cl]^-$, which was consistent with a molecular formula of $C_{25}H_{32}O_{11}$. The NMR data (Table 1) showed the presence of a furan moiety (δ_H 6.56, d, *J* = 1.4 Hz; 7.68, t, *J* = 1.4 Hz; and 7.72, br s), an α,β-unsaturated carbonyl moiety (δ_H 6.97, dd, *J* = 10.0, 4.4 Hz; 5.91, d, *J* = 10.0 Hz; δ_C 202.5), two methyls (δ_H 0.61, 1.20, each 3H, s), a lactone moiety (δ_C 171.6), and a β-glucopyranosyl group (δ_H 4.35, d, *J* = 7.6 Hz). The NMR data analysis indicated that **2** was structurally related to tinosporaside,[27] differing only in the presence of a hydroxy group at C-8 (δ_C 74.3) and an interchange of the substituents at C-1 and C-4. This hypothesis was shown to be correct by the HMBC cross-peaks from H-1 (δ_H 4.38) to C-5 (δ_C 42.3), C-9 (δ_C 40.0), and C-1′ (δ_C 105.1) and from H_3-19 (δ_H 1.20) to C-4 (δ_C 202.5).

The NOESY cross-peaks of H-10 (δ_H 2.44)/H_3-19 and H-10/H-12 (δ_H 5.71) suggested that H-10, H-12, and H_3-19 were cofacial, and they were assigned randomly as α-oriented. The correlation of H-1/H_3-20 (δ_H 0.61) supported the β-orientation of H-1 (Figure 3). The strong cross-peak of H-10/H-12

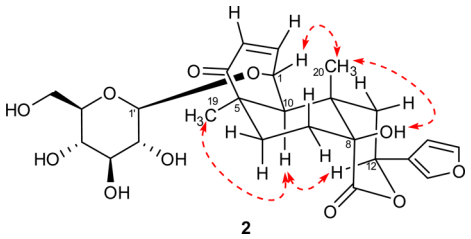

Figure 3. Selected NOESY correlations of **2**.

and the absence of a cross-peak of H-10/H_2-11 (δ_H 2.07, 2.27) in **2** suggested that OH-8 was β-oriented.[25] This was verified by the clear cross-peak of OH-8 (δ_H 6.03)/H_3-20. According to empirical rules,[28,29] the positive CE at 232 nm ($\Delta\varepsilon$ = +6.9) caused by the $\pi \rightarrow \pi^*$ electronic transition and the negative CE at 337 nm ($\Delta\varepsilon$ = −0.8) resulting from the n → π^* transition of an α,β-unsaturated carbonyl moiety both indicated that the configuration of **2** was 1*S*, 5*R*, 8*S*, 9*S*, 10*S*, 12*S*. The experimental ECD spectrum corresponded well with the calculated ECD spectrum for (1*S*,5*R*,8*S*,9*S*,10*S*,12*S*)-**2** (Figure 4), which

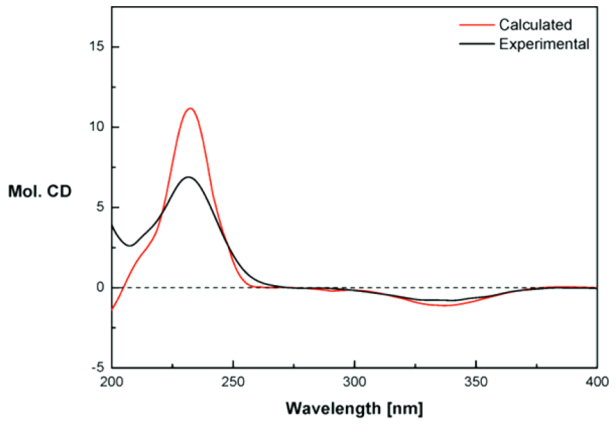

Figure 4. Comparison of experimental and calculated ECD spectra of **2**.

authenticated the configuration. Thus, the structure of **2** was shown to be an 18-norclerodane diterpenoid with a C-4 carbonyl group, which is rare because the carbonyl group is usually at C-1 or C-2.[21]

Tinosinenoside C (**3**) had a molecular formula of $C_{26}H_{36}O_{13}$, as determined by HRESIMS (*m*/*z* 591.1857 $[M + Cl]^-$, calcd

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for 591.1850) and ^{13}C NMR data. A β-glucopyranosyl moiety was recognized from the anomeric proton signal at δ_H 4.24 (d, J = 7.6 Hz). The ^1H NMR data (Table 1) of **3** displayed diagnostic signals for a typical furan moiety (δ_H 6.50, 7.47, and 7.55), a methyl group (δ_H 1.14), and a lactone moiety (δ_H 5.71, δ_C 175.2). The aforementioned data showed similarities to tinosineside A (**11**).[30] The substituents at C-1 and C-2 in **3** were hydroxy and acetoxy, respectively, compared to 1-acetoxy and 2-hydroxy in **11**. This regiochemistry was supported by the COSY cross-peaks of H-10 (δ_H 2.19)/H-1 (δ_H 3.88)/H-2 (δ_H 4.99)/H_2-3 (δ_H 1.77, 2.28) and HMBC cross-peaks from H-2 to C-10 (δ_C 40.1) and CH_3CO-2 (δ_C 173.7).

The relative configuration of **3** was constructed based on the coupling constants and the NOESY spectrum. The $^3J_{1,2}$ (3.2 Hz) and $^3J_{1,10}$ (8.4 Hz) values indicated that the orientations of H-1, H-2, and H-10 were β-axial, β-equatorial, and α-axial, respectively (Figure 5).[1] The cross-peaks of H-1/H-5 (δ_H 1.69)

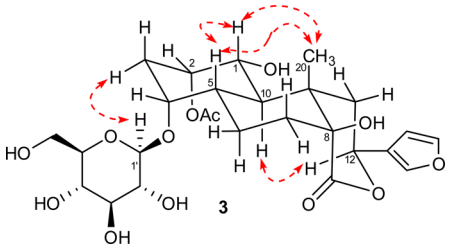

Figure 5. Selected NOESY correlations of **3**.

and H-5/H_3-20 (δ_H 1.14) suggested that H-5 and H_3-20 were β-oriented. The cross-peak of H-1′ (δ_H 4.24)/H-3α (δ_H 2.28) suggested that the glucopyranosyl moiety was α-oriented and that H-4 was β-oriented. The cross-peak of H-10/H-12 (δ_H 5.71) and the absence of the cross-peak of H-10/H_2-11 (δ_H 2.46, 2.55) revealed that H-12 was α-oriented and that OH-8 was β-oriented.[25] The experimental ECD spectrum of **3** conformed to the calculation spectrum for (1*R*,2*S*,4*R*,5*R*,8*S*,9*S*,10*R*,12*S*)-**3** (Figure 6). Therefore, the structure of **3** was defined as shown.

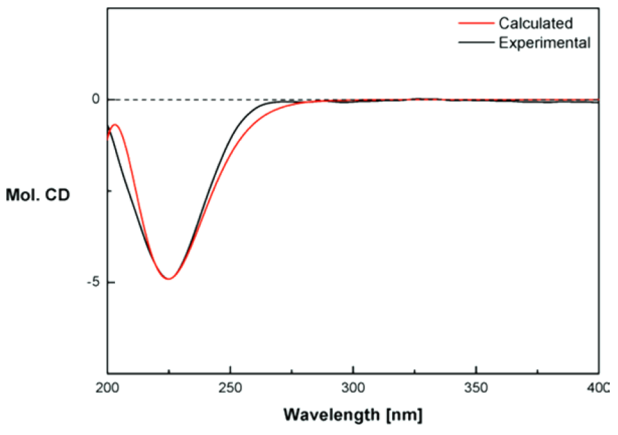

Figure 6. Comparison of experimental and calculated ECD spectra of **3**.

Tinosinenoside D (**4**) had a molecular formula of $C_{36}H_{44}O_{16}$ based on its ^{13}C NMR and HRESIMS data (m/z 755.2505 $[M + Na]^+$, calcd for 755.2522). In the ^{13}C NMR spectrum of **4**, in addition to the signals similar to those of compound **3**, 10 extra carbon signals were present, including one methoxy group (δ_C 56.8), eight sp^2 carbons (δ_C 113.0, 117.3, 117.4, 123.3, 130.3, 146.7, 150.1, and 151.0), and one ester moiety (δ_C 168.0). The ^1H NMR spectrum showed a cluster of typical signals at δ_H 7.17 (d, J = 8.5 Hz), 7.21 (dd, J = 8.5, 1.7 Hz), and 7.29 (d, J = 1.7 Hz), representing a 1,2,4-trisubstituted aromatic moiety, two *trans*-olefinic protons at δ_H 6.40 (d, J = 16.0 Hz) and 7.65 (d, J = 16.0 Hz), and one methoxy group at δ_H 3.84 (s). On the basis of the above data, the structure of **4** was similar to compound **3** but carrying a *trans*-feruloyl group, which was confirmed by 2D NMR data analysis. In the HMBC spectrum, the cross-peaks from H-4 (δ_H 4.85) to C-1′ (δ_C 168.0) located the feruloyl group at C-4, and the correlation from H-1″ (δ_H 4.97) to C-7′ (δ_C 150.1) linked the β-D-glucopyranosyl moiety to C-7′. The relative configuration of the diterpenoid moiety of **4** was established as being identical to that of **3** by analyzing their NMR data (Tables 1−3) and NOESY interactions (Figure 7). The $^3J_{2',3'}$ value (16.0 Hz) suggested a 2′,3′-*trans* olefinic bond. The structure of compound **4** was thus established as shown.

Compound **5**, 4-*epi*-tinosinenoside D, shared the same molecular formula as **4**, $C_{36}H_{44}O_{16}$, as was evident from an HRESIMS ion at m/z 755.2508 $[M + Na]^+$ (calcd for 755.2522) and ^{13}C NMR data. The NMR data (Tables 2 and 3) showed that the structure of compound **5** was similar to **4** except for the C-4 configuration. The $^3J_{3\beta,4}$ (11.0 Hz) and $^3J_{4,5}$ (11.0 Hz) values indicated that H-4 had an α-axial orientation, as could also be inferred from the NOESY cross-peak of H-4 (δ_H 4.79)/H-6α (δ_H 0.99) (Figure 8). Detailed 2D NMR data analyses indicated that the rest of the structure of **5** was the same as **4**. Hence, the structure of **5** was established as the 4-epimer of **4**.

Tinosinenoside E (**6**) also shared the molecular formula $C_{36}H_{44}O_{16}$ with **4**, as was established via ^{13}C NMR data and an HRESIMS sodium adduct ion at m/z 755.2501 $[M + Na]^+$ (calcd for 755. 2522). The NMR data (Tables 2 and 3) showed that compound **6** was also similar to **4**, except for a 2′,3′-*cis* olefinic unit as indicated by a distinctly smaller $^3J_{2',3'}$ value (13.0 Hz). The relative configurations of the C-1, C-2, C-4, C-5, C-8, C-9, C-10, and C-12 stereocenters of **6** were consistent with those of **4** according to the NOESY data and their similar NMR patterns. Thus, the structure of **6** was established as shown.

The molecular formula of tinosinenoside F (**7**) was $C_{36}H_{44}O_{16}$ based on the ^{13}C NMR data and an HRESIMS ion at m/z 755.2512 $[M + Na]^+$ (calcd for 755.2522). The NMR data (Tables 2 and 3) revealed that the structure of **7** was similar to **4**, but with interchanged acetoxy and hydroxy groups at C-1 (δ_C 77.1) and C-2 (δ_C 68.1), respectively. The COSY cross-peaks of H-10 (δ_H 2.71)/H-1 (δ_H 5.01)/H-2 (δ_H 4.10)/H_2-3 (δ_H 2.24, 1.90) and HMBC cross-peaks from H-1 to C-9 (δ_C 40.7) and CH_3CO-1 (δ_C 171.6) all supported this structural relation. The $^3J_{1,2}$ (3.0 Hz) and $^3J_{1,10}$ (10.5 Hz) values suggested that the orientations of H-1, H-2, and H-10 were β-axial, β-equatorial, and α-axial, respectively. The orientation of H-4 was β-equatorial, as indicated by the $^3J_{4,3}$ and $^3J_{4,5}$ values (both 3.0 Hz) and the NOESY cross-peak of H-4 (δ_H 4.97)/H-6β (δ_H 1.56). The structure of **7** was thus defined as shown.

The molecular formula of 2-deacetyltinosinenoside D (**8**) was defined as $C_{34}H_{42}O_{15}$ based on the ^{13}C NMR data and an HRESIMS sodium adduct ion at m/z 713.2405 $[M + Na]^+$ (calcd for 713.2416). Compound **8** was determined to be a deacetylated derivative of **4**, as its molecular mass was 42 amu fewer than that of compound **4**. A hydroxy group replacing the acetoxy group in **4** was attached to C-2 (δ_C 71.6) in **8**, which was verified by the chemical shift of H-2 (δ_H 3.93) and HMBC cross-peaks from H-2 to C-1 (δ_C 74.2), C-4 (δ_C 73.5), and C-10 (δ_C 37.5). Analysis of the coupling constants of H-1

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Table 2. ^{1}H NMR Data of Compounds 4−10

no.	4[a]	4[b]	5[a]	6[a]	6[b]	7[a]	8[a]	9[a]	9[b]	10[a]
1	3.92, dd (10.5, 3.0)	3.77, overlapped	3.95, dd (10.5, 3.0)	3.89, dd (10.5, 3.0)	3.74, overlapped	5.01, dd (10.5, 3.0)	3.77, dd (10.0, 3.0)	3.79, dd (10.5, 3.0)	3.62, m	3.79, td (10.0, 5.0)
2	5.05, q (3.0)	4.90, q (3.0)	5.19, q (3.0)	5.00, q (3.0)	4.88, q (3.0)	4.10, q (3.0)	3.93, q (3.0)	3.97, q (3.0)	3.82, m	1.81, m
3a	2.40, dt (16.0, 3.0)	2.14, m	2.24, ddd (13.5, 4.5, 4.0)	2.42, dt (16.0, 3.0)	2.16, m	2.24, dt (15.5, 3.0)	2.26, dt (15.5, 3.0)	2.22, dt (13.0, 4.0)	1.99, dd (8.5, 4.0)	1.98, m
3b	1.85, dt (16.0, 3.0)	1.87, m	1.70, ddd (13.5, 11.0, 2.5)	1.84, dt (16.0, 3.0)	1.87, dt (16.0, 3.0)	1.90, dt (15.5, 3.0)	1.83, dt (15.5, 3.0)	1.62, m	1.55, overlapped	1.65, m
4	4.85[c]	4.78, q (3.0)	4.79, td (11.0, 4.5)	4.86[c]	4.78, q (3.0)	4.97, q (3.0)	4.94, q (3.0)	4.98, overlapped	4.87, td (10.5, 5.0)	4.94, q (3.0)
5	1.84, m	1.79, m	1.79, m	1.82, m	1.77, m	1.94, m	1.78, m	1.70, qd (11.0, 4.0)	1.57, overlapped	1.78, m
6a	1.55, overlapped	1.44, m	1.91, m	1.52, dq (13.5, 4.0)	1.42, m	1.56, m	1.52, m	1.87, m	1.70, m	1.56, m
6b	1.53, overlapped	1.30, qd (13.5, 4.0)	0.99, m	1.40, qd (13.5, 4.0)	1.23, qd (13.5, 3.5)	1.51, m	1.45, qd (13.5, 4.0)	0.99, m	0.82, qd (13.5, 3.0)	1.39, qd (13.5, 3.5)
7a	2.31, dt (13.0, 3.0)	2.17, m	2.35, dt (13.0, 3.0)	2.30, m	2.16, m	2.32, br d (13.0)	2.30, dt (13.5, 3.0)	2.33, dt (12.5, 3.0)	2.16, br d (13.5)	2.30, dt (12.5, 3.5)
7b	1.72, td (13.0, 5.5)	1.54, td (13.5, 4.0)	1.61, td (13.0, 4.5)	1.69, m	1.52, td (13.0, 4.0)	1.72, td (13.0, 4.5)	1.70, td (13.5, 4.5)	1.59, m	1.43, td (13.5, 4.0)	1.68, m
10	2.45, t (11.0)	2.27, t (11.0)	1.99, dd (11.0, 10.5)	2.30, t (10.5)	2.11, dd (11.5, 10.5)	2.71, t (11.0)	2.39, dd (11.0, 10.5)	2.04, dd (11.0, 10.5)	1.86, t (10.5)	1.93, dd (11.0, 10.0)
11a	2.76, dd (14.0, 4.3)	2.70, dd (13.5, 4.0)	2.71, dd (14.0, 4.0)	2.75, dd (14.0, 4.0)	2.68, dd (13.0, 3.5)	2.67, dd (14.0, 13.0)	2.78, dd (13.5, 4.0)	2.74, dd (14.0, 4.0)	2.66, dd (13.0, 3.5)	2.74, dd (13.5, 4.0)
11b	2.54, dd (14.0, 12.5)	2.39, t (13.0)	2.48, dd (14.0, 12.5)	2.50, dd (14.0, 12.5)	2.37, t (13.0)	2.04, dd (14.0, 4.0)	2.51, dd (13.5, 12.5)	2.46, dd (14.0, 12.5)	2.33, dd (13.0, 12.5)	2.51, dd (13.5, 12.5)
12	5.80, dd (12.5, 4.3)	5.67, dd (12.0, 4.0)	5.70, dd (12.5, 4.0)	5.73, dd (12.5, 4.0)	5.59, dd (12.5, 3.5)	5.91, dd (13.0, 4.0)	5.91, dd (12.5, 4.0)	5.82, dd (12.5, 4.0)	5.67, dd (12.5, 3.5)	5.84, dd (12.5, 4.0)
14	6.52, m	6.50, m	6.49, dd (2.0, 0.5)	6.51, dd (1.5, 0.5)	6.52, m	6.55, m	6.53, br s	6.50, dd (1.5, 0.5)	6.48, dd (2.0, 1.0)	6.51, dd (1.5, 0.5)
15	7.49, t (1.5)	7.67, t (1.5)	7.47, dd (2.0, 1.5)	7.48, t (1.5)	7.66, t (1.5)	7.52, t (1.5)	7.47, br s	7.46, t (1.5)	7.65, dd (2.0, 1.5)	7.48, t (1.5)
16	7.58, br s	7.73, br s	7.54, dd (1.5, 0.5)	7.56, dd (1.5, 0.5)	7.72, br s	7.64, br s	7.57, br s	7.54, br s	7.66, br s	7.55, m
20	1.19, s	1.08, s	1.19, s	1.18, s	1.06, s	1.09, s	1.18, s	1.19, s	1.06, s	1.17, s
2′	6.40, d (16.0)	6.47, d (16.0)	6.42, d (16.0)	5.88, d (13.0)	5.82, d (13.0)	6.44, d (16.0)	6.41, d (16.0)	6.42, d (16.0)	6.54, d (16.0)	6.43, d (16.0)
3′	7.65, d (16.0)	7.55, d (16.0)	7.62, d (16.0)	6.90, d (13.0)	6.91, d (13.0)	7.64, d (16.0)	7.62, d (16.0)	7.61, d (16.0)	7.57, d (16.0)	7.62, d (16.0)
5′	7.29, d (1.7)	7.35, d (1.5)	7.25, br s	7.69, d (1.7)	7.57, d (2.0)	7.29, d (1.5)	7.27, d (1.0)	7.25, br s	7.37, d (2.0)	7.30, d (2.0)
8′	7.17, d (8.5)	7.11, d (8.5)	7.16, overlapped	7.11, d (8.5)	7.06, d (9.0)	7.16, d (8.5)	7.16, d (8.5)	7.16, overlapped	7.08, d (8.5)	7.17, d (8.5)
9′	7.21, dd (8.5, 1.7)	7.19, dd (8.5, 1.5)	7.16, overlapped	7.15, dd (8.5, 1.7)	7.22, dd (9.0, 2.0)	7.19, dd (8.5, 1.5)	7.18, dd (8.5, 1.0)	7.16, overlapped	7.20, dd (8.5, 2.0)	7.20, dd (8.5, 2.0)
1-Ac						2.02, s				
2-Ac	1.96, s	1.91, s	2.16, s	1.99, s	1.93, s					
6′-O-Me	3.84, s	3.77, s	3.89, s	3.86, s	3.74, s	3.85, s	3.85, s	3.89, s	3.81, s	3.86, s
Glc-1″	4.97, d (7.5)	4.98, d (7.5)	4.97, d (7.3)	4.97, d (7.5)	4.97, d (7.5)	4.96, d (7.5)	4.95, d (7.5)	4.96, d (7.5)	4.97, d (7.5)	4.96, d (7.5)
2″	3.51, dd (9.5, 7.5)	3.25, overlapped	3.51, dd (9.0, 7.3)	3.51, dd (9.0, 7.5)	3.25, overlapped	3.50, dd (9.5, 7.5)	3.51, dd (9.0, 7.5)	3.51, dd (9.0, 7.5)	3.25, overlapped	3.51, dd (9.0, 7.5)
3″	3.47, m	3.25, overlapped	3.47, dd (9.0, 8.5)	3.47, m	3.25, overlapped	3.46, m	3.47, dd (9.0, 8.0)	3.47, dd (9.0, 8.5)	3.25, overlapped	3.47, m
4″	3.41, m	3.15, m	3.39, dd (9.5, 8.5)	3.40, m	3.16, m	3.39, dd (9.5, 8.5)	3.39, dd (9.5, 8.0)	3.39, dd (9.5, 8.5)	3.15, m	3.39, dd (9.5, 8.5)
5″	3.44, m	3.33, m	3.44, m	3.44, m	3.32, m	3.43, m	3.43, m	3.43, m	3.32[c]	3.43, m
6″a	3.87, dd (12.0, 2.0)	3.65, dd (12.0, 2.0)	3.87, m	3.87, m	3.65, dd (12.0, 1.5)	3.87, dd (12.0, 2.0)	3.87, dd (12.0, 1.5)	3.87, dd (12.0, 1.5)	3.65, m	3.87, dd (12.0, 2.0)
6″b	3.68, dd (12.0, 5.0)	3.44, dd (12.0, 5.5)	3.69, dd (12.0, 5.0)	3.68, dd (12.5, 5.0)	3.44, dd (12.0, 5.5)	3.68, dd (12.0, 5.0)	3.68, dd (12.0, 5.0)	3.69, dd (12.0, 5.0)	3.43, dd (12.0, 6.0)	3.68, dd (12.0, 5.0)

[a] ^{1}H NMR were measured in MeOH-d_4 at 500 MHz. [b] ^{1}H NMR were measured in DMSO-d_6 at 500 MHz. [c] Signal overlapped by solvent peaks.

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Table 3. ^{13}C NMR Data of Compounds 4−10[a]

position	4	5	6	7	8	9	10
1	72.7	72.0	72.5	77.1	74.2	73.6	72.0
2	75.0	75.4	75.0	68.1	71.6	72.4	32.2
3	32.1	34.5	32.1	34.8	35.0	37.2	29.4
4	73.1	74.3	72.8	72.9	73.5	74.5	74.2
5	39.1	40.4	38.9	39.3	39.2	40.7	39.4
6	26.4	27.3	26.6	26.5	26.6	27.4	27.2
7	30.3	30.3	30.4	30.2	30.4	30.4	30.4
8	76.7	76.7	76.6	76.6	76.7	76.8	76.6
9	40.9	41.2	41.0	40.7	40.9	41.0	41.4
10	38.3	42.4	38.5	35.7	37.5	41.2	44.5
11	36.9	36.8	36.9	37.1	37.0	36.8	37.2
12	74.1	74.1	74.0	73.3	74.1	74.0	74.2
13	127.4	127.3	127.3	127.2	127.5	127.4	127.4
14	109.8	109.7	109.8	109.7	109.8	109.8	109.7
15	144.9	144.7	144.8	145.1	144.7	144.7	144.8
16	141.1	141.0	141.2	141.3	141.2	141.2	141.1
17	175.0	175.0	175.1	174.7	175.2	175.3	175.2
20	14.9	15.5	15.0	15.3	15.2	15.7	15.5
1′	168.0	168.3	167.6	168.5	168.6	168.3	168.3
2′	117.3	117.0	118.5	117.6	117.8	117.4	117.3
3′	146.7	146.3	144.6	146.4	146.3	146.1	146.4
4′	130.3	130.5	130.7	130.6	130.7	130.5	130.4
5′	113.0	112.6	115.2	112.8	113.0	112.5	112.9
6′	151.0	151.1	145.0	151.0	151.0	151.0	151.0
7′	150.1	150.2	150.0	150.0	150.0	150.1	150.1
8′	117.4	117.5	116.7	117.4	117.5	117.5	117.4
9′	123.3	123.5	125.9	123.3	123.3	123.5	123.4
1-Ac				171.6			
				21.6			
2-Ac	172.8	172.7	173.0				
	21.5	21.2	21.4				
6′-OMe	56.8	56.8	56.6	56.7	56.8	56.8	56.8
Glc-1″	102.1	102.2	102.3	102.2	102.3	102.2	102.2
2″	74.8	74.8	74.8	74.8	74.8	74.8	74.8
3″	77.8	77.9	77.8	77.8	77.9	77.9	77.8
4″	71.2	71.3	71.3	71.3	71.3	71.3	71.3
5″	78.2	78.3	78.2	78.3	78.3	78.3	78.3
6″	62.4	62.5	62.5	62.5	62.5	62.5	62.5

[a]^{13}C NMR were measured in MeOH-d_4 at 125 MHz.

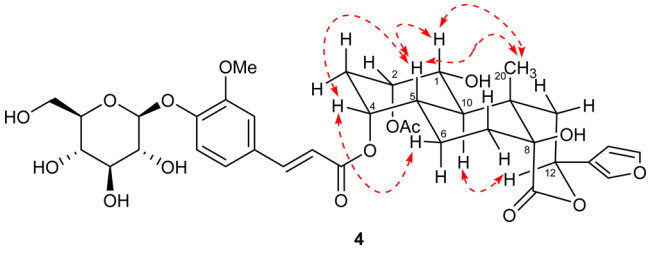

Figure 7. Selected NOESY correlations of 4.

(dd, J = 10.0, 3.0 Hz), H-2 (q, J = 3.0 Hz), and H-10 (dd, J = 11.0, 10.5 Hz) suggested that the orientations of H-1, H-2, and H-10 were β-axial, β-equatorial, and α-axial, respectively.[1] The $^3J_{4,3}$ and $^3J_{4,5}$ values (both 3.0 Hz) and the NOESY cross-peak of H-4 (δ_H 4.94)/H-6β (δ_H 1.52) revealed the β-equatorial orientation of H-4. Hence, the structure of compound **8** was elucidated.

The ^{13}C NMR data and HRESIMS ion at m/z 713.2405 [M + Na]$^+$ (calcd for 713.2416) showed that 4-*epi*-2-deacetyltinosinenoside D (**9**) had the same molecular formula $C_{34}H_{42}O_{15}$ as **8**.

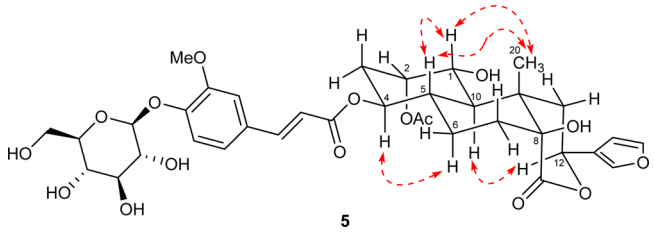

Figure 8. Selected NOESY correlations of 5.

The NMR data (Tables 2 and 3) of **9** were similar to those of compound **8**, except for a difference in the chemical shifts around C-4, which suggested opposite relative configurations of C-4. The $^3J_{3\beta,4}$ (10.5 Hz) and $^3J_{4,5}$ (10.5 Hz) values and the NOESY cross-peak of H-4 (δ_H 4.98)/H-6α (δ_H 0.99) confirmed the α-axial orientation of H-4. The structure of **9** was thus established as shown.

The molecular formula of 2-deacetoxytinosinenoside D (**10**) was defined as $C_{34}H_{42}O_{14}$ by the ^{13}C NMR data and an HRESIMS ion at m/z 697.2453 [M + Na]$^+$ (calcd for 697.2467), which was 16 amu fewer than that of **8**. Its NMR data (Tables 2 and 3) revealed that compound **10** was structurally related to **8**, the difference being the shielded H_2-2 (δ_H 1.81) and C-2 (δ_C 32.2) resonances, suggesting that **10** was the C-2 deoxy derivative of **8**. This was verified by an analysis of the COSY cross-peaks of H-10 (δ_H 1.93)/H-1 (δ_H 3.79)/H_2-2/H_2-3 (δ_H 1.98, 1.65). Thus, the structure of **10** was defined as shown.

Three known diterpenoid glucosides, tinosineside A (**11**),[30] 1-deacetyltinosposide A (**12**),[2] and tinosineside B (**13**),[30] were also isolated. Similar NMR and MS data analyses and comparisons with literature data were used to establish their structures.

All the isolates and the positive control, doxorubicin (IC_{50} 0.32 ± 0.04 μM), were assessed in terms of their cytotoxic effects against HeLa cells. Only 1-deacetyltinosposide A (**12**) showed mild cytotoxicity, with an IC_{50} value of 8.35 ± 0.60 μM; no signs of cytotoxicity were found for the other compounds ($IC_{50} \geq 10$ μM). Additionally, the NO production inhibitory activity of compounds **1**−**13** on LPS-activated N9 microglial cells was also tested; the results showed that none of these compounds had inhibitory activity ($IC_{50} \geq 100$ μM).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a PerkinElmer 341 polarimeter. UV absorption spectra were acquired on a PerkinElmer 650 spectrophotometer, and ECD spectra were measured in MeOH with a Chirascan spectrometer. IR spectra were obtained with KBr disks on a PerkinElmer Spectrum Two FT-IR spectrometer. NMR spectra were recorded on a 500 or 400 MHz Bruker AVANCE apparatus. HRESIMS data were acquired on a Thermo-Scientific Exactive mass spectrometer. Silica gel (200−300 mesh, Qingdao Marine Chem. Co., Ltd., China), C_{18} reversed-phase silica gel (50 μm, YMC, Japan), MCI gel (CHP20, 75−150 μm, MCC, Japan), and Sephadex LH-20 gel (Pharmacia Biotech, Sweden) were used for column chromatography (CC). Precoated silica gel plates (Qingdao Marine Chem. Co., Ltd., GF254) were used for analytical TLC. After spraying with a color reagent (10% H_2SO_4 in EtOH), heating revealed the spots. Semipreparative HPLC was performed on a YMC-Pack ODS-A column (250 × 20 mm, S-5 μm, Japan) with an LC3000 instrument (Chuang Xin Tong Heng Science and Technology Co., Ltd., Beijing, China) equipped with a UV3000 detector (Chuang Xin Tong Heng Science and Technology).

Plant Material. The stems of *T. sinensis* were collected in May 2014 in Guilin, Guangxi Province, China. The specimen (No. TS-2014016) was identified by Professor Shao-Qing Tang of the College of Life Science, Guangxi Normal University, and stored in the

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State Key Laboratory for Chemistry and Molecular Engineering of Medicinal Resources, Guangxi Normal University.

Extraction and Isolation. The air-dried stems of *T. sinensis* (17.3 kg) were extracted with 95% aqueous EtOH (3 × 100 L) under reflux. The extract (2.2 kg) was dispersed in H_2O and partitioned with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc fraction was concentrated to yield a dark brown gum (226.1 g), which was separated into seven fractions (Fr1−Fr7) by silica gel CC and gradient elution first with petroleum ether/acetone (10:1 to 1:1) and then with CH_2Cl_2/MeOH (8:1 to 2:1). Fraction Fr6 (24.8 g) was further fractionated by MCI CC (50:50 to 100:0 MeOH/H_2O) to afford eight subfractions (Fr6.1−Fr6.8). Subfraction Fr6.3 (3.4 g) was subjected to reversed-phase C_{18} (RP-C_{18}) CC (20:80 to 100:0 MeOH/H_2O) to yield eight subfractions (Fr6.3.1−Fr6.3.8). Subfraction Fr6.3.2 (232.7 mg) was purified by Sephadex LH-20 CC (MeOH) and then subjected to silica gel CC (CH_2Cl_2/MeOH, 20:1) to yield compound **2** (9.0 mg). The purification of subfraction Fr6.3.3 (282.0 mg) by semipreparative HPLC with 20:80 MeCN/H_2O (6 mL/min) as the isocratic solvent system yielded compound **1** (14.7 mg, t_R 43.7 min). Subfraction Fr6.3.6 (1.2 g) was fractionated by Sephadex LH-20 CC (MeOH) and purified by semipreparative HPLC (20:80 MeCN/H_2O, 6 mL/min) to yield compounds **12** (3.2 mg, t_R 23.9 min), **3** (18.6 mg, t_R 27.6 min), and **11** (173.2 mg, t_R 53.9 min). Subfraction Fr6.4 (1.2 g) was separated into four subfractions (Fr6.4.1−Fr6.4.4) via RP-C_{18} CC (30:70 to 100:0 MeOH/H_2O). Subfraction Fr6.4.2 (134.2 mg) was subjected to semipreparative HPLC (21:79 MeCN/H_2O, 6 mL/min) to yield compound **8** (16.8 mg, t_R 36.1 min). Compound **13** (33.2 mg, t_R 37.4 min) was purified from Fr6.4.4 (96.9 mg) by semipreparative HPLC (23:77 MeCN/H_2O, 8 mL/min). Subfraction Fr6.5 (5.0 g) was subjected to RP-C_{18} CC (30:70 to 100:0 MeOH/H_2O) to yield 11 subfractions (Fr6.5.1−Fr6.5.11). Subfractions Fr6.5.4 (270.7 mg) and Fr6.5.6 (376.7 mg) were purified via semipreparative HPLC (6 mL/min) and eluted with 25:75 MeCN/H_2O + 0.1% TFA to yield compounds **4** (47.4 mg, t_R 36.3 min) and **6** (30.7 mg, t_R 48.2 min). Subfraction Fr6.5.8 (244.4 mg) was subjected to Sephadex LH-20 CC (MeOH) and purified by semipreparative HPLC (25:75 MeCN/H_2O, 6 mL/min) to yield compound **9** (8.4 mg, t_R 25.1 min). Fr6.5.10 (557.9 mg) and Fr6.5.11 (292.6 mg) were purified via semipreparative HPLC (6 mL/min) and eluted with 25:75 MeCN/H_2O + 0.1% TFA to obtain compounds **7** (47.6 mg, t_R 52.1 min) and **10** (11.1 mg, t_R 58.7 min). Subfraction Fr6.6 (2.5 g) was subjected to silica gel CC (EtOAc/EtOH/H_2O, 64:2:1 to 8:2:1) and subsequently to semipreparative HPLC (45:55 MeOH/H_2O, 8 mL/min) to yield compound **5** (15.1 mg, t_R 65.1 min).

*Tinosinenoside A (**1**):* white, amorphous powder; $[\alpha]^{20}_D$ +2 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.17) nm; IR (KBr) ν_{max} 3416, 2931, 1738, 1381, 1161, 1068 cm^{-1}; ^1H NMR and ^{13}C NMR (MeOH-d_4), see Table 1; (+) HRESIMS *m*/*z* 559.1780 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{12}Na$ 559.1786).

*Tinosinenoside B (**2**):* white solid; $[\alpha]^{20}_D$ +47 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.03) nm; IR (KBr) ν_{max} 3428, 2924, 1641, 1425, 1066, 593 cm^{-1}; ^1H NMR and ^{13}C NMR (DMSO-d_6), see Table 1; (−) HRESIMS *m*/*z* 543.1643 $[M + Cl]^-$ (calcd for $C_{25}H_{32}O_{11}Cl$ 543.1639).

*Tinosinenoside C (**3**):* white, amorphous powder; $[\alpha]^{20}_D$ −14 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 209 (3.73) nm; IR (KBr) ν_{max} 3323, 2942, 1716, 1373, 1264, 1031, 603 cm^{-1}; ^1H NMR and ^{13}C NMR (MeOH-d_4), see Table 1; (−) HRESIMS *m*/*z* 591.1857 $[M + Cl]^-$ (calcd for $C_{26}H_{36}O_{13}Cl$ 591.1850).

*Tinosinenoside D (**4**):* white, amorphous powder; $[\alpha]^{20}_D$ +28 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 209 (5.12), 283 (4.69), 323 (4.37) nm; IR (KBr) ν_{max} 3437, 2944, 1712, 1375, 1260, 1133, 1071 cm^{-1}; ^1H NMR (MeOH-d_4 and DMSO-d_6), see Table 2; ^{13}C NMR (MeOH-d_4), see Table 3; (+) HRESIMS *m*/*z* 755.2505 $[M + Na]^+$ (calcd for $C_{36}H_{44}O_{16}Na$ 755.2522).

*4-epi-Tinosinenoside D (**5**):* white, amorphous powder; $[\alpha]^{20}_D$ +7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.42), 294 (4.23), 318 (4.22) nm; IR (KBr) ν_{max} 3434, 2932, 1709, 1383, 1258, 1128, 1074 cm^{-1}; ^1H NMR (MeOH-d_4), see Table 2; ^{13}C NMR (MeOH-d_4) see Table 3; (+) HRESIMS *m*/*z* 755.2508 $[M + Na]^+$ (calcd for $C_{36}H_{44}O_{16}Na$ 755.2522).

*Tinosinenoside E (**6**):* white, amorphous powder; $[\alpha]^{20}_D$ −55 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.51), 291 (4.13), 320 (4.13) nm; IR (KBr) ν_{max} 3390, 2942, 1717, 1382, 1264, 1136, 1066 cm^{-1}; ^1H NMR (MeOH-d_4 and DMSO-d_6), see Table 2; ^{13}C NMR (MeOH-d_4), see Table 3; (+) HRESIMS *m*/*z* 755.2501 $[M + Na]^+$ (calcd for $C_{36}H_{44}O_{16}Na$ 755.2522).

*Tinosinenoside F (**7**):* white, amorphous powder; $[\alpha]^{20}_D$ +3 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.71), 290 (4.33), 320 (4.31) nm; IR (KBr) ν_{max} 3437, 2939, 1717, 1378, 1254, 1140, 1071 cm^{-1}; ^1H NMR (MeOH-d_4), see Table 2; ^{13}C NMR (MeOH-d_4), see Table 3; (+) HRESIMS *m*/*z* 755.2512 $[M + Na]^+$ (calcd for $C_{36}H_{44}O_{16}Na$ 755.2522).

*2-Deacetyltinosinenoside D (**8**):* white, amorphous powder; $[\alpha]^{20}_D$ −17 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.30), 292 (4.24), 317 (4.25) nm; IR (KBr) ν_{max} 3424, 2937, 1704, 1380, 1258, 1136, 1072 cm^{-1}; ^1H NMR (MeOH-d_4), see Table 2; ^{13}C NMR (MeOH-d_4), see Table 3; (+) HRESIMS *m*/*z* 713.2405 $[M + Na]^+$ (calcd for $C_{34}H_{42}O_{15}Na$ 713.2416).

*4-epi-2-Deacetyltinosinenoside D (**9**):* white, amorphous powder; $[\alpha]^{20}_D$ +17 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.35), 293 (4.22), 319 (4.22) nm; IR (KBr) ν_{max} 3427, 2941, 1701, 1383, 1258, 1174, 1074 cm^{-1}; ^1H NMR (MeOH-d_4 and DMSO-d_6), see Table 2; ^{13}C NMR (MeOH-d_4), see Table 3; (+) HRESIMS *m*/*z* 713.2405 $[M + Na]^+$ (calcd for $C_{34}H_{42}O_{15}Na$ 713.2416).

*2-Deacetoxytinosinenoside D (**10**):* white, amorphous powder; $[\alpha]^{20}_D$ −20 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.35), 293 (4.22), 320 (4.23) nm; IR (KBr) ν_{max} 3428, 2941, 1707, 1380, 1257, 1131, 1070 cm^{-1}; ^1H NMR (MeOH-d_4), see Table 2; ^{13}C NMR (MeOH-d_4), see Table 3; (+) HRESIMS *m*/*z* 697.2453 $[M + Na]^+$ (calcd for $C_{34}H_{42}O_{14}Na$ 697.2467).

Acid Hydrolysis of Compounds 1−10. Each of **1**−**10** (each 1.0 mg) was added to 1.0 mL of 6% hydrochloric acid. The reaction mixture was refluxed at 80 °C for 4 h and extracted with EtOAc (3 × 2 mL) to remove the aglycone. The aqueous layer was subjected to silica gel CC (EtOAc/EtOH/H_2O, 7:4:1) to obtain the sugar fraction. The sugar fraction was subjected to HPLC (Jasco LC-4000) under the following conditions: a Shodex Asahipak NH2P-50 4E (250 mm × 4.6 mm, 5 μm) column; a Jasco OR-4090 optical rotation detector; and a 78:22 MeCN/H_2O mobile phase (1 mL/min). The retention time (t_R 11.8 min) and positive optical rotation of the sugar were compared with those of an authentic sample, confirming the sugar to be D-glucose.

ECD Calculations. The conformational analysis was initially performed using Confab[31] with the MMFF94 force field. The conformers chosen for the ECD calculations were from above 1% of the Boltzmann population. The selected conformer was optimized at B3LYP/6-311G** using DFT. It was further optimized in MeOH using the conductor-like polarizable continuum model calculation model. The ECD calculations were conducted using the TDDFT method at the B3LYP/6-311G** level in MeOH. After overlapping the Gaussian functions for each transition, the ECD spectrum was simulated in SpecDis. All calculations were performed with the Gaussian 09 program package.

Cytotoxicity Assay. Cytotoxicity was measured using the MTT assay.[32] In short, 8 × 10^3 HeLa cells per well (in 100 μL of culture medium) were seeded in 96-well plates (Corning). Cells were incubated with five concentrations (2.5, 5, 10, 20, and 50 μM) of each compound in triplicate at 37 °C for 48 h, and doxorubicin was used as a positive control. The MTT solution (20 μL, 5 mg/mL) was directly dropped into the proper wells. After 4 h, the formazan crystals of the surviving cells were dissolved by adding 150 μL of DMSO to each well. The absorbance values of each well at 570 nm were measured using a microplate spectrophotometer (iMark, Bio-Rad, USA). The IC_{50} values were calculated by the Logit method.

NO Production Measurement and Cell Viability Assay. The Griess reaction was used to measure both the accumulation of nitrite in the culture supernatants and the NO synthase activity.[33] The viability of the microglial cells was evaluated by the MTT assay.[33]

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00976.

Computational section and the ECD spectra of compounds **1**−**3** as well as the 1D NMR, 2D NMR, and HRESIMS spectra of compounds **1**−**10** (PDF)

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Notes

The authors declare no competing financial interest.

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