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Trigochinins A−C: Three New Daphnane-Type Diterpenes from *Trigonostemon chinensis*

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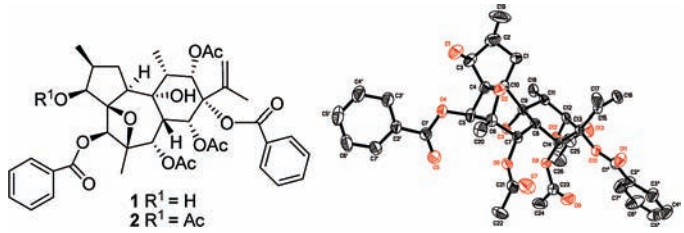
ABSTRACT



Three highly oxygenated new diterpenes, trigochinins A−C (1−3) were isolated from *Trigonostemon chinensis*. Their structures with the absolute configuration were determined by a spectroscopic method, X-ray crystallography, and CD analysis. This study suggested the revision of the C-6 stereochemistry of trigonothyrins A−C reported quite recently. Compound 3 showed significant inhibition against MET tyrosine kinase activity with a IC_{50} value of 1.95 μM.

The genus *Trigonostemon* (Euphorbiaceae) comprised of ca. 50 species grows mainly in the tropical and subtropical regions of Asia.[1] Previous chemical investigations on this genus have led to the isolation of an array of structurally interesting compounds, including the modified diterpenoids,[2−4] flavonoidal indole alkaloid,[5] and phenanthrenes.[6] The modified daphnane-type diterpenoids have been found to possess antiflea insecticide,[2,3] a cytotoxic,[3b] and acaricidal[3c] activities. In continuation of our research, two highly modified daphnane-type diterpenoids, trigochilides A and B with unique 12-carbon-containing polyketide appendages at C-16 forming a macro-lactone with C-3, were isolated from the twigs and leaves of *Trigonostemon chinensis* Merr.[7] In continuation, three highly oxygenated novel daphnane-type diterpenes, trigochinins A−C (**1**−**3**), were isolated from the twigs and leaves of *T. chinensis*. Their structures with the absolute configuration were established on the basis of

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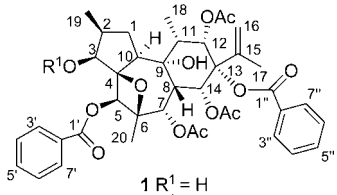
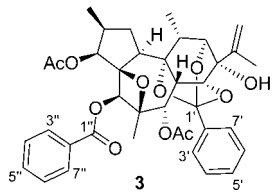
a spectroscopic method, X-ray crystallography, and CD analysis. The solid data of spectroscopic analysis and X-ray diffraction allowed revision of the C-6 stereochemistry of trigonothyrins A−C reported quite recently.

MET tyrosine kinase is a central mediator of cell growth, survival, motility, and morphogenesis during the development. Recently, accumulating evidence highlighted that the overexpression and/or dysregulation of MET tyrosine kinase have been widely documented in many human tumor types, and correlates with a poor prognostic outcome in patients. Therefore, MET tyrosine kinase has been considered as a versatile candidate for targeted cancer chemotherapy.[8]

Compound **3** showed significant inhibition on MET tyrosine kinase activity with a IC_{50} value of 1.95 μM, and SU11274 was used as the positive control with a IC_{50} value of 10.0 nM.

We present herein the isolation, structural elucidation, and biological evaluation of these compounds.





The plant material of *T. chinensis* was collected from Xishuangbanna Tropical Botanical Garden of China. The air-dried powder of leaves and twigs of *T. chinensis* (6 kg) was extracted with 95% EtOH at rt three times to give an ethanolic extract (314 g), which was partitioned between EtOAc and water to obtain the EtOAc soluble fraction E (91 g). Fraction E was separated on a column of MCI gel CHP20P (MeOH/H_2O, 4/6 to 9/1, v/v) to afford six subfractions 1a−1g. Fraction 1a (2.53 g) was separated over a column of reverse-phase silica gel (MeOH/H_2O, 7/3 to 9/1, v/v) to afford three major fractions 1a1 (850 mg), 1a2 (316 mg), and 1a3 (278 mg). Fraction 1a3 was subjected to a silica gel column eluted with petroleum ether/EtOAc (5:1, v/v) to afford compounds **1** (40 mg) and **2** (100 mg). Fraction 1b (340 mg) was chromatographed on a silica gel column (petroleum ether/EtOAc, 5/1, v/v), and the major component was then purified by silica gel chromatography eluted with CH_2Cl_2/MeOH (100/1, v/v) to give compound **3** (7 mg).

Trigochinin A (**1**),[9] obtained as colorless crystals, had a molecular formula of $C_{40}H_{46}O_{13}$ as determined by HRESIMS ion at *m/z* 757.2842 $[M + Na]^+$ (calcd for $C_{40}H_{46}O_{13}Na$, 757.2836) with 18 degrees of unsaturation. The IR absorptions implied the presence of hydroxyls (3568, 3446 cm^{-1}) and ester carbonyl groups (1755, 1741, 1724 cm^{-1}). In accordance with its molecular formula, all 40 carbons were well resolved as 40 carbon resonances in the ^{13}C NMR spectrum (Supporting Information, Section S2, Table 2), and were further classified by DEPT experiments as seven methyls, two methylenes (one olefinic), nineteen methines (five oxygenated and ten olefinic ones), and twelve quaternary carbons (five ester carbonyls, four oxygenated and three olefinic carbons). Two tertiary methyls at δ_H 1.95 (s, 3H) and 1.33 (s, 3H), two secondary methyls at δ_H 1.17 (d, J = 6.9 Hz, 3H) and 0.95 (d, J = 7.2 Hz, 3H), a terminal double bond at δ_H 5.49 (s, 1H) and 5.44 (s, 1H), three acetyl groups, and two benzoyl groups were distinguished by analysis of the NMR data (Supporting Information, Sections S1 and S2, Tables 1 and 2). The above functionalities accounted for 14 degrees of unsaturation, and the remaining four degrees of unsaturation required that compound **1** was tetracyclic in the diterpenoid core. Furthermore, two proton resonances at δ_H 3.61 (s), and 2.79 (d, J = 10.2 Hz), which did not correlate with any carbons in the HSQC spectrum, were only attributable to the presence of hydroxyls.

Comprehensive analysis of the 1D and 2D NMR spectra of **1**, especially the HMBC spectrum (Figure 1a), allowed the establishment of the typical A, B, and C rings of a daphnane-type diterpenoid.[10] In the HMBC spectrum, two hydroxyls resonating at δ_H 3.61 and 2.79 were assigned to C-9 and C-3 by the key correlations between 9-OH and C-9

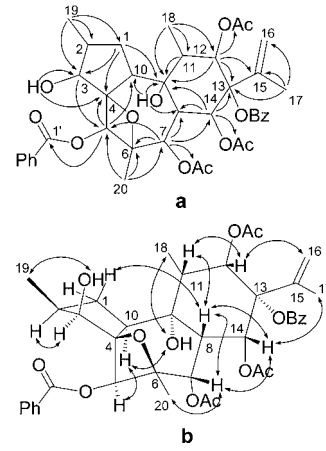


Figure 1. (a) Selected HMBC (H→C) and (b) ROESY (H↔H) correlations of **1**.

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(9) **Trigochinin A (1):** prisms; mp 255−257 °C; $[\alpha]^{21}_D$ −6.0 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 231.0 (4.50) nm; IR (KBr) ν_{max} 3568, 3446, 2983, 2931, 1755, 1741, 1724, 1452, 1375, 1275, 1234, 1119, 1026, 714 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) (see the Supporting Information, Tables 1 and 2); positive mode ESIMS *m/z* 757.2 $[M + Na]^+$, 1491.5 $[2M + Na]^+$; EIMS *m/z* 675 (10), 493 (5), 381 (27), 216 (11), 177 (13), 105 (100), 77 (8); HRESIMS *m/z* 757.2842 $[M + Na]^+$ (calcd for $C_{40}H_{46}O_{13}Na$, 757.2836).

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(δ_C 76.7) and between 3-OH and C-3 (δ_C 72.1), respectively; three acetoxyls were placed on C-7 (δ_C 78.9), C-12 (δ_C 72.7), and C-14 (δ_C 75.3) on the basis of the HMBC correlations from H-7, H-12, and H-14 to each corresponding carbonyl of the acetyls, respectively; one benzoyl group was attached to C-5 (δ_C 73.6) by the HMBC correlation from H-5 at δ_H 6.30 (s) to the carbonyl of OBz. According to the molecular formula, the presence of one additional ring in compound **1** was required, implying that an oxetane ring was most likely formed between two oxygenated quaternary carbons at δ_C 92.8 (C-4) and 84.8 (C-6) since they were severely downfield shifted. In consequence, the remaining oxygenated quaternary carbon resonance at δ_C 81.9 was tentatively assigned to C-13 bearing the only leftover benzoyloxy group by the HMBC correlations from H-12, H-14, and H-17 to C-13. To confirm this assignment, a single-crystal X-ray diffraction study (Figure 2) was conducted, which not only corroborated the

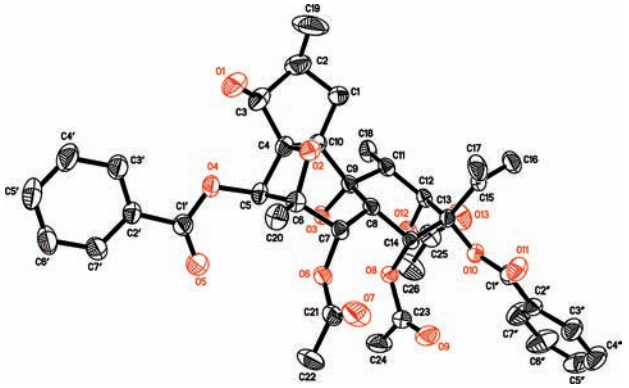

Figure 2. Single-crystal X-ray structure of **1**.

planar structure of compound **1** as assigned above, but also established the relative configuration of **1** unambiguously. The relative configuration and the conformation of **1** determined by single-crystal X-ray diffraction were consistent with those of **1** in solution as fixed by the ROESY experiment (Figure 1b).

Trigochinin B (**2**)[11] possessed the molecular formula of $C_{42}H_{48}O_{14}$ as determined by HRESIMS. Comparison of the ^{1}H and ^{13}C NMR data (Supporting Information, Sections S1 and S2, Tables 1 and 2) of **2** with those of **1** revealed that the only difference between the two compounds was the presence of one additional acetyl group in **2**, and this was supported by its molecular formula, which showed 42 mass units more than that of **1**. In the ^{1}H NMR spectrum of **2**, the H-3 at δ_H 5.22 (d, J = 10.4 Hz) was downfield shifted ca. $\Delta\delta$ 1.04 as compared with that for **1**, indicating that an acetoxy was located at the C-3 (δ_C 73.8) of **2**. As the result, both the C-2 (δ_C 31.0) and C-4 (δ_C 91.0) of **2** were upfield shifted ca. $\Delta\delta$ 1.8 as compared with those of **1** due to the γ-gauche effects from 3-OAc. The structure of **2** was further confirmed by a combined analysis of HMBC and ROESY spectra (Supporting Information, Section S3).

Trigochinin C (**3**)[12] showed the molecular formula $C_{38}H_{42}O_{11}$ as determined by the HRESIMS at *m/z* 697.2631 $[M + Na]^+$ (calcd for $C_{38}H_{42}O_{11}Na$, 697.2625). The IR absorptions implied the presence of hydroxyls (3560, 3450 cm^{-1}) and ester carbonyl groups (1736 cm^{-1}). Comparison of the ^{1}H and ^{13}C NMR data (Supporting Information, Sections S1 and S2, Tables 1 and 2) of **3** with those of **1** indicated that it was also a daphnane-type diterpenoid possessing a 4,6-oxetanyl group. In the HMBC spectrum (Supporting Information, Section S3), a hydroxy group resonating at δ_H 3.81 (br s, 1H) was assigned to C-13 by the key HMBC correlation between 13-OH and C-13 at δ_C 69.4; two acetoxy groups were placed at C-3 at δ_C 74.0 and C-7 at δ_C 77.0 on the basis of HMBC correlations from H-3 and H-7 to each corresponding acetyl carbonyl, respectively; the benzoyl group was attached to C-5 at δ_C 73.7 by the HMBC correlation between the carbonyl of OBz and H-5 at δ_H 6.15 (s). The remaining three oxygenated carbons were assigned to C-9 at δ_C 77.0, C-12 at δ_C 79.6, and C-14 at δ_C 76.2 by the multiple HMBC correlations of Me-18/C-9, Me-18/C-12, H-8/C-9, H-14/C-13, H-12/C-13, H-12/C-15, and H-7/C-14. A typical quaternary carbon at δ_C 108.8 assignable to C-1′ was indicative of the presence of an orthobenzoate,[3,4] and an 9,12,14-orthobenzoate was thus assigned, which was confirmed by the HMBC correlations from H-12, H-14, and H-3′ (or H-7′) to C-1′.

The relative stereochemistry of **3** was established by comparing its NMR data with those of **1** and ROESY experiment (Supporting Information, Section S3). The similar NMR patterns of the two compounds indicated that they share the same relative configuration in the daphnane diterpenoid core, which was confirmed by the observed key ROESY correlations from H-8 to H-7, H-11, and H-14, from H-11 to H-1, and from H-10 to H-3 and H-5. The ROESY correlations of H-14/H_3-17, and H_2-16/H-11 and H-12 revealed that the C-15-C-17 moiety at C-13 was β-oriented, and the 9,12,14-orthobenzoate was α-directed.

The absolute configuration of **1**−**3** was determined by applying the CD exciton chirality method.[13] The positive Cotton effect (λ_{max} = 233 nm) and the negative Cotton effect (λ_{max} = 211 nm) of **1**, which centered at around the UV maximum (λ_{max} = 231 nm, log ε 4.50) of a benzoate,[14] were recognized to be the exciton coupling between the benzoate moiety at C-13 and the Δ^{15} double bond, indicating that

(11) **Trigochinin B (2):** white powder; $[\alpha]^{21}_D$ +18.0 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 231.0 (4.43) nm; IR (KBr) ν_{max} 3583, 3433, 2976, 2933, 1757, 1736, 1452, 1375, 1277, 1234, 1119, 1028, 714 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) (see the Supporting Information, Tables 1 and 2); positive mode ESIMS *m/z* 799.3 $[M + Na]^+$; EIMS *m/z* 717 (9), 535 (5), 381 (11), 177 (12), 105 (100), 77 (8); HRESIMS *m/z* 799.2955 $[M + Na]^+$ (calcd for $C_{42}H_{48}O_{14}Na$, 799.2942).

(12) **Trigochinin C (3):** white powder; $[\alpha]^{21}_D$ + 42.0 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 230.8 (4.17) nm; IR (KBr) ν_{max} 3560, 3450, 2978, 2935, 1736, 1452, 1371, 1277, 1232, 1095, 1026, 716 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) (see Supporting Information, Tables 1 and 2); positive mode ESIMS *m/z* 697.2 $[M + Na]^+$; EIMS *m/z* 657 (33), 469 (13), 427 (11), 176 (34), 105 (100), 77 (35); HRESIMS *m/z* 697.2631 $[M + Na]^+$ (calcd for $C_{42}H_{48}O_{14}Na$, 697.2625).

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compound **1** had a positive chirality (Figure 3). The two chromophores were arranged in a clockwise manner to assign

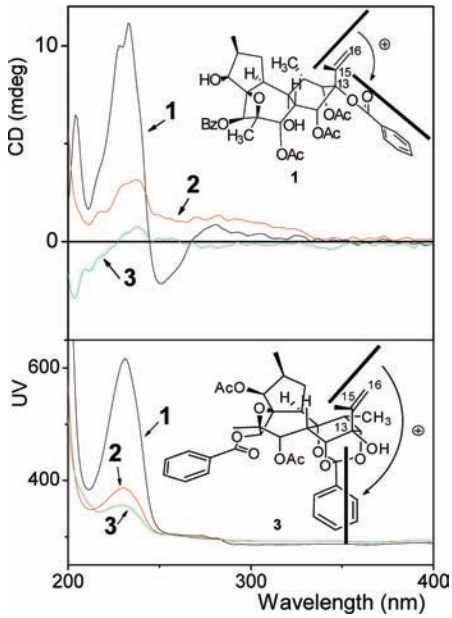

Figure 3. CD and UV spectra of compounds **1**−**3**. Stereoviews of compounds **1** and **3**. Bold lines denote the electric transition dipole of the chromophores for compounds **1** and **3**.

the absolute configuration of **1** as depicted. The CD split manner of compound **2**, which centered at the UV maximum (ca. λ_{max} = 231 nm) of a benzoate, was similar to that of **1**. The absolution configuration of compound **2** was thus defined as shown. The CD spectrum (Figure 3) of compound **3** showed high similarity to that of **2**. The first positive Cotton effect of **3** at 238 nm was readily distinguished, which is considered to be aroused from the exciton coupling between the 9,12,14-orthobenzoate and the Δ^{15} double bond. The clockwise manner of two coupling chromophores in the space allowed the assignment of its absolute configuration as shown.

The most interesting structural feature of compounds **1**−**3** is the oxetane ring that formed between two oxygenated quaternary carbons C-4 and C-6, which is unprecedented in the family of daphnane diterpenes until quite recently a similar 4,6-oxetane ring with a different stereochemistry at C-6 of trigonothyrins A−C[15] was reported. However, the extensive comparison of NMR data of trigochinins A−C with those of trigonothyrins A−C showed that the proton and carbon resonances of the 4,6-oxetane ring or its adjacent atoms of these two classes of compounds are very similar, and the solid fact that the structure of **1** was determined by X-ray diffraction indicated that the stereochemistry at C-6 of trigonothyrins A−C required revision as the same as that of compounds **1**−**3**.

In this study, compounds **1**−**3** were evaluated against MET tyrosine kinase activity according to the reported protocol.[16] In the enzyme-linked immunosorbent assay (ELISA) with purified MET tyrosine kinase, compound **3** exhibited significant inhibition against MET tyrosine kinase activity with a IC_{50} value of 1.95 μM, and (3*Z*)-*N*-(3-chlorophenyl)-3-({3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1*H*-pyrrol-2-yl}methylene)-*N*-methyl-2-oxoindoline-5-sulfonamide (SU11274) was used as the positive control with a IC_{50} value of 10.0 nM.[17]

Acknowledgment. Financial support of the National Natural Science Foundation (Grant No. 30630072; 30721005; 20932007) and National Science & Technology Major Project "Key New Drug Creation and Manufacturing Program" (Grant No. 2009ZX09301-001) of the People's Republic of China is gratefully acknowledged. We thank Prof. Y.-K. Xu of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences for the identification of the plant material.

Supporting Information Available: ^{1}H and ^{13}C NMR data of compounds **1**−**3**, selected HMBC and ROESY correlations of **2** and **3**, Experimental Section, ^{1}H, ^{13}C, and 2D NMR (HSQC, HMBC, and ROESY), EIMS and IR spectra of trigochinins A−C (**1**−**3**), and the X-ray crystallographic data for trigochinin A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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