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Trichiconins A–C, Limonoids with New Carbon Skeletons from *Trichilia connaroides*

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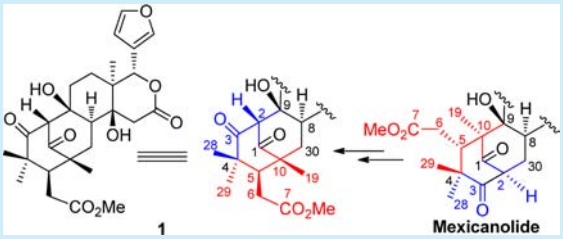
Supporting Information

ABSTRACT: Three limonoids, trichiconin A (**1**) possessing a new carbon skeleton of a rearranged A,B-ring system and trichiconins B (**2**) and C (**3**) featuring an unprecedented A,B,D-*seco* skeleton, were isolated from the twigs of *Trichilia connaroides*. The carbon scaffold of trichiconin A is designated as trichiconane. Their structures with absolute stereochemistry were determined by spectroscopic data, X-ray crystallography, and CD analysis. Compounds **2** and **3** showed modest anti-HIV activities.



Limonoids are the characteristic metabolites of the Meliaceae plants and possess diverse structures and significant biological activities.[1] Particularly, a large array of limonoids with a wide spectrum of biological activities, such as cytotoxic,[2] antimalarial,[3] antifeedant,[4] and anti-inflammatory[5] properties, have been isolated from the plants of the Meliaceae family in recent years. *Trichilia connaroides* (Wight et Arn.) Bentv. (Meliaceae), a tall tree that mainly grows in the southeast of Asia,[6] has been applied by local residents as a folk medicine to treat arthritis, pharyngitis, and tonsillitis.[6b] Previously, six limonoids and two degradation products of limonoids were isolated from this plant that grows in a different location.[6c] In the continuing search for structurally interesting and biologically important limonoids, trichiconin A (**1**) possessing a new carbon skeleton of a rearranged A,B-ring system and trichiconins B (**2**) and C (**3**) featuring an unprecedented A,B,D-*seco* skeleton (Figure 1) were isolated from the twigs of *T. connaroides*, which was collected from Guangxi Province of China. Compounds **2** and **3** showed modest anti-HIV activity with EC_{50} values of 5.9 ± 0.4 (selective index, SI > 4.2) and 3.6 ± 0.6 (SI = 3.5) μM, respectively. Herein, the isolation, structural elucidation, and anti-HIV evaluation of these limonoids are discussed.

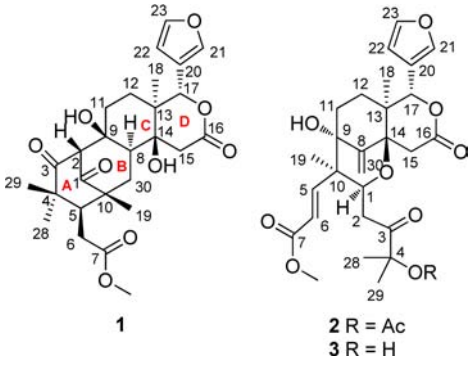


Figure 1. Structures of trichiconins A–C (**1**–**3**).

Trichiconin A (**1**)[7] was obtained as a colorless crystal. The molecular formula was assigned as $C_{27}H_{34}O_9$ by the HRESI(+)MS ion peak at *m*/*z* 525.2098 $[M + Na]^+$ (calcd 525.2101), requiring 11 indices of hydrogen deficiency. The IR spectrum suggested the presence of hydroxy (3492, 3437 cm^{-1}) and carbonyl (1730 cm^{-1}) groups. Analysis of the NMR data (Table 1) revealed the existence of two keto groups (δ_C 211.5 and 209.0), two ester carbonyls (δ_C 173.8 and 170.0), and a typical β-substituted furan ring. In addition, five methyls (one methoxy), five methylenes, four methines (one oxygenated), and five quaternary carbons (two oxygenated) were also distinguished. The aforementioned data suggested that compound **1** was a limonoid possessing a tetracyclic core.

Three proton-bearing fragments of C-5–C-6, C-11–C-12, and C-8–C-30 as drawn with the bold bond (Supporting Information (SI), S2A) were readily established by a $^1H-^1H$ COSY spectrum. Subsequently, these fragments with the quaternary carbons and oxygen atoms were connected to delineate the planar structure of **1** by the HMBC spectrum (SI, S2A). Two singlet proton signals that showed no correlation with any carbons in HSQC (SI, Figure S4) were assigned to 9-OH and 14-OH, respectively, by the HMBC correlations of 9-OH/C-9 and 14-OH/C-14. In the HMBC, the correlation networks of CH_3-18/C-12, C-13, C-14, and C-17; H-11/C-9; H-15/C-16; 9-OH/C-2 and C-8; and 14-OH/C-8 and C-15 defined the intact C and D rings of a typical limonoid for **1**. The β-substituted furan ring was then located at C-17 by the HMBC correlations of H-17/C-20, C-21, and C-22. The multiple HMBC correlations of

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compounds 1−3 in $CDCl_3$

	1		2		3	
no.	δ_H (multi, J in Hz)	δ_C	δ_H (multi, J in Hz)	δ_C	δ_H (multi, J in Hz)	δ_C
1		211.5	3.85 (d, 10.0)	76.0	3.91 (d, 10.1)	75.8
2	3.00 (s)	70.6	α 2.69 (dd, 16.5, 10.0)	36.7	α 2.81 (dd, 16.9, 10.1)	36.7
			β 1.87 (d, 16.5)		β 2.05 (d, 16.9)	
3		209.0		206.2		211.7
4		50.6		83.9		76.8
5	2.61 (d, 11.0)	45.0	7.33 (d, 16.0)	147.9	7.38 (d, 16.0)	147.6
6	α 2.44 (m)	32.5	5.94 (d, 16.0)	122.6	5.99 (d, 16.0)	122.9
	β 2.53 (m)					
7		173.8		166.9		166.9
8	1.88 (m)	38.3		146.9		147.0
9		81.2		76.3		76.4
10		48.1		52.8		52.8
11	α 2.45 (m)	30.9	α 1.49 (td, 13.8, 5.8)	35.7	α 1.51 (td, 13.5, 6.0)	36.0
	β 1.56 (d, 14.7)		β 1.94 (m)		β 1.98 (m)	
12	α 1.13 (m)	24.9	α 1.18 (dd, 13.0, 5.8)	29.6	α 1.21 (dd, 13.0, 6.0)	29.6
	β 1.86 (m)		β 2.00 (m)		β 2.02 (m)	
13		40.5		41.1		41.2
14		74.2		81.6		81.8
15	α 2.76 (d, 18.2)	39.5	α 2.91 (d, 18.0)	33.8	α 2.96 (d, 18.0)	33.9
	β 2.55 (m)		β 2.71 (d, 18.0)		β 2.78 (d, 18.0)	
16		170.0		169.7		169.6
17	5.79 (s)	79.2	5.53 (s)	79.4	5.53 (s)	79.4
18	0.93 (s)	15.0	0.80 (s)	14.0	0.84 (s)	14.1
19	1.15 (s)	19.7	0.94 (s)	16.3	0.98 (s)	16.5
20		120.9		121.0		121.0
21	7.40 (s)	141.1	7.43 (s)	140.7	7.44 (s)	140.8
22	6.36 (s)	110.1	6.37 (s)	109.8	6.38 (s)	109.8
23	7.40 (s)	143.1	7.38 (s)	143.0	7.39 (s)	143.1
28	1.19 (s)	23.3	1.42 (s)	22.5	1.31 (s)	25.7
29	0.81 (s)	20.9	1.30 (s)	23.8	1.30 (s)	26.6
30	2.11 (m)	40.2	*pro-E* 5.50 (s)	110.6	*pro-E* 5.54 (s)	110.7
			pro-Z 5.09 (s)		*pro-Z* 5.14 (s)	
7-OMe	3.75 (s)	52.5	3.78 (s)	51.9	3.81 (s)	52.0
4-OAc			2.02 (s)	21.2		
				170.7		
4-OH					3.38 (s)	
9-OH	3.34 (s)					
14-OH	4.60 (s)					

CH_3-28 (29)/C-3, C-4, and C-5; H-2/C-1, C-3, and C-10; H-5, H-6, and 7-OCH_3/C-7; and CH_3-19/C-1, C-5, and C-10 established the A ring. The key HMBC correlations of H-2/C-8, C-9, and C-11; 9-OH/C-2; and CH_3-19/C-30 constructed a unique B ring by featuring the unprecedented C-2−C-9 and C-10−C-30 bonds.

In the ROESY spectrum (SI, S2B), the cross-peaks of H-17/H-12β and 14-OH; 14-OH/H-15β; and CH_3-18/H-12α and H-8α allowed the assignments of CH_3-18 and the furan ring in an α-orientation and 14-OH in a β-direction, randomly. Subsequently, the ROESY correlations of H-5/CH_3-28 and H-8/H-5, H-15α, and CH_3-18 suggested that H-5 and H-8 were α-oriented. The H-2, CH_3-19, CH_3-29, and 9-OH were accordingly assigned to be β-configured by the ROESY correlations of CH_3-29/CH_3-19 and H-2, and H-2/9-OH. Finally, the structure of **1** was confirmed by a successful performance of single-crystal X-ray diffraction, which also determined its absolute configuration [the absolute structure parameter of −0.13(7)][8] (Figure 2). The conformation of **1** in solution ($CDCl_3$) as imaged by ROESY data was consistent with that of solid state as determined by X-ray study.

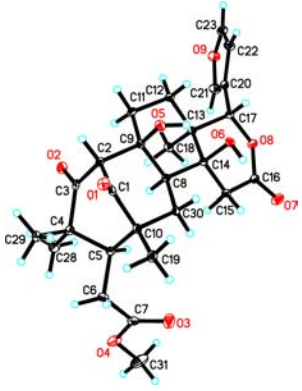

Figure 2. Single-crystal X-ray structure of **1**.

Trichiconin A (**1**) has a rearranged carbon skeleton without precedent among the known limonoid families. We propose to name this limonoid scaffold trichiconane.

Trichiconin B (**2**),[9] a colorless crystal, possessed the molecular formula of $C_{29}H_{36}O_{10}$ as determined by the HRESI(+)MS ion at

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m/z 567.2195 $[M + Na]^+$ (calcd 567.2206), requiring 12 indices of hydrogen deficiency. The presence of hydroxy and carbonyl groups was evident from the IR absorptions at 3479 and 1736 cm^{-1}, respectively. The ^{13}C NMR data (Table 1) showed 29 carbon resonances, which were classified by DEPT and HSQC spectra as six methyl, five methylene, seven methine, and 11 quaternary carbons. A methoxy, an acetyl, a 1,2-disubstituted double bond, an exocyclic double bond, and a typical β-substituted furan ring were discerned by the NMR data analysis (Table 1). This evidence suggested that compound **2** was also a limonoid. The functionalities of four carbonyls, two double bonds, and the furan ring accounted for nine indices of hydrogen deficiency, and the remaining three required the presence of three additional rings in **2**.

The intact C, δ-lactone D, and furan E rings of a limonoid for **2** were readily delineated by a comprehensive analysis of its 1D and 2D NMR data, in particular the HMBC spectrum (SI, S3A), in which the multiple correlations as drawn confirmed the connections of C−E rings. The furan ring was located at C-17 by the HMBC correlations of H-17/C-20, C-21, and C-22. The HMBC correlations of H_2-11/C-10 and CH_3-19/C-1, C-5, C-9, and C-10 allowed the rational attachments of C-1, C-5, C-9, and C-19 to the quaternary C-10 at δ_C 52.8. The HMBC correlations from H_2-30 to C-8, C-9, and C-14 fixed the exocyclic $\Delta^{8(30)}$ double bond in the C ring, and the key HMBC correlation from H-1 at δ_H 3.85 to the oxygenated quaternary C-14 at δ_C 81.6 established the 1,14-epoxy ring. In addition, the only hydroxy group was positioned at C-9 (δ_C 76.3) based on the chemical shift.[10] The HMBC correlations of H-5/C-6, and C-7 (δ_C 166.9), H-6/C-7, and OCH_3/C-7 established a linkage of $-CH{=}CHCO_2CH_3$. Similarly, the multiple HMBC correlations of H-1/C-2 and C-3, H_2-2/C-3, and CH_3-28(29)/C-3, and C-4 furnished a sequence of $-CH_2COC(CH_3)_2-$ and attached the C-2 to C-1. The remaining acetoxy group was only assignable to the C-4 at δ_C 83.9. Thus, the planar structure of **2** featuring an unprecedented A,B,D-seco skeleton was constructed.

The relative configuration of **2** was partially assigned by the coupling constants and ROESY spectrum. The Δ^5 double bond was assigned in an *E*-geometry by the coupling constant ($J_{5,6}$ = 16.0 Hz). In the ROESY spectrum (SI, S3B), the correlations of H_3-18/H-15α, H_3-18/H-21, and H-17/H-12β indicated that CH_3-18 and the furan ring were cofacial and arbitrarily assigned to be α-oriented. Consequently, the H-1 and CH_3-19 were fixed in an α-orientation by the ROESY correlations of H_3-18/$H_{pro\text{-}Z}$-30, H_3-19/$H_{pro\text{-}E}$-30, and H_3-19/H-1. The relative configurations of C-9 and C-14 could not be assigned by the available NMR data. To our delight, a qualified crystal was obtained in a mixture of petroleum ether/EtOAc (10:1). A single crystal X-ray diffraction analysis was thus performed successfully with the absolute structure parameter of −0.02(6),[8] which not only secured the planar structure but also determined its absolute configuration as 1*S*, 9*S*, 10*R*, 13*S*, 14*R*, and 17*R* (Figure 3).

Trichiconin C (**3**)[11] had a molecular formula of $C_{27}H_{34}O_9$ based on the HRESI(+)MS ion at *m/z* 525.2097 $[M + Na]^+$ (calcd 525.2101), which showed 42 mass units less than that of **2**, suggesting that it was likely the 4-*O*-deacetyl derivative of **2**. The NMR data of **3** highly resembled those of **2**, except for its C-3 at δ_C 211.7 shifted downfield ($\Delta\delta_C$ +5.5) and C-4 at δ_C 76.8 shifted upfield ($\Delta\delta_C$ −7.1) as compared with the counterparts of **2**, further indicating the presence of a hydroxy group at C-4 of **3**. This was confirmed by the key HMBC correlations from 4-O<u>H</u> to C-3, C-4, and C-28 (SI, Figure S21). The chemical shifts of C-3 and C-4, as well as the observation of 4-O<u>H</u> at δ_H 3.38

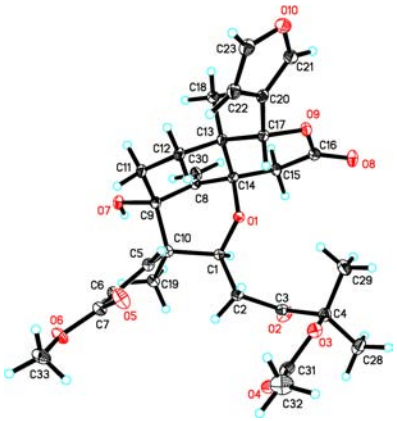

Figure 3. Single-crystal X-ray structure of **2**.

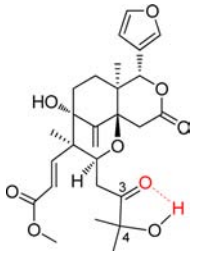

Figure 4. Formation of hydrogen bond in compound **3**.

(1H, s), suggested the formation of an intramolecular H-bond between 4-OH and the 3-keto group (Figure 4), which stabilized the proton of 4-OH and resulted in the obviously downfield shifted C-3 and upfield shifted C-4 as compared with those of **2** due to the electron-withdrawing and shielding effects of the H-bond, respectively. The relative stereochemistry of **3** was assigned to be identical to that of **2** by ^1H NMR data and ROESY spectra (Table 1; SI, Figure S22). Its absolute configuration was determined by a CD spectrum (Figure 5). The CD curves of **3**

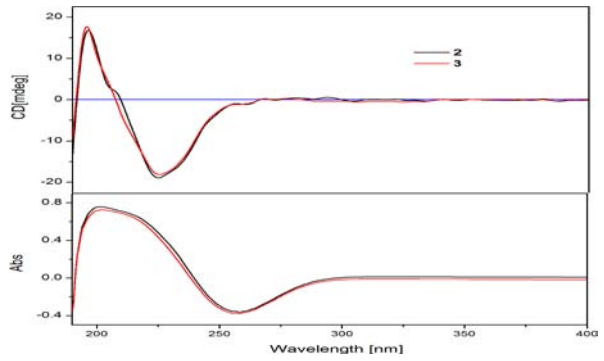

Figure 5. CD spectra of compounds **2** and **3**.

well matched those of **2**, indicating that they shared the same absolute stereochemistry as depicted.

Possible biosynthetic pathways for **1**−**3** were proposed as shown in Scheme 1. Compounds **1**−**3** shared a common precursor of a C-9 hydroxylated mexicanolide limonoid, which was then transformed to a derivative of methyl angolensate. The latter undergoing a base catalytic procedure would give two key intermediates **ia** and/or **ib**. The intermediate **ia** would finally be converted to **1** via a cascade of rearrangement and redox chemistry. The **ib** would produce **2** and **3** by the cleavage of the

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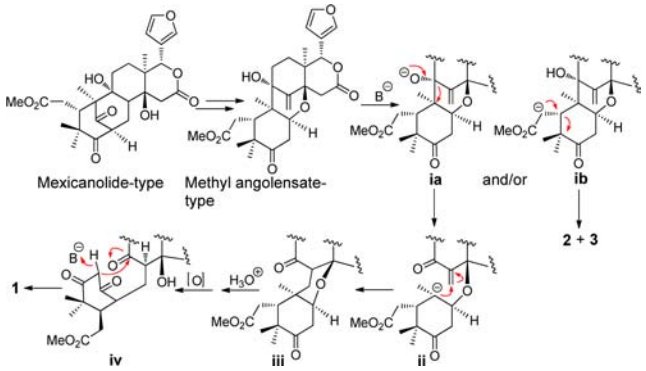
Scheme 1. Hypothetical Biosynthetic Pathways of 1−3



C-4−C-5 bond as the key step, in which **2** was an acetylated product of **3**.

Acquired immunodeficiency syndrome (AIDS) that is caused by the human immunodeficiency virus (HIV) has been a global threat to human lives. The continuing reports on drug resistance require the development of new, more potent anti-HIV drugs. Natural products are a well-known source of new anti-HIV agents with novel structures.[12] Trichiconins A−C (**1**−**3**) were tested in vitro for their anti-HIV activities on HIV-1 NL 4-3 infected MT4 cells. Both compounds **2** and **3** showed modest anti-HIV activities with EC_{50} values of 5.9 ± 0.4 (SI > 4.2) and 3.6 ± 0.6 (SI = 3.5) μM, respectively, where the anti-HIV drug NVP was used as the positive control (EC_{50} = 0.012 ± 0.02 μM).[13] Trichiconin A (**1**) was inactive.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, selected HMBC and ROESY correlations of **1** and **2**, and full spectroscopic data (NMR, MS, and IR) of compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(7) **Trichiconin A (1):** Colorless crystals; mp 219−220 °C; $[\alpha]^{20}_{D}$ +52.6 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.94) nm; IR (KBr) ν_{max} 3492, 3437, 2954, 1730, 1695, 1502, 1460, 1381, 1340, 1205, 1022, 872 cm^{-1}; for ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; ESI(+)MS *m*/*z* 1005.6 [2 M + H]$^+$, 1027.4 [2 M + Na]$^+$; HRESI(+)MS *m*/*z* 525.2098 [M + Na]$^+$ (calcd for $C_{27}H_{34}O_9Na$, 525.2101).

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(9) **Trichiconin B (2)**: Colorless crystals; mp 188−189 °C; $[\alpha]^{20}_{D}$ −82.1 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 219 nm (4.19); CD (MeOH) λ ($\Delta\varepsilon$) 197 (10.86), 208 (1.29), 225 (−12.35) nm; IR (KBr) ν_{max} 3479, 3147, 2951, 1736, 1647, 1439, 1385, 1254, 1203, 1161, 1030, 760 cm^{-1}; for ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; ESI(+)MS *m*/*z* 545.2 [M + H]$^+$, 1111.4 [2 M + Na]$^+$; HRESI(+)MS *m*/*z* 567.2195 [M + Na]$^+$ (calcd for $C_{29}H_{36}O_{10}Na$, 567.2206).

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(11) **Trichiconin C (3)**: Colorless oil; $[\alpha]^{20}_{D}$ −95.3 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 215 nm (4.15); CD (MeOH) λ ($\Delta\varepsilon$) 196 (8.46), 226 (−8.64) nm; IR (KBr) ν_{max} 3467, 3147, 2949, 1720, 1647, 1439, 1385, 1246, 1053, 876, 602 cm^{-1}; for ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; ESI(+)MS *m*/*z* 1005.5 [2 M + H]$^+$, 1027.5 [2 M + Na]$^+$; HRESI(+)MS *m*/*z* 525.2097 [M + Na]$^+$ (calcd for $C_{27}H_{34}O_9Na$, 525.2101).

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