Spirotrichilins A and B: Two Rearranged Spirocyclic Limonoids from *Trichilia connaroides*

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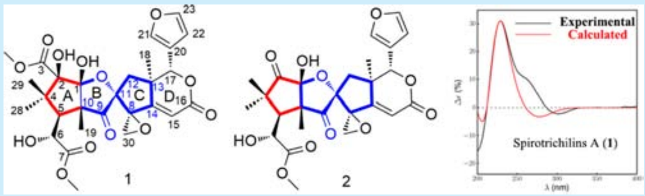
Supporting Information

ABSTRACT: Spirotrichilins A (**1**) and B (**2**), two novel limonoids with an unprecedented spiro[cyclopenta[*b*]furan-2,1′-cyclopentan] ring system in A/B/C rings, were isolated from the fruits of trichilia connaroides. Their planar structures and absolute configurations were established based on 1D-, 2D-NMR data, electronic circular dichroism (ECD) exciton chirality method and time-dependent density functional theory (TDDFT)/ECD calculation. A benzilic acid-like rearrangement in ring A was proposed as the key step in the plausible biogenetic pathway of **1** and **2**.



Limonoids, possessing 17*β*-furan ring tetranortriterpenoids structurally, are the characteristic secondary metabolites of Meliaceae family in the plant kingdom.[1] The limonoid family encompasses a diverse array of structural frameworks, which upon further rearrangements lead to the formation of highly oxygenated and structural complex *seco*-limonoids.[2] Not surprisingly, the unprecedented carbon skeletons and the wide spectrum of biological properties of limonoids have attracted great interest within the chemical and biological research communities.[2] Trichiconins,[2d] trichiconlides,[2e] and cipacinoids,[2f] several limonoids with novel skeletons, have been isolated and identified from Meliaceae plants, and the total synthesis of cipadonoid,[3] mexicanolides,[4] and limonin[5] has been achieved in recent years.

Trichilia connaroides (Wight et Arn.) Bentv (Meliaceae), a tall tree that mainly distributed in the southeast of China, has been applied by local residents as anti-inflammatory Chinese folk medicine to treat arthritis, pharyngitis, and tonsillitis.[6] The limonoids, isolated from the *Trichilia* genus, exhibit diverse and complex carbon scaffolds, particularly rearranged mexicanolide and phragmalin, trijugin, and 30-nortrijugin.[7] Various frameworks infer that the biosynthetic pathway of limonoids in plants of *Trichilia* genus is extremely active, which also suggests that other structurally diverse natural limonoid might exist. Thus, the fruits of *t. connaroides* were selected as the research subject in our continuing search for structurally interesting and biologically important limonoids. As a result, spirotrichilins A (**1**) and B (**2**), two novel limonoids with an unprecedented spiro[cyclopenta-[*b*]furan-2,1′-cyclopentan] ring system in A/B/C rings, were isolated and identified in our recent research. Their structures and absolute configurations were determined by 1D- and 2D-NMR, electronic circular dichroism (ECD) exciton chirality method, and time-dependent density functional theory (TDDFT)/ECD calculation. Compound **1** was evaluated for anti-inflammatory effect in the LPS-induced RAW264.7 cell line. Herein, we report the isolation, structural elucidation, and activity evaluation of compounds **1** and **2**.

Spirotrichilin A (**1**) was obtained as a colorless resin. The molecular formula was assigned as $C_{28}H_{32}O_{13}$ by the positive HRESIMS ion peak at m/z 599.1737 $[M + Na]^+$ (calcd 599.1735) and ^{13}C NMR data, corresponding 13 degrees of unsaturation. Analyses of the 1D-NMR data (methanol-d_4, Supporting Information, Table S1) revealed the existence of a typical *β*-substituted furan ring (δ_H 7.65, 7.58, 6.52), an olefinic proton singlet signal (δ_H 5.77), three ester carbonyls (δ_C 174.7, 172.5, 165.9), and a keto group (δ_C 215.4). Based on the combination of 1D-NMR and HSQC spectra, two methylenes (δ_H 3.47, δ_C 55.7; δ_H 2.31 d, δ_C 47.7), and three methines (δ_H 2.75, δ_C 49.4; δ_H 4.29, δ_C 71.8; δ_H 5.49, s, δ_C 82.2) were distinguished. The above-mentioned data indicated that compound **1** was a trijugin-type limonoid featuring a pentacylic core structure.[8]

From a biogenetic point of view, compound **1** possessed similar C, D rings, characteristic ternary oxygen ring, and furyl ring as those in the trijugin-type limonoid.[8] This deduction was confirmed by the key correlations from Me-18 (δ_H 1.28, s) to C-17 (δ_C 82.2), C-12 (δ_C 47.7), and C-14 (δ_C 169.4); from H-17 (δ_H 5.49, s) to C-20 (δ_C 121.6), C-13, and C-14; from H_2-12 [δ_H 2.31 d ($J = 15.0$) 2.15 d ($J = 15.0$)] to C-11 (δ_C 87.5), C-13, C-8, and C-14, which were similar to those of trichilin A[8b] (Figure 1). The presence of $\Delta^{14,15}$ downfield shifted C-11 from δ_C 85.5 to 87.5,[2f] and the characteristic hemiketal carbon signal of C-1 at δ_C 113.2 suggested that the ether linkage between C-14 and C-1 in trichilins was interrupted and formed a new ether between C-11 and C-1 (δ_C 113.2).[8] Key HMBC correlations from Me-19 (δ_H

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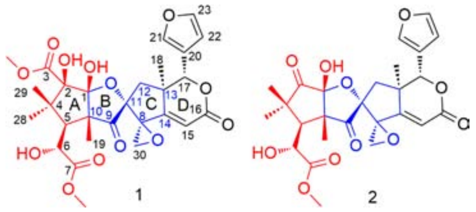

Figure 1. Structures of compounds **1** and **2**.

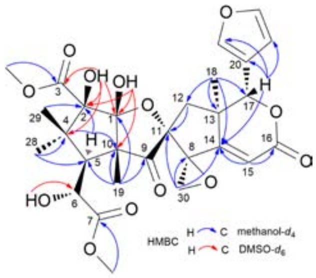

Figure 2. HMBC correlations of **1**.

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compounds **1**−**2**

	1[a]		**2**[b]	
no.	δ_H, milt (*J* Hz)	δ_C	δ_H, milt (*J* Hz)	δ_C
1		111.5		107.5
2		86.2		212.4
3		170.9		
4		48.5		47.1
5	2.61 d (8.0)	47.9	2.55 d (9.5)	46.1
6	4.17 d (8.0, 5.5)	69.9	4.30 d (9.5, 6.5)	71.4
7		172.7		172.7
8		63.2		64.6
9		213.3		213.0
10		56.4		55.6
11		86.6		87.3
12	2.20 d (14.5)	46.7	2.38 d (14.5)	47.9
	2.03 d (14.5)		2.03 d (14.5)	
13		41.7		43.1
14		167.6		167.3
15	5.63 s	107.7	5.68 s	109.6
16		163.0		163.6
17	5.39 s	79.8	5.51 s	81.3
18	1.16 s	14.5	1.25 s	21.1
19	1.17 s	20.0	1.23 s	10.7
20		120.0		121.6
21	7.72 s	141.2	7.75 d (1.5)	142.1
22	6.57 s	109.4	6.58 (1.5)	110.3
23	7.81 s	143.8	7.62 t (1.5)	144.6
28	0.99 s	27.0	1.32 s	28.8
29	1.07 s	19.6	1.25 s	21.7
30	3.23 d (6.5)	54.4	3.39 d (6.0)	55.5
	3.01 d (6.5)		3.13 d (6.0)	
OMe-3	3.65 s	51.1		
OMe-7	3.60 s	51.3	3.767 s	52.0
OH-1	6.33 s			
OH-2	5.51 s			
OH-6	5.71 d (5.5)		4.85 (6.5)	

[a] Obtained in DMSO-d_6. [b] Obtained in acetone-d_6. Assignments supported with 2D-NMR spectra.

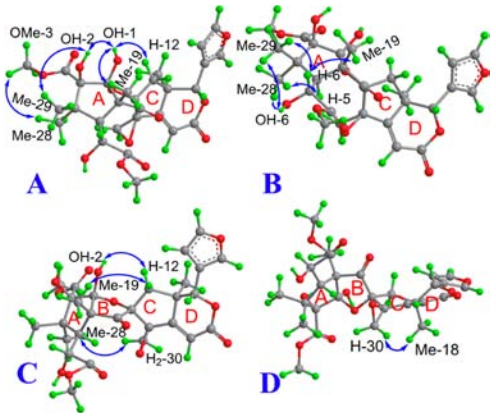

Figure 3. Key ROESY correlations of **1** (DMSO-d_6).

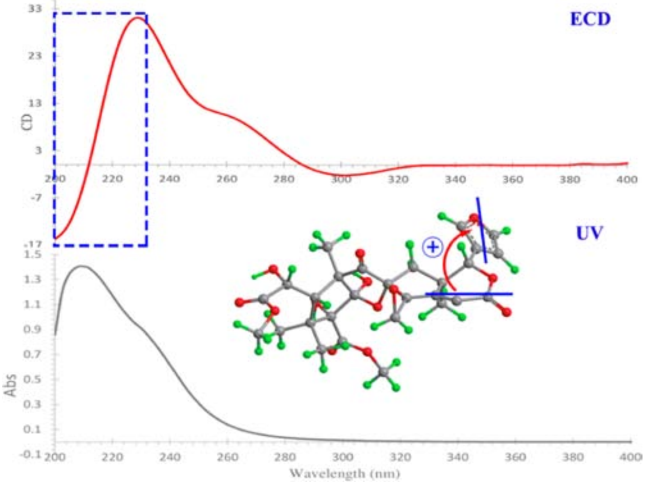

Figure 4. UV and ECD spectra of **1** (in MeOH).

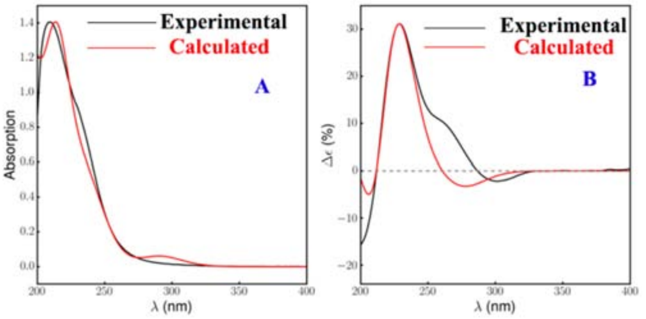

Figure 5. Calculated and experimental UV (A) and ECD (B) spectra of **1** (in MeOH).

1.17, s) to C-1 (δ_C 113.2), C-9 (δ_C 213.3), and C-10 (δ_C 56.4) indicated the formation of a novel 1,7-dioxadispiro[2.3.0.4.]-hendecane ring systems in B/C rings. Accordingly, the key point for structure determination was restricted to the construction of the left hemisphere of **1** (Figure 2). The obvious correlations (Figure 2) from an MeO signal (δ_H 3.79) to a carbonyl carbon at δ_C 172.5, from Me-28 (δ_H 1.25, s) and Me-29 (δ_H 1.16, s) to C-2 (δ_C 87.7), C-4 (δ_C 49.5), and C-5 (δ_C 49.4); Me-19 (δ_H 1.31, s) to C-1, C-5, and C-10 (δ_C 58.8), suggested that a novel pentacyclic ring A might be derived from oxidized cleavage of C-3/C-4 and formation of C-2/C-4. Because of the absence of proton signals in C-1, C-2, C-3, OH-1, and OH-2 in methanol-d_4

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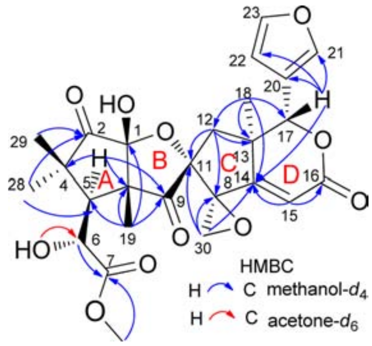


Figure 6. Key HMBC correlations of **2**.

solvent (Supporting Information, S25), above deduction cannot be confirmed completely and precisely.

To solve this uncertainty, the 1D- and 2D-NMR data (Table 1) in DMSO-d_6 solvent were resurveyed in a bid to obtain the signals of active hydrogen, particularly OH-1 and OH-2. As a result, three key exchangeable proton signals of OH-1 (δ_H 6.33), OH-2 (δ_H 5.51), and OH-6 [δ_H 5.71 d (5.5)] were distinguished, which were useful for further ensuring the rationality of rings A and B. The correlations from OH-2 to C-1, C-2, C-3, and C-4, and from OH-1 to C-1, C-2, and C-10 further confirmed the linkage of C-1 and C-2, and the formation of pentacyclic ring A, as well as the attachment of the MeO_2C-moiety to C-2 (Figure 2, red arrows). Thus, the planar construction of **1** was assigned as depicted.

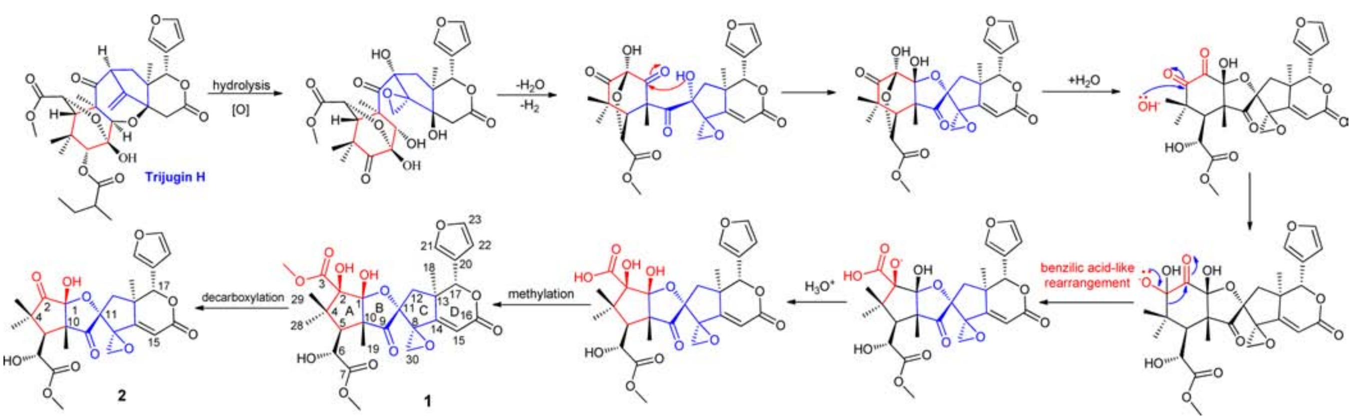
The relative configuration of **1** was assigned by ROESY spectrum (Figure 3). The ROESY correlations from OMe-3 to Me-28, from OH-2 to Me-29, and from OH-1 to Me-19 and H-12β allowed the assignment of OH-1, OH-2, Me-29, and Me-19 in a β-orientation and MeO_2C-moiety in an α-orientation (Figure 3A). The ROESY correlations of H-5/Me-28 indicated that Me-28 and H-5 were cofacial and assigned as α-orientated (Figure 3B). The C5−C6 bond was not rotated randomly upon steric hindrance in DMSO-d_6 solvent, which was confirmed by the ROESY correlations from H-6 to Me-29, Me-19; from OH-6 to Me-28, Me-29. Therefore, the relative relationship of H-5 and H-6 were confirmed as *trans*-axial bonds together with coupling value 8.0 Hz between H-5 and H-6 in ABX coupled system.[2f] In the double-spiral structure (blue part), the two planes of B and C rings were in a vertical state. C11−C12 bond and C8−C30 bond were in a β-orientation (Figure 3C) and an α-orientation, respectively, which were assigned by the ROESY correlations from H-12 to Me-19 and OH-1; from H_2-30 to Me-18 (Figure 3D). Therefore, the relative configuration of **1** was confirmed definitely.

To determine its absolute configuration, the ECD spectrum of **1** was measured in MeOH, which was dominated by a positive Cotton effect at λ_{max} 229 nm ($\Delta\varepsilon$ + 31.1) and a negative Cotton effect at λ_{max} 200 nm ($\Delta\varepsilon$ − 15.7) due to the transition interaction between two different chromophores of the α,β-unsaturated lactone and the furan ring. This evidence indicated a positive chirality for **1** (Figure 4) and determined the chirality of C-17 as *R*.[9] Subsequently, the former result was also confirmed by the well matched ECD data between calculated ECD data of 1*R*, 2*R*, 5*S*, 6*R*, 8*S*, 10*S*, 11*R*, 13*R*, 14*E*, and 17*R* and that of our isolated **1** (Figure 5).

Spirotrichilin B (**2**) was obtained as colorless powders. The molecular formula was determined as $C_{26}H_{28}O_{11}$ by the positive HRESIMS ion peak at m/z 539.1527 $[M + Na]^+$ (calcd 539.1524) and ^{13}C NMR data, requiring 13 degrees of unsaturation. The similar UV and ECD spectra (Supporting Information, Figure 1) infer that its structure is similar to that of **1**. Combining the preceding analyses of NMR data, compounds **1** and **2** exhibited similarity in the structure of B, C, and D rings, especially the spiro-B/C ring system, which was confirmed by the presence of OH-6 (δ_H 4.85, d, J = 6.5 Hz) in NMR data in acetone-d_6. As in the case of **1**, exhibition of an additional keto carbonyl (δ_C 212.4) and disappearance of an ester carbonyl group (δ_C 170.9) and a methoxy group carbon suggested that the methyl formate group in **1** was degraded in **2**, which was confirmed by the observation of HMBC correlations (Figure 6) from Me-28 (δ_H 1.32) and Me-29 (δ_H 1.25) to C-2 (δ_C 212.4). Thus, the planar structure of **2** was determined finally. The relative configuration of **2** was assigned to be identical to those of **1** by ^1H NMR and ROESY data. The ECD data of **2** well matched that of **1**, indicating that they shared the same absolute configuration, which was also determined by ECD exciton chirality method (Supporting Information, S26).

The hypothetical biosynthesis pathways of **1** and **2** are illustrated in Scheme 1. Trijugin-type limonoid (trijugin H) could be recognized as the origin, and the formation of oxygen heterocyclic ring (hemiketal formation reaction) and the benzilic acid-like rearrangement might play an important role in the formation of the backbone of **1** and **2** in ring A. Primarily, the formation of oxygen heterocyclic ring (ring B) undergoes the C30−O−C14 ester bond cleavage, C14−C15 dehydration, and new C1−O−C11 ester bond formation.

Scheme 1. Hypothetical Biosynthetic Pathways of 1 and 2



Subsequently, a nonenzymatic benzilic acid-like rearrangement[10] would lead to the formation of a five-membered A-ring and a new carboxyl group (COOH-3). Methyl esterification of it may lead to afford **1**; otherwise, the decarboxylation reaction of it may form **2**.

The limonoid class of compounds possesses not only a great variety of chemical structures, but also a wide range of biological properties such as anti-inflammatory,[11a−c] cytotoxic,[11e] and antiviral activities.[2f,8c,11d] Accordingly, these novel isolates were conducted through wide and preliminary investigations on their bioactivities. As a result, compound **1** showed weak anti-inflammatory activity in the LPS-induced RAW264.7 cell line with 25.89% and 37.13% inhibitory effects on NO production at 25 and 50 μM, respectively.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00738.

Experimental section; UV, ESIMS, HRESIMS, ECD, and 1D- and 2D-NMR spectra of **1** and **2** (PDF)

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Notes

The authors declare no competing financial interest.

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