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Trichiliton A, a Novel Limonoid from *Trichilia connaroides*

Xin Fang,[a,b] Yingtong Di,*[a] Zhaoliang Geng,[a] Chenjian Tan,[a] Jie Guo,[c] Jing Ning,[a] and Xiaojiang Hao*[a]

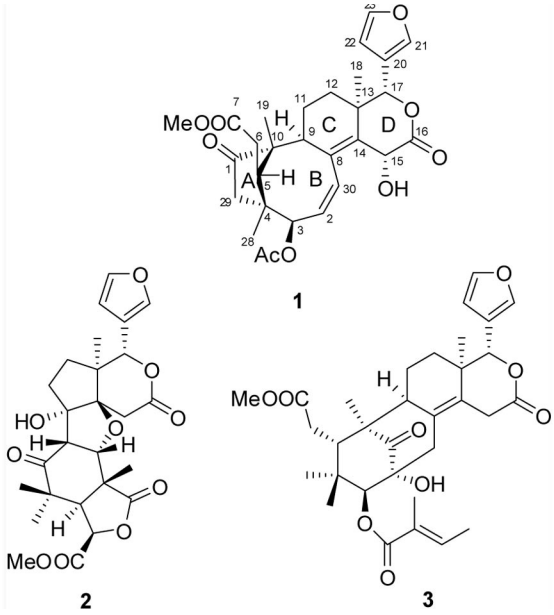
Keywords: Terpenoids / Natural products / Structure elucidation / Biosynthesis / Reaction mechanisms

Trichiliton A (**1**), a novel limonoid with an unprecedented bicyclo[5.2.1^{4,10}]decane motif in the tetranortriterpenoid core, has been isolated from the leaves of *Trichilia connaroides*. Its structure and absolute configuration have been elucidated by spectroscopic analysis and computational methods. The biosynthetic pathway of compound **1** is discussed and involves an alternative new routine from mexicanolide to phragmalin.

Introduction

Limonoids are a structurally diverse group of secondary metabolites mainly found in the Meliaceae, Rutaceae, Cneoraceae, and Simaroubaceae family and either contain or are derived from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton. They are classified on the basis of which of the four triterpenoid skeleton rings have been oxidatively opened. Until recently, about 1300 limonoids with more than 35 carbon frameworks had been isolated from the four families,[1] with the mexicanolide,[2] phragmalin,[3] and modified phragmalin-type limonoids[4] possessing characteristic bicyclo[3.3.1^{2,10}]nonane, tricyclo[3.3.1^{2,10}.1^{1,4}]decane and tricyclo[4.2.1^{10,30}.1^{1,4}]decane ring systems. Some limonoids with significant bioactivity have attracted continuous attention, highlighted by the recent successful total synthesis of azadirachtin.[5]

Trichilia connaroides (Wight et Arn.) Bentv. (Meliaceae) has traditionally been used as folk medicine in China for treating arthritis, pharyngitis, tonsillitis, and other ailments. It is known to produce structurally complex limonoids.[6] In the course of our search for structurally novel limonoids from the Meliaceae family, our group has reported a highly rearranged tetranortriterpenoid with a complex ring system obtained from this species.[7] A subsequent study led to the isolation of a novel limonoid, which we named trichiliton A (**1**), characterized by a previously unknown bicyclo-[5.2.1^{4,10}]decane ring system. In addition, two known limonoids, trijugin C (**2**) and $\Delta^{8,14}$-2-hydroxy-6-deoxyswietenine (**3**), were found to coexist with trichiliton A (**1**). In this paper we describe the structural elucidation, biosynthetic pathway, and cytotoxicity of trichiliton A (**1**).



1 **2** **3**

Results and Discussion

Trichiliton A (**1**) was isolated as a white amorphous solid. Its molecular formula, $C_{29}H_{34}O_9$, was determined by FAB(–)-HRMS (*m*/*z*: found 526.2209 $[M]^-$, calcd. 526.2203) and ESI-HRMS (*m*/*z*: found 565.1825 $[M + K]^+$, calcd. 565.1839), which indicated 13 degrees of unsaturation. The IR spectrum showed strong absorption bands corresponding to a hydroxy group (3433 cm^{-1}), carbonyls (1735 cm^{-1}), and double bonds (1628 cm^{-1}). The ^{1}H NMR spectrum

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences,
Kunming 650204, P. R. China
Fax: +86-871-5223070
E-mail: haoxj@mail.kib.ac.cn
diyt@mail.kib.ac.cn
[b] Graduate School of the Chinese Academy of Sciences, Beijing 100039, P. R. China
[c] Department of Natural Products Chemistry, Shenyang Pharmaceutical University,
103 Wenhua Road, Shenhe District Shenyang 110016, P. R. China

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(Table 1) also displayed coupling signals arising from double bonds [δ_H = 6.23 (d, J = 12.5 Hz), 5.71 ppm (dd, J = 12.5, 8.5 Hz)] and, in combination with HMQC NMR spectrum, 33 protons were unambiguously assigned to their corresponding carbon atoms. However, according to the molecular formula, one proton signal was not observed, which can presumably be attributed to a hydroxy group, as supported by the IR OH absorption band and a pseudomolecular ion peak $[M - H_2O]^-$ at m/z = 509 in the FAB(–)-MS spectrum.

Table 1. ^1H (500 MHz) and ^{13}C (100 MHz) NMR data for trichiliton A (**1**) in $CDCl_3$.

Atom no.	δ_H ppm (multi, J [Hz])	δ_C [ppm]	Atom no.	δ_H ppm (multi, J [Hz])	δ_C [ppm]
1		220.1	15	4.87 (s)	66.1
2	5.71 (dd, 12.5, 8.5)	125.8	16		173.9
3	5.20 (d, 8.5)	75.0	17	5.70 (s)	81.2
4		44.1	18	1.14 (s)	16.5
5	3.77 (br. d, 13.5)	37.4	19	1.00 (s)	22.6
6β	2.50 (dd, 16.5, 13.5)	33.5	20		120.4
6α	2.36 (dd, 16.5, 2.0)		21	7.53 (s)	141.9
7		174.5	22	6.47 (br. s)	109.8
8		139.0	23	7.46 (br. s)	143.1
9	2.80 (d, 5.5)	44.5	28	1.06 (s)	22.4
10		54.0	29α	2.10 (d, 17.5)	46.5
11α	1.76 (m)	19.1	29β	2.37 (d, 17.5)	
11β	1.91 (d, 15.0)		30	6.23 (d, 12.5)	137.4
12α	1.08 (m)	28.2	OMe	3.72 (s)	52.1
12β	1.28 (m)		OAc		169.8
13		38.7		2.04 (s)	20.4
14		134.2			

The ^{13}C NMR and DEPT spectra (Table 1) of **1** revealed 29 carbon signals, in agreement with the molecular formula, which included five methyls, four methylenes, ten methines (three oxygenated and five olefinic), and ten quaternary carbons (four carbonyls and three olefinic carbons). In addition to a methoxy [δ_H = 3.72 (s) ppm, δ_C = 52.1 ppm] and acetyl group [δ_H = 2.04 (s) ppm, δ_C = 20.4, 169.8 ppm], **1** contains 26 carbon atoms, including a β-substituted furan ring [δ_H = 7.53 (s), 6.47 (s), 7.46 (s) ppm, δ_C = 120.4, 141.9, 109.8, 143.1 ppm], which suggested that compound **1** is a tetranortriterpenoid. Furthermore, apart from the four double bonds and four carbonyl groups, the remaining five degrees of unsaturation indicated compound **1** to be pentacyclic.

By comprehensive analysis of the 2D NMR spectra of **1**, including the ^1H–^1H COSY, HMQC, and HMBC spectra, three partial structures **a** (C-2, C-3, C-30, and the acetyl group), **b** (C-8, C-9, C-11 to C-18, and C-20 to C-23), and **c** (C-1, C-10, C-19, C-28, C-29, C-4 to C-7, and OMe) were identified, as shown in Figure 1. Fragment **a** was easily established by ^1H–^1H COSY correlations of 30-H/2-H/3-H and the HMBC cross-peak of 3-H with 3-OAc. The second substructure **b** was assembled from six-membered lactone ring D fused to six-membered ring C at C-13 and C-14 and bearing a β-furyl ring and a methyl at C-17 and C-13, respectively. This was established by extensive comparison of the ^1H and ^{13}C NMR spectroscopic data of **1** with those of known D-seco-type limonoids[8] and further confirmed by 2D NMR analysis. Furthermore, with the identification of two oxygenated carbon atoms, the hydroxy was eventually located at C-15 in fragment **b**. This was supported by its chemical shifts [δ_H = 4.87 (s) ppm, δ_C = 66.1 ppm], identical to the literature data of other C-15 oxygenated limonoids.[9] The structure of partial structure **c** was initially determined by identification of the C1–C4–C5–C10–C29 cyclopentanone ring. The HMBC correlations from 29-H_2 to C-1, C-4, C-5, and C-10, and from 5-H to C-4, together with correlations from 19-H_3 to C-5, C-9, and C-10 allowed the construction of the cyclopentanone ring with a carbonyl group and a methyl (19-Me) at C-1 and C-10, respectively. ^1H–^1H COSY correlation of 5-H with 6-H and HMBC cross-peaks from 6-H to C-7 and from OMe to C-7 linked a side-chain containing C-6 and C-7 to the ring at C-5. The HMBC correlation of 28-H_3 with C-4 and C-29 indicated that 28-Me is attached to the cycloamyl ring at C-4.

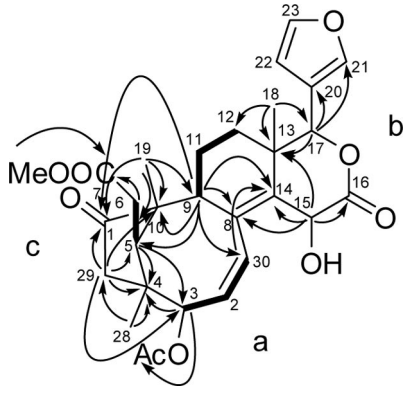


Figure 1. Partial structures **a**–**c** in **1**; ^1H–^1H COSY (−) and selected HMBC (H→C) correlations of **1**.

The linkage of the fragments **a**–**c** was accomplished by analysis of the HMBC correlations. Whereas the cross-peaks of 30-H/C-14 and 9-H/C-30 connected fragments **a** and **b** through the carbon–carbon bond of C-30–C-8, the correlations of 3-H/C-4, 29-H_2/C-3, and 5-H/C-3 linked fragments **a** and **c** together through the carbon–carbon bond of C-4–C-3. Similarly, the HMBC correlations of 9-H with C-1, C-5, and C-10 and of 19-H_3 with C-9 established the linkage of fragments **b** and **c** through the carbon–carbon bond of C-9–C-10. Thus, the gross structure of **1** was established.

The relative configuration of **1** was deduced from the analysis of its ROESY correlations in combination with molecular modeling studies. As shown in Figure 2, the observed correlations of 17-H with 5-H, 15-H, and OAc-3-H_3, together with the correlation of 15-H with OAc-3-H_3, indicated that 5-H, 15-H, 17-H, and OAc-3-H_3 are cofacial and thus were arbitrarily assigned a β orientation, which accordingly determined the α orientations of 3-H and 15-OH. The α assignment of 18-H_3 was deduced from the ROESY correlations of 18-H_3/22-H. However, the orientation of 9-H, the only remaining unknown orientation, could not be assigned by ROE data as the observed correlations of 9-H with 30-H, 11-H_2, and 19-H_3 were not sufficient to determine its direction. Therefore DFT calculations at the

HF/6-31G* and B3LYP/6-31G* levels of theory in GAUSSIAN 03[10] were conducted on the two structures of **1** corresponding to the α (**A**) and β (**B**) orientations of 9-H, as shown in Figure 3. The calculated distances of the proton pairs of the former are fully consistent with the corresponding ROESY data and thus 9-H was assigned the α orientation. Therefore the relative configurations of all the chiral centers of **1** were determined and are in agreement with its presumed biosynthetic origin, which will be discussed in detail later. Furthermore, the ROE correlations of 5-H with 17-H and 12β-H suggested that C-5, C-12, and C-17 are on the "upper" side of the system, whereas the ROE cross-peaks of 29α-H with 3-H and 2-H indicated that C-1, C-2, C-3, and C-29 are on the "lower" side. This observation suggested that the torsion angle between plane A and the plane incorporating C-3, C-4, C-8, C-9, and C-10 along the axis of the two bridgehead carbons, C-4 and C-10, is about 90° for the lowest energy, and the molecule should adopt a folded conformation. The optimized structure also showed that the eight-membered ring B adopts a chair conformation, which supports the above observation.

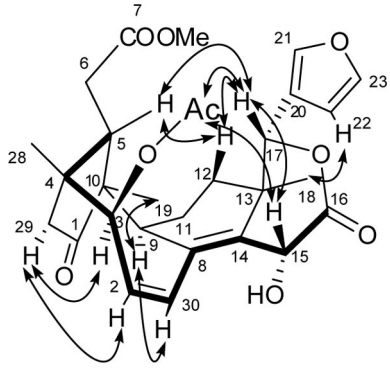
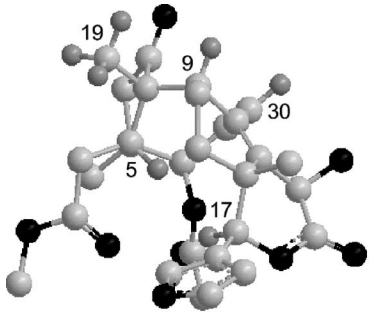
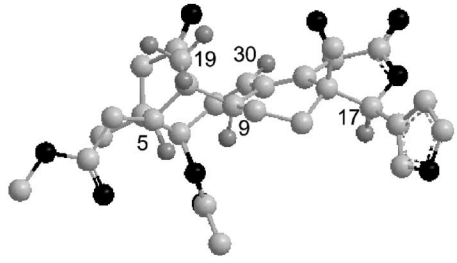


Figure 2. Key ROESY (H↔H) correlations of **1**.

Our initial attempt to assign the absolute configuration of **1** by use of the circular dichroic (CD) exciton chirality method failed. The UV absorption band (204 nm) of **1** suggested that the $\Delta^{2(30)}$ and $\Delta^{8(14)}$ double bonds are not completely conjugated, which might be caused by tension in the *anti* coplanar conformation of rings A and B. This means **1** possesses three chromophores, namely the two double bonds and the furan ring, which resulted in complex CD curves with different splitting patterns that are beyond investigation, and with the absence of CD analyses on suitable model compounds, the application of empirical CD rules is also problematic. Therefore we turned to computational methods. The possible presence of several conformers and their relative populations were taken into account to ensure that the calculation of the optical rotation was reliable. A SPARTAN 08[11] search for conformations of compound **1** was thus performed, which identified two stable conformations, I and II, with only different angles of rotation of the furan ring around the C-17–C-20 single bond. Reoptimization of the structure of the stable conformations was carried out at the HF/6-31G* and B3LYP/6-31G* levels of theory. Harmonic vibrational frequencies of each conformation were then calculated at the B3LYP/6-31G* level to confirm their stability. Then the potential energy surface (PES) was scanned, starting from the optimized geometry of conformation I and varying the dihedral angle C13–C17–C20–C22. The results exhibited only two minima, as shown in Figure 4, and suggested that the occurrence of the comparatively rapid interconversion of I and II in solution at room temperature is a logical process. The relative free energies and equilibrium populations at room temperature of the two conformations were also calculated and are listed in Table 2. Finally, the "self-consistent reaction field" method (SCRF) was employed to perform the OR calculation of the two major conformers of compound **1** in the gas phase at the B3LYP-SCRF/6-31G** level in





Proton pairs	Distances found in **A**	Distances found in **B**	ROE correlations
H-5/H-17	2.66 Å	6.30 Å	yes
H-5/H-9	3.60 Å	2.00 Å	no
H-9/H-30	2.90 Å	3.79 Å	yes
H-9/H_3-19	3.23 Å	3.86 Å	yes

Figure 3. The two possible DFT-optimized structures (A and B) found for trichiliton A (**1**). The calculated distances of the key proton pairs in the two structures are listed.

GAUSSIAN 03.[12] As shown in Table 2, the computed optical rotation value (–117.1°) of **1** is close to the experimental value (–70.8°), and thus the absolute configurations of **1** were assigned as 3*R*,4*R*,5*S*,9*S*,10*R*,13*R*,15*R*,17*R*, which is also in agreement with the proposed biosynthetic pathway.

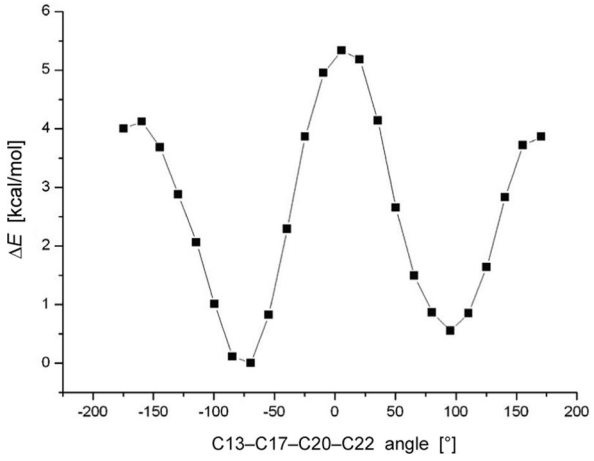
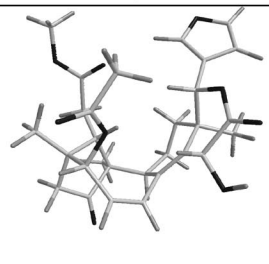
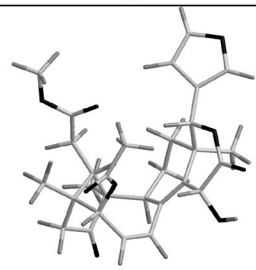


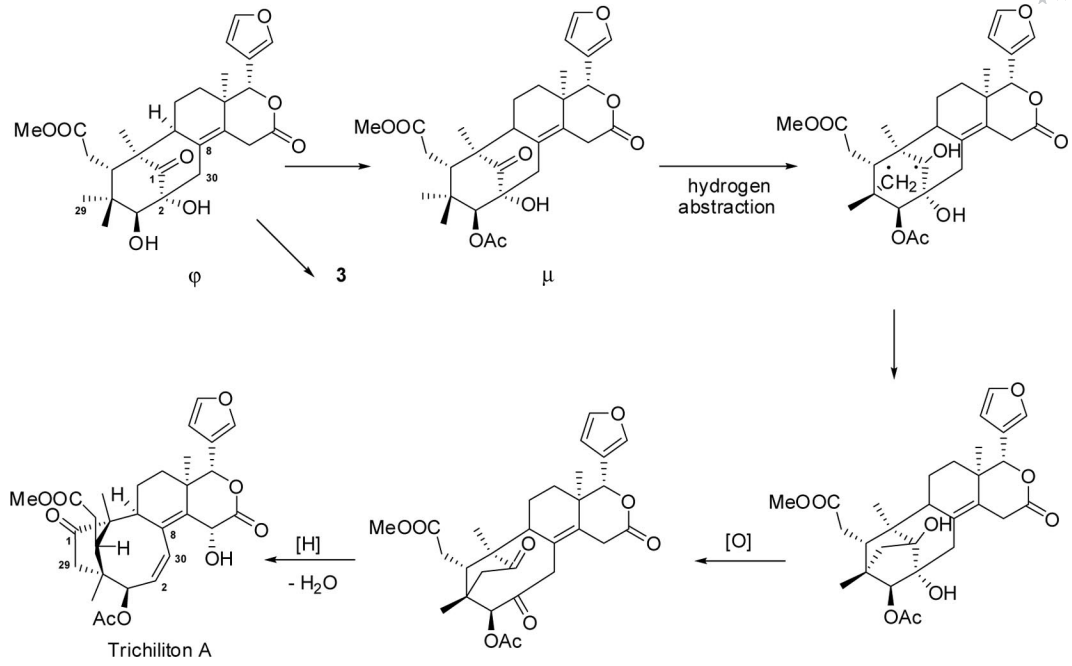
Figure 4. B3LYP/6-31G* PES scan with respect to the dihedral angle C12–C17–C20–C22 of trichiliton A (**1**).

Two known compounds were also isolated from the leaves of *Trichilia connaroides* and identified as trijugin C (**2**)[6d] and $\Delta^{8,14}$-2-hydroxy-6-deoxyswietenine (**3**)[13] by comparison of their spectroscopic data with literature data. To date trijugin C was only isolated from *Trichilia connaroides*. The full ^{13}C NMR spectroscopic data of $\Delta^{8,14}$-2-hydroxy-6-deoxyswietenine (**3**) is reported for the first time.

Trichiliton A (**1**) represents a new class of limonoid containing a previously unknown bicyclo[5.2.1^{4,10}]decane ring system, thus raising the interesting question of how it is formed in the plant. Structurally, its direct precursor should be a 1,2-dihydroxyphragmalin-type precursor like key intermediate φ in Scheme 1. The cleavage of the 1,2-diol followed by dehydration at C-2 to form the C-2–C-30 double bond could afford trichiliton A. However, we did not obtain phragmalin-type limonoids in this experiment, nor have there been any previous reports of the presence of this type of compound in the genus *Trichilia*. Instead, we isolated a mexicanolide-type limonoid, $\Delta^{8,14}$-2-hydroxy-6-deoxyswietenine (**3**), the precursor of which, 3-hydrolysate, could be biosynthesized into a phragmalin-type limonoid and serve as a precursor of trichiliton A (**1**). Interestingly, compound **1** has no oxy substituent at C-9. This implies its phragmalin precursor has no oxy substituent at C-9 either, which is indispensable in the radical biosynthetic pathway of phragmalin proposed by Taylor.[13] Therefore, we have suggested an alternative pathway for the biosynthesis of the phragmailin form of the mexicanolide-type limonoid, as shown in Scheme 1. The formation of the C-4–C-29–C-1 bridge is a result of the coupling reaction of the 1,29-biradical, which is produced by δ-hydrogen abstraction by the C-1 ketone (Norrish II type reaction). The structure of key intermediate μ was optimized at the B3LYP/6-31G* level of theory (see the Supporting Information) and the resulting key parameters associated with the δ-abstraction process are as follows: d = 2.65 Å, Δ = 86.3°, θ = 139.1°, ω = 3.7°, which are all close to the ideal values for abstraction (d = 2.72 Å, Δ = 90–120°, θ = 180°, ω = 0°).[14] The calculation suggested the δ-hydrogen abstraction by the C-1 ketone is possible when a certain enzyme is involved in catalyzing the Norrish II reaction. This mechanism could explain why compound **1** and other phragmalin-type limonoids have no hydroxy group at C-9, like those reported from the genus *Khaya*,[4,15] and confirms the previous hypothesis that the biosynthetic pathway for this species is probably different to that for other species of this genus.[6e] Note that the limonoids involved in the pathway mainly occur in the Swietenioideae subfamily of the Meliaceae family and are encountered less in the Melioideae subfamily to which the genus belongs. This finding might give some new insight into the position of the genus *Trichilia* in the Meliaceae family, especially its relationship with the genus *Toona* (Swiet-

Table 2. Calculated energies and optical rotations for trichiliton A (**1**).

Conformer	I	II	Exp
			
ΔG [kcal/mol]	0	0.40	
P	55.0 %	45 %	
$[\alpha]_D$	–123.4	–109.2	
Sum of $[\alpha]_D$	–117.1		–70.8



Scheme 1. Hypothetical biosynthetic pathway of trichiliton A.

enioideae subfamily).[16] The reinvestigation of the species of the phragmalin-type limonoids as "missing" links is now underway in the hope of finding further evidence of the suggested biosynthetic pathway.

Trichiliton A (**1**) was tested for in vitro cytotoxicity against an array of cell lines, including HL-60, SMMC-7721, A-549, and SK-BR-3, by using the MTT method.[17] However, compound **1** showed no cytotoxicity against any of the tumor cell lines detected (50% effective dose of clonal inhibition, $ED_{50} > 5$ μg/mL). The activity of the compound against wnt signaling was also analyzed by the reporter gene assay as this signaling is closely related to tumoragenesis,[18] however, it also showed no obvious activity against the signaling.

Conclusions

From the leaves of *Trichilia connaroides*, a novel limonoid trichiliton A (**1**) with a unique bicyclo[5.2.1^{4,10}]decane ring system has been isolated. Its structure was elucidated by extensive spectroscopic techniques and its absolute configuration was assigned by comparing its experimental optical rotation value with that obtained by computational methods.

The isolation of trichiliton A (**1**) in the plant is interesting and informative from both biosynthetic and chemotaxonmic points of view. We have suggested a biosynthetic pathway from the coexisting $\Delta^{8,14}$-2-hydroxy-6-deoxyswietenine, a mexicanolide-type limonoid, to trichiliton A (**1**), which involves an alternative new route from mexicanolide to phragmalin. This pathway in turn implies an unusual position of the species in the genus, and the genus in the Meliaceae family.

Experimental Section

General: Optical rotations were measured with a Perkin–Elmer model 241 polarimeter. UV spectra were recorded with a Shimadzu UV-250 spectrophotometer. IR spectra were recorded with a Bio-Rad FTS-135 spectrometer using KBr disks. ^{1}H and 2D NMR spectra were recorded with a Bruker DRX-500 instrument and the ^{13}C NMR spectra with a Bruker AM-400 spectrometer. Chemical shifts are reported using TMS as the internal standard. FAB-MS and FAB-HRMS spectra were measured with Finnigan MAT 90 and VG Auto Spec-3000 spectrometers, respectively. CD spectra were obtained with a JASCO 810 spectrophotometer. Column chromatography was performed on silica gel (90–150 μm, Qingdao Marine Chemical Inc.), Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany). Semi-peparative HPLC was performed with a Zorbax SB-C-18 (Agilent Co. Ltd. U.S.A.) column (i.d. 9.4 × 250 mm), developed with CH_3CN/H_2O (45:55, 20 min, flow rate 3.0 mL/min; detection, UV 210 nm) at 30 °C. Precoated silica gel GF_{254} and HF_{254} plates (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) were used for TLC.

Plant Material: The leaves of *Trichilia connaroides* were collected in March 2008 from Xishuanbanna, Yunnan Province, People's Republic of China. A voucher specimen (no. KUN 0620839) has been deposited at the Kunming Institute of Botany, Kunming, People's Republic of China.

Extraction and Isolation: The air-dried powder of the leaves (4.8 kg) of *T. connaroides* was extracted with 95% EtOH at room temperature to give a crude extract, which was further extracted with petroleum ether (PE), EtOAc, and *n*BuOH. The EtOAc-soluble fraction (28 g) was fractionated on an MCI gel column eluting with a $MeOH/H_2O$ gradient (4:6 to 9:1) to give five fractions. Fraction 2 (1.5 g) was extensively purified by chromatography through columns of silica gel and C-18 reversed-phase silica gel, and finally purified by semi-preparative HPLC to give Trichiliton **A** (**1**;

1.8 mg), trijugin C (**2**; 4.2 mg), and $\Delta^{8,14}$-2-hydroxy-6-deoxyswietenine (**3**; 4.6 mg).

Trichiliton A (1): A white amorphous power. $[\alpha]_D^{25}$ = –70.8 (c = 0.080, MeOH). UV (MeOH): λ_{max} = 204 nm. CD ($CHCl_3$): λ = 211 ($\Delta\varepsilon$ = –34.38), 241 ($\Delta\varepsilon$ = +5.27), 293 nm ($\Delta\varepsilon$ = +4.46). IR (KBr): $\tilde{\nu}$ = 3433, 2928, 1736, 1628, 1379, 1231, 1162, 1021 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data, see Table 1. MS (FAB, –): *m/z* = 526 $[M]^-$. HRMS (FAB, –): *m/z* = 526.2209 (calcd. for $[M]^-$ 526.2203). MS (ESI): *m/z* = 565 $[M + K]^+$. HRMS (ESI): *m/z* = 565.1825 (calcd. for $[M + K]^+$ 565.1839).

$\Delta^{8,14}$-2-Hydroxy-6-deoxyswietenine (3): A white amorphous power. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 217.4 (s, C-1), 174.0 (s, C-7), 169.5 (s, C-16), 167.3 (s, C-1′), 142.8 (d, C-23), 141.6 (d, C-21), 139.2 (d, C-3′), 133.1 (s, C-14), 129.0 (s, C-8), 125.6 (s, C-2′), 120.6 (s, C-20), 109.9 (d, C-22), 86.4 (d, C-3), 80.7 (d, C-17), 78.1 (s, C-3), 52.2 (q, OMe), 52.1 (s, C-10), 44.3 (t, C-30), 40.3 (d, C-5), 39.3 (s, C-4), 38.2 (s, C-13), 33.1 (t, C-6), 33.0 (t, C-15), 29.0 (t, C-12), 23.3 (q, C-28), 19.5 (q, C-29), 18.7 (t, C-11), 17.2 (q, C-18), 16.8 (q, C-19), 14.6 (q, C-4′), 12.4 (q, C-5′) ppm.

Cytotoxicity Bioassays: The following human tumor cell lines were used: HL-60, SMMC-7721, A-549, and SK-BR-3. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA) supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C.

The cytotoxicity assay was performed according to the MTT method. Briefly, adherent cells (100 μL) were seeded into 96-well microtiter plates and allowed to adhere for 12 h before drug addition whereas suspended cells were seeded just before drug addition with an initial density of 1×105 cells/mL. Each tumor cell line was exposed to the tested compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM in triplicate for 48 h with cisplatin as the positive control. After treatment, cell viability was measured and cell growth curve was plotted. IC_{50} values were calculated by the Reed and Muench method.

Supporting Information (see also the footnote on the first page of this article): IR, FAB-MS, ESI-MS, and 1D and 2D NMR spectra of **1**, 1D NMR spectra of trijugin C (**2**) and $\Delta^{8,14}$-2-hydroxy-6-deoxyswietenine (**3**), optimized standard orientation of trichiliton A (**1**) at the B3LYP/6-31G(d) level of theory.

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[1] H. P. Zhang, J. J. Tan, D. VanDerveer, X. Wang, M. J. Wargovich, F. Chen, *Phytochemistry* **2009**, *70*, 294–299.

[2] a) P. H. Coombes, D. A. Mulholland, M. Randrianarivelojosia, *Phytochemistry* **2005**, *66*, 1100–1107; b) B. S. Mootoo, R. Ramsewak, *J. Nat. Prod.* **1996**, *59*, 544–547; c) S. Kadota, L. Marpaung, T. Kikuchi, H. Ekimoto, *Chem. Pharm. Bull.* **1990**, *38*, 639–651; d) J. D. Connolly, R. McCrindle, K. H. Overton, *Tetrahedron* **1968**, *24*, 1489–1492.

[3] a) M. N. da Silva, M. S. P. Arruda, K. C. F. Castro, M. F. Das, G. F. da Silva, J. B. Fernandes, P. C. Vieira, *J. Nat. Prod.* **2008**, *71*, 1983–1987; b) J. Wu, Q. Xiao, J. S. Huang, Z. H. Xiao, S. H. Qi, Q. X. Li, S. Zhang, *Org. Lett.* **2004**, *6*, 1841–1844; c) D. E. U. Ekong, E. O. Olagbemi, *Tetrahedron Lett.* **1967**, *8*, 3525–3527.

[4] a) H. Zhang, O. A. Odeku, X. N. Wang, J. M. Yue, *Phytochemistry* **2008**, *69*, 271–275; b) M. Nakatani, S. A. M. Abdelgaleil, H. Okamura, T. Iwagewa, A. Sato, M. Doe, *Tetrahedron Lett.* **2000**, *41*, 6473–6477; c) L. R. Olmo, M. F. da Silva, E. Rodrigues Fo, P. C. Vieira, J. B. Fernandes, A. L. Pinheiro, E. F. Viela, *Phytochemistry* **1996**, *42*, 831–837.

[5] a) G. E. Veitch, E. Beckmann, B. J. Burke, A. Boyer, S. L. Maslen, S. V. Ley, *Angew. Chem. Int. Ed.* **2007**, *46*, 7629–7632; b) G. E. Veitch, E. Beckmann, B. J. Burke, A. Boyer, C. Ayats, S. V. Ley, *Angew. Chem. Int. Ed.* **2007**, *46*, 7633–7635; c) G. E. Veitch, A. Pinto, A. Boyer, E. Beckmann, J. C. Anderson, S. V. Ley, *Org. Lett.* **2008**, *10*, 569–572.

[6] a) K. K. Purushothaman, M. Venkatanarasimhan, A. Sarada, J. D. Connolly, D. S. Rycroft, *Can. J. Chem.* **1987**, *65*, 35–37; b) V. Mathuram, A. B. Kundu, *Indian J. Chem., Sect. B* **1990**, *29*, 970; c) A. Inada, M. Konishi, H. Murata, T. Nakanishi, *J. Nat. Prod.* **1994**, *57*, 1446–1449; d) H. P. Zhang, S. H. Wu, Y. M. Shen, Y. B. Ma, D. G. Wu, S. H. Qi, X. D. Luo, *Can. J. Chem.* **2003**, *81*, 253–257; e) X. N. Wang, C. Q. Fan, S. Yin, L. S. Gan, J. M. Yue, *Phytochemistry* **2008**, *69*, 1319–1327.

[7] Z. L. Geng, X. Fang, Y. T. Di, Q. Zhang, Y. Zeng, Y. M. Shen, X. J. Hao, *Tetrahedron Lett.* **2009**, *50*, 2132–2134.

[8] a) B. S. Mootoo, A. Ali, R. Motila, R. Pingal, A. Ramlal, A. Khan, W. F. Reynolds, S. McLean, *J. Nat. Prod.* **1999**, *62*, 1514–1517; b) M.-M. H. Tchuendem, J. F. Ayafor, J. D. Connolly, O. Sterner, *Tetrahedron Lett.* **1998**, *39*, 719–722; c) J. X. Cui, J. Wu, Z. W. Deng, P. Proksch, W. H. Lin, *J. Nat. Prod.* **2007**, *70*, 772–778; d) N. C. Veithch, G. A. Wright, P. C. Stevenson, *J. Nat. Prod.* **1999**, *62*, 1260–1263.

[9] a) D. A. Mulholland, S. E. Iourine, *Phytochemistry* **1998**, *47*, 1357–1361; b) I. T. Ismail, H. Ito, T. Hatano, S. Taniguchi, T. Yoshida, *Phytochemistry* **2003**, *64*, 1345–1349; c) I. T. Ismail, H. Ito, T. Hatano, S. Taniguchi, T. Yoshida, *Chem. Pharm. Bull.* **2004**, *529*, 1145–1147.

[10] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman Jr., J. A. Montgomery, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, *Gaussian 03, Revision D.01*, Gaussian, Inc., Wallingford, CT, **2005**.

[11] L. E. Overman, P. S. Tanis, *J. Org. Chem.* **2010**, *75*, 455–463.

[12] a) P. K. Kondru, W. Peter, D. N. Beratan, *J. Am. Chem. Soc.* **1998**, *120*, 2204–2205; b) D. Z. Liu, F. Wang, T. G. Liao, J. G. Tang, W. Steglich, H. J. Zhu, J. K. Liu, *Org. Lett.* **2006**, *8*, 5749–5752; c) H. M. Ge, C. H. Zhu, D. H. Shi, D. L. Zhang, Q. D. Xie, J. Yang, W. S. Ng, R. X. Tan, *Chem. Eur. J.* **2008**, *14*, 376–381.

[13] a) D. A. Mulholland, D. A. H. Taylor, *Phytochemistry* **1988**, *27*, 1741–1743; b) D. A. H. Taylor, *Phytochemistry* **1983**, *22*, 1297–1299.

[14] H. Ihmels, J. R. Scheffer, *Tetrahedron* **1999**, *55*, 885–907.

[15] a) L. R. V. Olmo, M. F. Das, G. F. da Silva, E. R. Fo, P. C. Vieira, J. B. Fernandes, A. L. Pinheiro, E. F. Vilela, *Phytochemistry* **1997**, *44*, 1157–1161; b) S. A. M. Abdelgaleil, H. Okamura, T. Iwagawa, A. Sato, I. Miyahara, M. Doe, M. Nakatani, *Tetrahedron* **2001**, *57*, 119–126.
[16] S. M. M. Agostinho, M. F. Das, G. F. da Silva, J. B. Fernandes, P. C. Vieira, A. L. Pinheiro, E. F. Vilela, *Biochem. Syst. Ecol.* **1994**, *22*, 323–328.
[17] M. C. Alley, D. A. Scudiero, A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker, M. R. Boyd, *Cancer Res.* **1988**, *48*, 589–601.
[18] N. Janssens, M. Janicot, T. Perera, *Investigational New Drugs* **2006**, *24*, 263–280.

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