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Note

Biscroyunoid A, an Anti-Hepatic Fibrotic 19-*nor*-Clerodane Diterpenoid Dimer with a C-16−C-12′ Linkage from *Croton yunnanensis*

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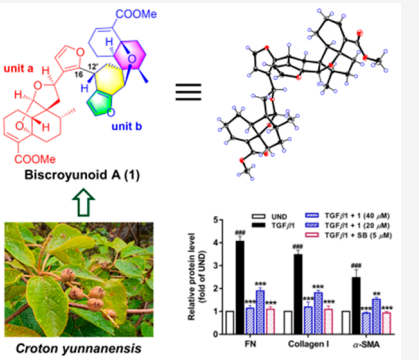
ABSTRACT: Biscroyunoid A (**1**), a 19-*nor*-clerodane diterpenoid dimer featuring a unique C-16−C-12′ linkage and containing an unusual 4,7-dihydro-5*H*-spiro[benzofuran-6,1′-cyclohexane] motif, together with its biosynthetic precursor, croyunoid A (**2**), were isolated from *Croton yunnanensis*. Their structures were determined by spectroscopic, computational, and single-crystal X-ray diffraction methods. Compound **1** exerted an antihepatic fibrosis effect in LX-2 cells via inhibition of TGFβ-Smad2/3 signaling.



Diterpenoid dimers are a small group of natural products mainly occurring in higher plants and soft corals.[1] So far, approximately 300 diterpenoid dimers, coupled mainly via direct C−C or C−O linkages, have been reported.[1,2] Structurally, the coupling between two monomers introduces an extra linkage or ring system into such compounds, which enhances their complexity. Biologically, their dual pharmacophores may simultaneously bind two different sites of the functional proteins, thereby potentially delivering an increased potency and selectivity.[3] Therefore, such compounds have aroused great interest in the scientific community in recent years. For instance, scospirosin B, a novel spiro *ent*-clerodane dimer with a 6/6/6/6/6-fused ring system, was demonstrated to be a potent immunosuppressive agent because of its selective inhibition of T-cell proliferation.[4] Aphadilactone C, a nemoralisin-type diterpenoid dimer with potent selective inhibition against diacylglycerol *O*-acyltransferase 1, was considered as a promising drug lead to treat metabolic diseases.[5] The total synthesis of hispidanin A, a unique asymmetric diterpenoid dimer, was simultaneously achieved by Yang's and Liu's groups with different strategies.[6,7]

Croton yunnanensis W. W. Smith (Euphorbiaceae) is a shrub distributed in the southwest provinces of mainland China.[8] Previous phytochemical investigations of this plant revealed diterpenoids and sesquiterpenoids as the major metabolites, of which some showed selective cytotoxicity against three tumor cell lines, SMMC-7721, HL-60, and A549.[9,10] In our continuing efforts toward obtaining novel diterpenoids from plants in the family Euphorbiaceae,[11−14] a 19-*nor*-clerodane diterpenoid dimer (**1**) featuring a C-16−C-12′ linkage, along with its precursor (**2**), were isolated from *C. yunnanensis*. Their structures (Figure 1) were elucidated by spectroscopic, computational, and single-crystal X-ray diffraction methods. Compound **1** exhibited moderate antihepatic fibrosis activity. Herein, we report the isolation, structure elucidation, a

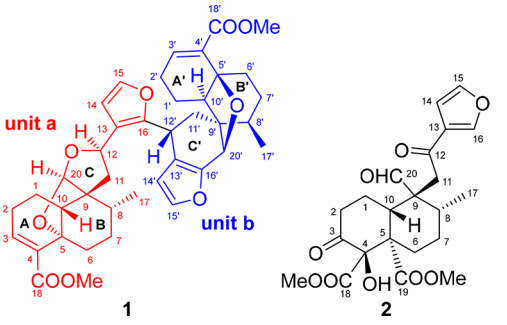


Figure 1. Structures of **1** and **2**.

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plausible biosynthetic pathway, and antihepatic fibrosis effects of **1** and **2**.

Biscroyunoid A (**1**) was isolated as colorless crystals. Its molecular formula, $C_{40}H_{46}O_9$, was deduced by HRESIMS ([M + Na]$^+$ m/z = 693.3046, calcd 693.3034). The ^{1}H NMR data (Table 1) displays two methyl doublets [δ_H = 0.89 (d, J = 5.9 Hz) and 1.03 (d, J = 6.5 Hz)], two methoxy groups [δ_H = 3.72 (s) and 3.73 (s)], three oxymethines [δ_H = 4.80 (dd, J = 9.0, 7.8 Hz), 4.98 (s), and 5.34 (s)], two olefinic protons [δ_H = 7.07 (dd, J = 6.0, 1.5 Hz) and 7.18 (dd, J = 6.3, 1.7 Hz)], and two 2,3-disubstituted furan rings [δ_H = 5.87 (d, J = 1.8 Hz), 6.31 (d, J = 1.9 Hz), 7.23 (d, J = 1.9 Hz), and 7.29 (d, J = 1.8 Hz)]. The ^{13}C and DEPT NMR spectra resolved 40 carbon signals, including two methyl ester groups, two furan rings, two trisubstituted double bonds, an acetal carbon, two methyls, 10 sp^3 methylenes, seven sp^3 methines (two oxygenated), two oxygenated sp^3 tertiary carbons, and two quaternary carbons. The aforementioned information implied that **1** consists of two *nor*-clerodane diterpenoid units.[15,16]

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data of 1 in $CDCl_3$ (J in Hz, δ in ppm)

no.	δ_H, multi.	δ_C	no.	δ_H, multi.	δ_C
1α	1.75, m	21.4	1′α	2.05, m	19.8
1β	1.66, m		1′β	1.43, m	
2α	2.30, m	26.1	2′α	2.01, m	25.8
2β	2.06, m		2′β	2.16, m	
3	7.18, dd (6.3, 1.7)	144.7	3′	7.07, dd (6.0, 1.5)	143.3
4		131.7	4′		132.3
5		86.9	5′		81.4
6α	2.61, dd (13.7, 6.5)	35.2	6′α	1.30, m	36.2
6β	1.25, m		6′β	2.56, dd (13.9, 6.7)	
7α	1.58, m	29.6	7′a	1.78, m	30.1
7β	1.72, m		7′b	1.70, m	
8	1.89, m	35.9	8′	1.81, m	41.1
9		57.9	9′		50.8
10	1.54, dd (13.4, 3.8)	52.7	10′	1.77, m	56.6
11a	2.11, dd (13.2, 7.8)	33.5	11′α	2.28, dd (14.6, 10.6)	33.8
11b	1.92, dd (13.2, 9.0)		11′β	2.02, dd (14.6, 5.4)	
12	4.80, dd (9.0, 7.8)	73.0	12′	4.55, dd (10.6, 5.4)	31.5
13		119.5	13′		119.6
14	6.31, d (1.9)	109.2	14′	5.87, d (1.8)	108.6
15	7.23, d (1.9)	141.2	15′	7.29, d (1.8)	142.8
16		152.0	16′		151.0
17	1.03, d (6.5)	17.2	17′	0.89, d (5.9)	17.5
18		166.1	18′		166.6
20	5.34, s	107.1	20′	4.98, s	70.4
OMe-18	3.72, s	51.5	OMe-18′	3.73, s	51.5

Detailed 2D NMR analysis further delineated the two *nor*-clerodane units, **a** and **b**, in **1**. The gross structure of unit **a** was determined readily to be the same as that of crotonyunnan A,[9] a 19-*nor*-clerodane diterpenoid previously isolated from this plant, by comparing directly their 2D NMR data (Figure 2). As for unit **b**, four spin systems, H-3′/H$_2$-2′/H$_2$-1′/H-10′, H$_2$-6′/H$_2$-7′/H-8′/H$_3$-17′, H$_2$-11′/H-12′, and H-14′/H-15′, were

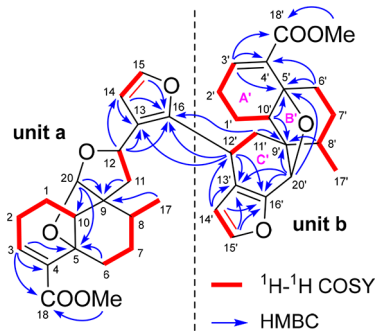

Figure 2. ^{1}H−^{1}H COSY and key HMBC correlations of **1**.

indicated by the ^{1}H−^{1}H COSY correlations observed (Figure 2). These fragments and the hydrogen-free carbons were further connected by heteronuclear multiple bond correlation (HMBC) analysis. The HMBC correlations from an olefinic proton (H-3′) to a hydrogen-free sp^2 carbon (C-4′) and an oxygenated tertiary carbon (C-5′), together with the correlation from a sp^3 methine (H-10′) to C-5′, were used to connect the $\Delta^{3'}$ and C-10′ via C-5′, thereby forming a six-membered carbon A′-ring. The methoxy carbonyl was attached to C-4′ by the HMBC correlation of H-3′/C-18′. A sp^3 methylene (C-6′) was further linked to C-5′ by the HMBC correlation from H$_2$-6′ to C-5′, while C-8′ and C-10′ were linked to a spiro quaternary carbon (C-9′) from the HMBC cross-peaks between H-8′ and H-10′ to C-9′, generating a six-membered carbon B′-ring. The HMBC correlations from a sp^3 methylene (H$_2$-11′) and an oxygenated methine (H-20′) to C-9′, together with the correlations from H-20′ and a sp^3 methine (H-12′) to two hydrogen-free sp^2 carbons (C-13′ and C-16′) were used to establish a six-membered carbon C′-ring. A 2,3-disubstituted furan ring was fused to the C′-ring at C-13′ and C-16′ by HMBC cross-peaks from H-14′ and H-15′ to C-13′ and C-16′. The strong HMBC correlation from H-20′ to the oxygenated tertiary carbon (C-5′) indicated the occurrence of an oxygen bridge between C-20′ and C-5′. Thus, the planar structure of unit **b** with a new 4-ethyl-3′,7′-dimethyl-spiro[cyclohexane-1,2′-bicyclo(4.4.0)decane] skeleton could be proposed. The connection of units **a** and **b** was achieved from the HMBC correlations of H-12′/C-13 and C-16 and H$_2$-11′/C-16, which enabled the gross structure of **1** with a unique C-16−C-12′ linkage to be constructed.

The relative configuration of **1** was established by NOESY NMR experiments. In unit **a**, the strong NOE cross-peaks of H-10/H-6β and H-8 suggested that they are axially oriented on the chair-conformation B-ring and were randomly assigned with a β-orientation (Figure 3). Consequently, the NOE correlations of H-7α/H-20, Me-17/H-20, and H-20/H-12 suggested H-20 and H-12 to be α-oriented. As for unit **b**, the

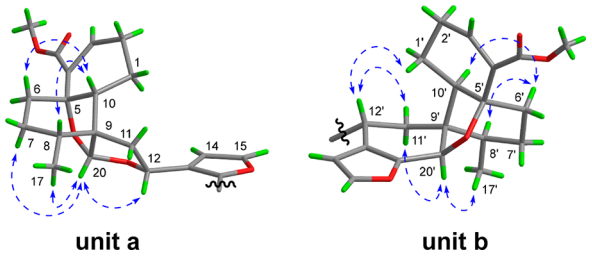

Figure 3. Key NOESY correlations of **1**.

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NOE correlations of H-12′/H-1′β, H-12′/H-11′β, and H-11′α/H-20′ indicated that H-12′, H-20′, and the C-9′−C-10′ bond are positioned axially on the six-membered C′-ring (half-chair conformation) and that H-12′ and H-20′ are *trans*-oriented. Thus, the B′-ring, C′-ring, and the tetrahydrofuran ring were found to be perpendicular to each other. Taking the B′-ring as the reference plane, the NOE correlations of H-20′/Me-17′ indicated Me-17′ as being β-oriented, while H-6′α/H-8′ and H-10′ designated these protons as α-oriented. The relationship between these two units could not be assigned by an NOE experiment because of the lack of diagnostic signals. However, the suitable crystals of **1** were obtained in a solvent system constituted by cyclohexane/ethyl acetate (8:1). Subsequent single-crystal X-ray diffraction allowed the successful determination of the structure of **1** with its absolute configuration as 5*S*, 8*R*, 9*R*, 10*R*, 12*R*, 20*R*, 5′*S*, 8′*R*, 9′*R*, 10′*R*, 12′*R*, and 20′*R* {[Flack parameter of 0.03(4)]} (Figure 4).

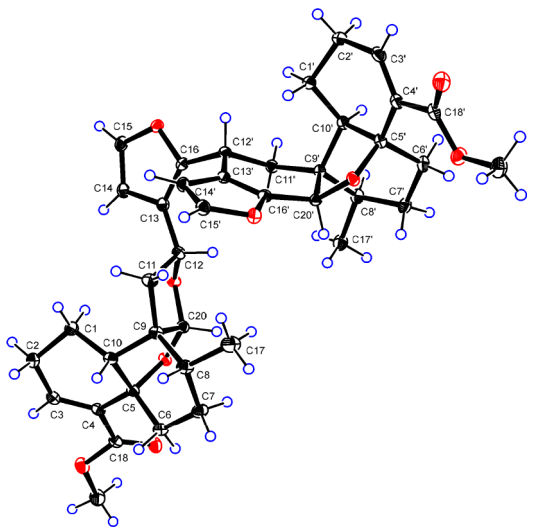

Figure 4. ORTEP diagram of **1**.

Croyunoid A (**2**) gave the molecular formula $C_{22}H_{26}O_9$. Its 1D NMR data (Table S1, Supporting Information) showed a close similarity to those of 3α,4β-dihydroxy-15,16-epoxy-12-oxo-cleroda-13(16),14-dien-9-al,[17] a known clerodane diterpenoid previously isolated from *Croton hovarum*, except for the presence of signals for two additional methoxy carbonyls (δ_H = 3.84 and 3.69; δ_C = 173.4, 171.0, 53.9, and 52.0) and a keto carbonyl (δ_C = 202.6) in **2**, instead of two methyl singlets and an oxygenated methine for the known compound. The two methoxy carbonyl groups were linked to C-4 and C-5 by HMBC correlations from OH-4 (δ_H = 4.15) to the carbonyl carbon (δ_C = 171.0, C-18) and from H-6β and H-10 (δ_H = 1.95 and 3.36) to the other carbonyl carbon (δ_C = 173.4, C-19), respectively. The additional keto carbonyl group was located at C-3 by HMBC cross-peaks from OH-4 and H_2-2 to δ_C = 202.6 and was further supported by the downfield-shifted carbon signals of C-2 (δ_C = 35.2) and C-4 (δ_C = 82.7) in **2**. The relative configuration of **2** was determined by analysis of its NOE data (Figure S1, Supporting Information), while its absolute configuration was determined as 4*R*,5*S*,8*R*,9*R*,10*R* by ECD calculations (see Supporting Information), which is consistent with the biogenesis of this compound class.

Putative biosynthetic pathways for **1** and **2** are proposed in Scheme 1. Briefly, enzyme-catalyzed cyclization of the initial diterpenoid precursor geranylgeranyl pyrophosphate (GGPP) could generate terpentedienol diphosphate (TDP),[18] which may then undergo a series of redox reactions to afford intermediate **i**. Then, C-5 of **i** successively could undergo decarboxylation and oxidation to yield an OH-5 intermediate **ii**. Reduction of 12-ketocarbonyl in **ii** followed by intramolecular acetalization between the aldehyde group (C-20) and 5,12-diol could generate intermediate **iv** (unit **a**). The intramolecular nucleophilic addition between C-16′ and the aldehyde group (C-20′) in **ii** followed by etherification between OH-5′ and OH-20′ could afford intermediate **v** (unit **b**). Finally, intermolecular nucleophilic addition between C-16 in **iv** and 12′-ketocarbonyl in **v** followed by reduction at C-12′ may then produce **1**. From a branch pathway, methyl esterification of **i** followed by oxidation of $\Delta^{3(4)}$ double bond could generate intermediate **vii**. Finally, **vii** may undergo acid-mediated epoxide ring-opening, followed by oxidation of OH-3 to afford **2**.

Compounds **1** and **2** were screened for antihepatic fibrosis activities in LX-2 cells by evaluating their regulation on transforming growth factor β 1 (TGFβ1)-stimulated liver fibrotic biomarkers. SB431542 was used as the positive control substance. As shown from an immunofluorescence assay (Figure 5A), the stimulation of TGFβ1 led to a significant increase of the fibronectin (FN) level in LX-2 cells, while treatment with **1** dose-dependently decreased the FN level without obvious cytotoxicity. The inhibitory effects of **1** on other liver fibrotic biomarkers, collagen I and α-SMA, were verified using a Western blot assay (Figure 5B).

Because TGFβ-Smad2/3 signaling is one of the most significant pathways activated in the progression of liver fibrosis,[19,20] the TGFβ1-triggered downstream variations were investigated further. As shown in Figure 6, **1** significantly inhibited the phosphorylation of Smad2/3 and then blocked Smad2/3 translocation from the cytoplasm to the nucleus, thereby suggesting its blockade in TGFβ-Smad2/3 signaling.

So far, about 20 naturally occurring dimeric clerodane diterpenoids have been reported. Notably, all of the previously reported clerodane dimers were postulated as being formed through intermolecular [2 + 2] cycloaddition, Diels−Alder [4 + 2] cycloaddition, etherification, or esterification of two monomers.[4,15,16,21,22] Biscroyunoid A (**1**), containing an unusual 4,7-dihydro-5*H*-spiro[benzofuran-6,1′-cyclohexane] monomer, represents the first example of a clerodane dimer coupled via a single C−C bond. It is possible that the particular structure of **1** renders it with better activity than its monomer (**2**), which makes it a promising lead structure for the development of an antihepatic fibrosis drug. Studies to investigate its in-depth cellular mechanisms are ongoing.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured on an X-4 melting instrument. Optical rotations were determined on a Rudolph Autopol I automatic polarimeter. ECD spectra were obtained on an Applied Photophysics Chirascan spectrometer, and UV spectra were obtained on a Shimadzu UV-2450 spectrophotometer. IR spectra were determined on a Bruker Tensor 37 infrared spectrophotometer. NMR spectra were measured on a Bruker AM-400 or AM-500 spectrometer at 25 °C. HRESIMS was performed on a Waters Micromass Q-TOF spectrometer. X-ray data were collected using an Agilent Xcalibur Nova X-ray diffractometer. Semipreparative HPLC was performed with a Shimadzu LC-20 AT instrument equipped with an SPD-M20A PDA detector. Purification by HPLC was performed on a YMC-pack

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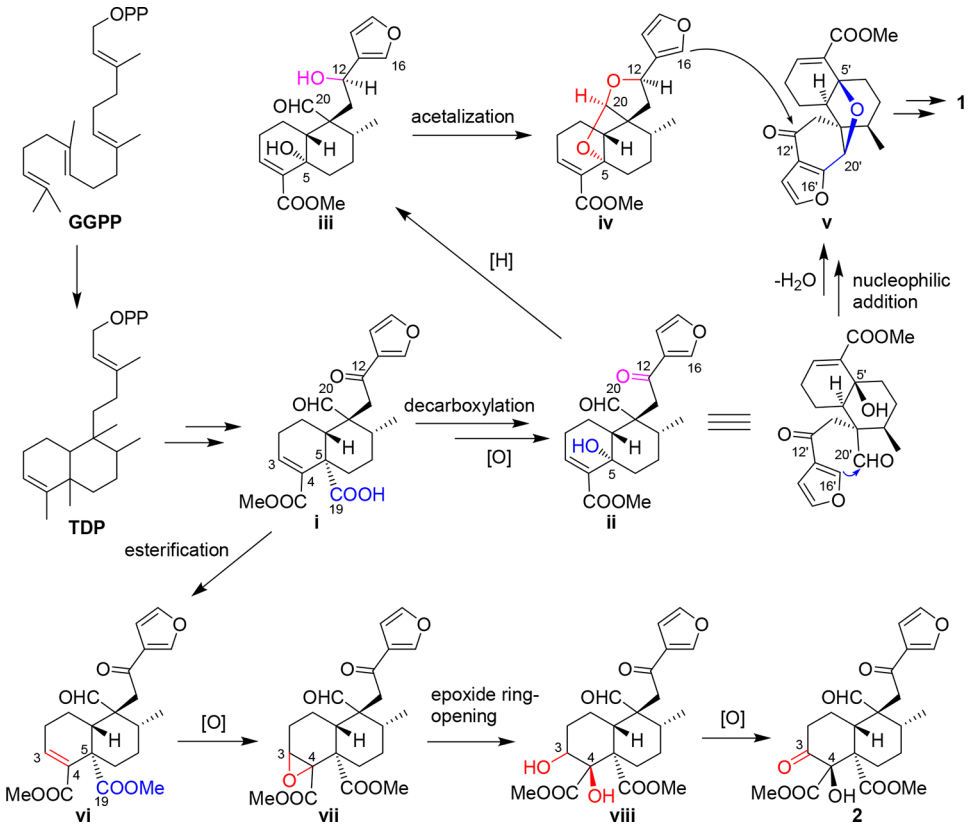
Scheme 1. Proposed Biosynthetic Pathways for 1 and 2



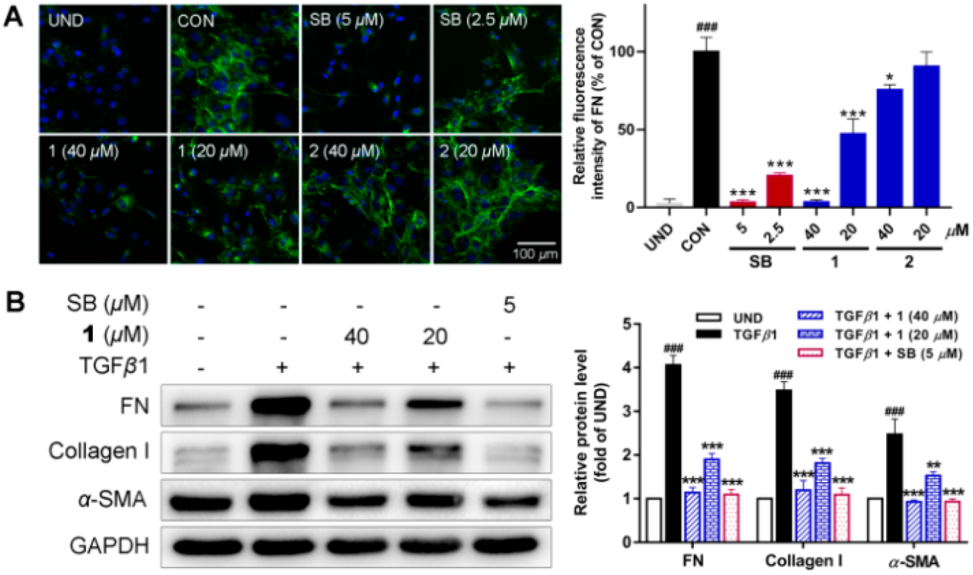


Figure 5. (A) Inhibitory effects of **1**, **2**, and SB431542 (SB) on FN (green) expression in an immunofluorescence assay. Blue signals represent the nucleus. (B) Inhibitory effects of **1** on fibrotic biomarkers in LX-2 cells induced by TGFβ1 (10 ng/mL). $^{\#\#\#}p < 0.001$ compared with untreated (UND) group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with TGFβ1 treated group ($n = 3$).

ODS-A column (250 × 10 mm, S-5 μm, 12 nm). Silica gel (Qingdao Haiyang Chemical Co., Ltd.), D101 macroreticular resin (Donghong Chemical Co., Ltd.), and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography (CC).

Plant Material. The leaves and twigs of *Croton yunnanensis* were collected in Xishuangbanna County, Yunnan Province, People's Republic of China (N21°93′16.04″, E101°26′29.51″), in June 2019. The plant was identified by Dr. You-Kai Xu, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, and a voucher specimen (YNBD-201906) has been deposited at the School of Pharmaceutical Sciences, Sun Yat-sen University.

Extraction and Isolation. The air-dried powder of *C. yunnanensis* (20 kg) was extracted using 95% EtOH (3 × 50 L) at room temperature. After evaporation of the solvent, the residue (1.0 kg) was suspended in H_2O and extracted with EtOAc (3 × 3 L). The EtOAc portion (630 g) was subjected to a passage over D101 macroporous resin (MeOH/H_2O, 45 → 100%) to give four fractions (Fr. I–IV). Fr. II (85 g) was chromatographed over silica gel eluted with petroleum

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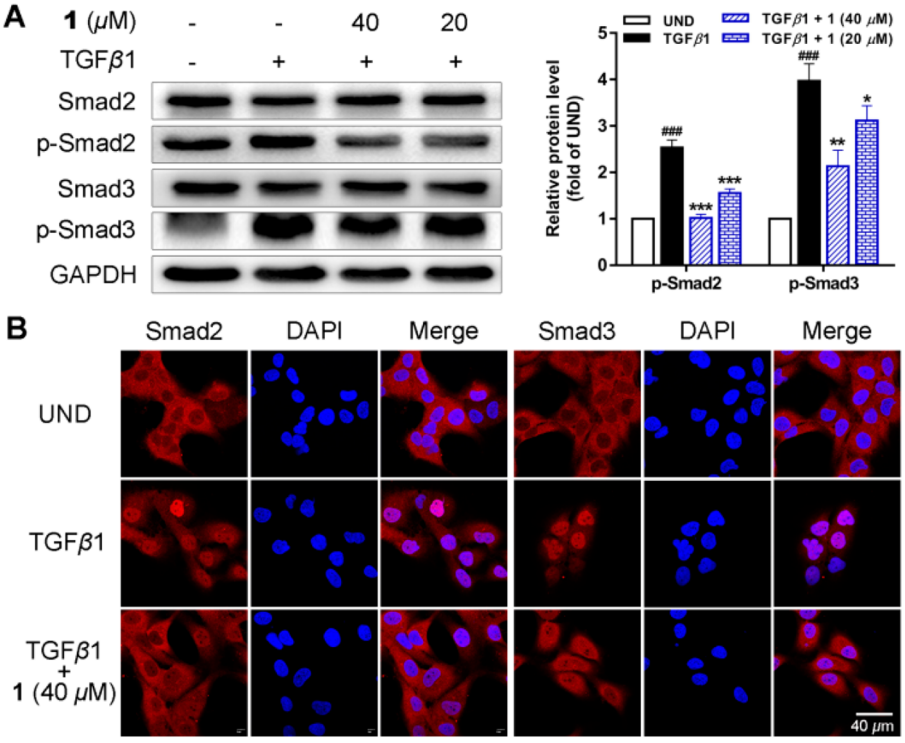

Figure 6. (A) Protein levels of Smad2/3 and p-Smad2/3 in LX-2 cells induced by TGFβ1 (10 ng/mL). $^{###}p < 0.001$ compared with UND group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with TGFβ1-treated group ($n = 3$). (B) Distributions of Smad2/3 proteins (red) in cytoplasm and nucleus (blue).

ether/EtOAc (10:1 → 0:1), to obtain five subfractions (Fr. IIa–Fr. IIe). Fraction IIb (6.5 g) was separated using a Sephadex LH-20 column (MeOH) to afford six fractions (Fr. IIb1–Fr. IIb6). Fr. IIb2 (120.0 mg) was purified by RP-HPLC equipped with a YMC-pack ODS-A column (CH_3CN/H_2O, 45:55, 3 mL/min) to give **2** (22.0 mg, t_R = 18.0 min, 0.0011‰). Fr. IIb5 (86.0 mg) was purified by RP-HPLC (CH_3CN/H_2O, 65:35, 3 mL/min) to afford **1** (9.6 mg, t_R = 16.5 min, 0.00048 ‰).

*Biscroyunoid A (**1**).* Biscroyunoid A was obtained as colorless block crystals, mp = 256–258 °C; $[\alpha]^{20}_D$ = −64.8 (c = 0.2, CH_3CN); UV (CH_3CN) λ_{max} (log ε) = 192 (3.69), 216 (3.62) nm; IR (KBr) ν_{max} = 2931, 2873, 1716, 1638, 1435, 1257, 1049, 745 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS m/z = 693.3046 $[M + Na]^+$ (calcd for $C_{40}H_{46}O_9Na^+$, 693.3034).

*Crystal Data for **1**.* Compound **1** was crystallized in a solvent mixture of cyclohexane/ethyl acetate (8:1) by slow evaporation at room temperature. $C_{40}H_{46}O_9$ (M = 670.77 g/mol): monoclinic, crystal size = 0.2 × 0.2 × 0.2 mm^3, space group $P2_1$ (no. 4), a = 11.15999(5) Å, b = 9.82325(4) Å, c = 15.42084(7) Å, β = 97.2988(4)°, V = 1676.848(13) $Å^3$, Z = 2, T = 99.99(10) K, μ(Cu Kα) = 0.759 mm^{-1}, D_{calc} = 1.328 g/cm^3, 34 197 reflections measured (5.778° ≤ 2θ ≤ 153.718°), 6906 unique (R_{int} = 0.0310, R_{sigma} = 0.0205), which were used in all calculations. The final R_1 was 0.0264 [$I > 2\sigma(I)$] and wR_2 was 0.0702 (all data). Flack parameter = 0.03(4). The crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Center (deposition number: CCDC 2160337).

*Croyunoid A (**2**).* Croyunoid A was obtained as a white amorphous powder; $[\alpha]^{20}_D$ = +8.3 (c = 0.1, CH_3CN); UV (CH_3CN) λ_{max} (log ε) = 195 (3.66), 216 (3.43), 252 (2.66) nm; ECD (c = 1.1 × 10^{-3} M, CH_3CN) λ_{max} ($\Delta\varepsilon$) = 287 (+2.16), 216 (−2.89) nm; IR (KBr) ν_{max} = 3455, 2954, 2927, 1722, 1674, 1438, 1276, 1157, 736 cm^{-1}; ^1H and ^{13}C NMR data, see Table S1; HRESIMS m/z = 457.1470 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_9Na^+$, 457.1469).

Immunofluorescence Assay. The detailed procedures of the immunofluorescence assay were performed by using our previous methods.[23]

Western Blot Analysis. Western blot assays were carried out as previously reported.[23]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.2c01054.

IR, MS, 1D and 2D NMR spectra of **1** and **2**, and ECD calculations of **2** (PDF)

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#W.L. and L.G. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Lin, L. G.; Ung, C. O. L.; Feng, Z. L.; Huang, L.; Hu, H. *Planta Med.* **2016**, *82*, 1309−1328.
(2) Sun, C. P.; Chang, Y. B.; Wang, C.; Lv, X.; Zhou, W. Y.; Tian, X. G.; Zhao, W. Y.; Ma, X. C. *Bioorg. Chem.* **2021**, *116*, 105356.
(3) Hadden, M. K.; Blagg, B. S. J. *Anti-Cancer Agents Med. Chem.* **2008**, *8*, 807−816.
(4) Li, X. R.; Yan, B. C.; Hu, K.; He, S.; Sun, H. D.; Zuo, J.; Puno, P. T. *Org. Lett.* **2021**, *23*, 5647−5651.
(5) Liu, J.; He, X. F.; Wang, G. H.; Merino, E. F.; Yang, S. P.; Zhu, R. X.; Gan, L. S.; Zhang, H.; Cassera, M. B.; Wang, H. Y.; Kingston, D. G. I.; Yue, J. M. *J. Org. Chem.* **2014**, *79*, 599−607.
(6) Li, F.; Tu, Q.; Chen, S.; Zhu, L.; Lan, Y.; Gong, J.; Yang, Z. *Angew. Chem., Int. Ed.* **2017**, *56*, 5844−5848.
(7) Deng, H.; Cao, W.; Liu, R.; Zhang, Y.; Liu, B. *Angew. Chem., Int. Ed.* **2017**, *56*, 5849−5852.
(8) Flora of China Editorial Committee. *Flora of China*, Vol. *44*; Science Press: Beijing, 1996; p 128.
(9) Su, X. M.; Liang, Q.; Hu, J. X.; Zhang, X. M.; Jia, R. L.; Xu, W. H. *Bioorg. Med. Chem.* **2021**, *51*, 116495.
(10) You, H. M.; Zhang, J. R.; Zhao, Z. H.; Wang, W.; Zhang, L. Z.; Jiang, Y. T.; Wang, Y.; Huang, X. Z.; Jiang, Z. Y.; China, J. *Chin. Mater. Med.* **2019**, *44*, 4648−4652, DOI: 10.19540/j.cnki.cjcmm.20190809.201.
(11) Yuan, F. Y.; Pan, Y. H.; Yin, A. P.; Li, W.; Huang, D.; Yan, X. L.; Wu, S. Q.; Tang, G. H.; Pu, R.; Yin, S. *Org. Chem. Front.* **2022**, *9*, 775−780.
(12) Huang, D.; Pan, Y. H.; Yuan, F. Y.; Li, W.; Yan, X. L.; Zou, M. F.; Tang, G. H.; Yin, S. *Org. Lett.* **2021**, *23*, 9602−9605.
(13) Li, W.; Wang, R. M.; Pan, Y. H.; Zhao, Y. Y.; Yuan, F. Y.; Huang, D.; Tang, G. H.; Bi, H. C.; Yin, S. *Org. Lett.* **2020**, *22*, 4435−4439.
(14) Song, Q. Q.; Rao, Y.; Tang, G. H.; Sun, Z. H.; Zhang, J. S.; Huang, Z. S.; Yin, S. *J. Med. Chem.* **2019**, *62*, 2060−2075.
(15) Li, W.; Huang, C.; Liu, Q.; Koike, K. *J. Nat. Prod.* **2017**, *80*, 2478−2483.
(16) Zhao, X. T.; Lei, C.; You, J. Q.; Zhao, T.; Yu, M. H.; Shi, X. L.; Hu, X.; Hou, A. J. *Bioorg. Chem.* **2021**, *112*, 104916.
(17) Krebs, H. C.; Ramiarantsoa, H. *Phytochemistry* **1996**, *41*, 561−563.
(18) Hamano, Y.; Kuzuyama, T.; Itoh, N.; Furihata, K.; Seto, H.; Dairi, T. *J. Biol. Chem.* **2002**, *277*, 37098−37104.
(19) Feng, X. H.; Derynck, R. *Annu. Rev. Cell Dev. Biol.* **2005**, *21*, 659−693.
(20) Heldin, C. H.; Moustakas, A. *Cell Tissue Res.* **2012**, *347*, 21−36.
(21) Merritt, A. T.; Ley, S. V. *Nat. Prod. Rep.* **1992**, *9*, 243−287.
(22) Li, R.; Morris-Natschke, S. L.; Lee, K. H. *Nat. Prod. Rep.* **2016**, *33*, 1166−1226.
(23) Li, S.; Gan, L.; Tian, Y. J.; Tian, Y.; Fan, R. Z.; Huang, D.; Yuan, F. Y.; Zhang, X.; Lin, Y.; Zhu, Q. F.; Tang, G. H.; Yan, X. L.; Yin, S. *Bioorg. Chem.* **2021**, *114*, 105222.