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•Original article•

Highly oxygenated clerodane furanoditerpenoids from the leaves and twigs of *Croton yunnanensis*

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[ABSTRACT] The phytochemical investigation of the leaves and twigs of *Croton yunnanensis* resulted in the isolation of eight new clerodane furanoditerpenoids, named croyunfuranoids A−H (**1**−**8**), along with three known analogs (**9**−**11**). The structures of these compounds were elucidated using spectroscopic analyses, and their absolute configurations were determined through a combination of electronic circular dichroism (ECD) calculations and single-crystal X-ray diffraction. Notably, Croyunfuranoid D (**4**) is identified as a rare 18,19-*dinor*-clerodane diterpenoid. Additionally, the structure of a previously reported diterpenoid, crotonyunnan B, was revised. All isolated compounds were evaluated for their inhibitory activities on nitric oxide (NO) production in LPS-induced RAW 264.7 macrophages. Among them, compounds **5** and **6** demonstrated significant inhibitory effects, with IC_{50} values of 20.33 ± 2.31 and 22.80 ± 1.31 μmol·L^{-1}, respectively.

[KEY WORDS] *Croton yunnanensis*; Euphorbiaceae; Clerodane furanoditerpenoid; NO inhibitory activity
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Introduction

The genus *Croton* (Euphorbiaceae), predominantly found in tropical and subtropical regions, comprises approximately 1300 species [1]. Many *Croton* species have been traditionally used in folk medicines and are well-recognized as a rich source of diterpenoids [2−4], particularly clerodane diterpenoids [5]. To date, over 200 clerodane diterpenoids have been isolated from *Croton* species [6−7]. In recent years, these diterpenoids have garnered significant interest due to their unique structures and diverse biological activities, including PXR agonistic, antitumor, and anti-inflammatory properties [8−11].

Croton yunnanensis W. W. Smith, a shrub widely distributed in Yunnan and Sichuan Provinces of mainland China [12], has been the subject of previous phytochemical studies that identified diterpenoids and sesquiterpenoids as its main constituents [13−15]. For example, crotonines A−H, a series of eight structurally diverse clerodane diterpenoids, have exhibited notable hypoglycemic activity [13]. Additionally, crotonyunnan E, a 15,16-*dinor*-*ent*-pimarane diterpenoid with a 6/6/6-fused ring system, has shown selective cytotoxicities against SMMC-7721, HL-60, and A549 cell lines [14]. In our earlier work, we isolated a unique 19-*nor*-clerodane diterpenoid diterpenoid dimer from *C. yunnanensis* with antihepatic fibrosis activity [16]. Continuing our search for structurally interesting diterpenoids from this plant, we isolated eight new clerodane furanoditerpenoids, designated as croyunfuranoids A−H (**1**−**8**), along with three known analogs (**9**−**11**) were isolated (Fig. 1). This paper reports the isolation, structural elucidation, and biological activities of these compounds.

Result and Discussion

Compound **1**, obtained as colorless crystals, has the molecular formula $C_{22}H_{28}O_9$ as determined by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) data showing an *m/z* 459.1620 $[M + Na]^+$ (Calcd. for $C_{22}H_{28}O_9Na^+$, 459.1626), corresponding to nine indices of hydrogen deficiency (IHD). The ^1H nuclear magnetic resonance (NMR) data (Table 1) displayed a methyl doublet [δ_H

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[Δ]These authors contributed equally to this work.
These authors have no conflict of interest to declare.

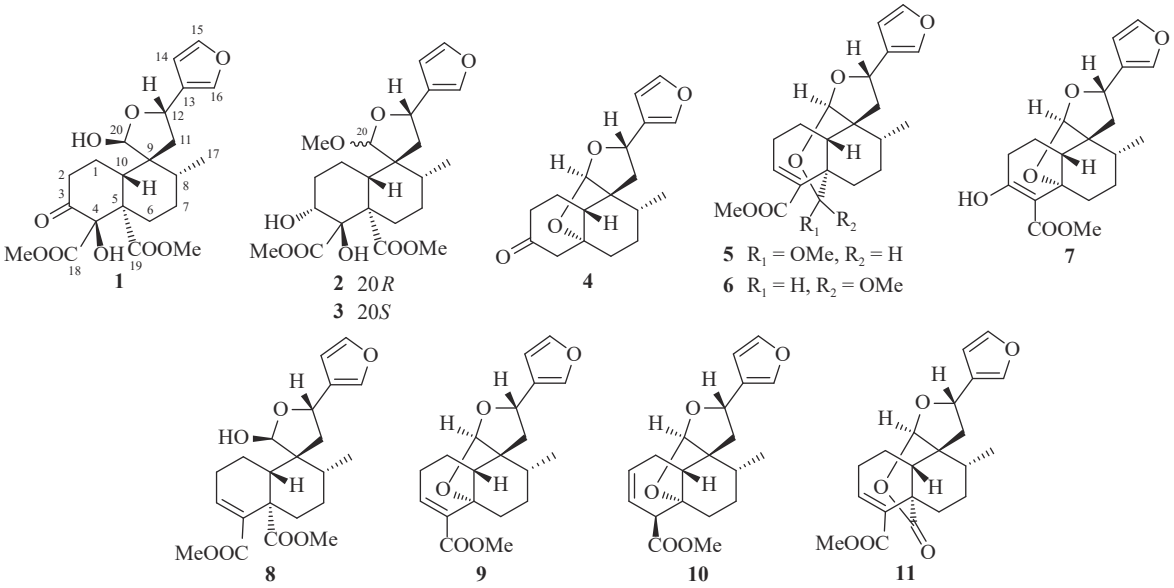


Fig. 1 Structures of compounds 1−11.

0.96 (3H, d, J = 6.3 Hz)], two methoxyl groups [δ_H 3.56 (3H, s) and 3.82 (3H, s)], two oxymethine protons [δ_H 5.09 (1H, dd, J = 11.5, 6.3 Hz) and 5.25 (1H, br s)], two exchangeable protons [δ_H 3.16 (1H, br s) and 4.21 (1H, s)], a β-substituted furan ring [δ_H 6.40 (1H, br s), 7.38 (1H, br s), and 7.40 (1H, br s)], and a series of aliphatic multiplets. The ^{13}C NMR spectrum, combined with DEPT and HSQC experiments, displayed 22 carbon signals, including a ketocarbonyl, two methyl ester groups, a β-substituted furan ring, a hemiacetal carbon, a methyl group, five sp^3 methylenes, three sp^3 methines (one oxygenated), an oxygenated sp^3 tertiary carbon, and two quaternary carbons. As this accounted for six IHDs, the remaining IHDs indicated the presence of three additional rings in the structure of compound **1**.

The 2D structure assignment for compound **1** was accomplished through the interpretation of 2D NMR data (Fig. 2). In the ^{1}H−^{1}H correlation spectroscopy (COSY) spectrum, three main spin systems were observed: H_2-2/H_2-1/H-10, H_2-6/H_2-7/H-8/H_3-17, and H_2-11/H-12. These fragments and the quaternary carbons were connected by analyzing the heteronuclear multiple bond correlation (HMBC) data. HMBCs of H_2/C-3 and C-4; H_2-6/C-5; H_3-17/C-7, C-8, and C-9; and H-10/C-4, C-5, and C-9 suggested the presence of a 6/6 carbocyclic ring system. The methyl ester groups were attached to C-4 and C-5, respectively, as indicated by HMBCs from 4-OH to C-18 and from H2-6 and H-10 to C-19. The construction of a 2-hydroxytetrahydrofuran ring was confirmed by HMBCs of H_2-11 and H-20/C-9 and H-12 and 20-OH/C-20. Finally, the furanyl moiety was connected to C-12 based on HMBCs from H-12 to C-13, C-14, and C-16. Thus, the planar structure of compound **1** was established as depicted.

The relative configuration of compound **1** was elucidated using nuclear overhauser effect spectroscopy (NOESY) data. The nuclear overhauser effect (NOE) interactions between H-7α/H-20 and H-8/H-10 (Fig. 3) indicated that H-7α, H-8, H-10, and C-9−C-20 bond occupied the axial positions of the chair conformational six-membered B-ring, suggesting that the A/B rings were *trans*-fused. Consequently, H-8 and H-10 were assigned β-orientations, while H-7α and the C-9−C-20 bond were α-oriented. The NOE correlation of 4-OH with H-10 indicated that 4-OH was β-oriented. Given that the tetrahydrofuran ring (C-ring) was perpendicular to the A/B-plane, the NOE interactions of H-1β with H-12 and H-7α with H-20 indicated both C-12 and C-20 were *S** configurations. The structure of compound **1**, including its absolute configuration (4*R*,5*S*,8*R*,9*R*,10*R*,12*S*,20*S*), was confirmed by X-ray crystallographic data (Fig. 4), with a Flack parameter = 0.06 (13). Compound **1** was thus assigned the trivial name croyunfuranoid A.

Compounds **2** and **3** were determined to have the same molecular formula ($C_{23}H_{32}O_9$), based on HR-ESI-MS analysis. The ^{1}H and ^{13}C NMR data (Table 1) of these compounds revealed structural features similar to those of compound **1**, with notable differences: the presence of an additional methoxy group and the replacement of the ketone group in compound **1** with a hydroxy group in compounds **2** and **3**. These differences were confirmed by HMBCs of 20-OMe/C-20 and 3-OH/C-3, as well as the ^{1}H−^{1}H COSY correlation of H_2-2/H-3 (Fig. 2). Detailed 2D NMR analyses supported that compounds **2** and **3** shared the same planar structure as compound. In the NOESY spectrum, the relative stereochemistry of the A/B rings in compounds **2** and **3** was found to be identical to that of compound **1**, as indicated by their similar NOESY data. The only stereochemical difference between compounds **2** and **3** was at the chiral center C-20. The *R** configuration of C-20 in compound **2** was supported by NOE correlations of H-12/H-20 and 20-OMe/19-OMe, while the correlations of H-12/20-OMe and H-20/H-7α indicated an S* configuration for C-20 in compound **3** (Fig. 3). Finally, the absolute configurations (3*R*,4*R*,5*S*,8*R*,9*R*,10*R*,12*S*,20*R* for

Table 1 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of 1−3 in $CDCl_3$ (*J* in Hz)

No.	1		2		3	
	δ_C	δ_H, multi.	δ_C	δ_H, multi.	δ_C	δ_H, multi.
1*α*	22.8	3.03, m	17.9	2.67, m	19.0	2.62, m
1*β*		2.15, m		1.63, m		1.71, m
2*α*	36.3	2.42, m	30.1	1.96, m	32.0	1.89, m
2*β*		2.80, m		2.13, m		2.01, m
3	203.5		73.2	3.74, m	74.4	3.73, m
4	82.9		78.2		79.8	
5	54.4		52.4		52.9	
6*α*	27.0	1.54, m	29.5	1.43, m	27.8	1.75, m
6*β*		1.78, m		1.78, m		1.99, m
7*α*	29.2	1.99, m	28.9	2.03, m	30.1	1.92, m
7*β*		1.61, m		1.79, m		1.57, m
8	41.8	1.47, m	43.0	1.41, m	41.9	1.40, m
9	53.2		51.0		53.7	
10	44.9	2.51, br d (12.8)	47.0	2.00, m	47.4	2.06, m
11a	43.5	2.32, dd (13.1, 6.3)	45.9	2.12, dd (13.6, 7.4)	44.0	2.21, dd (13.1, 6.8)
11b		1.91, dd (13.1, 11.5)		2.06, dd (13.6, 9.3)		1.82, dd (13.1, 10.5)
12	70.6	5.09, dd (11.5, 6.3)	68.6	4.66, dd (9.3, 7.4)	70.2	4.88, dd (10.5, 6.8)
13	125.0		126.2		125.2	
14	108.8	6.40, br s	109.0	6.46, br s	108.8	6.42, br s
15	143.3	7.38, br s	143.3	7.37, br s	143.3	7.38, br s
16	139.6	7.40, br s	139.5	7.41, br s	139.5	7.41, br s
17	17.5	0.96, d (6.3)	18.2	1.20, d (6.7)	17.8	0.95, d (6.8)
18	171.6		173.9		173.4	
19	173.3		176.8		176.9	
20	99.8	5.25, br s	107.2	4.83, s	106.0	4.63, s
18-OMe	53.8	3.82, s	53.3	3.82, s	52.9	3.77, s
19-OMe	51.1	3.56, s	52.1	3.76, s	52.4	3.80, s
20-OMe			56.9	3.33, s	54.1	3.19, s
3-OH				4.67, d (12.0)		6.56, d (12.0)
4-OH		4.21, s		3.56, s		3.15, s
20-OH		3.16, br s				

compound **2**; 3*R*,4*R*,5*S*,8*R*,9*R*,10*R*,12*S*,20*S* for compound **3**) were confirmed by single-crystal X-ray crystallographic data (Fig. 4). Thus, the structures of compounds **2** and **3** were established as depicted, and they were given the trivial names croyunfuranoid B and croyunfuranoid C, respectively.

Compound **4**, a white amorphous powder, was determined to have the molecular formula $C_{18}H_{22}O_4$ based on HR-ESI-MS data ($[M + Na]^+$ *m/z* 325.1406, Calcd. for 325.1410). The 1D NMR data (Table 2) revealed the presence of a ketocarbonyl group, a *β*-substituted furan ring, a quaternary carbon, an oxygenated sp^3 tertiary carbon, four sp^3 methines (two oxygenated), six sp^3 methylenes, and a methyl group. This data indicated that compound **4** possesses structural features characteristic of a *dinor*-clerodane diterpenoid. The planar structure of rings B−D in compound **4** was determined to be the same as that in crotonyunnan A (**9**) [14], as confirmed by detailed 2D NMR analyses (Fig. 2). For ring A, the ketone group (δ_C 209.5) was assigned at C-3, supported by

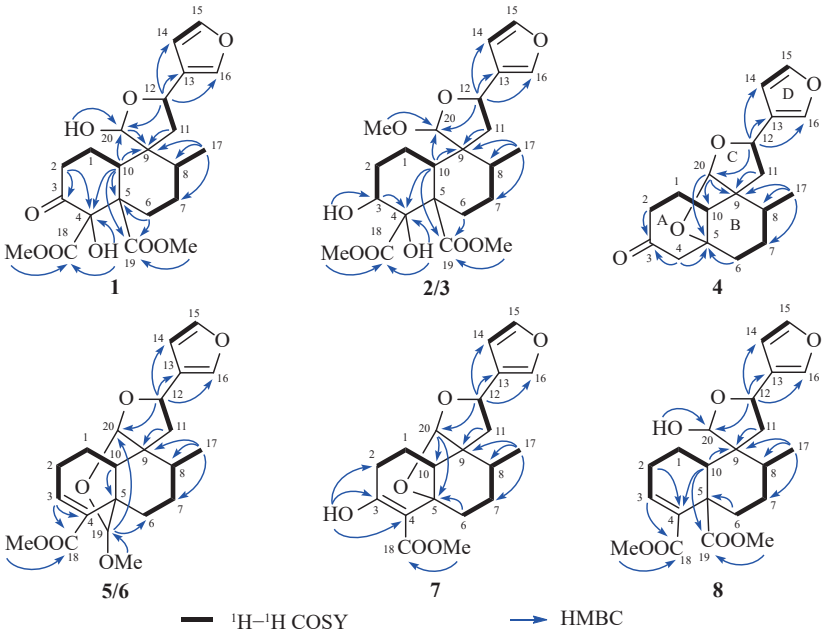


Fig. 2 Key ^{1}H–^{1}H COSY and HMBCs of compounds 1–8.

HMBC from H_2-2 to C-3. The HMBCs from an isolated methylene group (CH_2-4) to an oxygenated sp^3 tertiary carbon (C-5) further supported that compound **4** is an 18,19-*dinor*-clerodane diterpenoid. NOESY correlations of H-1*β*/H-12, H-7*α*/H-20, H-8/H-10, and H-10/H-11a indicated that compound **4** possesses the same relative configuration as compound **9** (Fig. 3). The absolute configuration (5*R*,8*R*,9*R*,10*R*,12*S*,20*R*) of compound **4** was determined by comparing its experimental and simulated electronic circular dichroism (ECD) spectra (Fig. 5). Consequently, the structure of compound **4** was established as depicted and named croyunfuranoid D.

Compounds **5** and **6** were determined to have the same molecular formula $C_{22}H_{28}O_6$, based on HR-ESI-MS. The 1D NMR data (Table 2) for both compounds closely resembled those of crotonolide D and isocrotonolide D [17], with the main difference being the presence of an additional methoxy group. This methoxy group was located at 19-OH, as indicated by the HMBC (Fig. 2) of the methoxy protons (δ_H 3.41 in compound **5**; δ_H 3.46 in compound **6**) to C-19 (δ_C 102.7 in compound **5**; δ_C 104.4 in compound **6**). The relative stereochemistry of **5** and **6** was established to be the same as that of crotonolide D and isocrotonolide D, respectively, based on comparisons of their 1D NMR and NOESY data (Fig. 3). Thus, the structures of compounds **5** and **6** were deduced as depicted and given the trivial names croyunfuranoid E and croyunfuranoid F, respectively.

Compound **7** was found to have the molecular formula $C_{20}H_{24}O_6$ as determined by HR-ESI-MS with an ion at *m*/*z* 383.1462 $[M + Na]^+$ (Calcd. for $C_{20}H_{24}O_6Na^+$, 383.1465), which is 16 mass units more than that of compound **9**. The ^{1}H and ^{13}C NMR data (Table 3) of compound **7** showed high similarity to those of compound **9**, except for the replacement of an sp^2 methine (δ_C 144.5, C-4) in compound **9** with an oxygenated, hydrogen-free sp^2 carbon (δ_C 176.7) and the presence of an additional hydroxy group (δ_H 13.08, 3-OH) in compound **7**. This suggested that compound **7** was a 4-hydroxylated derivative of compound **9**, which was further supported by HMBCs from 3-OH to C-2, C-3, and C-4 (Fig. 2). The relative configuration of compound **7** was established to be the same as that of compound **9** based on NOESY data comparisons (Fig. 3), and its absolute configuration was assigned as 5*S*,8*R*,9*R*,10*R*,12*S*,20*R* through ECD calculations (Fig. 5). Compound **7** was given the trivial name croyunfuranoid G.

Compound **8** was isolated as a white amorphous powder. Its molecular formula $C_{22}H_{28}O_7$, was determined by HR-ESI-MS data. The 1D NMR data (Table 3) for compound **8** showed high similarity to those of compound **1**, with the key difference being the replacement of the ketone group (C-3) and an oxygenated sp^3 tertiary carbon (C-4) in compound **1** by a trisubstituted double bond in compound **8**. This structural difference was confirmed by HMBCs from H_2-2 and H-10 to C-4, along with the ^{1}H–^{1}H COSY correlation of H_2-2/H-3 (Fig. 2). The absolute configuration (5*S*,8*R*,9*R*,10*R*,12*S*,20*S*) of compound **8** was determined by comparing its ECD data with those of compound **1**. Compound **8** was given the trivial name croyunfuranoid H.

By comparing their 1D NMR data with those reported in the literature, compounds **9**–**11** were identified as crotonyunnan A (**9**), crotonyunnan B (**10**) [14], and dihydrocroverin (**11**) [18]. The absolute configurations of crotonyunnan A (**9**) and B (**10**) were previously assigned as 5*S*,8*R*,9*R*,10*R*,12*S*,20*R* and 4*S*,5*S*,8*R*,9*R*,10*S*,12*S*,20*R*, respectively, based on comparisons of their experimental and simulated ECD data from the literature. In this study, the structures of com-

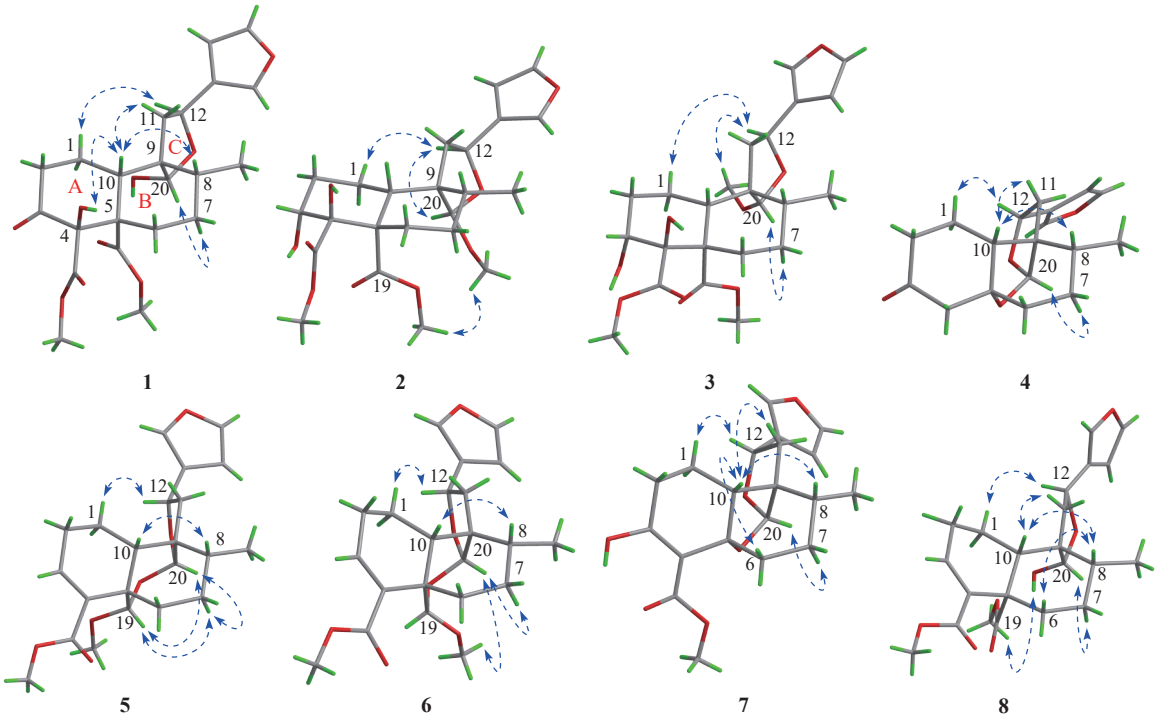


Fig. 3 Key NOESY correlations of compounds 1−8.

pounds **9** and **10**, including their absolute configurations, were confirmed by X-ray crystallographic data (Fig. 5), leading to the revision of the absolute configuration of previously published crotonyunnan B to 4*S*,5*S*,8*R*,9*R*,10*R*,12*S*,20*R*.

Compounds **1**−**11** were evaluated for their inhibitory activities on nitric oxide (NO) production in LPS-induced RAW 264.7 macrophages using the Griess assay, with quercetin serving as the positive control (IC_{50} 17.65 ± 1.33 μmol·L^{-1}). Under subtoxic concentrations (50 μmol·L^{-1}), compounds **2**, **5**, and **6** demonstrated moderate inhibitory activities, with IC_{50} values of 31.85 ± 3.40, 20.33 ± 2.31, and 22.80 ± 1.31 μmol·L^{-1}, respectively. Other compounds were inactive, showing inhibition rates of less than 50% at 50 μmol·L^{-1}. Comparative analysis of the structural features of the active compounds (**5** and **6**) suggested that the presence of a 19-OMe group might enhance the activity. Additionally, all compounds were tested for their inhibitory activities against *α*-glucosidase, *α*-amylase, and lipase at a concentration of 250 μmol·L^{-1}, but none exhibited significant activity, with inhibition rates below 30% (Table S1).

Clerodane diterpenoids represent a large group of natural products found in several hundred plant species across various families and in other taxonomic groups. In this study, eight new clerodane furanoditerpenoids (**1**−**8**) and three known analogs (**9**−**11**) were isolated from *C. yunnanensis*. Their structures were elucidated using spectroscopic analyses, ECD calculations, and single-crystal X-ray diffraction. The structure of the known compound crotonyunnan B was revised. Compounds **5** and **6** exhibited moderate inhibitory effects on NO production. Notably, dihydrocroverin (**11**), a known clerodane diterpenoid with a substantial yield of 0.175‰ isolated in this study, could serve as a chemotaxonomic marker for *C. yunnanensis*.

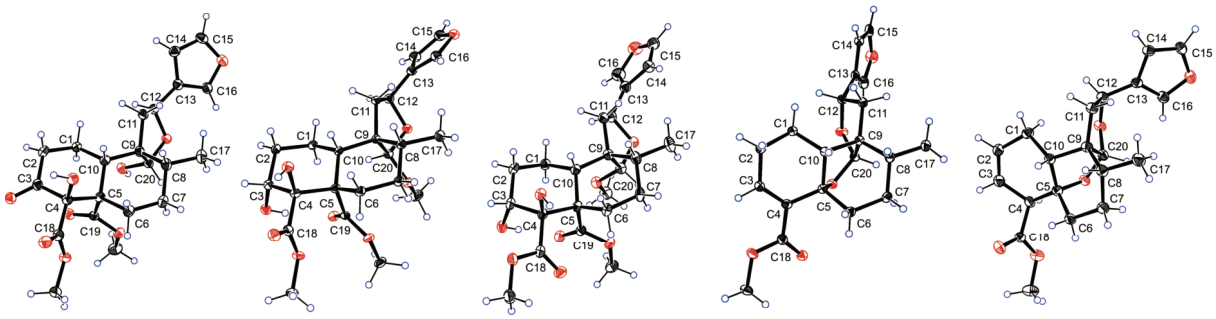
Experimental

General experimental procedures

Melting points were measured using an X-4 melting instrument. X-ray crystallographic data were collected with an Agilent Xcalibur Nova X-ray diffractometer. Optical rotations were determined using a Rudolph Autopol I automatic polarimeter. ECD spectra were recorded on an Applied Photophysics Chirascan spectrometer, and UV data were collected using a Shimadzu UV-2450 spectrophotometer. Infrared (IR) spectra were obtained with a Bruker Tensor 37 IR spectrophotometer. NMR spectra were measured on a Bruker AM-500 spectrometer at 25 °C. HR-ESI-MS data were acquired with a Waters Micromass Q-TOF spectrometer. Semi-preparative high-performance liquid chromatography (HPLC) was performed using a Shimadzu LC-20 AT instrument equipped with an SPD-M20A PDA detector. Purification by HPLC was conducted on a YMC-Pack ODS-A column (250 mm × 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

Croton yunnanensis was collected in June 2019 from Xishuangbanna County, Yunnan Province, China. The plant was identified by Dr. XU Youkai from Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen (No. YNBD-201906) has been stored in our laboratory for reference.



1 **2** **3** **9** **10**

Fig. 4 Single-crystal X-ray structures of 1−3, 9, and 10.

Extraction and isolation

The dried leaves and twigs of *C. yunnanensis* (20.0 kg) were powdered and extracted with 95% ethanol (3 × 50 L) at room temperature, yielding a crude extract (1.0 kg). This extract was suspended in water and partitioned with ethyl acetate (3 × 3 L). The ethyl acetate fraction (630.0 g) was separated using D101 macroporous resin CC (MeOH/H_2O, 45%−100%), resulting in four fractions (I−IV). Fr. III (66.0 g) was further purified by silica gel CC, eluted with CH_2Cl_2/MeOH (100 ∶ 1−5 ∶ 1), producing five subfractions (Frs. IIIa−IIIe). Subfraction IIIa (14.0 g) was subjected to a Sephadex LH-20 column, eluted with methanol, yielding five fractions (Frs. IIIa1−IIIa5). From Fr. IIIa2 (850.0 mg), further purification using silica gel CC (petroleum ether/acetone, 15 ∶ 1−1 ∶ 2), followed by RP-HPLC (CH_3CN/H_2O, 70 ∶ 30, 3 mL·min^{-1}), resulted in compounds **9** (56.5 mg, t_R 13.0 min) and **4** (12.0 mg, t_R 14.0 min). Colorless crystals of compounds **10** (360.0 mg) and **11** (3.5 g) were recrystallized from Fr. IIIa1 (1.2 g) and Fr. IIIa3 (5.9 g), respectively, using a CH_2Cl_2/MeOH (1 ∶ 10) system. Fr. IIIa4 (290.0 mg) was purified by RP-HPLC (CH_3CN/H_2O, 65 ∶ 35, 3 mL·min^{-1}), yielding compounds **7** (6.0 mg, t_R 11.5 min), **5** (18.0 mg, t_R 13.0 min), and **6** (30.5 mg, t_R 13.5 min). Fr. IIIa5 (180.0 mg) was separated by RP-HPLC (CH_3CN/H_2O, 75 ∶ 25, 3 mL·min^{-1}), yielding compounds **3** (18.5 mg, t_R 8.5 min) and **2** (22.0 mg, t_R 10.0 min). Fraction IIIc (2.5 g) was purified by silica gel CC (petroleum ether/EtOAc, 10 ∶ 1−1 ∶ 1), yielding compounds **1** (235.0 mg) and **8** (55.0 mg).

Identification of new compounds

*Croyunfuranoid A (**1**)*

Colorless crystals, mp: 196−198 °C; $[\alpha]_D^{20}$ +34 (*c* 0.1, CH_3CN); UV (CH_3CN) λ_{max} (log ε): 209 (3.74) nm; IR (KBr) ν_{max}: 3452, 2955, 1723, 1239, 1022 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); HR-ESI-MS at *m/z* 459.1620 $[M + Na]^+$ (Calcd. for $C_{22}H_{28}O_9Na^+$, 459.1626).

*Croyunfuranoid B (**2**)*

Colorless crystals, mp: 201−202 °C; $[\alpha]_D^{20}$ −23 (*c* 0.1, CH_3CN); UV (CH_3CN) λ_{max} (log ε): 195 (3.79) nm; IR (KBr) ν_{max}: 3417, 2952, 1725, 1691, 1245, 1025 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); HR-ESI-MS at *m/z* 475.1936 $[M + Na]^+$ (Calcd. for $C_{23}H_{32}O_9Na^+$, 475.1939).

*Croyunfuranoid C (**3**)*

Colorless crystals, mp: 203−205 °C; $[\alpha]_D^{20}$ −35 (*c* 0.1, CH_3CN); UV (CH_3CN) λ_{max} (log ε): 195 (3.79) nm; IR (KBr) ν_{max}: 3350, 2953, 1735, 1697, 1242, 1026 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); HR-ESI-MS at *m/z* 475.1937 $[M + Na]^+$ (Calcd. for $C_{23}H_{32}O_9Na^+$, 475.1939).

*Croyunfuranoid D (**4**)*

White amorphous powder; $[\alpha]_D^{20}$ −23 (*c* 0.1, CH_3CN); UV (CH_3CN) λ_{max} (log ε): 210 (3.66) nm; IR (KBr) ν_{max}: 2394, 1717, 1160, 1024 cm^{-1}; ECD (*c* 3.3 × 10^{-4} mol·L^{-1}, CH_3CN) λ_{max} ($\Delta\varepsilon$): 208 (+ 2.72), 286 (−0.33); ^{1}H and ^{13}C NMR data (Table 2); HR-ESI-MS at *m/z* 325.1406 $[M + Na]^+$ (Calcd. for $C_{18}H_{22}O_4Na^+$, 325.1410).

*Croyunfuranoid E (**5**)*

White amorphous powder; $[\alpha]_D^{20}$ −113 (*c* 0.2, CH_3CN); UV (CH_3CN) λ_{max} (log ε): 209 (3.75) nm; IR (KBr) ν_{max}: 2930, 1713, 1255, 1024 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 2); HR-ESI-MS at *m/z* 411.1774 $[M + Na]^+$ (Calcd. for $C_{22}H_{28}O_6Na^+$, 411.1778).

*Croyunfuranoid F (**6**)*

White amorphous powder; $[\alpha]_D^{20}$ −154 (*c* 0.2, CH_3CN); UV (CH_3CN) λ_{max} (log ε): 208 (3.76) nm; IR (KBr) ν_{max}: 2931, 1733, 1255, 1026 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 2); HR-ESI-MS at *m/z* 411.1774 $[M + Na]^+$ (Calcd. for $C_{22}H_{28}O_6Na^+$, 411.1778).

*Croyunfuranoid G (**7**)*

White amorphous powder; $[\alpha]_D^{20}$ −123 (*c* 0.2, CH_3CN); UV (CH_3CN) λ_{max} (log ε): 205 (3.82) nm; IR (KBr) ν_{max}: 3418, 2952, 1710, 1250, 1021 cm^{-1}; ECD (*c* 8.3 × 10^{-4} mol·L^{-1}, CH_3CN) λ_{max} ($\Delta\varepsilon$): 208 (+ 0.50) , 252 (−8.46); ^{1}H and ^{13}C NMR data (Table 3); HR-ESI-MS at *m/z* 383.1462 $[M + Na]^+$ (Calcd. for $C_{20}H_{24}O_6Na^+$, 383.1465).

*Croyunfuranoid H (**8**)*

White amorphous powder; $[\alpha]_D^{20}$ −57 (*c* 0.1, CH_3CN); UV (CH_3CN) λ_{max} (log ε): 209 (3.68) nm; IR (KBr) ν_{max}: 3451, 2954, 1717, 1260, 1216, 1024 cm^{-1}; ECD (*c* 7.4 × 10^{-4} mol·L^{-1}, CH_3CN) λ_{max} ($\Delta\varepsilon$): 208 (+ 0.50) , 227 (−18.11); ^{1}H and ^{13}C NMR data (Table 3); HR-ESI-MS at *m/z* 427.1724 $[M + Na]^+$ (Calcd. for $C_{22}H_{28}O_7Na^+$, 427.1727).

*X-ray crystallographic analyses of **1**−**3**, **9**, and **10***

Crystals of compounds **1**−**3**, **9**, and **10** were obtained by slow evaporation from a CH_3CN/H_2O (8 ∶ 1) solution at room temperature. The X-ray crystallographic data for these compounds have been deposited at the Cambridge Crystallo-

Table 2 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of 4−6 in $CDCl_3$ (*J* in Hz)

No.	4		5		6	
	δ_C	δ_H, multi.	δ_C	δ_H, multi.	δ_C	δ_H, multi.
1*α*	22.7	2.07, m	21.2	2.36, m	22.1	1.92, m
1*β*		2.30, m		1.86, m		1.97, m
2*α*	39.1	2.63, m	27.0	2.38, m	26.6	2.29, m
2*β*		2.29, m		2.15, m		2.11, m
3	209.5		140.2	7.02, dd (5.2, 2.2)	137.5	6.74, dd (5.6, 2.0)
4	48.2	*α* 2.81, d (16.9)	134.8		138.7	
		β 2.40, d (16.9)				
5	89.7		40.9		41.0	
6*α*	38.9	1.75, m	35.2	2.51, dd (13.2, 4.4)	32.4	2.35, m
6*β*		1.57, m		1.22, m		1.01, m
7*α*	30.3	1.41, m	29.8	1.73, m	31.2	1.80, m
7*β*		1.81, m		1.66, m		1.61, m
8	35.5	1.93, m	38.0	1.71, m	38.2	1.63, m
9	60.6		46.4		50.6	
10	51.4	1.76, dd (14.3, 6.0)	47.0	1.35, dd (13.2, 2.4)	49.8	1.41, m
11a	34.1	2.31, dd (13.6, 7.6)	38.3	2.14, d (8.0)	39.0	2.16, dd (13.0, 10.6)
11b		2.12, dd (13.6, 7.6)				2.13, dd (13.0, 5.6)
12	76.1	5.27, t (7.6)	70.6	5.07, t (8.0)	73.9	5.12, dd (10.6, 5.6)
13	128.8		127.9		125.3	
14	108.3	6.30, br s	108.6	6.38, br s	108.6	6.44, br s
15	143.6	7.37, br s	143.5	7.39, br s	143.3	7.39, br s
16	138.5	7.33, br s	139.1	7.40, br s	139.7	7.42, br s
17	17.6	0.95, d (6.7)	16.6	0.99, d (5.8)	16.9	0.97, d (6.3)
18			167.0		167.9	
19			102.7	4.96, s	104.4	4.87, s
20	106.9	5.46, s	99.2	5.25, s	98.3	5.26, s
18-OMe			51.2	3.71, s	51.4	3.71, s
19-OMe			56.9	3.41, s	56.0	3.46, s

graphic Data Centre (CCDC) under the following deposition numbers: 2277128 (**1**), 2277132 (**2**), 2277133 (**3**), 2277138 (**9**), and 2277141 (**10**). These data can be accessed free of charge *via* www. ccdc.cam.ac.uk/data_request/cif.

*Crystal data for croyunfuranoid A (**1**)*: $C_{22}H_{28}O_9$ (*Mr*: 436.44): monoclinic, space group $P2_1$ (no. 4), a = 7.6266(3) Å, b = 16.3043(5) Å, c = 8.6285(3) Å, β = 105.271(4)°, V = 1035.04(7) Å^3, Z = 2, T = 100.00(10) K, μ(CuKα) = 0.914 mm^{-1}, *Dcalc* = 1.400 g·cm^{-3}, 19910 reflections measured (10.628° ≤ 2Θ ≤ 158.138°), and 4320 unique (R_{int} = 0.0635, R_{sigma} = 0.0461) which were used in all calculations. Final R_1 = 0.0427 (I > 2σ (I)), final wR_2 = 0.1110 (all data), and flack parameter = 0.06(13).

*Crystal data for croyunfuranoid B (**2**)*: $C_{23}H_{32}O_9$ (*Mr*: 452.48): orthorhombic, space group $P2_12_12_1$ (no. 19), a = 7.50420(10) Å, b = 8.92480(10) Å, c = 31.9116(4) Å, V = 2137.23(5) Å^3, Z = 4, T = 100.00(10) K, μ(CuKα) = 0.903 mm^{-1}, *Dcalc* = 1.406 g·cm^{-3}, 21929 reflections measured (5.538° ≤ 2Θ ≤ 157.6°), and 4503 unique (R_{int} = 0.0759, R_{sigma} = 0.0484) which were used in all calculations. Final R_1 = 0.0373 (I > 2σ (I)), final wR_2 = 0.1015 (all data), and flack parameter = −0.16(9).

*Crystal data for croyunfuranoid C (**3**)*: $C_{23}H_{32}O_9 \cdot H_2O$ (*Mr*: 470.50): monoclinic, space group $P2_1$ (no. 4), a = 7.7276(2) Å, b = 9.0039(2) Å, c = 17.2974(4) Å, β = 101.263(2)°, V = 1180.35(5) Å3, Z = 2, T = 100.00(10) K,

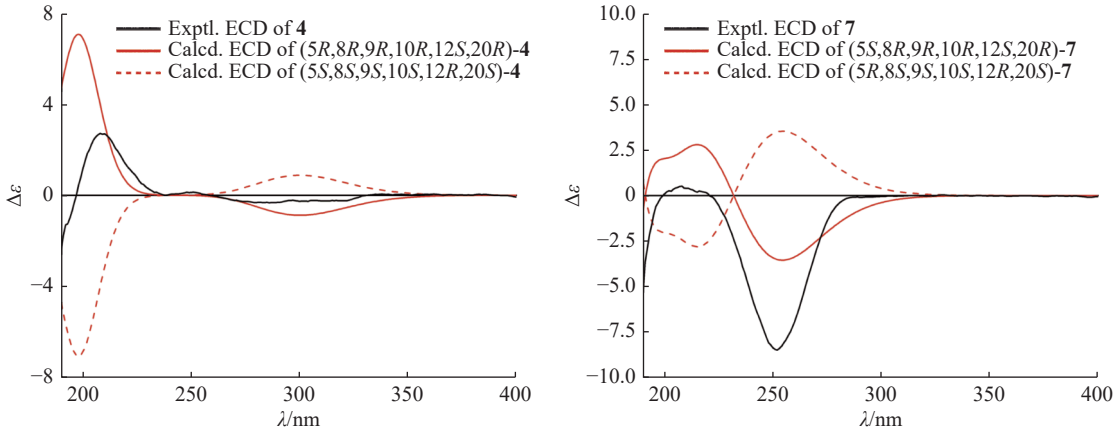


Fig. 5 Experimental and calculated ECD spectra of 4 and 7.

Table 3 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of 7 and 8 in $CDCl_3$ (*J* in Hz)

No.	7		8	
	δ_C	δ_H, multi.	δ_C	δ_H, multi.
1α	20.3	2.03, m	20.8	2.45, m
1β		1.86, m		1.98, m
2α	30.6	2.46, ddd (17.7, 4.3, 2.3)	28.8	2.42, m
2β		2.31, m		2.18, m
3	176.7		139.8	6.90, dd (5.1, 2.1)
4	101.0		137.3	
5	86.9		47.5	
6α	36.7	2.57, dd (13.3, 6.1)	34.3	2.64, dt (13.2, 3.8)
6β		1.30, ddd (13.3 12.0, 6.1)		1.10, td (13.2, 4.5)
7α	30.7	1.49, m	30.0	2.38, m
7β		1.81, m		1.59, m
8	35.4	1.91, m	43.1	1.40, m
9	59.2		53.7	
10	54.2	1.64, dd (13.4, 3.5)	52.6	1.69, br d (11.4)
11a	34.1	2.30, dd (13.5, 7.7)	43.7	2.30, dd (13.1, 7.0)
11b		2.09, dd (13.5, 7.7)		1.83, dd (13.1, 10.5)
12	75.5	5.24, t (7.7)	70.4	5.13, dd (10.5, 7.0)
13	129.2		125.3	
14	108.4	6.29, br s	108.9	6.43, br s
15	143.5	7.36, br s	143.3	7.39, br s
16	138.3	7.31, br s	139.5	7.42, br s
17	17.5	0.96, d (6.6)	17.8	0.98, d (6.8)
18	173.4		167.0	
19			175.5	
20	107.2	5.52, s	99.5	5.29, d (2.4)
18-OMe	51.7	3.81, s	51.5	3.70, s
19-OMe			51.6	3.61, s
3-OH		13.08, s		
20-OH				2.31, d (2.4)

μ(CuKα) = 0.869 mm^{-1}, Dcalc = 1.324 g·cm^{-3}, 23736 reflections measured (5.21° ≤ 2Θ ≤ 157.26°), and 4962 unique (R_{int} = 0.0833, R_{sigma} = 0.0505) which were used in all calculations. Final R_1 = 0.0434 (I > 2σ (I)), final wR_2 = 0.1148 (all data), and flack parameter = −0.01(11).

*Crystal data for crotonyunnan A (**9**)*: $C_{20}H_{24}O_5$ (*Mr*: 344.39): orthorhombic, space group $P2_12_12_1$ (no. 19), *a* = 8.91730(10) Å, *b* = 11.12010(10) Å, *c* = 16.9043(2) Å, *V* = 1676.25(3) Å^3, *Z* = 4, *T* = 100.01(10) K, μ(CuKα) = 0.796 mm^{-1}, *Dcalc* = 1.365 g·cm^{-3}, 18012 reflections measured (9.52° ≤ 2Θ ≤ 153.786°), and 3448 unique (R_{int} = 0.0617, R_{sigma} = 0.0341) which were used in all calculations. Final R_1 was 0.0381 (I > 2σ (I)), final wR_2 = 0.1052 (all data), and flack parameter = −0.06(8).

*Crystal data for crotonyunnan B (**10**)*: $C_{20}H_{24}O_5$ (*Mr*: 344.16): triclinic, space group P_1 (no. 1), *a* = 11.2756(2) Å, *b* = 11.3703(3) Å, *c* = 14.9826(3) Å, α = 78.387(2)°, β = 67.911(2)°, γ = 75.669(2)°, *V* = 1711.92(7) Å3, *Z* = 1, *T* = 100.00(10) K, μ(CuKα) = 0.779 mm^{-1}, *Dcalc* = 1.335 g·cm^{-3}, 63883 reflections measured (6.414° ≤ 2Θ ≤ 158.326°), and 13439 unique (R_{int} = 0.0906, R_{sigma} = 0.0602) which were used in all calculations. Final R_1 = 0.0486 (I > 2σ (I)), final wR_2 = 0.1721 (all data), and flack parameter = −0.09(9).

Cell culture

RAW 264.7 macrophages were obtained from the Cell Bank of the Shanghai Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, Shanghai, China). The cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg·mL^{-1} streptomycin. The cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cell viability was measured using the MTT assay.

Measurement of NO production

The inhibitory activities of compounds **1**−**11** on LPS-induced NO production were measured using the Griess reaction. RAW 264.7 macrophages were cultured in 96-well plates at a density of 5 × 10^4 cells/well. The cells were then incubated with or without 1.0 μg·mL^{-1} LPS in the absence or presence of the test compounds for 24 h. Following incubation, 50 μL of culture supernatant was mixed with an equal volume of Griess reagent (1% sulfanilamide, 0.1% *N*-1-naphthylethylenediamine dihydrochloride in 5% phosphoric acid) and incubated for 10 min at room temperature in the dark. The absorbance was then measured at 540 using a microplate reader. The percentage of inhibition was calculated using the formula: Inhibition (%) = [1 − ($A_{LPS + sample}$ − $A_{untreated}$)]/(A_{LPS} − $A_{untreated}$) × 100. All experiments were performed in triplicate, and the data were expressed as the mean ± standard deviation (SD) values.

α-Glucosidase inhibitory assay

α-Glucosidase (10 μL·mL^{-1} in PBS at a concentration of 10 mmol·L^{-1}, pH 7.4; 250 μL) and the sample solution (50 μL) were mixed and incubated at 37 °C for 15 min. Then, 25 μL of 5 mmol·L^{-1} *p*-NPG solution was added, and the mixture was incubated for an additional 30 min at 37 °C. After this incubation, 300 μL of distilled water was added to dilute the solution, and 100 μL of the diluted solution was used to measure the absorbance at 405 nm. The positive control was acarbose. Acarbose was used as the positive control. Each assay was performed in triplicate, and the results were represented as means ± SD.

α-Amylase inhibitory assay

α-Amylase (1 mg·mL^{-1} in PBS at a concentration of 10 mmol·L^{-1}, pH 7.4; 20 μL) and the sample solutions (50 μL) were mixed and incubated at 37 °C for 10 min. Then, 30 μL of 1% starch solution was added, and the mixture was incubated at 37 °C for another 10 min. The reaction was then terminated by adding 30 μL of DNS and heating the mixture at 100 °C for 10 min. After cooling to room temperature, 300 μL of distilled water was added to dilute the solution, and 100 μL of the diluted solution was used to measure the absorbance at 540 nm. Acarbose was used as the positive control. Each assay was conducted in triplicate, and the results were represented as mean ± SD.

Lipase inhibitory assay

The compounds and lipase were prepared in PBS (10 mmol·L^{-1}, pH 7.4). An emulsion consisting of 0.4% Triton X-100 and 0.1% Arabic gum in PBS was mixed with the substrate 4-nitrophenyl palmitate (*p*-NPP, 2 mmol·L^{-1} in 2-propanol) in a ratio of 9 ∶ 1. Lipase (1 mg·mL^{-1}, 50 μL) and the compounds (100 μL) were mixed and incubated at 37 °C for 30 min. Then, 50 μL of the *p*-NPP/emulsion mixture was added to the solution and incubated at 37 °C for 2 h. The solution was then diluted with 100 μL of distilled water, and the absorbance was measured at 405 nm. Orlistat was used as the positive control. Each assay was performed in triplicate, and the results were represented as mean ± SD.

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