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Crocrassins A and B, two novel sesquiterpenoids with an unprecedented carbon skeleton from *Croton crassifolius*

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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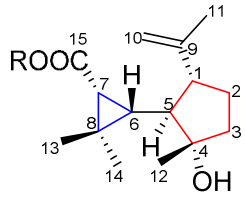
Crocrassins A (1) and B (2), two novel sesquiterpenoids possessing a unique skeleton, were isolated from a Chinese medicinal plant *Croton crassifolius*. Their structures were assigned on the basis of detailed spectroscopic analyses (especially HRESIMS and 2D NMR) with their absolute configuration being established via single-crystal X-ray diffraction studies as well as chemical transformation.

The genus *Croton* (Euphorbiaceae), composed of more than 1300 species globally, is mainly growing in tropical and subtropical regions,[1] with about 21 species distributed in the southern part of China.[2] It grows in sandy and wet soil, and on damp areas near river banks. Several species have long been used in Chinese folkloric medicine to alleviate dysmenorrhea, as a purgative, and to treat dyspepsia and malaria.[3] Previous phytochemical investigations on genus *Croton* have been carried out and several types of compounds were obtained, including diterpenes, sesquiterpenes, triterpenes, alkaloids, flavanones, lignans, polyphenolic compounds, phenylbutanoids, and peptides.[1,3] Collectively, some compounds possess antimicrobial, anti-inflammatory, antibacterial, antimalarial, and cytotoxic activities.[1]

Croton crassifolius Geisel, an undershrub up to 30–50 cm height, is mainly found in the southern parts of China and also distributed in Vietnam, Laos, and Thailand.[4] The roots of *C. crassifolius*, known as "Jiguxiang" in traditional Chinese medicine, have been long used for treatment of snake bites, stomach ache, sternalgia, joint pain, as well as pharyngitis, jaundice, and rheumatoid arthritis.[5] In previous studies, a number of clerodane-type diterpenes and sesquiterpenes were reported from this species.[6] As part of our continuing work on searching for structurally interesting bioactive compounds from traditional herbal medicine, further phytochemical investigation on this plant results in the isolation of two novel sesquiterpenoids with an unprecedented carbon skeleton via connection of a single carbon-carbon bond to form a cyclopropylcyclopentane (Fig. 1). This paper describes the isolation and structural elucidation of compounds **1** and **2** by extensive spectroscopic and single-crystal X-ray crystallographic analyses, as well as chemical transformation.



crocrassin A (**1**): R=H
crocrassin B (**2**): R=CH_3

Fig. 1 Structures of crocrassin A (**1**) and crocrassin B (**2**).

The air-dried and powdered roots of *C. crassifolius* (9.5 kg) were exhaustively extracted with 95% EtOH at room temperature, and the residue (962 g) obtained by concentrating the EtOH extract in vacuo was suspended in water and then partitioned with EtOAc and *n*-BuOH successively. The EtOAc-soluble portion (731 g) was applied to silica gel CC eluted with increasing polarities of petroleum ether-acetone mixtures (40:1 to 0:1) to afford combined fractions A-F. The fraction C was further separated by CC over silica gel and Sephadex LH-20 to yield **1** (4 mg) and **2** (2 mg).

Compound (**1**)‡§ was obtained as a colorless crystal, $[\alpha]_D^{25}$ + 60 (c 0.10, $CHCl_3$). It exhibited a quasimolecular ion peak at *m/z* 275.1616 corresponding to $[M + Na]^+$ in the HRESIMS analysis which provided the molecular formula $C_{15}H_{24}O_3$ (calcd for $[M + Na]^+$ *m/z* 275.1618), indicating four degrees of unsaturation. The IR spectrum of **1** displayed characteristic absorption bands for a carboxylic acid group (1697 cm$^-$

[1]),[7] double bond (3072, 1642 cm^{-1}),[8] and hydroxyl (3391 cm^{-1}) functionalities.

Analysis of the ^{1}H NMR data of **1** indicated the presence of two terminal olefinic protons at δ_H 4.64 (dd, J = 2.4, 1.8) and 4.79 (d, J = 2.4), an olefinic methyl at δ_H 1.67, three tertiary methyls at δ_H 1.20, 1.26, and 1.31, as well as signals with several complex multiplets from δ_H 1.20–2.5. The ^{13}C NMR and DEPT spectra revealed 15 carbon resonances, which were distinguished to be four methyls, three methylenes (including one olefinic carbon), four methines, together with four quaternary carbons (including one carbonyl, one oxygenated sp^3 carbon, one olefinic carbon). From the number of carbon resonances in the ^{13}C NMR, in combination with the molecular formula, it could be inferred that compound **1** could be a sesquiterpenoid. Moreover, deducting 2 degrees of unsaturation accounted for one carbonyl and one olefinic bond, the remaining two degrees of unsaturation were indicative of the bicyclic ring system of **1**.

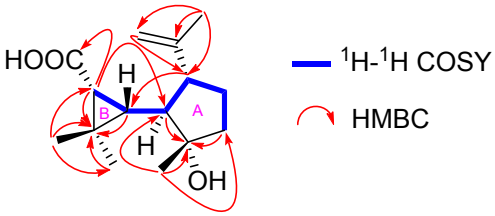


Fig. 2 Key ^{1}H-^{1}H COSY and HMBC correlations for **1**.

The planar bicycle carbon skeleton of **1** was established by the 2D NMR experiments, including ^{1}H-^{1}H COSY, HMQC and HMBC spectra. The proton and protonated carbon signals in the NMR spectra of **1** were unequivocally assigned by the HMQC experiment. In the ^{1}H-^{1}H COSY spectrum, homonuclear coupling correlations from H_2-3 through H_2-2, H-1, and H-5 to H-6, and then to H-7, suggested the presence of one key fragment (C-3/C-2/C-1/C-5/C-6/C-7). This structural fragment, along with other isolated groups, was connected through quaternary carbons by further examination of HMBC data (Fig. 2), especially correlations arising from the tertiary methyl protons. The protons of H_2-3 (δ_H 1.68, 1.79) and H-5 (δ_H 2.34) showed significant HMBC correlations to C-4 (δ_C 81.6), which suggested that C-1, C-2, C-3, C-4, and C-5 constructed a five-membered carbon ring A. In the HMBC spectrum, two singlet methyls CH_3-13 (δ_H 1.20) and CH_3-14 (δ_H 1.31), together with signals of two methines of C-6 (δ_H 0.97) and C-7 (δ_H 1.48), showed significant correlations to an sp^3 quaternary carbon at δ_C 26.5 (C-8). These HMBC correlations established a cyclopropane ring B, as shown in Fig 2. The connected position of the above determined two rings was established according to the following key correlations: in the ^{1}H-^{1}H COSY spectrum, H-5 correlated to H-6; in the HMBC, H-1 correlated to C-6, and H-7 correlated to C-5. Thus, ring A and ring B were connected by a C–C σ-bond at C-5 and C-6. The C-4 position of **1** was substituted by a hydroxyl based on the HMBC correlations of CH_3-12 (δ_H 1.26), H_2-3, and H-5 with the oxygenated quaternary carbon at δ_C 81.6. An isopropenyl group (δ_H 1.67, 4.64, and 4.79; δ_C 18.4, 111.8, and 147.0) was attached to the C-1 position (δ_C 55.4) due to the HMBC correlations of H-10a, H-10b, and CH_3-11 with C-1. The carboxylic acid group was assigned to be C-7 by the HMBC correlation of H-7 with C-15 (δ_C 178.2). Therefore, the planar structure of **1** was constructed as shown in Fig. 1.

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of **1** and **2**

	1[a]		**2**[a]	
no.	δ_H, mult, (J in Hz)	δ_C	δ_H, mult, (J in Hz)	δ_C
1	2.45 m	55.4 d	2.41 m	55.6 d
2a	1.81 m[b]	28.1 t	1.79 m[b]	28.3 t
2b	1.69 m[b]		1.68 m[b]	
3a	1.79 m[b]	41.8 t	1.78 m[b]	41.8 t
3b	1.68 m[b]		1.65 m[b]	
4		81.6 s		81.6 s
5	2.34 dd (10.8, 8.4)	48.0 d	2.27 dd (11.4, 8.4)	48.1 d
6	0.97 dd (10.8, 9.0)	39.4 d	0.90 dd (11.4, 9.6)	38.5 d
7	1.48 d (9.0)	28.3 d	1.48 d (9.6)	28.2 d
8		26.5 s		25.0 s
9		147.0 s		147.2 s
10a	4.79 d (2.4)	111.8 t	4.70 d (1.8)	111.0 t
10b	4.64 dd (2.4, 1.8)		4.58 d (1.8)	
11	1.67 brs	18.4 q	1.67 brs	18.5 q
12	1.26 s	25.1 q	1.27 s	25.1 q
13	1.20 s	29.1 q	1.16 s	29.1 q
14	1.31 s	16.3 q	1.33 s	16.2 q
15		178.2 s		172.1 s
OMe			3.55 s	51.2 q

[a]Measured in $CDCl_3$. [b]Singal pattern unclear due to overlapping.

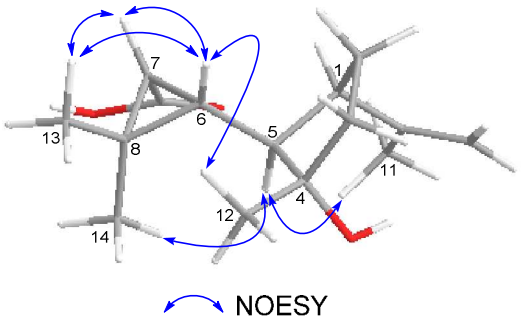


Fig. 3 Key NOESY correlations for **1**

The relative stereochemistry of **1** was established by interpretation of its NOESY spectrum (Fig. 3). In the NOESY spectrum, the cross peaks of H-6/H-7, H-7/H_3-13 demonstrated that H-6, H-7, and H_3-13 were oriented on the same side of the cyclopropane ring B, while H_3-14 and COOH were oriented on the opposite side. Consequently, the cross peaks of H-5/H_3-11, H-1/H_3-12 confirmed that H-1 and H_3-12 were on the same face of cyclopentane ring A, while H-5 was on the opposite face. The relative configuration between the conjoined bicyclic ring systems in **1** was defined by the observation of NOESY correlations between H-6 and H_3-12, between H-5 and H_3-14, which could only be accommodated by the C-5*R**/C-6*S** relative configuration shown in Fig. 3.[9]

After repeated attempts, a suitable single crystal of **1** was obtained from petroleum ether-acetone solution. The X-ray crystallographic data (Cu Kα) (Fig. 4) corroborated the planar structure and relative configuration of **1** elucidated via NMR data and further allowed the assignment of its absolute configuration as 1*R*, 4*S*, 5*R*, 6*S*, 7*R* [The refined Flack parameter is 0.07(14)].[10] Thus, the structure of **1** was established and named crocrassin A.

Compound (**2**)¶ was obtained as a colorless gum with $[\alpha]_D^{25}$ + 80 (c 0.10, $CHCl_3$). Its molecular formula $C_{16}H_{26}O_3$ was determined on the

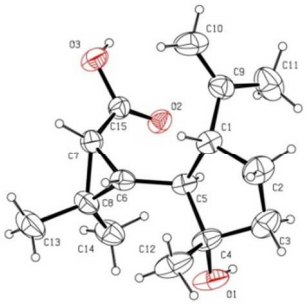


Fig. 4 X-ray structure of compound **1**.

basis of the HRESIMS at *m/z* 289.1775 $[M + Na]^+$ (calcd 289.1774), indicating four degrees of unsaturation and 14 mass units heavier than that of **1**. The IR spectrum of **2** indicated the presence of hydroxyl group (3439 cm^{-1}), carbonyl group (1728 cm^{-1}). The similar ^1H and ^{13}C NMR spectra revealed that **2** is an analogue of **1**. A careful comparison of the NMR data for compounds **1** and **2** (Table 1) suggested that they had the same carbon skeleton including the cyclopropane and cyclopentane moieties, which were connected by a C–C σ-bond. The major difference in the ^1H NMR of **2** compared to **1** was the presence of an additional oxymethyl group in **2** with the spectral data at δ_H 3.55 (s, 3H), which was further corroborated by the ^{13}C NMR spectrum as an additional signal compared to **1** was detected at δ_C 51.2. In addition, the ^{13}C NMR spectrum of **2** indicated that the carbonyl resonance was moved from δ_C 178.2 in **1** upfield to δ_C 172.1 in **2**, which demonstrated that the carboxylic acid group in **1** was esterified in **2**. Further examination of the ^1H-^1H COSY, HSQC, HMBC, and NOESY spectra confirmed the structure and relative configuration of **2** as shown in Fig. 1.

Unfortunately, we failed to obtain the single crystal of **2** for determining its absolute configuration. To determine the absolute configuration of **2**, compound **1** was methyl esterified using excess ethereal diazomethane in diethyl ether to give the compound **2**. Thus, the absolute configuration of **2** was identical to that of **1**, and deduced as 1*R*, 4*S*, 5*R*, 6*S*, 7*R*. Finally, the structure of **2** was established and named crocrassin B.

To the best of our knownledge, crocrassins A (**1**) and B (**2**) are the first examples of a novel carbon skeleton via connection of a single carbon-carbon bond to form a cyclopropylcyclopentane in the family of sesquiterpenoids.

Both **1** and **2** were evaluated for their cytotoxicity against four human cancer cell lines, HepG2, SGC-7901, K562, and K562/ADM, using the MTT method. Unfortunately, no significant activity was detected.

We are grateful to the National Natural Science Foundation of China (No. 21102065), the Natural Science Foundation of Gansu Province, China (No. 1208RJYA029) and the Fundational Research Funds for the Central Universities (No. lzujbky-2013-72 and No. lzujbky-2012-83) for financial support of this research.

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental procedures, NMR, HRESIMS, and IR spectra of compounds **1** and **2**. Crystallographic data for the structure of **1,** CCDC reference number 995584. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c000000x/

‡ Crocrassin A (**1**): colorless crystals; mp 113–115 °C; $[\alpha]_D^{25}$ + 60 (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 3391, 3072, 2953, 2926, 1696, 1453, 1438, 1376, 923, 739 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data, see Table 1; HRESIMS *m/z* 275.1616 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1618).

§ Crystal data for compound **1**: $C_{15}H_{24}O_3$, M_r = 252.34, orthorhombic, a = 111.2310(5) Å, b = 17.3234(7) Å, c = 31.7240(19) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 6172.2(5) Å^3, T = 293(2) K, space group $P2_12_12_1$, Z = 16, μ (CuKα) = 0.590 mm^{-1}, 39759 reflections measured, 11886 independent reflections (R_{int} = 0.0573). The final R_1 value was 0.0702 ($I > 2\sigma(I)$). The final w$R(F^2)$ value was 0.1736 ($(I > 2\sigma(I))$. The final R_1 value was 0.0950 (all data). The final w$R(F^2)$ value was 0.2074 (all data). The goodness of fit on F^2 was 1.020. Crystallographic data of crocrassin A (**1**) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC 995584). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB 21EZ, U.K.; fax (+44) 1223-336-033; or desposit@ccdc.cam.ac.uk).

¶ Crocrassin B (**2**): colorless gum; $[\alpha]_D^{25}$ + 80 (*c* 0.10, $CHCl_3$);IR (KBr) ν_{max} 3439, 2951, 1728, 1437, 1438, 1376, 928, 885 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data, see Table 1; HRESIMS m/z 289.1775 $[M + Na]^+$ (calcd for $C_{16}H_{26}O_3Na$, 289.1774).

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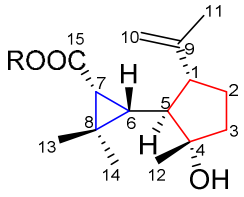

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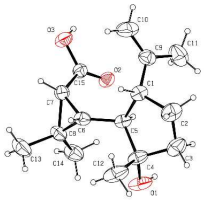

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