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Dicarabrones A and B, a Pair of New Epimers Dimerized from Sesquiterpene Lactones via a [3 + 2] Cycloaddition from *Carpesium abrotanoides*

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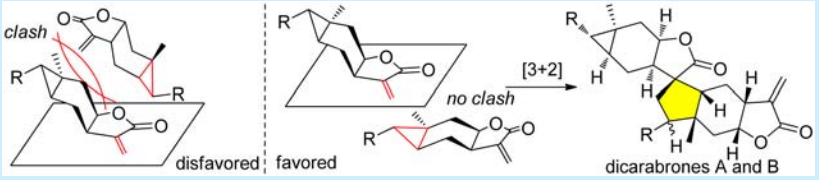
Supporting Information



ABSTRACT: Dicarabrones A and B, a pair of epimers possessing a new skeleton featuring a cyclopentane ring connecting two sesquiterpene lactone units, were isolated from the whole plant of *Carpesium abrotanoides* L. Their full structures were established on the basis of spectroscopic data and were further confirmed by single-crystal X-ray crystallography. They were presumably biosynthesized from two sesquiterpenoid monomers through a [3 + 2] cycloaddition. Dicarabrones A and B showed moderate effects on HL-60 cells with IC_{50} values of 9.1 and 8.2 μM, respectively.

In the past decade, considerable attention has been paid to the [3 + 2] cycloaddition reaction of cyclopropane, a versatile and efficient approach for the construction of five-membered rings that are prevalent frameworks in natural products.[1−4] More recently, the [3 + 2] cycloaddition reaction of cyclopropane has been proven efficient, and general strategies to construct bridged [*n*.2.1] (*n* = 2−4) carbocyclic skeletons, especially that of [3.2.1] octane, exist widely in terpenoids and alkaloids.[5−11] The importance of the [3 + 2] cycloaddition reaction of cyclopropane is demonstrated by its exclusive and extensive use in the synthesis of complex natural products such as (−)-allosecurinine,[12] (+)-virgatusin,[13] and (+)-isatisine A.[14]

The effective use of cyclopropane ring strain is pivotal for the construction of complex systems. Only a specific substitution pattern at the cyclopropane ring allows for particularly mild, efficient, and selective transformations.[1−4] On the other hand, various catalysts, mainly transition metals, have been employed to activate the reactions.[15] Therefore, seeking new catalysts is the focus of ongoing investigations.

In traditional Chinese medicine, the fruits of *Carpesium abrotanoides* L., known as "Nan-He-Shi" in Chinese, are used as important insecticides in Northern China.[16] Previous investigations on this species led to the isolation of a number of novel sesquiterpenoids.[17,18] Some of them, in particular, carabrone,[18] carpesiolin,[18] and telekin,[18] have been demonstrated as potent cytotoxic agents.

In our continuing effort to search for bioactive constituents from *C. abrotanoides*, dimeric sesquiterpenoids attracted our attention due to their biological and pharmacological activities that differ from the corresponding monomers.[19,20] Guided by LC−MS detection, a pair of dimeric sesquiterpenoid epimers, dicarabrones A (**1**) and B (**2**), were identified. These two compounds possessed a new skeleton with a cyclopentane ring connecting two monomeric units. The unusual carbon skeleton was presumably formed by a [3 + 2] cycloaddition reaction. Herein, the LC−MS-guided isolation, structural characterization, proposed biogenetic pathways, and biological evaluation of these two compounds are presented.

The air-dried whole plant of *C. abrotanoides* (50 kg) was extracted three times with 95% ethanol at room temperature to afford a crude extract (3.29 kg). The extract was further partitioned between CH_2Cl_2 and H_2O, giving a CH_2Cl_2-soluble fraction (1.66 kg). The CH_2Cl_2-soluble fraction was fractionated over MCI gel ($EtOH/H_2O$, from 50 to 95%) to yield fractions A−C. Fraction B (300 g) was then subjected to column chromatography (CC) over silica gel eluted with CH_2Cl_2/MeOH (50:1 to 10:1) in a stepwise manner to give seven subfractions (B1−B7). The subfractions were screened by LC−MS to seek the potential molecular weights of dimeric sesquiterpenes. The results showed that the protonated

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molecular ions of the peaks at the retention times of 16.33 and 16.80 min in subfraction B2 (56 g) were both 497 (Figure S1), which might be ascribed to the dimeric sesquiterpenes, with the molecular weights of the monomeric sesquiterpenes from the title plant ranging from 250 to 268.[17,18] Subsequently, subfraction B2 was selected for purification by CC over Sephadex LH-20 (MeOH) and then preparative HPLC (CH_3CN/H_2O from 30 to 60%), yielding **1** (12 mg) and **2** (18 mg) (Figure 1).

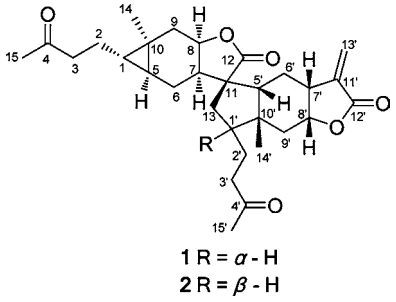

Figure 1. Structures of dicarabrones A (**1**) and B (**2**).

1 was obtained as a white lamellar crystal, and its molecular formula was assigned to be $C_{30}H_{40}O_6$ from the quasi-molecular positive ion at m/z 519.2712 $[M + Na]^+$ (calcd for $C_{30}H_{40}O_6Na$, 519.2723) in the ESI-HRMS, requiring 11 degrees of unsaturation. Two characteristic absorptions at 1752 and 1713 cm^{-1} in the IR spectrum indicated the presence of ester and ketone carboxyl groups. The ^{13}C NMR and DEPT spectra gave 30 carbon resonances including 4 methyls, 10 methylenes, 8 methines, and 8 quaternary carbons. From these resonances, two ketone carboxyls (δ_C 208.9, 208.3) and two ester carboxyls (δ_C 181.7, 170.3) could be clearly identified. The ^1H NMR spectrum of **1** exhibited two sets of oxygenated methylene protons at δ_H 4.56 (ddd, J = 2.4, 7.5, 10.2 Hz) and 4.69 (ddd, J = 3.4, 7.2, 10.7 Hz), two methyl singlets at δ_H 0.95 and 1.07, and another two methyl singlets at δ_H 2.16 and 2.17 (Table 1). These characteristic signals, with the MS data, suggested that **1** might be a dimeric sesquiterpene lactone.

Two protons at δ_H 0.42 (ddd, J = 4.2, 7.2, 7.2 Hz) and 0.30 (ddd, J = 4.2, 8.4, 8.8 Hz) (Table 1), showing $^1H-^1H$ COSY correlations between themselves and HMBC correlations with C-14 (δ_C 18.9) (Figure 2), were indicative of a three-membered ring moiety. The signals of δ_C 208.9 and 30.2, along with that of δ_H 2.16 (3H, s), suggested the presence of an acetyl group, which was attached to C-3 as inferred from the HMBC experiment (Figure 2). These signals showed great similarities to the data of the known carabrone[21] except for those of a quaternary carbon (δ_C 53.4) and a methylene (δ_C 35.0) rather than an exocyclic double bond between C-11 and C-13 in carabrone observed for **1**. Therefore, moiety A was proposed from these spectroscopic data, as shown in Figure 2.

Apart from the signals of moiety A, the remaining ones appearing at δ_C 208.3 and 30.1 and δ_H 2.17 (3H, s) also indicated the existence of another acetyl group, and those olefinic carbons at δ_C 123.7 and 139.0 (C-11′ and C-13′) and exocyclic olefinic protons at δ_H 5.67 and 6.30 (H_2-13′) suggested the existence of an α-methylene lactone.[21] The proton resonating at δ_H 1.38 (m) and 2.00 (dd, 5.7, 13.1 Hz) (Table 1) showed correlations with C-14′ (δ_C 24.4) in the HMBC spectrum, indicating a 1,5-*seco*-carabrone unit for moiety B (Figure 2).

Table 1. ^1H and ^{13}C NMR Data for 1 and 2 in $CDCl_3$

	1		2	
no.	δ_H mult. (J in Hz)	δ_C	δ_H mult. (J in Hz)	δ_C
1	0.42 (ddd, 4.2, 7.2, 7.2)	34.0	0.39 (ddd, 4.3, 7.2, 7.2)	34.4
2	1.57 m	23.5	1.62 m	23.5
3	2.53 (t, 7.3)	43.7	2.51 (t, 7.4)	43.7
4		208.9		209.0
5	0.30 (ddd, 4.2, 8.4, 8.8)	23.9	0.29 (ddd, 4.3, 7.7, 8.5)	23.7
6α	1.88 m	24.2	1.88 m	25.4
6β	0.63 (ddd, 8.8, 13.6, 13.7)		0.70 (ddd, 8.5, 13.6, 13.6)	
7	2.28 overlapped	38.6	2.31 overlapped	39.2
8	4.56 (ddd, 2.4, 7.5, 10.2)	76.9	4.61 (ddd, 2.3, 7.3, 9.8)	75.6
9α	2.41 overlapped	38.0	2.34 overlapped	37.6
9β	0.84 (dd, 10.2, 14.2)		0.96 (dd, 9.8, 14.0)	
10		15.8		16.1
11		53.4		53.5
12		181.7		181.6
13	1.66 m	35.0	1.99 overlapped	31.0
			1.62 overlapped	
14	1.07 s	18.9	1.04 s	19.1
15	2.16 s	30.2	2.15 s	30.2
1′	1.38 m	50.4	2.45 overlapped	47.2
2′	1.48 m	23.3	1.72 overlapped	23.1
			1.30 overlapped	
3′	2.40 (t, 7.2)	42.8	2.44 (t, 7.7)	43.2
4′		208.3		208.6
5′	2.00 (dd, 5.7, 13.1)	56.3	1.99 overlapped	51.5
6′	1.72 overlapped	28.3	1.71 overlapped	36.0
			1.36 overlapped	
7′	3.06 m	37.1	3.01 m	36.6
8′	4.69 (ddd, 3.4, 7.2, 10.7)	75.3	4.73 (ddd, 3.1, 7.3, 10.5)	75.4
9′α	1.32 (dd, 10.9, 13.0)	38.1	1.33 overlapped	31.4
9′β	2.27 overlapped		1.90 overlapped	
10′		41.2		42.8
11′		139.0		139.2
12′		170.3		170.1
13′a	6.30 (d, 2.0)	123.7	6.29 (d, 2.0)	123.4
13′b	5.67 (d, 2.0)		5.68 (d, 2.0)	
14′	0.95 s	24.4	1.14 s	28.2
15′	2.17 s	30.1	2.13 s	30.2

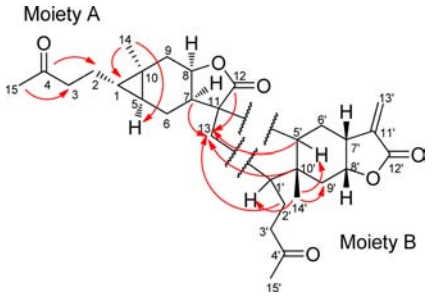

Figure 2. Key HMBC correlations (C → H) of **1**.

The connection between moieties A and B was established by the $^1H-^1H$ COSY correlation between H_2-13 and H-1′ and the HMBC correlations (Figure 2) between H_2-13 (δ_H 1.66) and C-7 (δ_C 38.6), C-12 (δ_C 181.7), C-2′ (δ_C 23.3), C-5′ (δ_C 56.3), and C-10′ (δ_C 41.2), which confirmed one C−C linkage

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between C-13 and C-1′ and the other one between C-11 and C-5′. Thus, a cyclopentane ring was constructed.

The relative configuration of **1** was inferred from the ROESY experiment. The cross-peaks of H_3-14/H-5, H-5/H-6α, H-6β/H_2-13, H_2-13/H_3-14′, H_3-14′/H_2-2′, H_3-14′/H-5′, and H_3-14′/H-7′ suggested that H_3-14, H-5, and H-6α are on the same face, while H-6β, H_2-13, H_3-14′, H_2-2′, H-5′, and H-7′ are on the other face of the molecule. The structure of **1**, including the absolute configuration (1*S*,5*S*,7*R*,8*R*,10*R*,11*R*,1′*S*,5′*R*,7′*R*,8′*R*,10′*R*), was finally confirmed by an X-ray diffraction experiment (Figure 3).

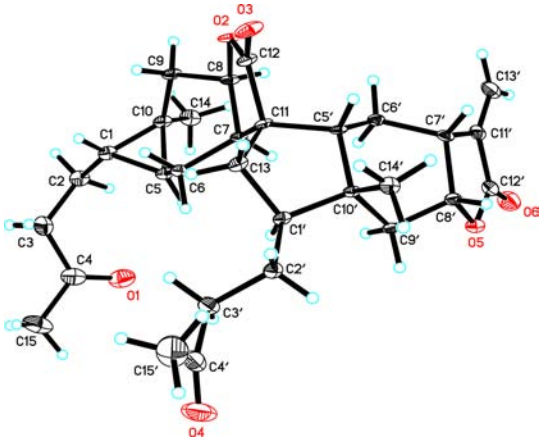


Figure 3. ORTEP drawing for **1**.

2 was obtained as a white lamellar crystal. The HRESIMS indicated a molecule formula of $C_{30}H_{40}O_6$ (*m*/*z* 519.2714, calcd for $C_{30}H_{40}O_6Na$, 519.2723), the same as that of **1**. The spectroscopic data of **2** were very similar to those of **1**, except for the major difference observed for the chemical shift of H-1′ (δ_H 1.38 for **1** vs 2.45 for **2**). The ROESY correlations indicated that most chiral carbons in **2** maintained the same configurations as those in **1**. However, due to the overlapping of the key ROESY correlations, the stereochemistry of the cyclopentane moiety, including H_2-13, H-1′, and H-5′, could not be determined. The stereochemistry was finally determined as (1*S*,5*S*,7*R*,8*R*,10*R*,11*R*,1′*R*,5′*R*,7′*R*,8′*R*,10′*R*) by X-ray diffraction analysis, as shown in Figure 4. Therefore, the structure of **2** was established as a C-1′ epimer of **1**.

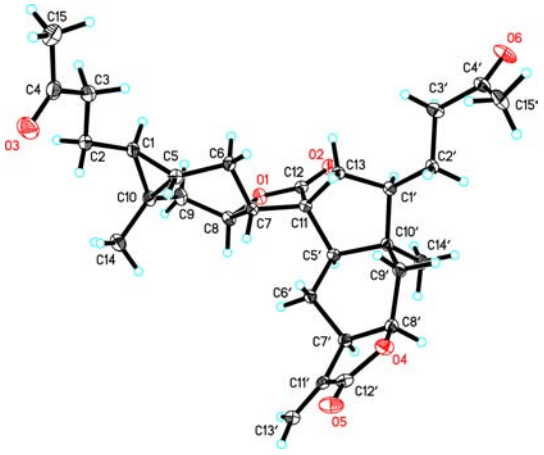
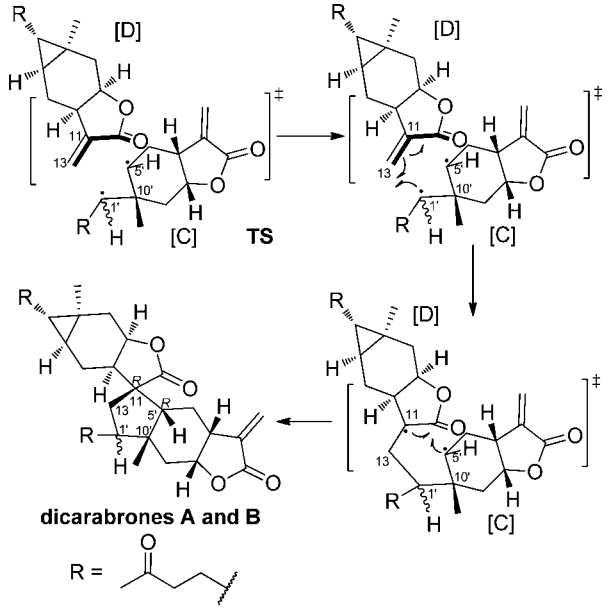


Figure 4. ORTEP drawing for **2**.

The skeletons of dicarabrones A and B feature a cyclopentane ring connecting two sesquiterpene units. The formation of the cyclopentane ring looks quite unique. So far, a number of structurally complicated dimeric sesquiterpinoids have been isolated, and most of them were proposed to be biosynthesized by a [4 + 2] or [2 + 2] cycloaddition of two monomers.[22] In our case, the monomer possesses both a three-membered ring and a double bond; therefore, the cyclopentane ring could be envisioned to arise from a [3 + 2] cycloaddition reaction starting from a three-membered ring in one molecule and a double bond in the other (Scheme 1).

Scheme 1. Proposed Biosynthetic Pathway for Dicarabrones A (1) and B (2)



Previous studies revealed that a required decrease in the activation barrier could be achieved by electron-donating and electron-accepting functionalities installed vicinally at the three-membered ring system.[1−4] The chemical bond between the donor- and acceptor-substituted carbon atoms of the cyclopropane could readily be cleaved heterolytically and can easily be rationalized by a zwitterionic relationship. However, the reactive zwitterionic intermediate would not be rationalized in our case due to the absence of significant donor and acceptor groups. Moreover, the zwitterionic intermediate could not be located in the model reaction after many attempts when we performed theoretical calculations for this [3 + 2] reaction using the M062X method.[23,24] Therefore, this reaction might take place via radicals rather than zwitterionic intermediates.

In our proposal (Scheme 1), the bond between C-1′ and C-5′ could be cleaved to form a radical intermediate C with one radical ready at C-5′, which was then trapped with the double bond in D. Due to steric hindrance in the structure, C-1′ would form a new bond with C-13 first, and subsequently, C-5′ formed the other new bond with C-11. Therefore, a five-membered ring was introduced to fuse two monomers C and D.

An empirical transition state model is proposed to explain the stereochemistry outcomes (Scheme 1). To minimize unfavorable steric interactions with the bulky six-membered ring fused to the lactone ring in D, the intermediate C would

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prefer to approach the double bond from the bottom face of D, which results in an *R* configuration of the newly created chiral C-11. At the same time, to avoid the steric repulsions caused by methyl group at C-10′, D would attack C from the reverse side of the methyl group, affording an *R* configuration for C-5′. In addition, the free rotation of the substituent R around C-1′ resulted in two possible configurations at C-1′ when the bond C-13/C-1′ was formed.

In an attempt to anticipate the plausible mechanism, we performed theoretical calculations using the M062X method.[23,24] The results showed that a triplet state intermediate was identified with a free energy of 60.2 kcal/mol, higher than that of the sesquiterpenoid monomer, indicating that it is very unstable (details in Figure S2). Moreover, the subsequent [3 + 2] cycloaddition of this intermediate with another sesquiterpenoid monomer required very significant activation free energy. Given the fact that Diels−Alder cycloaddition reactions catalyzed by biomolecules have indeed been realized,[25−27] we assume that nature might also make use of the enzyme to catalyze such a [3 + 2] cycloaddition reaction.

The basic cytotoxic activities of sesquiterpene lactone were principally ascribed to the introduction of an α-methylene-γ-lactone moiety into the molecules.[28,29] Compounds **1** and **2**, with their monomer carabrone, were evaluated for their cytotoxic effects on A549 and HL-60 cancer cell lines. Compounds **1** and **2** showed selective activities against HL-60 cells with IC_{50} values of 9.1 and 8.2 μM, respectively, with weak activity on A549 cells (IC_{50} > 10 μM). The monomer carabrone did not show toxicity against either A549 or HL-60 cancer cell lines.

To the best of our knowledge, dicarabrones A and B represent the first examples of dimeric carabrone sesquiterpene lactones. The existence of a cyclopentane ring in the molecules highlights the unique skeleton, which, from the perspective of biosynthesis, might be constructed from two carabrones via a [3 + 2] cycloaddition with the help of an enzyme. Our finding discloses an interesting phenomenon in the plant kingdom and provides several clues for scientists to seek effective agents to catalyze the valuable [3 + 2] cycloaddition reactions.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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