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Carpedilactones A–D, Four New Isomeric Sesquiterpene Lactone Dimers with Potent Cytotoxicity from *Carpesium faberi*

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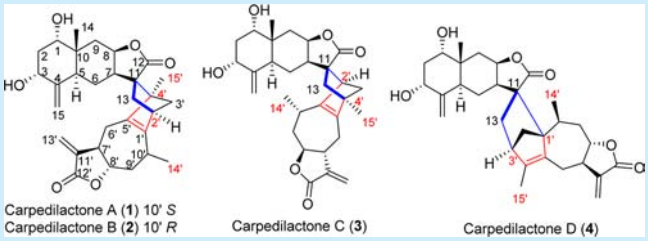
Supporting Information

ABSTRACT: Four new isomeric sesquiterpene lactone dimers, carpedilactones A–D (**1**–**4**), were isolated from the acetonic extract of *Carpesium faberi*. Among them, **1**–**3** are the first three 2,4-linked exo-Diels–Alder adducts between a eudesmanolide dienophile and a guaianolide diene. The absolute configurations of **1**–**4** were unambiguously established by Cu Kα X-ray crystallographic analyses. Compounds **1**–**4** exhibited potent cytotoxicities against human leukemia (CCRF-CEM) cells with IC_{50} value of 0.14, 0.32, 0.35, and 0.16 μM, respectively.



The genus *Carpesium*, belonging to the family Compositae, comprises about 21 species worldwide. Most of them are distributed in Asia, especially in the southwest of China.[1] In previous investigations, it was discovered that this genus is rich in diverse sesquiterpene lactones with cytotoxic, antiplasmodial, and anti-inflammatory activities.[2]

Carpesium faberi, native to the southwest of China and Japan, has long been used as a folk medicine for its hemostatic, antiparasitic, anti-inflammatory, and detoxifying properties.[1,2a] To date, only several sesquiterpene lactones and diterpenes were discovered from this species.[2a] Meanwhile, a series of sesquiterpene lactone dimers (SLDs) with significant anti-inflammatory and cytotoxic activities were continually discovered from the Compositae plants; especially, tens of SLDs with novel skeletons were discovered from the genus *Inula* and *Ainsliaea* by our group in recent years.[3] These results greatly inspired our interest to search for other structurally unique bioactive compounds from the genus *Carpesium*, which led to the isolation of carpedilactones A–D (**1**–**4**) (Figure 1), four isomeric exo-Diels–Alder adducts between a eudesmanolide dienophile and a guaianolide diene from *C. faberi*. Herein, we describe the isolation, structural elucidation, and the cytotoxicities of the four dimers.

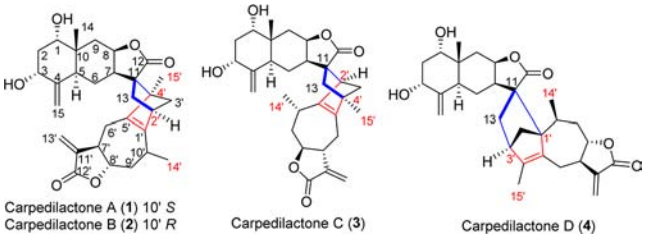


Figure 1. Chemical structures of **1**–**4**.

The whole plant of *C. faberi*, collected at ZunYi, Guizhou province of China, was extracted with acetone at room temperature to provide the crude extract (750 g), which was chromatographed on a silica gel column using gradient pertroleum ether/EtOAc (10:0 to 0:10) to afford 17 fractions (Fr. 1–17). Fraction 15 (51.0 g) was subjected to RP-C18 column and monitored by TLC using Dragendorff's spray reagent. The positive subfractions were further purified by RP-C18 HPLC using CH_3CN/H_2O (43:57) as the eluent to provide 60.5 mg of **1**, 24.8 mg of **2**, 5.1 mg of **3**, and 26.2 mg of **4**. Interestingly, **1**–**4** showed a false-positive reaction to Dragendorff's reagent. Although the mechanism is still unclear, this reaction was helpful for us to trace SLDs rapidly in our work. Compounds **1**–**4** proved to be the natural products by detecting their existence in the freshly prepared acetone extract by using HPLC-ESIMS (Supporting Information).

Carpedilactone A (**1**), $[\alpha]^{25}_{D}$ +95.4 (*c* 0.283, CH_3OH), was obtained as an optically active colorless prismatic crystal. Its molecular formula $C_{30}H_{38}O_6$ was determined by positive HRESIMS at *m*/*z* 517.2591 ($[M + Na]^+$, calcd 517.2561), requiring 12 degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl groups (3435, 3363 cm^{-1}) and a carbonyl group (1759 cm^{-1}).

From analyses of the ^1H and ^{13}C NMR spectra of **1**, together with the examination of HSQC and HMBC experiments, 30 carbon signals of the ^{13}C NMR spectrum were identified as three CH_3, nine CH_2 (including two sp^2 carbons), nine CH (four oxygenated ones), and nine quaternary carbons (assigned

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as two carbonyl groups, three sp^3, and four sp^2 carbons) (Tables S1 and S2, Supporting Information). With the characteristic signals of sesquiterpene lactone, such as one α-methylene-γ-lactone functionality (δ_H 6.13, Ha-13′; δ_H 5.69, Hb-13′; δ_C 172.3/119.8, C-12′/13′), one downfield shifted signal of the carbonyl group of lactone (δ_C 183.8, C-12), and two oxygenated CH (δ_H 4.95/4.31, H-8/8′; δ_C 79.3/83.0, C-8/8′), it could be speculated that **1** might be a dimeric sesquiterpene lactone.

The planar structure of **1** was constructed by detailed analyses of $^1H-^1H$ COSY, HSQC-TOCSY, and HMBC spectra (Figure 2). Three proton-bearing structural fragments,

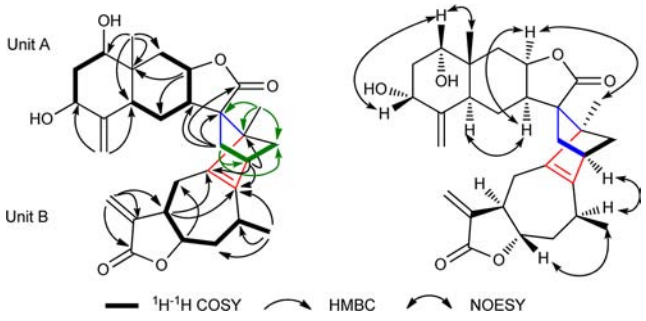


Figure 2. Selected NMR correlations of **1**.

corresponding to H-1/H_2-2/H-3, H-5/H_2-6/H-7/H-8/H_2-9, and H_2-6′/H-7′/H-8′/H_2-9′/H-10′/H_3-14′ (Figure 2), were observed by analyses of the $^1H-^1H$ COSY and HSQC-TOCSY spectra. The HMBC spectrum of **1** exhibited the correlations of H_3-14/C-1, C-5, C-9, and C-10, of H-8/C-6 and C-10, of H_2-15/C-3 and C-5, of H_2-13/C-7, C-11, and C-12, of H-2′/C-1′, C-4′, and C-5′, of H-7′/C-5′, C-1′, C-4′, and C-9′, of H-8′/C-6′, of H_2-13′/C-7′, C-11′, and C-12′, and of H_3-14′/C-1′, C-9′, and C-10′ as depicted with arrows from H to C. Thus, the monomeric units A and B were deduced as a eudesmanolide moiety similar to granilin (**5**)[4] (Scheme 1) and a guaianolide moiety. The key HSQC-TOCSY correlations of H_2-13/H-2′/H_2-3′ as well as the clearly observable HMBC correlations of H_3-15′/C-11, C-3′, and C-4′, and of H_2-13/C-2′ and C-3′ indicated that units A and B should be linked via two C−C single bonds (between C-13 and C-2′, C-11 and C-4′) probably arised from Diels−Alder [4 + 2] cycloaddition of the granilin unit as the dienophile and the guaianolide unit as the diene.

The relative configurations of **1** were deduced from interpretation of the observed NOESY correlations (Figure 2). In unit A (eudesmanolide moiety), the key NOESY correlations of H_3-14/H-1 and H-1/H-3 indicated they are in the same face and were arbitrarily assigned as β-oriented, while the correlations of H-5/H-7 and H-7/H-8 place them on the opposite side, in agreement with those of granilin (**5**).[4] In unit B (guaianolide moiety), the NOESY correlation of H-8′ with H_3-14′, without the correlation with H-7′, established the relative configuration of H-7′, H-8′, and 14′-CH_3 as shown in Figure 2. The relative configuration of C-11 was secured through observations of the NOESY correlations of H-8/H_3-15′ and H-2′/H-10′, suggesting that the CH_2-13 should be β-oriented. However, the relative configurations of C-2′ and C-4′ remained unresolved because there are two possible stereoisomers (*exo*- or *endo*-type). In order to solve this issue, it was necessary to execute a Cu Kα X-ray diffraction experiment.

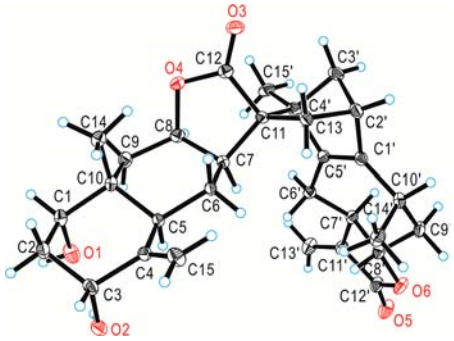
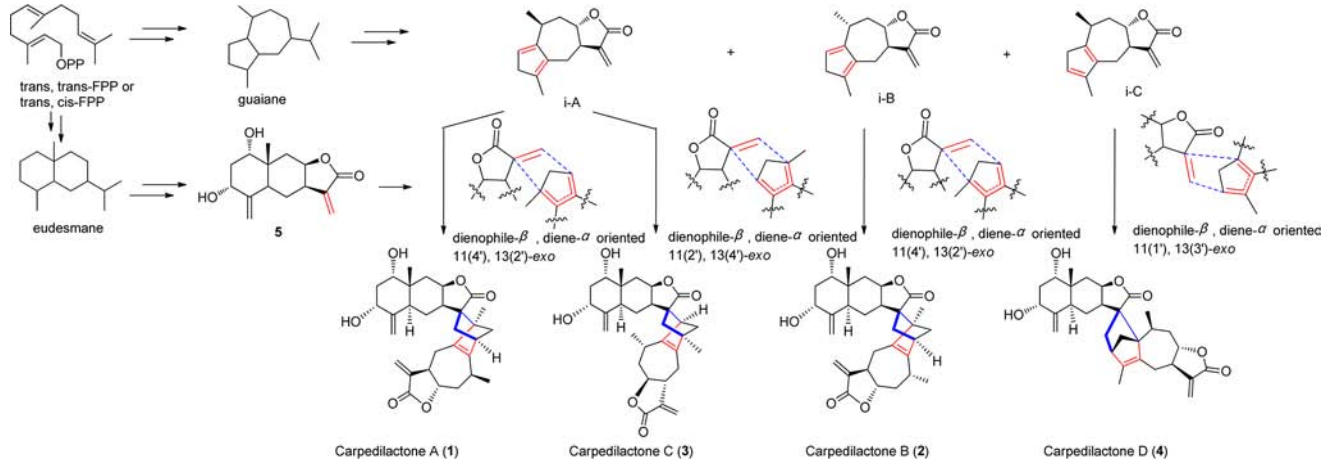


Figure 3. X-ray structure of **1**.

After trying several solvent systems, the single crystal of **1** was obtained from acetone/acetonitrile/water (2:1:1). Cu Kα X-ray crystallographic analysis established unambiguously the absolute configurations of **1** to be 1*S*, 3*R*, 5*R*, 7*R*, 8*R*, 10*R*, 11*S*, 2′*S*, 4′*R*, 7′*R*, 8′*S*, 10′*S* (Figure 3).[5] Accordingly, the structure of **1** was fixed and named carpedilactone A.

Carpedilactone B (**2**), an optically active colorless prismatic crystal with $[\alpha]^{25}_D$ +15.1 (*c* 0.27, CH_3OH), was assigned the same molecular formula $C_{30}H_{38}O_6$ as that of **1** due to positive HRESIMS at *m*/*z* 517.2577 ($[M + Na]^+$, calcd 517.2561).

Scheme 1. Proposed Biogenetic Pathway for Carpedilactones A−D (1−4)



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The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**. In the ^{1}H NMR spectrum, the main differences between them were the downfield shifted doublet methyl (H_3-14′) at δ_H 1.30 in **2**, in contrast to δ_H 1.18 in **1**, and the upfield shifted H-8′ and H-10′ at δ_H 4.07 and 2.54 in **2**, in contrast to δ_H 4.31 and 2.94 in **1**. In the ^{13}C NMR spectrum, the chemical shifts of C-10′, C-2′, and C-8′ were changed from δ_C 33.6, 48.2, and 83.0 in **1** to δ_C 30.4, 44.1, and 85.5 in **2**, respectively. The evidence indicated that **2** should be the C-10′ epimer of **1**, which was supported by the key NOESY correlations of H-10′/H-8′ and H-2′/H_3-14′. The structure of **2** was further confirmed by Cu Kα X-ray diffraction (Figure 4),[6] and its absolute configurations were unambiguously determined. Therefore, the absolute configuration of C-10′ of **2** was assigned as *R*.

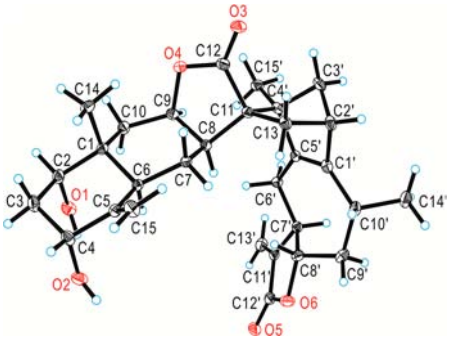

Figure 4. X-ray structure of **2**.

Carpedilactone C (**3**), obtained as a colorless prismatic crystal with $[\alpha]^{25}_D$ −2.3 (*c* 0.14, CH_3OH), had the molecular formula $C_{30}H_{38}O_6$ deduced from positive HRESIMS at *m*/*z* 495.2756 ($[M + H]^+$, calcd 495.2741).

Thirty carbon resonances were observed in the ^{13}C NMR spectrum, together with the characteristic NMR signals for a granilin unit and a guaianolide unit, implying that compound **3** is also a sesquiterpenoid lactone dimer similar to **1** and **2**. Differently, the two monomeric units of **3** were linked via two C−C single bonds between C-13 and C-4′, and between C-11 and C-2′, on the basis of the key ^{1}H−^{1}H COSY correlation (Figure 5) of H-2′/H_2-3′, and HMBC correlations (Figure 5)

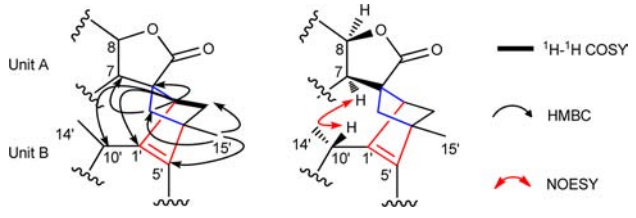

Figure 5. Selected NMR correlations of **3**.

of H_2-13/C-11, C-7, C-5′, and C-15′, of H_3-15′/C-13, C-3′, C-4′ and C-5′, and of H-2′/C-13, C-1′, C-4′, and C-10′, which furnished an additional cyclohexene ring. Thus, compound **3** was identified as the diastereomer of **1**.

Interestingly, a transannular NOESY correlation of H-10′ (δ_H 2.67) with H-7 (δ_H 2.01) (Figure 5) was clearly observed, suggesting that **3** was a stacked arrangement of the sesquiterpene monomers.[7] Finally, Cu Kα X-ray single crystal diffraction analysis not only confirmed the above deduction but also assigned the absolute configurations of **3** to be 1*S*, 3*R*, 5*R*, 7*R*, 8*R*, 10*R*, 11*S*, 2′*R*, 4′*S*, 7′*R*, 8′*S*, 10′*S* (Figure 6).[8]

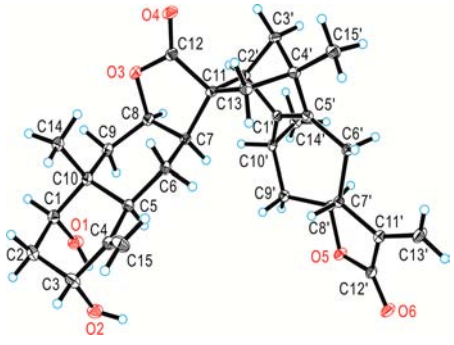

Figure 6. X-ray structure of **3**.

Carpedilactone D (**4**), $[\alpha]^{25}_D$ +65.8 (*c* 0.343, CH_3OH), was obtained as an optically active colorless column crystal. The HRESIMS (*m*/*z* 495.2779, $[M + H]^+$, calcd 495.2741) of **4** indicated the molecular formula of $C_{30}H_{38}O_6$, which is identical to those of **1**−**3**.

Extensive interpretation of the ^{1}H and ^{13}C NMR spectra showed that compound **4** possessed the same granilin unit and guaianolide unit as those of **1** and **3**, but possibly had a different linkage type for two units. The key ^{1}H−^{1}H COSY and HSQC-TOCSY correlations of H_2-13/H-3′/H_2-2′ as well as the HMBC correlations of H_2-13/C-11, C-12, C-7, C-4′, of H-3′/C-2′, C-13, of H_3-15′/C-3′, C-4′, C-5′, and of H_3-14′/C-1′, C-9′, C-10′, indicated that two monomeric units A and B were connected via two C−C single bonds between C-13 and C-3′ and between C-11 and C-1′, different from the linkage types of **1**−**3** between C-13 and C-11 of the granilin unit and C-2′ and C-4′ of the guaianolide unit (Figure 7).

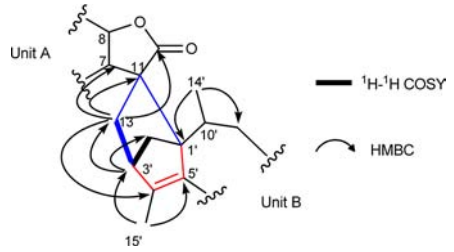

Figure 7. Selected NMR correlations of **4**.

Due to the lack of available NOESY correlations, the relative configurations of the additional cyclohexene moiety could not be confirmed. After several attempts, a suitable colorless crystal was obtained from acetone/methanol/water (3:3:1). Thus, the structure of **4** was established, and its absolute configurations were assigned to be 1*S*, 3*R*, 5*R*, 7*R*, 8*R*, 10*R*, 11*S*, 1′*S*, 3′*S*, 7′*R*, 8′*S*, 10′*S* (Figure 8)[9] by Cu Kα X-ray single crystal diffraction analysis.

So far, many sesquiterpene dimers have been reported from the Compositae species as well as other plants.[3,10] Biogenetically, these dimers may be derived from the enzymatic Diels−Alder cycloaddition reaction of homo- or heterosesquiterpene units. The isolation and identification of some natural Diels−Alderases, such as solanapyrone synthase, macrophomated synthase, and spnF, have afforded additional evidence.[11] In this study, it is a remarkable fact that either the 2,4-linkage of **1**−**3**

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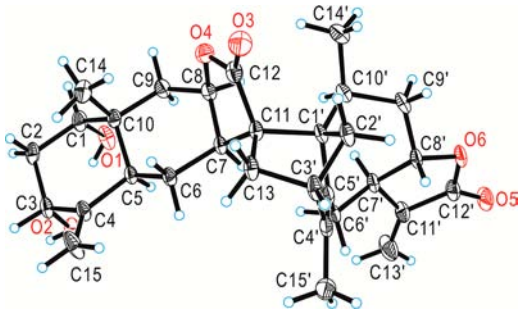

Figure 8. X-ray structure of **4**.

or the 1,3-linkage of **4**, the four dimers possessing the same exostereochemistry, is different from the endoproducts of the Diels–Alder addition according to the "Endo-Rule".[12] The interesting results suggested that the biosynthesis of **1**–**4** might be associated with an exo-Diels–Alderase. Considering the coexisting eudesmanolide and guaianolide constituents in the tilted plant, a possible biogenetic pathway for **1**–**4** was proposed in Scheme 1, in which the Diels–Alder cycloaddition is a key reaction. Therefore, the total synthesis of **1**–**4** or the search for the possible exo-Diels–Alderase will be an interesting topic.

Moreover, carpedilactones A–D (**1**–**4**) and the monomer granilin (**5**) were evaluated for cytotoxicity against four human cancer cell lines (A549, BEL 7409, HLF, and CCRF-CEM) by MTT assay with DOX (doxorubicin) as a positive control.[13] Compounds **1**–**4** showed moderate cytotoxicities against the first three cell lines, which are better than that of **5**. Interestingly, compounds **1**–**4** exhibited potent inhibition against human leukemia (CCRF-CEM) with IC_{50} values of 0.14, 0.32, 0.35, and 0.16 μM, respectively, much stronger than that of **5** with 4.65 μM (Table S3, Supporting Information). The above mentioned data revealed a simple and clear SAR; the dimeration can significantly improve the cytotoxicity of the monomer granilin (**5**), but it cannot be excluded that the α-methylene-γ-lactone skeleton in guaianolide exerts the function effectively. Thus, the detailed QSAR and molecular mechanisms of action of these dimers need to be explored in our future work.

In conclusion, carpedilactones A–C (**1**–**3**) are the first three 2, 4-linked eudesmanolide-guaianolide heterosesquiterpene lactone dimers, which might be biosynthesized via exo-Diels–Alder [4 + 2] cycloaddition. Meanwhile, considering their potent cytotoxicity, carpedilactones A–D (**1**–**4**) will also be potential antileukemia leads.

■ ASSOCIATED CONTENT

Supporting Information

The extraction scheme, compound characterization, spectroscopic data, the X-ray data, and CIF files for **1**–**4** are included herein. 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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