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# Cytotoxic 2, 4-Linked Sesquiterpene Lactone Dimers from *Carpesium faberi* Exhibiting NF-κB Inhibitory Activity

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**Six new 2, 4-linked exo/endo sesquiterpene lactone dimers, faberidilactones A–E (1–5) and endodischkuhriolin (6), were isolated from *Carpesium faberi*. The characteristic chemical shift of Ha-3′ and diagnostic negative/positive CE can be used to assign the exo/endo stereochemistry of those dimers. Compounds 1–3, 5 and 6 exhibited potent cytotoxicity with $IC_{50}$ value in the range of 1.11–8.50 μM against the four human tumor cell lines (CCRF-CEM, K562, HL-60 and HCT116 cells). Moreover, 1, 3 and 5 (exo-SLDs) showed potent NF-κB inhibitory effect with inhibition ratio of 65.98, 53.27 and 71.45 %, respectively.**

Sesquiterpene lactone dimers (SLDs) represent the class of natural products that have $C_{30}$ framework biosynthetically derived from two homo or hetero sesquiterpene monomers.[1] Most of them were formed by biosynthetic Diels-Alder [4+2] cycloaddition, exhibiting region/stereo selectivity. Since the first SLD Absinthin was isolated from *Artemisia absinthium* in 1950s, more than 150 SLDs have been discovered from the family Compositae. Their diverse skeletons, complex stereochemistry and potent antitumor activity have drawn the attention of chemists and pharmacologists.[1-4]

Structurally, the region/stereo selective SLDs can also be classified into several subclasses according to their linkages, such as 2, 4-/1, 3-/1, 4-linked SLDs. Before our investigation, there were four 2, 4-linked SLDs isolated from the family Compositae, but only one of them, dischkuhriolin,[5] was unambiguously elucidated as an exo-SLD *via* X-ray crystallographic data. An overview of reports on the structure elucidation of these Diels-Alder adducts revealed that it was a hard work to resolve the relative and absolute configuration of the three newly formed stereogenic centers of C-11, C-2′ and C-4′ (or namely exo/endo orientation), due to the lack of sufficient NOESY correlations and suitable crystals for X-ray single crystal diffraction.[6-8]

In our previous works, three 2, 4-linked exo-type eudesmanolide-guaianolide SLDs, carpedilactones A–C, were isolated from the title plant, and their absolute configuration were also determined by Cu–Kα X-ray crystallographic analysis.[9] Since the corresponding 2, 4-linked endo-type SLDs were never obtained from nature, it's difficult to summary the spectral difference between the exo and endo SLDs. Fortunatelly, in our continuing work, a pair of 2, 4-linked exo/endo diastereoisomeric carabranolide-guaianolide dimers and three exo congeners, faberidilactones A–E (**1–5**), together with endodischkuhriolin (**6**), the endo diastereoisomer of dischkuhriolin[5] (Fig. 1), were isolated from the same plant. Compounds **1**–**5** are the first five SLDs with new carabranolide-guaianolide skeleton formed by the enzymatic Diels-Alder [4+2] addition.[9,10] Furthermore, detailed analysis of the spectra of **1**–**6** led to the findings of two spectrographic features: the characteristic chemical shift of Ha-3′ and diagnostic negative/positive Cotton effect (CE), which can be simply used to elucidate the exo/endo configuration of those 2, 4-linked SLDs. Herein, we described the isolation, structure elucidation and NF-κB inhibitory activity of **1**–**6**.

Faberidilactone A (**1**), isolated as an optically active colourless oil with $[\alpha]^{25}_{D}$ +40.4 (*c* 1.288, $CH_3OH$), was

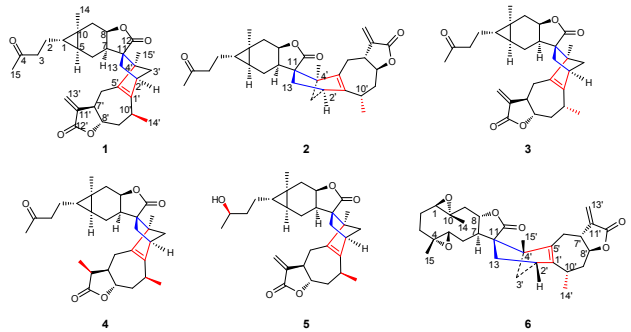

**Fig. 1** Chemical structures of **1**–**6**.

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preliminarily determined as a dimeric sesquiterpene lactone, based on the molecular formula $C_{30}H_{38}O_5$ (HRESIMS at *m/z* 479.2833 $[M + H]^+$, calcd 479.2792) and typical $^1H$ and $^{13}C$ NMR signals for SLDs (Tables S1 and S2, ESI†), including one *α*-methylene-*γ*-lactone functionality ($\delta_H$ 6.11, d, *J*=3.5 Hz, Ha-13′; $\delta_H$ 5.69, d, *J*=3.5 Hz, Hb-13′; $\delta_C$ 171.8/119.8, C-12′/13′), one downfield shifted signal of carbonyl group of lactone ($\delta_C$ 184.0, C-12), and two characteristic oxygenated CH of lactone ($\delta_H$ 4.83, m, H-8; 4.32, m, H-8′; $\delta_C$ 77.4, C-8; 83.0, C-8′).

Comprehensive NMR analysis resulted in the assembly of two subunits A and B. Unit A was assigned as a carabrone moiety[11] due to the four typical multiplets in the most upfield region: $\delta_H$ 0.30 (1H, m, H-5), 0.43 (1H, m, H-1), 0.66 (1H, m, Hb-6), 0.88 (1H, m, Hb-9), as well as the characteristic signals for a $CH_3CO$- group at $\delta_H$ 2.13 (3H, s, $H_3$-15)/$\delta_C$ 30.0 (C-15), 211.5 (C-4). Further evidences come from 2D NMR analysis. A proton-bearing structural fragment of $H_2$-3/$H_2$-2/H-1/H-5/$H_2$-6/H-7/H-8/$H_2$-9 was constructed by analysis of the $^1H$-$^1H$ COSY and HSQC-TOCSY experiments (Fig. 2). The HMBC correlations of H-1/C-2, C-3 and C-10; of H-5/C-1, C-2, C-10 and C-14; of H-7/C-6, C-8, C-11 and C-12; of H-8/C-6, C-9 and C-12; of $H_2$-13/C-11 and C-12; of $H_3$-14/C-1, C-5, C-9 and C-10; of $H_3$-15/C-4 and C-3 were observed in HMBC spectrum (Fig. 2). Similarly, the unit B was established as a guaianolide moiety according to the long proton-proton correlations system $H_2$-6′/H-7′/H-8′/$H_2$-9′/H-10′/$H_3$-14′ in $^1H$-$^1H$ COSY and HSQC-TOCSY spectra, combined with the key HMBC correlations of H-2′/C-4′, C-5′ and C-10′; of H-7′/C-8′, C-9′ and C-11′; of H-8′/C-6′ and C-10′; of $H_2$-13′/C-7′, C-11′ and C-12′; of $H_3$-14′/C-1′, C-9′ and C-10′; of $H_3$-15′/C-3′, C-4′ and C-5′(Fig. 2). Therefore, compound **1** was proposed to be formed by a carabrone and a guaianolide.

The linkage of the two units *via* two C–C single bonds between C-13/C-2′ and C-11/C-4′ was deduced from the $^1H$-$^1H$ COSY correlations of $H_2$-13/H-2′/$H_2$-3′, and the key HMBC correlations of H-2′/C-13; of $H_3$-15′/C-11; of $H_2$-13/C-1′, C-2′ and C-3′ (Fig. 2). Thus, the planar structure of **1** was constructed as shown in Fig. 2, probably formed from a Diels-Alder [4+2] cycloaddition of the carabrone unit as the dienophile and the guaianolide unit as the diene.

The relative configuration of asymmetry carbons in **1** was concluded from the NOESY correlations. In unit A, the H-7 was arbitrarily assigned as the *α*-orientation under the biogenetic consideration, then the key NOESY correlations of H-7/H-8/$H_3$-14/H-5/$H_2$-2, but not with H-1 (Fig. 2), indicated that H-7, H-8, $H_3$-14, H-5 and $H_2$-2 were in the same face and were assigned as *α*-orientation, while H-1 was assigned as *β*-orientation, in agreement with those of carabrone. In unit B, the NOESY

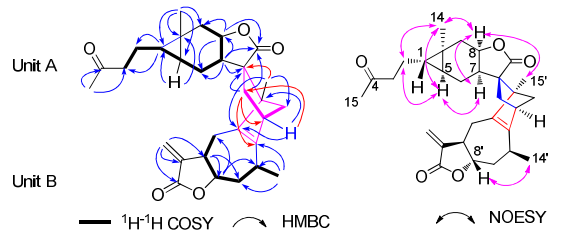


**Fig. 2** Selected NMR correlations of **1**.

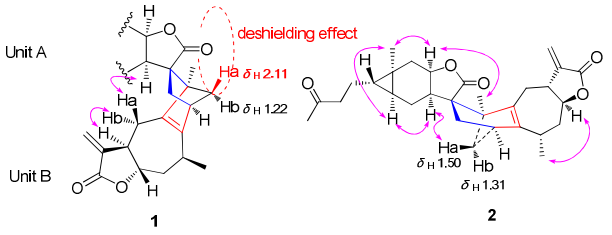


**Fig. 3** Selected NOESY correlations ( ) of **1** and **2**, and the deshielding effect of carbonyl group of C-12.

correlation of H-8′ with $H_3$-14′, but not with H-7′ (Fig. 2), showed that H-8′ and $H_3$-14′ were in the same face, while H-7′ was at the opposite side. Particularly, the relative configuration of the spiro-atom C-11 was resolved through the key NOESY correlation of H-8/$H_3$-15′ (Fig. 2), suggesting that the $CH_2$-13 should be *β*-oriented. Meanwhile, the key NOESY correlations of Ha-6′/H-7; of Hb-6′/H-7′ (Fig. 3) were found out, indicating that **1** was a stacked arrangement of two sesquiterpene monomers (i. e., exo orientation) (Fig. 3).

Interestingly, faberidilactone B (**2**), an endo diastereoisomer of **1**, was co-isolated from the same plant. **2** was an optically active colourless oil with $[\alpha]^{25}_D$ +124.3 (*c* 0.05, $CH_3OH$). HRESIMS data at *m/z* 501.2591 ($[M+Na]^+$, cald 501.2611) of **2** implied its molecular formula was $C_{30}H_{38}O_5$, same as that of **1**. Detailed 1D and 2D NMR analysis indicated that **2** had the same planar structure as **1** (Tables S1 and S2, ESI†). In addition, the relative configuration of C-11 was also resolved by the NOESY correlation of H-8/$H_3$-15′ (Fig. 3), exhibiting that the $CH_2$-13 was *β*-oriented. Differently, the key NOESY correlation of Ha-3′/H-7 (Fig. 3) showed that **2** was the stretched arrangement between two monomers, and **2** must be the endo diastereoisomer of **1**.

Inspecting the differences between **1** and **2** on $^1H$ NMR data (Tables S1 and S2, ESI†), we found the most obvious change was the chemical shift of Ha-3′, i. e., the chemical shift of Ha-3′ was upfield shifted from $\delta_H$ 2.11 in **1** to $\delta_H$ 1.50 in **2** (Fig. 3), indicating that the downfield chemical shift of Ha-3′ in **1** was the result of the deshielding effect of carbonyl at C-12. Thus, the structures of **1** and **2** were established as shown in Fig. 3.

In order to solve the absolute configuration of **1** and **2**, a theoretical calculation of their electronic circular dichroism (ECD) spectra was performed using time-dependent density-functional theory (TD-DFT) at the B3LYP/TZVP level with the PCM model in methanol solution (ESI†). As shown in Fig. 4, the ECD spectra of model A and model B were well in accordance with the experimental CD spectrum of **1** and **2**, respectively. Subsequently, the absolute configuration of **1** was assigned as 1*S*, 5*S*, 7*R*, 8*R*, 10*R*, 11*S*, 2′*S*, 4′*R*, 7′*R*, 8′*S*, 10′*S*. Differently, the chirality of C-2′ and C-4′ in **2** were assigned as 2′*R*, 4′*S*.

Obviously, the exo-SLDs (**1**, **3**–**5** and carpedilactones A–C[9] (Their CD spectra attached in ESI†)) showed an intense negative CE around 214 nm, while the endo-SLDs **2** and **6** showed an intense positive CE around 228 nm (Fig. 4 and ESI†), demonstrating that the conformationally flexible side chain at C-1, the carbonyl chromophore at C-4 and the double bond between C-11′ and C-13′ had hardly any effect on their spectra,

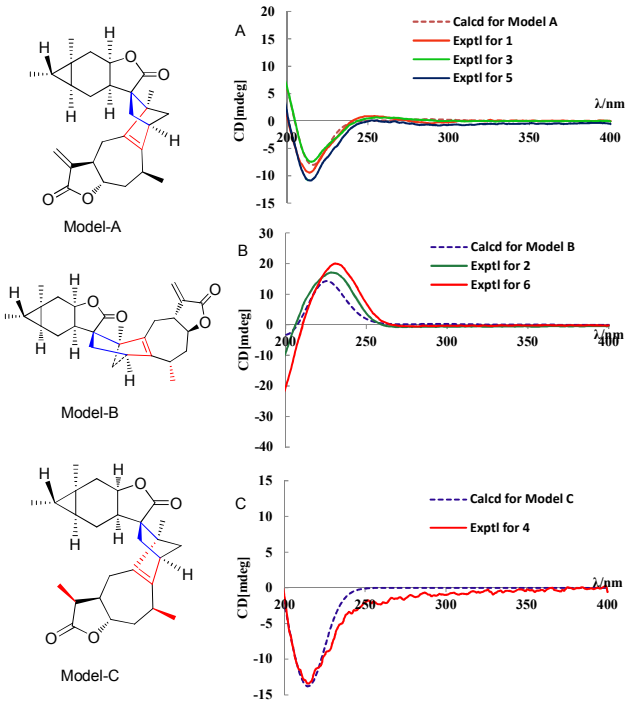


**Fig. 4** Model-**A** (simplified model structure of **1**, **3** and **5**), Model-**B** (simplified model structure of **2**), and Model-**C** (simplified model structure of **4**) in which the butan-2-one or the butan-2-ol side-chain were replaced by a methyl group, respectively, since these side-chain contribute only weakly to the observed CD spectra of compounds **1**–**5;** (A) Calculated and experimental ECD spectra of **1**, **3** and **5**. (B) Calculated and experimental ECD spectra of **2** and **6**. (C) Calculated and experimental ECD spectra of **4**.

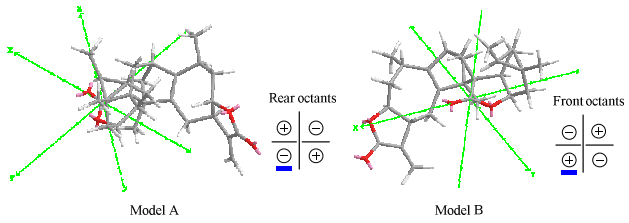


**Fig. 5** Model A and Model B viewed along the O=C bond on axes as employed in application of the octant rule.

which agreed with the theoretical basis that only if the strongly absorbing chromophores located near in space and close in energy, very intense CD couplets would be arose.[12,13] Thus, we deduced that their opposite CE was only associated with the isolated carbonyl chromophore at C-12, i. e., other chromophores did not participate at all in the chromophoric systems, so their opposite CE could be successfully explained by the ketone octant rule[12].

As shown in Fig. 5, it is apparent that the contribution of all atoms and groups nearly cancel each other with the exception of the unit B. The unit B of Model A in bottom left corner in rear octants justified the negative sign observed in the CD spectrum, on the contrary, the unit B of Model B in bottom left corner in front octants confirmed the positive sign observed in the CD spectrum. Hence, the CD spectrum of 2, 4-linked SLDs is an extraordinary reporter of the exo/endo configuration.

Faberidilactone C (**3**), was isolated as an optically active

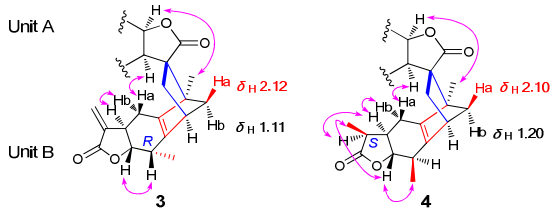


**Fig. 6** Selected NOESY correlations ( ) of **3** and **4**.

colourless oil with $[\alpha]^{25}_{D}$ +38.0 (*c* 0.05, $CH_3OH$). From the observation of 1D and 2D NMR spectra of **3** (Tables S1 and S2, $ESI^+$), it was discovered that the chemical structure of **3** was the epimer of **1** at C-10′, which were supported by the NOESY correlation of H-10′/H-8′ (Fig. 6). Therefore, the absolute configuration of C-10′ was confirmed as *R*.

Faberidilactone D (**4**), was isolated as a colourless oil with $[\alpha]^{25}_{D}$ -0.56 (*c* 0.3, $CH_3OH$). The positive HRESIMS at *m/z* 481.2985 ($[M+H]^+$, cald 481.2949) of **4** indicated a molecular formula of $C_{30}H_{40}O_5$, which was two hydrogen atoms more than that of **1**. The $H_2$-13′ was disappeared and an additional doublet methyl was present in $^1$H NMR spectra (Tables S1 and S2, $ESI^+$), required that the $\Delta^{11'(13')}$ exocyclic double bond was reduced to the methyl. This interpretation was also supported by the HMBC correlations of $H_3$-13′ with C-11′, C-12′ and C-7′ (Fig. 6). Furthermore, the relative configuration of $CH_3$-13′ was elucidated as $\beta$-orientation by the key NOESY correlations of $CH_3$-13′ with H-8′ and of H-11′ with H-7′ (Fig. 6). Thus, the absolute configuration of C-11′ was determined as *S*.

Faberidilactone E (**5**), isolated as an optically active colourless oil with $[\alpha]^{25}_{D}$ +54.3 (*c* 0.077, $CH_3OH$). The HRESIMS and NMR spectra of **1** and **5** (Tables S1 and S2, $ESI^+$) suggested that these two compounds are a keto-nealcohol pair, which were supported by the following evidences: (1) the carbonyl of ketone at $\delta_C$ 211.5 in **1** was replaced by the oxygenated methine at $\delta_C/\delta_H$ 68.3/3.72 in **5**; (2) the singlet acetyl methyl at $\delta_H$ 2.13 (s, $H_3$-15) in **1** transferred to the doublet methyl at $\delta_H$ 1.14 (d, $H_3$-15) in **5**; (3) the $^1$H-$^1$H COSY correlations of $H_3$-15/H-4/$H_2$-3/$H_2$-2/H-1 in **5**; (4) the HMBC correlations of $H_3$-15 with H-4 and H-3 in **5**, revealing that the carabrone moeity (unit A) has changed to carabrol.[14] Subsequently, the *R*-configuration of C-4 was confirmed by the modified Mosher's method (Fig. 7, $ESI^+$).[15]

Although the NMR data of endodischkuhriolin (**6**) (Tables S1 and S2, $ESI^+$) was closely similar to dischkuhriolin[5], there were some obvious differences, i. e., in $^1$H NMR, the upfield chemical shift of Ha-3′ at $\delta_H$ 1.47 in **6**, in contrast to that of Ha-3′ ($\delta_H$ 2.46) in dischkuhriolin; in NOESY, the correlation of

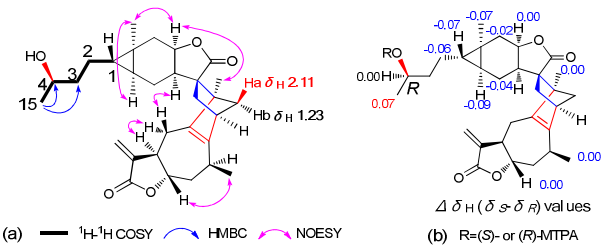


**Fig. 7** (a) Selected NMR correlations of **5**. (b) $\Delta\delta_H$ ($\delta_S$-$\delta_R$) values obtained for the MTPA esters of **5**.

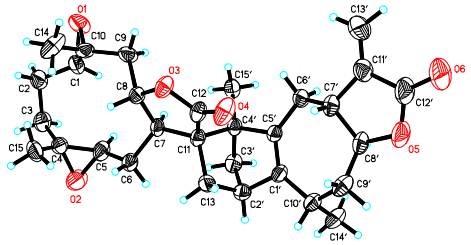

**Fig. 8** X-ray crystallographic structure of **6**.

H-7/Ha-3′ was observed in **6**, instead of the correlation of H-5/H-8′ in dischkuhriolin, revealing that **6** was the endo-type diastereoisomer of dischkuhriolin. For unambiguous confirmation of the stereochemical structure of **6**, a single-crystal X-ray diffraction experiment was performed, and the absolute configuration was assigned as 1*R*, 4*R*, 5*R*, 7*R*, 8*S*, 10*R*, 11*S*, 2′*R*, 4′*S*, 7′*R*, 8′*S*, 10′*S* (Fig. 8, CCDC-1035615, ESI†), which confirmed our above deduction.

Compounds **1**–**3**, **5** and **6** were found to be cytotoxic against the four cell lines (CCRF-CEM, K562, HL-60 and HCT116 cells) with $IC_{50}$ value in the range of 1.11-8.50 $\mu$M, which better than that of the dimer without $\alpha$-methylene-$\gamma$-lactone **4** and the two monomers carabrone **7** and 4*R*-carabrol **8** (Table S3, ESI†). Mechanism studies[16] showed that the three exo-SLDs **1**, **3** and **5** had potent NF-κB inhibitory activity with inhibition ratio of 65.98, 53.27 and 71.45 %, respectively, which stronger than that of the endo-SLD **2** with inhibition ratio of 38.59 %. Furthermore, **1**, **3** and **5** dose-dependently suppressed TNF-$\alpha$-induced phosphorylation of the NF-κB p65 subunit and degradation of IκB$\alpha$ in bEnd.3 cells (Fig. 9A–9D, ESI†). These above data indicated that: (1) the cytotoxicity of the SLDs **1**–**3** and **5** was attributed to the function of $\alpha$-methylene-$\gamma$-lactone acting as alkylating agent and dimeration; (2) **1**, **3** and **5** (exo-SLDs)-induced cytotoxicity might be attributed to suppressing NF-κB activation, while the endo-SLD **2** might not be restricted to this mechanism of action.

In conclusion, six new 2, 4-linked SLDs, faberidilactones A–E (**1**–**5**) and endodischkuhriolin (**6**), were isolated from *Carpesium faberi*. Notably, the spectrographic results of **1**–**6**, dischkuhriolin[5] and carpedilactones A–C[9] discovered that the 2, 4-linked exo-SLD was the stacked arrangement of two monomers, whereas the endo SLD was the stretched arrangement between two monomers, as well as their two spectrographic features. Meanwhile, we believed that the two features could be applied to the determination of exo/endo stereochemistry of 1, 3-linked SLDs, since they possess the similar exo/endo norbornylene core scaffold and the same carbonyl chromophore at C-12.

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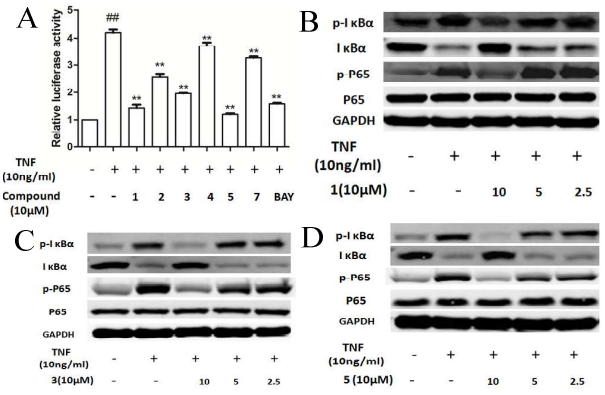


**Fig. 9** (A) Compounds **1**–**5** and **7** inhibited TNF-$\alpha$-induced NF-κB activation. ## $p < 0.01$, relative to control group; ** $p < 0.01$, relative to TNF-$\alpha$ treatment group. (B–D) Compounds **1**, **3** and **5** (2.5–10 $\mu$M) dose-dependently suppressed TNF-$\alpha$-induced phosphorylation of the NF-κB p65 subunit and degradation of IκB$\alpha$ in bEnd.3 cells.

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