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# Octacyclic and decacyclic *ent*–abietane dimers with cytotoxic activity from *Euphorbia fischeriana* steud.

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ABSTRACT

A novel Diels-Alder adduct possesses a 6/6/6/5/6/6/6/6 octacyclic skeleton featured with bicyclo[2.2.2]octane moiety, biseupyiheoid A (**1**), along with another decacyclic 6/6/6/3/5/6/5/6/6/6 fused diterpenoid dimer, bisfischoid C (**2**), were isolated from *Euphorbia fischeriana*. Their structures were determined by spectroscopic, X-ray crystallographic approaches, and quantum mechanical calculations. The structural features of **1** and **2** were hypothesized to involve intramolecular Diels-Alder reactions with different coupling patterns. Dimer **1** showed antiproliferative activity through apoptosis activation in LoVo cells.



Diterpenoids originated from geranylgeranyl pyrophosphate (GGPP) are a large group of highly diversified terpenoids with typical skeletons including acyclic, mono-, bi-, tri-, and tetracyclic systems [1]. A small subset of diterpenoids comprises dimeric diterpenoids featured with at least 40 carbons in their structures, in which the two monomeric blocks are linked together directly or through an oxygen atom [2]. Diterpenoid dimers possess increased complex ring systems and more chiral centers compared to their monomeric analogues, which poses a great challenge for their structural determination.

The genus *Euphorbia* (Euphorbiaceae) has been regarded as a valuable resource of bioactive diterpenoids with unique structural diversity [3]. For example, euphopias A–C isolated from *E. helioscopia* are rearranged jatrophane diterpenoids with anti-inflammatory potentials [4]; pedrolide is a polycyclic diterpene obtained from *E. pedroi* showed the inhibition effects on P-glycoprotein [5]. Some plants of *Euphorbia* genus, including *E. ebracteolate* and *E. fischeriana*, have been used as traditional Chinese medicines for thousands of years. With the aim of discovering more intriguing diterpenoids from medicinal plants of the genus *Euphorbia* [6,7], biseupyiheoid A (**1**, Fig. 1), an unprecedented dimeric *ent*–abietane containing spirocyclic 6/6/6/5/6/6/6/6 ring systems, and a decacyclic 6/6/6/3/5/6/5/6/6/6 skeletal *ent*–abietane dimer, bisfischoid C (**2**), were identified from the roots of *E. fischeriana*. The biological assay revealed that **1** showed the significant cytotoxic activity by apoptosis-induced effect in human colon carcinoma LoVo cells.

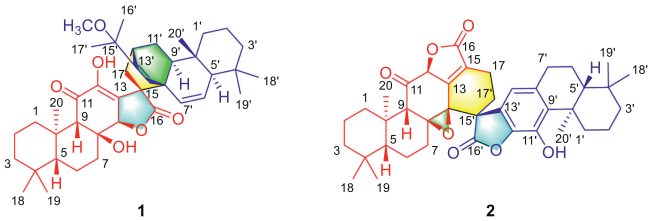

**Fig. 1.** Structures of dimeric diterpenoids **1** and **2**.

Compound **1**, obtained as a yellow oil, has a molecular formula of $C_{41}H_{58}O_6$ with 13° of unsaturation, as deduced from its HRESIMS (*m/z* 669.4129 $[M+Na]^+$, calcd. 669.4126). The $^1H$ NMR data (Table S1 in Supporting information) exhibited eight tertiary methyls at $\delta_H$ 0.56 (s, 3H), 0.80 × 3 (s, 9H), 0.85 (s, 3H), 0.93 (s, 3H), 1.33 (s, 3H), and 1.36 (s, 3H), and a methoxy group at $\delta_H$ 3.26, three olefinic signals at $\delta_H$ 5.43 (br s), 5.87 (dd, $J$ = 10.2, 2.9 Hz) and 5.99 (dd, $J$ = 10.2, 1.7 Hz). In addition, a proton associated with the oxygenated carbon at $\delta_H$ 5.41 (s) and a number of

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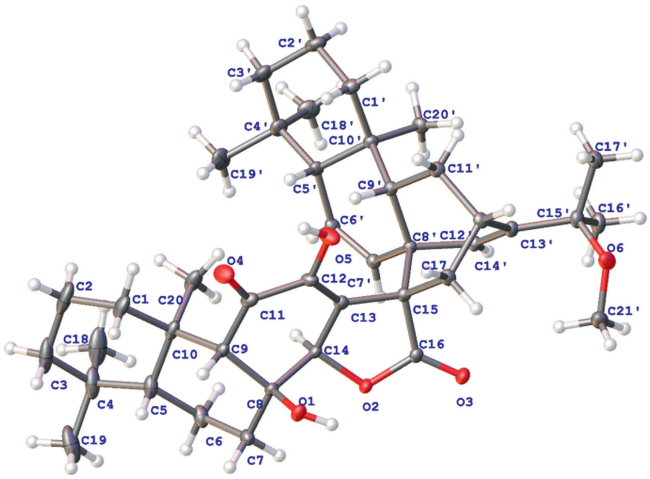



**Fig. 2.** X-ray crystallographic structure of **1** (CCDC number: 2121291).

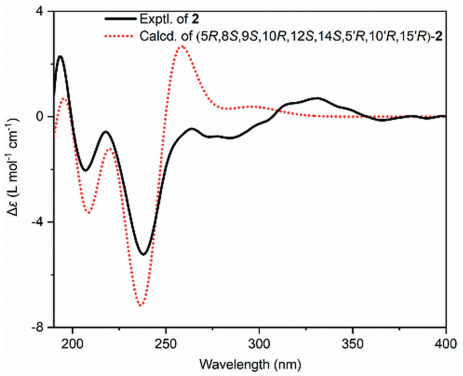



**Fig. 3.** Calculated and experimental ECD spectra of **2** at the CAM-B3LYP/def2-tzvp level.

**Table 1**
Cytotoxic activities of **1** and **2**.

| Cell line | $IC_{50}$ ± SD (μmol/L) | | |
|---|---|---|---|
| | **1** | **2** | Cisplatin[a] |
| LoVo | 6.7 ± 1.3 | > 20 | 1.7 ± 0.2 |
| A549 | 10.4 ± 1.6 | > 20 | 5.3 ± 0.8 |
| MCF-7 | > 20 | > 20 | 7.9 ± 1.2 |
| HepG2 | > 20 | > 20 | 1.8 ± 0.2 |

[a] Positive control.

protons at $\delta_H$ 1.0−3.0 were also observed, suggesting the existence of a terpenoid backbone. The $^{13}C$ NMR and DEPT spectra (Table S1 in Supporting information) displayed 41 carbon resonances, including nine methyls, ten methylenes, nine methines (three olefinic and one oxygenated), and thirteen nonprotonated carbons (two oxygenated, three olefinic, and two carbonyls). Three double bonds and two carbonyl moieties accounted for five degrees of unsaturation, suggesting the existence of eight rings in the structure of **1**.

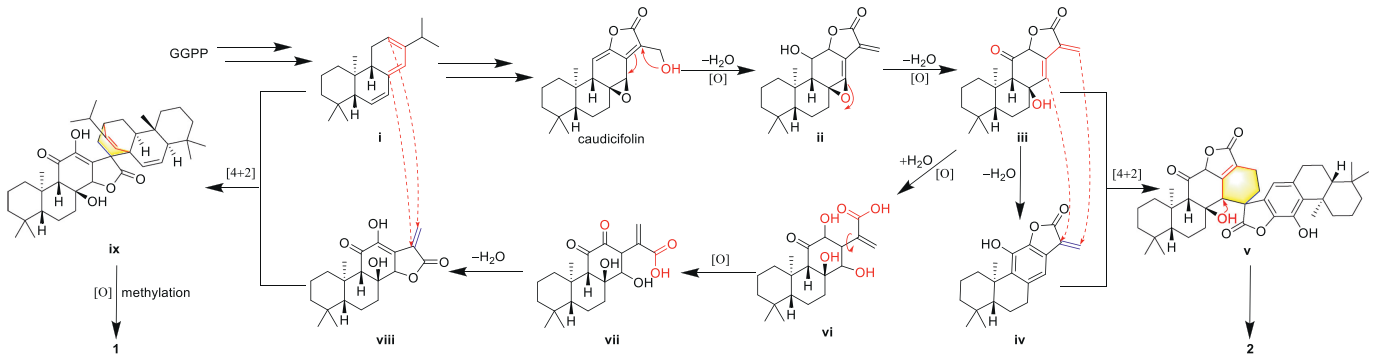
The planar structure of **1** was determined by its 2D NMR spectra (Fig. S1 in Supporting information). The $^1H$–$^1H$ COSY spectrum shows $H_2$–1/$H_2$–2/$H_2$–3 and H-5/$H_2$–6/$H_2$–7 correlations, together with long-range correlations from $H_3$–20 to C-1, C-5, C-9, and C-10, from H-9 to C-8, C-10, C-11, C-12, and C-14 in the HMBC spectrum, allowing in three fused six-membered rings as shown in Fig. 1. In addition, HMBC correlations of H-14/C-12 and C-13, $H_2$–17/C-12, C-15, and C-16 were observed, indicating that a block of abietane (part A) was present in **1**. The cross-peaks of $H_2$–1′/$H_2$–2′/$H_2$–3′, H-5′/H-6′/H-7′, and H-9′/$H_2$–10′/H-11′ in the $^1H$–$^1H$ COSY spectrum, as well as cross-peaks from $H_3$–20′ to C-1′, C-5′, C-9′, and C-10′, from H-9′ to C-8′ and C-14′, from $H_3$–16′ to C-13′, from $OCH_3$–15′ to C-15′ in the HMBC spectrum, confirmed the presence of another abietane part with a methoxy at C-15′ (part B) in the structure of **1**. The $^1H$–$^1H$ COSY correlation of H-12′/$H_2$–17 and HMBC correlations of H-14′/C-15 and H-9′/C-15 suggested that part A was linked with the part B *via* two single bonds of C-17 and C-15 with C-12′ and C-8′. Taking together, compound **1** was confirmed to be an abietane dimer with a bicyclo[2.2.2]octane moiety.

The relative configuration of **1** was partially fixed by the NOESY spectrum (Fig. S1). The correlation of $H_3$–20/H-14 indicated their same orientation. The relative configuration of the rigid bicyclo[2.2.2]octane was established by correlations of H-9′/H-17 and $H_3$–20′/H-14′. As for the C-8 and C-15, their configuration was a challenge, due to the lack of corresponding signals. Fortunately, a signal crystal of **1** was successfully obtained from a mixed solvent system (MeOH/$CH_2Cl_2$, 5:1), and X-ray diffraction was carried out with Cu K$\alpha$ radiation (Fig. 2), which not only confirmed the proposed structure but also determined the absolute configuration of **1** as 5*R*, 8*S*, 9*S*, 10*R*, 14*R*, 15*S*, 5′*R*, 8′*R*, 9′*S*, 10′*S*, 12′*S*. Finally, **1** was conducted as the first example of a novel class of diterpenoid dimer based on *ent*–abietane, and it was named biseupyiheoid A.

The molecular formula of compound **2** was deduced as $C_{40}H_{48}O_7$ by analysis of the HRESIMS (*m/z* 641.3477 $[M+H]^+$, calcd. 641.3473), $^{13}C$ NMR, and DEPT spectra (Table S1). Compound **2** was also a dimeric diterpenoid that shares the same skeleton with bisfischoids A and B [8], as evidenced by its detailed 2D NMR analysis (Fig. S2 in Supporting information). The relative configuration of C-12 in **2** was determined by the cross-peak of H-12/$H_3$–20 in the NOESY spectrum (Fig. S2). Although the correlations of $H_2$–17′/H-14 were observed, the relative configuration of C-15′ and C-8 (C-14) could not be clearly assigned. Therefore, four possible isomers of **2** that differed in the configurations of C-8 (C-14) and C-15′ (Fig. S3 in Supporting information) have resorted to quantum chemical calculations of NMR and DP4+ analysis [9–11]. The results show that isomer 8*S**,14*S**,15′*R**−**2** with DP4+ possibility of 100% agrees well with the experimental data. Finally, the structure of **2** was elucidated as shown in Fig. 1 by ECD calculations (Fig. 3).

Although **1** and **2** have different backbones, it should be noted that the linkage connecting the two blocks of dimers **1** and **2** are both cyclohexene rings. It is reasonable to speculate that the dimerization reaction in the formation of **1** and **2** was the Diels-Alder-like [4 + 2] cycloaddition [12]. A biosynthetic pathway starting from GGPP is proposed in Scheme 1. The first key intermediate produced from GGPP was **i** with a triene moiety in the *ent*–abietane framework. Enzyme-dependent oxidation and esterification reactions in plants could yield caudicifolin, a major diterpenoid monomer exited in *E. fischeriana* [6], from intermediate **i**. The oxidation at C-12 and the dehydration at C-17 would be able to give **ii** with a diene fragment. The highly reactive epoxy moiety could be cleaved and oxidized to form intermediate **iii**. Further dehydration reactions can give **iv** with an aromatic ring. The [4 + 2] cycloaddition between **iii** and **iv** affords **v** that readily to form **2** [13,14]. The key intermediate **iv** may undergo hydrolysis and oxidation to give **vi**. The oxidation of OH-12 and the subsequent keto-enol tautomerism and esterification reactions formed **viii**. Another [4 + 2] reaction between **i** and **viii** produced **ix**, which produced **1** eventually.

*ent*–Abietanes in the genus *Euphorbia* have long been known for their promising cytotoxic activities [15,16]. The cytotoxic activities of **1** and **2** against four human cancer cells derived from diverse organs were evaluated. The results (Table 1) revealed that dimer **1** ($IC_{50}$ = 6.7 μmol/L) possesses an anti-proliferative effect against colon carcinoma LoVo cells, while dimer **2** displayed no cytotoxicity. Subsequently, the apoptosis-inducing activity for compound **1** was evaluated, and the result revealed that **1** could induce the




**Scheme 1.** Proposed biogenetic pathway for **1** and **2**.

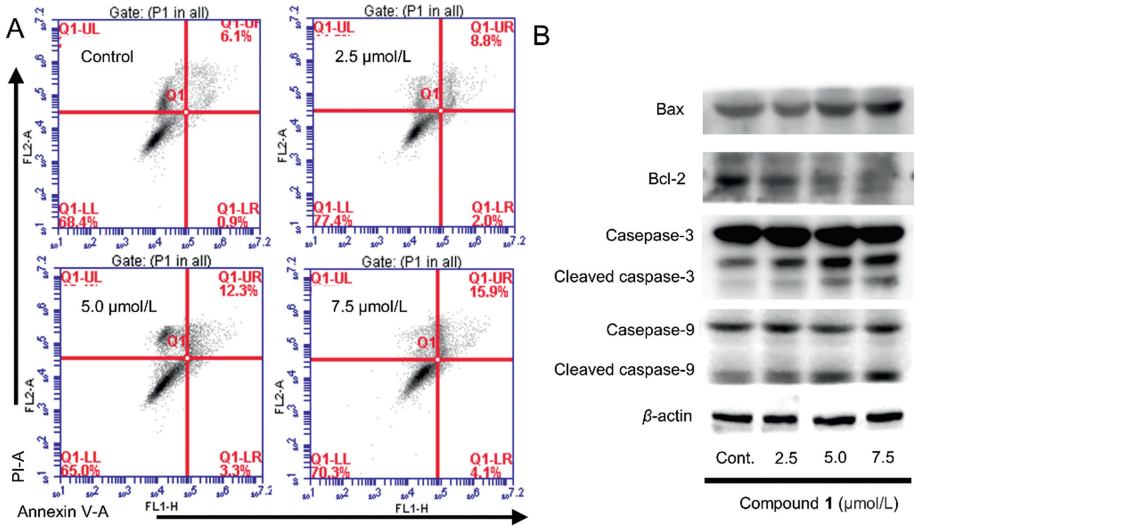



**Fig. 4.** (A) Cell apoptosis induction effects of dimer **1** analyzed by annexin V-FITC/PI flow cytometry. (B) Compound **1** regulated the expression of apoptosis-related proteins in LoVo cells.

apoptotic cell death of LoVo cells in a dose-dependent manner (Fig. 4A). Dimer **1** was further investigated for the expression levels of apoptosis-related markers in LoVo cells. As shown in Fig. 4B, compound **1** up-regulated levels of Bax, cleaved caspase 3, and cleaved caspase 9, and down-regulated the level of Bcl-2 dose-dependently.

In conclusion, biseupyiheoid A (**1**) and bisfischoid C (**2**), two spiro *ent*–abietane dimers with 6/6/6/5/6/6/6/6 and 6/6/6/3/5/6/5/6/6/6 fused ring systems, respectively, were obtained from *E. fischeriana*. Dimeric diterpenoids **1** and **2** possessed the skeletons derived from Diels-Alder cycloaddition in different patterns, which further expanded the structural diversity of diterpenoids from the genus *Euphorbia*. Dimer **1** displayed prominent cytotoxicity and showed significant apoptosis-inducing activities in LoVo cells mediated by regulating Bax, caspase 3, caspase 9, and Bcl-2.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2022.03.003.

## References

[1] J.R. Hanson, T. Nichols, Y. Mukhrish, M.C. Bagley, Nat. Prod. Rep. 36 (2019) 1499–1512.
[2] L.G. Lin, C.O.L. Ung, Z.L. Feng, et al., Planta Med. 82 (2016) 1309–1328.
[3] A. Vasas, J. Hohmann, Chem. Rev. 114 (2014) 8579–8612.
[4] Q.Q. Shi, X.J. Zhang, T.T. Wang, et al., Org. Lett. 22 (2020) 7820–7824.
[5] R.J. Ferreira, G. Spengler, A. Orthaber, et al., Org. Lett. 23 (2021) 274–278.
[6] C.J. Wang, Q.L. Yan, Y.F. Ma, et al., J. Nat. Prod. 80 (2017) 1248–1254.
[7] Y.L. Wei, C. Wang, Z.B. Cheng, et al., J. Nat. Prod. 80 (2017) 3218–3223.
[8] C.P. Sun, Y.B. Chang, C. Wang, et al., Bioorg. Chem. 116 (2021) 105356.
[9] N. Grimblat, M.M. Zanardi, A.M. Sarotti, J. Org. Chem. 80 (2015) 12526–12534.
[10] W.Y. Wang, J. Yang, Y.Y. Liao, et al., J. Nat. Prod. 83 (2020) 1998–2003.
[11] W.Y. Wang, J. Yang, Y.Y. Liao, et al., J. Nat. Prod. 83 (2020) 1157–1166.
[12] B. Liu, S.M. Fu, C.Y. Zhou, Nat. Prod. Rep. 37 (2020) 1627–1660.
[13] L. Gao, C. Su, X.X. Du, et al., Nat. Chem. 12 (2020) 620–628.
[14] Q.B. Chen, J. Gao, C. Jamieson, et al., J. Am. Chem. Soc. 141 (2019) 14052–14056.
[15] X.L. Yan, J.S. Zhang, J.L. Huang, et al., Phytochemistry 166 (2019) 112064.
[16] Y. Wang, X.Q. Ma, S.S. Yan, et al., Cancer Res. 69 (2009) 7302.