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Maoecrystal Z, a Cytotoxic Diterpene from *Isodon eriocalyx* with a Unique Skeleton

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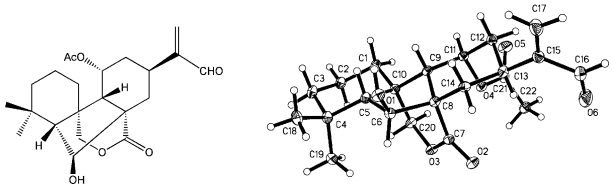
ABSTRACT



A novel diterpene with an unprecedented tetracyclic 6,7:8,15-di-*seco*-7,20-olide-6,8-cyclo-*ent*-kaurane skeleton, named maoecrystal Z (1), has been isolated from the leaves of a Chinese medicinal herb, *Isodon eriocalyx* (Labiatae). Its structure was determined by comprehensive NMR and MS spectroscopic analysis coupled with single-crystal X-ray crystallographic diffraction. Compound 1 exhibited comparable inhibitory effect against human K562 leukemia, MCF7 breast, and A2780 ovarian tumor cells with IC_{50} = 2.90, 1.63, and 1.45 μg/mL and with camptothecin and paclitaxel as the positive controls.

Plants of the genus *Isodon* (= *Rabdosia*) in the Labiatae family are well-known as a rich natural resource of diterpenoids. More than 600 diterpenoids, mainly *ent*-kauranoids, have been reported from these herbs since the first investigation in 1910. A number of those isolated diterpenoids have been found to have potent antitumor activities with low toxicity.[1] They are therefore being studied as candidates for anticancer drugs.[1] Continuous phytochemical investigations have discovered many interesting compounds with novel chemical structures, such as 1:1 complexes of natural *ent*-kauranoids (Diter-Complex-RA),[2] a natural equimolecular mixture of two epimeric *ent*-kauranoids (irroratin A),[3] 8,15-*seco*-*ent*-kauranoids (rubescensin U),[4] 6,7:8,15-*seco*-*ent*-kauranoids (laxiflorins F and G),[5] 15,16-*seco*-*ent*-kauranoid (rubescensin S),[6] 20-*nor*-*ent*-kauranoid (rubescensin N),[7] symmetric and asymmetric *ent*-kauranoid dimers (maoecrystal M, enanderinanin J, xindongnins M−O, and lushanrubescensin J),[8−11] novel *ent*-abietanoids (laxiflorins N−O, micranthin C),[12,13] and 6,7-*seco*-6-*nor*-15(8→9)-*abeo*-5,8-epoxy-*ent*-kauranoid (maoecrystal V).[14]

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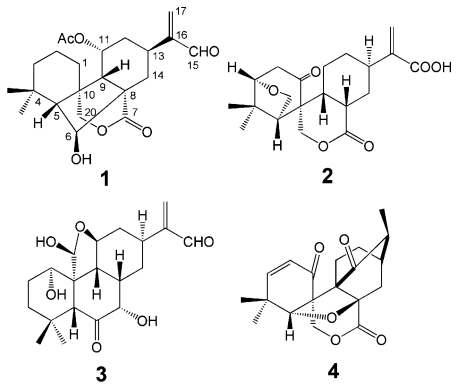
Isodon eriocalyx (Dunn.) Hara, a perennial herb or shrub widely distributed in southwestern China, is one of a few intensively studied *Isodon* species. This is partly because it has long been used as folk medicine for the treatment of sore throat, inflammation, influenza, hypertension, and dermatophytosis. Its major constituent, eriocalyxin B, which possesses two bioactive α,β-unsaturated ketone moieties, is regarded as one of the most potent cytotoxic diterpenes in hundreds of known *ent*-kauranoids.[1] Furthermore, it has been proven to be a rich source of novel *ent*-kauranoids with interesting structural features of daedal oxygenation and cleavage patterns. So far, more than 50 diterpenes including over 30 new ones have been isolated and identified from this herb in recent years.[14,15] Our further investigation on the bioactive ingredients of this herb has led to the isolation of a novel diterpene (**1**) with an unprecedented backbone. Its structure was established according to its NMR and MS spectral data, and confirmed by X-ray diffraction,[16] to be 6β-hydroxy-11α-acetoxy-6,7:8,15-di-*seco*-7,20-*olide*-6,8-cyclo-*ent*-kaur-16-en-15-aldehyde. In addition, compound **1** was tested for its cytotoxic activity against three human tumor cell lines, K562, MCF7, and A2780. This paper reports the structure elucidation and cytotoxicity of compound **1**.

Leaves of *I. eriocalyx* (Dunn.) Hara were collected from Weishan Prefecture of Yunnan Province, China, in October 2002 and identified by Prof. H. W. Li. Dried leaf powder (1.1 kg) was extracted with 70% aqueous acetone (3 × 3 L × 24 h) and then concentrated in vacuo to give a crude extract (40 g), which was suspended in water and partitioned with AcOEt. The AcOEt part (15 g) was separated and concentrated in vacuo to dryness and then resuspended in methanol (50 mL). The methanol extract was subjected to a SUPELCO Diaion HP-20 column, eluted with aqueous methanol gradient. The 90% methanol partition was collected and concentrated in vacuo. The residue was loaded onto a silica gel column and then eluted with a gradient of chloroform/methanol to yield four fractions. The chloroform/methanol 9:1 fraction was separated by repeated silica gel column chromatography to afford a colorless crystalline, maoecrystal Z (**1**) (8 mg, 0.0073‰).



Maoecrystal Z (**1**), $[\alpha]_D$ 18.5−119.05 (*c* 0.98, CH_3OH),[17] was isolated as colorless cubes ($Me_2CO-MeOH-H_2O$, 90:9:1). Its positive ESIMS showed a $[M + H]^+$ at *m*/*z* 391, a $[M + Na]^+$ at *m*/*z* 413, and a $[M + K]^+$ at *m*/*z* 429, respectively, corresponding to a molecular formula of $C_{22}H_{30}O_6$, as determined by HR-ESIMS ($[M + Na]^+$ found 413.1955, calcd 413.1935). The IR spectrum demonstrated absorptions at 1743, 1711, and 1689 cm^{-1}, indicating the existence of three carbonyl groups: lactone, acetoxyl ketone, and conjugated ketone, respectively. The ^1H and ^{13}C NMR (including DEPT) spectra (vide Table 1) showed characteristic signals of two tertiary methyl groups [δ_H 1.05 (3H, s) and 1.23 (3H, s), δ_C 34.8 (CH_3) and 21.6 (CH_3)], an α,β-unsaturated aldehyde moiety [δ_H 5.87 (1H, s), 6.21 (1H, s), 9.53 (1H, s); δ_C 134.0 (CH_2), 154.5 (C), 194.8 (C)], a δ-lactone residue [δ_H 4.13 (1H, ABd, J = 12.0 Hz), 4.65 (1H, ABd, J = 12.0 Hz); δ_C 72.1 (CH_2), 175.7 (C)], two oxymethines [δ_H 4.35 (1H, t, J = 7.0 Hz), 5.56 (1H, br s); δ_C 74.2 (CH), 68.6 (CH)], and an acetoxyl group [δ_H 2.03 (3H, s); δ_C 21.4 (CH_3), 170.1 (C)]. In addition, in the high-field region of the ^{13}C NMR and DEPT spectra, five methylenes, three methines, and three quaternary carbons were exhibited. These data were compared with those of previously isolated diterpenoids from this plant. The moieties of 6,7-*seco*-7,20-olide and 8,15-*seco*-16-en-15-aldehyde in an *ent*-kaurane skeleton were then considered for compound **1**. Further comparison of the NMR data of **1** with those of two known diterpenoids, laxiflorin G (**2**),[5] and rubescensin U (**3**),[6] confirmed its structural features of 6,7:8,15-di-*seco*-7,20-olide-*ent*-kaur-16-en-15-aldehyde. However, compound **1** is neither a normal 6,7-*seco*-7,20-olide-*ent*-kaurane nor a common 8,15-*seco*-*ent*-kaurane. Its C-6 is an oxymethine instead of an oxymethylene, which indicated that C-6 is

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(16) Colorless cube crystals of maoecrystal Z, crystallized from acetone/methanol/H_2O, belong to the monoclinic space group P21. Crystal data: $C_{22}H_{30}O_6$, M = 390.48, a = 6.4400(17) Å, b = 14.431(3) Å, c = 11.2310(18) Å, β = 90.988(9)°, V = 1043.6(4) $Å^3$, Z = 2, d = 1.243 g/cm^3, linear absorption coefficient μ = 0.893 cm^{-1}. A colorless cube of dimensions 0.06 × 0.04 × 0.02 mm was used for X-ray measurements on a Rigaku Saturn CCD area detector with graphite monochromated Mo Kα radiation. Of the 8051 reflections that were collected, 2609 were unique (R_{int} = 0.052); equivalent reflections were merged. Data were collected and processed using CrystalClear (Rigaku). The structure was solved by direct methods and expanded using Fourier techniques. All calculations were performed using the CrystalStructure crystallographic software package except for refinement, which was performed using SHELXL-97 (Sheldrick, G. M. University of Gottingen, Gottingen, Germany, 1997). Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition no. CCDC 609437). Copies of these data can be obtained, free of charge, on application to the CCDC via the Internet at www.ccdc.com.ac.uk/conts/retrieving.html (or 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44 1223 336033, e-mail: deposit@ccdc.cam.ac.uk).

(17) Maoecrystal Z (**1**): colorless cubes ($Me_2CO-MeOH-H_2O$, 90:9:1); $[\alpha]_D$ 18.5−119.05 (*c* 0.98, CH_3OH); positive ESIMS *m*/*z* (rel int) 391 ($[M + H]^+$), 413 ($[M + Na]^+$), 429 ($[M + K]^+$); HR-ESIMS found 413.1955, calcd 413.1935 for $C_{22}H_{30}O_6-Na$; IR (KBr) λ_{max} 3445, 2947, 1743, 1711, 1689, 1433, 1372, 1222, 1161, 1103, 1038 cm^{-1}.

Table 1. ^{1}H and ^{13}C NMR Assignments and Two-Dimensional NMR Correlations of **1**[a]

position	δ_H (mult)	δ_C (mult)	HMBC (H−C)	ROESY (H−H)
1α	1.61(br d, J = 13.2 Hz)	32.5 (CH_2)	2, 3, 5, 9, 10	11β, 20b
1β	1.12 (m)			5β, 9β
2α	1.37 (overlapped)	19.7 (CH_2)	1, 3, 4, 10	19, 20a
2β	1.37 (overlapped)			
3α	1.47 (m)	42.1 (CH_2)	1, 2, 4, 5, 18, 19	
3β	1.17 (m)			5β
4		33.2 (C)		
5β	1.65 (d, J = 7.0 Hz)	66.3 (CH)	1, 3, 4, 6, 9, 10, 18, 19	1β, 3β, 9β
6α	4.35 (t, J = 7.0 Hz)	74.2 (CH)	4, 5, 7, 9	19, 20a
7		175.7 (C)		
8		53.3 (C)		
9β	1.89 (s)	52.6 (CH)	1, 5, 6, 8, 10, 12, 20	1β, 5β, 12β, 14β
10		42.9 (C)		
11β	5.56 (br s)	68.6 (CH)	8, 13, OAc	1α, 20b
12α	2.22 (br d, J = 13.6 Hz)	36.5 (CH_2)	9, 11, 13	11β, 13α
12β	1.43 (m)			9β, 17a
13α	3.60 (br t, J = 12.4 Hz)	29.2 (CH)		12α, 14α, 17a
14α	2.73 (dd, J = 12.8, 1.6 Hz)	31.9 (CH_2)	6, 8, 9, 12, 13	13α
14β	2.05 (m)			9β, 17a
15	9.53 (s)	194.5 (CH)	13, 16, 17	17b
16		154.5 (C)		
17a	6.21 (s)	134.0 (CH_2)	13, 15, 16	12β, 13α, 14β
17b	5.87 (s)			15
18	1.23 (3H, s)	34.8 (CH_3)	3, 4, 5, 19	19
19	1.05 (3H, s)	21.6 (CH_3)	3, 4, 5, 18	2α, 6α, 18, 20a
20a	4.65 (d, J = 12. 0 Hz)	72.1 (CH_2)	1, 5, 9, 10	2α, 6α, 19
20b	4.13 (d, J = 12. 0 Hz)			1α, 11β
OAc	2.03 (3H, s)	21.4 (CH_3)	OAc	
		170.1 (C)		

[a] Data were recorded in C_5D_5N on Bruker AM-400 MHz (^{1}H, ^{13}C, and DEPT) and Bruker DRX-500 MHz spectrometers (HMBC, ROESY); chemical shifts (δ are given in parts per million with reference to the most downfield signal of C_5D_5N (δ 8.71 ppm) for ^{1}H and to the center peak of the most downfield signal of C_5D_5N (δ 149.9 ppm) for ^{13}C.

bonded to an adjacent carbon after the cleavage from C-7. Furthermore, compound **1** has three quaternary carbons, one more than those of common 8,15-*seco*-*ent*-kaurane. Therefore, to determine the structure of **1**, the key was to assign the C-6 bonding and the additional quaternary carbon.

In recent years, some 8,15-*seco*-*ent*-kauranes have been reported, in which C-15 was oxygenated to either carboxy group or aldehyde. In this case, C-15 was oxygenated to aldehyde. This could be proven by the HMBC correlations between the aldehyde proton/carbon and its coupled olefinic proton/carbon and a methine carbon/proton (C-13). Therefore, C-8 should be free. However, from the HMBC cross-peaks (see Table 1) between the extra quaternary carbon with a methine proton (H-9), a methylene proton (H-14), and an oxymethine proton (H-11), it can be concluded that the quaternary carbon was ascribable to C-8. This suggested that C-8 must be bonded carbon to carbon once again after cleaving from C-15. Thus, similar cleavages coupled with a new bonding might happen to both C-6 and C-8. A carbon to carbon bond between C-6 and C-8 was thereby deduced, which was supported by the HMBC couplings between H-6 and C-7/C-9, H-9/H-14, and C-6. This led to a completely unique skeleton: 6,7:8,15-di-*seco*-7,20-olide-6,8-cyclo-*ent*-kaurane. A nor-diterpene previously reported from the same plant resource, maoecrystal V (**4**), 16(*R*)-methyl-1,15-dioxo-6,7-*seco*-6-*nor*-15(8→9)-*abeo*-5,8-epoxy-*ent*-kaur-2-en-7,20-oilde, had almost the same skeleton as that of compound **1**, except for the key C-6. In the known analogue, C-6 was cut out and replaced by an oxygen atom. An oxygen bridge (C-5-O−C-8) was assigned as its unique structural feature. Biogenetically, it was possible for C-6 to be linked with C-8 after the cleavages at C-6/C-7 and C-8/C-15. This assignment unequivocally explained all the correlations observed in the HMBC spectrum. The acetoxy group was located at C-11 according to HMBC coupling between H-11 and the carbonyl carbon. However, the direct HMBC evidences for 6,8-*cyclo* moiety, e.g., the correlations of H-5/C-8 and H-6/C-8, were unfortunately not observed. To confirm compound **1** is such a novel diterpene with a completely unique skeleton, further solid evidence like X-ray diffraction was necessary.

After many attempts with different solvents, a single crystal of **1** was finally obtained from Me_2CO−MeOH−H_2O (90:9:1) and then analyzed by an X-ray diffraction experiment (Figure 1).[16] The result confirmed that **1** possessed the above-deduced 6,7:8,15-di-*seco*-7,20-olide-6,8-cyclo-*ent*-kaurane skeleton. The relative stereochemistry of **1** was also determined by X-ray analysis. The α-orientation of H-6 was in agreement with the ROESY correlation between H-6 and Me-19, H-20 (Table 1). The α-oriented acetoxyl at C-11 was also confirmed by the NOE between H-1α and H-11.

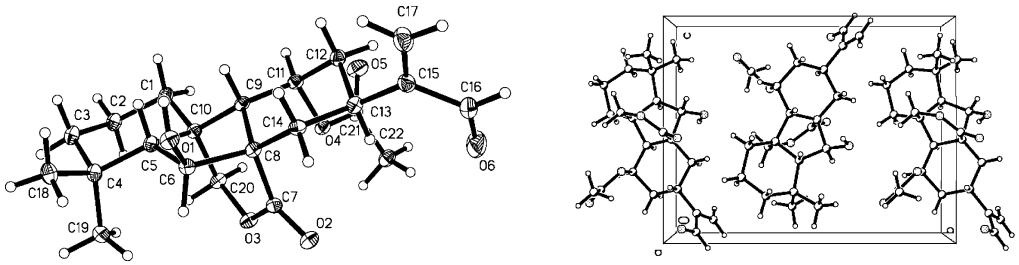
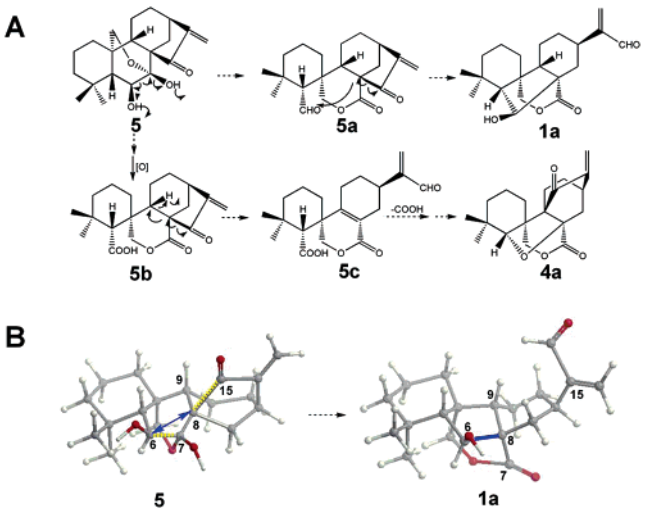


Figure 1. X-ray crystallographic structure of maoecrystal Z (**1**).

Although the absolute configuration of **1** was not individually determined, compound **1** was supposed to have the *ent*-configuration due to its close biogenetical relationship with those known *ent*-kauranoids from the same herb resources which absolute configuration had been determined before.[18] Consequently, compound **1** was determined as 6β-hydroxy-11α-acetoxy-6,7:8,15-di-*seco*-7,20-olide-6,8-cyclo-*ent*-kaur-16-en-15-aldehyde and was given the common name maoecrystal Z.[15]

An in vivo biosynthesis study of **1** from a commonly occurring 7,20-epoxy-*ent*-kaurane (**5**) would allow us to better understand the structural evolution among *ent*-kaurane diterpenoids of *Isodon* plants. A biogenetic pathway is proposed for the skeleton of **1** and 4 (Scheme 1). The

Scheme 1



cleavage at C-6/C-7 in **5** results in the form of **5a**. Further cleavage at C-8/C-15 and rearrangement of **5a** produces the novel skeleton **1a** (Scheme 1A). These cleavages have been proved by some oxygenation reactions.[18] At the same time, it is also possible for **1a** that these cleavages happen simultaneously, since the β-orientation of the hydroxy group at C-6 remains identical to that of **5** (Scheme 1B). This reaction might be biochemically generated and enzymatically catalyzed, which is another interesting topic to be investigated. The recently reported nor-diterpenoid, maoecrystal V (**2**), seems have a similar biosynthesis pathway. If the aldehyde group of **5a** was further oxygenated to a carboxy group (**5b**), the consequent decarboxylation and rearrangement might lead to the formation of **4a**, the skeleton of compound **2**. To our knowledge, compound **1** is by far one of the most modified naturally occurring *ent*-kauranoids from *Isodon* species.

Compound **1** was evaluated for its cytotoxicities against three human tumor cell lines, K562 leukemia, MCF7 breast, and A2780 ovarian, using the same bioassay method as previously described.[19] The bioassay results presented in Table 2 showed that compound **1** exhibited comparable inhibitory activity, with IC_{50} in the low μg/mL range, against all the human tumor cells. The bioassay data are presented in Table 2.

Table 2. Cytotoxicity of Compound **1**[a]

	IC_{50}		
cells	**1** (μg/mL)	camptothecin (μM)	paclitaxel (nM)
K562	2.90 ± 0.28	0.26 ± 0.05	
MCF7	1.63 ± 0.34		2.87 ± 0.22
A2780	1.45 ± 0.03		4.52 ± 0.51

[a] Data are presented as mean ± sem of three independent experiments).

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Supporting Information Available: 1D and 2D NMR spectra of maoecrystal Z (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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