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Maoecrystal V, Cytotoxic Diterpenoid with a Novel C_{19} Skeleton from *Isodon eriocalyx* (Dunn.) Hara

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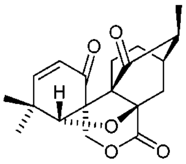
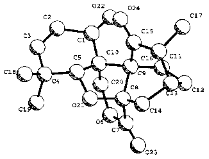
ABSTRACT



Maoecrystal V (**1**)



Maoecrystal V (1), a novel C_{19} diterpenoid possessing a unique 6,7-*seco*-6-*nor*-15(8→9)-*abeo*-5,8-epoxy-*ent*-kaurane skeleton, was isolated from the leaves of a Chinese medicinal herb, *Isodon eriocalyx*. Its structure was determined by comprehensive NMR and MS spectroscopic analysis and confirmed by single-crystal X-ray diffraction study. Compound 1 showed remarkable inhibitory activity toward HeLa cells with $IC_{50} = 0.02\ \mu g/mL$ (*cis*-platin: $IC_{50} = 0.99\ \mu g/mL$).

Since 1976, in the continuous search for bioactive constituents from the abundant diterpenoids (mainly *ent*-kauranoids) from the genus *Isodon* (= *Rabdosia*) plants of the Labiatae family, our group has photochemically investigated more than 50 *Isodon* species distributed in China. About 400 new diterpenoids with a diversity of highly oxygenated structures have been isolated and characterized, most of which have been recently summarized.[1] A number of these isolated diterpenoids were found to have potent antitumor activities with very low toxicity. They are therefore promising candidates of anticancer agents being studied in our laboratory. Another achievement of our studies is the discovery of many interesting novel compounds 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] 6,7:8,15-*seco*-*ent*-kauranoids (laxiflorins F and G),[4] 15,16-*seco*-*ent*-kauranoid (rubescensin S),[5] symmetric and asymmetric *ent*-kauranoid dimers (maoecrystal M,[6] enanderinanin J,[7] xingdongnins M−O[8]), and novel *ent*-abietanoids (laxiflorins N−O,[9] micranthin C[10]).

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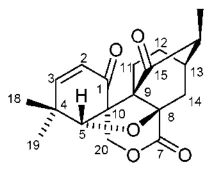
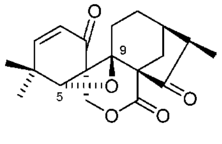
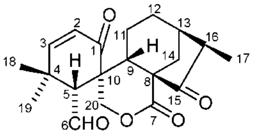
Isodon eriocalyx (Dunn.) Hara, a perennial herb or shrub widely distributed in southwestern China, has long been used as a folk medicine for the treatment of sore throat, inflammation, influenza, hypertension, and dermatophytosis.[11] As a representative *Isodon* species being rich in *ent*-kauranoids of different oxygenation and cleavage patterns, *I. eriocalyx* has been intensively investigated, and so far about 50, including over 30 new *ent*-kauranoids, have been isolated and identified.[1] About 10 years ago, we studied the leaves of this plant collected from Jiangchuan prefecture of Yunnan province, which led to the isolation of a number of new diterpenoids.[12−14] Among them, compound **1**, named maoecrystal V, was not published due to the failure to obtain a suitable single crystal for X-ray crystallographic analysis (mainly because of the limited amount) to verify its unprecedented backbone, which had been established according to its NMR and MS spectral data. Recently, a single crystal of compound **1** was achieved and the X-ray diffraction experiment was successfully performed, which unveiled the uncommon structure of the compound. In addition, compound **1** was tested for its cytotoxic activity against five human tumor cell lines (K562, A549, BGC-823, CNE, HeLa). Described in this paper are the structure elucidation and cytotoxicity of compound **1**.



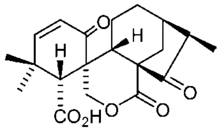
1



1a



Epi-eriocalyxin A (2)



2a

Leaves of *I. eriocalyx* (Dunn.) Hara were collected from Jiangchuan Prefecture of Yunnan Province, China, on September 10, 1994, and identified by Prof. H. W. Li. Dried powdered leaves (11.9 kg) were extracted with CH_3OH (4 × 40 L) under reflux and then concentrated in vacuo to give a crude extract (978 g), which was suspended in water and successively partitioned with petroleum ether (60−90 °C) and AcOEt. The AcOEt part (395 g) was chromatographed on a silica gel column (1.6 kg) eluting with gradient $CHCl_3$ in Me_2CO to yield seven fractions (I−VII). Fraction II was decolored with an MCI-gel CHP-20P column (90% aqueous CH_3OH as the eluent) and subjected to further silica gel column chromatography to afford a colorless crystalline, maoecrystal V (**1**) (5 mg).

Maoecrystal V (**1**), $[\alpha]_D^{23.6}$ −92.9° (*c* 0.70, CH_3OH), was isolated as colorless needles ($Me_2CO-MeOH-H_2O$, 95:4:1). Its EIMS showed a molecular ion peak at *m/z* 330 ([M]+), and positive ESIMS displayed $[M + Na]^+$ at *m/z* 353,[15] corresponding to a molecular formula of $C_{19}H_{22}O_5$, as determined by HR-ESIMS (found 353.1359, calcd 353.1364). The IR spectrum[15] demonstrated absorptions at 1754, 1720, and 1684 cm^{-1}, indicating the existence of three carbonyl groups for lactone, saturated ketone, and conjugated ketone, respectively. Observed in the ^1H and ^{13}C NMR (including DEPT) spectra (vide Table 1) were two tertiary and a secondary methyl group [δ_H 1.04 (3H, s) and 1.21 (3H, s), 1.09 (3H, d, $J = 7.3$ Hz); δ_C 30.4 (q), 18.4 (q) and 15.0 (q)], an α,β-unsaturated ketone moiety [δ_H 5.99 (1H, ABd, $J = 10.3$ Hz), 6.54 (1H, ABd, $J = 10.3$ Hz); δ_C 127.2 (d), 156.7 (d), 194.8 (s)], a δ-lactone residue [δ_H 4.73 (1H, ABd, $J = 12.3$ Hz), 4.32 (1H, ABd, $J = 12.3$ Hz); δ_C 69.5 (t), 169.5 (s)], an oxymethine [δ_H 4.66 (1H, s); δ_C 85.5 (d)], an oxygen-containing quaternary carbon [δ_C 84.6 (s)], and an isolated ketone [δ_C 211.7 (s)]. In addition, the high-field region of the ^{13}C NMR and DEPT spectra exhibited three methylenes, two methines, and three quaternary carbons. Comparison of all these spectral data with those of previously isolated diterpenoids[1] from this plant suggested that compound **1** is a norditerpenoid that shares structural features with the known *ent*-kaurane diterpenoid, epi-eriocalyxin A (**2**).[12] Considering that C-6 of this class of diterpenoids was easily oxidated from aldehyde to carboxylic acid (e.g., maoecrystals N and O[12]), compound **1** was presumably derived from **2** through oxidation (**2a**) and then decarboxylation at C-6. Further comparison of the NMR spectral data of **1** with those of **2** revealed that **1** still differed from **2** by the obvious replacements of two methines by an oxymethine and an oxyquaternary carbon, respectively.

From the HMBC cross-peaks (see Table 1) between the oxymethine proton/carbon and two geminated methyl carbons/protons (Me-18 and Me-19) and similar interactions between the former and the oxymethylene carbon/protons (C-20/H_2-20), it can be concluded that the oxymethine was ascribable to C-5. Biogenetically, C-9 of *ent*-kauranoids can be oxygenated from a methine to an oxyquaternary carbon, which was also plausible in **1** from the $^1H-^{13}C$ long-range correlations between H_2-11 and H_2-14 and the oxyquaternary carbon in

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(15) Maoecrystal V (**1**): colorless needles (from acetone); mp 248 °C; $[\alpha]_D^{23.6} = -92.9°$ (*c* 0.70, CH_3OH); EIMS *m/z* (rel int) 330 (39, $[M]^+$), 315 (20), 302 (100), 287 (6), 272 (30), 257 (59), 244 (28), 229 (54), 215 (24), 199 (20), 189 (24), 175 (16), 161 (20), 147 (20), 133 (23), 115 (22), 105 (31), 96 (54), 91 (61), 77 (52), 69 (54), 55 (65); positive ESIMS *m/z* (rel int) 353 (100, $[M+Na]^+$); HR-ESIMS found 353.1359, calcd for $C_{19}H_{22}O_5$-Na 353.1364; UV (MeOH) λ_{max} (log ϵ): 225.2 (3.9) nm; IR (KBr) ν_{max} 2964, 2928, 1754, 1720, 1684, 1176, 1129 cm^{-1}.

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

position	δ_H (mult, J, Hz)	δ_C (mult)	COSY (H−H)	HMBC (H−C)	ROESY (H−H)
1		194.8 (s)			
2	5.99 (ABd, 10.3)	127.2 (d)	3	4, 10	3
3	6.54 (ABd, 10.3)	156.7 (d)	2	1, 4, 5, 18	2
4		38.3 (s)			
5β	4.66 (s)	85.5 (d)		4, 9, 18, 19, 20	18
7		169.5 (s)			
8		84.6 (s)			
9		56.9 (s)			
10		52.4 (s)			
11	2.14 (2H, m)	18.7 (t)	12α,12β	8, 9, 12, 15	20a, 20b
12α	1.48 (m)	18.2 (t)	11, 12β	11, 16	12β, 13α
12β	1.73 (m)		11, 12α, 13α		12α
13α	1.88 (m)	32.9 (d)	12β, 14β, 16α		14β, 16α
14α	1.77 (d, 14.1)	34.9 (t)	14β	7, 8, 12, 13, 16	14β
14β	3.28 (dd, 4.8, 14.1)		13α, 14α	7, 8, 9, 13, 16	13α, 14α
15		211.7 (s)			
16α	2.31 (m)	48.3 (d)	13α, 17	12, 15, 17	13α
17	1.09 (3H, d, 7.3)	15.0 (q)	16α	13, 15, 16	
18	1.04 (3H, s)	30.4 (q)		3, 4, 5, 19	
19	1.21 (3H, s)	18.4 (q)		3, 4, 5, 18	
20a	4.73 (ABd, 12.3)	69.5 (t)	20b	1, 5, 7, 9, 10	11
20b	4.32 (ABd, 12.3)		20a	1, 5, 7, 9, 10	11

[a] Data were recorded in C_5D_5N on Bruker AM-400 MHz (^{1}H, ^{13}C) and Bruker DRX-500 MHz spectrometers (COSY, HMBC, REOSY); chemical shifts (δ) are given in parts per million with references 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.

its HMBC spectrum. Accordingly, the oxyquaternary carbon in **1** was originally assigned to C-9. The molecular formula of **1**, corresponding to 9 double-bond equivalents, implied the existence of an oxygen bridge between C-5 and C-9 in **1**. Thus, the structure of **1** was originally proposed as **1a**. However, in the course of full assignments of all proton and carbon resonances of **1**, HMBC interactions between H_2-11 and C-15, instead of between H_2-14 and C-15, were unexpectedly observed, which suggested a migrated C-9−C-15 connection rather than the normal C-8−C-15 connection in **1**. Meanwhile, the aforementioned oxygen bridge (C-5−O−C-9) also was reassigned to C-5−O−C-8. Compound **1** should therefore have a rearranged 15(8→9)-*abeo-ent*-kaurane skeleton. This adjustment unequivocally explained all the correlations observed in the HMBC spectrum. Because **1** is a novel diterpenoid possessing such a completely unique skeleton, further solid evidence such as X-ray diffraction or chemical transformation was necessary.

Fortunately, after many attempts with different solvents, a single crystal of compound **1** was finally obtained from Me_2CO−MeOH−H_2O (95:4:1) and an X-ray diffraction experiment (Figure 1)[16] was realized, which confirmed that

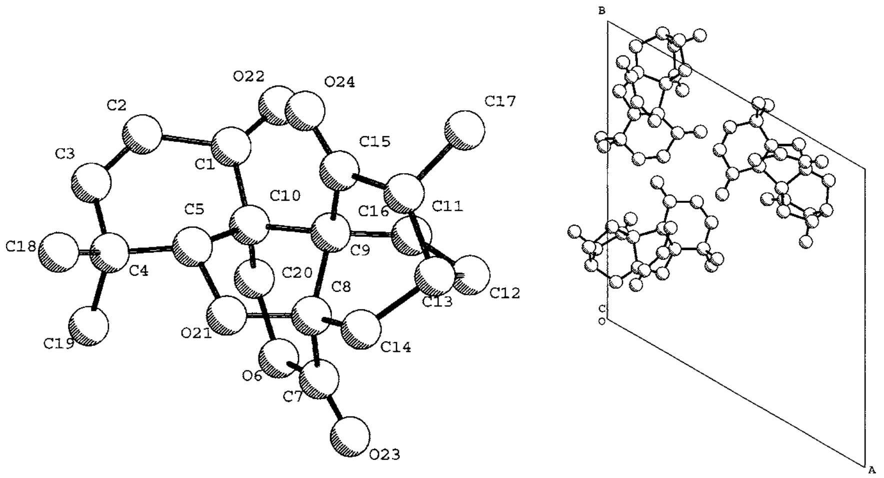
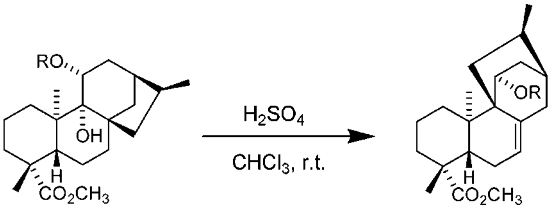


Figure 1. X-ray crystallographic structure of maoecrystal V (**1**).

1 has the above-deduced 6,7-*seco*-6-*nor*-15(8→9)-*abeo*-5,8-epoxy-*ent*-kaurane skeleton. The relative stereochemistry of **1** was also established by X-ray analysis. The β-orientation of H-5 was in agreement with the ROESY correlation (Table 1) between H-5 and Me-18. The β-oriented Me-17 causes an upfield shift for C-12 in comparison to those compounds with an α-oriented Me-17 (from ca. δ_C 30 to δ_C 18.2), due to the γ-steric compression effect between Me-17 and H-12β.[12] Consequently, compound **1** was determined as 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 and given the trivial name maoecrystal V.

Two 15(8→9)-*abeo*-*ent*-kauranoids, **5** and **6** (compounds **2** and **11** in the literature),[17] were synthesized through acid-catalyzed rearrangement of the 9α-hydroxy-11α-acetoxy and 9α,11α-dihydroxy derivatives of methyl (−)-kaur-9(11)-en-19-oate (**3** and **4**), in which the two oxy-substituents at C-9 and C-11 were indispensable for the reaction (Scheme 1). Since **1** could reasonably be argued to derive from **2**, such an acid-catalyzed rearrangement could not be realized due to the lack of any oxy substituent at C-9 and C-11 in **2**. Thus, this reaction might be biochemically generated and enzyme-catalyzed, which is another interesting topic to be investigated. An in vivo biosynthesis study of **1** from **2** would allow us to better understand the structural evolution among *ent*-kaurane diterpenoids of *Isodon* plants. Compound **1** is by far the most modified naturally occurring *ent*-kauranoid from *Isodon* species.

Scheme 1



3 R = Ac
4 R = H

5: R = Ac (96% yield)
6: R = H (8% yield)

Compound **1** was evaluated for its cytotoxicity toward five human tumor cell lines, viz., K562, A549, BGC-823, CNE, and HeLa, using the same bioassay method as previously described.[18] Interestingly, **1** only showed remarkable inhibitory activity against HeLa cells with $IC_{50} = 0.02$ μg/mL (*cis*-platin: $IC_{50} = 0.99$ μg/mL). In contrast, **1** was almost noncytotoxic toward the other four cell lines, indicating that its cytotoxicity is highly selective (only exhibiting potent cytotoxicity against gynecologic cancer cells) (Table 2), which is warrant for further investigation.

Table 2. Cytotoxicity of Compound **1**

	IC_{50} (μg/mL)				
test substance	K562	A549	BGC-823	CNE	HeLa
1	6.43×10^4	2.63×10^5	1.47×10^4	nd[a]	0.02
cis-platin	0.38	1.61	0.25	2.31	0.99

[a] Not determined.

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Supporting Information Available: One- and Two-dimensional NMR spectra of maoecrystal V (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Crystal data: $C_{19}H_{22}O_5$, $M = 330.38$, trigonal system, space group $P3_1$, $a = b = 15.409$ (1), $c = 6.605$ (1) Å, $\gamma = 120°$, $V = 1358.2$ (2) Å^3, $Z = 3$, $d = 1.216$ g/cm^3. A crystal of dimensions 0.05 × 0.10 × 0.20 mm was used for measurements on a MAC DIP-2030K diffractometer with a graphite monochromator (ω-2θ scans, $2\theta_{max} = 50.0°$), Mo Kα radiation. The total number of independent reflections measured was 1571, of which 1569 were observed ($|F|^2 \geq 8\sigma|F|^2$). The crystal structure was solved by the direct method SHELX-86 (Sheldrick, G. M. University of Gottingen: Gottingen, Germany, 1985) and expanded using difference Fourier techniques, refined by the program and method NOMCSDP (Lu, Y.; Wu, B. M. *Chin. Chem. Lett.* **1992**, *3*, 637−640) and full-matrix least-squares calculations. Final indices: $R_f = 0.081$, $R_w = 0.099$ ($w = 1/\sigma|F|^2$). Crystallographic data for the structure of **2** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 249099). 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).

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