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Diterpenoids from *Isodon eriocalyx*

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Two new diterpenoids, Maoecrystal U and *epi*-maoecrystal P, together with nine known diterpenoids and eight other known compounds were isolated from *Isodon eriocalyx* (). The structures of two new diterpenoids were elucidated as 6 β -acetoxy-15 β -hydroxy-3 α ,20-epoxy-*ent*-kaur-16-ene-1,7-dione and 16(S)-methoxymethy-6 β ,7 β -dihydroxy-7 α ,20-epoxy-*ent*-kaur-2,3-ethenylene-1,15-dione on the basis of spectroscopic evidence, especially by 2D NMR techniques.

INTRODUCTION

Isodon eriocalyx (Dunn) Hara (*Labiatae*) () is widely distributed in Yunnan Province, P.R. China.¹ It has long been used as folk medicine to treat sore throat and inflammation² as well as to reduce blood pressure.³ A series of new *ent*-kaurene diterpenoids have been isolated from *I. eriocalyx* collected in different regions of Yunnan Province.^{4,5,7-10} In order to find more efficient biological substances, *I. eriocalyx*, collected in Zhongdian Prefecture of Yunnan Province, was subjected to phytochemical investigations.

The aerial parts were extracted with ethanol. Then the ethanolic extract was chromatographed on a silica gel column as described in the experimental section. Nineteen compounds were obtained and their structures were determined by physical and spectral data.

RESULTS AND DISCUSSION

Compound **1** was obtained from acetone as colorless plates or hexahedral crystals. Its molecular formula was established as C₂₂H₂₈O₆ by EI MS (ion at *m/z* 388, M⁺) and ¹³C NMR (including DEPT). An examination of its physical and spectral characteristics revealed it to be a new diterpenoid which was named Maoecrystal U. It exhibited absorption due to secondary hydroxyl (3494, 1261 cm⁻¹), acetoxy (1737 cm⁻¹), saturated six-membered ketone (1737 cm⁻¹), and alkylene (1666 cm⁻¹) functions in its IR spectrum. It showed the absence of conjugated functionality because of no UV absorption.

In the ¹H NMR spectrum, the C₂₀-methylene appeared as two AB doublets at δ 4.67 and 4.09 (*J* = 9.9 Hz). Two broad peaks at δ 5.16 and 4.98 (*J* = 2.5 Hz), which indicated that

there was an double bond in the molecule, belonged to C₁₇-methylene. The two methyls appeared as singlets at δ 1.23 and 1.02. The proton of 6 α -acetoxy appeared at δ 5.53 (d, *J* = 12.6 Hz). The ¹³C NMR spectrum exhibited two six-membered-ring ketone peaks at δ 207.9 and 205.3, an acetoxy carbonyl signal at δ 169.6, and double bond carbons at δ 152.6 and 107.1. All of these pointed out that compound **1** was kaurene type.

In careful comparison ¹H NMR and ¹³C NMR data of **1** with those of maoecrystal **3**⁴, it indicated that **1** had the same skeleton as that of **3**: 3 α ,20 α -epoxy-*ent*-kaur-16-ene-1,7-dione (Table 1).

Because **1** and **3** had the same formula C₂₂H₂₈O₆ and both had hydroxyl and acetoxy groups, we concluded that the only difference between **1** and **3** was the sites of hydroxyl and acetoxy groups.

By analyzing the HMBC of **1** carefully, we found that H-6 and carbonyl of acetoxy group had correlations, which showed that the acetoxy group was at C-6. In addition, the proton δ_{H} 4.81 (1H, t, *J* = 2.5 Hz) had correlations with C-7, C-9, C-16, C-17 in HMBC, which indicated that the proton was H-15, so the hydroxyl was at C-15.

The relative configurations of substitutes were confirmed from the NOESY. In NOESY, there were the NOE correlations between H-6 α and H-19 α , H-15 α and H-14 β ; all of these can establish 6-OAc and 15-OH as β -orientation. Therefore, the structure of **1** was deduced as 6 β -acetoxy-15-hydroxy-3 α ,20 α -epoxy-16-ene-*ent*-kaur-1,7-dione.

Compound **2** was obtained from acetone as colorless needles, which had a molecular formula of C₂₁H₂₈O₆ deduced from EI MS (ion at *m/z* 376) and ¹³C NMR (including DEPT). From its physical and spectral characteristics, it was also recognized as a new diterpenoid, named as *epi*-maoecrystal P. Its IR spectrum showed peaks at 3332, 1350-1110 cm⁻¹ (hydroxyls), 1716 cm⁻¹ (ketone), and 1663 cm⁻¹ (α,β -unsaturated

Table 1. ^1H , ^{13}C NMR (comparing with **3**, **20** respectively) Data of Compounds **1** and **2**^a

Proton No.	Compounds		Carbon No.	Compounds			
	1	2		1	3	2	20
2	—	5.87d (10.1)	1	207.6s	210.2s	196.8s	197.3s
2 α	2.75dd (10.8, 4.1)	—	2	41.6t	42.1t	127.1d	127.4d
2 β	2.69dd (10.8, 4.1)	—	3	76.8d	77.3d	161.2d	160.6d
3	—	6.68d (10.1)	4	37.5s	38.1s	35.8s	36.2s
3 β	3.65s	—	5	47.9d	51.5d	57.0d	59.3d
5 β	1.86d (12.6)	2.00d (8.8)	6	73.5d	71.9d	73.0d	73.6d
6 α	5.53d (12.6)	3.93d (8.8)	7	205.3s	208.8s	95.3s	96.3s
9 β	2.70s	1.82dd (4.8, 13.4)	8	57.0s	56.6s	60.2s	61.1s
11	1.28m	2.29m	9	33.5d	35.0d	48.1d	48.1d
11'	1.31m	2.26m	10	51.1s	51.8s	46.4s	46.3s
12	1.43m	2.15m	11	20.4t	20.8t	19.1t	19.3t
12'	1.47m	2.18m	12	32.7t	32.8t	29.5t	20.0t
13 α	2.89d (8.0)	1.15d (8.2)	13	38.8d	40.2d	30.0d	29.7d
14 α	1.73d (11.8)	1.28dd (8.2, 4.3)	14	34.6t	35.9t	25.2t	28.1t
14 β	1.60m	1.36dd (8.2, 4.3)	15	74.6d	74.8d	221.2s	222.5s
15 α	4.81m	—	16	152.6s	151.6s	58.4d	56.6d
16 β	—	2.40s	17	107.1t	108.3t	71.7t	69.0t
17a	5.16s	3.50dd (4.4, 9.4)	18	29.0q	29.5q	29.9q	30.3q
17b	4.98d (2.7)	3.44d (9.4)	19	22.9q	23.1q	24.6q	34.2q
18	1.03s	1.23s	20	62.0t	62.4t	65.5t	65.6t
19	1.21s	1.37s	OAc	169.6s	170.3s	—	—
20 α	4.67d (9.9)	4.28d (10.1)	CH ₃	20.6q	21.0q	—	—
20 β	4.08d (9.9)	3.99d (10.1)	OCH ₃	—	—	59.0q	58.6q
OCH ₃	—	3.33s					
CH ₃							
OAc	2.15s						

^a Chemical shifts (ppm), multiplicity, and coupling constants (Hz in parentheses).**1**, **2**, **3** in CDCl₃; **20** in C₅D₅N.

ketone, which was also supported by the strong UV absorption at 241 nm). A couple of AB doublets at δ 4.28 and 3.99 (d, J = 10.1 Hz) in the ^1H NMR were the feature of C₂₀-methylene. The two methyls at δ 1.37(s) and 1.23(s) suggested that they were connected with C-4. Proton H-6 α appeared at δ 3.93 (dd, J = 8.8, 11.9 Hz) obviously. By the analysis of ^{13}C NMR data, an oxidized carbon (C-20) formed hemi-acetal with C-7. All the facts above revealed that **2** closely resembled maoecrystal **P** (compound **20**),⁵ differing only in the downfield shift of C-12 (δ 29.5) data (Table 1).

The big downfield shift of C-12 (δ 29.5) of compound **1** could only be explained by the absence of γ -steric compression effect of 16 β -CH₂OCH₃ and 12 β -H. In addition, there was significant NOE correlation between H-16 with H-12 β , which indicated the H-16 was β -oriented, so the C-16 was α -orientation. Thus, **2** was elucidated as 16(S)-methoxy-methy-6 β ,7 β -dihydroxy-7 α ,20-epoxy-*ent*-kaur-2,3-ethenylene-1,15-dione.

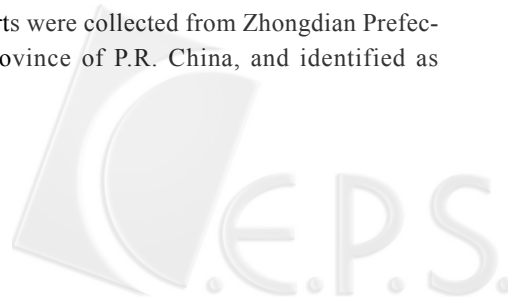
EXPERIMENTAL SECTION

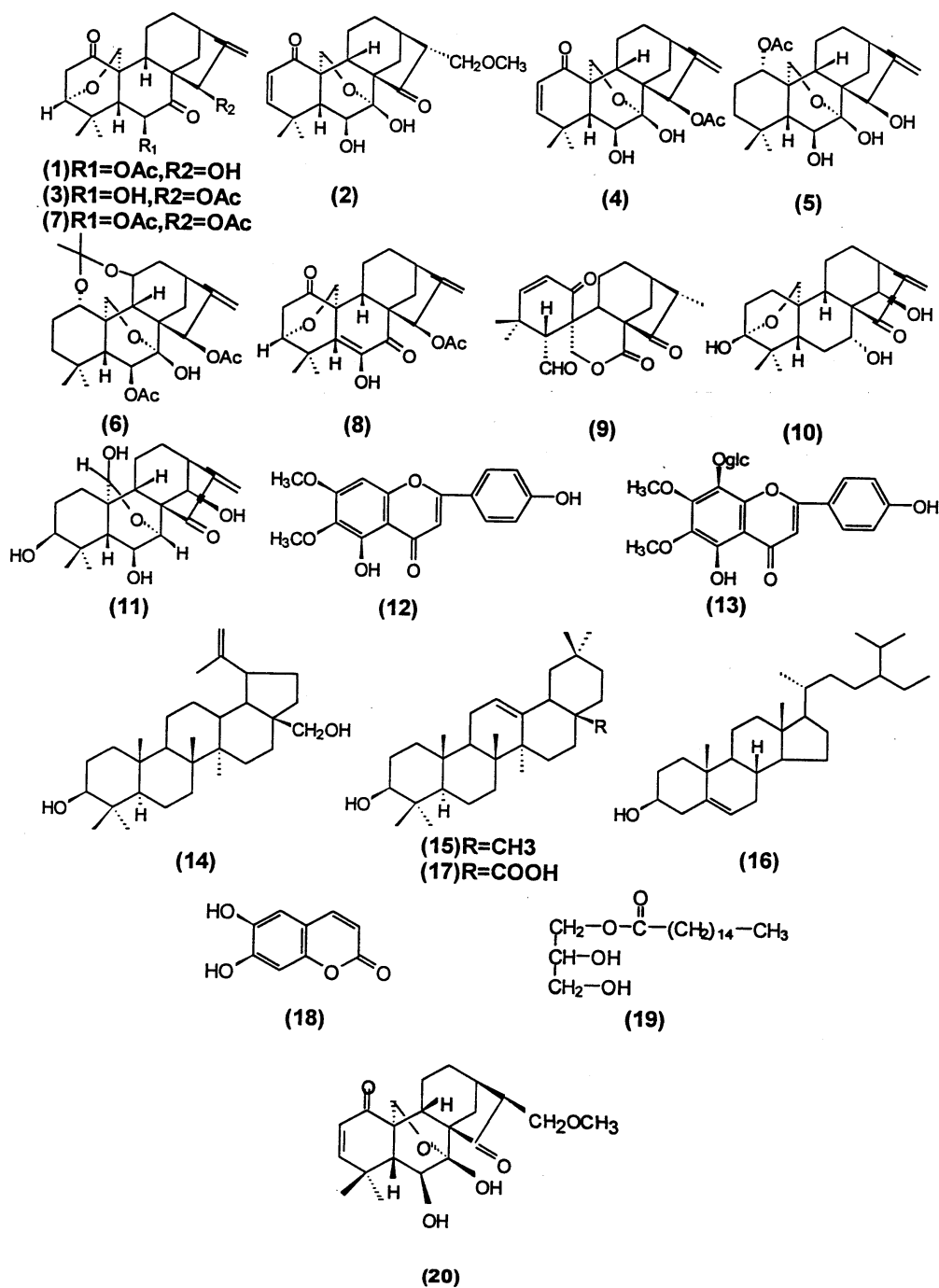
Instrumental

Melting points were determined on XRC-1 micro-melting-point spectroscopy and were uncorrected. CD spectra were measured on a JASCO-20 spectrophotometer. IR spectra were recorded on a Bio-Rad FTS spectrometer and UV spectra on an UV-210A spectrometer. ^1H - and ^{13}C - NMR spectra were recorded on a Bruker AM-400 MHz or a Bruker AM-500 MHz, using TMS as internal standard, and MS spectra on a VG Auto Spec-3000 spectrometer. Acme's silica gel G, produced by the Oceanic and Chemical Plant in Qingdao, was used for TLC, and silica gel (200-300 mesh), from the same source for column chromatography.

Plant Material

The aerial parts were collected from Zhongdian Prefecture of Yunnan Province of P.R. China, and identified as





Isodon eriocalyx by Professor Zhong-Wen Lin, Kunming Institute of Botany, Academia Sinica, P.R. China. A voucher specimen (No. 9706) was deposited at the Herbarium in Kunming Institute of Academia Sinica, Kunming 650204, P.R. China.

Extraction and Isolation

The dried aerial parts of *I. eriocalyx* (2.65 kg) were extracted with industrial ethanol under reflux ($3 \times 7L$) for three hours each time. After concentration of the combined extracts

under reduced pressure, the residue (540 g) was diluted with hot water and the water-insoluble material removed by filtration through celite. The filtrate (450 g) was extracted with petroleum ether (60-90 °C), ethyl acetate and n-butanol.

The ethyl acetate extract (135 g) was chromatographed on a silica gel column (6.5×150 cm, 200-300 mesh, 600 g) and eluted with petroleum ether- $CHCl_3$ (2:1, 2000-15000 mL), $CHCl_3$ (15000-18000 mL), and $CHCl_3$ -acetone (6:1, 18000-24000 mL) respectively, to yield three fractions, P1 (35 g), P2 (45 g) and P3 (50 g). The fraction P1 was further sepa-

rated by column chromatography on silica gel (200-300 mesh, 300 g) and eluted with ether-CHCl₃ (2:1-2:3, 1000 mL each elute) **6**⁵ (6 mg), **16**⁶ (1 g), **19** (2.5 g), **3**⁴ (26.5 g), **7**⁷ (180 mg) were obtained, fraction P2 was further separated by column chromatography on silica gel (200-300 mesh, 400 g) and eluted with CHCl₃-acetone (7:1, 4:1, 1:1, 1000 mL each elute), three fractions were obtained. Fraction 1 (11 g, CHCl₃-acetone 7:1) was subjected to column chromatography on silica gel (200-300 mesh, 100 g) and eluted with CHCl₃-acetone (9:1, 8:1, 500 mL each elute), **1** (90 mg), **2** (36 mg), **4**⁴ (38 mg), **5**⁴ (200 mg), **8**⁸ (180 mg), **9**⁹ (1.6 g), **10**¹⁰ (300 mg), **11**¹⁰ (1 g) were obtained. Fraction 2 (13 g, CHCl₃-acetone 4:1) was purified on the silica gel column (200-300 mesh, 100 g) and eluted with CHCl₃-acetone (3:1, 500 mL), obtaining **13**¹¹ (14 mg), **14**¹² (260 mg) and **17** (2 g). Fraction 3 (8 g, CHCl₃-acetone 2:1) was chromatographed on silica gel column (200-300 mesh, 90 g) and eluted with CHCl₃-acetone **12**¹³ (20 mg) and **15**¹⁴ (300 mg). Fraction P3 was subjected to column chromatography on silica gel (200-300 mesh, 300 g) and eluted with CHCl₃-methanol (15:1, 1000 mL), and **18**¹⁵ (7 mg) was obtained.

The known compounds **3-19** were identified by comparing either their corresponding properties (m.p., MS, IR, ¹H and ¹³C NMR) with values in the literature or authentic samples.

Compound **1**: colorless plate or hexahedral crystals; m.p. 250-252.5 °C; [α]_D¹⁸ = -112.9° (c 0.62, CHCl₃); IR γ_{\max} (KBr) cm⁻¹: 3494, 2955, 1737, 1666, 1391, 1261, 1068; EIMS (CHCl₃) *m/z* (rel. int. %): 388 [M]⁺ (65), 370 [M-H₂O]⁺ (4.5), 360 (23), 346 [M-Ac]⁺ (9), 328 [M-OAc]⁺ (56), 318 (14), 300 (96), 258 (67), 243 (42), 231 (100), 229 (94), 215 (99), 201 (37), 185 (48), 175 (33), 159 (31), 145 (39), 133 (28), 17 (28), 105 (44), 91 (71).

Compound **2**: colorless needles; m.p. 222-224.5 °C; [α]_D²⁵ = -14.2 °C (c 0.60, CHCl₃); UV λ_{\max} (CHCl₃): 241 nm (log ϵ 3.5); IR: 3332, 2934, 1716, 1663, 1502, 1377, 1308, 1265, 1219, 1172, 1094; FABMS (FAB⁺) (CHCl₃) *m/z* (rel. int. %): 377 [M+H]⁺ (100), 359 [M-H₂O]⁺ (56), 327 (2), 99 (13), 83 (27).

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Key Words

Isodon eriocalyx; Diterpenoids; Kauran-type; Maoecrystal U; *epi*-Maoecrystal P.

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