Diterpenoids from *Isodon eriocalyx*†

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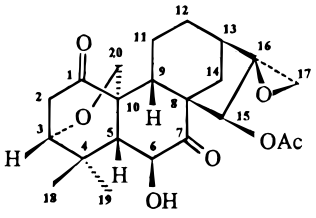
Three new diterpenoids, eriocalyxins C−E (**1**−**3**), were isolated from *Isodon eriocalyx*. Their structures were elucidated as 6*β*-hydroxy-15*β*-acetoxy-3α,20-epoxy-16*β*,17-epoxy-*ent*-kaur-1,7-dione (**1**), 1α,7*β*-dihydroxy-6*β*,15*β*-diacetoxy-7,20-epoxy-*ent*-kaur-16-ene (**2**), and 15*β*-acetoxy-1,6-dioxo-6,7-*seco*-*ent*-kaur-2,16-dien-7,20-olide (**3**), respectively, by means of spectroscopic methods, including one- and two-dimensional NMR techniques.

Isodon eriocalyx (Dunn) Kudo (Labiatae) is widely distributed in Yunnan Province of the People's Republic of China.[1] It has been used in Chinese folk medicine to treat sore throats and inflammation. Previous investigations have shown that many bioactive diterpenoids have been isolated from this species, collected in different regions.[2−9] To search for novel constituents, we reinvestigated this plant, collected in Heqing County of Yunnan Province. From the dried leaves of *I. eriocalyx*, three new *ent*-kaurane diterpenoids, eriocalyxins C−E (**1**−**3**), were isolated together with eleven known diterpenoids, maoecrystals A−D,[2] odonicin,[3,11] eriocalyxins A and B,[4] maoecrystals O,[9] R, and T,[10] enmenin,[12,13] and other four compounds, 3,4-dihydroxycinnamic ethyl ester, cirsimaritin, *β*-sitosterol, and ursolic acid. In this paper, we describe the isolation and structure elucidation of the three new diterpenoids, **1**−**3**.



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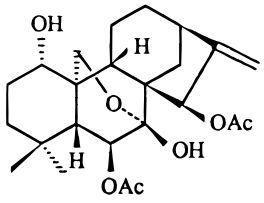

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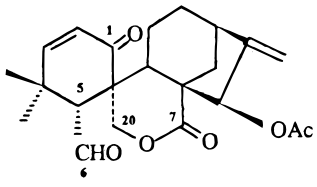

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The molecular formula of eriocalyxin C (**1**) was determined as $C_{22}H_{28}O_7$ by HREIMS (M^+ *m*/*z* 404.1825, calcd 404.1835), in which the molecular ion (*m*/*z* 404) was 16 amu greater than that of maoecrystal A.[2] The IR spectrum showed the presence of hydroxyl, ketone, and acetyl groups (3380−3350, 1730−1710 cm^{-1}), along with the absence of the characteristic absorption for an exomethylene unit at ca. 1650 cm^{-1}. Further study showed that the ^1H, ^{13}C, and DEPT NMR spectra of **1** were very similar to those of maoecrystal A, indicating the two compounds to have the same carbon skeleton. The only difference was that **1** had one oxygenated quaternary carbon (*δ* 67.8, s) and one oxygenated methylene (*δ* 47.5, t) instead of a C-16 exomethylene carbon unit. Inspection of the $^1H-^{13}C$ COSY and COLOC spectra of **1** showed that the methylene proton signals at *δ* 2.92 and 2.83 correlated with the quaternary carbon signal at *δ* 67.8, while the latter carbon signal revealed cross-peaks with the methylene proton signal at *δ* 1.95 (H-14α) and the two methine proton signals at *δ* 1.88 (H-13) and *δ* 6.69 (H-15). Thus, the quaternary carbon at *δ* 67.8 and the methylene at *δ* 47.5 could be assigned to C-16 and C-17, respectively. According to the molecular formula of **1** and its unsaturation, a C-16, C-17 epoxy group was found to be present. The main C−H long-range correlations of **1** are shown in Figure 1.

The C-6 hydroxyl group of **1** was assigned to the *β*-orientation on the basis of the coupling constant, $J_{5\beta,6\alpha}$ = 11.8 Hz. Comparison of the ^{13}C NMR data of **1** with those of maoecrystal A revealed that almost all the chemical shifts of the two compounds were closely coincident, except for C-12, C-13, C-14, C-16, and C-17, suggesting a common stereochemistry. On the basis of the *γ*-effect resulting in a change of the chemical shift of C-12 from *δ* 32.9 in maoecrystal A to *δ* 28.7 in **1**, the epoxy ring (C-16 and C-17) should be in the *β*-orientation.[9] From a NOESY experiment of **1**, the NOE effects between H-17a and H-15α, H-15α and one (H-14*β*) of the C-14 methylene protons, and H-17b and H-13α confirmed the above deduction. The major NOE correlations in **1** are shown in Figure 2. Therefore, **1** was deduced as 6*β*-hydroxy-15*β*-acetoxy-3α,20-epoxy-16*β*,17-epoxy-*ent*-kaur-1,7-dione.

Eriocalyxin D (**2**) showed a molecular ion peak at *m*/*z* 434.2328 in its HRMS, corresponding to a molecular formula of $C_{24}H_{34}O_7$ (calcd 434.2305), which is the same as that of maoecrystal F.[3] The ^1H, ^{13}C, and DEPT NMR spectra of these two compounds were very similar, with the only difference being that the signals of H-1*β* at *δ* 4.93 (1H, dd, *J* = 10.0, 4.0 Hz) and C-1 at *δ* 74.5 in maoecrystal F were shifted upfield to *δ* 3.76 (1H, dd, *J* = 10.9, 5.2 Hz) and *δ* 73.3 in **2**. Also, the H-15 signal at *δ* 4.99 (1H, t, *J* = 2.5 Hz) in maoecrystal F shifted downfield to *δ* 6.21 (1H, brs) in **2**. This evidence suggested that a hydroxyl group

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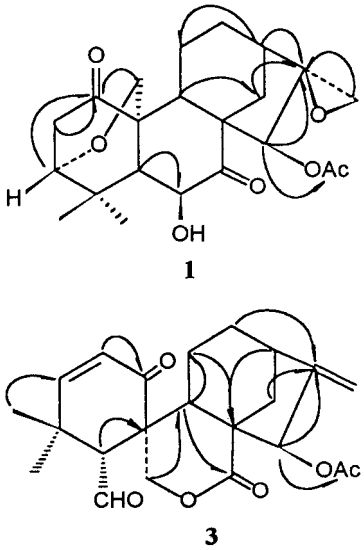

Figure 1. COLOC (H to C) correlations for **1** and **3**.

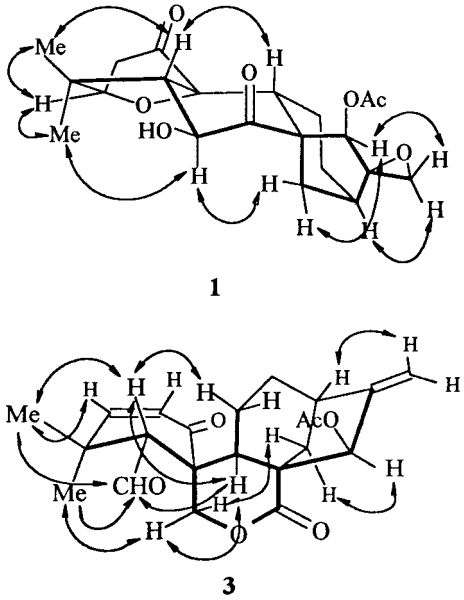

Figure 2. Major NOE correlations in **1** and **3**.

should be assigned to C-1 and an acetyl group to C-15 in **2**. In the COLOC spectrum of **2**, cross-peaks between H-6 (δ 5.01) and an acetyl carbonyl (δ 171.3), and between H-15 (δ 6.21) and another acetyl carbonyl (δ 171.1), could be observed. The unambiguous assignments of the ^{1}H and ^{13}C NMR data of **2** were made by a combination of NMR techniques, including ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, and COLOC spectra. Therefore, **2** was structurally determined as 1α,7β-dihydroxy-6β,15β-diacetoxy-7,20-epoxy-*ent*-kaur-16-ene.

Eriocalyxin E (**3**) had a molecular formula $C_{22}H_{26}O_6$ from its HRMS (M^+ *m*/*z* 386.1734, calcd 386.1729). Its mass spectrum showed that the molecular ion (*m*/*z* 386) was 42 amu greater than that of eriocalyxin A.[4] The UV absorption at 228.5 nm and IR bands at 1720, 1700, and 1650 cm^{-1} indicated the presence of α, β-unsaturated ketone, ester carbonyl, and exomethylene groups. The ^{1}H, ^{13}C, and DEPT NMR spectra of **3** were very similar to those of eriocalyxin A except for those derived from the D-ring, suggesting that both compounds are based on the same carbon skeleton. Instead of an α-methyl group at C-16 and a carbonyl at C-15 in eriocalyxin A, an exomethylene group at C-16 and an acetyl group at C-15 in **3** were determined by NMR

Table 1. ^{13}C NMR Data for Eriocalyxins C−E (**1**−**3**) in Pyridine-d_5

carbon	**1**	**2**	**3**[a]
1	209.7 (s)	73.3 (d)	197.6 (s)
2	42.2 (t)	30.4 (t)	156.5 (d)
3	77.5 (t)	39.5 (t)	125.5 (d)
4	38.1 (s)	33.9 (s)	36.0 (s)
5	51.6 (d)	55.7 (d)	57.9 (d)
6	71.9 (d)	75.6 (d)	200.1 (d)
7	208.9 (s)	95.4 (s)	172.5 (s)
8	58.1 (s)	52.1 (s)	50.9 (s)
9	39.7 (d)	46.5 (d)	37.2 (d)
10	51.8 (s)	41.6 (s)	49.9 (s)
11	22.2 (t)	19.3 (t)	16.9 (t)
12	28.7 (t)	32.2 (t)	32.1 (t)[b]
13	34.9 (d)	37.1 (d)	36.3 (d)
14	35.7 (t)	27.4 (t)	31.5 (t)[b]
15	75.5 (d)	75.4 (d)	81.6 (d)
16	67.8 (s)	159.1 (s)	153.9 (s)
17	47.5 (t)	108.6 (t)	110.5 (t)
18	29.5 (q)	32.7 (q)	31.5 (q)
19	23.1 (q)	22.3 (q)	24.6 (q)
20	62.5 (t)	63.9 (t)	67.8 (t)
OAc	169.3	171.3	169.7
	20.4	171.1	20.9
		21.98	
		21.40	

[a] Recorded in $CDCl_3$. [b] Assignments exchangeable.

spectral analysis. In the COLOC spectrum of **3**, H-15 at δ 5.57 correlated with the acetyl carbonyl at δ 169.7 and C-16 at δ 153.9, while the C-16 signal correlated with H-12 at δ 2.07 and H-14 at 2.31. The structure of ring D in **3** is commonly present in *ent*-kaurane diterpenoids, especially those isolated from *Isodon* species. The unambiguous ^{13}C NMR data of **3** are listed in Table 1. The γ-effect of C-15-OAc caused the chemical shift of C-9 in ^{13}C NMR spectrum to shift upfield from δ 42.7 as in eriocalyxin A to δ 37.2 in **3**, indicating the configuration of the OAc at C-15 to be β-oriented.[14] From NOESY experiment of **3**, the NOE effect between the H-15 and one (H-14β) of the C-14 methylene protons confirmed the H-15 in an α-configuration. The major NOE effects of **3** are shown in Figure 2. Therefore, **3** was determined as 15β-acetoxy-1,6-dioxo-6,7-*seco*-*ent*-kaur-2,16-dien-7,20-olide.

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were determined on a Kofler apparatus. The optical rotations were measured with a Horiba Sepa-300 polarimeter. UV spectra were recorded in MeOH on a Shimadzu UV-210A spectrometer. IR spectra were recorded on Perkin-Elmer 577 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer with TMS as internal standard. EIMS and HRMS were recorded on a VG Auto Spec 3000 instrument.

Plant Material. The leaves of *I. eriocalyx* were collected in Heqing County, Yunnan Province, People's Republic of China, in September 1993 and were identified by Prof. H.-W. Li of Kunming Institute of Botany. A voucher specimen (KIB-93-09-01, Lin) has been deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Academia Sinica, Kunming, People's Republic of China.

Extraction and Isolation. Dried and powdered leaves (5 kg) were extracted with 95% EtOH (2000 mL × 3) by refluxing for 2 h, and then the solvent was removed in vacuo. The residue was partitioned in H_2O and extracted with petroleum ether and EtOAc (1500 mL × 3), respectively. The EtOAc extract (180 g) was subjected to column chromatography over silica gel eluted with $CHCl_3$−Me_2CO (gradient elution) to afford 12 fractions (F1−F12). Fractions F4, F5, and F7 were chromatographed further over silica gel eluted with petroleum

ether−Me_2CO (from 10:1 to 1:1 gradient elution), to afford a further 10 fractions F41−F410, F51−F510, and F71−F710. Fraction F44 was purified over silica gel with cyclohexanes−EtOAc (7:3 and 6:4) and $CHCl_3$−2-propanol (20:1) to yield **3** (40 mg). Fractions F54 and F73 were separated over silica gel with $CHCl_3$−2-propanol (15:1 and 12:1 to 10:1), then purified with $CHCl_3$−benzene−2-propanol (15:5:1 and 12:5:1) to yield **1** (15 mg), and **2** (30 mg), respectively. Fractions F3, F6, F8, and the rests of F4−F5 and F7 were repeatedly chromatographed over silica gel and recrystallized to afford eriocalyxins A (10 mg) and B (20 mg), maoecrystals A (8 g), B (20 g), C (50 mg), and D (1 g), maoecrystals O (15 mg), R (20 mg), and T (30 mg), odonicin (2 g), enmenin (20 mg), 3,4-dihydroxycinnimic ethyl ester (20 mg), cirsimaritin (50 mg), *β*-sitosterol (20 mg), and ursolic acid (10 g).

Eriocalyxin C (1): white crystals; mp 191.5−192.5°; $[\alpha]_D^{22}$ −67.1° (*c* 0.26, MeOH); UV end absorption; IR (KBr) ν_{max} 3380−3350, 3020, 2930, 2860, 1730−1710 (br), 1440, 1360, 1215, 1085, 1050, 970, 950, 930, 900 cm^{-1}; ^1H NMR (pyridine-d_5) δ 6.69 (1H, brs, H-15α), 5.02 (1H, d, J = 11.8 Hz, H-6α), 4.86 (1H, d, J = 9.4 Hz, H-20a), 4.17 (1H, d, J = 9.4 Hz, H-20b), 3.77 (1H, dd, J = 3.4, 1.8 Hz, H-3*β*), 3.30 (1H, d, J = 7.8 Hz, H-9*β*), 2.92 (1H, d, J = 4.5 Hz, H-17a), 2.83 (3H, overlapped, H_2-2 and H-17b), 2.14 (1H, m, H-11α), 1.95 (3H, s, OAc), 1.95 (1H, m, H-14α), 1.88 (1H, brd H-5*β*), 1.87 (1H, m, H-13α), 1.71 (3H, s, Me-19), 1.67 (1H, dd, J = 10.5, 5.3 Hz, H-11*β*), 1.54 (1H, m, H-12α), 1.25 (1H, m, H-12*β*), 1.23 (3H, s, Me-18); ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 404 $[M]^+$ (10), 386 $[M - H_2O]^+$ (10), 368 $[M - 2H_2O]^+$ (20), 344 (100), 326 (50), 288 (25), 245 (28), 231 (50), 213 (63); HRMS *m*/*z* 404.1825 $[M^+]$, calcd for $C_{22}H_{28}O_7$ 404.1835.

Eriocalyxin D (2): colorless crystals; mp 208−210°; $[\alpha]_D^{23}$ −64.6° (*c* 0.27, MeOH); UV end absorption; IR (KBr) ν_{max} 3520, 3440, 2940, 2850, 1710, 1360, 1260−1210 (br.), 1060, 940 cm^{-1}; ^1H NMR (pyridine-d_5) δ 6.21 (1H, brs, H-15α), 5.83 (1H, d, J = 7.8 Hz, H-6α), 5.24 (1H, brs, H-17a), 5.11 (1H, brs, H-17b), 4.83 (1H, d, J = 9.6 Hz, H-20a), 4.38 (1H, d, J = 9.6 Hz, H-20b), 3.76 (1H, dd, J = 10.9, 5.2 Hz, H-1*β*), 2.56 (1H, m, H-13α), 2.23 (1H, m, H-9*β*), 2.29, 2.12 (each 3H, s, 2×OAc), 2.19 (1H, m, H-11α,), 2.20 (2H, overlapped, H-12α and H-14α), 2.02 (1H, m, H-14*β*), 2.01 (1H, m, H-11*β*), 1.90 (1H, d, J = 7.8 Hz, H-5*β*), 1.83 (2H, m, H_2-2), 1.50 (1H, m, H-12*β*), 1.37 (2H, m, H_2-3), 1.19 (3H, s, Me-18), 0.93 (3H, s, Me-19); ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 434 $[M]^+$ (35), 392 (100), 332 (55), 314 (30), 227 (60); HRMS *m*/*z* 434.2328 $[M^+]$, calcd for $C_{24}H_{34}O_7$ 434.2305.

Eriocalyxin E (3): colorless crystals; mp 178−179.5°; $[\alpha]_D^{23}$ +92.7° (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ϵ) 228.5 nm (3.62); IR (KBr) ν_{max} 3420, 2980, 2960, 2860, 1720, 1700, 1650, 1470, 1360, 1280, 1240, 1220, 1150, 1120, 1090, 1070, 1040, 990, 900 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.94 (1H, d, J = 2.8 Hz, H-6), 6.53 (1H, d, J = 10.2 Hz, H-2), 5.88 (1H, d, J = 10.2 Hz, H-3), 5.57 (1H, brs, H-15α), 5.09 (1H, brs, H-17a), 4.90 (1H, brs, H-17b), 4.86 (1H, d, J = 11.0 Hz, H-20a), 4.60 (1H, d, J = 11.0 Hz, H-20b), 3.18 (1H, d, J = 2.8 Hz, H-5*β*), 2.71 (1H, brs, H-13α), 2.45 (1H, dd, J = 11.0, 5.5 Hz, H-9*β*), 2.32 (1H, d, J = 12.7 Hz, H-14α), 2.20 (3H, s, OAc), 2.16 (1H, dd, J = 12.7, 6.0 Hz, H-14*β*), 2.06 (1H, td, J = 12.1, 7.6 Hz, H-12α), 1.40 (3H, overlapped, H_2-11 and H-12*β*), 1.35 (3H, s, Me-18), 1.23 (3H, s, Me-19); ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 386 $[M]^+$ (10), 344 (5), 326 (8), 316 (80), 298 (60), 287 (40), 269 (15), 257 (40), 135 (100); HRMS *m*/*z* 386.1734 $[M^+]$, calcd for $C_{22}H_{26}O_6$ 386.1729.

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