Cytotoxic *ent*-Kaurane Diterpenoids from *Isodon eriocalyx*

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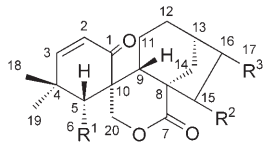
Five new *ent*-kaurane diterpenoids, *epi*-maoecrystal N (**1**), eriocalyxin G (**2**), maoecrystal W (**3**), maoecrystal X (**4**), and maoecrystal Y (**5**), along with 22 known ones, were isolated from *Isodon eriocalyx* (DUNN.) HARA., and their structures were determined by spectroscopic methods. All diterpenoids, except for **3** and **13**, were evaluated for inhibition of the K562, T-24, Me180, QGY-7701, and BIU87 cell lines (*Table 2*).

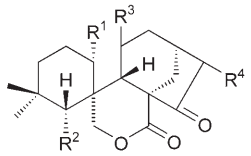
Introduction. – The plants of the genus *Isodon* are well-known as abundant resources of naturally occurring *ent*-kauranoid diterpenes, with a diversity of oxygenated and cleavage patterns. Since 1976, with the aim of searching for bioactive constituents from this genus, our group has phytochemically investigated more than 50 *Isodon* species distributed in China, and *ca.* 400 new diterpenoids have been isolated and characterized [1]. Many interesting novel diterpenoids, such as 1:1 complexes of natural *ent*-kauranoids (Diterp-Complex-RA) [2], a natural equimolecular mixture of two epimeric *ent*-kauranoids (irroratin A) [3], novel *ent*-abietanoids (laxiflorins N–O [4], micranthin C [5]), symmetric and asymmetric *ent*-kauranoid dimers (maoecrysral M [6], enanderinanin J [7], xindongnins M–O [8]), 6,7:8,15-seco-*ent*-kauranoids (laxiflorins F and G) [9], and a 15,16-seco-*ent*-kauranoid (rubescensin S) [10] were found. A number of these new diterpenoids were found to exhibit potent antitumor activities, with often minimal toxicities. They are, therefore, promising candidates as anticancer agents being studied in our laboratory.

Isodon eriocalyx (DUNN.) HARA., indigenous to different areas within the Yunnan Province, is one of this species chemically studied in our group, and more than 30 new *ent*-kauranoids have been isolated so far from this plant [11]. In a re-investigation of the chemical constituents of *I. eriocalyx* collected in the Lijiang prefecture, Yunnan Province, we have isolated 27 *ent*-kaurane diterpenoids (**1**–**27**), including five new ones (**1**–**5**). Herein, we present the isolation and structural elucidation of these compounds, as well as their cytotoxicities towards the K562, T-24, Me180, QGY-7701, and BIU87 cell lines.

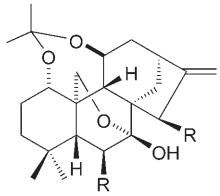
Results and Discussion. – *Structure Elucidation. Epi*-maoecrystal N (**1**), isolated as a colorless powder, exhibited the $[M+1]^+$ peak at *m/z* 361 in the ESI mass spectrum, consistent with the molecular formula $C_{20}H_{24}O_6$, which was confirmed by the HR-ESI-MS data of $[M+Na]^+$ at *m/z* 383.1475 (calc. 383.1470 for $C_{20}H_{24}NaO_6^+$). The analysis of



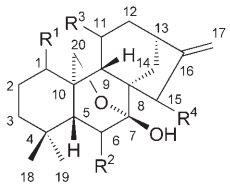
	R^1	R^2	R^3
1	COOH	=O	β-Me
2	CHO	β-OH	α-Me
7	CHO	=O	α-Me
8	COOH	=O	α-Me
28	CHO	β-OH	$=CH_2$



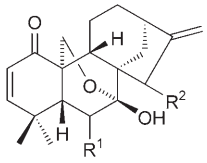
	R^1	R^2	R^3	R^4
3	AcO	COOH	β-OH	α-Me
9	H	CHO	α-OH	β-Me
10	AcO	CHO	H	α-Me
29	AcO	COOH	H	α-Me



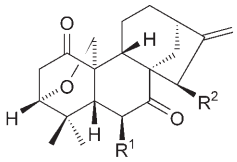
24 R = AcO
25 R = OH



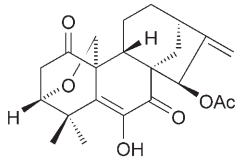
	R^1	R^2	R^3	R^4
4	α-OH	β-OH	H	β-AcO
5	α-OH	β-AcO	α-OH	β-AcO
18	α-OH	β-AcO	β-AcO	β-OH
19	=O	β-AcO	H	β-AcO
20	=O	β-OH	H	β-AcO
21	α-AcO	β-AcO	H	β-OH
22	β-OH	β-AcO	H	β-AcO
23	H	β-OH	α-AcO	=O
26	α-OH	β-OH	α-AcO	β-OH
27	α-AcO	β-AcO	=O	β-AcO
30	α-OH	β-AcO	H	β-AcO
31	α-OH	β-OH	α-OH	β-OH



	R^1	R^2
6	β-OH	=O
15	β-OH	β-OH
16	β-OH	β-AcO
17	β-AcO	β-AcO



	R^1	R^2
11	OH	AcO
12	OH	OH
13	AcO	OH



14

1D- and 2D-NMR spectra, and comparison of the NMR data with those of maoecrystal N (**8**) [12], established the structure of **1**[1]).

The ^{1}H-NMR spectrum of **1** displayed two Me *singlets* (δ(H) 1.37, 1.32), one Me *doublet* (δ(H) 0.95, $J = 7.0$ Hz), one CH=CH group (δ(H) 5.94 and 6.58 (2*d*, $J =$ 10.1 Hz each)), and two *doublets* for O−CH_2 (δ(H) 5.63 and 5.00 (2*d*, $J = 11.4$ Hz each)). The ^{13}C-NMR and DEPT spectra (*Table 1*) showed 20 C-atoms, including three Me, four CH_2, and six CH groups, and seven quaternary C-atoms. Considering the structure types of diterpenoids isolated from this plant, together with characteristic lactone carbonyl (δ(C) 170.1) and O−CH_2 resonances (δ(C) 70.2), it was reasonable to deduce that **1** is a 6,7-seco-7,20-olide-type *ent*-kauranoid. Detailed comparison of the

[1]) For systematic names, see *Exper. Part*.

^{1}H- and ^{13}C-NMR data of **1** with those of maoecrystal N (**8**) revealed that they were very similar, except that C(12) (δ(C) 20.2) and C(17) (δ(C) 11.5) were shifted by 9 and 5 ppm, respectively, to higher field, indicating that **1** is the (16*R*) epimer of maoecrystal N. This deduction was confirmed by the HMBC correlations of Me(17) with C(13) (δ(C) 32.6) and C(15) (δ(C) 217.1), and the ROESY correlation of H–C(16) with H_α–C(13) (*Fig. 1*).

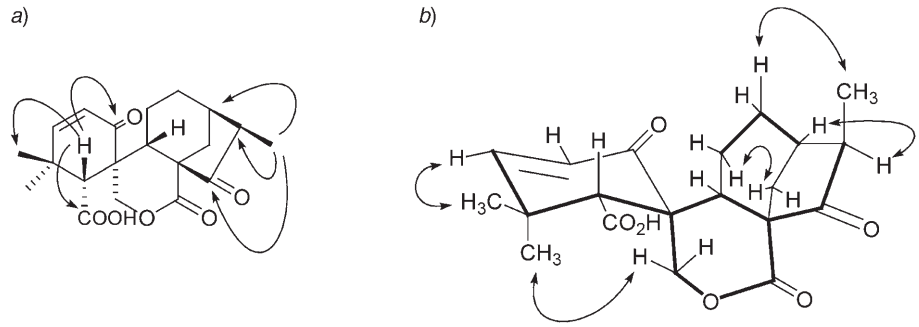


Fig. 1. *Selected HMBC* (a) *and ROESY* (b) *correlations of* **1**

Eriocalyxin G (**2**) was obtained as a colorless powder, and the molecular formula was inferred from its pseudomolecular $[M+Na]^+$ signal at *m/z* 369 in the ESI mass spectrum, and from its ^{13}C-NMR data (*Table 1*). The analysis of the NMR data of **2** indicated a 6,7-seco-7,20-olide-type *ent*-kauranoid, which only differed from tose of laxiflorin D (**28**) [13] at C(16) and C(17). The C(16)=CH_2(17) bond in **28** was replaced by a CH (δ(H) 1.59, δ(C) 50.4) and a Me group (δ(H) 1.07, δ(C) 20.2) in **2**. The orientation of Me(17) was inferred to be α, judging from ROESY correlations of Me(17) with both H_α–C(15) and H_α–C(13).

Maoecrystal W (**3**), a colorless powder, has the molecular formula $C_{22}H_{30}O_8$, as deduced from its $[M-1]^-$ FAB-MS signal at *m/z* 421. Inspection of the ^{1}H- and ^{13}C-NMR spectra of **3** disclosed the same 6,7-seco-7,20-olide-type *ent*-kaurane skeleton as compounds **1** and **2**. The NMR data (*Table 1*) of **3** were very similar to those of maoecrystal O (**29**) [12], the only difference being an additional 11-OH group in **3**. This deduction was confirmed by HMBC correlations of H–C(11) (δ(H) 4.44 (*m*)) with C(10) (δ(C) 44.4) and C(13) (35.6). H–C(11) was shown to be α-orientated through ROESY correlations with H–C(14) (δ(H) 2.16) and the 1-OAc group (δ(H) 2.18).

Maoecrystal X (**4**), a colorless powder, had the molecular formula $C_{22}H_{32}O_6$, as inferred from the $[M+Na]^+$ signal at *m/z* 415.2092 (calc. 415.2096 for $C_{22}H_{32}NaO_6^+$) in the HR-ESI mass spectrum. Extensive 1D- and 2D-NMR experiments, and comparison of NMR data with those of eriocalyxin D (**30**) [14], pointed to structure **4** as for its metabolite.

The ^{1}H-NMR spectrum of **4** exhibited two Me *singlets* (δ(H) 1.26, 1.18), one acetyl Me group (δ(H) 2.28), and two vinylic *singlets* at δ(H) 5.12 and 5.24. The ^{13}C-NMR data of **4** accounted for 22 C-atoms, including three characteristic CH (δ(C) 56.6, 46.1, 37.1 due to C(5), C(9), and C(13), resp.), three quaternary C-atoms (δ(C) 33.9, 52.1, and 41.1, assignable to C(4), C(8), and C(10), resp.), two Me *singlets* (δ(C) 33.7 and 22.6 attributable to C(18) and C(19), resp.), a C(16)=CH_2(17) moiety (δ(C) 160.9,

Table 1. *^{13}C-NMR Spectroscopic Data of Compounds* **1**–**5**

Position	**1**	**2**	**3**	**4**	**5**
1	198.9 (*s*)	199.4 (*s*)	77.1 (*d*)	73.9 (*d*)	73.6 (*d*)
2	124.9 (*d*)	125.4 (*d*)	25.1 (*t*)	30.7 (*t*)	29.1 (*t*)
3	158.0 (*d*)	157.0 (*d*)	40.1 (*t*)	39.8 (*t*)	39.1 (*t*)
4	36.2 (*s*)	36.3 (*s*)	34.5 (*s*)	33.9 (*s*)	33.7 (*s*)
5	55.1 (*d*)	58.8 (*d*)	58.9 (*d*)	56.6 (*d*)	55.2 (*d*)
6	173.5 (*s*)	202.0 (*d*)	175.9 (*s*)	74.8 (*d*)	75.6 (*d*)
7	170.1 (*s*)	175.6 (*s*)	171.3 (*s*)	97.0 (*s*)	95.8 (*s*)
8	59.2 (*s*)	55.1 (*s*)	60.0 (*s*)	52.1 (*s*)	51.6 (*s*)
9	43.6 (*d*)	37.9 (*d*)	49.4 (*d*)	46.1 (*d*)	49.8 (*d*)
10	50.7 (*s*)	49.9 (*s*)	44.4 (*s*)	41.1 (*s*)	42.7 (*s*)
11	18.0 (*t*)	17.1 (*t*)	66.3 (*d*)	19.4 (*t*)	66.2 (*d*)
12	20.2 (*t*)	32.2 (*t*)	42.0 (*t*)	32.3 (*t*)	41.3 (*t*)
13	32.6 (*d*)	38.7 (*d*)	35.6 (*d*)	37.1 (*d*)	37.2 (*d*)
14	32.7 (*t*)	31.5 (*t*)	29.8 (*t*)	27.7 (*t*)	27.9 (*t*)
15	217.1 (*s*)	88.0 (*d*)	215.5 (*s*)	75.2 (*d*)	75.7 (*d*)
16	48.8 (*d*)	50.4 (*d*)	51.5 (*d*)	160.9 (*s*)	160.0 (*s*)
17	11.5 (*q*)	20.2 (*q*)	17.1 (*q*)	107.6 (*t*)	108.3 (*t*)
18	32.0 (*q*)	31.2 (*q*)	34.7 (*q*)	33.7 (*q*)	32.9 (*q*)
19	24.9 (*q*)	23.9 (*q*)	24.5 (*q*)	22.6 (*q*)	22.3 (*q*)
20	70.2 (*t*)	68.8 (*t*)	68.4 (*t*)	63.8 (*t*)	65.8 (*t*)
*Me*CO	–	–	22.0 (*q*)	22.2 (*q*)	22.0 (*q*)
Me*C*O	–	–	171.6 (*s*)	171.3 (*s*)	171.1 (*s*)
*Me*CO	–	–	–	–	21.4 (*q*)
Me*C*O	–	–	–	–	171.1 (*s*)

107.6), one O–CH$_2$ (δ(C) 63.8, C(20)), and one hemiacetal-type quaternary C-atom (δ(C) 97.0, C(7)), implying that **4** is a 7,20-epoxy-*ent*-kauranoid. Compared with the known eriocalyxin D (**30**), the NMR data of **4** displayed only one instead of two AcO group(s). An OH group was located at C(6) based on HMBC correlations between H–C(6) (δ(H) 4.24) and both C(4) and C(8) (*Fig. 2*). An AcO group was attached to C(15) to account for the observed HMBC correlations of H–C(15) (δ(H) 6.44) with AcO (δ(C) 171.3), C(17), C(9), and C(7), respectively. From the ROESY spectrum (*Fig. 2*), H–C(1), H–C(6), and H–C(15) of eriocalyxin X (**4**) were determined to be β-, α-, and α-oriented, respectively, as in eriocalyxin D.

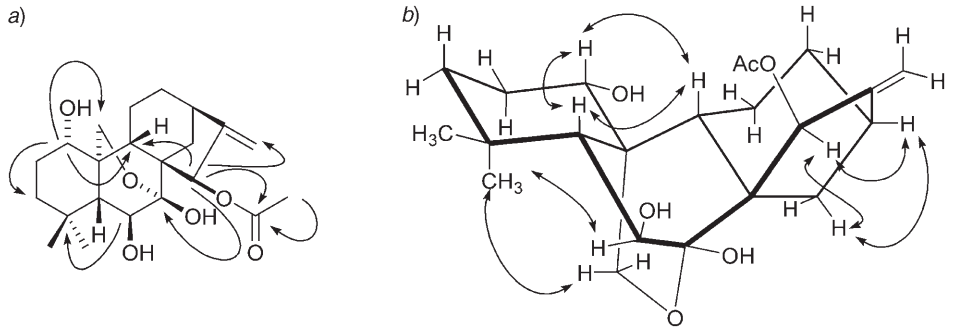


Fig. 2. *Selected HMBC* (a) *and ROESY* (b) *correlations of* **4**

Maoecrystal Y (**5**), a colorless powder, showed its $[M+Na]^+$ signal at *m/z* 473 in the ESI mass spectrum. Together with NMR data (*Table 1*), the molecular formula $C_{24}H_{34}O_8$ was deduced, as further confirmed by HR-ESI-MS ($[M+Na]^+$) at *m/z* 473.2142 (calc.: 473.2151 for $C_{24}H_{34}NaO_8^+$). The ^{1}H- and ^{13}C-NMR data of **5** suggested that this compound was a 7,20-epoxy-*ent*-kauranoid, too. A detailed comparison of the NMR data of **5** with those of nervosanin B (**31**) [15] displayed a close resemblance, except for two extra AcO groups in **5**. One of these was positioned at C(6) through HBMC long-range correlations of H–C(6) (δ(H) 5.92 (d, J = 8.1 Hz)) with C(4) (δ(C) 33.7), C(8) (δ(C) 51.6), and AcO (δ(C) 171.1). The other AcO unit was located at C(15) based on HMBC correlations between H–C(15) (δ(H) 6.31) and C(7) (δ(C) 95.8), C(9) (δ(C) 49.8), C(13) (δ(C) 37.2), C(17) (δ(C) 108.3), and AcO (δ(C) 171.1). The relative configuration of **5** was shown to be identical with that of nervosanin B by a ROESY experiment.

The structures of the known compounds **6**–**27** were established as eriocalyxin B (**6**) [16], eriocalyxin A (**7**) [16], maoecrystal N (**8**) [12], rabdosichuanin B (**9**) [17], maoecrystal L (**10**) [18], maoecrystal A (**11**) [19], laxiflorin L (**12**) [20], maoecrystal U (**13**) [21], coetsoidin A (wang) (**14**) [22], maoecrystal C (**15**) [19], maoecrystal B (**16**) [19], odonicin (**17**) [23], ternifolin (**18**) [24], maoecrystal D (**19**) [19], maoecrystal S (**20**) [25], maoecrystal F (**21**) [26], trichokaurin[2]) (**22**) [27], longikaurin E (**23**) [28], maoecrystal T (**24**) [25], wikstroemioidin B (**25**) [29], sodoponin (**26**) [30], and shikokianidin (**27**) [31] by comparison of their spectral data with literature values.

Cytotoxity. All diterpenoids, except for **3** and **13**, were tested for their cytoxicities against the K562, T-24, Me180, QGY-7701, and BIU87 cell lines, with cisplatin as positive control [32][33]. As shown in *Table 2*, compounds **6** and **23**, both with an α,β-unsaturated ketone in ring *D*, exhibited the strongest cytotoxicities against K562, with IC_{50} values of 0.51 and 0.75 ± 0.01 μg/ml, respectively; the activities of compounds **7**, **12**, and **15** were moderate. Compounds **10** and **26** showed only weak activities. Compounds **6**, **7**, and **23** showed notable inhibitory effects against the growth of T-24 cells.

Table 2. *Cytotoxic Activities of Selected Compounds from* Isodon eriocalyx. Compounds not listed showed only weak or no activities towards the five cell lines tested.

Compound	IC_{50} [μg/ml]				
	K562	T-24	Me180	QGY	BIU87
Cisplatin[a])	3.38 ± 0.12	2.28 ± 0.12	0.78 ± 0.01	2.14 ± 0.02	2.93 ± 0.03
6	0.51 ± 0.01	4.59 ± 0.38	8.03 ± 0.30	1.02 ± 0.11	3.48 ± 0.25
7	1.74 ± 0.03	2.72 ± 0.13	11.23 ± 0.23	2.92 ± 0.05	26.92 ± 0.60
10	18.53 ± 0.24	13.10 ± 0.22	> 100	9.61 ± 0.42	43.87 ± 1.02
12	1.11 ± 0.01	32.58 ± 0.22	6.95 ± 0.13	10.39 ± 0.31	48.32 ± 2.04
15	1.23 ± 0.02	> 100	3.52 ± 0.33	> 100	n.d.[b])
16	> 30	> 100	1.27 ± 0.06	17.11 ± 0.49	n.d.
23	0.75 ± 0.01	4.56 ± 0.23	4.25 ± 0.54	3.11 ± 0.33	3.86 ± 0.31
26	23.66 ± 0.24	> 100	2.30 ± 0.06	9.34 ± 0.51	19.99 ± 0.29

[a]) Positive control. [b]) Not determined.

[2]) Also known as *enmenin*.

Moreover, Compound **6** demonstrated stronger activity than cisplatin against QGY-7701. Only compounds **6** and **23** obviously inhibited the proliferation of BIU87 cells. Compounds not listed in *Table 2* showed very weak or no inhibitory effects against the above five cell lines.

All the tested substances, except for compounds **6** and **23**, lack the α-methylidene five-membered ketone, which is thus, thought to be very important for activity, and showed weak activity as expected [1]. This indicates that a *Michael* acceptor in ring *D* of the 15-oxo-*ent*-kaurene series is essential for antitumor activity.

Experimental Part

General. Semi-prep. HPLC: *Agilent 1100*, with *Zorbax SB-C18* column. Prep. HPLC: *Shimadzu LC-8A* liquid chromatograph. UV Spectra: λ_{max} (log ε) in nm. Optical rotation: *Horiba SEAP-300* spectropolarimeter. IR Spectra: *Bio-Rad-FtS-135* spectrometer, KBr pellets; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. Mass spectra: *VG Autospec-3000* spectrometer; in *m/z* (rel. %).

Plant Material. The leaves of *Isodon eriocalyx* (Dunn.) Hara. were collected in Lijing Prefecture, Yunnan Province, P. R. China, in September 2002, and were identified by Prof. *Zhong-Wen Lin*. A voucher specimen (KIB-2002-092 Lin) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The powdered, air-dried leaves of *I. eriocalyx* (8.5 kg) were extracted with 70% aq. Me_2CO (3 × 48 l) at r.t. overnight. The extract was partitioned between H_2O and AcOEt. The org. layer was evaporated (242 g), and the extract was chromatographed on *MCI*-gel *CHP 20P* (MeOH/H_2O 9 : 1, then MeOH). The fraction eluted with MeOH/H_2O 9 : 1 (219 g) was subjected to column chromatography (CC) (1.5 kg SiO_2, 200 – 300 mesh; $CHCl_3$/Me_2CO 1 : 0, 9 : 1, 8 : 2, 7 : 3, 6 : 4, and 0 : 1) to afford fractions (Fr.) *1 – 6*. *Fr. 1* was submitted to repeated CC (SiO_2; 1. petroleum ether/AcOEt 8 : 2 and 7 : 3; 2. cyclohexane/i-PrOH 9 : 1; 3. RP-18) followed by semi-prep. and prep. HPLC to yield compounds **6** (1.092 g), **7** (551 mg), **11** (1.7 g), **14** (23 mg), **16** (4.313 g), and **20** (61 mg). In the same way, *Fr. 2* yielded compounds **9** (109 mg), **13** (2 mg), **15** (914 mg), **18** (705 mg), **19** (35 mg), **21** (13 mg), and **24** (17 mg). Compounds **1** (5 mg), **8** (351 mg), **10** (56 mg), **17** (17 mg), **22** (238 mg), **23** (101 mg), **25** (2 mg), and **26** (46 mg) were isolated from *Fr. 3*. Compounds **2** (2 mg), **3** (13 mg), **4** (17 mg), **5** (153 mg), **12** (213 mg), and **27** (13 mg) were obtained analogously from *Fr. 4*.

epi-*Maoecrystal N* (=*(16*R*)-7,20-Epoxy-1,7,15-trioxo-16-methyl-6,7-seco*-ent-*kaur-2-en-6-oic Acid*; **1**). Colorless powder. UV (MeOH): 226 (3.95). $[\alpha]_D^{26.9} = +94.6$ ($c = 0.148$, C_5H_5N). IR (KBr): 3441, 2965, 2937, 2875, 1740, 1726, 1690, 1634, 1466, 1391, 1375, 1260, 1222, 1199. ^{1}H-NMR (400 MHz, C_5D_5N): 0.95 (*d*, *J* = 7.0, Me(17)); 1.32 (*s*, Me(19)); 1.37 (*s*, Me(18)); 1.37 (overlapping, H_a–C(12)); 1.64 – 1.70 (*m*, H_b–C(12)); 1.71 – 1.75 (*m*, CH_2(11)); 2.37 – 2.41 (*m*, H–C(13)); 2.42 – 2.50 (overlapping, H–C(9), H–C(16)); 2.64 (*d*, *J* = 12.4, H_a–C(14); 2.97 (*d*, *J* = 12.4, H_b–C(14)); 3.43 (*s*, H–C(5)); 5.00 (*d*, *J* = 11.4, H_a–C(20)); 5.63 (*d*, *J* = 11.4, H_b–C(20)); 5.94 (*d*, *J* = 10.1, H–C(2)); 6.58 (*d*, *J* = 10.1, H–C(3)). ^{13}C-NMR (100 MHz, C_5D_5N): see *Table 1*. ESI-MS: 361 ($[M+1]^+$), 383 ($[M+Na]^+$). HR-ESI-MS: 383.1475 ($C_{20}H_{24}NaO_6^+$; calc. 383.1470).

Eriocalyxin G (=*(15*R*,16*S*)-7,20-Epoxy-1,7-dioxo-16-methyl-6,7-seco*-ent-*kaur-2-en-6-al*; **2**). Colorless powder. UV (MeOH): 227 (3.88). $[\alpha]_D^{27.5} = +113.9$ ($c = 0.139$, C_5H_5N). IR (KBr): 3417, 2929, 2872, 2752, 1735, 1715, 1657, 1460, 1377, 1250, 1224, 1103. ^{1}H-NMR (400 MHz, C_5D_5N): 1.07 (*d*, *J* = 7.0, Me(17)); 1.18 (*s*, Me(19)); 1.19 (*s*, Me(18)); 1.24 – 1.27 (*m*, H_a–C(12)); 1.52 – 1.56 (*m*, CH_2(11)); 1.59 (overlapping, H–C(16), H–C(13)); 1.80 – 1.84 (*m*, H_b–C(12)); 2.30 (*d*, *J* = 12.3, H_a–C(14)); 2.39 (*d*, *J* = 5.5, 12.3, H_b–C(14)); 2.93 (*dd*, *J* = 12.1, 6.0, H–C(9)); 3.19 (*d*, *J* = 4.7, H–C(5)); 4.12 (*d*, *J* = 6.0, H–C(15)); 4.96 (*d*, *J* = 10.6, H_a–C(20)); 5.55 (*d*, *J* = 10.6, H_b–C(20)); 5.93 (*d*, *J* = 10.1, H–C(2)); 6.45 (*d*, *J* = 10.1, H–C(3)). ^{13}C-NMR (100 MHz, C_5D_5N): see *Table 1*. ESI-MS: 369 ($[M+Na]^+$). HR-ESI-MS: 369.1676 ($C_{20}H_{26}NaO_5^+$; calc. 369.1677).

Maoecrystal W (=*(1α,11β,16*S*)-1-Acetoxy-7,20-epoxy-11-hydroxy-7,15-dioxo-16-methyl-6,7-seco*-ent-*kauran-6-oic Acid*; **3**). Colorless powder. UV (MeOH): 202 (3.37). $[\alpha]_D^{17.4} = +107.9$ ($c = 0.3985$, C_5H_5N). IR (KBr): 3490, 2956, 2932, 2878, 1737, 1714, 1688, 1482, 1455, 1410, 1372, 1275, 1230, 1063, 1042, 690. ^{1}H-NMR (500 MHz, C_5D_5N): 0.99 (*d*, *J* = 7.7, Me(17)); 1.10 (*s*, Me(18)); 1.25 (*s*, Me(19)); 1.43 – 1.47 (*m*, CH_2(3)); 1.53 – 1.56 (*m*, CH_2(12)); 1.98 – 2.02 (*m*, H–C(13)); 2.05 – 2.09 (*m*, CH_2(2)); 2.14 – 2.20 (*m*, H_a–C(14)); 2.24 – 2.29 (*m*, H–C(16)); 2.52 (*d*, *J* = 11.5, H–C(9)); 2.62 – 2.66 (*m*, H_b–C(14)); 4.39 (br. *s*, H–C(5)); 4.42 – 4.47 (*m*,

H–C(11)); 5.40 (*d*, *J* = 12.6, H_a–C(20)); 5.63–5.68 (*m*, H–C(1)); 5.75 (*d*, *J* = 12.6, H_b–C(20)). ^{13}C-NMR (100 MHz, C_5D_5N): see *Table 1*. ESI-MS: 421 ($[M-1]^-$). HR-ESI-MS: 421.1872 ($C_{22}H_{29}O_8^-$; calc. 421.1862).

Maoecrystal X (=(*1α,6β,7β,15*R)-*7α,20-Epoxy-1,6,7-trihydroxy*-ent-*kaur-16-en-15-yl Acetate*; **4**) Colorless powder. UV (MeOH): 204 (3.75). $[\alpha]_D^{27.4} = -105.6$ (*c* = 0.336, C_5H_5N). IR (KBr): 3447, 2934, 2864, 1718, 1659, 1637, 1496, 1455, 1371, 1260, 1164, 1146, 1071, 1053. ^{1}H-NMR (400 MHz, C_5D_5N): 1.18 (*s*, Me(19)); 1.26 (*s*, Me(18)); 1.43–1.48 (*m*, CH_2(3)); 1.52–1.57 (*m*, CH_2(12)); 1.78 (*d*, *J* = 8.3, H–C(5)); 1.87–1.91 (*m*, CH_2(2)); 2.03–2.08 (*m*, H_a–C(11)); 2.12 (*dd*, *J* = 15.5, 5.5, H_b–C(14)); 2.21–2.26 (*m*, H_b–C(11)); 2.22 (*d*, *J* = 15.5, H_a–C(14)); 2.28 (*s*, AcO)); 2.60 (overlapping, H–C(9), H–C(13)); 3.80 (*t*, *J* = 9.7, H–C(1)); 4.24 (*dd*, *J* = 8.3, 3.4, H–C(6)); 4.40 (*d*, *J* = 9.2, H–C(20)); 4.84 (*d*, *J* = 9.2, H–C(20)); 5.12 (br. *s*, H_a–C(17)); 5.24 (br. *s*, H_b–C(17)); 6.44 (br. *s*, H–C(15)). ^{13}C-NMR (100 MHz, C_5D_5N): see *Table 1*. EI-MS: 392 (5, M^+), 330 (22), 314 (43), 227 (48), 146 (69), 119 (85), 91 (100), 85 (72), 81 (71), 69 (36), 55 (51). HR-ESI-MS: 415.2092 ($C_{22}H_{32}NaO_6^+$; calc. 415.2096).

Maoecrystal Y (=*1α,6β,7β,11α,15*R)-*7α,20-Epoxy-1,7,11-trihydroxy*-ent-*kaur-16-en-6,15-diyl Diacetate*; **5**). Colorless powder. UV (MeOH): 203 (3.73). $[\alpha]_D^{27.5} = -103.7$ (*c* = 0.254, C_5D_5N). IR (KBr): 3432, 2930, 1735, 1711, 1632, 1374, 1063, 1038. ^{1}H-NMR (400 MHz, C_5D_5N): 0.94 (*s*, Me(18)); 1.20 (*s*, Me(19)); 1.35–1.39 (*m*, CH_2(2)); 1.74–1.78 (*m*, CH_2(2)); 1.88–1.93 (overlapping, H_a–C(12), H–C(5)); 2.14 (*s*, AcO); 2.18 (*d*, *J* = 4.5, 11.8, H_a–C(14)); 2.28 (*s*, AcO); 2.43 (*d*, *J* = 3.8, H–C(9)); 2.61 (*dd*, *J* = 14.6, 9.8, H_b–C(12)); 2.78 (*dd*, *J* = 9.8, 4.5, H–C(13)); 3.34 (*d*, *J* = 11.8, H_b–C(14)); 3.98 (*dd*, *J* = 11.6, 3.2, H–C(1)); 4.30 (*d*, *J* = 9.3, H_a–C(20)); 4.64–4.68 (*m*, H–C(11)); 5.10 (br. *s*, H_a–C(17)); 5.20 (*d*, *J* = 9.3, H_b–C(20)); 5.32 (br. *s*, H–C(13)); 5.92 (*d*, *J* = 8.1, H–C(6)); 6.31 (br. *s*, H–C(15)). ^{13}C-NMR (100 MHz, C_5D_5N): see *Table 1*. ESI-MS: 473 ($[M+Na]^+$). HR-ESI-MS: 473.2142 ($C_{24}H_{34}NaO_8^+$; calc. 473.2151).

Cytotoxicity. Cytotoxicities were determined by means of the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium hydrobromide) and the SRB (sulforhodamin B) assays [32][33], cisplatin being used as positive controls. The K562 cells (5×10^4 cells/ml) were treated at various drug concentrations (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M) in a 96-well plate for 44 h. Then, 10 µl of MTT soln. (5 g/l) were added to each well, and the plate was incubated for 4 h at 37° with 5% CO_2. The absorbance of each well was measured with a microplate reader (*Bio-Tek EL-340*) at 570 and 630 nm.

Exponentially growing T-24, Me180, QGY-7701, or BIU87 cells (4×10^4 cells/ml) were separately placed on a 96-well microplate. Cultures were pre-incubated for 24 h at 37° with 5% CO_2. Then, 10 µl of control or test soln. was put into each well, and the plate was incubated for an additional 48 h. At the end of exposure, the cells were fixed by addition of 50 µl of cold 50% trichloroacetic acid at 4° for 1 h. After washing with tap water, the cells of each well were stained with 50 µl of a 0.4% SRB soln. in 1% AcOH for 30 min. Then, the cultures were rinsed with 1% AcOH. Finally, 10 mM unbuffered *Tris* soln. (150 µl) was added to each well, and the optical density was measured at 570 nm.

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