

Four New Cucurbitacins from the Fruit of *Momordica charantia*

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Four new 5 β ,19-epoxycucurbitacins, kuguacins T–W (**1**–**4**, resp.), along with nine known cucurbitane derivatives, **5**–**13**, were obtained from the fresh fruit of *Momordica charantia*. Structures of the new metabolites were elucidated as 5 β ,19-epoxy-25-hydroxycucurbitane-3,7,23-trione (**1**), 5 β ,19-epoxy-3,7-dioxo-23,24,25,26,27-pentanorcucurbitan-22-oic acid (**2**), 5 β ,19-epoxy-3 β -hydroxycucurbit-24-ene-7,23-dione (**3**), and 5 β ,19-epoxy-25-hydroxycucurbit-23-ene-3,7-dione (**4**), by extensive spectroscopic investigations, which were confirmed by a single-crystal X-ray diffraction analyses in the case of compound **4**.

Introduction. – The fruit of *Momordica charantia* L. (Cucurbitaceae), called *kugua* in Chinese, is a popular vegetable in the south of China. Tissues of this plant, such as fruits, leaves, and stem, are used as a traditional Chinese medicine for the treatment of toothache, diarrhea, furuncles, and diabetes. In addition, antidiabetic properties have been reported.

In recent years, cucurbitane-type compounds from *M. charantia* have been shown to possess biological properties, such as antidiabetes [1–3], anticancer [4–9], agonist/antagonist [10], antimalarial [11], and antioxidant activities [12][13]. Previous phytochemical investigations have disclosed a series of new cucurbitane triterpenoids, as well as the anti-HIV activities of some cucurbitacins, isolated from fruit, root, leaf, and stem of *M. charantia* [14–17]. In our search for bioactive metabolites, a further study of the fruit led to the isolation of four new cucurbitacins, **1**–**4**, which possess a 5 β ,19-epoxycucurbitane skeleton, and nine known ones, **5**–**13** (Fig. 1), kuguacin L (**5**) [14], karavilagenin D (**6**) [18], (23*E*)-3 β ,7 β ,25-trihydroxycucurbita-5,23-dien-19-al (**7**) [19], (23*E*)-3 β ,7 β -dihydroxy-25-methoxycucurbita-5,23-dien-19-al (**8**) [19], kuguacin R (**9**) [14], 5 β ,19-epoxycucurbita-6,23-diene-3 β ,19,25-triol (**10**) [20], 5 β ,19-epoxycucurbita-6,23-dien-3 β -ol (**11**) [21], kuguacin E (**12**) [15], and kuguacin P (**13**) [14].

Results and Discussion. – Kuguacin T (**1**) was isolated as colorless needles with the molecular formula C₃₀H₄₆O₅, deduced from the positive-ion HR-ESI-MS (m/z 509.3212 ([*M*+Na]⁺, C₃₀H₄₆NaO₅⁺; calc. 509.3242)) and ¹³C-NMR data. In the ¹H-NMR spectrum, signals of seven Me groups (δ (H) 0.78 (s, 3 H), 0.99 (s, 3 H), 1.00 (d, *J* = 6.4, 3 H), 1.14 (s, 3 H), 1.27 (s, 3 H), and 1.53 (s, 2 \times 3 H), an isolated *AB* system (δ (H)

Table 1. ^1H - and ^{13}C -NMR Data of Compounds **1**–**4**. In (D₅)pyridine; δ in ppm; J in Hz.

Position	1		2		3		4	
	$\delta(\text{H})^{\text{a}}$	$\delta(\text{C})^{\text{b}}$	$\delta(\text{H})^{\text{c}}$	$\delta(\text{C})^{\text{d}}$	$\delta(\text{H})^{\text{c}}$	$\delta(\text{C})^{\text{d}}$	$\delta(\text{H})^{\text{a}}$	$\delta(\text{C})^{\text{b}}$
1	2.01–2.04 (m), 1.59–1.62 (m)	24.7 (t)	2.03–2.08 (m), 1.62–1.66 (m)	24.2 (t)	1.70–1.74 (m), 1.51–1.74 (m)	18.5 (t)	2.03–2.07 (m), 1.59–1.62 (m)	24.7 (t)
2	2.78–2.82 (m), 2.30–2.34 (m)	36.0 (t)	2.73–2.77 (m), 2.25–2.29 (m)	35.6 (t)	1.82–1.86 (m), 1.76–1.80 (m)	27.3 (t)	2.91–2.95 (m), 2.37–2.41 (m)	36.1 (t)
3		213.0 (s)		213.8 (s)	3.58 (s)	76.4 (d)		213.1 (s)
4		49.8 (s)		49.4 (s)		38.5 (s)		49.8 (s)
5		91.9 (s)		91.3 (s)		89.1 (s)		91.9 (s)
6	2.90, 2.75 (AB, J = 18.1)	50.1 (t)	2.70, 2.47 (AB, J = 18.3)	49.3 (t)	2.96, 2.48 (AB, J = 17.9)	50.9 (t)	2.92 (overlap), 2.77 (overlap)	50.1 (t)
7		212.5 (s)		213.3 (s)		212.8 (s)		212.6 (s)
8	2.77 (br. s)	62.9 (d)	2.66 (br. s)	62.6 (d)	2.73 (s)	63.0 (d)	2.72 (s)	62.9 (d)
9		46.9 (s)		46.6 (s)		47.1 (s)		47.0 (s)
10	3.01 (dd, J = 10.4, 6.5)	41.3 (d)	2.90 (dd, J = 10.5, 6.5)	41.0 (d)	2.67 (dd, J = 11.1, 6.5)	41.0 (d)	3.02 (dd, J = 9.9, 6.8)	41.3 (d)
11	1.49–1.52 (m), 1.29–1.32 (m)	22.9 (t)	1.64–1.67 (m), 1.45–1.48 (m)	22.7 (t)	1.60–1.64 (m), 1.24–1.27 (m)	22.3 (t)	1.49–1.52 (m), 1.30–1.34 (m)	22.9 (t)
12	1.58–1.61 (m), 1.50–1.53 (m)	30.7 (t)	1.71–1.74 (m), 1.55–1.59 (m)	30.4 (t)	1.59–1.62 (m), 1.49–1.62 (m)	30.7 (t)	1.55–1.58 (m), 1.47–1.50 (m)	30.6 (t)
13		46.1 (s)		45.8 (s)		46.1 (s)		46.0 (s)
14		49.1 (s)		48.6 (s)		49.1 (s)		49.1 (s)
15	1.73–1.75 (m), 1.39–1.42 (m)	34.7 (t)	1.60–1.63 (m), 1.24–1.26 (m)	34.2 (t)	1.72–1.76 (m), 1.35–1.38 (m)	34.8 (t)	1.73–1.76 (m), 1.36–1.38 (m)	34.8 (t)
16	1.81–1.85 (m), 1.23–1.25 (m)	28.2 (t)	1.86–1.89 (m), 1.39–1.41 (m)	26.6 (t)	1.82–1.85 (m), 1.26–1.28 (m)	28.3 (t)	1.85–1.89 (m), 1.25–1.27 (m)	28.0 (t)
17	1.45–1.49 (m)	49.7 (d)	1.87–1.89 (m)	46.5 (d)	1.45–1.49 (m)	50.0 (d)	1.44–1.46 (m)	49.3 (d)
18	0.78 (s)	15.5 (q)	0.84 (s)	15.8 (q)	0.81 (s)	15.5 (q)	0.78 (s)	15.6 (q)
19	3.65 (d, J = 8.7), 3.55 (d, J = 8.7)	79.5 (t)	3.65 (d, J = 8.8), 3.47 (d, J = 8.8)	79.4 (t)	3.89, 3.57 (AB, J = 8.5)	79.2 (t)	3.67 (d, J = 8.7), 3.57 (d, J = 8.7)	79.5 (t)
20	2.18–2.22 (m)	32.7 (d)	2.40–2.42 (m)	42.4 (d)	2.15–2.17 (m)	33.4 (d)	1.44–1.47 (m)	36.6 (d)
21	1.00 (d, J = 6.4)	20.1 (q)	1.14 (d, J = 6.8)	17.2 (q)	1.02 (d, J = 5.5)	20.0 (q)	0.96 (d, J = 5.7)	18.9 (q)
22	2.72–2.75 (m), 2.42–2.44 (m)	52.3 (t)		179.6 (s)	2.54 (br. d, J = 6.4), 2.14–2.16 (m)	51.8 (t)	2.17–2.20 (m), 1.80–1.84 (m)	39.4 (t)

Table 1 (cont.)

Position	1		2		3		4	
	$\delta(\text{H})^a$	$\delta(\text{C})^b$	$\delta(\text{H})^c$	$\delta(\text{C})^d$	$\delta(\text{H})^e$	$\delta(\text{C})^d$	$\delta(\text{H})^f$	$\delta(\text{C})^b$
23		211.1 (s)				200.6 (s)	5.94 (br. s)	124.1 (d)
24	2.85 (overlap)	56.0 (t)			6.23 (s)	124.9 (d)	5.94 (br. s)	141.8 (d)
25		69.5 (s)				154.2 (s)		69.8 (s)
26	1.53 (s)	30.5 (q)			1.83 (s)	27.3 (q)	1.58 (s)	30.9 (q)
27	1.53 (s)	30.2 (q)			2.22 (s)	20.6 (q)	1.58 (s)	30.9 (q)
28	1.27 (s)	17.8 (q)		17.2 (q)	1.28 (s)	21.2 (q)	1.28 (s)	17.9 (q)
29	1.14 (s)	25.3 (q)		25.5 (q)	0.97 (s)	26.2 (q)	1.19 (s)	25.4 (q)
30	0.99 (s)	21.3 (q)		21.2 (q)	0.90 (s)	21.5 (q)	0.98 (s)	21.3 (q)

^{a)} Recorded at 400 MHz. ^{b)} Recorded at 100 MHz. ^{c)} Recorded at 500 MHz. ^{d)} Recorded at 150 MHz.

CH₂(6)) to δ (C) 91.9 (C(5)), 41.3 (C(10)), and 212.5 (C(7)), and from δ (H) 3.65 and 3.55 (*d*, *J* = 8.7, 1 H each, CH₂(19)) to δ (C) 91.9 (C(5)), 41.3 (C(10)), and 62.9 (C(8)) confirmed the deduction. In line with the configuration of cucurbitane compounds, the 5,19-epoxy ring is assumed to be β -oriented. Thus, the structure of **1** was elucidated as 5 β ,19-epoxy-25-hydroxy-cucurbitane-3,7,23-trione.

The molecular formula of kuguacin U (**2**) was determined as C₂₅H₃₆O₅ by the positive-ion HR-ESI-MS spectrum (*m/z* 417.2609 ([*M* + H]⁺)). The ¹H-NMR spectrum of **2** displayed signals of five Me groups (δ (H) 0.84 (*s*), 0.97 (*s*), 1.04 (*s*), 1.12 (*s*), and 1.14 (*d*, *J* = 6.8)) and two CH₂ groups (δ (H) 2.47, 2.70 (*AB*, *J* = 18.3, CH₂(6)) and 3.65, 3.47 (*d*, *J* = 8.8, CH₂(19))). The ¹³C-NMR and DEPT spectra showed signals for 25 C-atoms, including those of five Me, eight CH₂ and four CH groups, and eight quaternary C-atoms. By detailed comparison of the 1D-NMR data with those of **1** showed that both compounds possessed the same structure in rings *A*–*D*. The differences were the absence of the side chain consisting C(23), C(24), C(25), C(26), and C(27), and the presence of a COOH group (δ (C) 179.6 (C(22)) in **2**, suggesting a 5 β ,19-epoxy-23,24,25,26,27-pentanorcucurbitane skeleton for **2**. These differences could be explained as the result of an oxidative cleavage between C(22) and C(23) of **1**. HMBCs δ (H) 1.14 (*d*, *J* = 6.8, H–C(21))/ δ (C) 42.4 (C(20)), 179.6 (C(22)), and 46.5 (C(17)) also confirmed this deduction. Thus, compound **2** was determined as 5 β ,19-epoxy-3,7-dioxo-23,24,25,26,27-pentanorcucurbitan-22-oic acid.

Kuguacin V (**3**) was obtained as white powder with the molecular formula C₃₀H₄₆O₄, as deduced from the HR-ESI-MS (*m/z* 471.3453 ([*M* + H]⁺), for C₃₀H₄₇O₄⁺; calc. 471.3474) and ¹³C-NMR data. The IR spectrum showed absorptions for OH (3509 cm⁻¹), isolated C=O (1693 cm⁻¹), and conjugated C=O (1619 cm⁻¹) functionalities. The ¹H-NMR spectrum of **3** displayed signals of seven Me groups (δ (H) 0.81 (*s*), 0.90 (*s*), 0.97 (*s*), 1.02 (*d*, *J* = 5.5 Hz), 1.28 (*s*), 1.83 (*s*), and 2.22 (*s*)), an olefinic H-atom at δ (H) 6.23 (*s*), and two *AB* systems (δ (H) 2.96, 2.48 (*AB*, *J* = 17.9, 1 H each, CH₂(6)) and 3.89, 3.57 (*AB*, *J* = 8.5, 1 H each, CH₂(19))). A comparison of the ¹³C-NMR and DEPT data of **3** with those of kuguacin E [15] revealed that both compounds possessed the same structures in rings *A*–*D*, except the presence of four additional C-atoms (δ (C) 124.9 (*d*), 154.2 (*s*), 27.3 (*q*), and 20.6 (*q*)) in **3** instead of a Me linked at C(23) in kuguacin E. In 2D-NMR spectra, HMBCs from δ (H) 2.54 (*br. d*, *J* = 12.5, H_a–C(22)) and 2.14–2.16 (*m*, H_b–C(22)) to δ (C) 33.4 (C(20)), 200.6 (C(23)), and 124.9, and from the olefinic H-atom signal at δ (H) 6.22 (*s*, 1 H), correlated in the HSQC spectrum with the resonance at δ (C) 124.9 (*d*), to the signals at δ (C) 51.8 (*t*, C(22)), 200.6 (*s*, C(23)), 154.2 (*s*), 27.3 (*q*), and 20.6 (*q*), further ascribed the four signals to C(24) (δ (C) 124.9 (*d*)), C(25) (δ (C) 154.2 (*s*)), C(26) (δ (C) 27.3 (*q*)), and C(27) (δ (C) 20.6 (*q*)). Me(26) and Me(27) were distinguished by a ROESY spectrum, in which the resonance at δ (H) 6.22 (*s*, H–C(24)) correlated with the signal at δ (H) 1.83 (*s*, Me(26)). Therefore, compound **3** was identified as 5 β ,19-epoxy-3 β -hydroxycucurbit-24-ene-7,23-dione.

The positive-ion HR-ESI-MS of kuguacin W (**4**) exhibited a molecular-ion peak at *m/z* 493.3273 ([*M* + Na]⁺), in accordance with the molecular formula C₃₀H₄₆NaO₄. Its IR spectrum showed absorptions attributable to OH (3521 cm⁻¹) and isolated C=O (1714 cm⁻¹) groups. In ¹H-NMR spectrum, signals of a CH₂ group (δ (H) 3.67, 3.57 (*d*, *J* = 8.7), as well as of seven Me groups were observed. Comparison of ¹H-

and ^{13}C -NMR data of **4** with those of **1** showed that both compounds possessed the same structures in rings *A*–*D*, with the differences in the side chain, which could be presumed by the replacement of $\text{C}(23)=\text{O}$ and CH_2 (24) groups in **1** by a $\text{C}(23)=\text{C}(24)$ moiety in **4**. Obvious HMBs from Me signals at $\delta(\text{H})$ 1.58 (*s*, Me(26), MeC(27)) to the signals at $\delta(\text{C})$ 69.8 (*s*, C(25)) and 141.8 (*d*, C(24)), and from the two CH_2 signals at $\delta(\text{H})$ 2.17–2.20 (H_a -C(22)) and 1.80–1.84 (H_b -C(22)) to the signals at $\delta(\text{C})$ 124.1 (*d*, C(23)), 141.8 (*d*, C(24)), 36.6 (*d*, C(20)), and 18.9 (*q*, C(21)), confirmed the above deduction. The single-crystal X-ray crystal structure (Fig. 2) of **4** established the proposed structure, and compound **4** was identified as 5 β ,19-epoxy-25-hydroxy-cucurbit-23-ene-3,7-dione.

Extracts of *M. charantia* also showed anticancer activities, and can be used as a dietary supplement for prevention of breast cancer [22]. *M. charantia* leaf extracts displayed antitumor activity by suppressing rat prostate cancer progression *in vitro* and *in vivo* [23]. Some of the cucurbitacins isolated from *M. charantia* were assayed for their cytotoxicities against five human tumor cell lines (HL-60, A-549, SK-BR-3, PANC-1, and SMMC-7721) by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method [24], using cisplatin as positive control. The results of the cytotoxicity assays (Table 2) revealed that only **7** and kuguacin J exhibited moderate or weak cytotoxic activities ($IC_{50} > 12.01 \mu\text{M}$) towards some cancer cell lines, whereas most of the tested compounds showed no significant activity, with IC_{50} values higher than $40 \mu\text{M}$. It is interesting that there is a minor but significant difference between compound **7** and the inactive metabolite **8**, consisting in the replacement of the OH group at C(25) in **7** by a MeO group in **8**.

This work was financially supported by NSFC (81373288), the Natural Science Foundation of Yunnan Province (2008CD158), the Western Doctoral Foundation of Chinese Academy of Sciences (J.-C. Chen), the Foundation of State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences (P2008ZZ23, P2010ZZ14), and the Cooperative Project of Guangdong Province and CAS (2009B091300135).

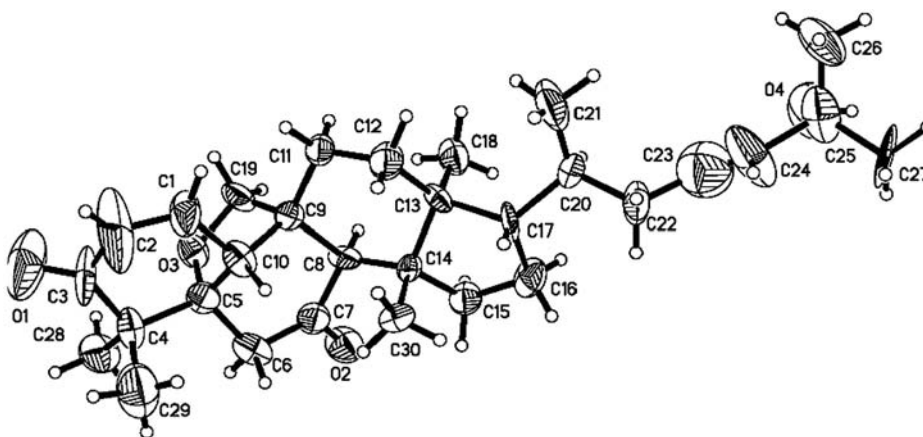


Fig. 2. X-Ray crystal structure of **4**

Table 2. Cytotoxic Activities of Tested Cucurbitacins (IC_{50} [μM])

Compound	HL-60	A-549	SK-BR-3	PANC-1	SMMC-7721
1	> 40	> 40	> 40	> 40	> 40
2	> 40	> 40	> 40	> 40	> 40
4	> 40	> 40	> 40	> 40	> 40
5	> 40	> 40	> 40	> 40	> 40
7	> 40	> 40	> 40	> 40	> 40
8	12.01	17.81	16.57	37.64	> 40
Kuguacin G	> 40	> 40	> 40	> 40	> 40
Kuguacin H	> 40	> 40	> 40	> 40	> 40
Kuguacin I	> 40	> 40	> 40	> 40	> 40
Kuguacin J	13.54	20.81	14.58	21.77	33.11
Kuguacin K	> 40	> 40	> 40	> 40	> 40
Kuguacin N	> 40	> 40	> 40	> 40	> 40
Cisplatin	1.67	19.36	29.70	17.38	37.97

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2 , 200–300 mesh; *Qingdao Marine Chemical Inc.*, Qingdao, P. R. China), or *Lichroprep RP-18* gel (40–63 μm ; *Merck*, DE-Darmstadt). Fractions were monitored by TLC, and spots were visualized by heating silica-gel plates sprayed with 15% H_2SO_4 in H_2O . M.p.: *Tech X-4* digital display micromelting point apparatus; uncorrected. Optical rotations: *PerkinElmer 241* polarimeter. UV Spectra: *Shimadzu* double-beam *210A* spectrophotometer. IR Spectra: *Bio-Rad FTS-135* spectrometer with KBr pellets. ^1H -, ^{13}C -, and 2D-NMR Spectra: *Bruker AM-400* or *Bruker DRX-500* instruments, with TMS as internal standard. ESI-MS: *Bruker HCT Esquire 3000* spectrometer. HR-ESI-MS: *Agilent 6210 TOF LC/MS*; m/z .

Plant Material. The fresh fruits (140 kg) were collected at Midu County, Yunnan Province, P. R. China, in October 2007. The sample was identified by Prof. *Shu-Kun Chen*, and a voucher specimen (No. KIB 2007-10-14) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy Sciences, Kunming, P. R. China.

Extraction and Isolation. The fresh fruit (140 kg) was cut, and then extracted with acetone (3×50 l, each time for 3 d) at r.t. After removal of the solvent under reduced pressure, a residue (1,540 g) was obtained. This extract was dissolved in H_2O (3 l) and then extracted with AcOEt (3×6 l) to furnish a residue (249 g), which was then subjected to CC (SiO_2 ; $\text{CHCl}_3/\text{MeOH}$ 1:0, 20:1, 0:1) to yield *Frs. I–III*. *Fr. II* (157 g) was then purified by CC (SiO_2 ; $\text{CHCl}_3/\text{MeOH}$ 100:1, 50:1, 30:1, 20:1) to afford *Frs. A–D*. *Fr. B* (10 g) was subjected to CC (SiO_2 ; $\text{CHCl}_3/\text{MeOH}$ 1:0, 50:1) to give *Frs. B1–B3*, monitored by TLC. *Fr. B2* (3 g) was submitted to repeated CC (SiO_2 ; $\text{CHCl}_3/\text{MeOH}$ from 100:1 \rightarrow 30:1; and *RP-18*; $\text{MeOH}/\text{H}_2\text{O}$ from 60:40 \rightarrow 75:25), followed by CC (*Sephadex LH-20*; MeOH) to yield compounds **4** (14 mg) and **13** (21 mg). *Fr. C* (40 g) was separated by CC (SiO_2 ; $\text{CHCl}_3/\text{MeOH}$, from 100:1 \rightarrow 20:1) into *Frs. C1–C4*. *Fr. C1* (5 g) was subjected to CC (SiO_2 ; $\text{CHCl}_3/\text{MeOH}$ from 50:1 \rightarrow 30:1), and further purified by CC (*RP-18*; $\text{MeOH}/\text{H}_2\text{O}$ from 55:45 \rightarrow 75:25) to give pure **1** (23 mg), **3** (34 mg), and **12** (11 mg). Compound **2** (91 mg) was crystallized from *Fr. C2* (7 g) in MeOH . Compounds **5** (7 mg), **6** (71 mg), **8** (107 mg), and **11** (27 mg) were isolated from *Fr. C3* (25 g) by CC (SiO_2 ; $\text{CHCl}_3/\text{MeOH}$ from 40:1 \rightarrow 20:1; *RP-18*; $\text{MeOH}/\text{H}_2\text{O}$ from 55:45 \rightarrow 70:30, and then *Sephadex LH-20*; MeOH). *Fr. D* (11 g) was by CC (SiO_2 ; $\text{CHCl}_3/\text{MeOH}$ from 30:1 \rightarrow 10:1) into *Frs. D1–D4*. Compound **7** (1100 mg) was crystallized from *Fr. D2* (6 g) in MeOH . *Fr. D3* was further subjected to repeated CC (SiO_2 ; $\text{CHCl}_3/\text{MeOH}$ from 30:1 \rightarrow 20:1; and *RP-18*; $\text{MeOH}/\text{H}_2\text{O}$ from 50:50 \rightarrow 70:30) to furnish a mixture (231 mg) of compounds **9** and **10**, with a ratio of ca. 1:1 as determined by ^{13}C -NMR spectroscopy.

Kuguacin T (= 5 β ,19-Epoxy-25-hydroxycucurbitane-3,7,23-trione = (5R,8S,9R,10S,13R,14S,17R)-Decahydro-17-[(2R)-6-hydroxy-6-methyl-4-oxoheptan-2-yl]-4,4,13,14-tetramethyl-2H-5,9-(epoxymethano)cyclopenta[a]phenanthrene-3,7(4H,6H)-dione; **1**). Colorless needles (MeOH). M.p. 193–195 $^\circ$.

$[\alpha]_D^{23} = +1.1$ ($c = 0.09$, MeOH). UV: 202. IR: 3460, 2962, 2878, 1710, 1466, 1383. ^1H - and ^{13}C -NMR: see Table I. ESI-MS (pos.): 509 ($[M + \text{Na}]^+$), 468 ($[M - \text{H}_2\text{O}]^+$). HR-ESI-MS: 509.3212 ($[M + \text{Na}]^+$, $\text{C}_{30}\text{H}_{46}\text{NaO}_5^+$; calc. 509.3242).

Kuguacin U (5 β ,19-Epoxy-3,7-dioxo-23,24,25,26,27-pentanorcucurbitan-22-oic Acid = (2S)-2-[(5R,8S,9R,10S,13R,14S,17R)-Decahydro-4,4,13,14-tetramethyl-3,7-dioxotetra-2H-5,9-(epoxymethano)cyclopenta[a]phenanthren-17-yl]propanoic Acid; **2**). Colorless needles (MeOH). M.p. 295–296°. $[\alpha]_D^{23} = +14.8$ ($c = 0.07$, MeOH). UV 203. IR: 3424, 2976, 2877, 1710, 1451, 1387, 1033. ^1H - and ^{13}C -NMR: see Table I. ESI-MS (pos.): 439 ($[M + \text{Na}]^+$), 855 ($[2M + \text{Na}]^+$). HR-ESI-MS: 417.2609 ($[M + \text{H}]^+$, $\text{C}_{25}\text{H}_{37}\text{O}_5^+$; calc. 417.2640).

Kuguacin V (5 β ,19-Epoxy-3 β -hydroxycucurbit-24-ene-7,23-dione = (3S,5R,8S,9R,10S,13R,14S,17R)-Dodecahydro-3-hydroxy-4,4,13,14-tetramethyl-17-[(2R)-6-methyl-4-oxohept-5-ene-2-yl]-2H-5,9-(epoxymethano)cyclopenta[a]phenanthren-7(6H)-one; **3**). White powder. $[\alpha]_D^{23} = -26.6$ ($c = 0.09$, pyridine). UV: 220. IR: 3509, 2955, 2876, 1693, 1619, 1444, 1036. ^1H - and ^{13}C -NMR: see Table I. ESI-MS (pos.): 493 ($[M + \text{Na}]^+$), 963 ($[2M + \text{Na}]^+$). HR-ESI-MS: 471.3453 ($[M + \text{H}]^+$, $\text{C}_{30}\text{H}_{47}\text{O}_4^+$; calc. 471.3474).

Kuguacin W (5 β ,19-Epoxy-25-hydroxycucurbit-23-ene-3,7-dione = (5R,8S,9R,10S,13R,14S,17R)-Decahydro-17-[(2R,4E)-6-hydroxy-6-methylhept-4-en-2-yl]-4,4,13,14-tetramethyl-2H-5,9-(epoxymethano)cyclopenta[a]phenanthrene-3,7(4H,6H)-dione; **4**). Colorless needles (MeOH). M.p. 113–115°. $[\alpha]_D^{23} = -15.7$ ($c = 0.04$, MeOH). UV: 220. IR: 3521, 2958, 1714, 1466, 1373. ^1H - and ^{13}C -NMR: see Table I. ESI-MS (pos.): 494 ($[M + \text{H} + \text{Na}]^+$), 452 ($[M - \text{H}_2\text{O}]^+$). HR-ESI-MS: 493.3273 ($[M + \text{Na}]^+$, $\text{C}_{30}\text{H}_{46}\text{NaO}_4^+$; calc. 493.3293).

Cytotoxic Assays. Cytotoxic activities against human promyelocytic leukemia (HL-60), human hepatocellular carcinoma (SMMC-7721), carcinomic human alveolar basal epithelial (A-549), human breast cancer (SK-BR-3) cells, and human pancreatic adenocarcinoma (PANC-1) cells were tested according to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazoliumbromide (MTT) method [24]. Cisplatin was used as reference compound to evaluate the cytotoxicity of tested compounds against the five cell lines, resp. Briefly, cells were plated in 96-well plates 24 h before treatment and continuously exposed to different concentrations of tested compounds. After incubation for 48 h, MTT soln. (40 μM , 20 μl) was added to each well, which were incubated for a further 4 h. Then, 20% sodium dodecyl sulfate (100 μl) was added to each well. After 12 h at r.t., the IC_{50} values were calculated by the Reed–Muench method [25].

X-Ray Crystal Structure Analyses of 4. $\text{C}_{30}\text{H}_{46}\text{O}_4$; M_r 468.65; orthorhombic system; space group $P2_12_12_1$; $a = 6.583(3)$ Å, $b = 10.929(5)$ Å, $c = 18.986(9)$ Å, $V = 1359.1(11)$ Å³, $Z = 2$, $D_{\text{calc}} = 1.145$ g/cm³, crystal dimensions 0.23 \times 0.17 \times 0.12 mm; measurements on a Bruker APEX II diffractometer with a graphite monochromator (ω scans, $2\theta_{\text{max}} = 56.78^\circ$), MoK_α radiation. The total number of independent reflections measured was 7724, of which 4342 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: $R_1 = 0.0925$, $wR_2 = 0.1654$. The crystal structure of **4** was solved by the direct method SHELXS-97 and expanded using difference Fourier techniques, refined by the program SHELXL-97 and the full-matrix least-squares calculations. Crystallographic data for the structure has been deposited with the Cambridge Crystallographic Data Centre (deposition No. CCDC-899214). These data can be obtained free of charge via http://www.cam.ac.uk/data_request/cif.

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Received February 14, 2014