# 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 chrantia*. 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 $C_{30}H_{46}O_5$, deduced from the positive-ion HR-ESI-MS (*m*/*z* 509.3212 ($[M+Na]^+$, $C_{30}H_{46}NaO_5^+$; calc. 509.3242)) and $^{13}$C-NMR data. In the $^1$H-NMR spectrum, signals of seven Me groups ($\delta$(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 ($\delta$(H)

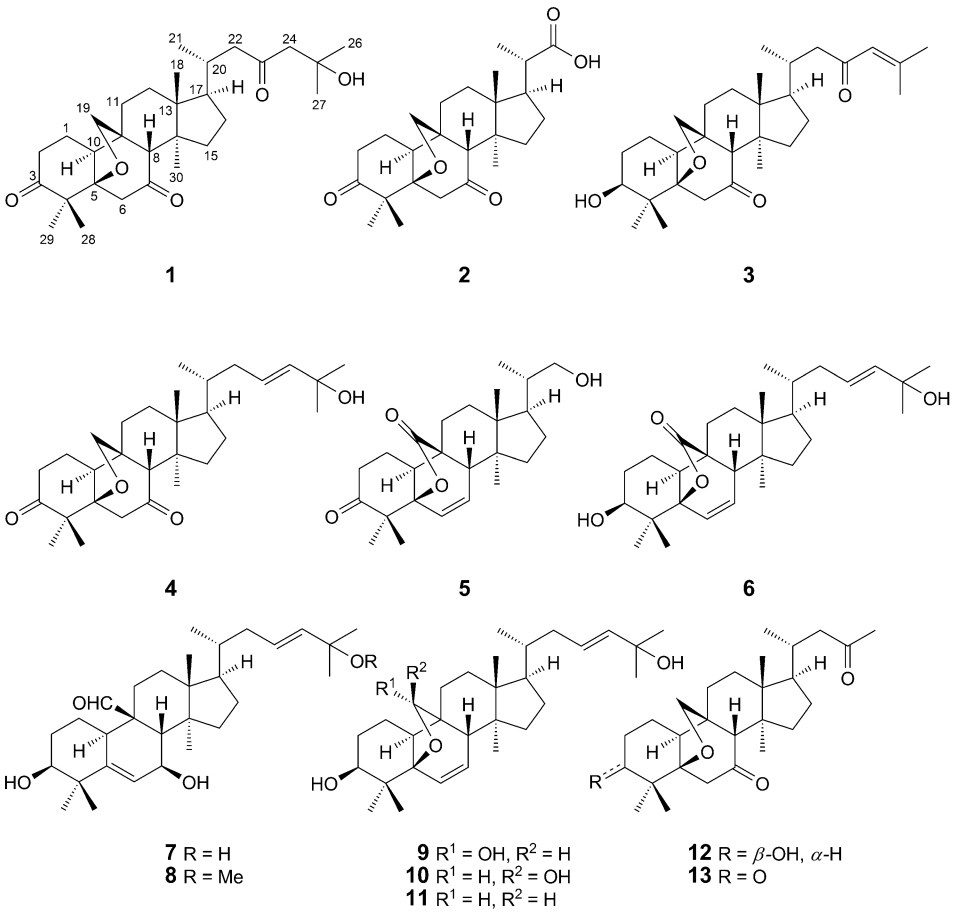


Fig. 1. *Chemical structures of compounds* **1**–**13**

2.75, 2.90 (*AB*, $J = 18.1$, $CH_2(6)$), and a $CH_2$ group ($\delta$(H) 3.55, 3.65 (*d*, $J = 8.7$, $CH_2(19)$) were observed (*Table 1*). The $^{13}$C-NMR and DEPT spectra revealed the presence of 30 C-atoms including those of seven Me, ten $CH_2$, and four CH groups, and nine quaternary C-atoms, which indicated a triterpene compound. A typical $^1$H-NMR signal at $\delta$(H) 3.01 (*dd*, $J = 10.4$, 6.5, 1 H), which was tentatively ascribed to H–C(10), suggested a cucurbitane skeleton [14]. Further, comparison of the NMR data of **1** with those of kuguacin H indicated that these two compounds were structurally very similar with the exception of the signals ascribed to ring *B* [14]. Comparison of $^1$H- and $^{13}$C-NMR data of **1** with those of kuguacin H showed that the differences can be rationalized by the replacement of an CHO group at C(9) and a C(5)=C(6) bond in kuguacin H by a $CH_2OH$ group at C(9), a tertiary ether group at C(5), and a $CH_2(6)$ group in **1**. Considering that the signals of C(19) and C(5) were markedly downshifted to $\delta$(C) 79.5 (C(19)) and $\delta$(C) 91.9 (C(5)), respectively, an 5,19-epoxy functionality was proposed. Obvious HMBCs observed from $\delta$(H) 2.90 and 2.75 (*AB*, $J = 18.1$, 1 H each,

Table 1. *$^1$H- and $^{13}$C-NMR Data of Compounds* **1**–**4**. In ($D_5$)pyridine; $\delta$ in ppm; $J$ in Hz.

| Position | **1** | | **2** | | **3** | | **4** | |
|---|---|---|---|---|---|---|---|---|
| | $\delta$(H)[a]) | $\delta$(C)[b]) | $\delta$(H)[c]) | $\delta$(C)[d]) | $\delta$(H)[c]) | $\delta$(C)[d]) | $\delta$(H)[a]) | $\delta$(C)[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** | |
|---|---|---|---|---|---|---|---|---|
| | $\delta$(H)[a]) | $\delta$(C)[b]) | $\delta$(H)[c]) | $\delta$(C)[d]) | $\delta$(H)[c]) | $\delta$(C)[d]) | $\delta$(H)[a]) | $\delta$(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*) | 1.12 (*s*) | 17.2 (*q*) | 1.28 (*s*) | 21.2 (*q*) | 1.28 (*s*) | 17.9 (*q*) |
| 29 | 1.14 (*s*) | 25.3 (*q*) | 1.04 (*s*) | 25.5 (*q*) | 0.97 (*s*) | 26.2 (*q*) | 1.19 (*s*) | 25.4 (*q*) |
| 30 | 0.99 (*s*) | 21.3 (*q*) | 0.97 (*s*) | 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.

$CH_2$(6)) to $\delta$(C) 91.9 (C(5)), 41.3 (C(10)), and 212.5 (C(7)), and from $\delta$(H) 3.65 and 3.55 (2*d*, $J = 8.7$, 1 H each, $CH_2$(19)) to $\delta$(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 $\beta$-oriented. Thus, the structure of **1** was elucidated as 5$\beta$,19-epoxy-25-hydroxy-cucurbitane-3,7,23-trione.

The molecular formula of kuguacin U (**2**) was determined as $C_{25}H_{36}O_5$ by the positive-ion HR-ESI-MS spectrum (*m*/*z* 417.2609 ($[M+H]^+$)). The $^1$H-NMR spectrum of **2** displayed signals of five Me groups ($\delta$(H) 0.84 (*s*), 0.97 (*s*), 1.04 (*s*), 1.12 (*s*), and 1.14 (*d*, $J = 6.8$)) and two $CH_2$ groups ($\delta$(H) 2.47, 2.70 (*AB*, $J = 18.3$, $CH_2$(6)) and 3.65, 3.47 (*d*, $J = 8.8$, $CH_2$(19))). The $^{13}$C-NMR and DEPT spectra showed signals for 25 C-atoms, including those of five Me, eight $CH_2$ 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 ($\delta$(C) 179.6 (C(22)) in **2**, suggesting a 5$\beta$,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 $\delta$(H) 1.14 (*d*, $J = 6.8$, H–C(21))/$\delta$(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$\beta$,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 $C_{30}H_{46}O_4$, as deduced from the HR-ESI-MS (*m*/*z* 471.3453 ($[M+H]^+$), for $C_{30}H_{47}O_4^+$; calc. 471.3474) and $^{13}$C-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 $^1$H-NMR spectrum of **3** displayed signals of seven Me groups ($\delta$(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 $\delta$(H) 6.23 (*s*), and two *AB* systems ($\delta$(H) 2.96, 2.48 (*AB*, $J = 17.9$, 1 H each, $CH_2$(6)) and 3.89, 3.57 (*AB*, $J = 8.5$, 1 H each, $CH_2$(19))). A comparison of the $^{13}$C-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 ($\delta$(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 $\delta$(H) 2.54 (br. *d*, $J = 12.5$, $H_a$–C(22)) and 2.14 – 2.16 (*m*, $H_b$–C(22)) to $\delta$(C) 33.4 (C(20)), 200.6 (C(23)), and 124.9, and from the olefinic H-atom signal at $\delta$(H) 6.22 (*s*, 1 H), correlated in the HSQC spectrum with the resonance at $\delta$(C) 124.9 (*d*), to the signals at $\delta$(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) ($\delta$(C) 124.9 (*d*)), C(25) ($\delta$(C) 154.2 (*s*)), C(26) ($\delta$(C) 27.3 (*q*)), and C(27) ($\delta$(C) 20.6 (*q*)). Me(26) and Me(27) were distinguished by a ROESY spectrum, in which the resonance at $\delta$(H) 6.22 (*s*, H–C(24)) correlated with the signal at $\delta$(H) 1.83 (*s*, Me(26)). Therefore, compound **3** was identified as 5$\beta$,19-epoxy-3$\beta$-hydroxy-cucurbit-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 $C_{30}H_{46}NaO_4$. Its IR spectrum showed absorptions attributable to OH (3521 cm$^{-1}$) and isolated C=O (1714 cm$^{-1}$) groups. In $^1$H-NMR spectrum, signals of a $CH_2$ group ($\delta$(H) 3.67, 3.57 (*d*, $J = 8.7$), as well as of seven Me groups were observed. Comparison of $^1$H-

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 C(23)=O and $CH_2$ (24) groups in **1** by a C(23)=C(24) moiety in **4**. Obvious HMBCs from Me signals at $\delta$(H) 1.58 (*s*, Me(26), MeC(27)) to the signals at $\delta$(C) 69.8 (*s*, C(25)) and 141.8 (*d*, C(24)), and from the two $CH_2$ signals at $\delta$(H) 2.17 – 2.20 ($H_a$–C(22)) and 1.80 – 1.84 ($H_b$–C(22)) to the signals at $\delta$(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$\beta$,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*-tetraazolium 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 ($IC_{50} > 12.01$ μM) towards some cancer cell lines, whereas most of the tested compounds showed no significant activity, with $IC_{50}$ values higher than 40 μM. 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).

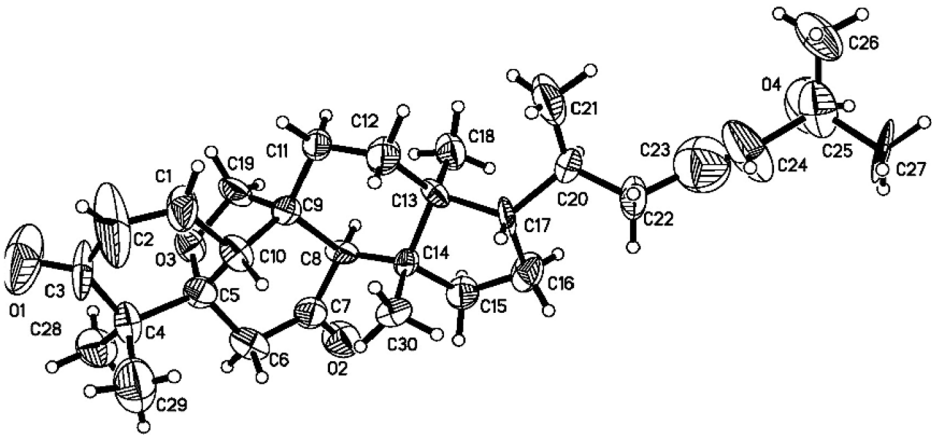


Fig. 2. *X-Ray crystal structure of* **4**

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. $^1$H-, $^{13}$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 ($SiO_2$; $CHCl_3$/MeOH 1:0, 20:1, 0:1) to yield *Frs. I–III*. *Fr. II* (157 g) was then purified by CC ($SiO_2$; $CHCl_3$/MeOH 100:1, 50:1, 30:1, 20:1) to afford *Frs. A–D*. *Fr. B* (10 g) was subjected to CC ($SiO_2$; $CHCl_3$/MeOH 1:0, 50:1) to give *Frs. B1–B3*, monitored by TLC. *Fr. B2* (3 g) was submitted to repeated CC ($SiO_2$; $CHCl_3$/MeOH from 100:1 → 30:1; and *RP-18*; MeOH/$H_2O$ from 60:40 → 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 ($SiO_2$; $CHCl_3$/MeOH, from 100:1 → 20:1) into *Frs. C1–C4*. *Fr. C1* (5 g) was subjected to CC ($SiO_2$; $CHCl_3$/MeOH from 50:1 → 30:1), and further purified by CC (*RP-18*; MeOH/$H_2O$ from 55:45 → 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 ($SiO_2$; $CHCl_3$/MeOH from 40:1 → 20:1; *RP-18*; MeOH/$H_2O$ from 55:45 → 70:30, and then *Sephadex LH-20*; MeOH). *Fr. D* (11 g) was by CC ($SiO_2$; $CHCl_3$/MeOH from 30:1 → 10:1) into *Frs. D1–D4*. Compound **7** (1100 mg) was crystallized from *Fr. D*2 (6 g) in MeOH. *Fr. D3* was further subjected to repeated CC ($SiO_2$; $CHCl_3$/MeOH from 30:1 → 20:1; and *RP-18*; MeOH/$H_2O$ from 50:50 → 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* (*=5β,19-Epoxy-25-hydroxycucurbitane-3,7,23-trione = (5*R,*8*S,*9*R,*10*S,*13*R,*14*S,*17*R*)-Decahydro-17-[(2*R*)-6-hydroxy-6-methyl-4-oxoheptan-2-yl]-4,4,13,14-tetramethyl-2*H*-5,9-(epoxymethano)cyclopenta[a]phenanthrene-3,7(4*H,*6*H*)-dione*; **1**). Colorless needles (MeOH). M.p. 193–195°.

$[\alpha]_D^{23} = +1.1$ ($c = 0.09$, MeOH). UV: 202. IR: 3460, 2962, 2878, 1710, 1466, 1383. $^1$H- and $^{13}$C-NMR: see *Table 1*. ESI-MS (pos.): 509 ($[M+Na]^+$), 468 ($[M - H_2O]^+$). HR-ESI-MS: 509.3212 ($[M+Na]^+$, $C_{30}H_{46}NaO_5^+$; calc. 509.3242).

*Kuguacin U* (*5β,19-Epoxy-3,7-dioxo-23,24,25,26,27-pentanorcucurbitan-22-oic Acid* = (2S)-*2-[(5*R,*8*S,*9*R,*10*S,*13*R,*14*S,*17*R*)-Decahydro-4,4,13,14-tetramethyl-3,7-dioxotetra-2*H-*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. $^1$H- and $^{13}$C-NMR: see *Table 1*. ESI-MS (pos.): 439 ($[M+Na]^+$), 855 ($[2M+Na]^+$). HR-ESI-MS: 417.2609 ($[M+H]^+$, $C_{25}H_{37}O_5^+$; calc. 417.2640).

*Kuguacin V* (*5β,19-Epoxy-3β-hydroxycucurbit-24-ene-7,23-dione* = (*3*S,*5*R,*8*S,*9*R,*10*S,*13*R,*14*S,*17*R*)-Dodecahydro-3-hydroxy-4,4,13,14-tetramethyl-17-[(2*R*)-6-methyl-4-oxohept-5-ene-2-yl]-2*H-*5,9-(epoxymethano)cyclopenta[*a*]*phenanthren-7(6*H*)-one*; **3**). White powder. $[\alpha]_D^{23} = -26.6$ ($c = 0.09$, pyridine). UV: 220. IR: 3509, 2955, 2876, 1693, 1619, 1444, 1036. $^1$H- and $^{13}$C-NMR: see *Table 1*. ESI-MS (pos.): 493 ($[M+Na]^+$), 963 ($[2M+Na]^+$). HR-ESI-MS: 471.3453 ($[M+H]^+$, $C_{30}H_{47}O_4^+$; calc. 471.3474).

*Kuguacin W* (*5β,19-Epoxy-25-hydroxycucurbit-23-ene-3,7-dione* = (*5*R,*8*S,*9*R,*10*S,*13*R,*14*S,*17*R*)-Decahydro-17-[(2*R*,*4*E)-6-hydroxy-6-methylhept-4-en-2-yl]-4,4,13,14-tetramethyl-2*H-*5,9-(epoxymethano)cyclopenta[*a*]*phenanthrene-3,7(4*H,*6*H*)-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. $^1$H- and $^{13}$C-NMR: see *Table 1*. ESI-MS (pos.): 494 ($[M+H+Na]^+$), 452 ($[M - H_2O]^+$). HR-ESI-MS: 493.3273 ($[M+Na]^+$, $C_{30}H_{46}NaO_4^+$; 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-dimethylthiahiazol-2-y1)-2,5-diphenyl-2*H*-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**. $C_{30}H_{46}O_4$; $M_r$ 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, $V = 1359.1(11)$ Å$^3$, $Z = 2$, $D_{calc} = 1.145$ g/cm$^3$, crystal dimensions $0.23 \times 0.17 \times 0.12$ mm; measurements on a *Bruker APEX II* diffractometer with a graphite monochromator ($\omega$ scans, $2\theta_{max} = 56.78°$), Mo$K_\alpha$ 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.

## REFERENCES


[1] L. Harinantenaina, M. Tanaka, S. Takaoka, M. Oda, O. Mogami, M. Uchida, Y. Asakawa, *Chem. Pharm. Bull.* **2006**, *54*, 1017.

[2] M.-J. Tan, J.-M. Ye, N. Turner, C. Hohnen-Behrens, C.-Q. Ke, C.-P. Tang, T. Chen, H.-C. Weiss, E.-R. Gesing, A. Rowland, D. E. James, Y. Ye, *Chem. Biol.* **2008**, *15*, 263.

[3] A. C. Keller, J. Ma, A. Kavalier, K. He, A.-M. B. Brillantes, E. J. Kennelly, *Phytomedicine* **2011**, *19*, 32.

[4] Z.-J. Zhu, Z.-C. Zhong, Z.-Y. Luo, Z.-Y. Xiao, *Acta Pharm. Sin.* **1990**, *25*, 898.

[5] T. Akihisa, N. Higo, H. Tokuda, M. Ukiya, H. Akazawa, Y. Tochigi, Y. Kimura, T. Suzuki, H. Nishino, *J. Nat. Prod.* **2007**, *70*, 1233.

[6] C.-R. Chen, Y.-W. Liao, L. Wang, Y.-H. Kuo, H.-J. Liu, W.-L. Shih, H.-L. Cheng, C.-I Chang, *Chem. Pharm. Bull.* **2010**, *58*, 1639.
[7] P. Pitchakarn, S. Suzuki, K. Ogawa, W. Pompimon, S. Takahashi, M. Asamoto, P. Limtrakul, T. Shirai, *Cancer Lett.* **2011**, *306*, 142.
[8] X. Wang, W. Sun, J. Cao, H. Qu, X. Bi, Y. Zhao, *J. Agric. Food Chem.* **2012**, *60*, 3927.
[9] C. Hsu, C.-L. Hsieh, Y.-H. Kuo, C.-j. Huang, *J. Agric. Food Chem.* **2011**, *59*, 4553.
[10] J. Zhang, Y. Huang, T. Kikuchi, H. Tokuda, N. Suzuki, K.-i. Inafuku, M. Miura, S. Motohashi, T. Suzuki, T. Akihisa, *Chem. Biodiversity* **2012**, *9*, 428.
[11] C. Ramalhete, F. P. da Cruz, D. Lopes, S. Mulhovo, V. E. Rosário, M. Prudêncio, M.-J. U. Ferreira, *Bioorg. Med. Chem.* **2011**, *19*, 7474.
[12] K.-W. Lin, S.-C. Yang, C.-N. Lin, *Food Chem.* **2011**, *127*, 609.
[13] C.-H. Liu, M.-H. Yen, S.-F. Tsang, K.-H. Gan, H.-Y. Hsu, C.-N. Lin, *Food Chem.* **2010**, *118*, 751.
[14] J.-C. Chen, W.-Q. Liu, L. Lu, M.-H. Qiu, Y.-T. Zheng, L.-M. Yang, X.-M. Zhang, L. Zhou, Z.-R. Li, *Phytochemistry* **2009**, *70*, 133.
[15] J. Chen, R. Tian, M. Qiu, L. Lu, Y. Zheng, Z. Zhang, *Phytochemistry* **2008**, *69*, 1043.
[16] J.-C. Chen, L. Lu, X.-M. Zhang, L. Zhou, Z.-R. Li, M.-H. Qiu, *Helv. Chim. Acta* **2008**, *91*, 920.
[17] J.-Q. Liu, J.-C. Chen, C.-F. Wang, M.-H. Qiu, *Molecules* **2009**, *14*, 4804.
[18] H. Matsuda, S. Nakamura, T. Murakami, M. Yoshikawa, *Heterocycles* **2007**, *71*, 331.
[19] M. O. Fatope, Y. Takeda, H. Yamashita, H. Okabe, T. Yamauchi, *J. Nat. Prod.* **1990**, *53*, 1491.
[20] S. Begum, M. Ahmed, B. S. Siddiqui, A. Khan, Z. S. Saify, M. Arif, *Phytochemistry* **1997**, *44*, 1313.
[21] C.-I Chang, C.-R. Chen, Y.-W. Liao, H.-L. Cheng, Y.-C. Chen, C.-H. Chou, *J. Nat. Prod.* **2006**, *69*, 1168.
[22] R. B. Ray, A. Raychoudhuri, R. Steele, P. Nerurkar, *Cancer Res.* **2010**, *70*, 1925.
[23] P. Pitchakarn, K. Ogawa, S. Suzuki, S. Takahashi, M. Asamoto, T. Chewonarin, P. Limtrakul, T. Shirai, *Cancer Sci.* **2010**, *101*, 2234.
[24] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.
[25] L. J. Reed, H. A. Muench, *Am. J. Hyg.* **1938**, *27*, 493.

*Received February 14, 2014*