# Four Novel Diterpenoids from *Crinipellis* sp. 113

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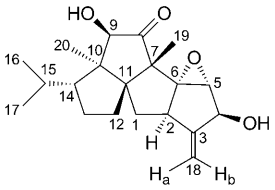
Four novel diterpenoids, namely (4$\beta$)-4,4-*O*-dihydrocrinipellin A (**1**), (4$\beta$,8$\alpha$)-4,4-*O*,8,8-*O*-tetrahydrocrinipellin B (**2**), crinipellin C (**3**), and crinipellin D (**4**), along with three known ones, (3$\beta$,4$\beta$)-3,3-*C*,4,4-*O*-tetrahydrocrinipellin A (**5**), (4$\beta$)-4,4-*O*-dihydrocrinipellin B (**6**), and phlebiakauranol alcohol, were isolated from the fungal strain *Crinipellis* sp. 113. Their structures were elucidated by spectroscopic analyses, including 1D- and 2D-NMR experiments, and by HR-Q-TOF mass spectrometry. Antitumor and antibacterial assays with the novel compounds **1**–**4** were carried out, showing moderate activities against HeLa cells and no effects on the growth of tested bacteria or yeast.

**Introduction.** – *Crinipellis stipitaria* (Agaricales) is a fungus that grows on both the dead and living parts of grasses. Previously, one antibacterial metabolite named 'crinipellin' was isolated from this species without structure elucidations [1]. Some years later, an investigation of several strains of this fungus led to the isolation of several 'crinipellin'-related compounds, namely crinipellin A, crinipellin B, and 9-*O*-acetylcrinipellin A. According to the molecular formula, 9-*O*-acetylcrinipellin A was determined to be the previous 'crinipellin' [1][2]. In addition to these antibiotically active compounds, two inactive accompanying substances, dihydrocrinipellin B and tetrahydrocrinipellin A, were also found [2]. Recently, we isolated a fungal strain, named 113, from Zhujiangyuan in Yunnan Province, China. This strain was determined to be *Crinipellis* sp. based on its ITS sequence of rDNA (ITS1-5.8S-ITS2). We now report the isolation from this strain and structure determination of four novel diterpenoids, namely (4$\beta$)-4,4-*O*-dihydrocrinipellin A[1]) (**1**), (4$\beta$,8$\alpha$)-4,4-*O*,8,8-*O*-tetrahydrocrinipellin B[1]) (**2**), crinipellin C[1]) (**3**), and crinipellin D[1]) (**4**), along with three known ones, (3$\beta$,4$\beta$)-3,3-*C*,4,4-*O*-tetrahydrocrinipellin A (**5**) [2], (4$\beta$)-4,4-*O*-dihydrocrinipellin B (**6**) [2], and phlebiakauranol alcohol [3], and the antitumor and antibacterial activities of compounds **1**–**4**.

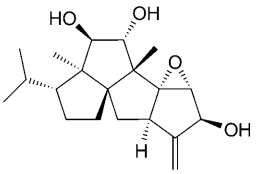
**Results and Discussion.** – 1. *Structure Elucidation.* The morphological properties of the isolate 113 were examined after incubation for 14 d on potato/dextrose agar (PDA) medium at 28°. This organism was identified to be *Crinipellis* sp. according to its ITS sequence of rDNA (ITS1-5.8S-ITS2). This fungal strain 113 was extracted with AcOEt/

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

MeOH/AcOH 80:15:5. After evaporation of the organic solvents, the crude extract was partitioned between $H_2O$ and AcOEt (1:1) until the organic layer was colorless. The organic phase was concentrated to a brown syrup, and the latter purified by repeated column chromatography (*RP-18*, *Sephadex LH-20*, and silica gel) to afford compounds **1**–**6** and phlebiakauranol alcohol. Their structures were elucidated on the basis of spectroscopic data, including $^1$H- and $^{13}$C-NMR, DEPT, HSQC, HMBC, $^1$H,$^1$H-COSY, and ROESY.




**1**[1])

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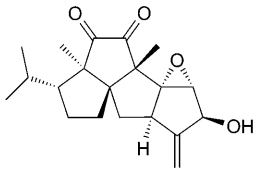


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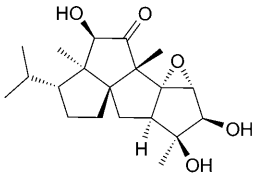


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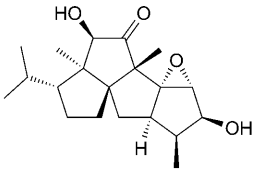
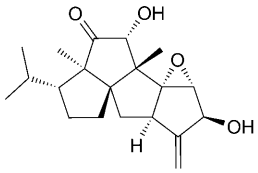



**5**

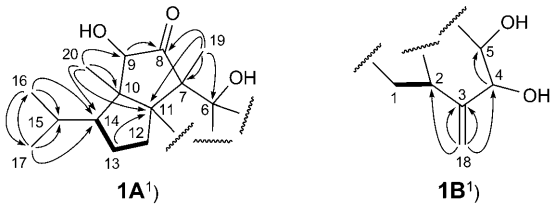



**6**

Compound **1** was deduced to have the molecular formula $C_{20}H_{28}O_4$ based on the HR-Q-TOF-MS data (*m/z* 355.2729 ($[M+Na]^+$)). The IR spectrum indicated the presence of OH groups (3330 $cm^{-1}$) and of a C=O group (1745 $cm^{-1}$). The $^{13}$C-NMR spectra of **1** (*Table 1*) displayed six quaternary C-atoms (one O-bearing, one olefinic, and one C=O), six CH (three O-bearing), four $CH_2$ (one olefinic), and four Me groups. Inspection of the $^1$H,$^1$H-COSY, HMQC, and HMBC data readily revealed a crinipellin-type structure for **1** [2]. The HMBCs from the H-atoms of four Me groups to the corresponding C-atoms, along with the $^1$H,$^1$H-COSYs $CH_2$(12)/$CH_2$(13)/H–C(14) established fragment **1A** (*Fig. 1*). Furthermore, the HMBC spectra showed that the exocyclic $CH_2$(18) was correlated with C(2), C(3), and C(4), and H–C(4) was correlated with C(5). In combination with the $^1$H,$^1$H-COSY $CH_2$(1)/H–C(2), this led to the establishment of fragment **1B** (*Fig. 1*). Finally, the HMBCs from H–C(2) and H–C(4) to C(6), and from $CH_2$(12) to C(1) implied that the fragments **1A** and **1B** were linked through three C–C single bonds. Additionally, the chemical shifts of C(5) ($\delta$(C) 64.6) and C(6) ($\delta$(C) 81.6) were shifted upfield compared to regular O-bearing CH groups and/or quaternary C-atoms, indicating the presence of an epoxy group between C(5) and C(6). This was further supported by HR-Q-TOF-MS data, which indicated the presence of four rather than five O-atoms in **1**. The relative configuration of **1** was determined by the analysis of the ROESY plot. The presence of the NOE correlations H–C(14) ↔ Me(19) ↔ H–C(5), and H–C(4) ↔ H–C(2) ↔ H–C(9) ↔ Me(20) indicated that H–C(5), H–C(14), and Me(19) were on the same side and in $\beta$-orientation, while H–C(2), H–C(4), H–C(9), and Me(20) were in $\alpha$-orientation (*Fig. 2*). Thus, the structure of compound **1** was established to be 5b,6-epoxydecahydro-4,7-dihydroxy-

Table 1. *$^{13}$C-NMR Data* ((D$_5$)pyridine) *of Compounds* **1**–**4**. $\delta$ in ppm.

| | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| $CH_2$(1) | 39.5 (*t*) | 39.3 (*t*) | 37.8 (*t*) | 33.3 (*t*) |
| CH(2) | 47.7 (*d*) | 49.6 (*d*) | 46.1 (*d*) | 52.2 (*d*) |
| C(3) | 158.2 (*s*) | 159.1 (*s*) | 155.1 (*s*) | 82.6 (*s*) |
| CH(4) | 75.3 (*d*) | 75.2 (*d*) | 73.3 (*d*) | 79.1 (*d*) |
| CH(5) | 64.6 (*d*) | 64.5 (*d*) | 62.6 (*d*) | 63.6 (*d*) |
| C(6) | 81.6 (*s*) | 84.0 (*s*) | 79.1 (*s*) | 79.8 (*s*) |
| C(7) | 51.8 (*s*) | 45.8 (*s*) | 50.1 (*s*) | 51.0 (*s*) |
| C(8) or CH(8) | 217.5 (*s*) | 86.5 (*d*) | 209.8 (*s*) | 217.1 (*s*) |
| CH(9) or C(9) | 86.5 (*d*) | 86.5 (*d*) | 210.1 (*s*) | 85.4 (*d*) |
| C(10) | 55.0 (*s*) | 52.3 (*s*) | 60.3 (*s*) | 54.1 (*s*) |
| C(11) | 63.3 (*s*) | 64.2 (*s*) | 64.3 (*s*) | 61.0 (*s*) |
| $CH_2$(12) | 34.5 (*t*) | 36.2 (*t*) | 32.8 (*t*) | 33.5 (*t*) |
| $CH_2$(13) | 24.5 (*t*) | 26.3 (*t*) | 29.3 (*t*) | 23.2 (*t*) |
| CH(14) | 53.4 (*d*) | 52.3 (*d*) | 54.5 (*d*) | 52.3 (*d*) |
| CH(15) | 29.7 (*d*) | 29.0 (*d*) | 31.2 (*d*) | 28.5 (*d*) |
| Me(16) | 20.9 (*q*) | 21.0 (*q*) | 21.2 (*q*) | 19.7 (*q*) |
| Me(17) | 26.5 (*q*) | 26.2 (*q*) | 22.5 (*q*) | 25.2 (*q*) |
| $CH_2$(18) or Me(18) | 116.2 (*t*) | 114.2 (*t*) | 115.9 (*t*) | 20.5 (*q*) |
| Me(19) | 16.8 (*q*) | 21.3 (*q*) | 14.8 (*q*) | 15.6 (*q*) |
| Me(20) | 17.5 (*q*) | 18.3 (*q*) | 8.9 (*q*) | 16.2 (*q*) |




**1A**[1]) **1B**[1])

Fig. 1. *Selected HMBCs* (H → C) *and* $^1$*H,*$^1$*H-COSYs* (—)*, and the fragment structures* **1A** *and* **1B** *of compound* **1**

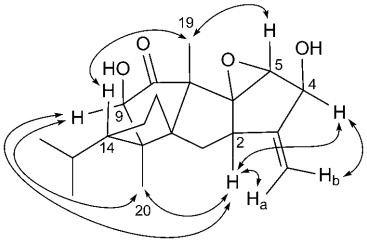



Fig. 2. *Selected NOE correlations* (H ↔ H) *of compound* **1**[1])

3-isopropyl-3a,5a-dimethyl-8-methylene-1*H*-pentaleno[1,6a-*a*]pentalen-5(5a*H*)-one, named (4$\beta$)-4,4-*O*-dihydrocrinipellin A[1]).

Compound **2** had the molecular formula $C_{20}H_{30}O_4$ as derived from the HR-Q-TOF-MS data (*m/z* 357.2853 ($[M+Na]^+$)). The structure of **2** was established by comparison of its NMR data with those of compound **1**. The spectroscopic data of both compounds were similar (*Tables 1* and *2*), except for the addition of an OH group at C(8) of **2** along with the absence of a C=O group. The relative configurations of **2** were established from the same ROESY correlations as in **1**. The NOE correlations between H–C(8) and Me(19) showed that H–C(8) was in $\beta$-orientation. Therefore, compound **2** was determined to be 5b,6-epoxydodecahydro-3-isopropyl-3a,5a-dimethyl-8-methylene-1*H*-pentaleno[1,6a-*a*]pentalene-4,5,7-triol, named (4$\beta$,8$\alpha$)-4,4-*O*,8,8-*O*-tetrahydrocrinipellin B[1]).

Table 2. *$^1$H-NMR Data* ((D$_5$)pyridine) *of Compounds* **1**–**4**. $\delta$ in ppm, *J* in Hz.

| | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| $H_\alpha$–C(1) | 1.95 (*t*, *J* = 13.8) | 2.26 (*dd*, *J* = 7.5, 13.3) | 2.42 (*dd*, *J* = 7.2, 14.0) | 2.33 (*dd*, *J* = 7.2, 13.2) |
| $H_\beta$–C(1) | 2.48 (*dd*, *J* = 6.1, 13.8) | 1.74 (*t*, *J* = 13.3) | 2.07 (*t*, *J* = 14.0) | 1.99–2.01 (*m*) |
| H–C(2) | 3.19 (*dd*, *J* = 7.5, 12.5) | 3.16 (*dd*, *J* = 7.5, 12.4) | 2.72 (*dd*, *J* = 7.2, 13.0) | 2.73 (*dd*, *J* = 7.2, 13.2) |
| H–C(4) | 4.81 (*s*) | 4.85 (*s*) | 4.75 (*s*) | 4.38 (*s*) |
| H–C(5) | 3.85 (*s*) | 3.78 (*s*) | 3.90 (*s*) | 3.89 (*s*) |
| H–C(8) | – | 4.21 (*d*, *J* = 9.3) | – | – |
| H–C(9) | 4.91 (*s*) | 4.13 (*d*, *J* = 9.3) | – | 4.91 (*s*) |
| $H_\alpha$–C(12) | 1.44–1.49 (*m*) | 1.35–1.38 (*m*) | 1.63–1.66 (*m*) | 1.60–1.64 (*m*) |
| $H_\beta$–C(12) | 1.86–1.88 (*m*) | 1.78–1.82 (*m*) | 2.02–2.05 (*m*) | 1.95–1.98 (*m*) |
| $H_\alpha$–C(13) | [a]) | 1.41–1.44 (*m*) | 1.38–1.41 (*m*) | [a]) |
| $H_\beta$–C(13) | 1.47–1.50 (*m*) | 1.50–1.53 (*m*) | 1.83–1.86 (*m*) | 1.51–1.54 (*m*) |
| H–C(14) | 1.79–1.81 (*m*) | 2.30 (*td*, *J* = 8.8, 2.5) | 1.76 (*q*, *J* = 9.8) | 1.83 (*td*, *J* = 10.0, 2.3) |
| H–C(15) | 2.50–2.53 (*m*) | 2.42 (*qd*, *J* = 6.8, 2.5) | 1.48–1.50 (*m*) | 2.56 (*qd*, *J* = 6.9, 2.3) |
| Me(16) | 0.87 (*d*, *J* = 6.9) | 0.90 (*d*, *J* = 6.8) | 0.73 (*d*, *J* = 6.7) | 0.88 (*d*, *J* = 6.9) |
| Me(17) | 0.97 (*d*, *J* = 6.9) | 1.09 (*d*, *J* = 6.8) | 0.72 (*d*, *J* = 6.7) | 0.98 (*d*, *J* = 6.9) |
| $H_a$–C(18) or Me(18) | 5.29 (*s*) | 5.25 (*s*) | 5.18 (*s*) | 1.66 (*s*) |
| $H_b$–C(18) | 5.46 (*s*) | 5.49 (*s*) | 5.35 (*s*) | |
| Me(19) | 1.19 (*s*) | 1.22 (*s*) | 1.31 (*s*) | 1.24 (*s*) |
| Me(20) | 1.36 (*s*) | 1.31 (*s*) | 1.20 (*s*) | 1.33 (*s*) |

[a]) The same as $H_\beta$–C(13).

Compound **3** was determined to have the formula $C_{20}H_{26}O_4$ by its HR-Q-TOF-MS (*m/z* 353.2302 ($[M+Na]^+$)) and NMR ($^1$H, $^{13}$C, and DEPT) data. The $^1$H- and $^{13}$C-NMR spectra (*Tables 1* and *2*) showed that **3** had a similar structure as **1**, except for a signal at $\delta$(C) 210.1 (C(9)) which pointed to the presence of a C=O instead of an OH group. The relative configurations of **3** were validated based on the same ROESY correlations as in **1**. Therefore, compound **3** was determined to be 5b,6-epoxydecahydro-7-hydroxy-3-isopropyl-3a,5a-dimethyl-8-methylene-4*H*-pentaleno[1,6a-*a*]pentalene-4,5(5a*H*)-dione, named crinipellin C[1]).

Compound **4** was established as having a molecular formula $C_{20}H_{30}O_5$ by its HR-Q-TOF-MS (*m/z* 373.2409 ($[M+Na]^+$)) and NMR ($^1H$, $^{13}C$, and DEPT) data. The $^1H$- and $^{13}C$-NMR data of **4** (*Tables 1* and *2*) were similar to those of **1**, except for the signals at $\delta$(C) 82.6 (C(3)) and 20.5 (C(18)) due to the addition of an OH group at C(3) along with the absence of a C(3)=C(18) bond. The relative configurations of **4** were determined based on the same ROESY correlations as in **1**. The NOE correlation Me(18)/Me(20) indicated that Me(18) was in $\alpha$-orientation. Therefore, compound **4** was determined to be 5b,6-epoxydecahydro-4,7,8-trihydroxy-3-isopropyl-3a,5a,8-trimethyl-1*H*-pentaleno[1,6a-*a*]pentalen-5(5a*H*)-one, named crinipellin D[1] (=(3$\beta$,4$\beta$)-3,3-*C*,4,4-*O*-tetrahydro-3-hydroxycrinipellin A).

2. *Biological Studies.* At 10 μg/l, compounds **1**–**4** exhibited growth-rate inhibitions against HeLa cells of 28.1, 24.4, 34.4, and 26.7%, respectively, measured by the MTT method. The antibacterial activities of compounds **1**–**4** were tested against bacteria (*Escherichia coli* (CMCC (B) 44103), *Bacillus subtilis* (CMCC (B) 63501), *Bacillus pumilus* (CMCC (B) 63202), and *Staphylococcus aureus* (CMCC (B) 26003)), and yeast (*Candida albicans* (AS 2.538)) by using the Oxford plate-assay system. Two replicates were performed for each compound at the concentration 0.3 mg/ml with the loading volume 100 μl. The results showed that compounds **1**–**4** had no effects on the growth of the tested bacteria or yeast at 30 μg/plate.

The crinipellins, isolated from different strains of the fungus *Crinipellis stipitaria* (Agaricales), are the first tetraquinane-type diterpenoids [1][2]. All of them share the 12-isopropyl-4,8,11-trimethyltetracyclo[6.6.0.0$^{1,11}$.0$^{3,7}$]tetradecane (=dodecahydro-3-isopropyl-3a,5a,8-trimethyl-1*H*-pentaleno[1,6a-*a*]pentalene) skeleton, and belong to a very small family. Previously, only five members have been reported [2]. However, these molecules provided a formidable challenge for organic synthesis due to their unique backbone composed of four fused five-membered rings, particularly, three contiguous quaternary chiral C-atoms (C(1), C(8), and C(11)) [4–8]. The present work affords further structure diversity to this small family of diterpenoids by reporting four new compounds, in particular one with two vicinal C=O groups and two with two vicinal OH groups. These four novel crinipellins showed moderate growth-inhibitory activities against HeLa cell but no inhibitory effects on the growth of tested bacteria which may be due to the lack of an enone structure unit; this is consistent with the behavior reported for (3$\beta$,4$\beta$)-3,3-*C*,4,4-*O*-tetrahydrocrinipellin A (**5**) and (4$\beta$)-4,4-*O*-dihydrocrinipellin B (**6**) [2]. This work further demonstrated that the fermentation of macrofungi can be an efficient approach to obtain new bioactive natural products [9][10].

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## Experimental Part

*General.* TLC: precoated silica gel ($SiO_2$) *GF$_{254}$* plates (0.20–0.25 mm; *Qingdao Marine Chemical Factory*). Column chromatography (CC): $SiO_2$ (200–300, and 80–100 mesh; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China), $SiO_2$ *GF$_{254}$* (*Merck*), *RP-18* (*Merck*), and *Sephadex LH-20* (*Amersham Biosciences*). Optical rotations: *Perkin-Elmer-341* polarimeter; in MeOH. IR Spectra: *Nicolet FT-IR 360*; KBr pellets; $\tilde{\nu}$ in cm$^{-1}$. $^1H$- and $^{13}C$-NMR Spectra: *Bruker-DRX-500* spectrometer, at 600 and 150 MHz,

resp.; in ($D_5$)pyridine; $\delta$ in ppm rel. to $Me_4Si$, $J$ in Hz. HR-Q-TOF-MS: *Bruker-Daltonics-BioTof-Q* spectrometers; in $m/z$.

*Isolation and Fermentation of the Fungal Strain.* The fungus was isolated from Zhujiangyuan of Yunnan Province, China. This strain was identified as *Crinipellis* sp. by partial sequence analysis of the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rDNA gene. A nucleotide-to-nucleotide BLAST query of the *NCBI* (*National Center of Biotechnology Information*) database yielded *Crinipellis stipitaria* strain PB302 as the closest match to the ITS rDNA of strain 113 (96%). The strain 113 was grown on PDA (potato/dextrose agar) plates at 28° for 14 d in the dark. Every plate contained PDA media (20 ml), and a total of 3 l was cultivated under the above fermentation conditions.

*Extraction and Isolation.* The cultured agar was chopped, diced, and extracted with 3 l of AcOEt/MeOH/AcOH 80:15:5 at r.t. overnight. The org. soln. was collected through filtration, and the remaining agar residue was extracted exhaustively with the same solvent until the filtrate was colorless. The combined filtrate, upon concentration, yielded crude extract as brown syrup. The brown syrup was partitioned between $H_2O$ and AcOEt 1:1 until the AcOEt layer was colorless. The combined org. layers were concentrated to yield the AcOEt extract (2.3 g). The extract was subjected to MPLC (*RP-18* $SiO_2$ (145 g), 30, 50, 70, and 100% acetone/$H_2O$ (2 l each): *Fractions a–k*. *Fr. d* (500 mg) was separated by CC (*Sephadex LH-20*, MeOH): *Frs. d.1* and *d.2*. *Fr. d.1* (190 mg) was purified by MPLC (*RP-18* $SiO_2$ (30 g), 30% acetone/$H_2O$) and then subjected to CC (*Sephadex LH-20*, acetone): *Frs. d.1.a* and *d.1.b*. *Fr. d.1.a* (40 mg) was purified by MPLC (*RP-18* $SiO_2$ (30 g), 30% acetone/$H_2O$), then subjected to CC (*Sephadex LH-20*, acetone), and further purified by CC ($SiO_2$, $CHCl_3$/MeOH 150:1): **6** (20 mg). *Fr. d.1.b* (60 mg) was purified by MPLC (*RP-18* $SiO_2$ (30 g), 30% acetone/$H_2O$), and then by CC ($SiO_2$, $CHCl_3$/MeOH 160:1): **4** (34 mg). *Fr. d.2* (200 mg) was purified by MPLC (*RP-18* $SiO_2$ (30 g), 30% acetone/$H_2O$) and then subjected to CC (*Sephadex LH-20*, acetone): *Frs. d.2.a* and *d.2.b*. *Fr. d.2.a* (29 mg) was purified by CC (*Sephadex LH-20*, acetone), CC ($SiO_2$, petroleum ether/acetone 25:1), and again CC (*Sephadex LH-20*, acetone): **1** (5 mg). *Fr. d.2.b* (7 mg) was purified by CC ($SiO_2$, $CHCl_3$/MeOH 40:1): **2** (5 mg). *Fr. e* (160 mg) was subjected to CC (*Sephadex LH-20*, MeOH), and purified by CC ($SiO_2$, petroleum ether/acetone 6:1): phlebiakauranol alcohol (6 mg). *Fr. f* (180 mg) was subjected to CC (*Sephadex LH-20*, MeOH): *Frs. f.1* and *f.2*. *Fr. f.1* (45 mg) was twice purified by MPLC (*RP-18* $SiO_2$ (30 g), 42 and 35% acetone/$H_2O$): **5** (5 mg). *Fr. f.2* (20 mg) was purified by MPLC (*RP-18* $SiO_2$ (30 g), 45% acetone/$H_2O$) and then CC ($SiO_2$, petroleum ether/acetone 25:1): **3** (5 mg).

*(4β)-4,4*-O-*Dihydrocrinipellin A* (= rel-*(1a*R,*2*S,*3a*R,*4a*R,*7*R,*7a*R,*8*R,*9a*S,*9b*R*)-Octahydro-2,8-dihydroxy-7a,9a-dimethyl-3-methylene-7-(1-methylethyl)-3*H,5H-*pentaleno[6′a,1′:5,6]pentaleno[1,6a*-b*]oxiren-9(9a*H*)-one*; **1**): Colorless oil. $[\alpha]_D^{20} = -67.67$ ($c = 0.600$, MeOH). IR (KBr): 3330, 2958, 2873, 1745, 1594, 1451, 1383, 1029. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. HR-Q-TOF-MS: 355.2729 ($[M+Na]^+$, $C_{20}H_{28}NaO_4^+$; calc. 355.1885).

*(4β,8α)-4,4*-O,*8,8*-O-*Tetrahydrocrinipellin B* (= rel-*(1a*R,*2*S,*3a*R,*4a*R,*7*R,*7a*R,*8*R,*9*R,*9a*R,*9b*R*)-Decahydro-7a,9a-dimethyl-3-methylene-7-(1-methylethyl)-3*H,5H-*pentaleno[6′a,1′:5,6]pentaleno[1,6a*-b*]oxirene-2,8,9-triol*; **2**): Colorless oil. $[\alpha]_D^{20} = +2.2$ ($c = 0.500$, MeOH). IR (KBr): 3380, 2957, 2871, 1457, 1046, 906. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. HR-Q-TOF-MS: 357.2853 ($[M+Na]^+$, $C_{20}H_{30}NaO_4^+$; calc. 357.2042).

*Crinipellin C* (= rel-*(1a*R,*2*S,*3a*R,*4a*R,*7*R,*7a*R,*9a*S,*9b*R*)-Octahydro-2-hydroxy-7a,9a-dimethyl-3-methylene-7-(1-methylethyl)-3*H,*8*H-*pentaleno[6′a,1′:5,6]pentaleno[1,6a*-b*]oxirene-8,9(9a*H*)-dione*; **3**): Colorless oil. $[\alpha]_D^{20} = -53.2$ ($c = 0.500$, MeOH). IR (KBr): 3427, 2960, 2930, 2872, 1746, 1384, 1030. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. HR-Q-TOF-MS: 353.2302 ($[M+Na]^+$, $C_{20}H_{26}NaO_4^+$; calc. 353.1729).

*Crinipellin D* (= rel-*(1a*R,*2*R,*3*S,*3a*R,*4a*R,*7*R,*7a*R,*8*R,*9a*S,*9b*R*)-Octahydro-2,3,8-trihydroxy-3,7a,9a-trimethyl-7-(1-methylethyl)-3*H,5H-*pentaleno[6′a,1′:5,6]pentaleno[1,6a*-b*]oxiren-9(9a*H*)-one*; **4**): Colorless oil. $[\alpha]_D^{20} = -24.35$ ($c = 3.400$, MeOH). IR (KBr): 3384, 2961, 2874, 1747, 1455, 1384, 1064, 1032. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. HR-Q-TOF-MS: 373.2409 ($[M+Na]^+$, $C_{20}H_{30}NaO_5^+$; calc. 373.1991).

*Biological Studies.* Cytotoxicities of compounds **1**–**4** were investigated by using the human cancer cell line HeLa, following the MTT (=2-(4,5-dimethylthiazol-2-yl)-3,5-dimethyl-2*H*-tetrazolium bromide) standards [11], and Cisplatin (= DDP = *cis*-diamminedichloroplatinum) was used as a pos. control in this experiment.

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