Cycloheximide Derivatives from the Fruits of *Dipteronia dyeriana*

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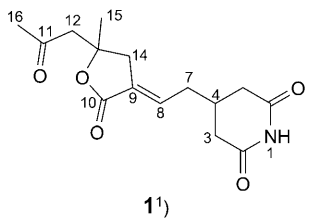
The phytochemical investigation of *Dipteronia dyeriana* (Aceraceae) resulted in the isolation and identification of three new cycloheximide derivatives: dipteronines A–C (**1**–**3**). Their structures were elucidated based on the 1D- and 2D-NMR spectra. Dipteronine A (**1**) is the first example of a 10,11-secocycloheximide. Cytotoxic and antifungal activities of these isolates were also evaluated.

Introduction. – *Dipteronia* is a genus endemic to China and includes only two species, *Dipteronia sinensis* OLIV. and *Dipteronia dyeriana* HENRY. In previous studies, some cytotoxic triterpenoid ester saponins and lignans have been isolated from the branches of *D. dyeriana* [1]. In continuation of our studies on this plant, three new cycloheximides, dipteronines A–C[1]) (**1**–**3**), were isolated from its fruits. Cycloheximide (=4-{(2*R*)-2-[(1*S*,3*S*,5*S*)-3,5-dimethyl-2-oxocyclohexyl]-2-hydroxyethyl}piperidine-2,6-dione), a glutarimide antibiotic possessing cytotoxic and fungicidal activity, is exclusively applied in biochemical research as a potent inhibitor of eukaryotic protein synthesis [2][3]. Therefore, we also evaluated the inhibitory effects of dipteronines A–C on human leukemia K562, human hepatoma HepG2, lung adenocarcinoma A549, human breast cancer MCF-7 cells, and the fungus *Candida albicans*. This article reports the structure elucidation of these new compounds and the results of the bioassay.



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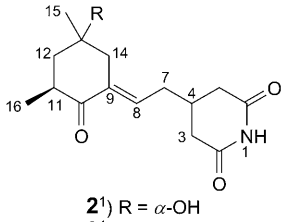


2[1]) R = α-OH
3[1]) R = β-OH

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

Results and Discussion. – Dipteronine A (**1**), a colorless oil, was determined to have the molecular formula $C_{15}H_{19}NO_5$ on the basis of HR-ESI-MS data (*m/z* 292.1190 ($[M-H]^-$)), indicating seven degrees of unsaturation. The IR spectrum of **1** showed absorptions for imide (3437 cm^{-1}) and CO (1704 cm^{-1}) groups. A glutarimide moiety in **1** was deduced from the signals at δ(C) 175.0 (*s*, C(2) and C(6)), 38.1 and 38.0 (*t*, C(3) and C(5)), and 31.1 (*d*, C(4)) in the ^{13}C-NMR spectrum (*Table*)[1]) [3]. The NMR spectra of **1** also showed the presence of two more CO groups (δ(C) 207.7 (*s*, C(11)) and 171.9 (*s*, C(10))) and a trisubstituted C=C bond (δ(H) 6.61–6.65 (*m*, H–C(8)); δ(C) 137.1 (*d*, C(8)) and 131.9 (*s*, C(9))). Six out of seven degrees of unsaturation being accounted for inferred that **1** contained an additional ring besides the glutarimide one.

Table. *^{13}C- and ^{1}H-NMR Data* (125 and 500 MHz, resp.; CD_3OD) *of* **1**–**3**[1]). Chemical shifts δ in ppm, *J* in Hz.

	1		**2**		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
C(2)	175.0		175.2[a])		175.3[b])	
CH_2(3)	38.0[c])	2.63–2.67, 2.36–2.39 (2*m*)	38.1[d])	2.61–2.62, 2.33–2.34 (2*m*)	38.2	2.62–2.65, 2.33–2.34 (2*m*)
H–C(4)	31.3	2.36–2.39 (*m*)	31.6	2.33–2.34 (*m*)	31.5	2.31–2.32 (*m*)
CH_2(5)	38.1[c])	2.63–2.67), 2.36–2.39 (2*m*)	38.2[d])	2.64–2.65, 2.37–2.38 (2*m*)	38.2	2.62–2.63, 2.37–2.38 (2*m*)
C(6)	175.0		175.1[a])		175.2[b])	
CH_2(7)	35.6	2.26–2.28 (*m*)	33.4	2.22–2.25 (*m*)	33.4	2.21–2.22 (*m*)
H–C(8)	137.1	6.61–6.65 (*m*)	135.8	6.45 (br. *t*, *J* = 7.0)	136.1	6.43 (br. *t*, *J* = 7.5)
C(9)	131.9		138.2		138.1	
C(10)	171.9		204.8		205.6	
C(11) or H–C(11)	207.7		41.4	2.48–2.49 (*m*)	40.1	2.73–2.74 (*m*)
CH_2(12)	53.0	3.00 (*s*)	46.3	1.68 (br. *t*, *J* = 13.5, H_α), 2.11 (*ddd*, *J* = 13.5, 7.0, 2.0, H_β)	45.3	1.67 (br. *t*, *J* = 13.5, H_α), 2.00 (*ddd*, *J* = 13.5, 6.5, 3.0, H_β)
C(13)	83.2		70.0		69.4	
CH_2(14)	37.8	2.95 (br. *d*, *J* = 17.5, H_a), 2.75 (br. *d*, *J* = 17.5, H_b)	41.5	2.68 (*d*, *J* = 15.6, H_β), 2.52 (*d*, *J* = 15.6, H_α)	41.4	2.75–2.76 (*m*, H_β), 2.37–2.38 (*m*, H_α)
Me(15)	27.9	1.43 (*s*)	28.1	1.34 (*s*)	30.7	1.32 (*s*)
Me(16)	31.1	2.14 (*s*)	16.4	1.11 (*d*, *J* = 7.0)	15.9	1.08 (*d*, *J* = 7.0)

[a–d]) Assignments with the same superscript are interchangeable.

The ^{1}H,^{1}H-COSY plot of **1** exhibited a partial structure **a** (C(4)–C(7)–C(8)) (*Fig. 1*), which was attached to the glutarimide moiety through C(4). Based on the HMBC cross-peaks Me(16)/C(11) and C(12), Me(15)/C(12), C(13), and C(14), H–C(8)/C(10) and C(14), and CH_2(7)/C(9) (*Fig. 1*), a structure moiety C(16)–C(11)–C(12)–C(13)(–C(15))–C(14)–C(9)(–C(8))–C(10) was confirmed, which was attached to the segment **a** through C(8). Given that the structure of **1** required an additional ring, the signals for a C–O group at δ(C) 83.2 (*s*, C(13)) should arise from a cyclic ester involving the CO of C(10), thus from a five-membered lactone

ring. The ROESY correlation δ(H) 2.95 (br. *d*, $J = 17.5$, H_a–C(14))/δ(H) 2.26–2.28 (*m*, CH_2(7)) established the (*E*)-configuration of the C(8)=C(9) bond. Thus, the planar structure of **1** was established. Dipteronine A (**1**) might be a racemate because its optical rotation value was zero.

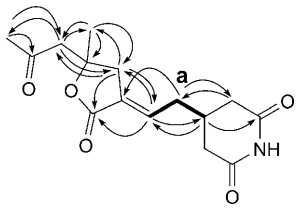
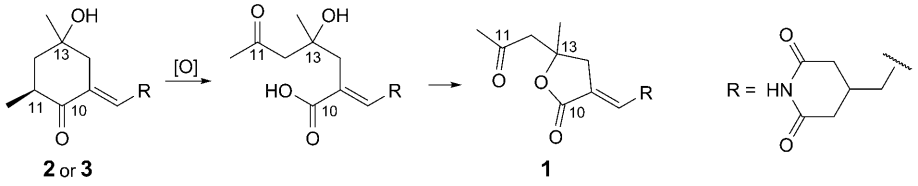


Fig. 1. *Key* $^1H,^1H$*-COSY* (—) *and HMBC* (H → C) of **1**

A possible biogenetic pathway to **1** is shown in the *Scheme*. Dipteronine A (**1**) could be generated from dipteronine B (**2**) or C (**3**), two cycloheximide derivatives also isolated from this plant. Dipteronine A (**1**) is the first example of a 10,11-secocycloheximide.

Scheme. *Plausible Biogenetic Path for Dipteronine* A (**1**)[1])



Dipteronine B (**2**), a colorless oil, had a molecular formula $C_{15}H_{21}NO_4$, as suggested from the HR-ESI-MS (*m*/*z* 278.1393 ([*M* – H]$^-$)), indicating six degrees of unsaturation. The IR spectrum showed characteristic absorptions for OH (3625 cm^{-1}), imide (3446 cm^{-1}), and CO (1703 cm^{-1}) groups. The ^{1}H- and ^{13}C-NMR spectra of **2** (*Table*) showed high analogy to those of anhydrocycloheximide (=4-{(2*E*)-2-[(3*S*,5*S*)-3,5-dimethyl-2-oxocyclohexylidene]ethyl}piperidine-2,6-dione) [3], except that **2** had a signal of an O-bearing quaternary C-atom at δ(C) 70.0 (*s*, C(13)) instead of a CH signal. This suggested that **2** and anhydrocycloheximide differ structurally only in the presence of an OH substituent in **2**, which was located at C(13) based on the HMBCs. The ROESY correlation δ(H) 2.52 (*d*, $J = 15.6$, H_α–C(14))/δ(H) 2.22–2.25 (*m*, CH_2(7)) established the (*E*)-configuration of the C(8)=C(9) bond (*Fig. 2*). The relative configuration of **2** was determined by the coupling constants and the ROESY data. In the ^{1}H-NMR spectrum, the large vicinal coupling constant between H–C(11) and H_α–C(12) ($J(11,12\alpha) = 13.5$ Hz) was characteristic of their *trans* diaxial relationship [4], and H–C(11) was arbitrarily assigned as β-orientated. The correlation H_α–C(12)/H_α–C(14) indicated that H_α–C(14) was also axial, and the correlations Me(15)/H–C(11), H_β–C(12), and H_β–C(14) implied that Me(15) was β-orientated.

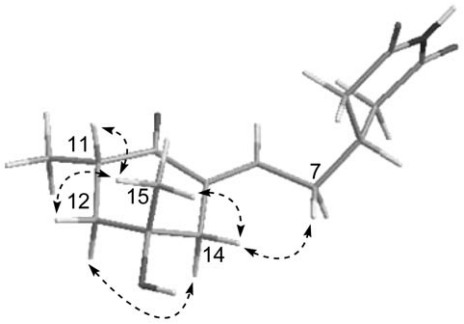


Fig. 2. *Key ROESY correlations of* **2**

Dipteronine C (**3**), a colorless oil, had the same molecular formula $C_{15}H_{21}NO_4$ as **2**, as deduced from the HR-ESI-MS (*m/z* 314.1156 $[M+Cl]^-$). The IR spectrum also showed characteristic absorptions for OH (3649 cm^{-1}), imide (3432 cm^{-1}), and CO (1688 cm^{-1}) groups. The ^1H- and ^{13}C-NMR data of **3** (*Table*) were very close to those of **2**. Extensive analysis of the HMBC spectrum of **3** revealed that both **3** and **2** possessed the same planar structure. In the ROESY plot of **3**, the crucial correlations Me(15)/H_α–C(12) and H_α–C(14) implied that Me(15) was α-orientated.

All compounds **1**–**3** were tested for their biological activity against K562 and HepG2 cell lines as well as the fungus *Candida albicans*. Compounds **1** and **3** were also tested against A549 and MCF-7 cell lines. As a result, only compound **3** showed weak inhibitory effects on A549 cells, with an IC_{50} value of 119.7 μM as compared to 5-fluorouracil ($IC_{50} = 0.208$ μM). A former study suggested that the OH group at C(8) of cycloheximide is important for its cell-cycle inhibitory activity [5].

This work was supported by grants from the *Ministry of Education of China through its 111 & 985 Projects* (B08044 & CUN 985-3-3), the *Ministry of Science and Technology of China* (2005DKA210006), and the *Knowledge Innovation Project of the Chinese Academy of Sciences*. We thank Dr. *Qian-Yun Sun* and the staff of the Key Laboratory of Chemistry for Natural Products of Guizhou Province and the Chinese Academy of Sciences for performing the cytotoxic (A549 and MCF-7 cells) and fungicidal screens.

Experimental Part

General. Anal. TLC: pre-coated silica-gel F_{254} plates (*Qingdao Meigao Chemical Co.*); detection by spraying with 5% H_2SO_4 in EtOH, followed by heating. Prep. TLC: silica gel F_{254} (*Qingdao Meigao Chemical Co.*). Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Meigao Chemical Co.*), *C-18* silica gel (40–75 μm; *Fuji Silysia Chemical Ltd.*), *MCI* gel (70–150 μm; *Mitsubishi Chemical Corporation*), and *Sephadex-LH-20* gel (*GE Healthcare Bio-Xciences AB*). HPLC: *Agilent-1200* system, *Zorbax-SB-C_{18}* semiprep. column (9.4 × 250 mm, 5 μm). Optical rotations: *Jasco DIP370* automatic digital polarimeter. UV Spectra: *Shimadzu Double-Beam-210A* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* spectrophotometer; KBr pellets; in cm^{-1}. NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers; δ in ppm rel. to Me_4Si, *J* in Hz. MS: *VG-Autospec-3000* magnetic-sector instrument and *API-Qstar-Pulsar* instrument; in *m/z*.

Plant Material. *Dipteronia dyeriana* was collected from Pingbian County, Yunnan Province, P. R. China, in October 2007, and identified by *C.-L. L.* of the Kunming Institute of Botany, Chinese Academy

of Sciences, P. R. China. A voucher specimen (PB0701) was deposited with the Laboratory of Ethnobotany, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The air-dried fruits of *D. dyeriana* (6 kg) were extracted three times with 90% EtOH under reflux (4, 3, and 3 h). The EtOH extract was concentrated, and the residue (900 g) was suspended in petroleum ether, AcOEt, and H_2O. The AcOEt-soluble portion (17 g) was subjected to CC (*MCI* gel, MeOH/H_2O 70:30) to yield a fraction (9 g) which was subjected to medium-pressure liquid chromatography (MPLC; MeOH/H_2O 5:95 → 95:5). The MeOH/H_2O 35:65 portion was subjected to CC ($CHCl_3$/MeOH 20:1 and 10:1) to yield **1** (7.3 mg) and another fraction. The latter was subjected to CC (*Sephadex LH-20*, MeOH), prep. TLC ($CHCl_3$/AcOEt/MeOH 2:3:1; AcOEt/MeOH/H_2O 75:25:1), and then to semiprep. HPLC (MeOH/H_2O 40:60): **2** (5.9 mg) and **3** (4.3 mg).

Dipteronine A (=*4-{(2*E*)-2-[Dihydro-5-methyl-2-oxo-5-(2-oxopropyl)furan-3(2*H*)-ylidene]ethyl}piperidine-2,6-dione*; **1**): Colorless oil. $[\alpha]_D^{25.1} = 0$ ($c = 0.42$, MeOH). UV (MeOH): 221 (3.69). IR: 3437, 2973, 2931, 1703, 1266, 1152. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 292.1190 ($[M-H]^-$, $C_{15}H_{18}NO_5^-$; calc. 292.1184).

Dipteronine B (rel-*4-{(2*E*)-2-[(3*R*,5*R*)-5-Hydroxy-3,5-dimethyl-2-oxocyclohexylidene]ethyl}piperidine-2,6-dione*; **2**): Colorless oil. $[\alpha]_D^{20.2} = +23.1$ ($c = 0.20$, MeOH). UV (MeOH): 238 (3.70). IR: 3625, 3446, 1703, 1533, 1258. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 278.1393 ($[M-H]^-$, $C_{15}H_{20}NO_4^-$; calc. 278.1392).

Dipteronine C (rel-*4-{(2*E*)-2-[(3*R*,5*S*)-5-Hydroxy-3,5-dimethyl-2-oxocyclohexylidene]ethyl}piperidine-2,6-dione*; **3**): Colorless oil. $[\alpha]_D^{20.4} = -62.8$ ($c = 0.21$, MeOH). UV (MeOH): 239 (3.56). IR: 3649, 3432, 1688, 1626, 1376, 1264. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 314.1156 ($[M+Cl]^-$, $C_{15}H_{21}ClNO_4^-$; calc. 314.1159).

Activity Assay. The inhibitory activity of compounds **1**–**3** against K562 [6], HepG2 [6], A549 [7], and MCF-7 cells [8], and the fungus *Candida albicans* [9] were measured by the methods described earlier.

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Received January 9, 2009