

Cycloheximide Derivatives from the Fruits of *Dipteronia dyeriana*

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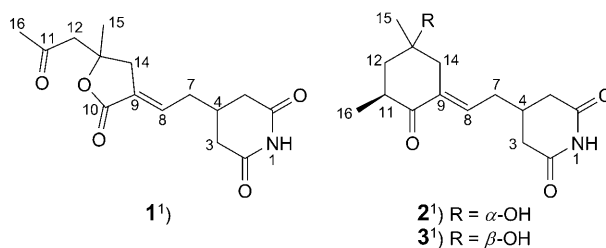
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The phytochemical investigation of *Dipteronia dyeriana* (Aceraceae) resulted in the isolation and identification of three new cycloheximide derivatives: dipteronines A–C (**1**–**3**). Their structures were elucidated based on the 1D- and 2D-NMR spectra. Dipterone 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–C¹⁾ (**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.



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

Results and Discussion. – Dipterone 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 $\delta(C)$ 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 (Table¹) [3]. The NMR spectra of **1** also showed the presence of two more CO groups ($\delta(C)$ 207.7 (*s*, C(11)) and 171.9 (*s*, C(10))) and a trisubstituted C=C bond ($\delta(H)$ 6.61–6.65 (*m*, H–C(8)); $\delta(C)$ 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 1H -NMR Data (125 and 500 MHz, resp.; CD_3OD) of **1**–**3**¹). Chemical shifts δ in ppm, J in Hz.

	1		2		3	
	$\delta(C)$	$\delta(H)$	$\delta(C)$	$\delta(H)$	$\delta(C)$	$\delta(H)$
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 <i>m</i>)	38.1 ^d)	2.61–2.62, 2.33–2.34 (2 <i>m</i>)	38.2	2.62–2.65, 2.33–2.34 (2 <i>m</i>)
H–C(4)	31.3	2.36–2.39 (<i>m</i>)	31.6	2.33–2.34 (<i>m</i>)	31.5	2.31–2.32 (<i>m</i>)
CH ₂ (5)	38.1 ^c)	2.63–2.67), 2.36–2.39 (2 <i>m</i>)	38.2 ^d)	2.64–2.65, 2.37–2.38 (2 <i>m</i>)	38.2	2.62–2.63, 2.37–2.38 (2 <i>m</i>)
C(6)	175.0		175.1 ^a)		175.2 ^b)	
CH ₂ (7)	35.6	2.26–2.28 (<i>m</i>)	33.4	2.22–2.25 (<i>m</i>)	33.4	2.21–2.22 (<i>m</i>)
H–C(8)	137.1	6.61–6.65 (<i>m</i>)	135.8	6.45 (br. <i>t</i> , $J = 7.0$)	136.1	6.43 (br. <i>t</i> , $J = 7.5$)
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 (<i>m</i>)	40.1	2.73–2.74 (<i>m</i>)
CH ₂ (12)	53.0	3.00 (<i>s</i>)	46.3	1.68 (br. <i>t</i> , $J = 13.5$, H_a), 2.11 (<i>ddd</i> , $J = 13.5$, 7.0, 2.0, H_β)	45.3	1.67 (br. <i>t</i> , $J = 13.5$, H_a), 2.00 (<i>ddd</i> , $J = 13.5$, 6.5, 3.0, H_β)
C(13)	83.2		70.0		69.4	
CH ₂ (14)	37.8	2.95 (br. <i>d</i> , $J = 17.5$, H_a), 2.75 (br. <i>d</i> , $J = 17.5$, H_b)	41.5	2.68 (<i>d</i> , $J = 15.6$, H_β), 2.52 (<i>d</i> , $J = 15.6$, H_a)	41.4	2.75–2.76 (<i>m</i> , H_β), 2.37–2.38 (<i>m</i> , H_a)
Me(15)	27.9	1.43 (<i>s</i>)	28.1	1.34 (<i>s</i>)	30.7	1.32 (<i>s</i>)
Me(16)	31.1	2.14 (<i>s</i>)	16.4	1.11 (<i>d</i> , $J = 7.0$)	15.9	1.08 (<i>d</i> , $J = 7.0$)

^{a–d}) Assignments with the same superscript are interchangeable.

The 1H , 1H -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 CH₂(7)/C(9) (Fig. 1), a structure moiety C(16)–C(11)–C(12)–C(13)–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 ester ring, the signals for a C–O group at $\delta(C)$ 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 $\delta(\text{H})$ 2.95 (br. *d*, $J = 17.5$, $\text{H}_\alpha\text{-C}(14)) / \delta(\text{H})$ 2.26–2.28 (*m*, $\text{CH}_2(7)$) established the (*E*)-configuration of the $\text{C}(8)=\text{C}(9)$ bond. Thus, the planar structure of **1** was established. Dipterone A (**1**) might be a racemate because its optical rotation value was zero.

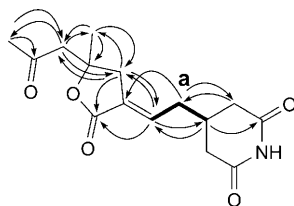
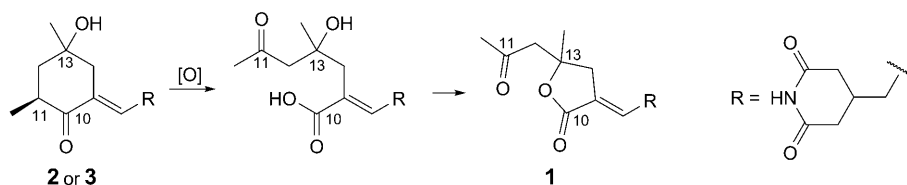


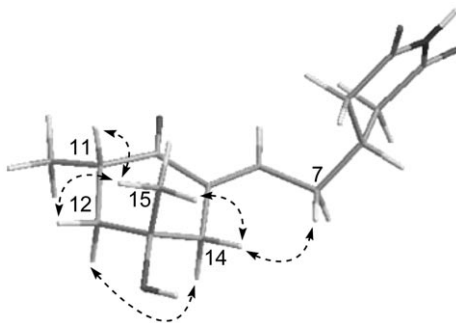
Fig. 1. Key $^1\text{H}, ^1\text{H}$ -COSY (—) and HMBC ($\text{H} \rightarrow \text{C}$) of **1**

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

*Scheme. Plausible Biogenetic Path for Dipterone A (**1**)¹*



Dipterone B (**2**), a colorless oil, had a molecular formula $\text{C}_{15}\text{H}_{21}\text{NO}_4$, as suggested from the HR-ESI-MS (m/z 278.1393 ($[M - \text{H}]^-$)), 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 ^1H - 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 $\delta(\text{C})$ 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 $\delta(\text{H})$ 2.52 (*d*, $J = 15.6$, $\text{H}_\alpha\text{-C}(14)) / \delta(\text{H})$ 2.22–2.25 (*m*, $\text{CH}_2(7)$) established the (*E*)-configuration of the $\text{C}(8)=\text{C}(9)$ bond (*Fig. 2*). The relative configuration of **2** was determined by the coupling constants and the ROESY data. In the ^1H -NMR spectrum, the large vicinal coupling constant between $\text{H}-\text{C}(11)$ and $\text{H}_\alpha\text{-C}(12)$ ($J(11,12_\alpha) = 13.5\text{ Hz}$) was characteristic of their *trans* diaxial relationship [4], and $\text{H}-\text{C}(11)$ was arbitrarily assigned as β -orientated. The correlation $\text{H}_\alpha\text{-C}(12) / \text{H}_\alpha\text{-C}(14)$ indicated that $\text{H}_\alpha\text{-C}(14)$ was also axial, and the correlations $\text{Me}(15) / \text{H}-\text{C}(11)$, $\text{H}_\beta\text{-C}(12)$, and $\text{H}_\beta\text{-C}(14)$ implied that $\text{Me}(15)$ 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 $119.7\text{ }\mu\text{M}$ as compared to 5-fluorouracil ($IC_{50} = 0.208\text{ }\mu\text{M}$). A former study suggested that the OH group at C(8) of cycloheximide is important for its cell-cycle inhibitory activity [5].

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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-Sciences AB). HPLC: Agilent-1200 system, Zorbax-SB-C $_{18}$ semiprep. column (9.4 $\times$ 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 H₂O. The AcOEt-soluble portion (17 g) was subjected to CC (MCI gel, MeOH/H₂O 70:30) to yield a fraction (9 g) which was subjected to medium-pressure liquid chromatography (MPLC; MeOH/H₂O 5:95 → 95:5). The MeOH/H₂O 35:65 portion was subjected to CC (CHCl₃/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 (CHCl₃/AcOEt/MeOH 2:3:1; AcOEt/MeOH/H₂O 75:25:1), and then to semiprep. HPLC (MeOH/H₂O 40:60): **2** (5.9 mg) and **3** (4.3 mg).

Dipteronine A (= 4-{(2E)-2-[Dihydro-5-methyl-2-oxo-5-(2-oxopropyl)furan-3(2H)-ylidene]ethyl}piperidine-2,6-dione; **1**): Colorless oil. $[\alpha]_{\text{D}}^{25.1} = 0$ ($c = 0.42$, MeOH). UV (MeOH): 221 (3.69). IR: 3437, 2973, 2931, 1703, 1266, 1152. ¹H- and ¹³C-NMR: Table. HR-ESI-MS: 292.1190 ($[M - H]^-$, C₁₅H₁₈NO₅⁻; calc. 292.1184).

Dipteronine B (rel-4-{(2E)-2-[(3R,5R)-5-Hydroxy-3,5-dimethyl-2-oxocyclohexylidene]ethyl}piperidine-2,6-dione; **2**): Colorless oil. $[\alpha]_{\text{D}}^{20.2} = +23.1$ ($c = 0.20$, MeOH). UV (MeOH): 238 (3.70). IR: 3625, 3446, 1703, 1533, 1258. ¹H- and ¹³C-NMR: Table. HR-ESI-MS: 278.1393 ($[M - H]^-$, C₁₅H₂₀NO₄⁻; calc. 278.1392).

Dipteronine C (rel-4-{(2E)-2-[(3R,5S)-5-Hydroxy-3,5-dimethyl-2-oxocyclohexylidene]ethyl}piperidine-2,6-dione; **3**): Colorless oil. $[\alpha]_{\text{D}}^{20.4} = -62.8$ ($c = 0.21$, MeOH). UV (MeOH): 239 (3.56). IR: 3649, 3432, 1688, 1626, 1376, 1264. ¹H- and ¹³C-NMR: Table. HR-ESI-MS: 314.1156 ($[M + Cl]^-$, C₁₅H₂₁ClNO₄⁻; 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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