Two New Trijugin-Type Limonoids from *Cipadessa cinerascens*

by **Zhi-Guo Zhang***[a]), **Yun-Tao Cheng**[b]), **Gui-Lin Hu**[a]), and **Guo-Neng Li**[a])

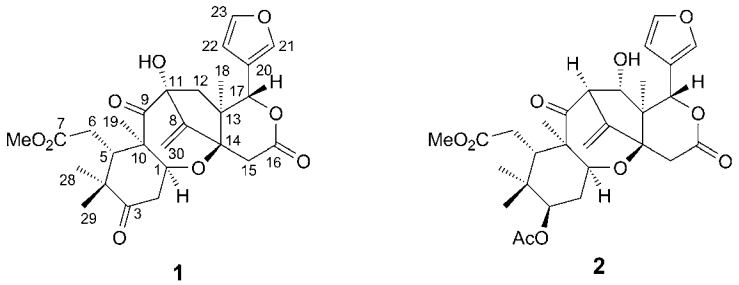
[a]) School of Light Industry, Zhejiang University of Science and Technology, Hangzhou 310023, P. R. China (phone: +86-571-85070788; fax: +86-571-85070785; e-mail: 107023@zust.edu.cn)

[b]) *Jiangsu Yuxiang Pharmaceutical Technology Co., Ltd.*, Yancheng 224500, P. R. China

Two new trijugin-type limonoids, cipatrijugins G and H (**1** and **2**, resp.) along with four known limonoids, were isolated from the leaves of *Cipadessa cinerascens*. All structures were elucidated on the basis of spectroscopic analyses, including IR, 1D- and 2D-NMR, and ESI-MS and HR-ESI-MS techniques.

Introduction. – Limonoids, a family of highly oxygenated nortriterpenoids, have attracted great attention for their complex structures and significant biological activities [1][2]. Recently, trijugin-type limonoids, characterized by a contracted five-membered ring *C* [3][4], have obtained renewed interest on the basis of both new found trichilin A [5] with a highly rearranged ring system, and their biogenetical relationships with cipadesin-type, methyl angolensate-type, and cipadonoid-type limonoids [1][6][7]. In our previous work, two novel trijugin-type limonoids, and a cipadesin-type limonoid were isolated from *Cipadessa cinerascens* [8]. Herein, we report the isolation and structure elucidation of two new trijugin-type limonoids cipatrijugin G and H (**1** and **2**, resp.) (*Fig. 1*) from the leaves of *C. cinerascens*. Besides, four known limonoids, cipadesin F [9], cineracipadesin A [10], cineracipadesin F [10], and cipadesin A [11], were found to co-exist with these two new ones, of which the first three were previously isolated from the leaves of *C. cinerascens*, and the last one was also reported from the seed of *C. cinerascens* [12].

Results and Discussion. – Cipatrijugin G (**1**) was isolated as white amorphous powder with a molecular formula $C_{27}H_{32}O_9$ deduced from the $[M+Na]^+$ ion peak at



1 **2**

Fig. 1. *Structures of cipatrijugins G* (**1**) *and H* (**2**)

m/*z* 523.1949 (calc. 523.1944) in HR-ESI-MS. Its IR absorption bands revealed the presence of OH (3428 cm^{-1}) and C=O groups (1736, 1685 cm^{-1}). The ^{13}C-NMR spectrum (*Table*) exhibited 27 C-atom signals including those of eleven quaternary C-atoms (four C=O and two olefinic C-atoms), six CH (two O-bearing and three olefinic ones), five CH_2, and five Me groups according to the DEPT spectrum. Except for a MeO group (δ(H) 3.68 (*s*); δ(C) 52.2), the remaining 26 C-atom signals including those of a β-substituted furan ring (δ(H) 7.42 (*s*, H–C(21)), 6.39 (*s*, H–C(22)), 7.51 (*s*, H–C(23)); δ(C) 121.4, 143.6, 108.1, 140.1) indicated a limonoid skeleton for compound **1**.

Table 1. *^{1}H- and ^{13}C-NMR* ((D$_6$)DMSO) *Assignments of Cipatrijugins G and H* (**1** and **2**, resp.). δ in ppm, *J* in Hz.

Position	**1**		**2**	
	δ(H)[a])	δ(C)[b])	δ(H)[a])	δ(C)[b])
1	4.33 (*dd*, *J* = 3.0, 3.0)	74.5	4.05 (*dd*, *J* = 3.0, 3.0)	72.1
2	2.95 (*dd*, *J* = 15.0, 3.0, H_α), 2.46 (*dd*, *J* = 15.0, 3.0, H_β)	41.5	2.21 (*ddd*, *J* = 15.0, 3.0, 2.5, H_α), 1.88 (*ddd*, *J* = 15.0, 3.0, 2.5, H_β)	29.8
3		210.0	4.82 (*dd*, *J* = 2.5, 2.5)	74.3
4		49.0		38.1
5	3.10 (*dd*, *J* = 3.4, 3.4)	42.9	3.19 (*d*, *J* = 4.0)	36.4
6	2.81 (*dd*, *J* = 17.0, 3.4, H_α), 2.38 (*dd*, *J* = 17.0, 3.4, H_β)	30.4	2.67–2.76 (*m*, H_α), 2.21–2.31 (*m*, H_β)	28.8
7		173.5		173.9
8		147.0		140.2
9		209.1		205.9
10		54.2		55.2
11		85.5	2.43 (*d*, *J* = 3.0)	71.2
12	1.42 (*d*, *J* = 14.5, H_α), 3.20 (*d*, *J* = 14.5, H_β)	46.8	5.58 (*d*, *J* = 3.0)	75.1
13		45.6		51.3
14		87.7		89.1
15	2.85–3.03 (*m*, 2 H)	34.6	2.84, 2.77 (2*d*, *J* = 15.0)	34.5
16		168.2		168.1
17		78.7	6.17 (*s*)	78.2
18	0.92 (*s*, 3 H)	17.7	1.06 (*s*, 3 H)	10.8
19	1.27 (*s*, 3 H)	18.6	1.14 (*s*, 3 H)	19.9
20		121.4		121.4
21	7.42 (*s*)	143.6	7.51 (*s*)	140.9
22	6.39 (*s*)	108.1	6.54 (*s*)	109.6
23	7.51 (*s*)	140.1	7.70 (*s*)	143.6
28	1.10 (*s*, 3 H)	22.0	0.99 (*s*, 3 H)	22.7
29	1.15 (*s*, 3 H)	25.2	0.87 (*s*, 3 H)	27.2
30	5.74, 5.30 (2 br. *s*)	113.6	5.29, 5.07 (2*s*)	115.2
COO*Me*	3.68 (*s*)	52.2	3.67 (*s*)	52.3
11-OH	4.83 (*s*)			
12-OH			3.71 (*s*)	
3-AcO			2.04 (*s*, 3 H)	20.7, 170.4

[a]) Recorded at 500 MHz. [b]) Recorded at 125 MHz.

A comprehensive analysis of the 1D- and 2D-NMR spectra of **1** revealed the presence of a lactone group (δ(C) 168.2), a C(8)=C(30) bond (δ(H) 5.74, 5.30 (both br. *s*, CH_2(30); δ(C) 147.0, 113.6) and the key HMBC of Me(19) with the ketone C-atom C (9) (*Fig. 2*) further confirmed that **1** was a trijugin-type limonoid [3][4]. An additional ketone group was located at C(3), as suggested by the HMBC of H–C(1), Me(28), and Me(29) with C(3), which is very rare in trijugin-type limonoids. A OH group was linked to C(11) based on its HMBC with C(11). The constitution of **1** was fully established as shown in *Fig. 1*.

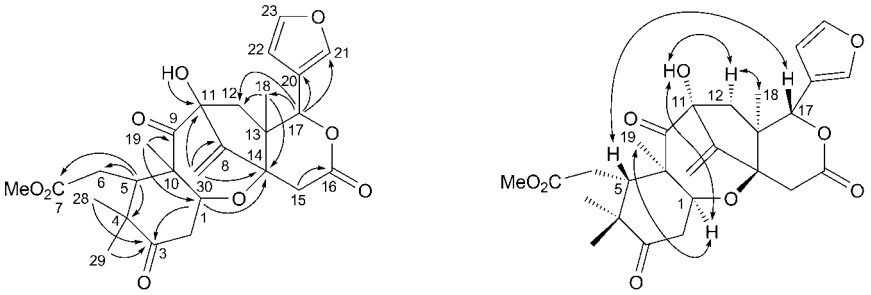


Fig. 2. *Selected HMBCs* (H → C) *and Key ROESY* (H ↔ H) *correlations of* **1**

The relative configuration of **1** was deduced from the analysis of its ROESY correlations. As shown in *Fig. 2*, the cross-peak H–C(5)/H–C(17) indicated that H–C(5) and H–C(17) were β-orientated. The correlation of H–C(1) to Me(19) and OH–C(11), as well as of OH–C(11) to H_α–C(12), and of H_α–C(12) to Me(18) indicated α-orientation for H–C(1), OH–C(11), Me(19), and Me(18). Therefore, the structure of compound **1** was finally established as depicted in *Fig. 1*.

Cipatrijugin H (**2**) was isolated as a white amorphous powder with the molecular formula $C_{29}H_{36}O_{10}$ deduced from the $[M+Na]^+$ ion peak at *m*/*z* 567.2220 (calc. 567.2206) in HR-ESI-MS. Its IR spectrum showed absorption bands similar to those of **1**. The ^{1}H- and ^{13}C-NMR data for **2** resemble those of **1**, except for the absence of the signals of an O-bearing quaternary C-atom, a CH_2 and a ketone C-atom, and the appearance of signals of a CH group (δ(H) 2.43 ((*d*, *J* = 3.0, H–C(11)); δ(C) 71.2 (C(11))), two O-bearing CH groups (δ(H) 4.82 (*dd*, *J* = 2.5, 2.5, H–C(3)), 5.58 (*d*, *J* = 3.0, H–C(12)), δ(C) 74.3 (C(3)), 75.1 (C(12)), and one additional AcO group (δ(H) 2.04 (*s*); δ(C) 20.7, 170.4). These data suggested that the oxo group was replaced by an AcO group, and the OH group at C(11) shifted to C(12) in **2**. The detailed 2D-NMR data confirmed our deduction. The HMBC cross-peak H–C(3)/C=O definitely evidenced the presence of the AcO group at C(3), and the OH group resonating at δ(H) 3.71 exhibited HMBC with C(12), confirming its location at C(12). The ROESY correlations H–C(3)/H–C(1) and OH–C(12)/Me(18) indiciated α-orientation of H–C(3) and OH–C(12), and corresponding β-orientation of AcO–C(3). The structure of compound **2** was thus elucidated as depicted.

The structures of known compounds were identified by comparison of their physical data with those in the literature.

This work was financially supported by the *National Natural Science Foundation of China* (Nos. 51206148 and 51106140), the *Zhejiang Provincal Natural Science Foundation of China* (Nos. Y1110642 and Y407311), and the *Pre-Research Special Foundation for Interdisplinary Subject* at Zhejiang University of Science and Technology (No. 2011JC01Z).

Experimental Part

General. Column chromatography (CC): *RP-18* silica gel and *MCI* gel (((SiO_2; 40–63 μm, *Merck*, Germany), and GF_{254} SiO_2 (200–300 mesh, *Qingdao Marine Chemical Co., Ltd.*, Qingdao, P. R. China). TLC: GF_{254} SiO_2 plates; detection with 254-nm UV light or visualized by spraying with 5% H_2SO_4 in EtOH and then heating. Semi-prep. HPLC: *Shimadzu* instrument (*LC-10A PUMP*, *SPD-10A* UV/VIS detector). Optical rotations: *Shanghai Cany Precision Instrument WWZ-2S* polarimeter. IR Spectra: *Thermo Nicolet Nexus 670* FT-IR spectrometer with KBr pellets; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker-500-Avance-III* spectrometer at 500 (^1H) and 125 MHz (^{13}C); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI- and HR-MS (pos.): *Bruker-Apex-III* mass spectrometer; in *m*/*z* (rel. %).

Plant Material. The leaves of *C. cinerascens* were collected in Guangxi, Zhuang Autonomous Region, of China and were purchased from the *Chinese Herb Transaction Center*, Anhui Province, P. R. China. The material was identified by Dr. *Gang Ren* (Zhejiang University, Hangzhou, P. R. China). A voucher specimen (No. CC 080703) is deposited with the School of Biological and Chemical Engineering, Zhejiang University of Science and Technology, P. R. China.

Extraction and Isolation. The air-dried powder of the leaves of *C. cinerascens* (5.0 kg) was extracted with 95% EtOH (3 × ; 5, 5 and 3 d) at r.t. to give a crude extract (421 g), which was dissolved in H_2O and further extracted with petroleum ether, AcOEt, and BuOH, resp. The AcOEt extract (201 g) was fractionated by CC (*MCI* gel; MeOH/H_2O 4:6 → 10:0) to yield five fractions, *Frs. I–V. Fr. III* (61 g) was then subjected to CC (SiO_2; $CHCl_3$/MeOH 1:0 → 1:1) to yield five fractions, *Frs. 1–5. Fr. 3* (11 g) was submitted to CC (*RP-18* (40–63 μm); MeOH/H_2O from 4:6 to 10:0), and then purified again by CC (SiO_2; $CHCl_3$/acetone 10:1 → 8:2) to afford **1** (12 mg), cineracipadesin F (5 mg), and cipadesin A (7 mg). *Fr. 4* (16 g) was subjected to CC (SiO_2; $CHCl_3$/acetone 10:1 → 7:3), and then further purified by semi-prep. HPLC (MeOH/H_2O 65:35) to afford **2** (9 mg), cipadesin F (14 mg), and cineracipadesin A (4 mg).

Cipatrijugin G (= *Methyl* rel-*[(4*R*,4a*S*,6*R*,7a*R*,8*S*,11a*S*,12a*R*)-4-(Furan-3-yl)dodecahydro-6-hydroxy-4a,7a,9,9-tetramethyl-13-methylidene-2,7,10-trioxo-4*H*-6,12a-methanopyrano[4,3-*b*][1]benzoxocin-8-yl]acetate*; **1**). White amorphous powder. $[\alpha]_D^{25} = -12.6$ ($c = 0.50$, $CHCl_3$). IR (KBr): 3428, 2977, 1736, 1685, 1366, 1042, 1024. ^1H- and ^{13}C-NMR ((D$_6$)DMSO): see the *Table*. ESI-MS: 501.2 ($[M+H]^+$). HR-ESI-MS: 523.1949 ($[M+Na]^+$, $C_{27}H_{32}NaO_9^+$; calc. 523.1944).

Cipatrijugin H (= *Methyl* rel-*[(4*R*,4a*S*,5*S*,6*R*,7a*R*,8*S*,10*R*,11a*S*,12a*S*)-10-(Acetyloxy)-4-(furan-3-yl)-dodecahydro-5-hydroxy-4a,7a,9,9-tetramethyl-13-methylidene-2,7-dioxo-4*H*-6,12a-methanopyrano[4,3-*b*][1]benzoxocin-8-yl]acetate*; **2**). White amorphous powder. $[\alpha]_D^{25} = -93.4$ ($c = 0.43$, $CHCl_3$); IR (KBr): 3433, 2951, 1746, 1689, 1376, 1232, 1124. ^1H- and ^{13}C-NMR ((D$_6$)DMSO): see the *Table*. ESI-MS: 545.2 ($[M+H]^+$). HR-ESI-MS: 567.2220 ($[M+Na]^+$, $C_{29}H_{36}NaO_{10}^+$; calc. 567.2206).

REFERENCES

[1] X. Fang, Y. T. Di, X. J. Hao, *Curr. Org. Chem.* **2011**, *15*, 1363.
[2] A. Roy, S. Saraf, *Biol. Pharm. Bull.* **2006**, *29*, 191.
[3] K. K. Purushothaman, M. Venkatanarasimhan, A. Sarada, J. D. Connolly, D. S. Rycroft, *Can. J. Chem.* **1987**, *65*, 35.
[4] I. S. Ismail, H. Ito, T. Hatano, S. Taniguchi, T. Yoshida, *Phytochemistry* **2003**, *64*, 1345.
[5] Z.-L. Geng, X. Fang, Y.-T. Di, Q. Zhang, Y. Zeng, Y.-M. Shen, X.-J. Hao, *Tetrahedron Lett.* **2009**, *50*, 2132.
[6] X. Fang, Q. Zhang, C.-J. Tan, S.-Z. Mu, Y. Lü, Y.-B. Lu, Q.-T. Zheng, Y.-T. Di, X.-J. Hao, *Tetrahedron* **2009**, *65*, 7408.

[7] X. Fang, Y.-T. Di, H.-P. He, H.-Y. Liu, Z. Zhang, Y.-L. Ren, Z.-L. Gao, S. Gao, X.-J. Hao, *Org. Lett.* **2008**, *10*, 1905.
[8] Z.-G. Zhang, K. Yao, G.-L. Hu, J. Zhang, *Helv. Chim. Acta* **2010**, *93*, 698.
[9] X.-H. Yuan, B.-G. Li, C.-X. Xu, M. Zhou, H.-Y. Qi, G.-L. Zhang, *Chem. Pharm. Bull.* **2007**, *55*, 902.
[10] X. Fang, Y.-T. Di, C.-S. Li, Z.-L. Geng, Z. Zhang, Y. Zhang, Y. Lu, Q.-T. Zheng, S.-Y. Yang, X.-J. Hao, *J. Nat. Prod.* **2009**, *72*, 714.
[11] A. C. Leite, J. B. Fernandes, M. F. G. F. da Silva, P. C. Vieira, *Z. Naturforsch., B* **2005**, *60*, 351.
[12] X. Fang, Y.-T. Di, G.-W. Hu, S.-L. Li, X.-J. Hao, *Biochem. Syst. Ecol.* **2009**, *37*, 528.

Received June 5, 2013