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Cipadonoid A, a Novel Limonoid with an Unprecedented Skeleton, from *Cipadessa cinerasecns*

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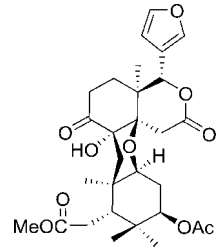
ABSTRACT



Cipadonoid A (1), a novel limonoid with an unprecedented skeleton, was isolated from the leaves of *Cipadessa cinerasecns*. Its structure and relative configuration were determined by spectroscopic analysis and computer modeling. 1 represents a new type of limonoid, characterized by a rearranged tetrahydropyranyl ring B incorporating usually exocyclic C-30. A possible biosynthetic pathway of 1 was also proposed.

Limonoids, with diverse structures and broad range of bioactivities, have been an attraction for both natural product and synthesis chemists.[1] In recent years, a number of new limonoids have been isolated from the Meliaceae family by several research groups,[2] including three novel B, D-seco limonoids from the species *Cipadessa cinerasecns*.[3]

C. cinerasecns is a shrub mainly growing in the southwest of China, whose leaves and roots have been used as primitive medicine for the treatment of stomachache, dysentery, rheumatism, malaria, scald, and itchy skin.[4] Previously, our group reported six limonoids from the species.[5] A subsequent study on the limonoids of leaves of *C. cinerasecns*[6] led to the isolation of a novel B, D-seco type limonoid, cipadonoid

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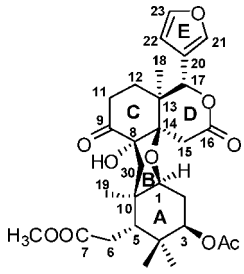
A (**1**), featuring a new tetrahydropyranyl ring B involving the carbon of common exomethylene group of C-30. Unlike other C-30 modified limonids, **1** was characterized with C-30 exomethylene group inserted between C-8 and C-10, instead of between C-8 and C-2 or C-8 and C-14.[7] We describe herein the isolation and structural elucidation of **1**.



The air-dried powder of the plant material (11.5 kg) was extracted with 95% EtOH three times. The extracts were combined and concentrated, followed by suspension in water. The water layer was further extracted with petroleum ether, $CHCl_3$, and *n*-BuOH. The $CHCl_3$ extract (500 g) was then subjected to silica gel column eluted with PE/EtOAc (from 1:0 to 1:1) and then PE/EtOAc/CH_3OH (from 1:1:0 to 1:1:1), giving 10 fractions (A1-A10). The fraction A6 (10.5g) was subjected to a MCI gel column (MeOH/H_2O 5:5 → 10:0) and further purified by Sephadex LH-20 and semipreparative HPLC to give **1** (4 mg).

1[8] was obtained as a white amorphous powder. The molecular formula was determined as $C_{29}H_{38}O_{10}$ from the $[M + Na]^+$ ion peak at *m*/*z* 569.2345 in HRESIMS due to 11 degrees of unsaturation. The IR absorption bands indicated the existence of hydroxyl (3432 cm^{-1}) and ketone (1728 cm^{-1}). The ^{13}C NMR spectra of **1** suggested that 6 out of 11 degrees of unsaturation come from two carbon−carbon double bonds and four carbonyls; thus, the remaining 5 degrees of unsaturation indicated compound **1** to be pentacyclic. The ^1H and ^{13}C NMR spectra of 1 (Table 1) (Py-d_6) showed the presence of an acetyl, a methyl ester, a lactone unit, a ketone carbonyl, five quaternary carbons (two oxygenated), four methines, six methylenes, and a methoxy group. Moreover, the existence of a β-substituted furan ring [δ_H 6.64 (s), 7.73 (s), 7.77 (s); δ_C 111.1, 122.2, 141.6, 143.7] and four tertiary methyl groups [δ_H 0.83 (s), 0.87 (s), 0.96 (s), 1.40 (s); δ_C 22.9, 22.3, 28.2, 15.6] suggested that compound **1** should be a limonoid.

Further insights were obtained from extensive comparison of ^1H and ^{13}C NMR data of **1** with those of known limonoids, especially cipadesin D(**2**).[5] The similarity of chemical shifts of the two compounds in A, D, and E rings suggested that both compounds share the same A, D, and E ring systems, as further confirmed by 2D NMR studies. Furthermore, the striking absence of a C-30 sp^2 methylene signal in **2** and the presence of a sp^3 C-30 methylene signal (δ_H 1.58, 3.00; δ_C 45.1) in **1** implied that the methylene group might be incorporated into a new ring. The HMBC correlations of H_2-30 /C-1, C-8, C-10 and C-14, together with Me-19/C-1 and C-10,indicated that three quaternary carbons C-8, C-10, and C-14 and a methine of C-1 were linked through C-30 as shown in Figure l. The two oxygenated carbons C-1 and C-14 were connected with a 1,14-oxide bridge, by HMBC correlations of H-1 (δ_H 3.90)/C-14 (δ_C 80.0). The above evidence suggested the presence of a tetrahydropyran ring involving C-30. Meanwhile, the $^1H-^1H$ COSY correlation (H-11—H-12) and HMBC correlations (H-11/ C-9, H-12/C-13, and Me-18/C-13, C-14) established a six-member ring C with a carbonyl at C-9. The remained important

Table 1. ^1H (500 MHz) and ^{13}C (100 MHz) NMR Assignments of Cipadonoid A (**1**)

no.	δ_H (multi, *J* in Hz)[a]	δ_C[a]	δ_H (multi, *J* in Hz)[b]	δ_C[b,c]
1	3.90 (t, 3.0)	72.8	3.66 (t, 3.0)	71.5
2α	2.12 (dt, 15.5, 3.0)	27.8	2.26 (dt, 15.5, 3.0)	27.1
2β	1.84 (dt, 15.5, 3.0)		1.62 (dt, 15.5, 3.0)	
3	4.97 (t, 3.0)	75.9	4.58 (t, 3.0)	75.4
4		38.4		37.4
5	2.43 (d, 8.8)	37.2	1.96 (dd, 6.5, 4.0)	36.2
6a	2.56 (d, 17.0)	30.0	2.20 [d]	29.2
6b	2.32 (dd, 17.0, 8.8)		2.19 [d]	
7		173.9		173.0
8		72.4		71.3
9		209.9		208.8
10		38.2		37.5
11α	3.00 [d]	32.4	2.49 (2H)[d]	31.5
11β	2.74 (dd, 19.0 7.0)			
12α	1.48 (dd, 12.5, 7.0)	25.9	1.30 (m)	25.0
12β	2.88 (dd, 12.5, 7.0)		2.50 [d]	
13		40.0		38.9
14		80.0		78.9
15a	3.66 (d, 18.5)	31.1	3.18 (d, 18.5)	30.1
15b	3.62 (d, 18.5)		2.90 (d, 18.5)	
16		170.3		169.6
17	6.07 (s)	79.9	5.53 (s)	78.8
18	1.40 (s)	15.6	1.06 (s)	15.1
19	0.83 (s)	22.9	0.85 (s)	21.6
20		122.2		121.0
21	7.77 (s)	141.6	7.60 (s)	140.9
22	6.64 (s)	111.1	6.50 (s)	110.4
23	7.73 (s)	143.7	7.69 (s)	143.4
28	0.96 (s)	28.2	0.67 (s)	27.7
29	0.87 (s)	22.3	0.90 (s)	22.5
30α	1.58 (d, 14.5)	45.1	1.17 (d, 14.5)	42.5
30β	3.00 (d, 14.5)		2.40 (d, 14.5)	
OMe	3.73 (s)	51.6	3.57 (s)	51.1
2-OAc		170.4		169.8
	2.21 (s)	21.6	2.08 (s)	21.3
8-OH	8.00 (s)		5.78 (s)	

[a] Recorded in Py-d_6. [b] Recorded in DMSO-d_6. [c] Recorded at 125 MHz. [d] Overlapped, without designating multiplicity.

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assignments were as follows: an acetoxyl was located at C-3 by the HMBC correlations of H-3/C-3-OAc, and a proton of hydroxyl resonance at δ_H 8.00 was assigned as OH-8 based on its HMBC correlations with C-8 and C-14. Therefore, the planar structure of **1** was established as shown in Figure 1.

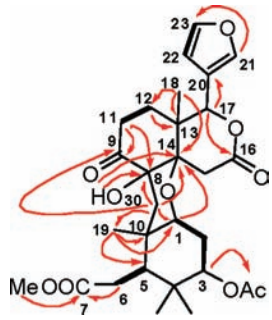


Figure 1. Key HMBC(→) and $^1H-^1H$ COSY(—) correlations of **1**.

The relative configuration of compound **1** was deduced from the analysis of ROESY correlations and the energy minimized molecular model using density functional theory (DFT) at the B3LYP/6−31G* basis set level in Gaussian 03.[9] As shown in ROESY data (Py-d_6), the correlations of H-1/Me-19, and H-3/Me-19 indicated that H-1, H-3, and Me-19 were cofacial, arbitrarily assigned as the α-oriented. The ROE correlations of H-5/H-30β and H-17/H-12β suggested H-5 and H-17 were β-oriented. Meanwhile, C-15 and Me-18 also took α-orientation due to NOE correlation of H-15a/H-1 and H-15b/Me-18. However, it was impossible to determined the configuration of 8-OH due to the absence of ROESY correlations of 8-OH signal and the other protons resonance in Py-d_6. Therefore, NMR experiments were reperformed in DSMO-d_6. And crucial NOE correlation between HO-8 and H-15b was observed, which revealed that HO-8 was α-oriented. The computer modeled 3D structure analysis of **1** was compatible with the aforementioned relative configurations as shown in Figure 2.

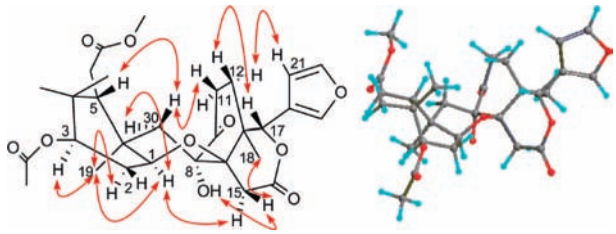


Figure 2. Key ROESY (↔) correlations and 3D computer modeling at B3LPY/6−31G* level of **1**.

The absolute configuration of **1** was assigned by the CD exciton chirality method.[10] The CD spectrum of **1** exhibited negative chirality resulting from the exciton coupling between the two different chromophores of the furan ring at 222 nm ($\Delta\varepsilon$ −1.16, $\pi \rightarrow \pi^*$ transition)[11] and the ketone at 220 nm ($\Delta\varepsilon$ −3.12, $\pi \rightarrow \pi^*$ transition),[12] indicating that the transition dipole monents of the two chromophores were oriented in a counterclockwise manner (Figure 3). The

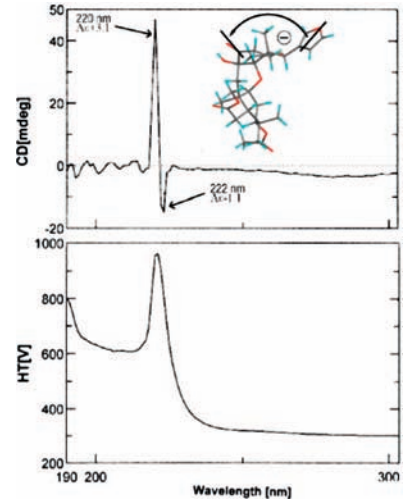
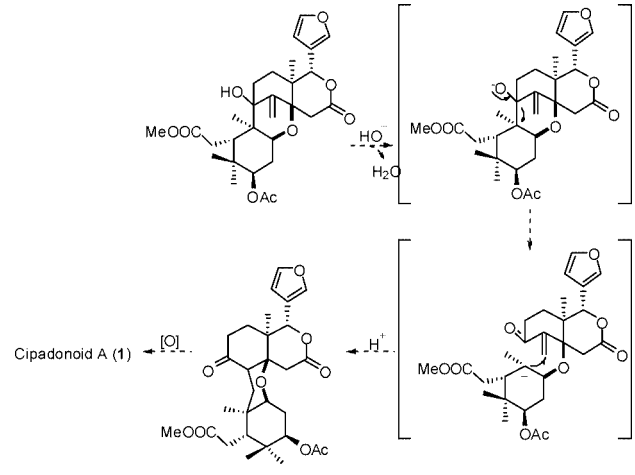


Figure 3. CD and UV spectra of **1**. Bold lines denote the electric transition dipole of the chromophores.

absolute configuration of **1** was thus assigned as 1*S*, 3*S*, 5*R*, 8*R*, 10*S*, 13*R*, 14*S*, 17*S*.

Biogenetically, **1** might be derived from the methyl angolensate type limonoid by the fact that configurations of all chiral centers were identical in both **1** and cipadesin D. Thus, a possible biosynthetic pathway for **1** was proposed as shown in Scheme 1.

Scheme 1. Hypothetical Biosynthetic Pathway of **1**



Cipadonoid A (**1**) was tested for the in vitro cytotoxicity against the P-388 (murine leukemia) cell lines by using the

MTT method.[13] However, it showed no activity against the cancer cell (50% effective dose of clonal inhibition, ED_{50} > 5 μg/mL).

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Supporting Information Available: Experimental section; ^{1}H and ^{13}C NMR assignments of **1** (in $CDCl_3$); IR, ESIMS, and 1D and 2D NMR spectra of **1**; standard orientation of cipadonoid A (1) at B3LYP/6−31G(d) level. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Cipadonoid A (**1**): white amorphous powder; $[\alpha]^{25}_D$ −10.0 (c 0.15 MeOH); UV (MeOH) λ_{max} nm 207; CD ($CHCl_3$) 220 nm ($\Delta\varepsilon$ −3.12), 222 nm ($\Delta\varepsilon$ −1.16); IR (KBr) 3432, 2924, 1728, 1628, 1376, 1276, 1076, 1041 cm^{-1}; ESI-MS *m*/*z* 569.4 $[M + Na]^+$; HRESI-MS *m*/*z* 569.2345, (calcd for $[M + Na]^+$ 569.2362).

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