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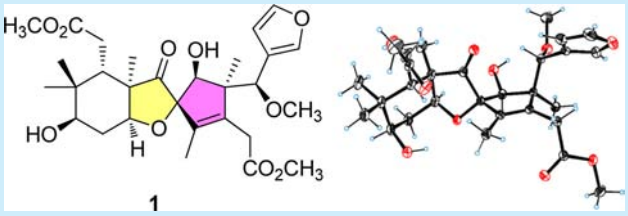
Cipacinoids A–D, Four Limonoids with Spirocyclic Skeletons from *Cipadessa cinerascens*

Jin-Hai Yu, Qun-Fang Liu, Li Sheng, Guo-Cai Wang, Jia Li, and Jian-Min Yue*

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, P.R. China

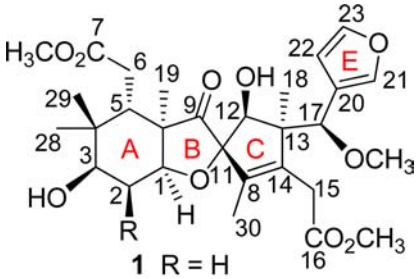
Supporting Information

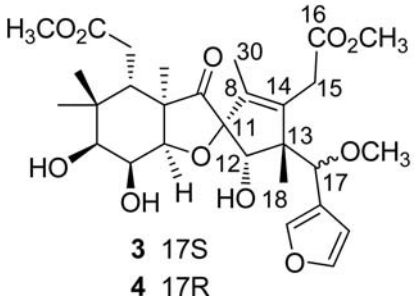
ABSTRACT: Four limonoids, cipacinoids A–D (**1**–**4**), with spirocyclic skeletons were isolated from *Cipadessa cinerascens*. It is particularly notable that compounds **1**–**3** had a 17*S*-configuration for the first time in the limonoid family. Their structures with absolute configurations were assigned by spectroscopic data, X-ray crystallography, and CD analysis. Compound **1** showed moderate protein tyrosine phosphatase 1B (PTP1B) inhibition.



The structurally diverse and biological significant limonoids that are mainly produced by the plant families of Meliaceae and Rutaceae have been attracting attention from the standpoint of both natural products and synthetic chemistry.[1] The plants of *Cipadessa* genus (Meliaceae) have delivered a large array of limonoids with a broad spectrum of biological activities.[2] The plant *Cipadessa cinerascens* (Pell.) Hand.-Mazz is a shrub or small tree which is widely distributed in the southwestern area of China, such as Guangxi, Guizhou, Sichuan, and Yunnan Provinces.[3] Its leaves and roots have been used as folk medicine for the treatment of cold, dysentery, stomachache, rheumatism, malaria, scald, and itchy skin.[4] Previously, a number of limonoids were isolated from two Chinese species of *Cipadessa* genus by our research group.[2a,e] In continuing the search for structurally diverse and biologically interesting metabolites from this plant genus, cipacinoids A–D (**1**–**4**) featuring unprecedented spirocyclic skeletons were isolated from the leaves of *C. cinerascens*. It is particularly noteworthy that compounds **1**–**3** possessed a 17*S*-configuration in the limonoid family for the first time. Herein, the isolation, structure elucidation, and biological evaluation of these limonoids are presented.

Cipacinoid A (**1**) was obtained as colorless crystals in methanol (mp 143–145 °C). It displayed a sodiated molecular ion peak at m/z 571.2517 $[M + Na]^+$ (calcd 571.2519) in the HRESI(+)MS analysis, which was consistent with the molecular formula of $C_{29}H_{40}O_{10}$ with 10 indices of hydrogen deficiency. The IR absorption bands revealed the presence of hydroxy (3523, 3432 cm^{-1}) and carbonyl (1743, 1726 cm^{-1}) groups. Analysis of the ^1H NMR data (Table 1) revealed the presence of a characteristic β-substituted furan ring (δ_H 7.45, 7.36 and 6.32), three methoxyls (δ_H 3.66, 3.59, 3.10, each 3H, s), and five tertiary methyls (δ_H 1.72, 1.26, 1.11, 0.93, 0.93, each 3H, s). The ^{13}C NMR data (Table 1) with the help of DEPT experiments showed the presence of 29 carbon resonances, including eight methyls (three methoxyls), three methylenes, eight methines (four oxygenated), and 10 quaternary carbons (one keto, two ester carbonyls, and one oxygenated). The aforementioned data and biogenetic reasoning suggested that cipacinoid A was likely a limonoid. Three carbonyls, one double bond, and the furan ring accounted for seven out of 10 indices of hydrogen deficiency, thus requiring the presence of three additional rings in cipacinoid A.





The planar structure of cipacinoid A was established by comprehensive analysis of 1D and 2D NMR spectra, particularly the HMBC spectrum (Figure 1A), in which the correlation networks of H_3-28(29)/C-3 (δ_C 75.5), C-4, and C-5; H_3-19/C-10, C-5 and C-1 (δ_C 80.9); H-1/C-2 and C-3; and

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds 1−4 in $CDCl_3$

	1		2		3		4	
no.	δ_H (mult, J, Hz)	δ_C	δ_H (mult, J, Hz)	δ_C	δ_H (mult, J, Hz)	δ_C	δ_H (mult, J, Hz)	δ_C
1	4.52, dd (3.3, 2.7)	80.9	4.65, d (3.6)	84.0	4.26, d (2.5)	85.6	4.34, d (2.7)	85.4
2a	2.27, dt (16.0, 2.7)	27.5	4.10, dd (3.6, 3.2)	65.0	3.96, dd, (2.5, 2.5)	65.2	3.99, dd, (2.8, 2.7)	65.2
2b	2.19, dt (16.0, 3.3)							
3	3.44, m	75.5	3.51 dd (10.0, 3.2)	79.8	3.45, dd (12.3, 2.5)	79.5	3.49, dd (11.9, 2.8)	79.4
4		39.5		41.2		40.9		40.9
5	2.58, dd (9.0, 2.3)	32.1	2.45, dd (8.8, 2.1)	31.9	2.65, dd (7.7, 3.2)	29.9	2.69, dd (7.3, 3.2)	29.3
6a	2.78, dd (16.5, 2.3)	29.5	2.76, dd (16.5, 2.1)	28.9	2.45, dd (16.8, 3.2)	28.8	2.44, dd (17.2, 3.2)	28.5
6b	2.39, dd (16.5, 9.0)		2.35, dd (16.5, 8.8)		2.28, dd (16.8, 7.7)		2.28, dd (17.2, 7.3)	
7		174.3		174.0		174.4		175.1
8		136.4		135.8		136.2		134.9
9		216.2		214.7		216.6		216.9
10		47.4		48.8		49.5		49.7
11		97.9		98.1		92.3		92.9
12	4.20, d (3.1)	91.6	4.22, d (2.9)	91.1	3.98, d (5.2)	82.3	3.87, d (5.8)	81.5
13		54.0		54.2		55.6		55.6
14		138.5		139.1		139.4		141.4
15a	2.71, d (16.5)	32.1	2.70, d (16.5)	32.0	2.88, d (16.0)	32.0	3.14, d (16.1)	32.5
15b	2.09, d (16.5)		2.09, d (16.5)		2.70, d (16.0)		3.03, d (16.1)	
16		171.0		171.0		170.8		170.9
17	4.90, s	79.9	4.83, s	79.8	4.38, s	80.6	4.34, s	81.2
18	1.26, s	17.2	1.25, s	17.2	1.32, s	18.7	1.23, s	19.9
19	1.11, s	14.0	1.14, s	13.9	1.10, s	14.0	1.12, s	13.8
20		121.9		121.9		122.3		123.3
21	7.36, m	142.0	7.35, m	142.0	7.37, m	142.6	7.36, m	142.0
22	6.42, dd (1.9, 0.8)	110.3	6.41, dd (1.8, 0.9)	110.3	6.33, m	110.7	6.34, m	111.3
23	7.45, dd (1.7, 1.7)	143.6	7.44, dd (1.7, 1.7)	143.6	7.41, dd (1.8, 1.8)	142.9	7.36, m	142.0
28	0.93, s	21.3	0.98, s	21.0	0.94, s	21.1	0.96, s	21.1
29	0.93, s	26.7	0.97, s	27.0	0.98, s	27.0	0.97, s	26.8
30	1.72, s	11.4	1.71, s	11.4	1.49, s	12.9	1.55, s	13.0
7-OCH_3	3.66, s	51.8	3.66, s	51.9	3.65, s	52.2	3.63, s	52.4
16-OCH3	3.59, s	52.0	3.59, s	52.0	3.61, s	52.0	3.67, s	52.0
17-OCH3	3.10, s	55.1	3.09, s	55.1	3.14, s	56.1	3.16, s	56.8
3-OH			3.14, d (10.0)		4.05, d (12.3)		4.04, d (11.9)	
12-OH	5.02, d (3.1)		4.95, d (2.9)		4.99, d (5.2)		4.07, d (5.8)	

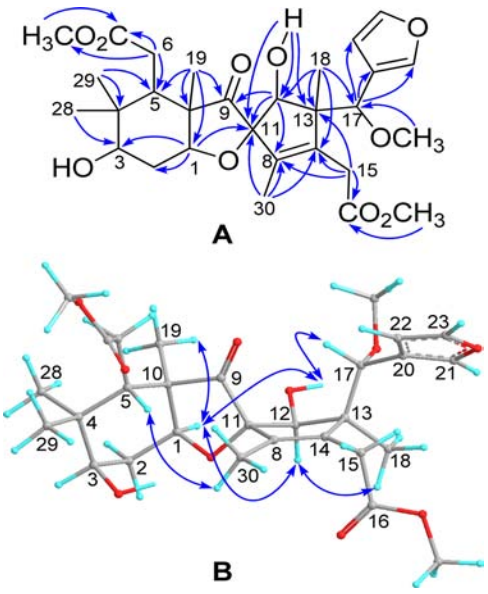

Figure 1. Key HMBC (A) and ROESY (B) correlations of 1.

H_2-6/C-5 and C-7 enabled the construction of the intact A-ring of a limonoid. The five-membered C-ring was established by the HMBC correlations of H_3-18/C-12 (δ_C 91.6), C-13 and C-14; H_3-30/C-8, C-11 (δ_C 97.9) and C-14; and H-12/C-8 and C-11. The B-ring was assembled to be a tetrahydrofuran ring by the key HMBC cross-peaks of H_3-19/C-10, C-1 and C-9 (δ_C 216.2); H-1/C-11; and H-12/C-9 and C-11. Subsequently, the HMBC correlations of H_2-15/C-8, C-13, C-14, and C-16; and 16-OCH_3/C-16 attached a methoxycarbonylmethyl moiety at C-14; and the HMBC correlations of H-6/C-5, C-7 and 7-OCH_3; and 7-OCH_3/C-7 indicated the presence of an additional methoxycarbonylmethyl moiety at C-5. Finally, the HMBC cross-peaks of H-17/C-20, C-21 and C-22; and 17-OCH_3/C-17 fixed the furan ring and a methoxy group to C-17 that was then linked to C-13 by the key HMBC correlation of H_3-18/C-17. An exchangeable proton signal at δ_H 5.02 was assigned to be a hydroxy group at C-12 by the key HMBC correlations of 12-OH/C-12 and was further supported by the coupling between H-12 and 12-OH ($J_{H\text{-}12,12\text{-}OH}$ = 3.1 Hz).

The relative configuration of **1** was mainly established by the examination of its ROESY data (Figure 1B). The strong correlation between H_3-19 and H-1 showed that they were cofacial and randomly assigned to be α-oriented. Subsequently, the ROESY correlations of H-1/H-12 and 12-OH and H_3-30/

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H-5 revealed the two planes of B- and C-rings were vertically arranged, and the latter correlation also indicated that H-5 was β-directed. In addition, the small coupling constants of $J_{2a,3/2b,3}$ = 2.7/3.3 Hz suggested that the H-3 was equatorially α-oriented. Furthermore, the ROESY correlation of 12-O*H*/H-17 and H-12/H_3-18 suggested 12-OH and H_3-18 were β- and α-configured, respectively. However, the stereochemistry at C-17 was left unassigned due to the absence of reliable data, although it was presumed to be *R*-configured biogenetically.[5] To our delight, compound **1** afforded high-quality crystals that allowed a successful performance of X-ray crystallography (Figure 2),

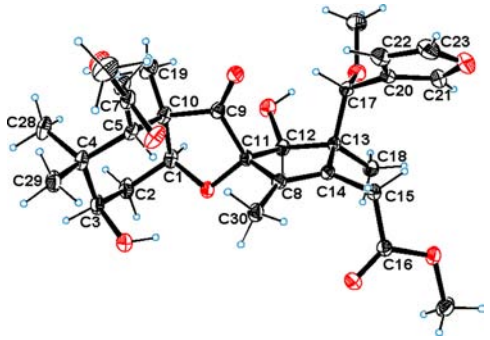

Figure 2. Single-crystal X-ray structure of **1**.

which not only confirmed the structure of **1** but also determined the absolute configuration as 1*S*,3*R*,5*S*,10*R*,11*S*,12*S*,13*S*,17*S* [absolute structure parameter −0.01(5)].[6] Thus, the structure of **1** was elucidated as shown. It is exceptionally noteworthy that compounds **1** possessed a 17*S*-configuration for the first time in the limonoid family.

Cipacinoid B (**2**) possessed a molecular formula of $C_{29}H_{40}O_{11}$ as determined by HRESI(+)MS at *m*/*z* 587.2470 $[M + Na]^+$ (calcd 584.2468). Comparison of its ^1H and ^{13}C NMR data (Table 1) with those of **1** revealed that their structures were closely related. The only difference was the presence of a hydroxy group at the C-2 of **2** as deduced from the proton and carbon resonances for the C-2 oxymethine (δ_H 4.10, dd, *J* = 3.6, 3.2 Hz; δ_C 65.0), which was supported by the couplings of H-2/H-1 (δ_H 4.65, d, *J* = 3.6 Hz) and H-2/H-3 (δ_H 3.51, dd, *J* = 10.0, 3.2 Hz). This assignment was further confirmed by the HMBC spectrum (Supporting Information, Figure S1A), in which the key correlations from H-1 (δ_H 4.65) and H-3 (δ_H 3.51) to C-2 (δ_C 65.0) were observed. The relative configurations in **2**, except for the C-2, were assigned the same as those of **1** on the basis of ^1H NMR data and ROESY spectrum (Supporting Information, Figure S1B). In particular, the key ROESY correlation between H-2 and H_3-19 indicated that H-2 was α-oriented. The CD spectrum of **2** (Figure 3) well matched that of **1**, and the absolute configuration of **2** was consequently assigned as depicted (1*R*,2*S*,3*S*,5*S*,10*R*,11*S*,12*S*,13*S*,17*S*).

Cipacinoid C (**3**), colorless crystals (in MeOH, mp 151−153 °C), was assigned a molecular formula $C_{29}H_{40}O_{11}$ by the HRESI(+)MS sodiated molecular ion peak at *m*/*z* 587.2461 $[M + Na]^+$ (calcd 587.2468). Comparison of its NMR data (Table 1) with those of **2** showed high similarities, and the main differences were the proton and carbon resonances associated with the spirocyclic center, e.g. the C-11 and C-12 carbon resonances of **3** were shifted upfield by $\Delta\delta_C$ 5.8 and 8.8 with respect to those of **2**, respectively. An analysis of the HMBC data (Supporting Information, Figure S2A) revealed that **3**

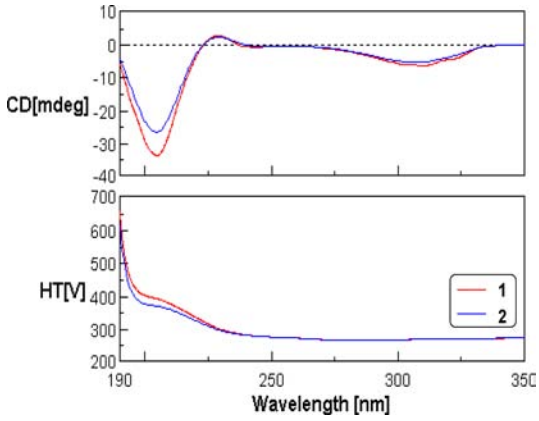

Figure 3. CD spectra of compounds **1** and **2**.

shared the same planar structure of **2**, in which the characteristic correlations of H-1/C-11 (δ_C 92.3); H-12/C-8, C-9, and C-11; and H_3-30/C-11 were observed. The remarkable variations of C-11 and C-12 carbon resonances of **3** were presumably resulted from the reversed C-11 configuration as compared with that of **2**. This assumption was verified by the ROESY data (Supporting Information, Figure S2B), in which the key correlations of H-1/H-30; and H-5/H-12 and 12-O*H* were evidenced. In addition, the 12-OH was assigned in an α-configuration by the ROESY correlation of 12-O*H*/H-17. Furthermore, the ROESY correlations of H_3-19/H-1 and H-2, and 3-O*H*/H-5 showed the stereochemistry of A-ring was identical to that of **2**.

Finally, A single crystal X-ray crystallography (Figure 4) confirmed the structure of **3**, and also determined the absolute

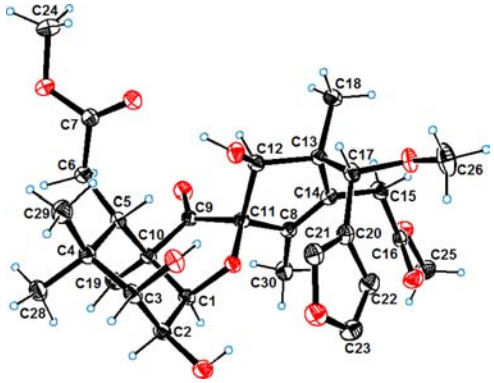

Figure 4. Single-crystal X-ray structure of **3**.

configuration as 1*R*, 2*S*, 3*S*, 5*S*, 10*R*, 11*R*, 12*S*, 13*S*, 17*S* [absolute structure parameter 0.05(4)]. The structure of **3** was thus established as depicted.

Cipacinoid D (**4**) shared the same molecular formula $C_{29}H_{40}O_{11}$ with **3** as determined by HRESI(+)MS ion peak at *m*/*z* 587.2466 $[M + Na]^+$ (calcd 587.2468). The 2D NMR data analysis, particularly the HMBC correlations (Supporting Information, Figure S3A) revealed that compound **4** shared a common planar structure with **3**. Its ^1H and ^{13}C NMR data (Table 1) also highly resembled those of **3**, and the slight changes were closely associated with the C-17, suggesting that they were likely the C-17 epimers. Further analysis of its ROESY data (Supporting Information, Figure S3B) showed that the relative configurations of the stereocenters from the A

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to C rings of **4** were identical to those of **3**, indicating that the only variation was the C-17 configuration. Comparing its NMR data with those of compound **3** (Table 1), the C-12 carbon resonance of **4** was more shielded due to the γ-gauche effects from 17-OCH_3, while the C-14 carbon resonance of **4** was more deshielded owing to the absence of 17-OCH_3 (Figure 5).

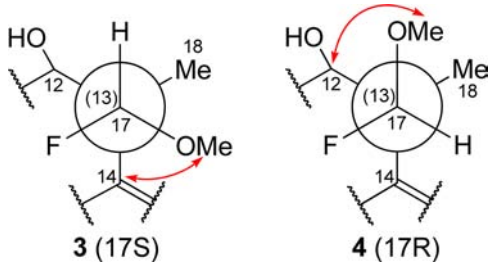

Figure 5. Newman projections along the C13/C17 bond for **3** and **4** (F stands for the furan ring; red arrow represents γ-gauche effect).

Further to this, the apparent variations in the CD curves of compounds **3** and **4** (Supporting Information, Figure S4) resulted from the reversed C-17 configuration. The structure of **4** was thus elucidated as depicted and named cipacinoid D.

PTP1B inhibitory evaluation: The protein tyrosine phosphatase 1B (PTP1B) plays an important role in cell regulation, growth, and the onset of human diseases. PTP1B overexpression stimulates the insulin-resistant phenotype in type 2 diabetes and obesity, which has thus been considered as a potential therapeutic target for type 2 diabetes and obesity.[7] Compounds **1**−**4** were assayed for the inhibitory effects on PTP1B enzyme by using an in vitro assay protocol and oleanolic acid was used as the positive control (IC_{50} = 2.3 μM).[8] Compound **1** showed moderate activity against PTP1B with IC_{50} value of 16.7 μM, and the others were inactive.

In summary, cipacinoids A−D (**1**−**4**) with an unprecedented spirocyclic skeleton were isolated from *C. cinerascens* for the first time. It is noteworthy that compounds **1**−**3** with a 17S-configuration are the first examples identified in the limonoid family and are structurally determined by the solid evidence of X-ray crystallography. This finding suggests that the formally identified D-ring demolished limonoids bearing either a 17R-OMe or a 17R-OAc, whose absolute configurations were tentatively assigned by comparing NMR data and biosynthetic consideration, should be reconsidered.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03487.

Experimental procedures, key HMBC and ROESY correlations of **2**−**4**, CD spectra for compounds **3** and **4**, and full spectra (NMR, MS, and IR) of compounds **1**−**4** (PDF)

X-ray data for compound **1** (CIF)

X-ray data for compound **3** (CIF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jmyue@simm.ac.cn. Tel: 86-21-5080-6718.

Notes

The authors declare no competing financial interest.

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