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Limonoids with Anti-HIV Activity from *Cipadessa cinerascens*

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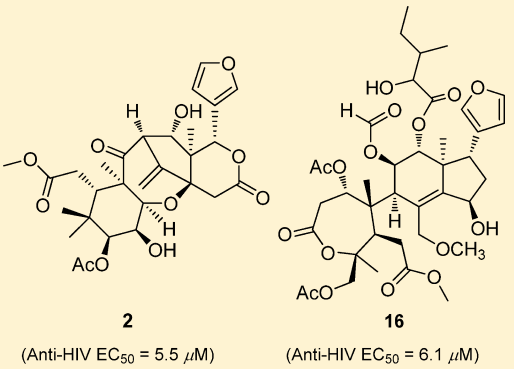
Supporting Information

ABSTRACT: Sixteen new limonoids, named ciparasins A–P (**1**–**16**), comprising three structural categories of trijugin-type (**1**–**7**), cipadesin-type (**8**–**15**), and prieurianin-type (**16**) compounds, as well as 15 known limonoid analogues (**17**–**31**), were isolated from *Cipadessa cinerascens*. Ciparasins E–G (**5**–**7**) were found to possess a rare γ-hydroxylbutenolide moiety at C-17. Ciparasins B (**2**) and P (**16**) showed significant anti-HIV activities, with EC_{50} values of 5.5 ± 0.6 (SI >7.2) and 6.1 ± 0.7 (SI >6.5) μM, respectively.



Plants in the genus *Cipadessa* (Meliaceae) biosynthesize structurally diverse limonoids, such as mexicanolides, methyl angolensates, trijugins, and cipadesins.[1−4] There are two species of this plant genus growing in mainland China, namely, *C. baccifera* and *C. cinerascens*.[5] The plant *C. cinerascens* (Pellegr.) Hand-Mazz., a shrub or small tree, is mainly distributed in the southwestern region of China, such as in Guangxi, Guizhou, Sichuan, and Yunnan Provinces.[5] Its leaves and roots have been applied in folk medicine to treat colds, dysentery, stomachache, rheumatism, malaria, and itchy skin.[6] In a previous study, 12 mexicanolide-type and a methyl angolensate-type limonoid were isolated from the seeds of *Cipadessa baccifera*.[3] In the present work, 16 new limonoids, named ciparasins A–P (**1**–**16**), including seven trijugins (**1**–**7**), eight cipadesins (**8**–**15**), and a prieurianin (**16**), along with 15 known limonoids (**17**–**31**), were isolated from the leaves of *C. cinerascens*. All the new limonoids were evaluated for anti-HIV activities, which has become a major research focus recently.[7−10] Ciparasins B (**2**) and P (**16**) showed significant anti-HIV activities with EC_{50} values of 5.5 ± 0.6 (SI >7.2) and 6.1 ± 0.7 (SI >6.5) μM, respectively. Herein, the isolation, structure elucidation, and biological evaluation of these limonoids are presented.

RESULTS AND DISCUSSION

Trijugin-Type Limonoids. Compound **1**, a white, amorphous powder, showed a molecular formula of $C_{31}H_{38}O_{12}$ as determined by the (+)-HRESIMS ion peak at m/z 625.2256 $[M + Na]^+$ (calcd 625.2255) and the ^{13}C NMR data, requiring 13 indices of hydrogen deficiency. Its IR absorption bands showed the presence of hydroxy (3524 cm^{-1}) and carbonyl (1733 cm^{-1}) groups. The ^1H and ^{13}C NMR data (Tables 1 and 2) exhibited diagnostic resonances for a β-substituted furan ring (δ_H 7.60, 7.44, and 6.56), an exocyclic double bond (δ_H 5.21 and 5.12), a keto group (δ_C 207.6), four ester groups (δ_C 175.1, 170.7, 170.5, and 168.4), two acetyl groups (δ_H 2.08 and 2.09, each 3H, s), and four tertiary methyls (δ_H 1.11, 1.05, 0.90, and 0.89, each 3H, s). These functionalities accounted for nine out of 13 indices of hydrogen deficiency, thus requiring the presence of four additional rings in **1**. The above-mentioned data together with biogenetic considerations suggested compound **1** to be a trijugin-type limonoid. The planar structure of **1** was delineated by analysis of the HMBC spectrum (Figure 1A), for which the carbon framework was the same as that of cipadesin D.[11] The only keto group was located at C-9 by the HMBC correlations of H-11, H-12, and H_3-19/C-9. In turn, the two acetoxy groups were linked to C-2 and C-3 by the HMBC correlations from H-2 (δ_H 5.21) and H-3 (δ_H 5.12) to each of the corresponding ester carbonyls, while OH-12 was assigned by the HMBC correlations from O<u>H</u>-12 (δ_H 2.59, which showed no HSQC correlation to any carbons) to C-12 (δ_C 76.4) and C-13 (δ_C 49.3).

The relative configuration of **1** was established by ROESY correlations (Figure 1B) and by comparing the NMR data with those of cipadesin D.[11] The H-1, H-2, and H-3 protons were assigned in an α-orientation by their coupling constants, which were closely comparable to those of cipadesin D. Subsequently, CH_3-28 and CH_3-19 were fixed as α-oriented by the ROESY correlations of H-2/H_3-19 and H-2/H_3-28.

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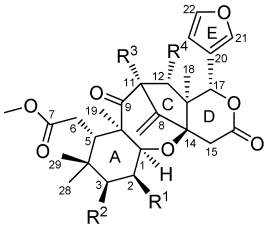
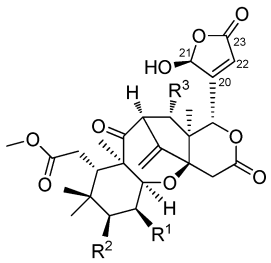
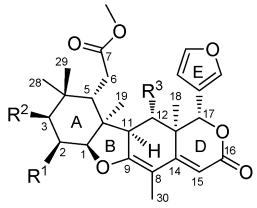


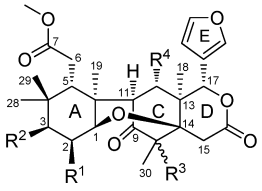
	R^1	R^2	R^3	R^4
1	OAc	OAc	H	OH
2	OH	OAc	H	OH
3	OH	OAc	H	OAc
4	H	OH	H	H
17	OAc	OAc	H	OAc
18	OAc	OAc	H	H
19	OAc	OAc	OH	H
20	H	OAc	H	OAc
21	H	OAc	H	OH
22	H	OAc	H	H



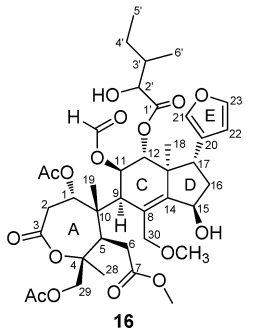
	R^1	R^2	R^3
5	H	OAc	H
6	OAc	OAc	OAc
7	H	OAc	OAc
23	OAc	OAc	H



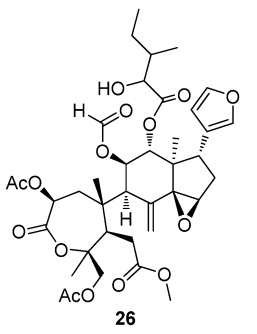
	R^1	R^2	R^3
13	OH	OAc	OH
14	OH	OH	OH
15	OH	OH	H
25	OAc	OAc	OH



	R^1	R^2	R^3	R^4
8	OAc	OAc	α-H	OH
9	H	OAc	α-H	OH
10	OH	OAc	α-H	OH
11	OAc	OAc	β-OH	OH
12	OAc	OAc	α-OH	OH
24	OAc	OAc	α-H	H



16



26

The ROESY correlations of H-5/H_3-29, H-5/H-12, and H-12/H-17 indicated CH_3-29, H-5, H-12, and H-17 to be β-directed. In turn, the ROESY correlations of H-1/H-15b, H-15b/$H_{pro\text{-}Z}$-30, and H-$30_{pro\text{-}E}$/H-11 suggested that the H-11 is α-oriented. Finally, the ROESY cross-peak of H-11/H_3-18 indicated that the CH_3-18 is also α-configured. Thus, the structure of **1** was assigned as depicted, and this compound was named ciparasin A.

Compound **2** displayed a sodiated molecular ion peak at m/z 583.2147 $[M + Na]^+$ (calcd 583.2150) in the (+)-HRESIMS corresponding to a molecular formula of $C_{29}H_{36}O_{11}$, which was consistent with the ^{13}C NMR data. Comparison of the ^1H and ^{13}C NMR data (Tables 1 and 2) with those of **1** revealed their structures to be closely related, and the only difference was the substitution at C-2. The H-2 resonance of **2** was shifted upfield by $\Delta\delta_H$ 1.13 as compared with that of **1**, suggesting that a hydroxy group is located at C-2. This was supported by the molecular weight and HMBC correlations from H-3 (δ_H 5.07) and H-1 (δ_H 4.08) to C-2 (δ_C 63.9) (Figure S13, Supporting Information). The small coupling constant of $J_{2,3}$ = 3.6 Hz, which was similar to that of **1**, indicated that OH-2 is also β-oriented. The structure of **2** (ciparasin B) was thereby elucidated as shown.

Compound **3** (ciparasin C) gave a molecular formula of $C_{31}H_{38}O_{12}$ as deduced from the ^{13}C NMR data and the (−)-HRESIMS ion peak at m/z 647.2326 $[M + HCO_2]^-$ (calcd 647.2345), indicative of being a structural isomer of **1**. The NMR data (Tables 1 and 2) showed the presence of two acetyl groups, of which one was apparently upfield shifted at δ_H 1.85 due to the shielding effect of the furan ring, suggesting that it is attached at C-12. Comparison of its NMR data with those of **2** revealed that they share a common A ring, indicating that the other acetoxy group is attached at C-3. This was confirmed by the 2D NMR spectra (Figures S20 and S21, Supporting Information), especially the HMBC spectrum, in which correlations from H-3 (δ_H 5.06) and H-12 (δ_H 5.52) to each of the corresponding ester carbonyls were observed. The relative configuration of **3** was established as the same as that of **1** from the very similar ^1H NMR coupling patterns. In particular, the small coupling constant of $J_{11,12}$ = 2.7 Hz suggested that the H-12 is β-oriented. Thus, the structure of **3** was assigned as shown.

Compound **4** (ciparasin D) had a molecular formula of $C_{27}H_{34}O_8$ as determined by the ^{13}C NMR data and (+)-HRESIMS ion peak at m/z 995.4409 $[2\ M + Na]^+$ (calcd 995.4400). Analysis of the ^1H and ^{13}C NMR data (Tables 1 and 2) revealed that **4** is also a structural congener of compounds **1**−**3**. The proton and carbon resonances of an oxymethine (δ_H 3.46, ddd, J = 7.5, 3.3, 2.9 Hz; δ_C 74.6) suggested that a hydroxy group is located at C-3, which was confirmed by the HMBC correlations of H_3-28 (H_3-29)/C-3 (δ_C 74.6) and O<u>H</u>-3 (δ_H 3.23)/C-3 and C-4 (δ_C 40.1) (Figure S29, Supporting Information). The small coupling constants of $J_{2a/2b,3}$ = 3.3/2.9 Hz suggested that OH-3 is axial and β-oriented. The structure of **4** was thus assigned as shown.

Compound **5** (ciparasin E), a white, amorphous powder, was found to possess a molecular formula of $C_{29}H_{36}O_{11}$, as determined by the (−)-HRESIMS ion peak at m/z 559.2174 $[M - H]^-$ (calcd 559.2185) and the ^{13}C NMR data. A comprehensive analysis of the NMR data revealed rings A−D of **5** to be identical to those of the coexistent cipatrijugin A (**22**),[12] while the characteristic β-substituted furan ring for a limonoid was absent, and concomitantly a γ-hydroxylbutenolide[13] was determined from the downfield signals at δ_H 6.15 and 6.29 (each 1H, s), suggesting this compound to be a trijugin-type limonoid bearing a γ-hydroxylbutenolide moiety at C-17. This was confirmed by the HMBC data (Figure 2A), in which key correlations of H-17/C-20, C-21 (δ_C 96.2), and C-23 and H-21/C-23 were observed. The relative configuration of **5** was assigned from the ROESY spectrum (Figure 2B), in which the correlations of H_3-19/H-1, H_3-19/H-2α, and H-2α/H_3-28 indicated that CH_3-19, H-2α, and CH_3-28 are cofacial, and these were assigned randomly as α-oriented. CH_3-29, H-5, H-12β, and H-17 were subsequently assigned as β-configured by the ROESY interactions of H_3-29/H-5, H-5/H-12β, and H-12β/H-17. The strong correlation of H-11/H_3-18, which was identical to that of **1**, revealed them to be α-oriented. When compared with **4**, the C-7 carbon resonance of **5** was apparently deshielded by $\Delta\delta_C$ 2.2, suggesting that a H-bond between the C-7 carbonyl and O<u>H</u>-21 was likely formed (Figure 2B), which stabilized the hemiacetal with a favorable β-configuration to yield a single C-21 epimer (normally, this occurs as a pair of epimers[1,13]) (Figures S34 and S35, Supporting Information). This was supported by the key ROESY correlations of H-21/H_2-12,

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Table 1. ^{1}H NMR Data of Compounds 1−5 (500 MHz, $CDCl_3$)

	1	2	3	4	5
position	(mult., J in Hz)	(mult., J in Hz)	(mult., J in Hz)	(mult., J in Hz)	(mult., J in Hz)
1	4.07, d (3.4)	4.08, m	4.09, d (3.3)	4.37, dd (3.3, 3.3)	4.17, dd (3.2, 3.2)
2a	5.21, dd (3.8, 3.4)	4.08, m	4.11, dd (3.8, 3.3)	2.27, ddd (15.6, 3.3, 2.9)	2.21, ddd (16.0, 3.2, 3.0)
2b				1.96, ddd (15.6, 3.3, 3.3)	1.88, ddd (16.0, 3.2, 3.0)
3	5.12, d (3.8)	5.07, d (3.6)	5.06, d (3.8)	3.46, ddd (7.5, 3.3, 2.9)	4.84, dd (3.0, 3.0)
5	2.85, m	2.76, dd (7.2, 2.1)	3.05, dd (7.2, 2.4)	2.89, m	2.92, m
6a	2.83, m	2.83, dd (18.1, 7.2)	2.86, dd (18.7, 2.4)	2.86, m	2.92, m
6b	2.31, m	2.29, dd (18.1, 2.1)	2.22, dd (18.7, 7.2)	2.31, dd (19.9, 5.0)	2.31, dd (19.5, 5.7)
11	3.38, d (3.0)	3.38, m	3.32, d (2.7)	3.54, dd (9.9, 4.6)	3.59, dd (10.1, 4.2)
12	5.44, dd (5.4, 3.0)	5.40, m	5.52, d (2.7)	α 1.78, dd (13.7, 9.9)	α 1.78, dd (13.4, 10.1)
				β 2.84, dd (13.7, 4.6)	β 2.77, dd (13.4, 4.2)
15a	2.81, d (17.2)	2.99,d (17.5)	3.09, d (17.5)	2.92, d (17.9)	2.83, d (10.4)
15b	2.75, d (17.2)	2.86,d (17.5)	2.88, d (17.5)	2.87, d (17.9)	2.83, d (10.4)
17	6.40, s	6.36, s	6.46, s	5.96, s	6.52, s
18	0.89, s	0.89, s	0.93, s	0.78, s	0.88, s
19	1.11, s	1.06, s	1.06, s	1.02, s	1.01, s
21	7.60, d (1.5)	7.60, dd (1.7, 0.9)	7.58, dd (1.7, 0.9)	7.52, m	6.15, s
22	6.56, d (1.8)	6.55, dd (1.9, 0.9)	6.42, dd (2.0, 0.9)	6.40, m	6.29, s
23	7.44, dd (1.8, 1.5)	7.45, dd (1.9, 1.7)	7.43, dd (2.0, 1.7)	7.43, dd (1.7, 1.7)	
28	1.05, s	1.00, s	1.03, s	1.00, s	0.95, s
29	0.90, s	0.91, s	0.86, s	0.85, s	0.79, s
30a	5.21, d (3.6)	5.35, d (1.2)	5.39, d (1.2)	5.42, s	5.47, s
30b	5.12, d (3.6)	5.19, d (1.2)	5.22, d (1.2)	5.23, s	5.25, s
OCH_3	3.67, s	3.67, s	3.67, s	3.65, s	3.68, s
OAc-2	2.09, s				
OAc-3	2.08, s	2.11, s	2.11, s		2.05, s
OAc-12			1.85, s		
OH-3				3.23, d (7.5)	
OH-12	2.59, d (5.4)	2.67, d (5.3)			
OH-21					5.96, brs

H-21/H_3-18, and H-22/H_3-18. The structure of **5** was thus elucidated as shown.

Compound **6** gave a molecular formula of $C_{33}H_{40}O_{15}$ as assigned by the (+)-HRESIMS sodiated ion peak at m/z 699.2278 $[M + Na]^+$ (calcd 699.2259) and its ^{13}C NMR data. Analysis of the ^{1}H and ^{13}C NMR data (Tables 2 and 4) revealed that **6** is also a trijugin-type limonoid, with a structure closely related to that of **5**. Compared with **5**, compound **6** incorporates two more acetoxy groups, which were located at C-2 and C-12 by the HMBC correlations from H-2 (δ_H 5.24) and H-12 (δ_H 5.45) to each of the corresponding ester carbonyls (Figure S46, Supporting Information). The H-2 proton was assigned as α-oriented by the small coupling constants of $J_{2,1/3}$ = 3.2/4.0 Hz. The H-12 proton was fixed in a β-orientation from the key ROESY correlation between H-5 and H-12. The relative configurations of the other stereocenters of **6** were assigned as being the same as those of **5** by the similar ^{1}H NMR coupling constants and from the ROESY data (Figure S47, Supporting Information), in which the presence of OH-21β was supported by the correlations of H-12/H-21, H-21/H_3-18, and H-22/H_3-18. Thus, the structure of **6** (ciparasin F) was assigned as shown.

Compound **7** gave a molecular formula of $C_{31}H_{38}O_{13}$, as deduced from its (+)-HRESIMS sodiated molecular ion peak at m/z 641.2207 $[M + Na]^+$ (calcd 641.2205) and its ^{13}C NMR data. Comparison of the ^{1}H and ^{13}C NMR data (Tables 2 and 3) with those of **6** revealed these compounds to be structural analogues, with the only difference occurring at the A ring, where a methylene (δ_H 2.23, 1.86; δ_C 30.0) in **7** was found in place of an oxymethine (δ_H 5.24; δ_C 65.3, CH-2) in **6**. This was confirmed by the key HMBC correlations of H-1 (δ_H 4.03) and H-3 (δ_H 4.87)/C-2 (Figure S55, Supporting Information). Similarly, OH-21 was assigned as β-configured by the diagnostic chemical shifts of C-7 and C-21 and the ROESY correlations of H-12β/H-21, H-21/H_3-18, and H_3-18/H-22 (Figure S56, Supporting Information). Thus, the structure of compound **7** (ciparasin G) was constructed as shown.

Cipadesin-Type Limonoids. Compound **8** was obtained as a white, amorphous powder. It afforded a molecular formula of $C_{31}H_{40}O_{12}$ as assigned by the (+)-HRESIMS ion peak at m/z 627.2426 $[M + Na]^+$ (calcd 627.2412) and from the ^{13}C NMR data, requiring 12 indices of hydrogen deficiency. The IR absorption bands revealed the presence of hydroxy (3509 cm^{-1}) and carbonyl (1744, 1713 cm^{-1}) groups. Analysis of the ^{1}H and ^{13}C NMR data (Tables 2 and 3) indicated the occurrence of a diagnostic β-substituted furan ring (δ_H 8.08, 7.49, and 6.69), a methoxy group (δ_H 3.70, 3H, s), two acetyls (δ_H 2.02 and 2.06, each 3H, s), a secondary methyl (δ_H 1.23, 3H, d, J = 7.2 Hz), four tertiary methyls (δ_H 0.88, 0.96, 1.04, and 1.13, each 3H, s), four ester carbonyls (δ_C 174.4, 170.7, 170.6, and 168.8), and a keto group (δ_C 210.6). This information, together with biogenetic reasoning, suggested **8** to be a cipadesin-type limonoid, thus sharing a common carbon skeleton with cipadesin A.[4] The planar structure of **8** was then constructed by analysis of the HMBC spectrum (Figure 3A). In particular, the HMBC correlations of H-11 and H_3-30/C-9 (δ_C 210.6) allowed the assignment of the only keto group to C-9, and the HMBC correlations from H-2 (δ_H 5.07) and H-3 (δ_H 5.05) to each of the corresponding ester carbonyls were used to locate the two

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Table 2. ^{13}C NMR Data of Compounds 1−16 (125 MHz)

position	1[a]	2[a]	3[a]	4[a]	5[a]	6[a]	7[a]	8[a]	9[a]	10[a]	11[b]	12[b]	13[a]	14[a]	15[a]	16[a]
1	73.8	76.2	76.3	74.8	71.6	74.0	72.0	76.5	75.8	78.9	75.4	75.9	88.4	90.4	90.6	70.0
2	65.4	63.9	64.1	31.4	29.7	65.3	30.0	66.2	27.7	63.7	66.7	66.6	66.8	64.8	65.0	39.1
3	74.4	78.1	78.3	74.6	74.2	74.1	74.2	75.1	75.3	78.2	75.5	75.4	78.9	79.7	79.7	170.3
4	38.9	39.2	39.6	40.1	38.6	39.3	38.6	39.3	37.9	39.5	39.1	39.0	39.9	40.8	40.6	83.4
5	37.2	37.2	35.5	37.0	38.1	35.5	35.6	37.8	38.1	37.6	38.3	37.8	35.9	34.8	33.9	46.4
6	29.3	29.4	28.5	29.8	29.6	28.5	29.1	30.1	30.6	30.2	29.9	29.7	26.4	31.2	31.9	32.3
7	175.1	175.2	173.8	174.4	176.6	175.6	176.0	174.4	174.7	174.5	174.5	174.3	175.3	175.2	173.9	173.3
8	142.5	142.3	142.1	144.2	142.8	140.7	140.8	44.2	44.2	44.2	73.5	73.7	101.9	103.3	103.3	130.1
9	207.6	208.0	205.7	209.6	209.6	204.2	205.3	210.6	211.4	210.6	213.2	211.7	159.7	158.8	161.6	41.4
10	55.4	55.2	56.6	55.3	55.2	56.8	58.5	46.3	44.9	45.6	46.4	45.3	47.6	47.8	46.6	46.2
11	70.9	70.8	69.1	58.3	58.4	69.2	69.2	67.4	66.9	67.26	68.4	67.4	55.7	55.7	50.2	77.4
12	76.4	76.0	76.3	35.6	34.9	76.0	75.7	67.4	67.4	67.31	66.7	66.9	69.5	69.7	31.2	78.9
13	49.3	49.4	49.6	45.8	45.9	49.2	49.3	46.0	45.7	45.9	45.4	45.2	44.1	44.2	37.9	48.7
14	89.3	89.5	88.4	89.3	87.2	87.9	87.3	80.0	79.8	80.7	83.3	81.5	162.7	162.2	162.0	159.8
15	33.6	34.1	34.4	34.4	34.7	33.8	33.7	39.0	39.6	39.0	34.9	35.6	105.3	106.3	105.5	69.8
16	168.4	169.0	168.8	168.1	167.7	167.0	167.0	168.8	168.9	168.8	171.7	171.4	165.9	165.7	166.3	37.7
17	79.6	79.7	78.4	79.6	78.4	77.4	77.2	79.1	78.9	79.2	81.3	80.9	77.9	78.0	80.1	46.1
18	10.2	10.3	11.7	17.9	17.1	11.2	11.1	14.7	14.8	15.1	15.3	15.3	11.3	11..2	16.6	15.6
19	19.1	19.1	19.6	19.9	19.7	19.4	18.4	18.5	19.1	18.5	17.6	18.4	21.1	20.4	19.5	18.1
20	121.8	121.7	121.1	121.7	164.2	163.5	163.6	121.2	121.2	121.1	121.6	121.2	122.6	122.5	120.4	121.4
21	140.1	140.1	140.4	140.0	96.2	96.1	96.0	141.6	141.8	141.7	141.8	141.9	141.4	141.4	141.1	140.7
22	109.2	109.1	108.8	108.4	119.9	120.7	120.6	110.3	110.3	110.3	110.7	110.8	109.7	109.7	109.8	110.9
23	143.4	143.4	143.4	143.5	169.3	169.3	169.3	143.4	143.4	143.5	142.7	142.6	145.1	145.3	143.1	142.5
28	22.8	22.9	22.9	22.7	22.7	22.7	22.7	21.9	22.0	21.8	21.1	20.9	21.7	21.9	21.7	28.2
29	27.9	27.8	27.5	28.4	27.2	27.3	27.2	27.9	28.1	27.9	27.4	27.4	26.4	26.9	26.9	67.9
30	114.3	114.3	114.8	114.9	115.5	116.0	115.5	10.5	10.7	10.7	25.5	20.9	9.8	9.8	9.6	68.9
OCOH-11																159.8
OCH_3-7	52.2	52.2	52.0	51.8	52.6	52.8	52.7	52.4	52.4	52.5	51.3	51.3	52.1	52.1	52.3	52.6
OCH_3-30																57.8
OAc-1																170.3/20.7
OAc-2	170.5/20.8		172.1/20.6			170.2/20.8		170.7/20.6			170.6/19.4	170.7/19.3				
OAc-3	170.7/20.5	171.7/20.7			170.8/20.7	170.8/20.8	170.8/20.3	170.6/20.9	170.8/21.0	171.1/20.8	171.3/19.4	171.0/19.4	171.6/21.1			
OAc-12			168.8/20.4			169.1/20.2	169.1/20.7									
OAc-29																170.3/20.8
1′																170.8
2′																76.4
3′																38.7
4′																23.8
5′																11.2
6′																14.3

[a]Data were measured in $CDCl_3$. [b]Data were measured in methanol-d_4.

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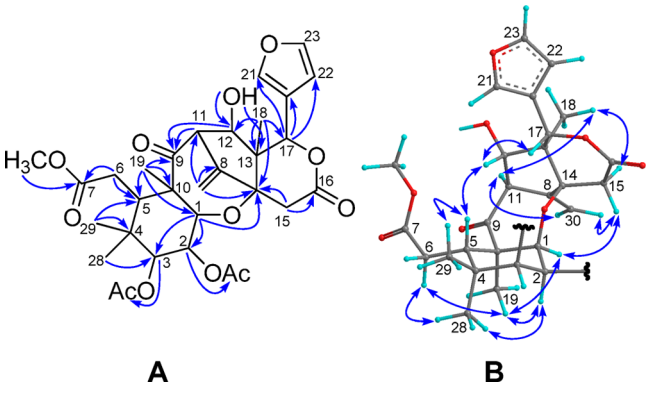

Figure 1. Key HMBC (A) and ROESY (B) correlations for **1** (OAc-2 and OAc-3 are not shown in B).

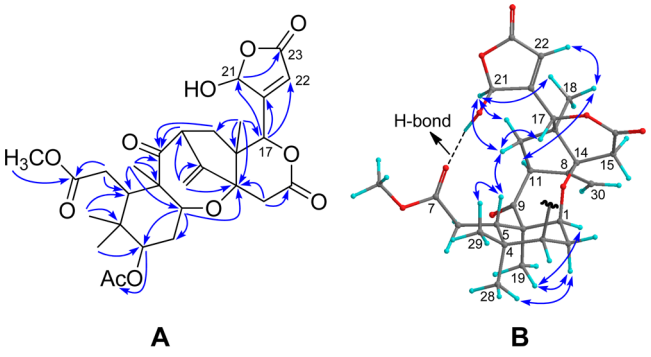

Figure 2. Key HMBC (A) and ROESY (B) correlations for **5** (OAc-3 is not shown in B).

acetoxy groups at C-2 and C-3, respectively. The HMBC cross-peaks of OH-12 (δ_H 1.97)/C-12 (δ_C 67.4) and C-13 supported the placement of the only hydroxy group at C-12. The relative configuration of **8** was established on the basis of the ROESY data (Figure 3B) and by analogy with that of cipadesin A. The stereocenters of the A ring were assigned as being the same as those of cipadesin A based on the highly similar coupling patterns of H-1, H-2, H-3, and H-5. The ROESY correlations of H-5/H-12 and H-12/H-17 indicated that H-12 and H-17 are β-oriented. In particular, the ROESY correlation of H_3-30/H-1 supported CH_3-30 as β-configured. Thus, the structure of **8** was elucidated as shown, and this compound was named ciparasin H.

Compounds **9** and **10** were assigned molecular formulas of $C_{29}H_{38}O_{10}$ and $C_{29}H_{38}O_{11}$ by the (+)-HRESIMS ion peak at m/z 569.2374 $[M + Na]^+$ (calcd 569.2357) and the (−)-HRESIMS ion peak at m/z 561.2327 $[M - H]^-$ (calcd 561.2341), respectively, as well as their ^{13}C NMR data. The ^1H and ^{13}C NMR data (Tables 2 and 3) of these compounds were closely comparable to those of **8**, with differences occurring at C-2. An additional methylene (δ_H 2.06, 2.04, δ_C 27.7) in **9** and a shielded oxymethine (δ_H 3.98, δ_C 63.7) in **10** were observed in place of the oxymethine (δ_H 5.07, δ_C 66.2, CH-2) of **8**; thus a CH_2-2 group was assigned for **9**, and a hydroxy group was placed at C-2 of **10**, which were consistent with their molecular weights. The HMBC correlations of H-1 (δ_H 3.47) and H-3 (δ_H 4.70)/C-2 (δ_C 27.7) in **9** (Figure S73, Supporting Information) and H-1 (δ_H 3.58) and H-3 (δ_H 5.07)/C-2 (δ_C 63.7) in **10** (Figure S82, Supporting Information) were observed to corroborate these conclusions. Finally, the OH-2 of **10** was assigned as β-configured as a result of the key ROESY correlation of H-2/H_3-19 (Figure S83, Supporting Information).

Table 3. ^1H NMR Data of Compounds 6−10 (500 MHz, $CDCl_3$)

	6	7	8	9	10
position	(mult., J in Hz)	(mult., J in Hz)	(mult., J in Hz)	(mult., J in Hz)	(mult., J in Hz)
1	4.03, d (3.2)	4.03, dd (2.9, 2.9)	3.71, d (4.0)	3.47, dd (4.1, 2.4)	3.58, d (4.1)
2a	5.24, dd (4.0, 3.2)	2.23, ddd (16.0, 3.0, 2.9)	5.07, dd (4.0, 3.0)	2.06, m	3.98, ddd (11.3, 4.1, 3.3)
2b		1.86, ddd (16.0, 3.0, 2.9)		2.04, m	
3	5.14, d (4.0)	4.87, dd (3.0, 3.0)	5.05, d (3.0)	4.70, t (3.0)	5.07, d (3.3)
5	3.03, dd (7.5, 2.2)	3.03, dd (7.8, 1.8)	2.70, dd (8.9, 1.6)	2.76, dd (8.7, 2.3)	2.60, dd (8.9, 2.2)
6a	2.83, dd (19.0, 2.2)	2.69, dd (19.0, 1.8)	2.44, dd (16.4, 8.9)	2.83, dd (16.3, 8.7)	2.43, dd (16.4, 8.9)
6b	2.29, dd (19.0, 7.5)	2.32, dd (19.0, 7.8)	2.24, dd (16.4, 1.6)	2.24, dd (16.3, 2.3)	2.22, dd (16.4, 2.2)
8			2.70, q (7.2)	2.68, q (7.2)	2.73, q (7.0)
11	3.36, d (2.7)	3.36, dd (2.7, 1.3)	2.48, s	2.51, s	2.52, s
12	5.45, d (2.7)	5.44, d (2.7)	4.59, d (3.6)	4.61, d (3.5)	4.60, s
15a	2.86, s	2.90, d (17.8)	2.67, s	2.74, d (17.7)	2.80, d (17.6)
15b	2.86, s	2.80, d (17.8)	2.67, s	2.65, d (17.7)	2.73, d (17.6)
17	6.60, s	6.62, s	6.36, s	6.39, s	6.35, s
18	1.01, s	0.98, s	1.04, s	1.05, s	1.07, s
19	1.11, s	1.04, s	0.96, s	0.90, s	0.93, s
21	5.90, s	5.91, s	8.08, dd (1.5, 0.9)	8.08, dd (1.5, 0.9)	8.09, dd (1.7, 0.9)
22	6.33, s	6.31, s	6.69, dd (1.8, 0.9)	6.69, dd; (1.8, 0.9)	6.68, dd (1.7, 0.9)
23			7.49, dd (1.8, 1.5)	7.49, dd (1.8, 1.5)	7.49, dd (1.7, 1.7)
28	1.07, s	0.97, s	1.13, s	1.03, s	1.08, s
29	0.78, s	0.77, s	0.88, s	0.90, s	0.86, s
30a	5.45, s	5.43, d (1.3)	1.23, d (7.2)	1.28, d (7.2)	1.34, d (7.2)
30b	5.27, s	5.25, d (1.3)			
OCH_3	3.67, s	3.66, s	3.70, s	3.70, s	3.71, s
OAc-2	2.11, s		2.06, s		
OAc-3	2.09, s	2.07, s	2.02, s	2.05, s	2.09, s
OAc-12	2.05, s	2.03, s			
OH-2					2.93, d (11.3)
OH-12			1.97, d (3.6)	1.63, d (3.5)	

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J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 4. ^{1}H NMR Data of Compounds 11−16 (500 MHz)

	11[a]	12[a]	13[b]	14[b]	15[b]	16[b]
position	(mult., J in Hz)	(mult., J in Hz)	(mult., J in Hz)	(mult., J in Hz)	(mult., J in Hz)	(mult., J in Hz)
1	4.56, d (4.3)	3.94, d (4.3)	4.28 d (4.5)	4.29, d (3.7)	4.30, d (3.7)	5.28, dd (9.7, 6.7)
2	5.17, dd (4.3, 3.2)	5.14, dd (4.3, 3.2)	4.14, dd (4.5, 3.0)	4.03, dd (3.7, 3.1)	4.06, dd (3.7, 3.1)	3.23, dd (18.7, 9.7)
						3.15, dd (18.7, 6.7)
3	5.02, d (3.2)	5.06, d (3.2)	5.00, d (3.0)	3.52, dd (8.7, 3.1)	3.52, dd (6.6, 3.1)	
5	2.60, dd (9.8, 2.1)	2.75, dd (10.0, 1.9)	2.62, d (9.4)	2.11, d (9.2)	2.09, d (9.4)	3.18, m
6a	2.57, dd (16.5, 9.8)	2.56, dd (16.5, 10.0)	3.03, d (16.9)	2.99, d (16.9)	2.40, dd (16.8, 9.4)	2.72, dd (17.7, 6.8)
6b	2.45, dd (16.5, 2.1)	2.44, dd (16.5, 1.9)	2.31, dd (16.9, 9.4)	2.37, dd (16.9, 9.2)	2.10, d (16.8)	2.45, dd (17.7, 3.4)
9						3.57, s
11	2.60, d (1.8)	2.64, d (1.6)	2.62, dd (10.0, 2.0)	2.61, dd (10.1, 2.0)	2.53, ddd (12.6, 4.6, 2.0)	5.67, s
12	4.50, d (1.8)	4.55, d (1.6)	4.36, dd (10.0, 1.6)	4.36, dd (10.1, 1.6)	α 1.28, dd (11.4, 4.6)	5.16, s
					β 2.14, dd (12.6, 11.4)	
15	2.99, d (17.4)	3.17, d (17.7)	5.65, s	5.69, s	5.65, s	4.99, d (5.2)
	2.87, d (17.4)	2.62, d (17.7)				
16α						2.11, m
16β						2.00, dd (13.7, 5.9)
17	6.75, s	6.64, s	5.20, s	5.17, s	5.04, s	3.32, dd (13.0, 5.9)
18	1.17, s	1.34, s	1.27, s	1.27, s	1.05, s	0.88, s
19	1.00, s	0.98, s	1.24, s	1.23, s	1.13, s	1.00, s
21	8.24, dd (1.7, 0.9)	8.24, dd (1.5, 0.7)	7.47, m	7.48, m	7.34, dd (1.5, 0.9)	7.27, d (1.4)
22	6.73, dd (1.8, 0.9)	6.72, dd (1.9, 0.9)	6.53, m	6.53, m	6.33, dd (1.9, 0.9)	6.24, m
23	7.56, dd (1.8, 1.7)	7.54, dd (1.9, 1.7)	7.51, m	7.52, m	7.42, dd (1.9, 1.5)	7.30, t (1.7)
28	1.18, s	1.18, s	1.06, s	0.98, s	0.97, s	1.60, s
29	0.85, s	0.87, s	0.78, s	0.93, s	0.92, s	4.23, d (12.2)
						4.17, d (12.2)
30	1.49, s	1.42, s	1.80, d (2.0)	1.80, d (2.0)	1.80, d (2.0)	α 4.35, d (10.8)
						β 4.70, d (10.8)
OCOH-11						8.07, s
OCH_3-7	3.72, s	3.72, s	3.72, s	3.74, s	3.73, s	3.42, s
OCH_3-30						3.72, s
OAc-1						2.04, s
OAc-2	1.96, s	2.00, s				
OAc-3	2.06, s	2.05, s	2.10, s			
OAc-29						2.11, s
OH-3				2.24, d (8.7)	2.54, d (6.6)	
OH-12			1.51, d (1.6)	1.51, d (1.6)		
2′						3.90, d (5.9)
3′						1.61, m
4′						1.15, m
						1.34, m
5′						0.82, t (7.4)
6′						0.66, d (6.8)

[a]Data were measured in methanol-d_4. [b]Data were measured in $CDCl_3$.

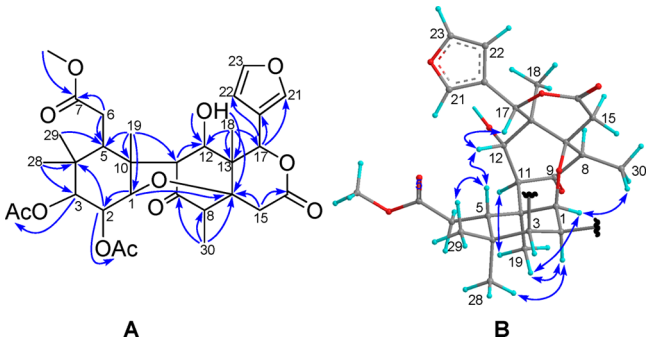

Figure 3. Key HMBC (A) and ROESY (B) correlations for 8 (OAc-2 and OAc-3 are not shown in B).

The structures of compounds 9 and 10 were thus identified as depicted and named ciparasins I and J, respectively.

Compound 11 (ciparasin K) displayed a molecular formula of $C_{31}H_{40}O_{13}$ as deduced from its (−)-HRESIMS ion peak at m/z 643.2449 $[M + HCO_2]^-$ (calcd 665.2451) and the ^{13}C NMR data. Analysis of the ^{1}H and ^{13}C NMR data (Tables 2 and 4) revealed that it is also a cipadesin-type limonoid, with a structure closely related to compound 8, with the difference being that an oxygenated quaternary carbon (δ_C 73.5) bearing a hydroxy group was evident at C-8 of 11, in place of the C-8 methine of 8. As a consequence, the $C\underline{H}_3$-30 of 11 appeared as a singlet proton signal as compared with a doublet in 8. This assignment was confirmed from the HMBC data (Figure S91, Supporting Information), and in particular the HMBC correlations from H_3-30 to C-8 (δ_C 73.5), C-9 (δ_C 213.2), and C-14 (δ_C 83.3) verified the presence of a hydroxy group at C-8. The OH-8 functionality was assigned a β-orientation from the key ROESY correlations of H_3-30/15a and H_3-30/H_3-18

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J. Nat. Prod. XXXX, XXX, XXX−XXX

(Figure S92, Supporting Information). Thus, the structure of **11** was established as depicted.

Compound **12** (ciparasin L) gave a common molecular formula of $C_{31}H_{40}O_{13}$ to **11**, as evidenced from the (+)-HRESIMS ion peak at *m/z* 643.2379 $[M + Na]^+$ (calcd 643.2361) and the ^{13}C NMR data. Analysis of the 2D NMR spectra (Figures S99 and S100, Supporting Information) revealed compound **12** to possess the same planar structure as **11**. Its NMR data (Tables 2 and 4) showed many similarities to those of **11**, especially the highly similar coupling patterns of H-1, H-2, H-3, H-5, H-11, H-12, and H-17, but suggested that it is the C-8 epimer of **11**. This assignment was confirmed by the strong ROESY correlation between H_3-30 and H-1 (Figure S101, Supporting Information). The structure of **12** was thus determined as shown.

Compound **13** (ciparasin M), a white, amorphous powder, displayed a molecular formula of $C_{29}H_{36}O_{10}$, as determined by the (+)-HRESIMS ion peak at *m/z* 545.2386 $[M + H]^+$ (calcd 545.2381) and from the ^{13}C NMR data. The UV spectrum showed a strong absorption maximum at 318 nm, suggesting the presence of an $\alpha,\beta,\gamma,\delta$-unsaturated lactone. Analysis of the NMR data (Tables 2 and 4) revealed the presence of a diagnostic β-substituted furan ring (δ_H 7.51, 7.47, and 6.53), an olefinic bond (δ_H 5.65), an allylic methyl (δ_H 1.80, 3H, d, J = 2.0 Hz), and four tertiary methyls (δ_H 1.27, 1.24, 1.06, and 0.78, each 3H, s). The aforementioned data as well as biogenetic considerations suggested that compound **13** is a cipadesin-type limonoid, with a structure similar to that of cipadesin C.[4] Analysis of the HMBC data (Figure 4A) was used to delineate

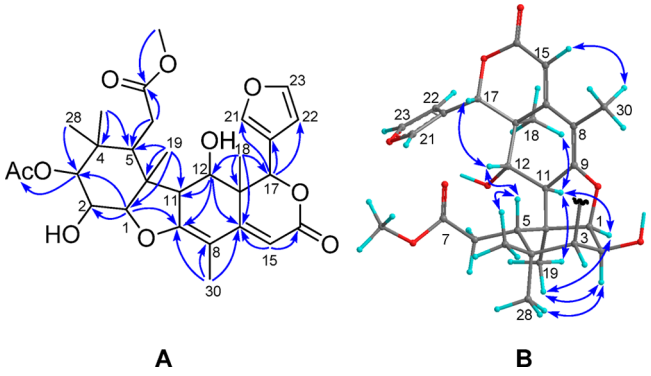

Figure 4. Key HMBC (A) and ROESY (B) correlations for **13** (OAc-3 is not shown in B).

the planar structure, representing a 2-deacetylated derivative of cipadesin C.[4] The relative configuration of **13** was established by comparing with cipadesin C and analysis of the ROESY data (Figure 4B). The coupling patterns and the coupling constants of H-1, H-2, H-3, and H-5 showed close similarities to those of cipadesin C, indicating that the A rings of these two compounds share the same relative configuration. Subsequently, H-11 was assigned in an α-orientation by the ROESY correlation of H-1/H-11, and H-12 was fixed in a β-configuration by the ROESY correlation of H-5/H-12. The structure of **13** was accordingly assigned as shown.

Compound **14** (ciparasin N), a white, amorphous powder, displayed a molecular formula of $C_{27}H_{34}O_9$, as established by the (+)-HRESIMS ion peak at *m/z* 503.2267 $[M + H]^+$ (calcd 503.2276) and the ^{13}C NMR data. Analysis of the NMR data (Tables 2 and 4) revealed the structure of **14** to be closely related to that of **13**, and the only difference was an OH-3 in **14** instead of an OAc-3 in **13**, which was consistent with its molecular weight and the shielded H-3 resonance. This assignment was confirmed by the HMBC correlations of H_3-28(H_3-29)/C-3 (δ_C 79.7) (Figures S117 and S118, Supporting Information). The small coupling constant of $J_{2,3}$ = 3.1 Hz permitted the assignment of OH-3 in a β-configuration. The structure of **14** was therefore elucidated as shown.

The protonated (+)-HRESIMS ion peak at *m/z* 487.2320 $[M + H]^+$ (calcd 487.2326) and the ^{13}C NMR data allowed the assignment of a molecular formula of $C_{27}H_{34}O_8$ for compound **15**. Comparison of the ^1H and ^{13}C NMR data (Tables 2 and 4) with those of **14** revealed that they are also structural analogues, and the only difference occurred in the C ring, where the oxymethine (δ_H 4.36, δ_C 69.7, CH-12) of **14** was replaced by a methylene (δ_H 2.14, 1.28, δ_C 31.2) in **15**. This was confirmed by the key HMBC correlation of H_3-18/C-12 (δ_C 31.2) (Figure S126, Supporting Information). The H-12β (δ_H 2.14) proton signal was readily distinguished by the large coupling constant of $J_{11,12\beta}$ = 12.6 Hz. Compound **15** (ciparasin O) was thus characterized as shown.

Prieurianin-Type Limonoid. Compound **16**, a white, amorphous powder, was assigned the molecular formula of $C_{39}H_{54}O_{16}$ from the sodiated (+)-HRESIMS ion peak at *m/z* 801.3310 $[M + Na]^+$ (calcd 801.3304) and its ^{13}C NMR data. The IR absorptions observed implied the presence of hydroxy (3448 cm^{-1}), carbonyl (1744 cm^{-1}), and olefinic (1638 cm^{-1}) groups. Analysis of the NMR data (Tables 2 and 4) showed the presence of a formate (δ_H 8.07, 1H, s; δ_C 159.8), a β-substituted furan ring (δ_H 7.30, 7.27, and 6.24), two oxymethylenes (δ_H 4.70, 4.35; δ_C 68.9, and 4.23, 4.17; δ_C 67.9), two methoxy groups (δ_H 3.72, 3.42, each 3H, s), two acetyl groups (δ_H 2.04, 2.11, each 3H, s), three tertiary methyls (δ_H 1.60, 1.00, and 0.88, each 3H, s), a secondary methyl (δ_H 0.66, 3H, d, J = 6.8 Hz), and a primary methyl (δ_H 0.82, 3H, t, J = 7.4 Hz). The above-mentioned data suggested that compound **16** is a prieurianin-type limonoid,[2] for which the NMR data (Tables 2 and 4) showed many similarities to those of the co-occurring mulavanin D (**26**).[14] Analysis of the NMR data, especially the HMBC correlations (Figure 5A), allowed the establishment of

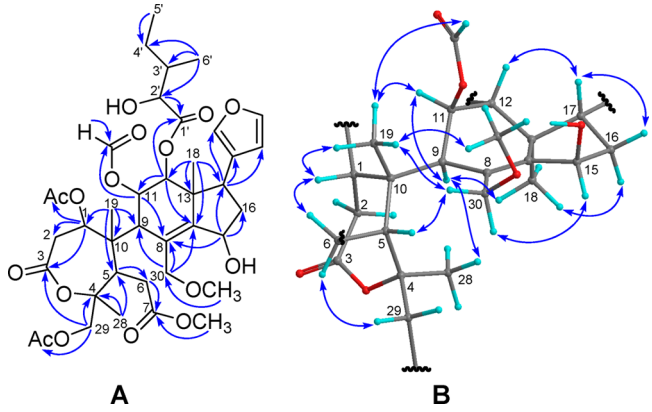

Figure 5. Key HMBC (A) and ROESY (B) correlations for **16** (C-1, C-12, and C-29 substituents, C-7 methoxycarbonyl, and furan ring are not shown in B).

the planar structure of **16**. The major differences from **26** occurred in the C and D rings, where a $\Delta^{8(14)}$ double bond was located from the HMBC correlations of H_2-30/C-8 and C-14 and H_3-18/C-14; a hydroxy group was assigned to C-15 by the HMBC correlations of H-15 (δ_H 4.99)/C-8, C-16, and C-17;

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J. Nat. Prod. XXXX, XXX, XXX−XXX

and a methoxy group was located at C-30 by the HMBC correlation of OC$\underline{H}_3$-30/C-30 (δ_C 68.9). In addition, a long-range HMBC correlation of H_2-29/C-4 (δ_C 83.4) allowed the establishment of the typical A ring of a seven-membered lactone, with two acetoxy groups attached at C-1 and C-29 from the HMBC correlations from H-1 (δ_H 5.28) and H_2-29 (δ_H 4.23, 4.17) to each of the corresponding ester carbonyls. Finally, the presence of a 3-methyl-2-hydroxylpentanoate group was confirmed by the HMBC correlations within the moiety, which was attached to C-12 by the key HMBC correlation from H-12 (δ_H 5.16) to the ester carbonyl (δ_C 170.3). The formate group was located at C-11 from the HMBC correlation from H-11 (δ_H 5.67) to the ester carbonyl (δ_C 159.8).

The relative configuration of **16** was established mainly from the examination of its ROESY data (Figure 5B). The ROESY correlations of H-1/H_3-19, H-1/H-6a, and H-6b/H_2-29 revealed them to be cofacial, and these were assigned arbitrarily in a β-orientation. In turn, the correlations of H_3-28/H-5, H_3-28/H-9, H-9/H-11, and H-9/H_3-18 indicated that they are α-oriented. The H-30β (δ_H 4.70) proton was determined from the ROESY correlations of H-5/H-30β, H_3-19/H-30β, and H_3-19/OC$\underline{H}_3$-30. In consequence, the OH-15 group was assigned as being β-configured from the strong ROESY correlation of H-30α/H-15. The H-12 proton was assigned as β-oriented by the ROESY correlation of H-12/H-17. The singlets of H-9, H-11, and H-12 indicated that the dihedral angles between H-11 and H-9 and between H-11 and H-12 are approximately 90°, and the C ring was assigned in a half-chair conformation. The stereochemistry within the 3-methyl-2-hydroxylpentanoate moiety was not assigned due to the lack of available data. Thus, the structure of **16** was characterized as shown, and this compound was named ciparasin P.

Fifteen known compounds were identified to be cipadesin D (**17**),[11] cipadesin H (**18**),[15] cipadesin E (**19**),[11] cipatrijugin D (**20**),[16] cipatrijugin H (**21**),[17] cipatrijugin A (**22**),[16] cipatrijugin G (**23**),[18] cipadesin B (**24**),[4] cipadesin C (**25**),[4] mulavanin D (**26**),[14] and five mexicanolide-type limonoids, khayasin T (**27**),[19] 3β-(isobutyryloxy)mexicanolide (**28**),[3] 2′*S*-methylbutanoylproceranolide (**29**),[3] 2′*R*-methylbutanoylproceranolide (**30**),[3] and febrifuin (**31**)[19] by spectroscopic analysis and comparing their NMR data with those reported.

All the new compounds were tested in vitro for anti-HIV activities on MTT cells infected by HIV-1 NL 4-3 with nevirapine as positive control.[20] Two compounds, ciparasins B (**2**) and P (**16**), showed significant anti-HIV activities, with each having an EC_{50} value of 5.5 ± 0.6 (SI >7.2) and 6.1 ± 0.7 (SI >6.5) μM, but only marginal cytotoxicity against MTT cells (both CC_{50} >40 μM). The other compounds were inactive.

In conclusion, 16 new limonoids, representing trijugin-type (**1**–**7**), cipadesin-type (**8**–**15**), and prieurianin-type (**16**) compounds, as well as 15 known limonoid analogues (**17**–**31**), were isolated from *Cipadessa cinerascens*. Ciparasins E–G (**5**–**7**) possess a rare C-17 γ-hydroxylbutenolide moiety, and ciparasins B (**2**) and P (**16**) showed significant anti-HIV activities. These findings are an important addition to the present knowledge on the structurally diverse and biologically important limonoid family.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were detected on an Autopol VI polarimeter at room temperature. UV spectra were measured on a Shimadzu UV-2550 UV–visible spectrophotometer. IR spectra were recorded on a Thermo IS5 spectrometer with KBr disks. NMR data were acquired on a Varian Mercury-500 spectrometer with TMS as internal standard. (+)- and (−)-ESIMS and (+)- and (−)-HRESIMS experiments were carried out on a Esquire 3000plus LCMS and a Waters Q-TOF Ultima Global mass spectrometer, respectively. Semipreparative HPLC was performed on a Waters 1525 pump equipped with a Waters 2489 detector (210 nm) and a YMC-Pack ODS-A column (250 × 10 mm, S-5 μm, 12 nm). Precoated silica gel GF254 plates (Qingdao Haiyang Chemical Co., Ltd., Qingdao, People's Republic of China) were used for TLC monitoring. D101-macroporous absorption resin (Sinopharm Chemical Reagent Co., Ltd., Shanghai, People's Republic of China), CHP20P MCI gel (75–150 μm, Mitsubishi Chemical Corporation), silica gel H (Qingdao Haiyang Chemical Co., Ltd., Qingdao), and C_{18} reversed-phase (RP-18) silica gel (20–45 μm, Fuji Silysia Chemical Ltd.) were used for column chromatography (CC). All solvents used for CC were of analytical grade (Shanghai Chemical Reagents Co., Ltd., Shanghai, People's Republic of China), and solvents used for HPLC were of HPLC grade (J & K Scientific Ltd., Shanghai, People's Republic of China).

Plant Material. The leaves of *Cipadessa cinerascens* was collected in July 2012 from Longlin County of Guangxi Province, People's Republic of China, and authenticated by Prof. Shao-Qing Tang of Guangxi Normal University. A voucher specimen has been deposited in Shanghai Institute of Materia Medica, Chinese Academy of Sciences (accession number: Cipcin-2012-1Y).

Extraction and Isolation. The air-dried powder of the plant leaves of *Cipadessa cinerascens* (6 kg) was extracted with 95% EtOH at room temperature three times to afford a crude extract (800 g). The extract was then dissolved in 2 L of water and partitioned with EtOAc (2 L × 3). The EtOAc extract (250 g) was subjected to CC over D101-macroporous absorption resin, eluted with EtOH–H_2O (50%, 80%, and 95%, v/v), to afford three fractions (A, B, and C). Fraction B (80%, 120 g) was subjected to passage over an MCI gel column, eluted with mixtures of MeOH–H_2O (50% to 100%, v/v), to give seven subfractions (B1–B7). Fraction B3 (60%, 5 g) was then separated by silica gel CC, eluted with petroleum ether–acetone (5:1 to 1:2, v/v), to produce three fractions (B3-1–B3-3). Fraction B3-1 was chromatographed by silica gel CC, eluted with $CDCl_3$–MeOH (150:1 to 100:1, v/v), to obtain three major components, and each of them was further purified by semipreparative HPLC, eluted with acetonitrile–water, to yield compounds **1** (43 mg), **8** (110 mg), and **9** (6.0 mg), respectively. Using similar procedures, fraction B3-2 yielded compounds **5** (5.0 mg), **6** (20 mg), **7** (4.0 mg), and **23** (6.0 mg). Fraction B4 (70%, 35 g) was separated by silica gel CC, eluted with petroleum ether–acetone (10:1 to 1:2, v/v), to obtain eight fractions (B4a–B4h). Fraction B4f (5 g) was subjected to silica gel CC, eluted with $CDCl_3$–MeOH (150:1 to 10:1, v/v), to give five major fractions (B4f-1–B4f-5), each of which was purified by semipreparative HPLC, using acetonitrile–water as the mobile phase, to afford compounds **10** (45 mg), **11** (10 mg), **12** (3.0 mg), **16** (11 mg), and **26** (10 mg). Fraction B4e was separated by RP-18 silica gel CC, eluted with MeOH–H_2O (50% to 70%, v/v), to obtain four fractions (B4e-1–B4e-4). Then, fraction B4e-1 was separated by silica gel CC, eluted with $CDCl_3$–MeOH (200:1 to 100:1, v/v), to yield compounds **13** (16 mg) and **17** (280 mg). Fraction B4e-2 was separated by silica gel CC, eluted with $CDCl_3$–MeOH (200:1 to 100:1, v/v), to collect the major components, and each of them was finally purified by semipreparative HPLC (acetonitrile–water) to obtain **2** (4.0 mg), **3** (5.0 mg), **4** (10 mg), **14** (8.0 mg), **15** (13 mg), **21** (10 mg), **24** (10 mg), and **25** (25 mg) in turn. Fraction B4c (5 g) was subjected to silica gel CC, eluted with $CDCl_3$–MeOH (200:1 to 100:1, v/v), to afford several major subfractions, and each of them was purified by semipreparative HPLC, using acetonitrile–water as the mobile phase, to afford compounds **18** (13 mg), **19** (3.5 mg), **20** (24 mg), and **22** (56 mg). Fraction B7 (90%, 3 g) was subjected to silica gel CC eluted with petroleum ether–acetone (15:1 to 1:2, v/v) to furnish five major fractions, and each was separated using repeated silica gel CC and finally purified by semipreparative HPLC, using acetonitrile–water as the mobile phase, to yield compounds **27** (18 mg), **28** (10 mg), **29** (8.0 mg), **30** (9.0 mg), and **31** (5.0 mg), sequentially.

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J. Nat. Prod. XXXX, XXX, XXX–XXX

Ciparasin A (1): white, amorphous power; $[\alpha]^{25}_{D}$ −1.0 (*c* 0.48, $CDCl_3$); IR (KBr) ν_{max} 3524, 2975, 1733, 1686, 1374, 1246, 1049 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1 and ^{13}C NMR ($CDCl_3$), see Table 2; (+)-ESIMS *m/z* 625.1 $[M + Na]^+$; (+)-HRESIMS *m/z* 625.2256 $[M + Na]^+$ (calcd for $C_{31}H_{38}O_{12}Na$, 625.2255).

Ciparasin B (2): white, amorphous power; $[\alpha]^{25}_{D}$ 12.9 (*c* 0.24, $CDCl_3$); IR (KBr) ν_{max} 3450, 2952, 1728, 1684, 1378, 1237, 1065 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1 and ^{13}C NMR ($CDCl_3$), see Table 2; (+)-ESIMS *m/z* 561.2 $[M + H]^+$, 1143.5 $[2 M + Na]^+$; (−)-ESIMS 559.5 $[M - H]^-$; (+)-HRESIMS *m/z* 583.2147 $[M + Na]^+$ (calcd for $C_{29}H_{36}O_{11}Na$, 583.2150).

Ciparasin C (3): white, amorphous power; $[\alpha]^{25}_{D}$ 3.0 (*c* 0.20, $CDCl_3$); IR (KBr) ν_{max} 3450, 2953, 1742, 1687, 1378, 1240, 1025 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1 and ^{13}C NMR ($CDCl_3$), see Table 2; (+)-ESIMS *m/z* 1227.5 $[2 M + Na]^+$; (−)-ESIMS 647.2 $[M + HCO_2]^-$; (−)-HRESIMS *m/z* 647.2326 $[M + HCO_2]^-$ (calcd for $C_{32}H_{39}O_{14}$, 647.2345).

Ciparasin D (4): white, amorphous power; $[\alpha]^{25}_{D}$ 5.4 (*c* 0.22, $CDCl_3$); IR (KBr) ν_{max} 3539, 3453, 2954, 1736, 1682, 1383, 1196, 1022 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1 and ^{13}C NMR ($CDCl_3$), see Table 2; (+)-ESIMS *m/z* 487.2 $[M + H]^+$, 995.5 $[2 M + Na]^+$; (−)-ESIMS 485.2 $[M - H]^-$; (+)-HRESIMS *m/z* 995.4409 $[2 M + Na]^+$ (calcd for $C_{54}H_{68}O_{16}Na$, 995.4400).

Ciparasin E (5): white, amorphous power; $[\alpha]^{25}_{D}$ 18.7 (*c* 0.30, $CDCl_3$); IR (KBr) ν_{max} 3441, 2956, 1760, 1736, 1683, 1379, 1256, 1032 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1 and ^{13}C NMR ($CDCl_3$), see Table 2; (+)-ESIMS *m/z* 583.2 $[M + Na]^+$, 1143.3 $[2 M + Na]^+$; (−)-ESIMS 1119.9 $[2 M - H]^-$; (−)-HRESIMS *m/z* 559.2174 $[M - H]^-$ (calcd for $C_{29}H_{35}O_{11}$, 559.2185).

Ciparasin F (6): white, amorphous power; $[\alpha]^{25}_{D}$ 1.5 (*c* 0.20, $CDCl_3$); IR (KBr) ν_{max} 3439, 2959, 1757, 1731, 1686, 1366, 1230, 1031 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 3 and ^{13}C NMR ($CDCl_3$), see Table 2; (+)-ESIMS *m/z* 699.1 $[M + Na]^+$; (−)-ESIMS 675.4 $[M - H]^-$; (+)-HRESIMS *m/z* 699.2278 $[M + Na]^+$ (calcd for $C_{33}H_{40}O_{15}Na$, 699.2259).

Ciparasin G (7): white, amorphous power; $[\alpha]^{25}_{D}$ 2.1 (*c* 0.24, $CDCl_3$); IR (KBr) ν_{max} 3432, 2957, 1761, 1727, 1685, 1381, 1257, 1074 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 3 and ^{13}C NMR ($CDCl_3$), see Table 2; (+)-ESIMS *m/z* 641.1 $[M + Na]^+$; (−)-ESIMS 617.5 $[M - H]^-$; (+)-HRESIMS *m/z* 641.2207 $[M + Na]^+$ (calcd for $C_{31}H_{38}O_{13}Na$, 641.2205).

Ciparasin H (8): white, amorphous power; $[\alpha]^{25}_{D}$ −52.7 (*c* 0.33, $CDCl_3$); IR (KBr) ν_{max} 3509, 3439, 2955, 1744, 1713, 1652, 1368, 1262, 1236, 1015 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 3 and ^{13}C NMR ($CDCl_3$), see Table 2; (+)-ESIMS *m/z* 605.3 $[M + H]^+$, 627.2 $[M + Na]^+$; (+)-HRESIMS *m/z* 627.2426 $[M + Na]^+$ (calcd for $C_{31}H_{40}O_{12}Na$, 627.2412).

Ciparasin I (9): white, amorphous power; $[\alpha]^{25}_{D}$ −30.2 (*c* 0.50, $CDCl_3$); IR (KBr) ν_{max} 3431, 2962, 1726, 1710, 1632, 1433, 1375, 1260, 1090, 1019 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 3 and ^{13}C NMR ($CDCl_3$), see Table 2; (+)-ESIMS *m/z* 547.3 $[M + H]^+$, 569.1 $[M + Na]^+$; (−)-ESIMS 591.9 $[M + HCO_2]^-$; (+)-HRESIMS *m/z* 569.2374 $[M + Na]^+$ (calcd for $C_{29}H_{38}O_{10}Na$, 569.2357).

Ciparasin J (10): white, amorphous power; $[\alpha]^{25}_{D}$ −17.5 (*c* 0.55, $CDCl_3$); IR (KBr) ν_{max} 3456, 2954, 1736, 1717, 1639, 1378, 1237, 1088, 1022 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 3 and ^{13}C NMR ($CDCl_3$), see Table 2; (+)-ESIMS *m/z* 563.2 $[M + H]^+$; (−)-HRESIMS *m/z* 561.2327 $[M - H]^-$ (calcd for $C_{29}H_{37}O_{11}$, 561.2341).

Ciparasin K (11): white, amorphous power; $[\alpha]^{25}_{D}$ −17.5 (*c* 0.27, $CDCl_3$); IR (KBr) ν_{max} 3567, 3452, 2953, 1738, 1719, 1643, 1370, 1263, 1237, 1090, 1029 cm^{-1}; ^1H NMR (CD_3OD), see Table 4 and ^{13}C NMR (CD_3OD), see Table 2; (−)-ESIMS *m/z* 665.6 $[M + HCO_2]^-$; (−)-HRESIMS *m/z* 665.2449 $[M + HCO_2]^-$ (calcd for $C_{32}H_{41}O_{15}$, 665.2451).

Ciparasin L (12): white, amorphous power; $[\alpha]^{27}_{D}$ −62.8 (*c* 0.30, $CDCl_3$); IR (KBr) ν_{max} 3447, 2955, 1737, 1639, 1376, 1256, 1085, 1030 cm^{-1}; ^1H NMR (CD_3OD), see Table 4 and ^{13}C NMR (CD_3OD), see Table 2; (+)-ESIMS *m/z* 643.1 $[M + H]^+$; (−)-ESIMS 1239.8 $[2 M - H]^-$; (+)-HRESIMS *m/z* 643.2379 $[M + Na]^+$ (calcd for $C_{31}H_{40}O_{13}Na$, 643.2361).

Ciparasin M (13): white, amorphous power; $[\alpha]^{25}_{D}$ −143.2 (*c* 0.28, $CDCl_3$); UV (MeOH) λ_{max} (log ε) 318 (4.54) nm; IR (KBr) ν_{max} 3436, 2940, 1739, 1726, 1656, 1584, 1396, 1240, 1111, 1042 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 4 and ^{13}C NMR ($CDCl_3$), see Table 2; (+)-ESIMS *m/z* 545.2 $[M + H]^+$, 1111.4 $[2 M + Na]^+$; (−)-ESIMS 589.5 $[M + HCO_2]^-$; (+)-HRESIMS *m/z* 545.2386 $[M + H]^+$ (calcd for $C_{29}H_{37}O_{10}$, 545.2381).

Ciparasin N (14): white, amorphous power; $[\alpha]^{25}_{D}$ −46.2 (*c* 0.29, $CDCl_3$); UV (MeOH) λ_{max} (log ε) 318 (4.40) nm; IR (KBr) ν_{max} 3547, 3428, 2949, 1730, 1708, 1576, 1397, 1257, 1110, 1050 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 4 and ^{13}C NMR ($CDCl_3$), see Table 2; (+)-ESIMS *m/z* 503.3 $[M + H]^+$, 1027.5 $[2 M + Na]^+$; (−)-ESIMS 547.3 $[M + HCO_2]^-$; (+)-HRESIMS *m/z* 503.2267 $[M + H]^+$ (calcd for $C_{27}H_{35}O_9$, 503.2276).

Ciparasin O (15): white, amorphous power; $[\alpha]^{25}_{D}$ −5.0 (*c* 0.32, $CDCl_3$); UV (MeOH) λ_{max} (log ε) 318 (4.26) nm; IR (KBr) ν_{max} 3439, 2948, 1733, 1707, 1578, 1396, 1256, 1113, 1050 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 4 and ^{13}C NMR ($CDCl_3$), see Table 2; (+)-ESIMS *m/z* 487.2 $[M + H]^+$, 995.5 $[2 M + Na]^+$; (−)-ESIMS 531.5 $[M + HCO_2]^-$; (+)-HRESIMS *m/z* 487.2320 $[M + H]^+$ (calcd for $C_{27}H_{35}O_8$, 487.2326).

Ciparasin P (16): white, amorphous power; $[\alpha]^{25}_{D}$ 2.6 (*c* 0.31, $CDCl_3$); IR (KBr) ν_{max} 3448, 2966, 1744, 1638, 1377, 1230, 1027 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 4 and ^{13}C NMR ($CDCl_3$), see Table 2; (+)-ESIMS *m/z* 801.3 $[M + Na]^+$; (−)-ESIMS 777.8 $[M - H]^-$; (+)-HRESIMS *m/z* 801.3310 $[M + Na]^+$ (calcd for $C_{39}H_{54}O_{16}Na$, 801.3304).

Anti-HIV and Cytotoxicity Assays. The anti-HIV and cytotoxic activities used for the limonoids isolated using MT-4 cell cultures have been described previously.[8] In brief, MT-4 cells were added to 96-well plates with serial dilutions of the test compounds. These cells were then infected by HIV-1 NL 4-3 at a final multiplicity of infection of 0.03. The assay plates were incubated in a humidified incubator at 37 °C under 5% CO_2. After 3 or 4 days, 10 mL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (5 mg/mL in PBS) was added into each well, and the plates were incubated for another 3 h at 37 °C. After this, a lysis buffer (10% Triton X-100 in acidified 2-propanol, v/v) was added to each well to lyse the cells and to solubilize the formazan crystal. A FLUOStar Optima plate reader (BMGLabtech) was used to read the plates at a wavelength of 570 nm. EC_{50} (50% effective concentration) is defined as the concentration of each compound achieving 50% protection on MT-4 cells against the HIV-induced cytopathic effect, and CC_{50} (50% cytotoxic concentration) is defined as the concentration of each compound killing 50% of the MT-4 cells. Both values were determined by Prism 5.0 software (GraphPad, SanDiego, CA, USA). The selectivity index (SI) is defined as the ratio of the values of CC_{50} to EC_{50}. Nevirapine was used as positive control, with a EC_{50} value of 0.1 ± 0.0 μM (SI >833.3). The test compounds with EC_{50} values of >100 μM are defined as inactive. The work on anti-HIV activity was performed in a biosafety level 3 facility, authorized by Public Health Agency of Canada, located at McGill AIDS Centre, Lady Davis Institute for Medical Research.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

IR, ESIMS, HRESIMS, 1D and 2D NMR spectra of compounds **1**−**16**. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00025.

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Notes

The authors declare no competing financial interest.

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J. Nat. Prod. XXXX, XXX, XXX−XXX

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