Tetrahedron 65 (2009) 7408–7414

Contents lists available at ScienceDirect

Tetrahedron

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Cipadonoids B–G, six new limonoids from *Cipadessa cinerascens*

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ARTICLE INFO

Article history:
Received 6 May 2009
Received in revised form 3 July 2009
Accepted 7 July 2009
Available online 10 July 2009

Keywords:
Limonoids
Absolute configuration
Chemical correlation
Computational method
Biogenetic relationship

ABSTRACT

Six new rings B,D-seco-type limonoids, cipadonoids B–G (**1**–**6**) were isolated from leaves of *Cipadessa cinerascens*. Their structure was elucidated by extensive spectroscopic methods, and that of **1** was confirmed by single-crystal X-ray diffraction. The absolute configuration of compounds (**2**–**6**), a class of cipadesin-type limonoids, was determined by CD exciton chirality method and chemical means, representing the first report of assignment of absolute configuration of such type compounds; a biosynthetic pathway of **2**–**6** was proposed and confirmed by chemical correlation and computational result. Compound **1** showed weak in vitro activity at the insect nicotinic acetylcholine receptor (nAChR).

1. Introduction

Limonoids are highly oxygenated and modified nortriterpenoids mainly found in the plants of the order Rutales, which either containing or derived from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton.[1] The most common structural feature of naturally occurring limonoids is a β-furyl ring at C-17 of the D-ring. This class of compounds has attracted continuous attention from biogenetic and synthetic points of view.[2,3]

Cipadessa cinerascens (Pell.) Hand.-Mazz, a shrub belonging to the Meliaceae family, is mainly distributed in the southwest of China. Its leaves, barks and roots are used as the folk medicine to treat stomachache, dysentery, rheumatism, malaria, scald, and skin itch.[4] Previous investigations on the chemical constituents of the species have yielded flavonoids, flavonoid glucosides, and six limonoids.[5] In the continuing search for novel limonoids from the Meliaceae family, we have recently reported from the species thirteen rings B,D-seco-type limonoids (including a novel skeleton one).[6] A reinvestigation on the same plant material led to the isolation of six new tetranortriterpenoids, cipadonoids B–G (**1**–**6**) and two known ones, cipadesin D (**7**) and cipatrijugin A (**8**).[5e,6b] Cipadonoid B (**1**) is a rare andirobin-type limonoid[7] incorporating a unique 2-en-1-one system in ring A; compounds **2**–**6** belong to a newly reported cipadesin-type limonoid,[5c,6c] in which rings A and B are connected via the carbon–carbon bond of C-10–C-11, and **5**,**6** also involve an unprecedented 2-oxabicyclo[3.2.2^{11,14}]nonane ring system. Biogenetically, compounds **2**–**6** might be derived from the methyl angolensate-type limonoid via pinacol rearrangement, which was confirmed by a computational study using DFT at B3LYP/6-31G* basis set level and chemical transformation. It is interesting to note that the presence and absence of $\Delta^{8(30)}$ double bond in the precursor lead to trijugin-type limonoid[8] and cipadesin-type limonoid, respectively. We describe herein the isolation, structural elucidation, biosynthetic pathway, and the bioactivity of compounds (**1**–**6**).

2. Results and discussion

2.1. Structural elucidation of cipadonoids B–G (1–6)

Cipadonoid B (**1**) was isolated as colorless crystals. Its molecular formula was deduced as $C_{27}H_{32}O_6$ from the HRESIMS, suggesting 12 degrees of unsaturation. The IR spectrum displayed carbonyl (1740 cm^{-1}), unsaturated lactone (1715 cm^{-1}), and α, β-unsaturated ketone (1667 cm^{-1}) absorption band maxima. The 1D NMR spectra revealed that 12 double-bond equivalents were occupied by five carbon–carbon double bonds and three carbonyls and indicated **1** to be quadricyclic. The ^{13}C spectra (Table 1) showed

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doi:10.1016/j.tet.2009.07.023

Table 1
^{1}H and ^{13}C NMR data of cipadonoid B (**1**) in $CDCl_3$[a]

No.	δ_H (mult, *J*)	δ_C	No.	δ_H (mult, J)	δ_C
1		203.5	14		166.1
2	6.79 (d, 10.0)	159.3	15		111.5
3	5.93 (d, 10.0)	127.0	16		166.2
4		37.1	17	5.31 (s)	79.6
5	2.85 (t)	43.5	18	0.99 (s)	18.5
6	2.47[b] (2H)	31.6	19	1.13 (s)	21.1
7		174.1	20		120.4
8		143.2	21	7.46 (br s)	141.0
9	2.43[b]	47.5	22	6.42 (br s)	111.0
10		50.6	23	7.39 (br s)	142.7
11α	1.77 (m)	20.9	28	1.13 (s)	30.1
11β	2.07 (d, 14.5)		29	1.13 (s)	23.9
12α	1.12[b]	29.4	30a	5.50 (s)	121.4
12β	1.40 (td, 14.5,4.0)		30b	5.04 (s)	
13		39.2	OMe	3.71 (s)	52.1

[a] Recorded at 500 and 100 MHz for ^{1}H and ^{13}C NMR, respectively.
[b] Overlapped, without designating multiplicity.

the presence of 27 carbon signals, apart from a methoxy [δ_H 3.71 (s), δ_C 52.1], the remaining 26 carbons including a β-substituted furan ring [δ_H 7.46 (br s, H-21), 6.42 (br s, H-22), and 7.39 (br s, H-23); δ_C 120.4, 141.0, 111.0, and 142.7] and an exocyclic methylene group [δ_H 5.50 (s) 5.04 (s), δ_C 121.4]. These facts, together with the aforementioned four rings framework suggested **1** to be a rare andirobin-type limonoid.[7] Furthermore, the existence of a 2-en-1-one system in ring A was indicated by a pair of doublets at δ 6.79 and 5.93 (J=10.0 Hz) in the ^{1}H NMR spectrum, and resonances at δ 203.5, 159.3, and 127.0 in the ^{13}C NMR spectrum assigned to C-1, C-2, and C-3, respectively. The planar structure of **1** was thus constructed by detailed 2D NMR analysis as shown in Figure 1. The single-crystal X-ray diffraction (Fig. 2) further confirmed it and allowed the determination of the relative configuration of **1**, of which the orientation information of Me-19, Me-28, and Me-29 was otherwise impossible to obtain due to their overlapped hydrogen signals in ROESY spectrum. To the best of our knowledge, cipadonoid B (**1**) is the first andirobin-type limonoid with 2-en-1-one system in ring A.

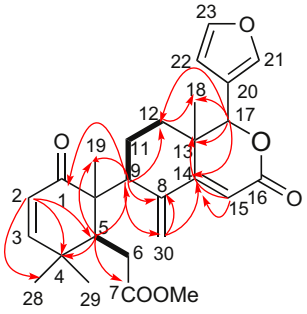


Figure 1. ^{1}H–^{1}H COSY (▬) and selected HMBC (⟶) correlations of **1**.

Cipadonoid C (**2**) was obtained as white amorphous powder. The HRESIMS spectrum of **2** indicated a molecular formula of $C_{29}H_{36}O_8$ by a pseudo-molecular ion peak at m/z 513.2470 $[M+H]^+$. The IR spectrum showed absorption bands at 1730 cm^{-1}, 1644 cm^{-1}, and 1240 cm^{-1} indicating the presence of carbonyl, unsaturated lactone, and ether, respectively. The ^{13}C and DEPT spectra (Table 2) exhibited 29 carbon signals including 7 methyls, 3 methylenes, 9 methines (3 oxygenated and 4 olefinic ones), and 10 quaternary carbons (3 carbonyls and 4 olefinic carbons). Apart from four double bonds and three carbonyl groups, the remaining five degrees of unsaturation indicated compound **2** to be pentacyclic. A

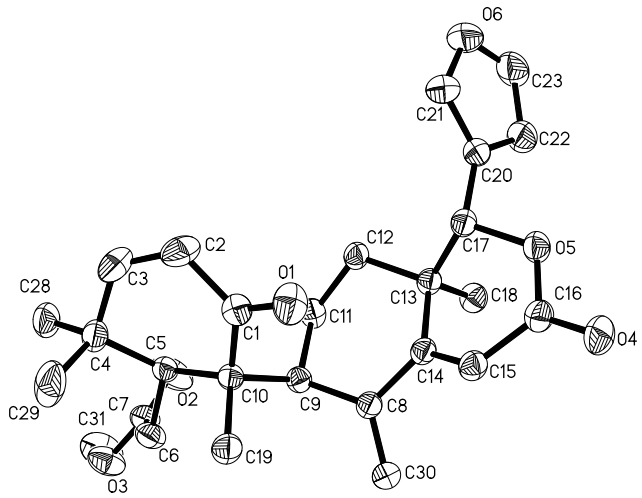


Figure 2. Single-crystal X-ray structure of **1**.

Table 2
^{13}C NMR ($CDCl_3$, 100 MHz) data of compounds **2**–**6**

No.	**2**	**3**[a]	**4**	**5**	**6**
1	86.5	86.3	83.3	75.8	75.1
2	25.0	25.3	25.0	27.8	28.3
3	76.0	76.1	74.9	75.2	75.2
4	37.8	38.4	38.2	38.0	38.6
5	35.1	35.0	35.2	37.1	36.8
6	32.2	32.4	31.8	30.2	29.6
7	174.1	175.3	175.6	174.0	174.5
8	101.0	101.3	104.3	44.1	42.7
9	163.2	161.1	158.5	210.3	212.1
10	44.5	48.2	52.4	45.3	50.5
11	50.4	56.5	80.1	64.1	85.5
12	31.3	71.4	70.5	70.0	70.7
13	37.3	45.3	44.1	45.4	45.3
14	163.9	162.0	160.2	79.7	78.4
15	103.5	105.9	107.1	39.3	39.2
16	166.7	164.8	165.2	168.2	167.8
17	80.1	78.0	77.4	78.8	79.1
18	16.6	14.2	16.3	16.3	17.0
19	20.1	18.6	11.8	18.8	16.6
20	120.4	122.1	120.5	121.0	120.7
21	141.0	143.5	142.6	141.9	142.2
22	109.9	112.0	111.1	109.8	109.9
23	143.0	144.6	143.7	143.0	142.8
28	21.4	21.7	22.0	22.1	22.4
29	26.1	26.1	27.0	28.2	28.0
30	9.5	10.4	10.6	10.4	10.9
OMe	52.1	52.1	52.3	52.1	52.0
3-OAc	171.0	170.9	170.7	171.0	171.1
	21.2	21.2	21.4	20.8	20.9
12-OAc		170.5	169.2	169.1	168.6
		20.2	20.1	20.7	20.4

[a] Measured in acetone-d_6.

extensive comparison of ^{1}H and ^{13}C NMR data of **2** with those of known rings B,D-seco-type limonoids[9] identified its A ring and D ring, which was further confirmed by 2D NMR analysis as shown in Figure 3. HMBC correlations from H_2-12 to C-9, C-14 as well as from H_3-30 to C-8, C-9, and C-14, together with ^{1}H–^{1}H COSY correlation of H_2-12/H-11 established a six membered ring C with C-30 exo-methyl at C-8. Rings A and C were connected through the carbon–carbon bond of C-10–C-11 as suggested by HMBC cross-peaks of H-5/C-11 and H_3-19/C-11. The *O*-acetyl group was located at C-3 by H-3/C-3–OAc. The planar structure of **2** was thus established as shown in Figure 3.

The relative configuration of **2** was established by ROESY experiments (Fig. 4) together with comparison with data of **2**–**5**. The ROESY correlations of H-11/ H-1, H_3-19, and H_3-18 indicated that

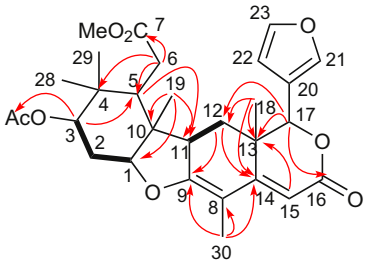


Figure 3. Selected HMBC (⟶) and ^{1}H–^{1}H COSY (▬) correlations of **2**.

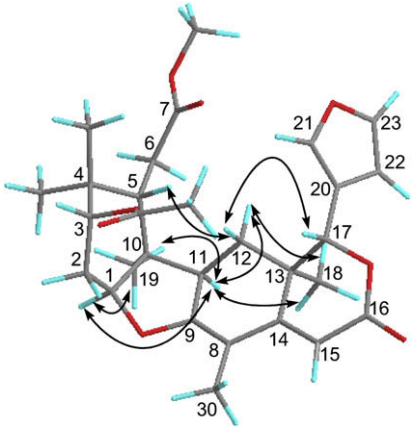


Figure 4. Key ROESY (⟷) correlations of compound **2**.

H-1, H-11, Me-18, and Me-19 were cofacial, arbitrarily assigned as the α-oriented. The β orientations of H-5, and H-17 were indicated by the ROESY correlations of H-12β with H-5 and H-17, in which H-12β was deduced from H-12α/H-11, H_3-18. However, the orientation of H-3 could not be determined simply from its NOE correlations with H_3-28, H_3-29, and H_2-2. Comparing 1D NMR data of compounds **2**–**5**, it shows that they share the similar ^{1}H and ^{13}C NMR chemical shift value at C-3, which suggested that they have the same relative configuration in C-3. Therefore, the α direction of H-3 in **2** was deduced by that of **5** and **6**, as was further confirmed by the hypothetical biogenetic pathway, which will be discussed in detail below.

The absolute configuration of **2** was determined by applying the CD exciton chirality method.[10] The UV spectrum of **2** showed an absorption band at λ_{max} 315 nm caused by the conjugated α,β,γ,δ-unsaturated δ-lactone (Woodward's rule gave ca. 313 nm).[11] Accordingly, the CD spectrum of **2** exhibited a positive first Cotton effect at 306 nm ($\Delta\varepsilon$ 0.204, $\pi\rightarrow\pi^*$ transition) and a negative second Cotton effect at 266 nm ($\Delta\varepsilon$ −3.68, $\pi\rightarrow\pi^*$ transition) due to exciton coupling between the two different chromophores of the α,β,γ,δ-unsaturated δ-lactone[12] and the furan ring,[12] respectively, indicating that the transition dipole moments of the two chromophores were oriented in a clockwise manner (Fig. 5). The absolute configuration of **2** was therefore assigned. To the best of our knowledge, cipadonoid C (**2**) represents the third example of limonoids with cipadesin-type skeleton found in nature and the first report of the determination of absolute configuration of this type limonoid.

Cipadonoid D (**3**) had molecular formula, $C_{31}H_{38}O_{10}$, as determined by HRESIMS. The 1D NMR spectral data of **3** exhibited close similarity to those of **2**, except for the absence of a methylene, as well as the presence of an additional oxygenated methine [δ_H 5.39 (d, 9.0); δ_C 71.4] and an *O*-acetyl group [δ_H 1.44 (s); δ_C 170.5, 20.2], which suggested that **3** is a *O*-acetyl derivative of **2**. The

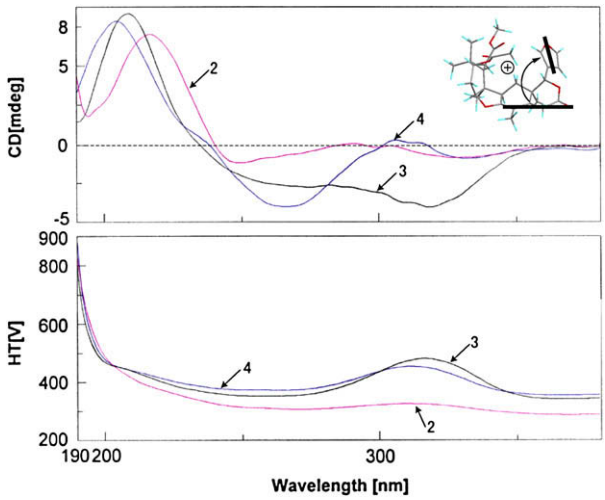


Figure 5. CD spectra of **2**–**4**. Bold lines denote the electric transition dipole of the chromophores for **2**.

HMBC correlation of H-12/C-3–OAc confirmed the deduction and located the *O*-acetyl group at C-12. The relative configuration of **3** was determined to be the same as **2** by similar chemical shifts and ROESY data, in which the relative orientation of 12-*O*-acetyl group was determined as β-oriented by the ROESY correlation of H-12/H-5, H-17. The large coupling constant ($J_{11,12}$=9.0 Hz) suggested a 1,2-diaxial relationship between H-11 and H-12, consistent with the previous α- and β-assignments for these two protons. The structure of **3** was established as depicted.

Comparison of the 1D and 2D NMR spectral data of cipadonoid E (**4**) to those of **3** showed close similarity except for a methine group at C-11 being substituted by a hydroxyl group [δ 3.19 (s)], which was supported by the down-field signals of C-10 and the HMBC correlations of the hydroxyl with C-9, C-11, and C-12. The relative orientation of OH-11 was determined as α-oriented by its ROE correlation with H_3-19. Cipadonoid E (**4**) was thus established to be 11α-hydroxycipadonoid D.

The absolute configurations of compounds **3** and **4** were identical to that of **2**, as determined by their similar CD curves in the CD spectra (Fig. 5) and nearly identical optical rotation values.

The HRESIMS spectrum of cipadonoid F (**5**) indicated a molecular formula of $C_{31}H_{40}O_{11}$, with 18 mass units higher than that of **3**, suggesting **3** to be dehydrate of compound **5**. By comparison of the 1D and 2D NMR spectral data of **5** with those of **3**, it revealed that they shared similar ring system and the difference was that the 1–14 ester bond in **3** was replaced by 1–9 ester bond in **5** as suggested by HMBC correlation of H-1/C-14, and the presence of C-9 carbonyl group in **5** by HMBC correlation of C-9/H-8, H-11, H-12, and H-30. The planar structure of **5** was thus elucidated as depicted in Figure 6. Compound **3** could be derived from **5** by dehydration, which was confirmed chemically as shown in Scheme 1.

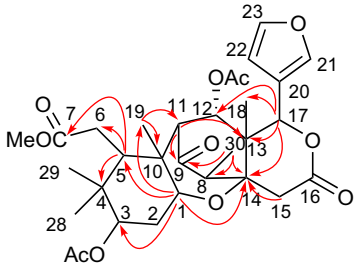
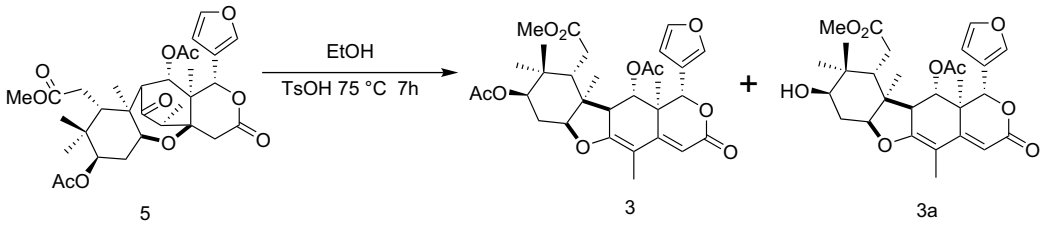


Figure 6. Selected HMBC (⟶) and ^{1}H–^{1}H COSY (▬) correlations of 5.



Scheme 1. Chemical conversion from **5** to **3**.

The relative configuration of **5** was established by ROESY experiment. As shown in Figure 7, the correlations of H-1/H_3-19, H_3-30, and H-3/H_3-19, H_3-30, and H-11/H_3-19, as well as H-22/H_3-18, suggested the stereochemistry of H-1α, H-3α, H-11α, Me-18α, Me-19α, and Me-30α. Meanwhile, the correlations of H-5/H-12, H-17 indicated the stereochemistry of H-5β, H-12β, and H-17β.

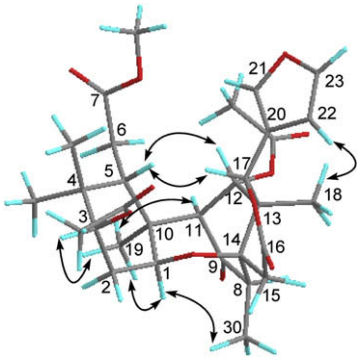


Figure 7. Key ROESY (⟷) correlations of compound **5.**

Cipadonoid G (**6**) was a hydroxyl derivative of **5**, as they shared the similar IR and 1D NMR spectrum and **6** was 16 mass units less than that of **5**, which was deduced from its molecular formula $C_{31}H_{40}O_{12}$ by HRESIMS. The main difference was that a quaternary carbon at C-11 being substituted by an α-hydroxyl group [δ 4.03 (s)], which was confirmed by the HMBC correlations of OH-11/C-9, C-11, and C-12, and ROESY correlation of OH-11/H_3-19. Cipadonoid G (**6**) was thus established to be 11α-hydroxycipadonoid F.

The aforementioned chemical transformation from **5** to **3** suggested that the absolute configuration of **5** was identical to that of **3**. The CD spectra of **5** and **6** showed a similar CD curves, which indicated the absolute configuration of **6** is identical to that of **5**, as supported by similar optical rotation values of the two compounds.

2.2. Hypothetical biogenetic route for cipadonoids C–G (2–6)

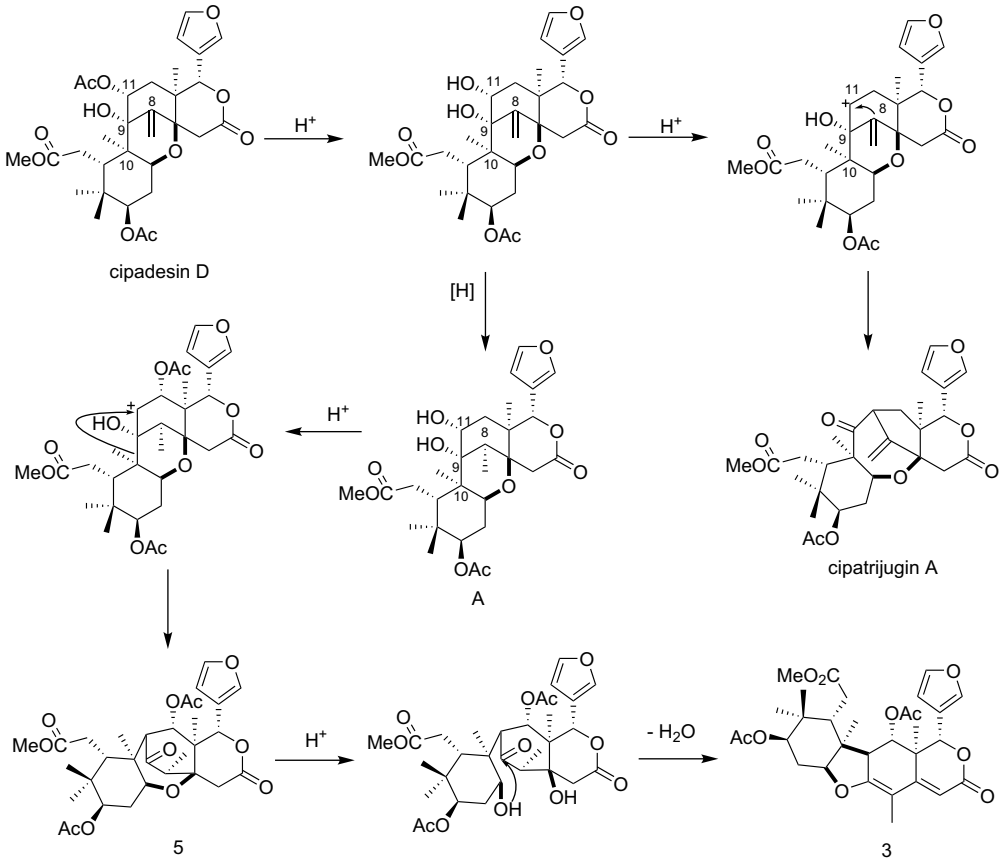
Structurally, cipadonoids C–G (**2**–**6**) and so far reported cipadesin-type limonoid, whose biogenetical pathway has not been discussed before,[5d,6c] belong to rings B,D-seco-type limonoid. However, they are different to common rings B,D-seco-type limonoid[9] in possessing C-30 methyl instead of a characteristic $\Delta^{8(30)}$ exocyclic double bond, which suggest a unique biogenetical pathway for them. A subsequence biosynthetic analysis showed that they might be derived from methyl 9,11-dihydroxyangolensate through a pinacol rearrangement. It's noteworthy that trijugin-type limonoid also supposed to be produced via a pinacol rearrangement of the same precursor.[8] Therefore, it should be an interesting and informative question of how methyl angolensate-type limonoid branch into cipadesin-type and trijugin-type limonoids in the plant.

Thus, a possible biosynthetic pathway from cipadesin D (**7**) to cipadonoids D (**3**), F (**5**), and cipatrijugin A (**8**) was proposed as shown in Scheme 2, in which the relative stability of C-9 and C-11 carbonium ion intermediates, leading to two possible migration routes in the mechanism, need to be settled firstly. Since the C-9 cation is a bridgehead carbonium ion, the relative stability of the two intermediates depends on whether the bicyclo[3.3.1]decane core display bridgehead-like behavior. Although the bridgehead-like behavior of other bicyclic systems has been discussed in detail,[13] the bicyclo[3.3.1]decane ring system was excluded and could not be rationalized from the literature. Therefore, the stability of these two intermediates was investigated by using DFT at B3LYP/6-31G* basis set level,[14] which showed the energy of the C-9 cation intermediate was 27.4 kcal/mol higher than that of C-11 cation intermediate. This is in agreement with the fact that we have never observed limonoid resulting from C-9 cation intermediate in nature. Then the relative migratory aptitude of C-10 and C-8 must be resolved, since their migration lead to cipadesin-type and trijugin-type limonoid, respectively. As the migratory aptitude sequence is vinyl group prior to alkyl group and tertiary carbon group prior to secondary carbon group, it is rational conclusion that the existence of $\Delta^{8(30)}$ double bond makes the relative migratory aptitude of C-8 higher than that of C-10, and the 8,30-single bond makes the relative migratory aptitude of C-8 lower than that of C-10. This explained well the characteristic C-30 methyl in cipadesin-type limonoid and $\Delta^{8(30)}$ double bond in trijugin-type limonoid. Structurally, the produced cipadonoid F (**5**) has a large ring strain due to 2-oxabicyclo[3.2.2[11,14]]nonane ring system, which could be taken away by the break of C-1,C-14 ether bond and the form of new C-1,C-9 ether bond, and then the form of C-9, C-8, C-14, and C-15 conjugative unsaturated system. The co-occurrence of the three type limonoids in *C. cinerascens* and the aforementioned chemical transformation from **5** to **3** (Scheme 1) supported the hypothetical biogenetic route. The biosynthetic sequences also predict the key intermediate A as a 'missing' link for further investigation.

Furthermore, our continuing investigation on the species for limonoids[6] revealed that it's a rich source of B,D-seco-type limonoids, with six different type limonoids being reported. The hypothesis of a biogenetic relationship of them has been depicted (see Supplementary data).

2.3. Bioactive evaluation of cipadonoids B–G (1–6)

Cipadonoids B–G (**1**–**6**) were tested for in vitro cytotoxicity against the P-388 (murine leukemia) cell lines by using the MTT method[15] and cipadonoid F (**5**) was selected to extensively evaluate its in vitro cytotoxicity against a serial of cell lines including HT29, HCT116, SW480, MDA-MB-231, MDA-MB-468, MCF-7, SMMC-7721, BEL-7402, MKN28, MKN45, SGF-7901, KB, RH30, SK-OV-3, Hela, HL-60, K562, and K562/A02 by using the MTT and SRB method.[16] However, no cytotoxicity was observed for all of the compounds (50% effective dose for clonal inhibition, ED_{50}>5 μg/mL). Furthermore, cipadonoids B (**1**) and D (**3**) were tested in vitro at insect nicotinic acetylcholine receptor (nAChR), and cipadonoid B (**1**) is a weak antagonist at the insect nAChR with pI50 value being 4.2; cipadonoid D (**3**) is inactive (pI50 value<4.0).



Scheme 2. Hypothetical biosynthetic pathway from cipadesin D to **5** and **3**.

3. Experimental section

3.1. General experimental procedures

Melting point was obtained on an X-4 apparatus and is uncorrected. Optical rotation was measured on a Perkin–Elmer model 241 polarimeter. IR spectrum was recorded on a Bio-Red FTS-135 spectrometer with KBr disc. ^{1}H and 2D NMR spectra were recorded on a Bruker DRX-500 instrument, while ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer. Chemical shifts were reported using TMS as internal standard. ESIMS and HRESIMS spectra were measured using a Finnigan MAT 90 instrument and VG Auto Spec-3000 spectrometer, respectively. CD spectra were obtained on a JASCO 810 spectrometer. Column chromatography was performed on silica gel (90–150 μm; Qingdao Marine Chemical Inc.), Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany). Semi-preparative HPLC was performed on a Zorbax SB-C-18 (Agilent Co. Ltd., U.S.A.) column (i.d. 9.4×250 mm). Precoated silica gel GF_{254} and HF_{254} plates (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) were used for TLC.

3.2. Plant material

The leaves of *C. cinerascens* were collected in July 2007 from Mengla, Yunnan Province of the People's Republic of China. The plant was identified by Prof. Cui of the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. Voucher specimen (NO. KUN 0596223) is deposited in Kunming Institute of Botany, Kunming, PR China.

3.3. Extraction and isolation

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/MeOH (from 1:1:0 to 1:1:1) to give 10 fractions (A1–A10). A2 (5.0 g) was subjected to an MCI gel column (MeOH/H_2O from 5:5 to 10:0), and further purified by Sephadex LH-20 and subjected to a silica gel column (acetone/$CHCl_3$ from 10:1 to 9:1) to give **1** (20.0 mg) and **3** (5.0 mg). A3 (10.0 g) was chromatographed over a silica gel column (acetone/$CHCl_3$ from 10:1 to 8:2) to afford **2** (40.0 mg) and **5** (1.0 g). A4 (8.0 g) was subjected to a C_{18} column (MeOH/H_2O from 1:9 to 10:0), in which a fraction that eluted with 40% MeOH was purified further by Sephadex LH-20 ($CHCl_3$/MeOH 1:1) to afford fraction B1. Fraction B1 was then further purified by semi-HPLC to give **4** (3.0 mg), and **6** (7.0 mg).

3.3.1. *Cipadonoid B (**1**)*

Colorless crystal (MeOH); mp 143–144 °C; $[\alpha]_D^{20}$ +294.4 (*c* 0.015, $CHCl_3$); CD (MeOH) 198 nm ($\Delta\varepsilon$ −6.53), 223 nm ($\Delta\varepsilon$ 20.88), 243 nm ($\Delta\varepsilon$ 0.34), 270 nm ($\Delta\varepsilon$ 25.41), 310 nm ($\Delta\varepsilon$ 1.30), 341 nm ($\Delta\varepsilon$ 4.81); IR (KBr) ν_{max} 3460, 3412, 2956, 1740, 1667, 1375, 1291, 1170, 1030, 1030 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; Positive ESIMS *m*/*z* 453.3 $[M+H]^+$ (100), 573.2 (13), 838.2 (28), 905.1 (62), 927.2 (26); Positive HRESIMS *m*/*z* 475.2097 $[M+Na]^+$, calcd 475.2096.

3.3.2. *Cipadonoid C (**2**)*

A white amorphous powder; $[\alpha]_D^{20}$ −136.6 (*c* 0.015, $CHCl_3$); CD (MeOH) 194 nm ($\Delta\varepsilon$ 1.76), 217 nm ($\Delta\varepsilon$ 7.03), 249 nm ($\Delta\varepsilon$ −1.20), 291 nm ($\Delta\varepsilon$ 0.02), 327 nm ($\Delta\varepsilon$ −0.87); IR (KBr): ν_{max} 3435, 1730,

1643, 1374, 1240, 1113, 1050 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; Positive ESIMS: *m/z* 513.5 $[M+H]^+$ (53), 586.3 (100); Positive HRESIMS: *m/z* 513.2470 $[M+H]^+$,calcd 513.2488.

Table 3
^1H (500 MHz, *J* in Hz) NMR data of cipadonoids C–G (**2**–**6**) in $CDCl_3$

No.	2	3[a]	4	5	6
1	4.22 (s)	4.29 (t, 3.0)	4.58 (t, 3.0)	3.41 (t, 3.0)	3.34 (t, 3.0)
2α	2.10 (s)	2.25 (m)	2.00 (dt, 3.0, 16.6)	2.06 (br, 2H)	2.15 (dt, 16.5, 3.0)
2β	2.52 (m)	2.22 (m)	2.37 (dt, 3.0, 16.6)		2.08 (dt, 16.5 3.0)
3	4.61 (s)	4.53 (t, 3.0)	4.65 (t, 3.0)	4.69 (t, 2.5)	4.69 (t, 3.0)
5	2.29 (d, 9.0)	2.31 (m)	2.15 (s)	2.87 (d, 10.0)	3.05 (d, 10.0)
6α	2.14 (s)	2.53 (d, 18.0)	2.32 (dd, 7.2, 18.1)	2.55 (d, 17.5)	3.51 (d, 18.0)
6β	2.42 (m)	2.28 (m)	2.91 (d, 18.1)	2.44 (dd, 17.5, 10.0)	2.33 (dd, 10, 18.0)
8				2.72 (d, 7.0)	2.81 (q, 7.1)
11	2.57 (d, 13.0)	2.98 (d, 9.0)		2.47 (s)	
12α	1.41 (dd, 4.0, 12.0)	5.39 (d, 9.0)	5.45 (s)	5.53 (s)	5.85 (s)
12β	1.81 (s)				
15α	5.59 (s)	5.56 (s)	5.82 (s)	2.64 (d, 17.0)	2.75 (d, 17.5)
15β				2.72[b]	2.65 (d, 17.5)
17	5.10 (s)	5.07 (s)	5.17 (s)	6.55 (s)	6.66 (s)
18	1.06 (s)	1.41 (s)	1.58 (s)	0.99 (s)	0.96 (s)
19	1.12 (s)	1.08 (s)	0.89 (s)	0.88 (s)	0.78 (s)
21	7.36 (br s)	7.48 (t, 1.5)	7.33 (br s)	8.10 (br s)	8.15 (br s)
22	6.35 (br s)	6.58 (d, 1.5)	6.51 (br s)	6.60 (br s)	6.67 (br s)
23	7.44 (br s)	7.39 (br s)	7.25 (br s)	7.42 (br s)	7.42 (br s)
28	1.01 (s)	0.95 (s)	0.94 (s)	1.01 (s)	1.00 (s)
29	0.80 (s)	0.68 (s)	0.73 (s)	0.86 (s)	0.88 (s)
30	1.78 (s)	1.82 (s)	1.91 (s)	1.29 (d, 7.0)	1.35 d (7.1)
OMe	3.79 (s)	3.64 (s)	3.72 (s)	3.75 (s)	3.77 (s)
3-OAc	2.06 (s)	2.01 (s)	2.07 (s)	2.06 (s)	2.11 (s)
12-OAc		1.44 (s)	1.57 (s)	1.87 (s)	1.86 (s)
11-OH			3.19 (s)		4.03 (s)

[a] Measured in acetone-d_6.
[b] Overlapped, without designating multiplicity.

*3.3.3. Cipadonoid D (**3**)*

A white amorphous solid; $[\alpha]_D^{20}$ −128.4 (*c* 0.058, MeOH); CD (MeOH) 210 nm (Δε 7.35), 276 nm (Δε −2.52), 283 nm (Δε −2.48), 320 nm (Δε −3.61); IR (KBr): ν_{max} 3434, 1724, 1661, 1587, 1369, 1250, 1052, 1025 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; Positive ESIMS: *m/z* 571.5 $[M+H]^+$ (62), 593.5 $[M+Na]^+$ (100); Positive HRESIMS: *m/z* 593.2371 $[M+Na]^+$, calcd 593.2362.

*3.3.4. Compound (**3a**)*

A white amorphous solid; $[\alpha]_D^{20}$ −78.6 (*c* 0.014, MeOH); IR (KBr) ν_{max} 3437, 1719, 1662, 1589, 1367, 1254, 1050, 1026 cm^{-1}; ^1H NMR data ($CDCl_3$, 500 MHz): 7.34 (1H, s, H-21), 7.32 (1H, s, H-23), 6.53 (1H, s, H-22), 5.81 (1H, s, H-15), 5.40 (1H, d, 9.0, H-12β), 5.18(1H, s, H-17β), 4.25 (1H, s, H-1α), 3.75 (3H, br s, OMe), 3.44 (1H, s, H-3α), 3.19 (1H, s, OH-3β), 2.75 (1H, br s, H-11α), 2.53 (1H, d, 17.5, H-6α), 2.36 (1H, dt, 16.0, 3.0, H-2β), 2.33 (1H, overlapped H-6β), 2.28 (1H, d, 7.5, H-5β), 2.15 (1H, dt, 16.0, 3.0, H-2α), 1.85 (3H, d, 2.5, Me-30), 1.47 (3H, s, Me-12-OAc), 1.45 (3H, s, Me-18), 1.04 (3H, s, Me-19),0.87 (3H, s, Me-28), 0.87 (3H, s, Me-29); ^{13}C NMR data ($CDCl_3$, 100 MHz): 174.8 (s, C-7), 170.5 (s, 12-OAc), 165.1 (s, C-16), 159.9 (s, C-9), 157.7 (s, C-14), 143.7 (d, C-23), 142.4 (d, C-21), 120.7 (s, C-20), 111.2 (d, C-22), 107.6 (d, C-15), 103.4 (s, C-8), 87.8 (d, C-1), 77.3 (s, C-17), 75.0 (d, C-3), 70.6 (d, C-12), 55.7 (d, C-11), 52.3 (q, OMe), 47.7 (s, C-10), 44.4 (s, C-13), 39.5 (s, C-4), 33.3 (d, C-5), 31.9 (t, C-6), 26.7 (t, C-2), 26.2 (q, C-29), 22.0 (q, C-28), 20.1 (q, Me-12-OAc), 18.3 (q, C-19), 13.6 (q, C-18), 10.1 (q, C-30); Positive ESIMS: *m/z* 551.5 $[M+Na]^+$ (43), 424.5 (21); Positive HRESIMS: *m/z* 551.2250 $[M+Na]^+$, calcd 551.2257.

*3.3.5. Cipadonoid E (**4**)*

A white amorphous solid; $[\alpha]_D^{25}$ −80.3 (*c* 0.104, MeOH); CD (MeOH) 205 nm (Δε 7.24), 266 nm (Δε −3.68), 306 nm (Δε 0.24), 332 nm (Δε −0.82); IR (KBr): ν_{max} 3436, 1724, 1661, 1369, 1025 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; Positive ESIMS: *m/z* 587.4 $[M+H]^+$ (100), 609.3 $[M+Na]^+$ (42), 515.3 (18), 625.4 (21); Positive HRESIMS: *m/z* 587.2495 $[M+H]^+$, calcd 587.2492.

*3.3.6. Cipadonoid F (**5**)*

A white amorphous solid; $[\alpha]_D^{25}$ −65.2 (*c* 0.011, $CHCl_3$); CD (MeOH) 212 nm (Δε 6.68), 250 nm (Δε −0.67), 285 nm (Δε 1.31), 326 nm (Δε −1.08); IR (KBr): ν_{max} 2982, 2952, 1739, 1370, 1253, 1234, 1091, 1025 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; Positive ESIMS: *m/z* 611.5 $[M+Na]^+$ (100), 589.3 $[M+H]^+$ (44), 469.4 (18), 529.4 (65), 606.4 (95), 643.4 (56), 662.3 (19), 679.4 (28); Positive HRESIMS: *m/z* 611.2485 $[M+Na]^+$ calcd 611.2468.

*3.3.7. Cipadonoid G (**6**)*

A white amorphous solid; $[\alpha]_D^{25}$ −80.3 (*c* 0.104, MeOH); CD (MeOH) 215 nm (Δε 8.45), 252 nm (Δε 0.87), 291 nm (Δε 3.42), 336 nm (Δε −0.03); IR (KBr): ν_{max} 3448, 1742, 1462, 1374, 1229, 1023 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; Negative ESIMS: *m/z* 603.5 $[M-H]^-$ (100), 635.4 (31), 675.3 (18); Positive HRESIMS: *m/z* 627.2433 $[M+Na]^+$, calcd 627.2417.

3.4. Chemical conversion from 5 to 3

Cipadonoid F (**5**) (30.0 mg) was added to EtOH (10 mL) and TsOH (5.0 mg), then heated to 70 °C at reflux under nitrogen and stirred for 7 h. The mixture was evaporated in vacuo to remove the solvent and the residue was dissolved in 10 mL CH_2Cl_2, and washed with 15 mL saturated aqueous $NaHCO_3$ solution, then 20 mL pure water. The combined CH_2Cl_2 phase was evaporated in vacuo and the residue was purified on an RP-18 column eluted with MeOH/H_2O (65:35) to give cipadonoid D (**3**) (9.0 mg), which was identified by ^1H NMR and EIMS spectra, and specific optical rotation $[\alpha]_D$, as well as **3a** (2.0 mg).

3.5. X-ray crystallographic analysis of 1

A colorless crystal of **1** was obtained in MeOH. Crystal data were obtained on a MAC DIP-2030K detector employing graphite monochromated Mo Kα radiation and operating in the ω scan mode. The structure was solved by direct methods and subsequent Fourier recycling, and refined with full-matrix least-squares calculations on F^2 using SHELX-97. All non-hydrogen atoms were refined anisotropically. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms. Crystallographic data for **1** has been deposited in the Cambridge Crystallographic Data Centre with the deposition number of CCDC 714981.

*3.5.1. X-ray data of **1***

$C_{27}H_{32}O_6$, *M*=452.53, tetragonal, space group $P4_1$, crystal dimensions 0.50×0.40×0.40 mm, Mo Kα radiation, *a*=*b*=10.556(4), *c*=21.818(8) Å, *d*=1.236 g/cm^{-3}, *V*=3044.0(0) $Å^3$; *Z*=4. The total number of independent reflections measured was 2322, of which 2266 were observed ($|F|^2 \geq 2\sigma|F|^2$). The final indices were R_1=0.0387, wR_2=0.1013, *S*=1.068.

3.6. Cytotoxicity bioassays

The in vitro assay at insect nicotinic acetylcholine receptor (nAChR) was performed according to the method in the previous report.[17]

Acknowledgements

We thank Prof. Ding, Prof. Geng, and Prof. Ye, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, and Prof. Dr.

Ernst R.F. Gesing, Bayer CropScience AG, for bioactivity testing. This work was financially supported by grants from the Ministry of Science and Technology (2009CB940900 and 2009CB522303), and National Natural Science Foundation (1130830114) of PRC.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.07.023.

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