

Three New Limonoids from *Cipadessa cinerascens*

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Three new limonoids, cipadesins D–F (1–3), together with 8,15-dihydroxy-13*E*-labdane, β -sitosterol and β -daucosterol, were isolated from the leaves and bark of *Cipadessa cinerascens*. Their structures were elucidated by spectral evidence. X-Ray crystallographic analysis confirmed the structure of 1.

Key words Meliaceae; *Cipadessa cinerascens*; limonoid; cipadesin D; cipadesin E; cipadesin F

The leaves and roots of *Cipadessa cinerascens* (PELL.) HAND-MAZZ (Meliaceae), a shrub distributed in Southwest China, are used for the treatment of rheumatism, malaria, scald and skin itch.¹⁾ From the leaves of *C. cinerascens* flavonoids and their glucosides were isolated.^{2–4)} From the leaves and bark of *C. cinerascens*, novel limonoids, cipadesins A–C, were obtained during our study.⁵⁾ The continued study led to the isolation of three new limonoids, cipadesins D–F (1–3), together with 8,15-dihydroxy-13*E*-labdane,⁶⁾ β -sitosterol and β -daucosterol.

Cipadesin D (1) was isolated as colorless crystal (MeOH). Its molecular formula C₃₃H₄₀O₁₃ was established from the quasi-molecular ion peak at m/z 667.2330 [M+Na]⁺ in the HR-ESI-MS. The IR peaks at 1749 and 1738 cm⁻¹, and ¹³C-NMR signals at δ 205.3, 173.7, 170.8, 170.4, 168.8 and 168.1 revealed the presence of a ketonic carbonyl group and five ester carbonyl groups. Besides a methoxy group (δ_{H} 3.69; δ_{C} 52.0) and three acetyl groups (δ_{H} 1.87, 2.09, 2.11; δ_{C} 168.8, 170.4, 170.8), the left twenty six ¹³C-NMR signals indicated that 1 may be a limonoid. The presence of β -substituted furan ring (δ_{H} 6.42, 7.44, 7.59; δ_{C} 108.8, 121.1, 140.4, 143.4) and four tertiary methyl groups as well as the HMBC experiment (Fig. 2) showed that 1 was trijugin type limonoid,^{7–9)} characterized by a five-membered ring C with an exocyclic carbonyl at C-9. Three acetyl groups were located at C-2, C-3 and C-12 by the HMBC cross signals between H-2 (δ_{H} 5.25), OAc (δ_{C} 170.4), between H-3 (δ_{H} 5.14), OAc (δ_{C} 170.8), and between H-12 (δ_{H} 5.55), OAc (δ_{C} 168.8). The relative stereochemistry of 1 was determined by NOESY experiments (Fig. 2). X-Ray crystallographic analysis of 1 (Fig. 3) confirmed the structure.

The UV, IR, ¹H- and ¹³C-NMR spectral data of cipadesins E, F (2, 3) were similar to those of 1, suggesting they are all limonoids.

Cipadesin E (2) was isolated as white powder. Its molecular formula C₃₁H₃₈O₁₂ was provided by the quasi-molecular

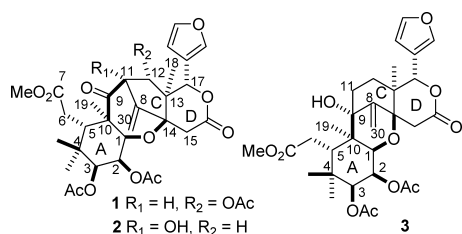


Fig. 1. Cipadesins D–F (1–3) Isolated from *Cipadessa cinerascens*

ion peak at m/z 625.2283 [M+Na]⁺ in the HR-ESI-MS. The presence of a ketonic carbonyl group and four ester carbonyl groups were recognized from the IR peak at 1746 cm⁻¹, and ¹³C-NMR signals at δ 210.7, 173.9, 170.8, 170.4 and 168.4. The HMBC correlations of H-12 (δ 1.55), H-19 (δ 1.14) and H-30 (δ 5.58, 5.28) with C-9 (δ 210.7) showed that 2 was trijugin type limonoid (Fig. 2). A hydroxyl group resonated at δ 4.72 gave HMBC correlations with C-9 and C-11, and NOESY correlation with H-12 α , allowing it to be assigned to C-11 α . The remaining structure was confirmed by HMBC and NOESY experiments (Fig. 2).

Cipadesin F (3) was isolated as white powder. Its molecular formula C₃₁H₄₀O₁₁ was concluded from the quasi-molecular ion peak at m/z 611.2471 [M+Na]⁺ in the HR-ESI-MS. Four ester carbonyl groups were revealed by the IR peak at 1739 cm⁻¹ and ¹³C-NMR signals at δ 175.0, 170.7, 170.2 and 169.9. HMBC experiment (Fig. 2) showed that 3 was methyl angolensate type limonoid,^{10,11)} characterized by a six-membered ring C connected with ring A by C-9 and C-10. The structure of 3 was determined by NMR data in DMSO-*d*₆. The hydroxyl group resonated at δ 4.72 was as-

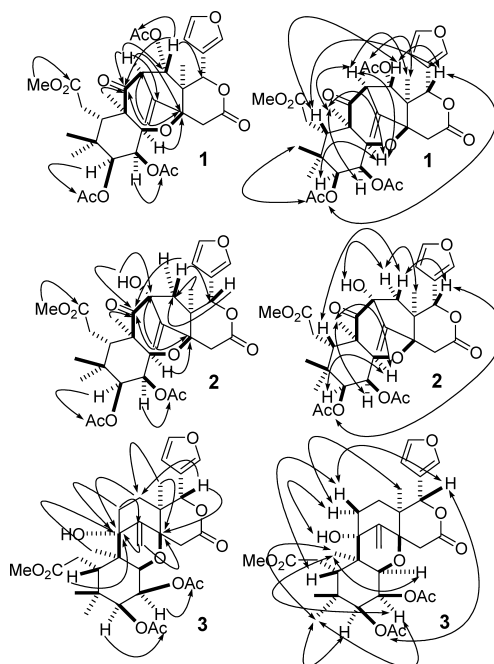


Fig. 2. Important HMBC and NOESY Correlations of 1–3 (HMBC: →; NOESY: ↔)

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Table 1. $^1\text{H-NMR}$ Data of Cipadesins D—F (1—3) in CDCl_3 (600 MHz)

| Position | 1 (multi., J in Hz) ^{a)} | 2 (multi., J in Hz) ^{b)} | 3 (multi., J in Hz) ^{a)} | 3 (multi., J in Hz) ^{a,c)} |
|-----------------|-------------------------------------|-------------------------------------|-------------------------------------|---------------------------------------|
| 1 | 4.08 (d, 3.1) | 4.05 (d, 3.2) | 3.48 (d, 4.6) | 3.42 (d, 4.6) |
| 2 | 5.25 (dd, 3.7, 3.1) | 5.24 (dd, 3.4, 3.2) | 5.17 (dd, 4.6, 3.0) | 5.06 (dd, 4.6, 3.0) |
| 3 | 5.13 (d, 3.7) | 5.14 (d, 3.4) | 5.05 (d, 3.0) | 4.91 (d, 3.0) |
| 5 | 3.13 (dd, 6.9, 2.2) | 2.95 (dd, 5.9, 3.5) | 2.91 (overlapped) | 2.79 (d, 10.0) |
| 6a | 2.89 (dd, 18.5, 2.2) | 2.84 (dd, 18.5, 5.9) | 3.04 (d, 17.1) | 3.04 (d, 17.0) |
| 6b | 2.25 (dd, 18.5, 6.9) | 2.33 (dd, 18.5, 3.5) | 2.43 (overlapped) | 2.36 (dd, 17.0, 10.0) |
| 11 α | 3.33 (d, 2.1) | | 1.38 (overlapped) | 1.49 (dt, 14.3, 5.8) |
| 11 β | | | 2.73 (m) | 2.39 (m) |
| 12 α | | 1.55 (d, 14.1) | 1.38 (overlapped) | 1.11 (m) |
| 12 β | 5.55 (d, 2.1) | 3.22 (d, 14.1) | 2.38 (overlapped) | 2.23 (overlapped) |
| 15 α | 2.85 (s) | 2.84 (s) | 2.52 (d, 17.8) | 2.24 (d, 17.8) |
| 15 β | 2.85 (s) | 2.84 (s) | 2.91 (d, 17.8) | 3.20 (d, 17.8) |
| 17 β | 6.51 (s) | 6.32 (s) | 5.79 (s) | 5.61 (s) |
| 18 | 0.94 (s) | 0.98 (s) | 0.84 (s) | 0.80 (s) |
| 19 | 1.12 (s) | 1.14 (s) | 0.90 (s) | 0.76 (s) |
| 21 | 7.59 (s) | 7.53 (s) | 7.43 (s) | 7.68 (s) |
| 22 | 6.42 (s) | 6.42 (d, 0.8) | 6.40 (s) | 6.49 (s) |
| 23 | 7.44 (s) | 7.45 (s) | 7.42 (s) | 7.71 (s) |
| 28 | 1.09 (s) | 1.09 (s) | 1.12 (s) | 1.03 (s) |
| 29 | 0.87 (s) | 0.90 (s) | 0.84 (s) | 0.72 (s) |
| 30a | 5.40 (s) | 5.58 (s) | 5.35 (s) | 5.32 (s) |
| 30b | 5.22 (s) | 5.28 (s) | 5.01 (s) | 5.12 (s) |
| OMe | 3.69 (s) | 3.67 (s) | 3.70 (s) | 3.61 (s) |
| OAc-2 β | 2.11 (s) | 2.12 (s) | 2.00 | 1.85 |
| OAc-3 β | 2.09 (s) | 2.08 (s) | 2.15 | 2.12 |
| OAc-12 α | 1.87 (s) | | | |

a) Assignments were based on HSQC and HMBC experiments, b) assignments were based on HMQC and HMBC experiments, c) in $\text{DMSO-}d_6$.

Table 2. $^{13}\text{C-NMR}$ Data of Cipadesins D—F (1—3) in CDCl_3 (150 MHz)

| C-atom | 1 ^{a)} | 2 ^{b)} | 3 ^{a)} | 3 ^{a,c)} |
|------------------|-----------------|-----------------|-----------------|-------------------|
| 1 | 74.0 | 73.6 | 74.8 | 74.2 |
| 2 | 65.5 | 65.3 | 66.4 | 67.1 |
| 3 | 74.5 | 74.2 | 76.2 | 76.2 |
| 4 | 39.2 | 39.1 | 39.2 | 39.2 |
| 5 | 35.6 | 37.2 | 36.3 | 36.2 |
| 6 | 28.4 | 29.3 | 31.1 | 31.0 |
| 7 | 173.7 | 173.9 | 175.0 | 174.8 |
| 8 | 142.2 | 147.0 | 149.2 | 149.4 |
| 9 | 205.3 | 210.7 | 78.6 | 77.7 |
| 10 | 56.5 | 53.4 | 50.7 | 50.3 |
| 11 | 69.1 | 85.9 | 34.8 | 33.3 |
| 12 | 76.5 | 47.2 | 30.2 | 30.6 |
| 13 | 49.5 | 45.0 | 41.2 | 41.0 |
| 14 | 88.2 | 87.3 | 81.7 | 81.8 |
| 15 | 33.8 | 33.7 | 33.7 | 34.3 |
| 16 | 168.1 | 168.4 | 169.9 | 170.1 |
| 17 | 78.3 | 79.5 | 80.0 | 79.9 |
| 18 | 11.5 | 17.6 | 13.6 | 13.9 |
| 19 | 19.7 | 19.3 | 16.2 | 16.8 |
| 20 | 121.1 | 121.8 | 121.2 | 121.7 |
| 21 | 140.4 | 139.7 | 140.1 | 140.1 |
| 22 | 108.8 | 108.5 | 109.5 | 110.4 |
| 23 | 143.4 | 143.6 | 143.0 | 144.0 |
| 28 | 22.8 | 22.8 | 21.7 | 21.7 |
| 29 | 27.4 | 27.6 | 27.1 | 27.4 |
| 30 | 114.8 | 114.1 | 109.5 | 110.4 |
| OCH ₃ | 52.0 | 52.0 | 51.9 | 52.0 |
| OAc-2 | 170.4, 20.8 | 170.4, 20.8 | 170.2, 21.4 | 170.0, 20.9 |
| OAc-3 | 170.8, 20.4 | 170.8, 20.4 | 170.7, 20.8 | 170.3, 21.4 |
| OAc-12 | 168.8, 20.4 | | | |

a) Assignments were based on HSQC and HMBC experiments, b) assignments were based on HMQC and HMBC experiments, c) in $\text{DMSO-}d_6$.

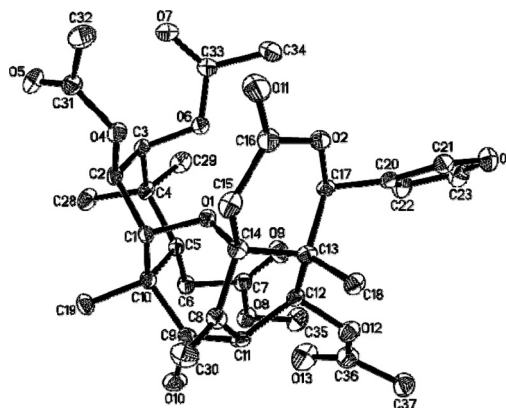


Fig. 3. ORTEP Diagram of 1

signed as HO-9 α on the basis of its correlations with C-8, C-9, C-10 and C-11 in HMBC experiment, and with H-11 α in NOESY experiment (Fig. 2). Two acetyl groups were placed at C-2 and C-3 by the HMBC cross signals between H-2, OAc, and between H-3, OAc. The relative stereochemistry of 3 was determined by NOESY experiment.

Experimental

General Melting points were measured on an X-6 melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 automatic polarimeter. UV and IR spectra were recorded on a Lambda 35 spectrometer and a Perkin-Elmer FT-IR spectrometer (KBr disk), respectively. Mass spectra were obtained on a Finnigan-LCQ^{DECA} mass spectrometer (ESI-MS) and a Bruker Daltonics Bio TOF-Q mass spectrometer (HR-ESI-MS). NMR spectra were carried out on a Bruker Avance 600 spectrometer with TMS as internal standard. Column chromatography was carried out on silica gel (200—300 mesh; Qingdao Haiyang Chemical Group Co., China), silica gel 60 (0.015—0.040 mm, Merck), MCI gel (75—150 μm , Mitsubishi) and RP-18 silica gel (Prepex 40—63 μm , Phenomenex). Semi-

preparative HPLC was carried out using a Perkin Elmer Series 200 HPLC system (Lichrocart RP-C₁₈ column, 10×250 mm, 5 μm). Solvents were distilled prior to use.

Plant Material Leaves and bark of *Cipadessa cinerascens* were collected in May 2004 at Panzhihua City, Sichuan Province of China, and identified by Prof. Zuo-Cheng Zhao at Chengdu Institute of Biology of the Chinese Academy of Sciences (CAS). A voucher specimen (No. A-186) was deposited in the Natural Products Research Center at Chengdu Institute of Biology, CAS.

Extraction and Isolation Air-dried and powdered leaves and bark (4.8 kg) were percolated with 95% EtOH (401×2, each 7 d) at room temperature. After evaporation of the solvents *in vacuo* at 50 °C, a residue (310 g) was obtained. This residue was suspended in H₂O (1 l) and extracted with CHCl₃ (11×3). The CHCl₃ extract (75 g) was partitioned between 95% aqueous MeOH and petroleum ether (60–90 °C) to yield the MeOH soluble fraction (45 g). β-Daucosterol (4 g) was obtained by crystallization of MeOH soluble fraction from MeOH, and the remnant was then subjected to silica gel column (φ8×L 25 cm, 800 g) and eluted with CHCl₃–EtOAc–MeOH (25:5:1) to give fractions A–C (A, 1.3 g; B, 12.2 g; C, 2.5 g). β-Sitosterol (580 mg) was obtained by crystallization of fraction A from MeOH. Fraction B was subjected to MCI gel column (φ5×L 12 cm, 150 g) and eluted with a gradient mixture of H₂O and MeOH (1:1, 3:7, 1:9, 0:1, each 2 l) to give fractions BA–BD. **1** (310 mg) was obtained by crystallization of fraction BB (2.7 g) from MeOH, and the remnant was then subjected to silica gel column (φ5×L 20 cm, 300 g) and eluted with CHCl₃–EtOAc–MeOH (50:5:1) to give fractions BBA–BBD. Fraction BBA (1.50 g) was further separated over silica gel 60 column (φ3.5×L 15 cm, 80 g) using petroleum ether (60–90 °C)–acetone (4:1) to give fractions BBAA and BBAB. Fraction BBAA was subjected to RP-18 silica gel column (φ2×L 20 cm, 40 g) and eluted with water–methanol (2:3) to give white powder (160 mg), which was divided into subfractions 1–3 by reversed phase HPLC (56% aqueous MeOH, 3 ml/min, 227 nm). **2** (9 mg) and **3** (12 mg) were obtained from subfraction 1 (28 mg) by HPLC (47% aqueous CH₃CN, 3 ml/min, 227 nm). Fraction BBB (170 mg) was further separated over silica gel 60 column (φ2×L 17 cm, 30 g) using petroleum ether (60–90 °C)–acetone (6:1) to give yellow oily residue (50 mg), from which 8,15-dihydroxy-13E-labdane (20 mg) was obtained by crystallization from MeOH at 4 °C.

Cipadesin D (**1**): Colorless needles (MeOH). mp 208.1–209.0 °C. ¹H-NMR data see Table 1. ¹³C-NMR data see Table 2. IR (KBr) cm⁻¹: 3445, 2990, 1749, 1738, 1686, 1372, 1244, 1073, 1003, 875, 607. UV λ_{max} (CHCl₃) nm (log ε): 241 (2.87). HR-ESI-MS (positive mode) *m/z* 667.2330 (Calcd for C₃₃H₄₀O₁₃Na [M+Na]⁺, 667.2361). [α]_D²⁵ –5.3°, [α]₅₇₈²⁵ –5.3°, [α]₅₄₆²⁵ –7.2°, [α]₄₃₆²⁵ –28.9°, [α]₃₆₅²⁵ –157.9° (*c*=0.15, CHCl₃).

Cipadesin E (**2**): White powder. ¹H-NMR data see Table 1. ¹³C-NMR data see Table 2. IR (KBr) cm⁻¹: 3445, 2976, 1746, 1686, 1634, 1379, 1248, 1164, 1083, 1048, 1021, 876, 602. UV λ_{max} (CHCl₃) nm (log ε): 240 (2.03). HR-ESI-MS (positive mode) *m/z* 625.2283 (Calcd for C₃₁H₃₈O₁₂Na [M+Na]⁺, 625.2255). [α]_D²⁵ –6.1°, [α]₅₇₈²⁵ –6.4°, [α]₅₄₆²⁵ –7.4°, [α]₄₃₆²⁵ –10.2°, [α]₃₆₅²⁵ –16.8° (*c*=0.39, CHCl₃).

Cipadesin F (**3**): White powder. ¹H-NMR data see Table 1. ¹³C-NMR data see Table 2. IR (KBr) cm⁻¹: 3451, 2953, 1739, 1635, 1384, 1250, 1198, 1168, 1049, 875, 602. UV λ_{max} (CHCl₃) nm (log ε): 240 (1.89). ESI-MS

(positive mode) *m/z* 611 [M+Na]⁺ (100). HR-ESI-MS (positive mode) *m/z* 611.2471 (Calcd for C₃₁H₄₀O₁₁Na [M+Na]⁺, 611.2463). [α]_D²⁵ –36.5°, [α]₅₇₈²⁵ –38.2°, [α]₅₄₆²⁵ –43.5°, [α]₄₃₆²⁵ –74.8°, [α]₃₆₅²⁵ –118.8° (*c*=0.42, CHCl₃).

X-Ray Crystallography of 1 Crystal data: C₃₃H₄₀O₁₃; *M*_w=644.65; dimensions 0.48×0.48×0.46 mm; monoclinic system, space group *P*2₁2₁2₁, *a*=10.770 (4) Å, *b*=11.174 (3) Å, *c*=13.377 (4) Å, α=β=γ=90°, *V*=1609.6 (11) Å³, *Z*=2, *d*=1.330 g/cm⁻³, λ=0.71073 Å, μ(Mo KR)=0.089 mm⁻¹, *F*(000)=684, *T*=296 (2) K. Of the 4231 reflections collected, 3886 were unique (*R*_{int}=0.0064). The structure was solved by direct method with SHELX 97¹²) and refined by fullmatrix least-squares on *F*². Final refinement: data/restraints/parameters=3886/1/424; *R*1=0.0644 (all data), *wR*2=0.1084 (all data); the Flack absolute structure parameter=0 (10), and *GOF*=0.945. The H coordinates were determined by calculated geometry. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.188 and –0.228 e⁻Å⁻³, respectively. CCDC 278633 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, U.K., fax: +44 1223 336033.

Supporting Information Available X-Ray data of **1**; HR-ESI-MS spectra; 1D and 2D NMR diagrams of **1**–**3**. This material is available free of charge via the Internet at <http://cpb.pharm.or.jp>.

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