Limonoids from *Cipadessa fruticosa*

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The fruits of *Cipadessa fruticosa* Bl. afforded two new limonoids: methyl 8α,30α-epoxide-3β-(2'-methylbutyryloxy)-1-oxomeliacate (cipadesin A) and methyl 21,23-dihydro-23-hydroxy-21-oxo-3β-tigloyloxy-1-oxomeliac-8(30)-enate (febrifugin A), along with the known limonoids cipadesin, khayasin T, febrifugin, ruageanin A and mexicanolide. Their structures were elucidated on the basis of spectroscopic methods.

Key words: Cipadessa fruticosa, Meliaceae, Limonoid, Cipadesin A, Febrifugin A

Introduction

Cipadessa fruticosa Bl. (Meliaceae) is widely cultivated in the southwest of China. This plant has been reported to contain *ent*-clerodanes and labdanes diterpenoids [1, 2], limonoids, sterols, sesquiterpenoids, heneicosene derivatives and one coumarin [3, 4]. Flavonoid glycosides [5, 6] were isolated from *C. cinerascens* and *C. boivinina* yielded sterols [7].

Limonoids are mainly found in plants belonging to the Meliaceae family. They have attracted considerable interest because of their biological properties and variety of structures [8]. In this paper, we report the isolation of two new limonoids **1** and **2**, along with five known limonoids: cipadesin (**3**) [3, 9], khayasin T (**4**) [10, 3], febrifugin (**5**) [3, 9, 11, 12], ruageanin A (**6**) [13] and mexicanolide (**7**) [14].

Results and Discussion

The ethyl acetate-soluble fraction of the dichloromethane extract of the fruits of *C. fruticosa* was purified by repeated column chromatography on silica gel and preparative HPLC to give the limonoids **1 – 7**.

Compound **1** had a molecular formula of $C_{32}H_{42}O_9$ as determined from the pseudo-molecular ion peak at *m/z* 593 $[M + Na]^+$ in the positive ESI mass spectrum and elemental analysis. The ^{1}H and ^{13}C NMR spectral data (Table 1 and 2) of **1** are similar to that cipadesin (**3**), a mexicanolide-type limonoid previously isolated from this plant [3]. They differed only with respect to an epoxide ring between C-8 and C-30 in **1** and the olefinic bond, in the same position, in **3**. The ^{1}H NMR spectrum of **1** indicated the presence of four tertiary methyl groups ($\delta_H = 0.80$, 0.82, 1.01 and 1.07), one methoxy singlet ($\delta_H = 3.72$), three downfield shifted signal attributed to a β-substituted furan ring ($\delta_H =$ 7.48, 7.43 and 6.46), two signals characteristic of proton attached to a carbon adjacent to an oxygen atom ($\delta_H = 5.17$, *s*, H-17 and $\delta_H = 5.10$, *d*, $J = 9.4$ Hz, H-3) and the proton on the epoxide ring ($\delta_H = 3.32$, *d*, $J = 2.4$ Hz, H-30). The ^{13}C NMR spectrum showed the presence of a ketone at $\delta_c = 214.3$ (C-1) and three ester carbonyls at $\delta_c = 172.1$ (C-16), 174.2 (C-7) and 175.9 (C-1'). In the ^{1}H-^{1}H COSY experiment the signal of H-30 showed cross peaks with the methine proton at $\delta_H = 3.56$ (*dd*, $J = 9.4$, 2.4 Hz, H-2), which correlated with the signal of H-30. This signal showed HSQC correlation with the signal at $\delta_c = 63.5$ attributed to C-30.

In the HMBC spectrum, the signal at $\delta_c = 214.3$ (C-1) showed correlations with the signals at $\delta_H = 1.07$ (*s*, H-19, 3H), 3.32 (*d*, $J = 2.4$ Hz, H-30) and 3.56 (*dd*, 9.4, 2.4 Hz, H-2). It was observed long-range correlations of $\delta_H = 5.17$ (*s*, H-17) with the signals at $\delta_c = 172.1$ (C-16), 120.1 (C-20) and 26.7 (C-18), suggesting the presence of a δ-lactone as D-ring. The α-configuration of epoxide ring at C8/C30 was determined by the small coupling constant of H-30 ($J =$ 2.4 Hz). The data above confirmed that limonoid **1** had a mexicanolide skeleton.

The 2-methylbutyryloxyl ester moiety at C-3 was characterized by the signals at $\delta_H = 2.58$ (*sextet*, $J = 6.8$ Hz, H-2'); 1.55 and 1.79 (*m*, H-3'); 0.97 (*t*, $J = 7.4$ Hz, H-4') and 1.25 (*d*, $J = 7.0$, H-5')

H	1	2	3	5
2	3.56 *dd* (9.4; 2.4)	3.51 *m*	3.49 *m*	3.52 *m*
3	5.10 *d* (9.4)	4.82 *d* (9.2)	4.81 *d* (9.3)	4.85 *d* (9.2)
5	3.23 *dd* (8.0; 3.4)	3.49 *brs*	3.42 *dd* (7.6; 4.1)	3.48 *m*
6	1.95 *m*	2.38 *m*	2.38 *m*	2.36 *m*
6	1.91 *m*			2.40 *m*
9	1.87 *m*	2.23 *m*	2.24 *m*	2.18 *m*
11	1.79 *m*	1.74 *m*	1.68 *m*	1.67 *m*
11	1.80 *m*			2.11 *m*
12	1.18 *m*	1.44 *m*	1.66 *m*	1.60 *m*
12	1.95 *m*	1.92 *m*	1.45 *m*	1.40 *m*
14	1.55 *m*	2.28 *m*	2.20 *m*	2.19 *m*
15	2.80 *dd* (15.8; 4.6)	2.80 *m*	2.88 *m*	2.84 *m*
15	3.67 *dd* (15.8; 5.7)		2.84 *m*	2.83 *brd* (19.7)
17	5.17 *s*	5.57 *s*	5.69 *s*	5.62 *s*
18	1.01 *s*	1.03 *s*	1.10 *s*	1.08 *s*
19	1.07 *s*	1.07 *s*	1.15 *s*	1.15 *s*
21	7.48 *m*	–	7.79 *m*	7.83 *m*
22	6.46 *dd* (1.8; 0.8)	7.34 *brs*	6.46 *dd* (1.8; 0.8)	6.48 *dd* (1.7; 0.7)
23	7.43 *t* (1.8)	6.21 *brs*	7.42 *t* (1.8)	7.43 *t* (1.7)
28	0.80 *s*	0.79 *s*	0.79 *s*	0.81 *s*
29	0.82 *s*	0.88 *s*	0.83 *s*	0.84 *s*
30	3.32 *d* (2.4)	5.30 *brd* (6.9)	5.38 *dd* (8.8; 1.9)	5.34 *brd* (7.1)
OMe	3.72 *s*	3.67 *s*	3.72 *s*	3.67 *s*
2'	2.58 *sextet* (6.8)	–	2.45 *m*	–
3'	1.55 *m*	6.92 *m*	1.45 *m*	6.93 *qq* (7.0; 1.4)
3'	1.79 *m*	–	1.66 *m*	–
4'	0.97 *t* (7.4)	1.82 *dd* (6.9; 1.2)	0.93 *t* (7.4)	1.74 *dd* (6.9; 1.4)
5'	1.25 *d* (7.0)	1.84 *t* (1.2)	1.14 *d* (7.0)	1.82 *t* (1.4)

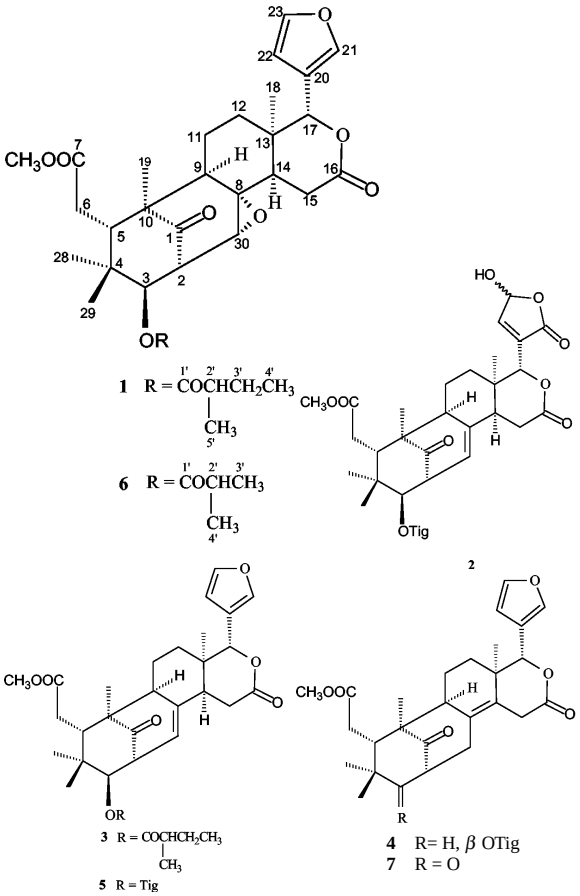
Table 1. ^{1}H NMR spectral data for compounds **1–3** and **5** (400 MHz, $CDCl_3$).

Resonances for **1–3** and **5** were confirmed by ^{1}H-^{1}H COSY, HSQC and HMBC spectra. Coupling constants (J in Hz) in parentheses.

and which showed correlations in the HSQC spectrum with $\delta_c = 41.5$ (C-2'), 26.5 (C-3'), 12.0 (C-4') and 17.4 (C-5'), respectively. The presence of this group in **1** was confirmed by comparison of its spectral data with those published for swietenin E, isolated from *Swietenia mahogoni* [10]. Its β-orientation was defined by the large coupling constant of H-3 ($J = 9.4$ Hz) [15]. Compound **1** was elucidated to be methyl 8α,30α-epoxide-3β-(2'-methylbutyryloxy)-1-oxomeliacate, named cipadesin A.

Compound **2** showed the pseudo molecular ion peak at *m/z* 607 $[M + Na]^+$, in the positive ESI mass spectrum, according to the molecular formula $C_{34}H_{40}O_{10}$, which was confirmed by elemental analysis. Their ^{1}H and ^{13}C NMR spectral data (Table 1 and 2) indicated that it was also a mexicanolide-type limonoid. This compound is similar to febrifugin (**5**), previously isolated from *Soymida febrifuga* [11] and *C. fruticosa* [3], except for the group attached at C-17. The signals at $\delta_H = 5.30$ (*brd*, $J = 6.9$ Hz, H-30) in the ^{1}H NMR spectrum and the ^{13}C NMR signals at $\delta_C = 137.9$ (C-8) and 123.4 (C-30) are characteristics of the olefinic linkage between C-8 and C-30 for limonoids with mexicanolide skeleton [16]. These attributions were confirmed by the correlation of H-30 with the signal at $\delta_C = 123.4$ (C-30) in the HSQC experiment. The tigloyl moiety at C-3 was defined by the signals at $\delta_H = 6.92$ (*m*, H-3'), which showed HSQC correlation with $\delta_C = 139.3$ (C-3'), and two signals of methyl group at $\delta_H = 1.82$ (*dd*, 6.9, 1.2 Hz) and 1.84 (*t*, 1.2 Hz) attributed to H-4' and H-5', respectively.

The characteristic signals for a furan ring at C-17, typical for limonoids of Meliaceae, were not observed in the ^{1}H and ^{13}C NMR spectral data of **2**. However, it showed two broad one-proton singlets at $\delta_H = 6.21$ (H-23) and 7.34 (H-22), which showed cross peaks in the ^{1}H-^{1}H COSY spectrum. These ^{1}H NMR signals showed further couplings to a broad singlet at $\delta_H = 5.57$, attributed to H-17. The ^{13}C NMR data indicated the presence of a hemicetal carbon at $\delta_C = 97.0$ (C-23), a α,β-unsaturated γ-lactone carbonyl at $\delta_C = 168.4$ (C-21) and two signals at $\delta_C = 135.4$ and 149.4, relating to the olefinic bond at C-20/C-22. The HSQC experiment established the correlation of the signal at $\delta_H = 6.21$ (*brs*, H-23) with $\delta_C = 97.0$ (C-23) and $\delta_H = 7.34$ (H-22) with the olefinic carbon at $\delta_C = 149.4$ (C-22). The data above indicated the presence of a γ-hydroxybutenolide function in **2**, which was confirmed by the comparison with the limonoid 7-deoxo-7α-acetoxykihadanin B, previously



isolated from *Trichilia elegans* [17]. The equilibrium between two epimeric forms at C-23 of this group accounts for the broadness of the ^{13}C signal of C-23, C-22 and with a lesser intensity at C-20. The occurrence of limonoids with γ-hydroxybutenolide at C-17 has already been reported in several members of the Meliaceae [17 – 20]. Compound **2** was characterized as methyl 21,23-dihydro-23-hydroxy-21-oxo-3β-tigloyloxy-1-oxomeliac-8(30)- enate, named febrifugin A.

Compound **3** showed spectral data identical to those published for cipadesin (**3**) [3]. However, the signal earlier reported to C-12/C-15 were inverted. These ^{13}C NMR signals were reassigned as shown in Table 2. The data reported to febrifugin (**5**) [11, 12] also presented mistaken. The ^{13}C NMR signals previously attributed to C-2, C-5, C-9, C-12, C-14, C-15, C-21 and C-23 were inconsistent with those observed to compound **5** (Table 2). These corrections were based on the ^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC and HMBC experiments of compounds **3** and **5** and confirmed by described data for structurally related compounds [10, 16].

Table 2. ^{13}C NMR spectral data for compounds **1 – 3** and **5** in $CDCl_3$ (100 MHz, $CDCl_3$).

C	1	2	3	5
1	214.3	217.3	217.1	217.2
2	48.8	49.1	48.9	49.1*
3	77.4	77.1	76.9	76.6
4	39.4	38.8	38.7	38.6
5	42.6	40.4	41.5	41.3*
6	33.5	33.0	32.9	32.9
7	174.2	173.7	174.0	174.0
8	60.7	137.9	138.4	138.5
9	56.0	56.4	56.8	56.8*
10	48.3	50.5	49.9	49.8
11	19.4	21.0	20.6	20.7
12	33.1	34.6	34.5*	34.5*
13	36.4	36.8	36.9	36.9
14	46.0	45.3	45.2	45.2*
15	34.1	29.2	29.7*	29.7*
16	172.1	167.9	169.3	168.9
17	78.8	77.1	76.9	77.0
18	26.7	22.5[a]	21.8	21.7
19	15.9	15.6	15.7	15.8
20	120.1	135.4	120.7	120.8
21	141.0	168.4	142.0	141.9*
22	110.3	149.4	109.7	109.7
23	143.1	97.0	142.9	143.0*
28	21.0	22.6[a]	22.4	22.6
29	22.5	20.8	20.6	20.2
30	63.5	123.4	122.8	123.1
OMe	52.4	52.2	52.1	52.1
1’	175.9	167.3	176.0	167.2
2’	41.5	127.7	40.8	127.5
3’	26.5	139.3	26.3	139.6
4’	12.0	14.7	11.4	14.6
5’	17.4	11.9	16.3	11.8

Resonances for **1 – 3** and **5** were confirmed by HSQC and HMBC spectra. * Data obtained in this study suggest that these resonances were previously incorrectly assigned.

The mexicanolide-type limonoids **4**, **6** and **7** were elucidated to be khayasin T [10], ruageanin A [13] and mexicanolide [14], respectively. It was possible through the comparison of their one and two-dimensional NMR spectral data with those previously reported to them.

Harms [21] classified the meliaceous genera into three subfamilies Cedreloideae, Swietenioideae and Melioideae (tribes Carapeae, Melieae, Turraeeae, Vavaeeae and Trichilieae). Pennington and Styles [22], in their more recent monograph, included Harms’ subfamily Cedreloideae, tribe Carapeae into the Swietenoideae and removed genus *Cipadessa* from Turraeeae classifying it in Trichilieae. Chemically, the family Meliaceae is distinguished by the frequent oc-

currence of limonoids. The mexicanolide group occurs widely in the genera of the Swietenioideae. The Melioideae appears to be the most prolific in production of A,B-*seco* limonoids but relatively poor in mexicanolide types [23, 24]. The latter group of compounds has been recorded in genera of the Harms' tribe Trichilieae. Thus, the present results obtained from *Cipadessa fruticosa* and those from literature [3], provide firm support for including *Cipadessa* in the Trichilieae.

Experimental Section

General

NMR: on a Bruker DRX 400, with TMS as internal standard; ESIMS: low resolution on a triple quadrupole Micromass Quattro LC instrument; IR: KBr, BOMEM, Hartmann & Braun/MB Series); UV: HP 8452A, diode array spectrophotometer; Preparative HPLC: on a Shimadzu LC-8A; the column used was Shim-pack Prep-Sil (H), 250 mm×20 mm, 5μ particle size, 100 Å pore diameter; detection on Shimadzu SPD-6AV; Elemental analysis: on a EA1108, CHNSO (Fisons).

Plant material

The fruits of *Cipadessa fruticosa* Bl. were collected in Viçosa, Minas Gerais, Brazil, and a voucher specimen (110.664) was deposited in the SPF Herbarium of Instituto de Cincias Biológicas-USP, São Paulo, Brazil.

Extraction and isolation of compounds

The powdered air-dried fruits (990 g) of *C. fruticosa* were subsequently extracted with hexane, CH_2Cl_2 and MeOH. The conc. CH_2Cl_2 extract (10.9 g) was submitted to vacuum chromatography over silica gel using a hexane-CH_2Cl_2-EtOAc-MeOH gradient. The ethyl acetate-soluble fraction (2.3 g), rich in limonoids, was chromatographed on silica gel, eluting with a hexane-CH_2Cl_2-acetone gradient to give 8 fractions (A-H). Fraction C was fractionated as above, using hexane-EtOAc gradient, affording 11 fractions. Fraction C-6 was twice chromatographed on silica gel, eluting with hexane-CH_2Cl_2-acetone (6:3:1) to give compounds **1** (8.5 mg) and **3** (19.4 mg). Fraction D was chromatographed as above, using hexane-EtOAc gradient, to afford 4 fractions. Fraction D-3 was twice chromatographed with a hexane-CH_2Cl_2-acetone gradient yielding a fraction containing compounds **6** and **7**, which was purified by HPLC (detection UV λ 220 nm), using hexane-*iso*-PrOH (85:15) with a flow rate of 1.5 ml min^{-1} to yield **6** (9.3 mg) and **7** (12.4 mg). Fraction E was twice subjected to column chromatography over silica gel, eluting with a hexane-CH_2Cl_2-acetone gradient affording 4 fractions. Fraction E-3 was purified by HPLC (detection UV λ 240 nm), using hexane-*iso*-PrOH (8:2) at 2.0 ml min^{-1} to give **2** (4.7 mg) and **5** (40.3 mg).

*Cipadesin A (**1**)*

Amorphous solid, $C_{32}H_{42}O_9$, $[\alpha]_D^{27}$ −103° ($CHCl_3$; c 0.9649); IR (KBr) $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 2973, 1730, 1459, 1385, 1266, 1184, 1145, 1026, 897, 738; UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 234 (5,1); ESIMS, *m/z* (rel. int.): 593 $[M + Na]^+$ (100): calcd. C 67.36, H 7.37; found C 67.40, H 7.30. ^{1}H NMR ($CDCl_3$): Table 1; ^{13}C NMR ($CDCl_3$): Table 2.

*Febrifugin A (**2**)*

Amorphous solid, $C_{34}H_{40}O_{10}$, $[\alpha]_D^{27}$ −121° ($CHCl_3$; c 0.2055); IR (KBr) $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3430, 2948, 1723, 1650, 1575, 1434, 1384, 1264, 1049, 882, 731; UV $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 236 (5,1); ESIMS, *m/z* (rel. int.): 607 $[M + Na]^+$ (100): calcd. C 65.75, H 6.85; found C 65.62, H 6.75. ^{1}H NMR ($CDCl_3$): Table 1; ^{13}C NMR ($CDCl_3$): Table 2.

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