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Article

Limonoids from *Cipadessa fruticosa* and *Cedrela fissilis* and their Insecticidal Activity

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O estudo químico dos frutos de *Cipadessa fruticosa* (Meliaceae) permitiu o isolamento dos limonóides cipadesina B (inédito) e swietemahonolídeo. Das raízes de *Cedrela fissilis* (Meliaceae) foi isolado o limonóide 3β-acetoxicarapina, inédito como produto natural, juntamente com os triterpenos ácido oleanólico e ácido oleanônico. Estes compostos, e outros seis mexicanolídeos anteriormente isolados de *C. fruticosa,* apresentaram atividade inseticida para as formigas cortadeiras *Atta sexdens rubropilosa*.

The chemical investigation of the fruits of *Cipadessa fruticosa* (Meliaceae) afforded the new limonoid cipadesin B and the known swietemahonolide. From the roots of *Cedrela fissilis* (Meliaceae) were isolated the limonoid 3β-acetoxycarapin, new as natural product, along with the triterpenes oleanolic and oleanonic acid. These compounds and other six mexicanolide limonoids previously isolated from *C. fruticosa* showed insecticidal activity against the leaf-cutting ants *Atta sexdens rubropilosa*.

Keywords: *Cedrela fissilis, Cipadessa fruticosa*, limonoids, triterpenes, insecticidal activity

Introduction

Cipadessa fruticosa Blume (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] We have recently reported the isolation of six mexicanolide limonoids from this plant.[5]

Cedrela fissilis Vell. (Meliaceae) is a valuable tree of timber industry. In Brazil, it can be found from Amazon forest up to north of Espírito Santo State. Previous investigation of fruits and seeds from this species afforded the limonoids fissinolide, mexicanolide and 3β-hydroxyisomexicanolide.[6] In a recent investigation we have isolated several gedunin limonoids from *C. fissilis*.[7]

Extracts and fractions from *C. fruticosa and C. fissilis* have been shown activity on leaf-cutting workers *Atta sexdens rubropilosa*.[8,9] Thus, the aim of this work was the chemical investigation of these Meliaceae species associated with biological assays against *Atta sexdens rubropilosa*. The study of the fruits of *C. fruticosa* allowed the isolation of a new mexicanolide limonoid: cipadesin B (**1**) and the known swietemahonolide (**2**). The limonoid 3β-acetoxycarapin (**3**) isolated from fruits of *C. fissilis*, is new as natural product and its spectral data are being described for the first time. The triterpenes oleanolic acid (**4**) and oleanonic acid (**5**) were also isolated from *C. fissilis*. The evaluation of the activity on leaf-cutting ants of these compounds, along with the following mexicanolide limonoids previously isolated from *C. fruticosa*: cipadesin A (**6**), ruageanin A (**7**), cipadesin (**8**), khayasin T (**9**), febrifugin (**10**) and mexicanolide (**11**)[5] was performed.

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Experimental

General experimental procedures

NMR: on Bruker DRX 400, with TMS as internal standard; ESIMS: low resolution on triple quadrupole Micromass Quattro LC instrument; IR: KBr, on BOMEM, Hartmann & Braun/MB Series; UV: on HP 8452A, diode array spectrophotometer; elemental analysis: on EA1108, CHNSO (Fisons).

Plant material

The roots of *Cedrela fissilis* Vell. were collected in São Carlos-SP, Brazil in 01/06/01 and identified by Dr. Maria Inês Salgueiro Lima from the Department of Botany, Universidade Federal de São Carlos, where a voucher specimen (6701) was deposited. 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 Ciências 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 concentrated 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), was chromatographed on silica gel, eluting with a hexane-CH_2Cl_2-acetone gradient to give 8 fractions (A-H). Fraction D was fractionated as above, using hexane-EtOAc gradient, affording 11 fractions. Fraction D-6 was chromatographed twice on silica gel, eluting with hexane-CH_2Cl_2-acetone (6:3:1) to give **1** (11.3 mg) and **2** (3.3 mg). Fraction E was chromatographed three times on silica gel, using hexane-EtOAc (7:3), affording **4** (36.5 mg) and **5** (4.3 mg). The isolation of limonoids **6**-**11** from fruits of *C. fruticosa* was recently reported.[5]

The hexane, CH_2Cl_2 and MeOH extracts from roots (320.9 g) of *C. fissilis* were obtained as described for *C. fruticosa*. Part of the CH_2Cl_2 extract (1.0 g) was fractionated on silica gel, eluting with a hexane-CH_2Cl_2-EtOAc-MeOH gradient, affording 9 fractions (A-I). Fraction I was chromatographed twice on silica gel with hexane-CH_2Cl_2-acetone gradient to afford **3** (12.1 mg).

Cipadesin B (***1***). Amorphous solid; mp 196 °C ; $[\alpha]_D^{27}$- 111.0° (*c* 0.20, $CHCl_3$); IR (film) ν_{max}/cm^{-1}: 3450, 2896, 1725, 1650, 1575, 1235, 1340, 900, 735; UV (CH_2Cl_2) λ_{max}/nm (log ε): 240 (5.2); ESIMS, *m/z* (rel. int.): 601 $[M + H]^-$ (100); Found: C, 63.85; H, 7.01. Calc. for $C_{32}H_{42}O_{11}$: C, 63.79; H, 6.98; ^{1}H and NMR (400 and 100 MHz, $CDCl_3$): Table 1.

3β-Acetoxycarapin (***3***). Amorphous solid; mp 132 - 133 °C; $[\alpha]_D^{27}$ - 13.1 ° (*c* 0.20, $CHCl_3$). IR (film)ν_{max}/cm^{-1}: 2950, 1727, 1626, 1504, 1378, 1238, 1022, 874, 735; UV (CH_2Cl_2) λ_{max}/nm (log ε): 250 (5.1); ESIMS, *m/z* (rel. int.): 535 $[M + Na]^+$ (100); Found: C, 68.0; H, 7.05. Calc. for $C_{29}H_{36}O_8$: C, 67.97; H, 7.03; ^{1}H and NMR (400 and 100 MHz, $CDCl_3$): Table 1.

Bioassay

The *A. sexdens rubropilosa* workers used in the assays were randomly removed from laboratory nests. They had a body mass of 20-25 mg. Before the assays the nests were supplied daily with leaves of *Eucalyptus* sp., oat seeds and occasionally with leaves of other plants such as *Hibiscus* sp., *Ligustrum* sp. or rose petals. Fifty ants were removed from the nests and put into five Petri dishes (ten ants each) for each treatment. During the assays the ants were maintained on an artificial diet prepared with glucose (50 g L^{-1}), bacto-peptone (10 g L^{-1}), yeast extract (1.0 g L^{-1}) and agar (15 g L $^{-1}$) in distilled water (100 mL).[23] The diet (0.4-0.5 g *per* dish) with the addition of compounds (experiment) or without (control) were offered daily in a small plastic cap. The control was prepared with the diet and the solvent. To ensure that undetectable remaining amounts of the solvent did not affect the ants, a comparison was made with another set of dishes in which water was used instead of solvent. As expected, the same survival rates were obtained with both systems (data not shown). The compounds were poured into the hot diet immediately after it was autoclaved. The final concentration of the compounds added to the diet was 100 μg mL^{-1}. During the assays the material was maintained in an incubator at a temperature of 25 (±1) ^{o}C and relative humidity ranging between 70-80%. The maximum length of observation was 25 days and the number of dead ants was registered daily.

The survival median 50% (S_{50}) was calculated and survival curves were compared by the computer-assisted software Graph-Pad™ using the log-rank test.

Results and Discussion

Compound **1**, isolated from fruits of *C. fruticosa*, had a molecular formula $C_{32}H_{42}O_{11}$ determined from the pseudo-molecular ion peak at *m/z* 601 $(M - H)^-$ in the negative ESI mass spectrum and elemental analysis. The ^{1}H and ^{13}C NMR spectral data (Table 1) of **1** are similar to that cipadesin A, a mexicanolide-type limonoid previously isolated from this plant.[5] They differed to each other only with respect to the γ-hydroxybutenolide moiety at C-17 of **1**. The ^{1}H NMR spectral data (Table 1) of limonoid **1** indicated the presence of four tertiary methyl groups (δ_H 0.79, 0.80, 1.05 and 1.06), one methoxyl group (δ_H 3.72), two signals characteristic of carbinolic hydrogen (δ_H 5.09, brs, H-17 and δ_H 5.10, d, *J* 8.9 Hz, H-3) and of the proton on the epoxide ring (δ_H 3.30, d, *J* 1.9 Hz, H-30).

In the HMBC correlation map, the signal at δ_C 214.3 (C-1) showed correlations with the signals at δ_H 1.06 (s,

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1** and **3** in $CDCl_3$. Resonances for **1** and **3** were confirmed by ^{1}H-^{1}H COSY, HSQC and HMBC spectra. Coupling constants (*J* in Hz) in parentheses

H	**1**	**3**	C	1	3
2	3.56 m	3.13 ddd (10.0; 4.6; 1.8)	1	214.3	218.4
3	5.10 d (8.9)	5.01 d (10.0)	2	48.8	46.2
5	3.23 m	3.27 dd (8.7; 2.4)	3	77.3	78.7
6	2.35 m	2.47 m	4	39.4	37.8
8	-	3.23 m	5	42.5	40.5
9	1.81 m	1.74 m	6	33.1	33.6
11	1.84 m	1.72 m	7	174.4	174.4
12	1.15; 2.16 m	1.40 m	8	60.5	34.3
14	1.52 m	-	9	55.7/55.8	48.7
15	2.86 m; 3.67 m	5.78 d (2.2)	10	48.3	51.1
17	5.09 brs	5.12 s	11	19.4	18.7
18	1.05 s	1.04 s	12	33.1	26.6
19	1.06 s	1.09 s	13	36.6/36.7	38.2
21	-	7.49 dd (1.5; 0.8)	14	46.0/46.1	170.4
22	7.34brs/ 7.37 brs	6.43 dd (1.5; 0.8)	15	34.3	112.6
23	6.19brs/ 6.25 brs	7.42 t (1.5)	16	171.4/171.8	164.5
28	0.79 s	0.81 s	17	77.0	81.1
29	0.80 s	0.84 s	18	26.4	18.0
30	3.30 d (1.9)	1.72 m	19	15.7	17.8
30	-	2.26 ddd (13.9; 4.6; 1.8)	20	133.4	119.9
O<u>Me</u>	3.72 s	3.72 s	21	171.4/171.8	141.2
<u>Me</u>COO	-	2.18 s	22	150.2/150.7	109.9
2’	2.58 m	-	23	96.9/97.4	143.0
3’	1.55 m	-	28	21.1	20.7
3’	1.76 m	-	29	22.5	24.0
4’	0.97 t (7.5)	-	30	63.5	34.9
5’	1.25 d (7.0)	-	Me<u>C</u>OO	-	170.1
			<u>Me</u>COO	-	20.4
			OMe	52.4	52.1
			1’	175.8	-
			2’	41.5	-
			3’	26.7	-
			4’	17.3	-
			5’	11.9	-

H-19, 3H), 3.30 (d, *J* 1.9 Hz, H-30) and 3.56 (m, H-2). It was observed long-range correlations of δ_H 5.09 (brs, H-17) with the signals at δ_C 171.4/171.8 (C-16), δ_C 133.4 (C-20) and δ_C 26.4 (C-18), suggesting the presence of a δ-lactone as D-ring.

The 2-methylbutyryloxyl ester moiety at C-3 was characterized by the signals at δ_H 2.58 (m, H-2’); 1.55 and 1.76 (m, H-3’); 0.97 (t, *J* 7.5 Hz, H-4’) and 1.25 (d, *J* 7.0 Hz, H-5’) and which showed correlations in the HSQC correlation map with δ_C 41.5 (C-2’), 26.7 (C-3’), 17.3 (C-4’) and 11.9 (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 mahagoni*.[10] Its β-orientation was defined by the large coupling constant of H-3 (*J* 8.9 Hz).[11]

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 **1**. However, it showed two broad one-proton singlets at δ_H 6.19/6.25 (H-23) and 7.34/7.37 (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 δ_H 5.09, attributed to H-17. The ^{13}C NMR data indicated the presence of a hemicetal carbon at δ_C 96.9/97.4 (C-23), an α,β-unsaturated γ-lactone carbonyl at δ_C 171.4/171.8 (C-21) and the signals at δ_C 133.4 and 150.2/150.7, relating to the olefinic bond at C-20/C-22. The HSQC experiment established the correlation of the signal at δ_H 6.19/6.25 (brs, H-23) with δ_C 96.9/97.4 (C-23) and δ_H 7.34/7.37 (H-22) with the olefinic carbon at δ_C 150.2/150.7 (C-22). The data above indicated the presence of a γ-hydroxybutenolide function in limonoid **1**. The duplication of the signals of this group indicated the presence of an epimeric mixture. Compound **1** was elucidated to be methyl 21,23-dihydro-23-hydroxy-21-oxo-8α,30α-epoxide-3β-(2’-methylbutyryloxy)-1-oxomeliacate, named cipadesin B.

Compound **3** was isolated from roots of *C. fissilis* and its complete spectral data are being described for the first time. Some ^{1}H NMR data of the synthetic derivative were published.[12-14] It showed the pseudo molecular ion peak

at *m/z* 535 $[M + Na]^+$, in the positive ESI mass spectrum, according to the molecular formula $C_{29}H_{36}O_8$, which was confirmed by elemental analysis. Their ^1H and ^{13}C NMR spectral data (Table 1) indicated that it was also a mexicanolide-type limonoid. The signals at δ_H 7.49, 6.43 and 7.42 in the ^1H NMR spectra indicated the presence of a β-substituted furan-ring. The signal at δ_H 5.78 (d, *J* 2.2 Hz, H-15) in the ^1H NMR spectrum and the ^{13}C NMR signals at δ_C 170.4 (C-14) and 112.6 (C-15) are characteristics of the olefinic linkage between C-14 and C-15 for limonoids with mexicanolide skeleton.[15] The position of the trisubstituted olefin at $\Delta^{14,15}$ was confirmed by the HMBC correlations of the signal of H-15 with the signals attributed to C-8 (δ_C 34.3), C-16 (δ_C 164.5) and C-13 (δ_C 38.2). The large allylic coupling (*J* 2.2 Hz) beetween H-15 and H-8 indicates that H-8 is β-disposed. It was confirmed by the ^1H-^1H COSY coupling of H-8 (δ_H 3.23, m) to H-15 and by its NOESY correlation with H-5. The acetoxyl group at C-3β was characterized by the singlet at δ_H 2.18 and the signal of the carbonyl group at δ_C 170.1, that showed HMBC correlation with the signal of H-3 (d, δ_H 5.01, *J* 10.0 Hz).

The mexicanolide limonoid swietemahonolide (**2**),[16] from fruits of *C. fruticosa* and the triterpenes oleanolic acid (**4**)[17] and oleanonic acid (**5**),[18] from roots of *C. fissilis* were characterized by comparison of their NMR spectral data with those previously reported in the literature.

Recently, we reported the biological activity of extracts and fractions of *C. fissilis* and *C. fruticosa* against the leaf-cutting ants *Atta sexdens rupropilosa*.[8,9] These studies showed that the fruits of *C. fruticosa* and roots of *C. fissilis* were active. In the continuation of these studies, the compounds **1**-**5** and six mexicanolide limonoids previously isolated from fruits of *C. fruticosa* (**6**-**11**)[5] were evaluated on the ants.

The results (Table 2) showed that all compounds assayed presented significant difference as compared to the control, according to the log-rank test ($p < 0.05$), which consider all tested period. In a recent work, we verified that several gedunin limonoids isolated from roots and leaves of *C. fissilis* were not active on *A. sexdens rubropilosa* workers.[7] Thus, the insecticidal activity of *C. fissilis* seems to be related to the presence of the oleanane triterpenoids. The activity previously verified for fruits of *C. fruticosa*[9] is probably due to the presence of mexicanolide-type limonoids, since these compounds showed significant activity on ants.

Limonoids have attracted considerable interest because of their biological properties. Indeed, *C-seco* limonoids, such as azadirachtin, are the most active insecticidal compound, possessing both antifeedant and growth-regulating activities.[19,20] The mexicanolide limonoids have been showed high activity against insects of *Lepidoptera* order,[15,21] but this is the first report of their activity on ants. The activity of oleanane triterpenes on leaf-cutting ants was already reported, showing their repellent action on *Atta cephalotes* workers in a feeding preferences assay.[22]

The results presented here suggest that the limonoids and triterpenes isolated from *C. fissilis* and *C. fruticosa* are promising in controlling leaf-cutting ants. In continuation of this research, these active compounds should be further evaluated in the field assays against lea-cutting ants.

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Table 2. Mortality (%) of *Atta sexdens rubropilosa* workers fed on compounds **1**-**11** at concentration of 100 μg mL^{-1}

Compound	Days										Survival median (S_{50})/ days
	1	2	3	6	8	10	14	17	21	25	
1	0	0	6	22	40	58	76	88	92	98	9 [a]
2	0	0	4	40	54	58	82	90	96	98	8 [a]
3	0	2	12	34	50	76	98	100	-	-	8 [a]
4	0	0	10	52	70	90	100	-	-	-	6 [a]
5	0	0	8	38	60	86	100	-	-	-	8 [a]
6	0	2	8	34	46	72	100				9 [a]
7	0	4	18	50	62	74	96	98	98	100	6 [a]
8	0	8	12	46	68	76	100	-	-	-	7 [a]
9	0	4	10	54	72	86	98	100	-	-	6 [a]
10	0	2	6	38	58	70	88	94	98	100	7 [a]
11	0	4	16	50	60	70	100	-	-	-	6 [a]
control (ethyl acetate)	0	0	6	22	36	50	74	90	94	96	10

[a] Significant difference according to the log-rank test ($p < 0.05$).

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