PERGAMON

Phytochemistry 59 (2002) 521–527

PHYTOCHEMISTRY

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Antifungal amides from *Piper arboreum* and *Piper tuberculatum*

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Received 10 May 2001; received in revised form 12 September 2001

Abstract

In continuation of our study of the Piperaceae we have isolated several amides, mainly those bearing isobutyl, pyrrolidine, dihydropyridone and piperidine moieties. Bioactivity-guided fractionation of extracts from leaves of *Piper arboreum* yielded two new amides, *N*-[10-(13,14-methylenedioxyphenyl)-7(*E*),9(*Z*)-pentadienoyl]-pyrrolidine (**1**), arboreumine (**2**) together with the known compounds *N*-[10-(13,14-methylenedioxyphenyl)-7(*E*)-pentaenoyl]-pyrrolidine (**3**) and *N*-[10-(13,14-methylenedioxyphenyl)-7(*E*), 9(*E*)-pentadienoyl]-pyrrolidine (**4**). Catalytic hydrogenation of **3** yielded the amide *N*-[10-(13,14-methylenedioxyphenyl)-pentanoyl]-pyrrolidine (**5**). We also have isolated six amides (**6–11**) and two antifungal cinnamoyl derivatives (**12, 13**) from seeds and leaves of *Piper tuberculatum*. Compounds **1**–**11** showed antifungal activity as determined by direct bioautography against *Cladosporium sphaerospermum* while compounds **3-4** and **6–13** also showed antifungal activity against *C. cladosporioides*. © 2002 Published by Elsevier Science Ltd.

Keywords: *Piper arboreum*; *Piper tuberculatum*; Piperaceae; Amides; Antifungal activity

1. Introduction

Chemical studies carried out on Brazilian Piperaceae species have revealed the occurrence of pyrones, lignoids and chromenes besides various amides bearing isobutyl, pyrrolidine, dihydropyridone and piperidine moieties (Das et al., 1996; Parmar et al, 1997; Alécio et al., 1998; Baldoqui et al., 1999; Navickiene et al., 2000). These amides have generated interest as a result of their potent insecticidal and antifungal properties (Miyako et al., 1989; Bernard et al., 1995; Alécio et al., 1998; Navickiene et al., 2000). In our previous papers, we have described the structures of the antifungal amides *N*-[7-(3′,4′-methylenedioxyphenyl)-2(*Z*),4(*Z*)-heptadienoyl]pyrrolidine, (3*Z*,5*Z*)-*N*-isobutyl- 8-(3′,4′-methylenedioxyphenyl)-heptadienamide isolated from leaves of *Piper hispidum* H. B. K. (Alécio et al., 1998) and 8(*Z*)-*N*-(12,13,14-trimethoxycinnamoyl)-Δ^3-pyridin-2-one from *Piper tuberculatum* Jacq. (Navickiene et al., 2000), besides eight known antifungal amides. In this paper, we describe the isolation, the structure elucidation and the evaluation of the antifungal activity of two new amides *N*-[10-(13,14-methylenedioxyphenyl)-7(*E*),9(*Z*)-pentadienoyl]-pyrrolidine (**1**), arboreumine (**2**) and nine (**3–11**) known antifungal amides *N*-[10-(13,14-methylenedioxyphenyl)-7(*E*)-pentaenoyl]-pyrrolidine (**3**), its derivative *N*-[10-(13,14-methylenedioxyphenyl)-pentanoyl]-pyrrolidine (**5**) and *N*-[10-(13,14-methylenedioxyphenyl)-7(*E*),9(*E*)-pentadienoyl]-pyrrolidine (**4**); besides pellitorine (**6**), $\Delta^{\alpha\beta}$dihydropiperine (**7**), piplartine (**8**), dihydropiplartine (**9**), *cis*-piplartine (or 8(*Z*)-*N*-(12,13, 14-trimethoxycinnamoyl)-Δ^3-pyridin-2-one) (**10**) and fagaramide (**11**). In addition to these amides we isolated two cinnamoyl derivatives, methyl 6,7,8-trimethoxydihydrocinnamate (**12**) and methyl *trans*-6,7,8-trimethoxycinnamate (**13**). The amides isolated from leaves of *Piper arboreum* (**1**–**4**) and the hydrogenated derivative of **3** (**5**) were active against the fungus *Cladosporium sphaerospermum*, and the compounds isolated from seeds and leaves of *Piper tuberculatum* **3, 4** and **6–13**

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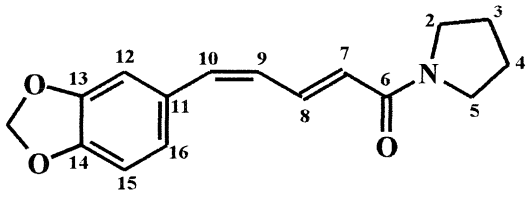
0031-9422/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0031-9422(01)00431-9



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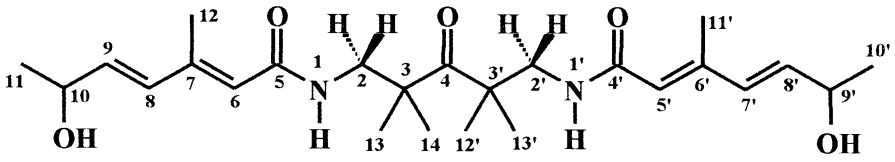

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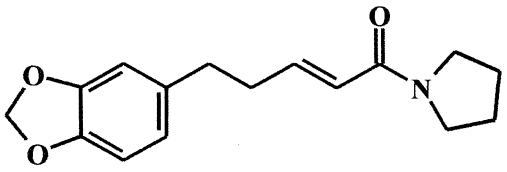

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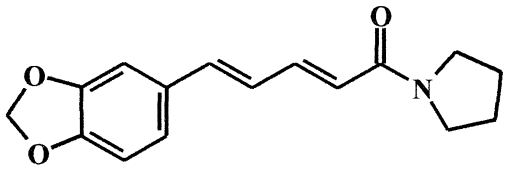

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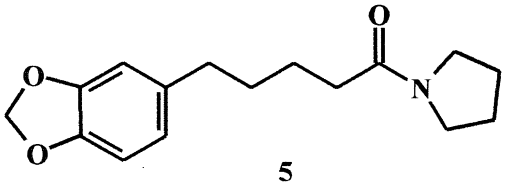

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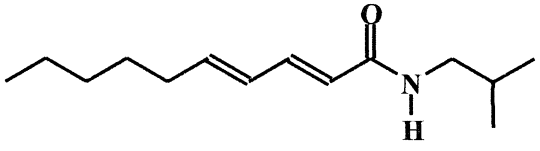

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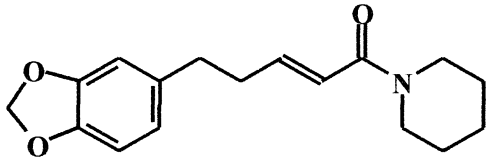

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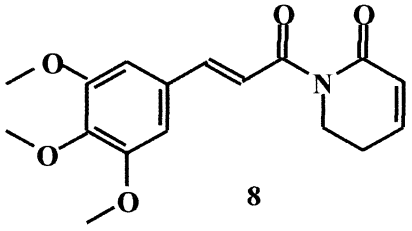

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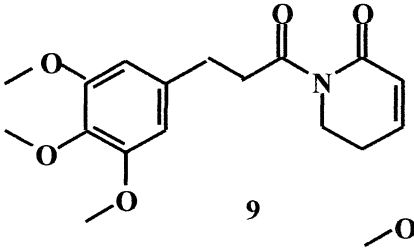

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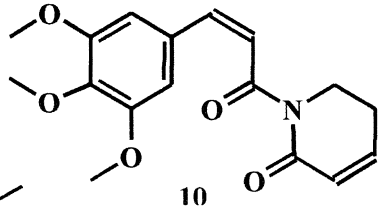

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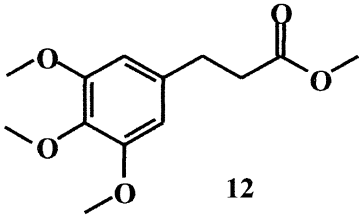

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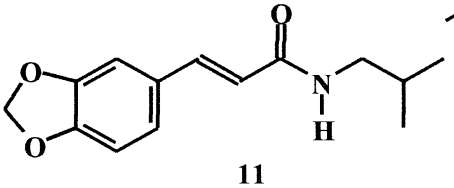

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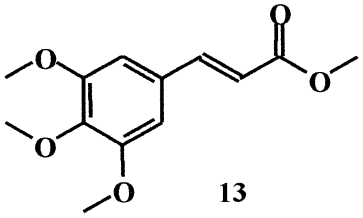

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(Navickiene et al., 2000) were also active against the fungi *Cladosporium sphaerospermum* and *C. cladosporioides*, as evaluated by direct bioautography (Homans and Fuchs, 1970).

2. Results and discussion

N-[10-(13,14-Methylenedioxyphenyl)-7(*E*),9(*Z*)-pentadienoil]-pyrrolidine (**1**) has a molecular formula of $C_{16}H_{17}NO_3$ as determined by analysis of the electrospray mass spectrum (ES–MS), elemental analyses and of the ^{13}C NMR spectroscopic data. The IR spectrum exhibited bands at 1637 (conjugated carbonyl group); 1600, 1492 and 1442 (aromatic); 1246, 1192 and 1036 cm^{-1}. Its ^1H NMR spectrum (Table 1) revealed the presence of a 13,14-methylenedioxyphenyl group by the signal at δ 5.93 (2H, *s*), and four olefinic protons which showed signals at δ 6.29 (1H, *d*, $J_{7,8}=14.3$ Hz), 7.77 (1H, *dd*, $J_{8,7}=14.3$ and $J_{8,9}=10.9$ Hz), 6.20 (1H, *dd*, $J_{9,8}=10.9$ and $J_{9,10}=11.4$ Hz) and 6.59 (1H, *d*, $J_{10,9}=11.4$ Hz) indicative of an α,β,γ,δ-unsaturated carbonyl system. The coupling constant values indicated that the double bonds possess the *E* and *Z* geometry (Shah et al., 1986; Alécio et al., 1998; Navickiene et al., 2000). The assignments of the *E* and *Z* configurations to hydrogens at C-7, C-8 and C-9, C-10, respectively were determined as follows; the doublet of doublets at δ 7.77 (H-8) showed coupling constants of 14.3 and 10.9 Hz typical of *trans-trans* configuration, which could be associated with an α,β unsaturated carbonyl system. The assignment of the *cis* configuration for the γ,δ double bond was based on the coupling constant value 11.4 Hz between the H-9 and H-10 (Cleyn and Verzele, 1975). The presence of a pyrrolidine moiety was determined by the signals at δ 3.52 (2H, *m*) and δ 1.90 (2H, m) attributed to H-2/H-5 and H-3/H-4, respectively. The ^{13}C NMR spectral data (Table 1) of **1** were in full agreement supporting the presence of this moiety. The low-field region of the ^{13}C NMR spectrum exhibited signals assigned to an amide carbonyl at δ 164.8, 10 sp^2 carbons, four sp^3 carbons [δ_C 46.5, 45.8, 26.0, and 24.2], and a typical methylenedioxy carbon at δ 100.7. The assignment of the remaining signals for the aromatic carbons were based on comparison with data described in the literature (Araújo-Junior et al., 1997; Alécio et al., 1998; Navickiene et al., 2000).

Table 1
^1H and ^{13}C NMR spectral data for amide **1** (ppm, $CDCl_3$)

Position	**1**	
	^1H δ [*m*, *J* (Hz), 200 MHz]	^{13}C δ (50 MHz)
2	3.52 (2H, *m*)	45.8
3	1.90 (2H, *m*)	26.0
4	1.90 (2H, *m*)	24.2
5	3.52 (2H, *m*)	46.5
6	–	164.8
7	6.29 (1H, *d*, $J_{7,8}=14.3$)	121.1
8	7.77 (1H, *dd*, $J_{8,7 \text{ and } 8,9}=14.3, 10.9$)	144.4
9	6.20 (1H, *dd*, $J_{9,8 \text{ and } 9,10}=10.9, 11.4$)	121.0
10	6.59 (1H, *d*, $J_{10,9}=11.4$)	134.9
11	–	127.0
12	6.78 (1H, *d*, $J_{12,16}=1.7$)	108.1
13	–	145.6
14	–	147.5
15	6.75 (1H, *d*, $J_{15,16}=8.1$)	108.7
16	6.82 (1H, *dd*, $J_{16,15 \text{ and } 16,12}=8.1, 1.7$)	122.1
OCH_2O	5.93 (2H, *s*)	100.7

Arboreumine (**2**) was shown to have the molecular formula $C_{25}H_{40}N_2O_5$ by analysis of both its ES–MS spectrum and its elemental analyses. Its IR spectrum revealed absorption bands at 3400, 1700, 1659 and 1630 cm^{-1} characteristic of hydroxyl, carbonyl, conjugated amide carbonyl and a conjugated double bond, respectively. Its ^1H NMR spectrum revealed the presence of two doublets at δ 2.15 (2H, $J_{2a,2b\sim2'a,2'b}=17.0$ Hz) and 2.37 (2H, $J_{2b,2a\sim2'b,2'a}=17.0$ Hz), attributed to H-2a, H-2′a and H-2b, H-2′b which are characteristic of methylene groups displaying geminal coupling. The presence of a hydroxyl group was further indicated by a signal assigned to a hydroxymethine hydrogen H-10, H-9′, at δ 4.29 (1H, *dq*, $J_{10,9\sim9',8'}=5.0$ Hz and $J_{10,11\sim9',10'}=6.5$ Hz). Three singlets of methyl protons at δ 1.83, 1.00, 0.95 and one doublet at δ 1.20 ($J_{11,10\sim10',9'}=6.5$ Hz) were assigned to H-12, H-11′; H-13, H-12′; H-14, H-13′ and H-11, H-10′, respectively. The signal at δ 2.80 was attributed to the hydrogen attached to a nitrogen, as confirmed by exchange with D_2O in the 1H NMR experiment. The methine protons H-6, H-5′; H-8, H-7′ and H-9, H-8′ showed signals at δ 5.81 (*q*, $J_{6,12\sim5',11'}=1.0$ Hz), 5.70 (*dd*, $J_{8,9\sim7',8'}=15.5$ Hz and $J_{8,12\sim7',11'}=1,0$ Hz) and 5.76 (*dd*, $J_{9,8\sim8',7'}=15.5$ Hz and $J_{9,10\sim8',9'}=5.0$ Hz), respectively (Table 2). Proton assignments were further confirmed by analysis of the DQCOSY spectrum. The spin system derived from H-8, H-7′; H-9, H-8′ and H-10, H-9′ was readily recognized by starting with the 1H doublet of doublets at δ 5.76 assigned to H-9, H-8′ ($J_{9,8\sim8',7'}=15.5$ Hz and $J_{9,10\sim8',9'}=5.0$ Hz) which showed cross-peaks with 1H doublet of doublets at δ 5.70 assigned to H-8, H-7′ ($J_{8,9\sim7',8'}=15.5$ Hz and $J_{8,12\sim7',11'}=1.0$ Hz) and with a ^1H doublet of quadruplet at δ 4.29 assigned to H-10, H-9′ ($J_{10,9\sim9',8'}=5.0$ Hz and $J_{10,11\sim9',10'}=6.5$ Hz). This last signal also showed a cross peak with the 3H doublet at δ 1.20 assigned to H-11, H-10′ ($J_{11,10\sim10',9'}=6.5$ Hz). The 2H doublet at δ 2.15 assigned to H-2a, H-2′a ($J_{2a,2b\sim2'a,2'b}=17.0$ Hz) showed cross-peak with the 2H doublet at δ 2.37 assigned to H-2b, H-2′b ($J_{2b,2a\sim2'b,2'a}=17.0$ Hz). The ^{13}C NMR spectral data were in agreement with this unusual structure (Table 2). Besides, the signals of some carbons and the positions of attachment of various functionalities were unambiguously assigned following interpretation of the HMBC spectrum. The carbon signals at δ 49.5 and 40.9

Table 2
^1H and ^{13}C NMR spectral data for amide **2** (ppm, $CDCl_3$)

Position	**2**	
	^1H δ [*m*, *J* (Hz), 500 MHz]	^{13}C δ^a (125 MHz)
1, 1′	2.80 (1H, *s*)	–
2a, 2′a	2.15 (1H, *d*, $J_{2a,2b\sim2'a,2'b}=17.0$)	49.5 (*t*)
2b, 2′b	2.37 (1H, *d*, $J_{2b,2a\sim2'b,2'a}=17.0$)	
3, 3′	–	40.9 (*s*)
4	–	198.3 (*s*)
5, 4′	–	163.9 (*s*)
6, 5′	5.81 (1H, *q*, $J_{6,12\sim5',11'}=1.0$)	126.1 (*d*)
7, 6′	–	[b]
8, 7′	5.70 (1H, *dd*, $J_{8,9\sim7',8'\text{ and }8,12\sim7',11'}=15.5, 1.0$)	128.6 (*d*)
9, 8′	5.76 (1H, *dd*, $J_{9,8\sim8',7'\text{ and }9,10\sim8',9'}=15.5, 5.0$)	135.6 (*d*)
10, 9′	4.29 (1H, *dq*, $J_{10,9\sim9',8'\text{ and }10,11\sim9',10'}=5.0, 6.5$)	67.3 (*d*)
11, 10′	1.20 (3H, *d*, $J_{11,10\sim10',9'}=6.5$)	23.4 (*q*)
12, 11′	1.83 (3H, *s*)	19.0 (*q*)
13, 12′	1.00 (3H, *s*)	22.7 (*q*)
14, 13′	0.95 (3H, *s*)	23.8 (*q*)

[a] Multiplicities of carbons (in parentheses) determined by a DEPT experiment.
[b] This signal was not observed in the ^{13}C NMR spectrum.

assigned to C-2, C-2′ and C-3, C-3′, respectively showed a correlation with the 3H singlets at δ 1.00, 0.95 (assigned to the two methyl groups attached to the carbon (C-3 and C-3′), whereas the cross-peak between the carbon signal at δ 198.3 and the 3H singlet at δ 0.95 (C-14 and C-13′) indicated the quaternary carbon C-3, C-3′ was adjacent to the carbonyl group C-4. At the same time, the carbonyl carbon at δ 198.3 (C-4) displayed a correlation with H-2a, H-2′a and H-2b, H-2′b (δ 2.15 and 2.37, respectively). These last protons also showed a cross-peak with the quaternary carbon C-3, C-3′ (δ 40.9). The cross-peaks between H-13, H-12′ and C-14, C-13′ and H-14, H-13′ and C-13, C-12′ were also observed. The carbon signals at δ 163.9 (C-5, C-4′), 126.1 (C-6, C-5′) and 40.9 (C-3, C-3′) showed a correlation with the 1H singlet at δ 2.80 (assigned to the hydrogen attached to the nitrogen atom). Irradiation of the proton resonance at δ 2.80 (N-H) in the 2D NOESY experiment caused an enhancement (9%) of the signal at δ 2.15 (H-2a, H-2′a), corroborating the adjacent relationship between the amide group and C-2, C-2′. The carbon signals at δ 67.3 (C-10, C-9′) and 135.6 (C-9, C-8′) showed cross-peak with the 3H doublet at δ 1.20 indicating the presence of the methyl group C-11, C-10′ adjacent to the hydroxymethine carbon. The $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl carbon system was unambiguously assigned by the correlation between the proton signal at δ 1.83 assigned to the methyl group at the position 12, 11′ with the carbon signals δ 126.1 (C-6, C-5′) and 128.6 (C-8, C-7′), whereas the quadruplet at δ 5.81 assigned to H-6, H-5′ ($J_{6,12\sim5',11'}=1,0$ Hz) showed a cross peak with the carbon signals at δ 163.9 (amide carbonyl carbon) and 128.6 (C-8, C-7′). Finally, HETCOR analyses were used to assign the signals for all proton-bearing carbons and the structure of amide **2** was determined as depicted.

The CH_2Cl_2:MeOH (2:1) extracts from the seeds and leaves of *P. tuberculatum* were fractionated by column chromatography on silica gel eluted with C_6H_{14} containing increasing amounts of EtOAc (up to 100%), followed by preparative thin-layer chromatography (TLC) to afford compounds **6**–**13**. Pellitorine (**6**) was obtained from fraction 13 from extract seeds, without any further purification, and it was identified by direct comparison of its spectroscopic data with the data previously described by Rosario et al., 1996. $\Delta^{\alpha,\beta}$dihydro piperine (**7**) and dihydropiplartine (**9**) had their ^1H NMR spectra published for the same compounds previously isolated from *Piper rugosum* (Maxwell and Rampersad, 1991) and *Piper guineense* (Parmar et al., 1997) and their ^{13}C NMR spectral data recently published (Navickiene et al., 2000). Piplartine (**8**) was isolated from seeds and leaves, and the ^1H and ^{13}C NMR data were similar those published (Duh et al., 1990; Filho et al., 1981). *Cis*-piplartine (**10**) had its ^1H and ^{13}C NMR spectral data recently published (Navickiene et al., 2000). Fagaramide (**11**) was isolated from leaf extract and its spectroscopic data were compared with those published (Isao, 1984). Methyl *trans*-6,7,8-trimethoxycinnamate (**13**) was previously described as a synthetic product (Settimj et al., 1976) and its spectroscopic data were similar to that obtained in this work.

The antifungal activity of compounds **1–13** was determined by means of direct bioautography on TLC plate (Homans and Fuchs, 1970). The detection limits of compounds **1–13** (Tables 3 and 4) were obtained according to methodology described (Rahalison et al., 1994). The compounds having no inhibition for *C. sphaerospermum* and *C. cladosporioides* growth at amounts higher than 10.0 μg were too weak to be considered.

The minimum amount of compounds **3**–**4**; **2** and **5**; and **1** required to inhibit growth of the fungus *C. sphaerospermum* on the TLC plates was determined as 0.1 μg, 5.0 μg and 10.0 μg, respectively. Compounds **3** and **4** showed strong antifungal activity, higher than the standards miconazole (0.5 μg) and nystatin (0.5 μg). These two compounds also showed high activity against *C. cladosporioides*, the amide **4** being 50 times more active when compared with the reference compounds. In the case of compounds **6**–**10**, **13** and **11**, **12** the minimum amount to inhibit the growth of the fungus *C. cladosporioides* on the TLC plates was determined as 5.0 and 10.0 μg, respectively. The higher limit detection of 5 μg values found for **6**–**10** and **13** indicated a moderate activity of these amides when compared to that observed with the standards miconazole and nystatin. The inhibition of fungal growth displayed for these compounds suggests that the pyrrolidine amides isolated from *P. arboreum* are substantially stronger antifungal agents than the

Table 3
Antifungal activity of amides **1–5** against *Cladosporium sphaerospermum*

Compound	Antifungal activity [a] (μg)
1	10.0
2	5.0
3	0.1
4	0.1
5	5.0

Positive controls: nystatin (0.5 μg).
[a] Minimum amount required for the inhibition of fungal growth on thin-layer chromatography (TLC) plates.

Table 4
Antifungal activity of compounds **6–13** against *Cladosporium cladosporioides*

Compound	Antifungal activity [a] (μg)
3	0.1
4	0.1
6	5.0
7	5.0
8	5.0
9	5.0
10	5.0
11	10.0
12	10.0
13	5.0

Positive controls: nystatin (0.5 μg) and miconazole (0.5 μg).
[a] Minimum amount required for the inhibition of fungal growth on (TLC) thin-layer chromatography plates.

piperidine, dihydropyridone and isobutyl amides from *P. tuberculatum*. It is important to note that the inhibition of *C. sphaerospermum* by dihydropyridone amide **9** appears to be higher than the standard nystatin [(0.1 μg) compared with nystatin (0.5 μg)] (Navickiene et al., 2000) while the inhibition of *C. cladosporioides* was lower than the standards (Table 4).

3. Experimental

3.1. Instrumentation and chromatography materials

Silica gel (Merck 230–400 mesh) was used for all column chromatography unless otherwise stated and solvents were redistilled prior to use. ^{1}H NMR spectra were recorded at 200 and 500 MHz and ^{13}C NMR at 50 and 125 MHz, using $CDCl_3$ as a solvent and TMS as reference. IR spectra were obtained on a Nicolet spectrometer. ES–MS were recorded on a VG Platform II spectrometer. HPLC separations were performed on a Varian PrepStar LC/UV/VIS model SD-1, using a reversed phase column (Supelcosil C_{18}; 21.2×250 mm) eluted with MeOH:H_2O (4:1), flow rate of 10.0 ml/min and detection at 254 nm. Elemental analyses were performed on a 2400 CHN Perkin-Elmer Analyser.

3.2. Plant material

Piper arboreum H. B. K. leaves were collected in Araraquara—SP, Brazil and identified by Dr. Guillermo E.D. Paredes (Universidad Nacional Pedro Ruiz Gallo, Peru). The voucher specimens (Cordeiro-1936) are deposited at Herbarium of Instituto de Biociências—USP, São Paulo—SP, Brazil.

Piper tuberculatum Jacq. seeds, leaves and stems were collected in the Campus do INPA—Manaus, Brazil, and identified by Dr. Guillermo E.D. Paredes (Universidad Nacional Pedro Ruiz Gallo, Peru). The voucher specimens (Kato-163) are deposited at Herbarium of Instituto de Biociências—USP, São Paulo—SP, Brazil.

3.3. Antifungal assays

The microorganisms used in the antifungal assays *C. sphaerospermum* (Penzig) SPC 491 and *C. cladosporioides* (Fresen) de Vries SPC 140 have been maintained at the Instituto de Botânica, São Paulo, SP, Brazil. For the antifungal assay—10.0 μl of solutions corresponding to 100.0 μg of crude extract and 10.0, 5.0, 1.0, 0.5, 0.1 μg of pure compound were applied to pre-coated TLC plates. TLC plates were developed with C_6H_{14}:EtOAc (7:3) for compounds **1–5** and with CH_2Cl_2:MeOH (97:3) for compounds **6–13**, after that the plates were dried for complete removal of solvents. The chromatograms were sprayed with a spore suspension of *C. sphaerospermum* or *C. cladosporioides* in glucose and salt solution (Rahalison et al., 1994) and incubated for 72 h in darkness in a moistened chamber at 25 °C. Clear inhibition zone appeared against a dark background indicating the minimal amount of **1–5** and **6–13** required for it (Tables 3 and 4). Nystatin and miconazole were used as positive controls whereas ampicillin and chloramphenicol were used as negative controls.

3.4. Extraction and isolation of constituents

A CH_2Cl_2 soluble part of the MeOH:H_2O (4:1) extract of leaves of *P. arboreum* was subjected to silica gel column chromatography (CC). The fractions obtained were further separated by silica gel (CC) to give the amides **1–4**.

The dried and powdered leaves of *P. arboreum* (2000.0 g) were extracted three times with 2000 ml of EtOH, during 2 days, at room temperature. The resulting EtOH extract was filtered and conc. in vacuo to afford 245.8 g of a green gum, which was solubilized in MeOH:H_2O (4:1), and extracted with C_6H_{14}, CH_2Cl_2 and EtOAc respectively. The resulting CH_2Cl_2 soluble

part (54.8 g) were applied to a silica gel column (300.0 g), and eluted with $CHCl_3$ containing increasing amounts of MeOH (up to 100%) to give 30 fractions. Fraction 19 (1.7 g) was applied to a C_{18} HPLC column (2.0 g) eluted with MeOH:H_2O (4:1). After that the fractions were purified by prep. TLC [CH_2Cl_2: $(CH_3)_2CO$ (92:02) and HOAc (1%), three elutions] yielding **1** (28.0 mg) and **3** (31.0 mg). Fraction 21 (0.5 g) was applied to a C_{18} column (2.0 g) eluted with MeOH:H_2O (4:1). The resulting fractions were purified by prep. TLC eluted with CH_2Cl_2:$(CH_3)_2CO$:MeOH (92:0.5:0.5) and HOAc (1%) to give **2** (8.0 mg). Fraction 23 (0.5 g) was applied to a C_{18} column (2.0 g) and eluted with MeOH:H_2O (4:1). The resulting fractions were purified by prep. TLC [CH_2Cl_2:C_6H_{14}: $(CH_3)_2CO$ (6:3:1) and HOAc (1%), three elutions] yielding **3** (83.0 mg) and **4** (30.0 mg).

The dried and powdered seeds and leaves of *P. tuberculatum* (24.3 g) were extracted 2× with 600 ml of CH_2Cl_2:MeOH (2:1), during 2 days (each extraction), at room temperature. The resulting extract of seeds was filtered and conc. in vacuo to afford 2.9 g of a green gummy residue. Part of this extract (2.0 g) was subjected to silica gel chromatography, eluted with C_6H_{14}:EtOAc (4:1) in gradient of polarity to give 36 fractions (15 ml). Fraction 21 (77.0 mg) was submitted to a prep. TLC [C_6H_{14}:EtOAc (3:2), three elutions] to yield **8** (33.0 mg) and **10** (25.0 mg). Fraction 19 (83.0 mg), submitted to prep. TLC [C_6H_{14}:EtOAc (75:25), three elutions], yielded **9** (7.0 mg). Fractions 8 (0.2 g) and 13 (39.0 mg) yielded compounds **6** (0.2 g) and **7** (18.0 mg), respectively, without any further purification. The leaf extract (56.9 g), submitted to CC (2000.0 g) [C_6H_{14}:EtOAc (4:1) in gradient of polarity] yielded 70 fractions. Fractions 26–62 (32.0 g) were pooled and submitted to CC (1000.0 g), with the same solvent system as described earlier. Fraction 66 (0.9 g) yielded the amide **8** (0.9 g), without further purification. Amide **11** (7.0 mg) was isolated from fraction 55 (0.2 g), which was further purified by reversed phase HPLC [MeOH:H_2O(4:1)]. Fraction 28 (0.4 g) was submitted to prep. TLC [C_6H_{14}:EtOAc (3:2), two elutions] yielding the cinnamoyl derivative **12** (66.0 mg) and fraction 67–68 (0.4 g) was purified by two prep. TLC [C_6H_{14}:EtOAc (4:1), two elutions and C_6H_{14}: EtOAc (7:3), one elution], respectively, to yield the cinnamoyl derivative **13** (13.0 mg).

3.5. *N-[10-(13,14-Methylenedioxyphenyl)-7(E),9(Z)-pentadienoil]-pyrrolidine (**1**)*

Found: C 70.7; H, 6.3; N, 5.1; O, 17.9%. $C_{16}H_{17}NO_3$ requires C, 70.8; H, 6.3; N, 5.2; O, 17.7%.

Amorphous solid. UV λ_{max} ^{MeOH} nm: 280 (2890); IR ν_{max} (KBr): 1637, 1600, 1492, 1442 and 1216 cm^{-1}; ES–MS *m*/*z* (rel. int.): 294 (48) [M + Na], 272 (100) [M + 1], 201 (50) and 135 (25); ^1H and ^{13}C NMR spectra (Table 1).

3.6. *Arboreumine (**2**)*

Found: C, 66.3; H, 9.1; N, 6.3; O, 17.7%. $C_{25}H_{40}N_2O_5$ requires C, 66.9; H, 8.9; N, 6.2; O, 17.8%.

Amorphous solid; UV λ_{max} ^{MeOH} nm: 240 (2607); IR ν_{max} (KBr): 3400, 1700, 1659, 1630 cm^{-1}; ^1H and ^{13}C NMR spectra (Table 2).

4. Unicited references

Bernard et al., 1995; Das et al., 1996

Acknowledgements

This work was funded by grants provided by FAPESP and CNPq/PADCT. This work was also supported by the State of São Paulo Research Foundation (FAPESP) within the BIOTA/FAPESP—The Biodiversity Virtual Institute Program (www.biotasp.org.br). M.F., M.J.K., M.C.M.Y. and V.S.B. are grateful to CNPq for research fellowships. RVS and HMDN thank CNPq and FAPESP for providing scholarships, respectively.

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