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Article

Alkamides and Phenethyl Derivatives from *Aristolochia gehrtii*

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Foram isolados de galhos de *Aristolochia gehrtii* a *cis*-*N*-feruloil-3-*O*-metildopamina, um derivado hemiacetálico do 5-hidroximetilfurfural, e vinte e dois compostos conhecidos. Entre estes compostos estão cinco lignanas, três terpenos, seis alcalóides dos quais cinco são alcamidas, dois derivados do ácido benzóico e seis derivados constituídos pelo grupo feniletil. As estruturas dos compostos isolados foram determinadas por métodos espectroscópicos e comparação de dados físicos e espectroscópicos dos compostos com aqueles disponíveis na literatura.

From the stems of *Aristolochia gehrtii*, the *cis*-*N*-feruloyl-3-*O*-methyldopamine and a hemiacetal derivative of 5-hydroxymethylfurfural were isolated, together with twenty-two known compounds. These compounds include five lignans, three terpenes, six alkaloids (five alkamides), two benzoic acid derivatives, and six phenethyl derivatives. The structures of the isolated compounds were determined by means of spectroscopic methods and comparison with literature data.

Keywords: *Aristolochia gehrtii*, Aristolochiaceae, *cis*-*N*-feruloyl-3-*O*-methyldopamine, phenylethyl derivatives

Introduction

As part of our continuing studies[1,2] on Brazilian *Aristolochia* species, the constituents of the stems of *Aristolochia gehrtii* Hoehne were examined. This study led to the isolation of (-)-eudesmin (**1**), (+)-methylpiperitol (**2**), (-)-hinokinin (**4**), cubebin (**5**), and sitosterol (**6**), whose occurrence is common in the Aristolochiaceae[3-6]. In addition, β-sitosteryl-D-glucoside (**7**), isovanillic acid (**8**), *p*-hydroxybenzoic acid (**9**) and piperitol (**3**) were isolated, together with thalipholine (**10**), 5-hydroxymethylfurfural (**11**), tyrosol (**12**), icariside D2 (**13**), salidroside (**14**), 3,4-dihydroxyphenethyl alcohol (**15**), thalictoside (**17**) and its corresponding aglycone (**16**). Although aliphatic nitro compounds are unusual in nature[7], the occurrence of nitrophenanthrene alkaloids is widespread in the Aristolochiaceae[8,9]. In addition, loliolide (**18**), *trans*- and *cis*-*N*-feruloyltyramine (**19**, **22**), *trans*-and *cis*-*N*-*p*-coumaroyltyramine (**20**, **23**) and *trans*-*N*-feruloyl-3-*O*-methyldopamine (**21**) were isolated. Structural elucidation of the new alkaloid *cis*-*N*-feruloyl-3-*O*-methyldopamine (**24**), isolated for the first time from a natural source, is discussed.

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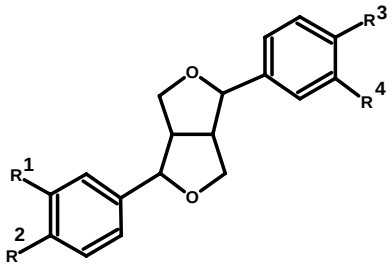
Experimental

General

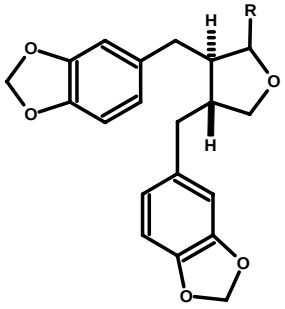
The NMR spectra were measured on a Bruker and a Varian spectrometers. ^{1}H NMR and ^{1}H-^{1}H COSY spectra were obtained at 200 and 500 MHz, ^{13}C NMR and DEPT were taken at 50 MHz. The mass spectra were obtained on an HP5970 spectrometer and on a Fisons Platform II by flow injection into the electrospray source. The instrument was operated in the positive ion mode. The IR spectra were obtained on a Nicolet-730 FT-IR spectrometer using KBr discs. UV absorption was measured in a Hewlett Packard 8452 A diode array spectrophotometer. TLC: Silica gel 60 PF_{254}. The purity of the solvents was checked using a Fisons 8060 gas chromatographer coupled to a Fisons VG Platform - column: Supelco, SPB-5 0.25 mm i.d. x 30 m, 0.25mm thickness; oven 100° → 20° min^{-1} → 250°; carrier gas: He (1.38 mL min^{-1}).

Plant material

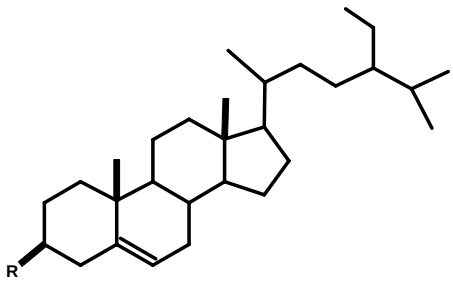
The plant material was collected in the Botanical Garden of São Paulo, São Paulo, SP, Brazil, and identified as *Aristolochia gehrtii* Hoehne by Dr. Condorcet Aranha. A voucher specimen was deposited at the herbarium of the Instituto Agronômico de Campinas, Campinas, SP, Brazil. The material was separated by plant parts, dried (~ 45º) and ground.



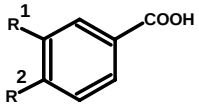
	R^1	R^2	R^3	R^4
1	OMe	OMe	OMe	OMe
2	-OCH_2O-		OMe	OMe
3	-OCH_2O-		OH	OMe



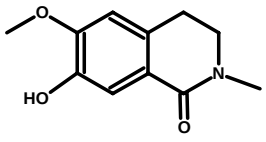
	R
4	=O
5	OH



	R
6	OH
7	OGlc



	R^1	R^2
8	OH	OMe
9	H	OH



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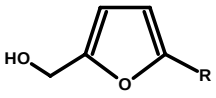
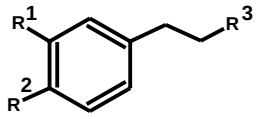
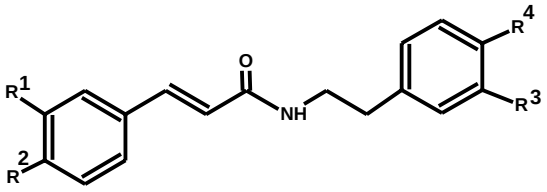


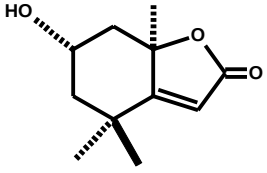
	R
11	CHO
11a	CH(OH)(OMe)



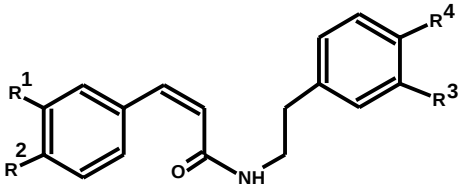
	R^1	R^2	R^3
12	H	OH	OH
13	H	OGlc	OH
14	H	OH	OGlc
15	OH	OH	OH
16	H	OH	NO_2
17	H	OGlc	NO_2



	R^1	R^2	R^3	R^4
19	OMe	OH	H	OH
20	H	OH	H	OH
21	OMe	OH	OMe	OH



18



	R^1	R^2	R^3	R^4
22	OMe	OH	H	OH
23	H	OH	H	OH
24	OMe	OH	OMe	OH

Isolation

Ground stems (850 g) were extracted exhaustively at room temperature with hexane, Me_2CO and EtOH successively and then individually concentrated. The crude acetone extract (4.0 g) was fractionated by CC (silica gel, 80.0 g, hexane-EtOAc gradient) leading to 51 fractions (15 mL). Frs. 16, 17, 18, 25, 30, 35, 36, 42, and 43 yielded **6** (16.2 mg), **5** (22.6 mg), **2** (86.0 mg), **1** (7.4 mg), **18** (9.0 mg), **9** (8.0 mg), **8** (7.7 mg), **7** (67.7 mg) and **15** (12.2 mg), respectively. After prep. TLC [PhMe-MeOH-HOAc (95:5:1)], fr. 12 yielded **4** (3.7 mg). Frs. 15, 21, and 27 by prep. TLC [PhMe-MeOH (95:5)] yielded **16** (11,5 mg), **3** (3.0 mg) and **12** (4.0 mg), respectively. Fr. 38 by TLC [$CHCl_3$-MeOH-NH_4OH (95:5:1)] afforded **19** (18.2 mg), **20** (9.4 mg) and **21** (10.0 mg). Frs. 39 and 40 by prep. TLC [$CHCl_3$-MeOH-NH_4OH (95:5:1)] yielded mixtures (1:5) of **19**+**22** (24.2 mg) and **20**+**23** (30.8 mg), respectively. After TLC [$CHCl_3$-MeOH (93:7)], fr. 41 afforded a mixture (1:4) of **21**+**24** (23.8 mg). Fr. 45 after prep. TLC [$CHCl_3$-MeOH-NH_4OH (80:20:1)] yielded **13** (13.3 mg), **14** (11.1 mg) and **17** (12.0 mg).

The ethanol extract of the stems (18.7 g) was fractionated

by partition ($CHCl_3$-H_2O). The organic fraction (855.0 mg) was concentrated and dissolved in hot MeOH. After cooling the resulting precipitate was separated and submitted to prep. TLC [PhMe-MeOH (9:1)] leading to the isolation of **11** (8.5 mg) and **11a** (3.0 mg). The aqueous fraction was extracted with EtOAc (8.5 g). A portion of this fraction (130.7 mg) was submitted to prep. TLC [$CHCl_3$-MeOH-NH_4OH (90:10:0.5)] affording 10 (12.5 mg).

β-sitosteryl-D-glucoside (7)

Colorless solid, mp. 292-297 ºC Me_2CO, decomp., lit. 298 ºC (EtOH)[10]. $[\alpha]_D$ –42.0º (pyridine, *c* 2.0), lit. $[\alpha]_D$ –40.1º (pyridine, *c* 1.3)[10]. Positive ES-MS *m/z* (rel. int.): 599 $[M+Na]^+$ (100), 577 $[M+H]^+$ (40), 161 (35).

IR ν_{max}/cm: 3423, 1595, 1485 (KBr). ^1H NMR (500 MHz, C_5D_5N) δ 5.40 (1H, m, H-6), 5.08 (1H, d, *J* 9.0 Hz, H-1'), 4.59 (1H, dd, *J* 12.0, 2.5 Hz, H-6'), 4.44 (1H, dd, *J* 12.0, 5.3 Hz, H-6'), 4.33 (1H, t, *J* 9.0 Hz, H-3'), 4.30 (1H, t, *J* 9.0 Hz, H-4'), 4.10 (1H, t, *J* 9.0 Hz, H-2'), 4.04-3.80 (2H, m, H-3, H-5'), 2.76 (1H, ddd, *J* 13.0, 5.5, 1.5 Hz, H-4), 2.53 (1H, br dd, *J* 13.0, 7.0 Hz, H-4), 2.18 (1H, br d, *J* 13.0 Hz, H-1), 2.03 (1H, m, H-12), 1.95 (1H, m, H-7), 1.89 (1H, m, H-16), 1.78 (2H, m, H-2, H-20), 1.72 (1H, m, H-25), 1.60 (1H, m, H-16), 1.48-1.24 (8H, m), 1.15 (2H, m, H-12, H-24), 1.04 (1H, m, H-1), 1.03 (1H, d, *J* 6.5 Hz, H-21), 0.98 (3H, s, H-19), 0.94 (1H, m, H-14), 0.94 (3H, t, *J* 7.5 Hz, H-29), 0.92 (3H, d, *J* 7.5 Hz, H-27), 0.91 (3H, t, *J* 7.0 Hz, H-26), 0.71 (3H, s, H-18). ^{13}C NMR (125 MHz, C_5D_5N) δ(C-1 to C-29, C-1' to C-6'): 37.2, 31.8*, 77.9**, 39.7, 140.7, 121.7, 31.9*, 50.1, 29.9, 36.6, 21.0, 39.0, 42.7, 56.5, 24.2, 28.2, 56.0, 12.2, 18.9, 36.1, 18.7, 33.9, 26.1, 45.8, 29.2, 19.1, 19.7, 23.1, 12.4, 102.3, 75.0, 78.3, 71.4, 78.1**, 62.5 (Values bearing the same sign may be reversed).

Thalipholine (**10**)

Yellow solid, mp. 210-211 ºC ($CHCl_3$), lit. 210-211ºC ($CHCl_3$)[11]. IR, UV and ^1H NMR: comparable with lit. values[11]. Positive ES-MS *m/z* (rel. int.): 208 $[M+H]^+$ (100), 151 (36). ^{13}C NMR (50 MHz, $CDCl_3$) δ 190.9 (C-1), 48.4 (C-3), 27.6 (C-4), 108.8 (C-5), 144.6 (C-6), 164.9 (C-7), 114.2 (C-8), 122.5 (C-9), 132.3 (C-10), 35.2 (NCH_3), 56.0 (OCH_3).

Hemiacetal (**11a**)

Colorless solid, mp. 35-37 ºC (MeOH). Positive ES-MS *m/z* (rel. int.): 159 $[M+H]^+$ (100), 157 $[M-1]^+$ (91), 143 (24). IR ν_{max}/cm: 3470, 1522, 1384, 1025 (KBr).

cis-N-*Coumaroyltyramine* (**23**)

Yellow solid, mp 248-251 ºC (MeOH). IR ν_{max}/cm: 3431, 1718, 1601, 1383 (KBr).

Positive ES-MS *m/z* (rel. int.): 284 $[M+H]^+$ (69), 306 $[M+Na]^+$ (100), 322 $[M+K]^+$ (76).

^1H NMR (200 MHz, CD_3OD) δ 2.65-2.78 (2H, m, H-7'), 3.40-3.49 (2H, m, H-8'), 5.58 (1H, m, NH), 5.79 (1H, d, *J* 12.6 Hz, H-8), 6.60 (1H, d, *J* 12.6 Hz, H-7), 6.68 (2H, d, *J* 8.5 Hz, H-3, H-5), 6.70 (2H, d, *J* 8.6 Hz, H-3', H-5'), 7.00 (2H, d, *J* 8.6 Hz, H-2', H-6'), 7.35 (2H, d, *J* 8.5 Hz, H-2, H-6). ^{13}C NMR (50 MHz, CD_3OD) δ 138.0 (C-7), 132.2 (C-2,6), 130.5 (C-2',6'), 121.6 (C-8), 116.7 (C-3,5), 116.0 (C-3', 5'), 42.2 (C-8'), 35.4 (C-7').

3.3.5. cis-N-*Feruloyl-3*-O-*methyldopamine (**24**)*

Yellow solid, mp 248-251 ºC (MeOH). IR ν_{max}/cm: 3384, 1718, 1598, 1515, 1271 (KBr). Positive ES-MS *m/z* (rel. int.): 344 $[M+H]^+$ (57), 366 $[M+Na]^+$ (62), 382 $[M+K]^+$ (45), 177 (100), 145 (62), 149 (43), 117 (74), 91 (66), 89 (96). ^1H NMR (200 MHz, CD_3OD) δ 2.62-2.67 (2H, m, H-7'), 3.39-3.48 (2H, m, H-8'), 3.79 (3H, s, OMe-3'), 3.82 (3H, s, OMe-3), 5.58 (1H, m, NH), 5.80 (1H, d, *J* 12.6 Hz, H-8), 6.58 (1H, d, *J* 12.6 Hz, H-7), 6.62 (1H, d, *J* 8.3 Hz, H-5'), 6.65 (1H, d, *J* 1.6 Hz, H-2'), 6.78 (1H, dd, *J* 8.3, 1.6 Hz, H-6'), 6.73 (1H, d, *J* 8.2 Hz, H-5), 6.85 (1H, dd, *J* 2.0, 8.2 Hz, H-6), 7.45 (1H, d, *J* 2.0 Hz, H-2).

Results and Discussion

Extracts from the stems of *A. gehrtii* were fractionated by chromatographic column, followed by preparative TLC, to afford the known lignans **1**-**5**[4,12-18], sitosterol (**6**) [19], benzoic acids **8**, **9**[20,21], isoquinolone alkaloid **10**[11,22,23], phenethyl derivatives **12**-**17**[7,24-27], and the terpene **18**[28], which were identified by comparison of their physical and spectroscopic (IR, UV, MS, ^1H and ^{13}C NMR) data with those reported in the literature. The absolute configurations of lignans **1** and **2** were established by comparison of their $[\alpha]_D$ values [*1*: -66.0° ($CHCl_3$, *c* 1.4), *2*: +74.8° ($CHCl_3$, *c* 2.5)] with those published in the literature [*1*: -64.2° ($CHCl_3$, *c* 1.1), *2*: +76.2° ($CHCl_3$, *c* 2.0)][15,16]. Compound **7** was identified as β-sitosteryl-D-glucoside by comparison of its ^1H and ^{13}C NMR data, as well as by comparison of its melting point[10,29]. From gCOSY, gTOCSY, gHMQC, and gHMBC experiments, it was possible to assign more feasible δ values for carbons and hydrogens, including C-3, C-1' to C-6' and H-3, H-1' to H-6', than those previously described in the literature[29]. The structure was further confirmed by acid hydrolysis, as reported by Ahmed *et al*[30], affording sitosterol and glucose.

Compound **11** was identified by comparison of its physical and spectroscopic data with those of 5-hydroxymethylfurfural, previously reported [21,31]. It was

also obtained as a hemiacetal derivative **11a**. The ES-MS of **11a** showed $[M+H]^+$ at *m/z* 159 (100%), corresponding to the addition product. The IR spectrum of **11a** did not display any carbonyl absorption. The formation of the hemiacetal could be explained by the addition of methanol to **11**, since methanol was used as a solvent to solubilize and purify **11**.

Besides three *trans*-cinnamoylamides **19**-**21**, three pairs of *cis*- and *trans*-isomers of cinnamoylamides (**19**+**22**, **20**+**23**, **21**+**24**) were isolated. Isomers **19**-**22** were identified by comparison of their spectroscopic data with those previously reported[32-35]. Best separation of the *cis* isomer **23** from the mixture (**20**+**23**) was achieved in a 5/1 *cis*/*trans* proportion, whereas isomer **24** from the mixture (**21**+**24**) was obtained in a 4/1 proportion. The structures of **23** and **24** were suggested by their electrospray mass spectra. The ES-MS of **23** displayed an $[M+Na]^+$ at *m/z* 306 (100%), $[M]^+$ at *m/z* 284 (corresponding to $C_{17}H_{17}NO_3$, 30 m less than **22**), and the ES-MS of **24** displayed an $[M]^+$ at *m/z* 344 (corresponding to $C_{19}H_{21}NO_5$, 30 μ more than **22**), with a base peak at *m/z* 177 (corresponding to a feruloyl moiety). The ^1H NMR spectra of both alkaloids showed signals of two *cis* olefinic hydrogens at ~ δ5.8 (1H, d, *J* 12.6 Hz) and 6.6 (1H, d, *J* 12.6 Hz) assigned to H-8 and H-7, respectively. In addition, two multiplets at ~ δ 2.6 and 3.4 for four methylene hydrogens were observed, which allowed us to establish its *cis* configuration. Comparison of their IR ($\nu_{C=O}$: 1718 cm^{-1}) and UV (λ_{max} 270 nm) spectra with structurally similar alkaloids, such as **22,** confirmed the *cis* configuration at C-7, 8. The main spectroscopic differences observed between the MS, ^1H and ^{13}C NMR data of **22** and **24** were due to the methoxyl group substituent at C-3. The substitution pattern of the aromatic rings was corroborated by ^1H-^1H COSY and NOE difference experiments, since they showed the correlations between the methoxyl hydrogens at δ 3.79 and δ3.82 with the aromatic hydrogens at δ 6.65 and δ 7.45, respectively. The correlations observed between H-7 and H-8 and NH (δ 5.52-5.60) confirmed the *cis* configuration established for **24**. Compound **23** had already been obtained from cell cultures of *Solarium khasianum*, and its TMSi derivative was identified by GC-MS[36].

To our knowledge, no phytochemical investigation has been carried out on this ornamental species. The occurrence of phenethyl derivatives (C_6-C_2) (**12**-**17**) is significant in this species. The co-occurrence of biosynthetic derivatives (**10**, **19**-**24**), which could be formed in this species by at least one unit C_6-C_2, is remarkable.

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