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## ARISTOLACTAMS AND FURTHER CONSTITUENTS FROM *ARISTOLOCHIA CHAMISSONIS*

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**ABSTRACT:** From the ethanol extract of the stems of *Aristolochia chamissonis*, spathulenol, sitosterol, b-sitosteryl-D-glucoside, kolavelool, 13-*epi*-2-oxo-kolavelool, *trans-N-p*-coumaroyltyramine, allantoin, aristolochic acid I, and aristolactam AII were isolated. The structures of aristolactam AII and piperolactam A have been revised by means of spectroscopic methods and chemical derivatizations.

**KEYWORDS:** Aristolochiaceae, *Aristolochia chamissonis*, diterpenes, *trans-N-p*-coumaroyltyramine, allantoin, aristolactam AII, piperolactam A

### Introduction

Aristolochiaceae family is a rich source of aristolochic acids and aristolactams. Aristolochic acids have been found only among the Aristolochiaceae, as well as in butterflies (Lepdoptheras belonging to the Troidine) that feed on such plants, whereas the aristolactams also occur in other botanical families<sup>1</sup>. In a previously paper we had reported the isolation of rearranged (4 $\oplus$  2)-*abeo*-clerodane and clerodane diterpenes from the hexane extract of stems of *Aristolochia chamissonis*<sup>3</sup>. In this paper we describe the isolation from the ethanol extract of spathulenol, sitosterol, kolavelool, 13-*epi*-2-oxo-kolavelool, previously isolated from *Aristolochia chamissonis*<sup>3</sup>. In addition, b-sitosteryl-D-glucoside, *trans-N-p*-coumaroyltyramine, allantoin, aristolochic acid I, and aristolactam AII were obtained. The structures of aristolactam AII and piperolactam A have been revised on the basis of the spectral data relating to aristolactam AII itself and its acetyl derivatives.

## Experimental

### General

The NMR spectra were measured on a Bruker spectrometer,  $^1\text{H}$  NMR and  $^1\text{H}$ - $^1\text{H}$  COSY spectra were obtained at 200 MHz,  $^{13}\text{C}$  NMR and DEPT were taken at 50 MHz, and  $^1\text{H}$ - $^{13}\text{C}$  COSY were optimized for  $J = 7$  Hz and 145 Hz. 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<sub>254</sub>.

### Plant material

The botanical material was identified and collected as previously described<sup>3</sup>.

### Isolation

Ground stems (850 g) were exhaustively extracted at room temperature with hexane,  $\text{Me}_2\text{CO}$  and EtOH successively, and then individually concentrated<sup>3</sup>.

The crude ethanol extract (12.2 g) of stems was washed with  $\text{CHCl}_3$  and then with MeOH. The insoluble residues from both solvents were separated, combined, and submitted to crystallization from MeOH yielding allantoin (1.0 g). The  $\text{CHCl}_3$  solution was concentrated (2.5 g) and fractionated by chromatographic column (silica gel, 50 g,  $\text{CHCl}_3$ : MeOH, gradient) leading to 30 fractions. Fractions 7, 10, 11, 12, 17, 19, 21, and 23 yielded (-)-13-epi-2-oxo-kolavelool, (42.8 mg), (-)-kolavelool (350.0 mg), spathulenol (4.8 mg), sitosterol (10.0 mg), aristolactam AII (12.0 mg), *trans*-*N*-*p*-coumaroyltyramine (17.1 mg), *b*-sitosteryl-*D*-glucoside (6.6 mg), and aristolochic acid I (14.5 mg), respectively. Aristolactam AII (8.2 mg) was submitted to acetylation ( $\text{Ac}_2\text{O}$ , pyridine, standard conditions). After preparative TLC ( $\text{CHCl}_3$ : MeOH 9:1) the crude product afforded mono-acetate derivative (2.4 mg) and di-acetate derivative (5.6 mg).

## Results and Discussion

Ethanol extract of the stems on chromatographic column gave nine known compounds. These compounds were identified by comparison of their physical (mp,  $a_D$ ) and spectroscopic (MS, IR, UV,  $^1\text{H}$  and  $^{13}\text{C}$  NMR) data with those of authentic samples isolated from *Aristolochia chamissonis*<sup>3</sup> and *A. gehrtii*<sup>8</sup>.

Analyses of the spectroscopic data (MS, IR, UV,  $^1\text{H}$  and  $^{13}\text{C}$  NMR) of the alkamide suggested two alternative structures for it (**1** or **2**). Compound **1** is known as aristolactam AII, previously isolated from eleven *Aristolochia* species and from *Pararistolochia flos-avis* (Aristolochiaceae), which structure identification were based mainly on the works of Achari *et al.* (1982, 1984)<sup>1,2</sup>, Crohare *et*

*al.* (1974)<sup>4</sup> and Priestap (1985, 1989)<sup>9, 10</sup>. From *Piper longum* (Piperaceae)<sup>5</sup> compounds **1** and **2** were isolated and named as piperolactam A and aristolactam A II, respectively, differing only by the position of the substituents at C-3 and C-4 (Tables 1 and 2).

Table 1: <sup>1</sup>H NMR spectral data for **1**, **1a**, **1b**, aristolactam AII<sup>4,5</sup> and piperolactam A<sup>5</sup> (δ, J in Hz)

H	<b>1</b> <sup>*</sup> (DMSO- <i>d</i> <sub>6</sub> , 200 MHz)	<b>1a</b> (DMSO- <i>d</i> <sub>6</sub> , 200 MHz)	<b>1b</b> (DMSO- <i>d</i> <sub>6</sub> , 200 MHz)	aristolactam AII <sup>4</sup> (CDCl <sub>3</sub> +DMSO- <i>d</i> <sub>6</sub> , 60 MHz)	aristolactam AII <sup>5</sup> (DMSO- <i>d</i> <sub>6</sub> , 100 MHz)	piperolactam A <sup>5</sup> (DMSO- <i>d</i> <sub>6</sub> , 500 MHz)
2	7.62 (s)	7.67 (s)	8.43 (s)	7.62 (s)	7.58 (s)	7.62 (s)
5	9.07 (m)	9.14 (m)	9.08 (m)	9.13 (m)	9.64 (m)	9.12 (d, J=8.0)
6	7.53 (m)	7.63 (m)	7.60 (m)	7.54 (m)	7.47 (m)	7.58 (m)
7	7.53 (m)	7.63 (m)	7.60 (m)	7.54 (m)	7.47 (m)	7.58 (m)
8	7.90 (m)	8.00 (m)	7.92 (m)	7.93 (m)	7.89 (m)	7.95 (d, J=8.0)
9	7.09 (s)	8.14 (s)	7.88 (s)	7.08 (s)	7.08 (s)	7.10 (s)
NH	10.79 (br s)			10.77 (s)		
OCH <sub>3</sub>	4.00 (s)	4.05 (s)	4.04 (s)	4.03 (s)	4.00 (s)	4.02 (s)
OH	10.05 (br s)	9.40 (br s)		10.22 (s)		10.81 (s)
OCOCH <sub>3</sub>			2.42 (s)			
NCOCH <sub>3</sub>		2.64 (s)	2.77 (s)			

\*Assignments were made with the assistance of <sup>1</sup>H-<sup>1</sup>H COSY.

Table 2: <sup>13</sup>C NMR spectral data for **1**, **1a**, **1b**, and aristolactam A II <sup>9</sup> (δ)

C	<b>1</b> * (DMSO- <b>1a</b> <i>d</i> <sub>6</sub> , 50 MHz)	(DMSO- <b>1b</b> (CDCl <sub>3</sub> + <i>d</i> <sub>6</sub> , 50 MHz)	aristolactam A II <sup>9</sup> (DMSO- <i>d</i> <sub>6</sub> , 25 MHz)	
1	120.5 ( <i>s</i> )	120.4	120.7	120.4
2	113.5 ( <i>d</i> )	113.9	120.7	113.5
3	152.3 ( <i>s</i> )	152.6	157.0	152.2
4	149.0 ( <i>s</i> )	150.7	144.9	148.9
4a	121.9 ( <i>s</i> )	122.2	122.0	121.9
4b	126.1 ( <i>s</i> )	126.7	126.5	126.1
5	126.9 <sup>a</sup> ( <i>d</i> )	126.9 <sup>a</sup>	126.9 <sup>a</sup>	126.8
6	125.4 <sup>a</sup> ( <i>d</i> )	126.5 <sup>a</sup>	127.3 <sup>a</sup>	125.3
7	127.4 <sup>a</sup> ( <i>d</i> )	127.7 <sup>a</sup>	128.0 <sup>a</sup>	127.3
8	129.1 ( <i>d</i> )	130.2	130.4	129.0
8a	134.9 ( <i>s</i> )	134.0	135.0	134.9
9	104.1 ( <i>d</i> )	112.1	115.8	104.0
10	135.4 ( <i>s</i> )	132.5	133.0	135.4
10a	122.4 ( <i>s</i> )	118.8	118.0	122.4
OCH <sub>3</sub>	59.6 ( <i>q</i> )	59.8	61.0	59.5
COCH <sub>3</sub>		170.7, 25.8	171.2, 168.9, 26.1, 20.9	
CO	168.7 ( <i>s</i> )	166.2	166.0	168.5

\* Multiplicity was established by DEPT pulse sequence.

<sup>a-b</sup> Assignments within the same column may be interchanged.

In order to unequivocally establish the structure for the isolated alkaloid from *A. chamissonis*, some 2D NMR experiments and chemical derivatisation were carried out. The correlations observed between protons and carbons by <sup>1</sup>H-<sup>13</sup>C COSY, and <sup>1</sup>H-<sup>13</sup>C COSY-LR (Figure 1) suggested structure **1** instead of **2** for this compound. This suggestion was corroborated by NOE difference experiments, which showed interaction between H-5 and methoxy protons, as well as between H-2 and hydroxy proton (Figure 2). Structure **1** was further confirmed by mono- (**1a**) and di-acetate (**1b**) derivatives. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of mono-acetate showed significant changes in the chemical shifts of CH-9 (Dd<sub>H</sub> = 1.05, Dd<sub>C</sub> = 8.0) in relation to **1**, whereas the spectra of the di-acetate showed that CH-2 and CH-9 had substantial changes in their chemical shifts (Dd<sub>H</sub> = 0.81,

0.79,  $Dd_C = 7.2, 11.7$ , respectively).

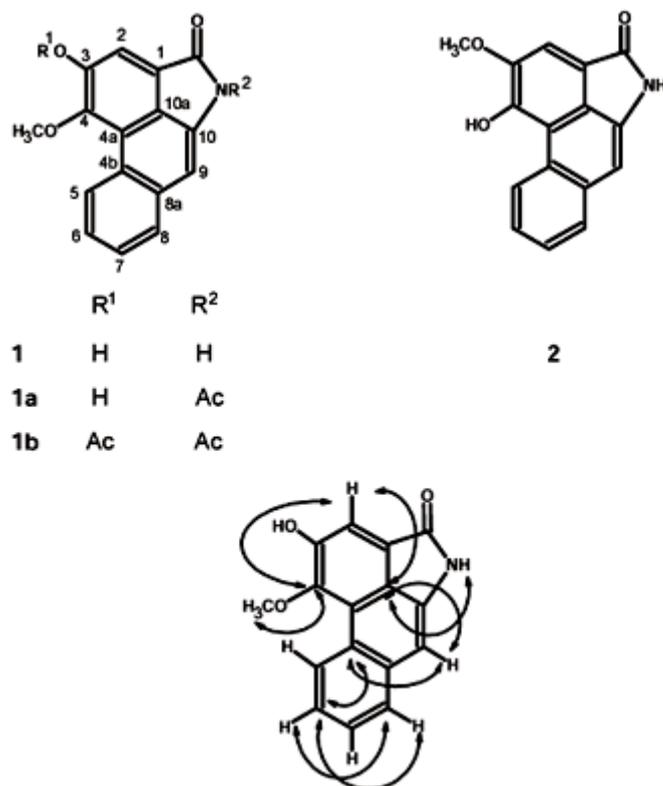


Figure 1: Long-range correlations observed in  $^1\text{H}$ - $^{13}\text{C}$  COSY spectrum of **1**.

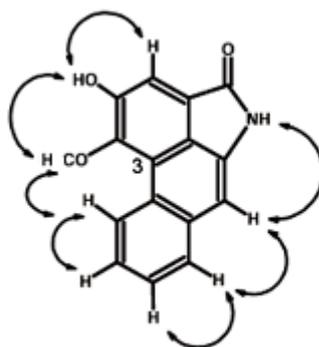


Figure 2: NOE correlations observed in NOESY spectrum of **1**.

## Conclusions

Analyses of spectroscopic data from **1** and its acetyl derivatives are in accordance with the reported structure for Aristolactam AII isolated from Aristolochiaceae and call for a revision of the structures of aristolactam AII and piperolactam A isolated from *Piper longum*.

It is important to note the isolation from *A. chamissonis* of phenanthrene compounds, which are very characteristic of the Aristolochiaceae family. Nearly forty aristolactamas and sixty aristolochic acid derivatives have been isolated from Aristolochiaceae species. Aristolochic acid I, for example, occurs in sixty species belonging to *Aristolochia* genus<sup>6,7</sup>.

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TSURUTA, A. Y., BOMM, M. D., LOPES, M. N., LOPES, L. M. X. Aristolactamas e Outros Constituintes Químicos de *Aristolochia chamissonis*. *Ecl. Quím. (São Paulo)*, v.27, p. , 2002.

**RESUMO:** Isolou-se do extrato etanólico de galhos de *Aristolochia chamissonis* espatulenol, sitosterol, sitosterol-b-D-glicosídeo, colavelool, 13-epi-2-oxo-colavelool, trans-N-p-coumaroiltiramina, alantoína, ácido aristolóquico I e aristolactama AII. As estruturas da aristolactama AII e da piperolactama A são revisadas com base em análises espectrométricas e derivatizações químicas.

**PALAVRAS-CHAVE:** Aristolochiaceae, *Aristolochia chamissonis*, diterpenos, trans-N-p-coumaroiltiramina, alantoína, aristolactama AII, piperolactama A.

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