

ISOLATION AND IDENTIFICATION OF THE ACIDIC COMPONENTS OF *ARISTOLOCHIA MACEDONICA* BORNM.\*

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Aristolochia acid I was isolated from the tubers of *A. macedonica* Bornm. and was identified by its methyl ester, UV and IR spectra and by TLC. Also by TLC the presence of traces of aristolochia acid II was proved. The peel of the tubers contained almost two times more aristolochia acids than the core.

Some species of the genus *Aristolochia* have been widely used in the traditional medicine of many nations. Recent pharmacologic research has confirmed their therapeutic value and for this reason their chemical composition is of great interest (1—3).

The greatest contribution to the investigation of the *Aristolochia* species has been made by Pailer et al. who have isolated and determined the structure of six aristolochia acids: aristolochia acid I (A I), aristolochia acid II (A II), aristolochia acid III (A III), aristolochia acid IIIa (A IIIa), aristolochia acid IV (A IV) and aristolochia acid IVa (A IVa) (4—6).

There are several *Aristolochia* species present in Yugoslavia. It was the aim of this work to determine the acidic components of *A. macedonica* Bornm., an indigenous species to Macedonia (7).

The plant material was collected on Mt. Kitka in the flowering season

For the isolation of the aristolochia acids we used the procedure given by Priestap et al. (8). After removing the fats with light petroleum, the plant material was extracted with methanol, which was then vacuum evaporated. The residue was basified and the tertiary bases extracted with ether. The aristolochia acids were then precipitated in the aqueous layer with HCl. Details of the method are given in the experimental part.

A I was isolated as the main component. The UV spectrum gave the characteristic absorptions: 253, 318 and 393nm (9, 10). The IR spectrum (Fig. 1) gave a number of bands: 1675 (C = O), 1535 (NO<sub>2</sub> antisymmetric stretching vibration), 1340 (NO<sub>2</sub> symmetric stretching vibration), 1270, 1030 and 940 cm<sup>-1</sup> (CH<sub>2</sub>O<sub>2</sub>) (6,8).

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In order to identify the other aristolochia acids, the crude acids were methylated with diazomethane. Again the main component in the precipitate was A I-Me.

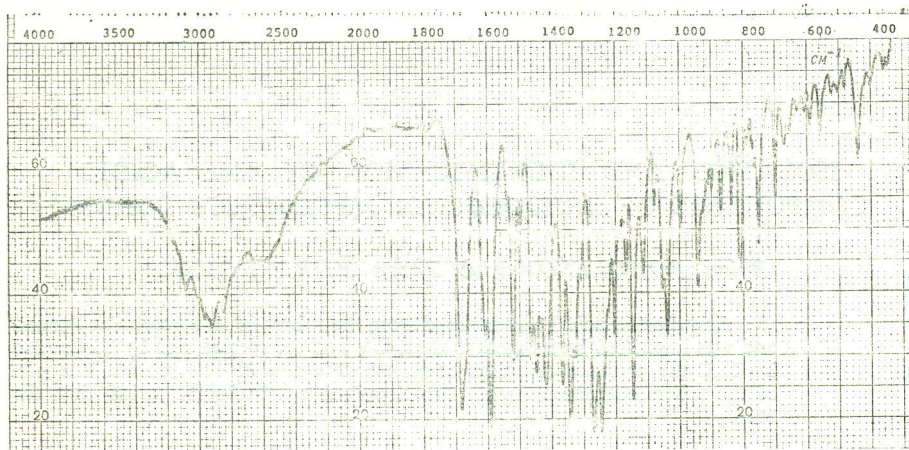


Fig. 1. IR spectrum of aristolochia acid I.

TLC of the filtrate with authentic samples of the methyl esters of A I, A II, A III and A IV, indicated that besides A I-Me, there were traces of A II-Me.

The size of the tubers enabled us to investigate separately their outer layer, the peel, and their inner layer, the core. We found that the amount of the crude aristolochia acids in the peel was about two times greater than in the core. However, no qualitative difference was noticed.

### Experimental

Melting points are uncorrected.

IR spectra were obtained using a Perkin Elmer 581 spectrophotometer in KBr discs.

UV spectra were recorded in absolute methanolic solution by a Beckman DB spectrophotometer.

TLC was carried out on glass plates coated with MN-silicagel GHR/UV<sub>245</sub>, and the chromatograms were developed with benzene-acetone-formic acid (96 : 3 : 1).

The dried and finely ground tubers, 500g, previously defatted with light petroleum (b. p. 40–70°C) were extracted for 70h with methanol in a Soxhlet apparatus. The solvent was evaporated under reduced pressure leaving a dark brown syrup, which was then suspended in water and ammonia (pH = 9,7) and the tertiary bases extracted with ether. To the aqueous layer 0.1 mol/dm<sup>3</sup> HCl was added and the acids extracted with ether. Extraction of the ethereal solution with 15% NaHCO<sub>3</sub>, followed by acidification of the

aqueous layer with 0.1 mol/dm<sup>3</sup> HCl (pH = 3), gave 7g of crude aristolochia acids (m.p. 256°C).

Several recrystallizations of the crude product from N,N-dimethylformamide/methanol gave yellow crystals of A I (m.p. 280—1°C).

Anal. for C<sub>17</sub>H<sub>11</sub>O<sub>7</sub>N (341.275)

Calcd.: C 59.82, H 3.22, N 4.10,

Found: C 59.61, H 3.65, N 4.90.

1 g of the crude product in absolute methanol was methylated with an ethereal solution of diazomethane. Several recrystallizations from N, N-dimethylformamide/methanol gave A I-Me.

Anal. for C<sub>18</sub>H<sub>13</sub>O<sub>7</sub>N (355.302)

Calcd.: C 60.84, H 3.68, N 3.94

Found: C 60.90, H 3.92, N 3.78.

The peel and the core were extracted using the same procedure. From 90 g peel 1.8 g crude acids were obtained (2%), and from 120 g core 1.32 g crude acids were obtained (1.12%).

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## РЕЗИМЕ

ИЗОЛАЦИЈА И ИДЕНТИФИКАЦИЈА НА КИСЕЛИТЕ КОМПОНЕНТИ ВО  
*ARISTOLOCHIA MACEDONICA* BORNM.

Бојан ПОДОЛЕШОВ и Зоран ЗДРАВКОВСКИ

Вршена е изолација и идентификација на киселите составни делови во *A. macedonica* Bornm. Изолирана е аристорохија киселина I и идентификувана е преку нејзиниот метил естер, УВ и ИЦ спектар, како и со хроматографска споредба со автентичен примерок. По пат на танко-слојна хроматографија докажани беа и трагови од аристорохија киселина II. Содржината на аристорохија киселините во лушките беше двапати поголема одошто во срцевината.

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