Constituents of *Aristolochia clematitis* L.

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Sesquiterpenic lactone aristolone, aristolochic acids I and II, aristolactam *N*-β-D-glucoside, β-sitosterol and its β-D-glucoside, aporphine alkaloid magnoflorine, ferulic and 4-coumaric acids and methyl ester of the latter were identified in the aerial part of *Aristolochia clematitis* L. Aristolone, sitosterol β-D-glucoside, and methyl 4-coumarate were found in this species for the first time.

The plants of the genus *Aristolochia* L. are known to contain phenanthrene derivatives of which predominantly aristolochic acids cause fagocytosis of leukocytes and reveal also antitumour [1] and genotoxic [2] activities. Moreover, phenylpropane derivatives [3] and alkaloids belonging to bisbenzylisoquinoline and aporphine [4, 5] groups were reported in this family.

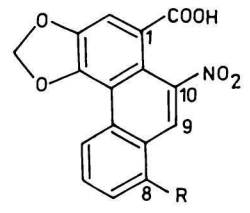
Therefore, we investigated constituents of *Aristolochia clematitis* L. growing wildly in our country. Aristolochic acids I and II (*I*, *II*), aristolactam *N*-β-D-glucoside (*III*), magnoflorine iodide (*IV*), aristolone (*V*), β-sitosterol, sitosterol β-D-glucoside (*VI*), ferulic (4-hydroxy-3-methoxycinnamic), 4-coumaric (4-hydroxycinnamic) acids, and methyl ester of the latter were isolated *via* extraction, chromatography on a silica gel-packed column and identified by physical methods.

The electron impact mass spectra of *I* and *II* reveal intense peaks of molecular radical ions and base peaks ($M_r - 46$) characterizing the cleavage of NO_2 groups; further fragmentation pattern was indicative of this type of compounds [6]; also the IR spectrum was in line with the structure.

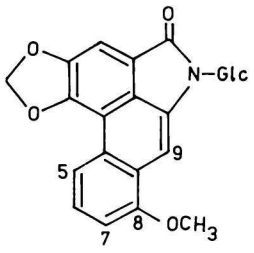
The UV and ^{1}H NMR spectra of compound *III* are indicative of aristolactams [7, 8]. The attachment of a saccharide unit to nitrogen was evidenced by the coupling constant with the anomeric saccharide proton $J = 9.5$ Hz [9]. The hexose, obtained by $LiAlH_4$ reduction of aristolactam in tetrahydrofuran was identified chromatographically and by comparison with the authentic sample (glucose).

Compound *IV*, the alkaloid of aporphine group, was identified according to

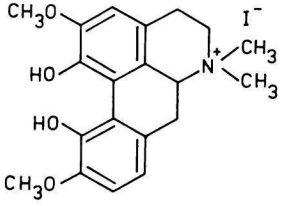
UV spectrum, optical rotation and by comparing with the specimen [10]. β-Sitosterol, isolated from the light petroleum extract was identified like the preceding substances.



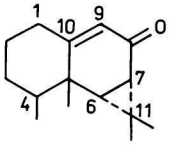
I R = OCH_3
II R = H



III



IV



V

The UV and IR spectra of compound *V* were characteristic of α,β-unsaturated ketones (λ_{max} = 239 nm, $\tilde{\nu}$ = 1660 and 1635 cm^{-1}). Its ^1H NMR spectrum agreed with that of aristolone. The suggested structure was also proved by its mass spectrum showing peaks associated with the molecular radical ion (m/z = 218.169) and further ones (cleavage of CH_3, C_3H_7, and C_3H_5O species) consistent with those of aristolone isolated previously from *A. debilis* [11].

Compound *VI* displayed a positive Liebermann—Burchard test, stretching vibrations of hydroxyl groups in the IR spectrum and fragments at m/z = 397 ($C_{29}H_{49}$) in the mass spectrum evidencing a steroidal skeleton. These data together with those of the ^1H NMR spectrum revealing also protons due to a hexose made it possible to identify this substance as sitosterol β-D-glucoside [12]. D-Glucose and β-sitosterol were identified chromatographically after alkali hydrolysis of *VI*.

Further compounds obtained from the petroleum ether extract were purified by preparative thin-layer chromatography and identified after analyzing their IR, ^1H NMR, and mass spectra and comparing their R_f values with those of

specimens as ferulic, 4-coumaric acids already reported in *A. argentina* [6] and methyl 4-coumarate, the latter being found in *A. clematitis* L. for the first time.

Experimental

The melting points were measured on a Kofler micro-hot stage, optical rotation was determined with a polarimeter Polamat A (Zeiss, Jena), the IR and UV spectra were recorded with model 377 (Perkin—Elmer) and Specord UV—VIS (Zeiss, Jena) apparatuses, in KBr and in methanol, respectively. The electron impact mass spectra and the ^{1}H NMR spectra (relative to tetramethylsilane) were taken with the respective AEI MS 902 S and Varian VXR 300 instruments. Silufol sheets UV_{254} and UV_{366} (Kavalier, CSFR) were employed for thin-layer chromatography and silica gel (Merck, Darmstadt) for column chromatography. Systems for thin-layer chromatography were: chloroform—methanol—benzene ($\varphi_r = 95:5:5$), chloroform—methanol ($\varphi_r = 9:1$), chloroform—benzene ($\varphi_r = 7:3$), methanol—water—ammonium hydroxide ($\varphi_r = 15:9:1$).

Extraction and isolation of compounds

The air-dried above-ground part of *Aristolochia clematitis* L. (2900 g) collected in Bratislava in 1988 was ground and successively extracted with petroleum ether and methanol. The extracts were separately evaporated to dryness; the methanolic one was suspended in water, the pH of which was adjusted with concentrated ammonium hydroxide to 9. The insoluble portion was filtered off and the filtrate was extracted with chloroform. The aqueous layer was acidified with concentrated hydrochloric acid to pH = 4 and extracted with chloroform, which was evaporated to dryness (682 mg) and separated by column chromatography on silica gel with chloroform—methanol (gradient elution). Fractions (100 cm^3 each) were combined according to the results of thin-layer chromatography after detection with UV light (254 or 366 nm), iodine vapours, ethanolic sulfuric acid, or Dragendorff reagent (alkaloids).

Combined fractions *6—9* ($CHCl_3$—MeOH, $\varphi_r = 9:1$) were crystallized from methanol to give yellow crystals of aristolochic acid I (42 mg), combined fractions *10—23* ($CHCl_3$—MeOH, $\varphi_r = 7:3$, crystallized from methanol) gave yellow crystals of aristolochic acid II (35 mg), combined fractions *40—62* ($CHCl_3$—MeOH, $\varphi_r = 6:4$) by the same procedure furnished white crystals of aristolactam *N*-β-D-glucoside (32 mg).

Concentrated aqueous solution of potassium iodide was added to the acidified aqueous layer and the aporphine alkaloids were extracted with chloroform. The solvent was distilled off under reduced pressure and the residue (45 mg) was crystallized from methanol and purified chromatographically on silica gel with chloroform—methanol ($\varphi_r = 1:2$). Fractions *25—30* were worked up and crystallized from methanol to yield magnoflorine iodide (28 mg).

The concentrated petroleum ether extract (586 mg) was chromatographed on silica gel with hexane—ethyl acetate ($\varphi_r = 2:1$) and crystallized from hexane to afford β-sitosterol (282 mg), m.p. = 130 °C, [*α*] (578 nm, 22 °C, $\varrho = 0.1$ g dm^{-3}, methanol) = −35° Further

elution with hexane—ethyl acetate ($\varphi_r = 2:1$) and ethyl acetate—methanol ($\varphi_r = 2:1$) gave aristolone (26 mg) and sitosterol β-D-glucoside (45 mg), respectively.

This portion contained also ferulic (45 mg), 4-coumaric (40 mg) acids and methyl 4-coumarate (40 mg). Aristolochic acid I (*I*), m.p. = 260—263 °C (Ref. [13] gives m.p. = = 275—278 °C), molecular radical ion, $m/z = 341.043$ (for $C_{17}H_{11}NO_7$ M_r(calc.) = = 341.053); UV, IR, and ^{1}H NMR spectra were in line with the structure and Ref. [6].

Aristolochic acid II (*II*), m.p. = 275—280 °C (Ref. [13] gives m.p. = 269—271 °C), molecular radical ion, $m/z = 311.045$ (for $C_{16}H_9NO_6$ M_r(calc.) = 311.043); UV, IR, and ^{1}H NMR spectra were in accordance with the structure and Ref. [6].

Aristolactam *N*-β-D-glucoside (*III*), m.p. = 325—330 °C (Ref. [13] gives m.p. = = 331—333 °C), [α] (578 nm, 23 °C, $\varrho = 0.02$ g dm^{-3}, MeOH) = −14°, molecular radical ion, $m/z = 455.131$ (for $C_{23}H_{21}NO_9$ M_r(calc.) = 455.121); UV, IR, mass, and ^{1}H NMR data were in agreement with the structure. Aristolactam *N*-β-D-glucoside was hydrogenolyzed with $LiAlH_4$ in tetrahydrofuran at 80 °C for 6 h and the hexose being isolated was identified as D-glucose [8].

Magnoflorine iodide (*IV*), m.p. = 273 °C, [α] (578 nm, 25 °C, $\varrho = 0.1$ g dm^{-3}, MeOH) = +195° The UV spectrum was identical with that published in [10]. Aristolone (*V*) was identified according to its UV, IR, mass, and ^{1}H NMR spectra.

Sitosterol β-D-glucoside (*VI*), m.p. = 285 °C (Ref. [12] gives m.p. = 295—298 °C), [α] (578 nm, $\varrho = 0.02$ g dm^{-3}, $CHCl_3$) = −40° was identified by its spectral (IR, mass, and ^{1}H NMR) data. This glucoside was hydrolyzed with dilute (25 %) sulfuric acid at 80 °C for 10 h according to Ref. [12]. Both β-sitosterol and D-glucose were identified chromatographically.

Methyl 4-coumarate, m.p. = 140 °C (Ref. [14] gives m.p. = 136—137 °C). Its UV, IR, and ^{1}H NMR spectra were in line with the structure.

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