Iridoid Glucosides from *Plantago altissima* L., *Plantago lanceolata* L., *Plantago atrata* Hoppe and *Plantago argentea* Chaix.

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Z. Naturforsch. **46c**, 963–965 (1991); received April 12, 1990

Plantago Species, Iridoids

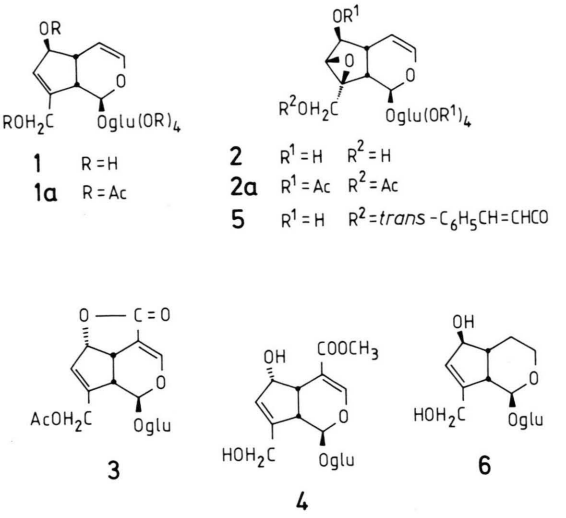
Plantago altissima L., *Plantago lanceolata* L., *Plantago atrata* Hoppe and *Plantago argentea* Chaix. were examined for iridoids. In all four species aucubin and catalpol were found. Globularin and methyl ester of the desacetylasperulosidic acid were isolated for the first time from Plantaginaceae plants (*P. altissima* and *P. lanceolata*). Asperuloside was found for the first time in *P. altissima* as well as dihydroaucubin in *P. atrata*.

Introduction

15 iridoid glucosides were found till now [1–9] in *Plantago* plants which are widely used in the folk medicine. We studied the iridoid composition of four *Plantago* species – *P. altissima, P. lanceolata, P. atrata* and *P. argentea* [10]. Aucubin (**1**) and catalpol (**2**) were shown in all these species [4] and asperuloside (**3**) in *P. lanceolata* [6] only.

Results and Discussion

Plantago altissima and *Plantago lanceolata*. Both plants are morphologically very close to each other. This was confirmed by the similar iridoid composition of both plants, from which five iridoid glucosides were isolated. The main constituents aucubin (**1**) and catalpol (**2**) were identified on the basis of ^{1}H NMR [11] and DCI-MS (NH_3) data, by comparison with authentic samples and transformation to acetates **1a** and **2a.** *P. lanceolata* contained more catalpol. In the mass spectra of **1** and **2** many peaks are accompanied with weak 2 u higher peaks which indicated the presence of dihydroaucubin and dihydrocatalpol. The low amount of these compounds did not allow their isolation in a pure state.



Asperuloside (**3**) was isolated from *P. altissima* and *P. lanceolata*. Its identification was carried out by ^{1}H NMR data [12] and DCI-MS (NH_3) data. This is the first isolation of **3** from *P. altissima*.

The forth iridoid on the basis of the ^{1}H NMR and MS spectra contained $-COOCH_3$, CH_2OH and OH groups. The ^{13}C NMR spectrum coincided with that of the methyl ester of the desacetylasperulosidic acid (**4**) [13], isolated from Rubiaceae plants [14–16]. This is the first isolation of **4** from Plantaginaceae plants. Both plants contained in the EtOAc-soluble part of the total MeOH extract an iridoid glucoside from a catalpol type with a *trans*-cinnamic substituent according to the ^{1}H NMR spectrum. The comparison of the ^{1}H NMR data of this compound with that of globularin (**5**) [17] as well as the DCI-MS (NH_3) data proved their identity. Globularin was found in Globulariaceae [17] and Lamiaceae [18] till now. This is the first isolation of **5** from Plantaginaceae plants.

Plantago atrata. From above ground parts as well as from roots of *P. atrata (P. montana, P. saxatilis)* [10] were isolated three iridoid glucosides. The main constituents **1** and **2** were identified as it was mentioned above. The DCI-MS (NH_3) data of the third compound showed a dihydroaucubin (**6**) structure. This was proved with the ^{1}H NMR spectrum which was identical with that of the rare iridoid **6** [3]. Besides from *P. asiatica* [3]

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0939–5075/91/0900–0963 $ 01.30/0

this compound was isolated recently from *P. carinata* [8].

Plantago argentea. The BuOH extract represented an almost pure aucubin–catalpol mixture, approximately in ratio 1:2, similarly to the published data [4].

The main iridoids in all studied plants were aucubin and catalpol. The presence only of C_9-iridoids in *P. atrata* and *P. argentea* gave reason to suggest that probably these plants were evolutionary higher then *P. altissima* and *P. lanceolata,* which contained C_9- and C_{10}-iridoids.

Materials and Methods

The ^1H NMR and ^{13}C NMR spectra were measured on a Bruker 250 MHz spectrometer. The DCI-MS (NH_3) spectra were obtained on a Varian MAT 44 mass spectrometer. The HPLC separations were achieved on a Perkin Elmer chromatograph with a RP-18 column Whatman ODS-3 (250 × 4.6 mm, 10 μm) and mobile phase MeOH–H_2O mixtures.

Plant material

Above ground parts of four *Plantago* species were collected in flowering as follows: *P. altissima* and *P. lanceolata* in Sofia region in July 1987, *P. atrata* in Vitosha mountain in September 1988 and *P. argentea* in Slavjanka mountain in July 1988. Only from *P. atrata* were collected roots too. Voucher specimens SOM 148259, 148262, 148263 and 148261, respectively, were deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences.

Isolation

Fresh aerial parts of *P. altissima* (2 kg), *P. lanceolata* (100 g), *P. atrata* (781 g), fresh roots (208 g) of *P. atrata* and dried aerial parts of *P. argentea* (46 g) were threefold extracted with MeOH. The MeOH concentrates were dissolved in H_2O and successively extracted with Et_2O, EtOAc and BuOH. The following purification was achieved as follows:

P. altissima. 17.6 g BuOH extract was separated by VLC on 360 g silica gel with $CHCl_3$–EtOH mixtures and EtOH. The first EtOH fraction (0.65 g) after filtration through 52 g aluminium oxide gave a 170 mg residue which was purified by HPLC with MeOH–H_2O (20:80) and flow rate 3 ml/min to give 13 mg **1** and 14 mg **4.** The second EtOH fraction (10.6 g) was treated with activated charcoal and washed with H_2O, H_2O–EtOH (9:1), EtOH and EtOH–acetone (1:1). 50 mg from the EtOH fraction (800 mg) were acetylated with pyridine–Ac_2O in the usual manner. The resultant acetate mixture was separated by preparative TLC on silica gel with Et_2O–petroleum ether (5:1) to give pure **1a** (16 mg) and **2a** (13.5 mg). 150 mg from the EtOH–acetone fraction (350 mg) were separated by HPLC with MeOH–H_2O (20:80) and flow rate 6 ml/min to give pure **1** (15 mg), **2** (12 mg) and **3** (13 mg).

The EtOAc extract (9 g) was separated by VLC on 360 g silica gel 60H with $CHCl_3$–EtOH mixtures. The fraction (850 mg) eluted with a solvent mixture (5:1–3:1) was repeatedly purified by VLC on 34 g silica gel with $CHCl_3$–MeOH–H_2O (60:15:4, lower layer). After elimination of the aromatic compounds by filtration through aluminium oxide the eluted with MeOH iridoid fraction was separated by HPLC with MeOH–H_2O (80:20) and flow rate 3 ml/min to give 18 mg pure **5.**

P. lanceolata. The BuOH (500 mg) and the EtOAc (0.25 g) fractions by TLC screening on silica gel and aluminium oxide as well as on HPLC showed the same iridoid composition as *P. altissima.*

P. atrata (aerial parts). 26 g BuOH fraction was treated with activated charcoal and washed with H_2O, H_2O–MeOH (9:1), MeOH and MeOH–acetone (1:1). 400 mg from the MeOH fraction (5.75 g) were purified by VLC on aluminium oxide with MeOH. The obtained iridoid mixture (88 mg) was additionally separated by HPLC with MeOH–H_2O (16:84) at flow rate 3.3 ml/min to give pure **2** (17 mg), **6** (6 mg) and **1** (20 mg).

P. atrata (roots). 5 g BuOH extract was treated with charcoal as it was mentioned above. The MeOH fraction (1.4 g) by TLC and HPLC comparison showed the same iridoid composition like the above ground parts.

P. argentea. The BuOH fraction (2 g) showed on TLC on silica gel and aluminium oxide the presence only of **1** and **2.** The DCI-MS (NH_3) of this fraction proved this composition.

Acknowledgements

We are grateful to Dr. A. Petrova from the Institute of Botany, Bulgarian Academy of Sciences for the botanical characterization of *P. lanceolata* and *P. altissima*. The project has been completed with the financial support of the Ministry of Culture, Sciences and Education under contract No. 346.

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