ASTROSIDE – A NEW ISOFLAVONE GLYCOSIDE FROM ASTRAGALUS AUSTRIACUS

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In a preceding paper [1] we reported the presence of a number of flavonoid compounds in various Astragalus species. Continuing an investigation of the flavonoid composition of Astragalus austriacus L., we have isolated in the pure state a flavonoid compound which we have called substance B. Substance B reduces Fehling's reagent only after acid hydrolysis, which indicates that it has a glucosidic nature, and gives a positive cyanidin reaction which shows its flavonoid character.

Table 1

Physicochemical Properties of Substance B and its Derivatives

Property	Substance B	Aglycone	Biochanin A	Demethylated aglycone	Genistein
Mp, °C	223—224.5	211—213	212—216	290—292	290—293
Formula	$C_{22}H_{22}O_{10}$	$C_{16}H_{12}O_5$	$C_{16}H_{12}O_5$	$C_{15}H_{10}O_5$	$C_{15}H_{10}O_5$
$[\alpha]_D^{20}$ degrees	—117.8 (*c* 0.98; Dimethyl formamide	—	—	—	—
Qualitative reactions					
Bryant's cyanidin reaction [2]	Yellow orange (in water)	Yellow orange (in octanol)	Yellow orange (in octanol)	—	—
Ferric chloride reaction	Purple red	Purple red	Purple red	Purple red	Purple red
Tauböcks-Wilson reaction [4, 5]	Yellow	Yellow	Yellow	Yellow	Yellow
With zirconyl nitrate [6]	Negative	Negative	Negative	Negative	Negative
With an ammoniacal solution of silver nitrate [7]	"	"	"	"	"
R_f value in the systems					
Butanol-acetic acid-water (4:1:5)	0.76	0.95	—	0.98	0.98
15% acetic acid	0.56	0.26	—	0.37	0.37

Quantitative acid hydrolysis showed that the glycoside is a monoglycoside. Acid hydrolysis gave the aglycone and the sugar component, which was identified as D-glucose by chromatography and by synthesis of the osazone. An idea of the properties of the glycoside and the aglycone is given by Table 1.

To determine the nature of the glycoside and the positions of the carbohydrate substituent and the free hydroxy groups, we investigated the substance spectroscopically in the UV region using ionizing and complex-forming reagents [8, 9] (Table 2, Fig. 1).

It can be seen from Table 2 that there are free hydroxy groups in position 5 of the glycoside and of the aglycone (from the bathochromic displacement of the long-wave band with zirconyl nitrate by 50–55 mμ), and the aglycone has a hydroxyl in position 7 (from a bathochromic shift of 10 mμ under the influence of sodium acetate) which is probably the one liberated on acid hydrolysis.

The negative reaction with an ammoniacal solution of silver nitrate shows that one of the o-hydroxy groups in ring B is substituted. The presence of absorption maxima in the UV spectrum of the glycoside and its aglycone at 260, 265, and 320 mμ (see Fig. 1) shows the isoflavonoid nature of the compound under study [10].

To confirm the isoflavonoid structure of the glycoside, the aglycone was subjected to alkaline hydrolysis, which gave formic acid. The IR spectrum of this compound (Fig. 2) has the absorption bands of carbonyl (1660 cm^{-1}), hydroxy (3420 cm^{-1}), and methoxy groups (2940 cm^{-1}). The aglycone obtained by acid hydrolysis was identified as biochanin A.

A comparison of the molecular rotation of the glycoside with the molecular rotation of phenyl glycosides [11], the IR-spectroscopic data, and the enzymatic splitting of the glycoside by the amylolytic enzyme of the fungus Aspergillus oryzae give grounds for assuming that the D-glucose is attached to the aglycone by a β-glycosidic link and is present in the glycoside in the pyranose form.

Table 2

Spectroscopic Characteristics of the Glycoside and its Aglycone

Medium	Bands	Glycoside		Aglycone	
		λ_{max} mμ	Δλ, mμ	λ_{max} mμ	Δλ, mμ
2×10^{-5} M in absolute ethanol	I	320	—	320	—
	II	265	—	260	—
The same + sodium acetate	I	320	0	330	10
	II	265	0	270	10
The same + zirconyl nitrate	I	375	55	370	50
	II	265	0	270	10
The same + zirconyl nitrate and citric acid	I	320	0	320	0
	II	265	0	265	5

Consequently, the glycoside may be characterized as biochanin A 7-β-D-glucopyranoside, or 5-hydroxy-4'-methoxyisoflavone 7-β-D-glucopyranoside, and we have called it astroside.

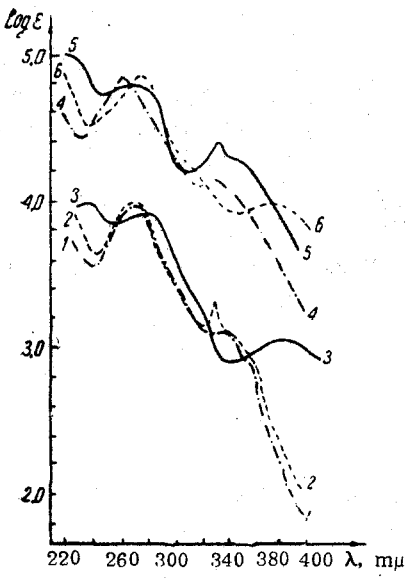

Fig. 1. UV absorption spectra:

1) Glycoside in ethanol; 2) glycoside in ethanol + zirconyl nitrate; 3) glycoside in ethanol + zirconyl nitrate and citric acid; 4) aglycone in ethanol; 5) aglycone in ethanol + zirconyl nitrate; 6) aglycone + ethanol + zirconyl nitrate and citric acid.

Experimental

Isolation of astroside. One kilogram of the comminuted herb Astragalus austriacus collected in the period of full flowering in the Zaporazhe Oblast was extracted three times with 10-*l* portions of 96% ethanol. The alcoholic extracts were evaporated in vacuum, 0.5 *l* of hot distilled water was added to the residue, and the ethanol was distilled off completely. The aqueous extract was allowed to stand for 12 hr in a refrigerator for the precipitation of the chlorophyll and other accompanying

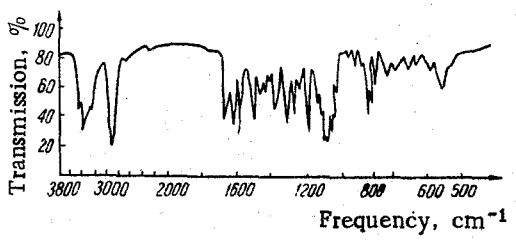

Fig. 2. IR absorption spectrum of the glycoside (mull in paraffin oil).

substances. The deposit was filtered off and the filtrate was purified by treatment with five or six 100-ml portions of chloroform. Evaporation of the aqueous solution gave a substance in the form of a white powder which, after three recrystallizations from methanol, had mp 223°–224.5° C (Kofler block).

Found, %: C 59.19; 59.48; H 4.78; 5.05; OCH_3 7.32; 7.46. Calculated for $C_{22}H_{22}O_{10}$, %: C 59.19; H 4.91; OCH_3 6.92.

Astroside forms small white plate-like crystals soluble in ethanol, methanol, pyridine, dimethylformamide and dioxane, and insoluble in water, chloroform, and ether. The cyanidin reaction gives a yellow orange coloration and ferric chloride gives a purple red coloration.

Acid hydrolysis of astroside. A solution of 0.1325 g of astroside in 40 ml of 50% ethanol containing 5% of sulfuric acid was heated on a boiling water bath for 4 hr. The aglycone liberated was filtered off and recrystallized from 50%

ethanol. The yield of aglycone was 0.0812 g (61.2%). It formed acicular crystals with mp 211°–213° C. We identified the aglycone by demethylation.

Demethylation of the aglycone. A solution of 0.1 g of the aglycone in 75 ml of pyridinium chloride, obtained by saturating anhydrous pyridine with gaseous hydrogen chloride, was heated in an atmosphere of nitrogen for 4 hr on a sand bath at 110°–120° C [12]. After the completion of the reaction, the mixture was diluted with 200 ml of distilled water. The crystals which separated out were filtered off and dried. The substance obtained formed small white acicular crystals with mp 290°–292° C which were identical with genistein in their chromatographic behaviour and melting points. Genistein was obtained by the acid hydrolysis of sophoricoside, which was kindly given to us by V. I. Litvinenko.

Investigation of the carbohydrate component. The acid hydrolyzate was neutralized with KU-2 ion-exchange resin in the OH-form and was analyzed by paper chromatography for its content of sugars in several systems in the presence of standard samples. After visualization with aniline phthalate and diphenylamine reagents, the sugar under investigation was identified as D-glucose. The osazone (mp 202°–202.5° C) gave no depression of the melting point with glucose osazone.

Enzymatic hydrolysis of astroside. With heating, 0.02 g of astroside was dissolved in 2 ml of ethanol and the solution was made up with water to 20 ml. The cooled solution was then treated with 0.02 g of a preparation from the fungus Aspergillus oryzae and left at 33° C for 24 hr. The enzyme was precipitated by boiling and the hydrolysis products were analyzed for their content of sugars in the butanol-acetic acid-water(4:1:5) system. D-Glucose was found.

Alkaline degradation of the aglycone. A solution of 50 mg of the aglycone in 30 ml of 20% caustic potash was heated on a boiling water bath for 30 min, cooled, and neutralized with 10% sulfuric acid to pH 4–5, after which it was extracted 5–6 times with 10-ml portions of ether. The ethereal extracts were evaporated and the residue was dissolved in the minimum amount of ethanol and chromatographed in the water-saturated butanol system. Various phenols and acids were used as reference samples. On treating the chromatograms with an alcoholic solution of Bromophenol Blue, formic acid was found.

The aqueous solution after extraction with ether was acidified and steam-distilled. Formic acid was found in the distillate by qualitative reaction and by chromatography. The results given confirm the isoflavone structure of astroside.

Summary

1. A new isoflavone glycoside, astroside, has been isolated from Astragalus austriacus L.

2. Astroside has been characterized as biochanin A-7-β-D-glucopyranoside, or 5-hydroxy-4'-methoxyisoflavone 7-β-D-glucopyranoside.

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