HIRSUTRIN – A NEW GLYCOSIDE FROM THE FLOWERS OF GOSSYPIUM HIRSUTUM

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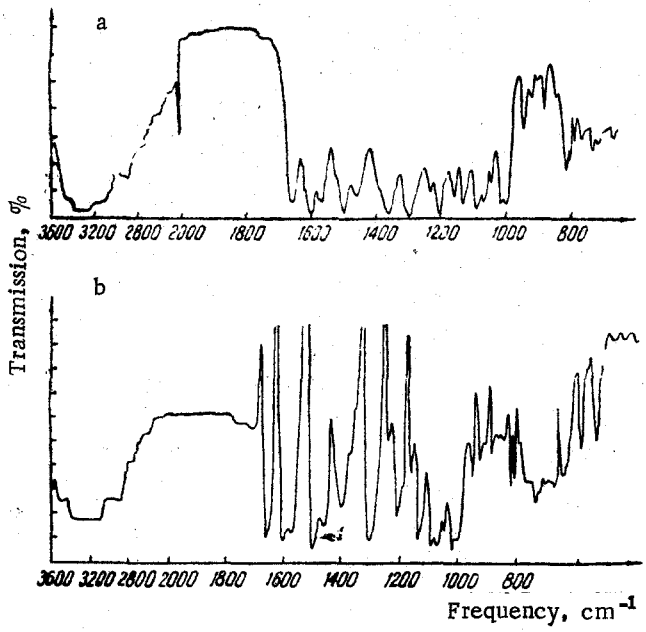
Continuing a chemical investigation of the flowers of cotton of variety 108-F [1], we have isolated a new glycoside hirsutrin $C_{21}H_{20}O_{12}$, an isomer of isoquercitrin.

Hirsutrin has mp 220°–222° C, $[\alpha]_D^{20}$ –72.46° (pyridine), UV spectrum: λ_{max} 359, 256 mμ (ethanol). Acetylation of the glycoside gave an octaacetate $C_{37}H_{36}O_{20}$. The methylation of hirsutrin with dimethyl sulfate led to the 5,7,3',4'-tetramethyl ether $C_{25}H_{28}O_{12}$, the UV spectrum of which had λ_{max} 345, 250 mμ (ethanol), and its methylation with diazomethane led to the 7,3',4'-trimethyl ether $C_{24}H_{26}O_{12}$ with UV spectrum λ_{max} 365, 253 mμ (ethanol).



IR spectra of hirsutrin: ordinary (a) and differential (b).

The hydrolysis of hirsutrin with 5% hydrochloric acid, with the enzyme of the snail Helix plectrotropis, and with the enzyme of Aspergillus oryzae, gave the aglycone quercetin and D-glucose.

The position of the attachment of the D-glucose to the quercetin, position 3, was established by the exhaustive methylation of the hirsutrin with its subsequent hydrolysis, giving the 5,7,3',4'-tetramethyl ether of quercetin.

The enzymatic degradation of hirsutrin by the fungus Aspergillus oryzae shows that the glycoside linkage in it is a β-linkage.

The properties of hirsutrin are very similar to those of isoquercitrin (quercetin 3-glucoside) and hyperin (quercetin 3-galactoside) [2,3]. Having no samples of these flavonols for direct comparison, we recorded the IR spectra of hirsutrin, the ordinary and the differential spectra (figure, a and b), which also showed that it differs somewhat from isoquercitrin and hyperin in the 1010–700 cm^{-1} region.

To determine the size of the oxide ring of the D-glucose in hirsutrin we made use of the method of comparing molecular rotations proposed by Kovalev and Litvinenko [2]. The presence of four maxima in the 1010–1100 cm^{-1} region and the molecular rotation with a correction factor of –190.4° shows that the D-glucose is in the pyranose form.

From the results obtained, the structure quercetin 3-β-D-glucopyranoside has been proposed for hirsutrin. Consequently, the difference between hirsutrin and isoquercitrin is only in the spatial arrangement of the D-glucose.

Experimental

Hirsutrin. When a methanolic extract of cotton flowers, after elimination of the quercimeritrin and quercetin 3'-glucoside, was diluted with water and allowed to stand for a long time, it deposited bright yellow crystals. These were recrystallized from mixtures of methanol and water (1:1) and pyridine and water (1:10) and dried at 105° C for 2 hr, mp 220°–221° C, $[\alpha]_D^{20}$ −72.46° (c 0.80, pyridine), R_f 0.70 [butan-1-ol–acetic acid–water (4:1:5)].

Found, %: C 52.35; H 4.65. Calculated for $C_{21}H_{20}O_{12} \cdot H_2O$, %: C 52.28; H 4.56.

The octaacetate. A solution of 0.3 g of hirsutrin in 5 ml of acetic acid was treated with 1 g of dry sodium acetate. After the mixture was heated for 2 hr and diluted with ice water, yellow oil formed which crystallized on standing. With two recrystallizations from methanol containing water, followed by drying for 2 hr at 110° C, the substance had mp 156°–157° C.

Found, %: C 54.54; H 4.64. Calculated for $C_{37}H_{36}O_{20} \cdot 0.5\ H_2O$, %: C 54.38; H 4.57.

The 5, 7, 3', 4'-tetramethyl ether. A mixture of 1 g of hirsutrin, 50 ml of dry acetone, 5 g of potassium carbonate, and 10 ml of dimethyl sulfate was heated for 3 hr. Then another 3 g of potassium carbonate, 30 ml of acetone, and 5 ml of dimethyl sulfate were added. After heating for an hour, white crystals of the methyl ether deposited on the walls of the flask. The melting point of the dried crystals was 219°–220° C (from aqueous acetone).

Found, %: C 55.90; H 5.80. Calculated for $C_{25}H_{28}O_{12} \cdot H_2O$, %: C 55.76; H 5.57.

The 7, 3', 4'-trimethyl ether. 40 ml of a saturated ethereal solution of diazomethane was added to 0.37 g of hirsutrin in 40 ml of a mixture of absolute ethanol and dry acetone (1:1). The mixture was left in a refrigerator for five days, the ether that deposited was recrystallized from dilute ethanol and dried at room temperature. The sample of the substance contracted at 152° C and melted at 205°–206° C; it gave a green coloration with ferric chloride.

Found, %: C 52.15; H 5.50. Calculated for $C_{24}H_{26}O_{12} \cdot 2.5\ H_2O$, %: C 52.26; H 5.62.

Acid hydrolysis. A mixture of 2.6 g of hirsutrin and 70 ml of 5% solution of hydrogen chloride in aqueous ethanol was heated on a water bath under reflux for 2 hr. The reaction mixture was evaporated and the aglycone which deposited was recrystallized from a mixture of ethanol and water (1:1) and dried at 150° C, mp 310°–312° C (decomp.).

Found, %: C 59.69; H 3.20. Calculated for $C_{15}H_{10}O_7$, %: C 59.60; H 3.30.

Hydrolysis with snail enzyme. A suspension formed by shaking 0.5 g of hirsutrin with 200 ml of water was treated with the pancreatic juice of the snail Helix plectotropis and a few drops of toluene. The reaction mixture was placed in a thermostated vessel at 36°–38° C for 7 days. The aglycone obtained was identical with quercetin.

Hydrolysis with fungal enzyme. A mixture of 20–30 mg of hirsutrin with approximately the same amount of the enzyme from Aspergillus oryzae was carefully shaken with a small amount of water and placed in a thermostated vessel at 35° C. After two days the yellow crystals that had deposited were dissolved in methanol and chromatographed in the butan-1-ol–acetic acid–water (4:1:5) system.

In contrast to the initial hirsutrin spot (R_f 0.70), which did not fluoresce in UV light, the quercetin spot (R_f 0.72) was formed with a bright greenish yellow fluorescence.

Quercetin. Pentaacetate, mp 197°–198° C [ethanol–water (5:1)].

Found, %: C 58.49; H 4.19. Calculated for $C_{25}H_{20}O_{12}$, %: C 58.59; H 3.90.

Pentamethyl ether, mp 152°–153° C (from acetone).

5, 7, 3', 4'-Tetramethyl ether. A mixture of 2.5 g of the 5, 7, 3', 4'-tetramethyl ether of hirsutrin and 3% aqueous sulfuric acid was heated for 1 hr and then cooled. Green needles deposited, and were crystallized from ethanol with the addition of a small amount of water. The crystals were dried at 105° C and had mp 193°–194° C.

Isolation of glucose. The mother liquor remaining after the enzymatic hydrolysis of hirsutrin and the separation of quercetin was evaporated to small bulk and freed from enzymes. The clear water-ethanol solution was concentrated to small bulk and diluted with a fivefold volume of butan-1-ol. A creamy powder with mp 130°–140° C, R_f 0.36 [ethyl acetate–pyridine–water (2:1:2)], was formed. A mixture with D-glucose gave no depression of the melting point. Its IR spectrum was identical with the IR spectrum of D-glucose. The osazone, mp 197°–198° C, was obtained in the usual way from the sugar obtained in the acid hydrolysis of hirsutrin.

Summary

A new glycoside, hirsutrin, with the structure of quercetin 3-β-D-glucopyranoside, has been obtained from the flowers of Gossypium hirsutum. It differs from isoquercitrin in the spatial arrangement of the D-glucose.

REFERENCES

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