FLAVONOL GLYCOSIDES OF THE FLOWERS OF A THIN-LEAVED COTTON PLANT OF THE SPECIES Gossypium barbadense

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According to many workers [1, 2], the composition of natural compounds (alkaloids, flavonoids, etc.) in plants depends on variety and species differences and also on the soil and climatic conditions.

We have previously studied the flavonoids of the flowers of the cotton plant of variety 108-F (G. hirsutum) growing in the northern parts of Uzbekistan. It was of interest to determine the composition of the flavonoids in varieties cultivated in the south of the republic.

Below we give the results obtained in an investigation of the flavonoids of the flowers of cotton variety 5904-I (G. barbadense) collected in August 1967 in the "Surkhan" collective farm, Surkhan-Dar'ya region.

The total amount of flavonols was determined by Wilson's method in Guseva and Nestyuk's modification [3], and also gravimetrically (from the aglycone) [4]. The leaves contained about 2% of flavonoids and the flowers more than 5%. A paper chromatogram in the butan-1-ol–acetic acid–water (4:1:5) system showed that the flowers contained four and the leaves two flavonols.

The flavonols from the leaves were identified as hirsutin and hybridin [5, 6], and three of the flavonols from the flowers were identified as quercimeritrin, quercetin 3'-glucoside, and hirsutrin [7, 8], which have been extracted from other species of cotton. All the flavonols mentioned above were isolated in the individual state according to differences in solubility in methanol, acetone, and pyridine. About 40% of the total flavonols of the flowers was represented by a flavonol with R_f 0.22. This flavonol gives some color reactions for chalcones, and we previously erroneously assumed that it was a chalcone glucoside [9].

The results of a study of the physicochemical properties of this compound and of the NMR spectrum of its acetate showed that it is a glucoside of gossypetin. The heptaacetate and the heptamethyl ether of the flavonol with R_f 0.22 described previously [9] must be regarded as the hexaacetate and hexamethyl ether.

The melting points of the flavonol and many of its derivatives are similar to those for gossypin (Table 1), which was isolated by T. R. Seshadri et al. from the flowers of the cotton plant Gossypium indicum [10] and from the flowers of Hibiscus vitifolius [11].

It can be seen from the table that the properties of the cotton flavonol differ slightly from those of gossypin. A difference is also shown by their IR spectra (Fig. 1).

Since both flavonols are monosides and they have the same aglycone and the same sugar, their difference consists in a difference in the position of the glucose in the aglycone or in a difference in the conformations of the glucose, or both together.

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TABLE 1. Physicochemical Properties of Gossypin and the Flavonol of the Cotton Plant

Properties	Gossypin	Cotton flavonol
Empirical formula	$C_{21}H_{20}O_{13}$	$C_{21}H_{20}O_{13}$
mp, °C	230 (decomp.)	228—229 (203) (without decomp.)
R_f [butan-1-ol–acetic acid–water (4 : 1 : 5)]	0,25 (fluoresces in UV light)	0,22 (does not fluoresce in UV light)
UV spectrum (nm)		
C_2H_5OH	385, 265*	385, 260
$+CH_3COONa$	400, 280	395, 265
$+H_3BO_3$	400, 275	395, 265
$+AlCl_3$	440, 255	443, 270
mp, °C of:		
the pentamethyl ether	196—198	—
the tetramethyl ether	—	191—192
the acetyl derivative	120	233—234
Hydrolysis with A. oryzae	—	Hydrolyzes
Acid hydrolysis	7% HCl	
Aglycone	Gossypetin	
Sugar	Glucose	

*We obtained these figures in the study of a sample of gossypin kindly given to us by T. R. Seshadri.

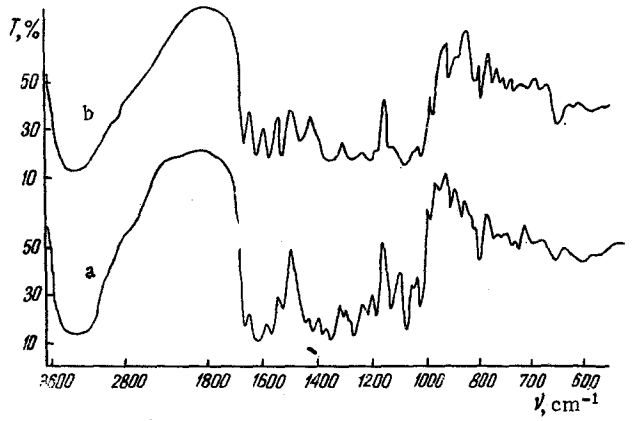

Fig. 1. IR spectra of gossypin (a) and gossypetin β-D-glycopyranoside (b).

NMR spectroscopy proved to be the most effective method of determining the position of attachment of the glucose to the aglycone molecule in the cotton flavonol. It is known that flavonoid compounds possess a very low solubility in the usual solvents for NMR, and therefore their acetyl or trimethylsilyl derivatives are usually used to obtain spectra [12]. The NMR spectra of acetylated derivatives of the cotton flavonol and its aglycone (gossypetin) in $CDCl_3$ are given in Fig. 2.

By analogy with the spectra of related compounds [12, 13], the assignment of the resonance lines in the spectrum of gossypetin can be made in the following way. In the 2.3 ppm region (δ scale) there are three peaks with relative intensities of 1 : 1 : 4 corresponding to the signals of the protons of acetyl groups, while the acetyl groups in the 3, 7, 3', and 4' positions are magnetically equivalent and resonate at 2.28 ppm. Because of the descreening action of the carboxy group, the acetyl group in the C-5 position gives a peak at 2.38 ppm. The signal at 2.33 ppm relates to an acetyl group at C-8. Three groups of signals are present in the weak-field region. The singlet at 6.97 ppm corresponds to a proton in the C-6 position of ring A. A doublet at 7.32 ppm (partially masked by traces of $CHCl_3$) is due to a proton at C-5' of ring B. A multiplet at 7.6 ppm represents the superposition of the signals of protons at C-2' and C-6'. The spin-spin coupling constant ($H_{5'}H_{6'}$) is 6.5 Hz.

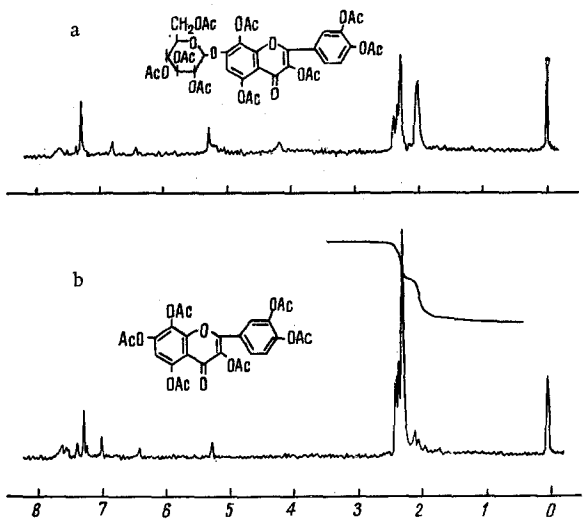

Fig. 2. NMR spectra of the acetate of gossypetin 7-β-D-glucopyranoside (a) and of gossypetin acetate (b).

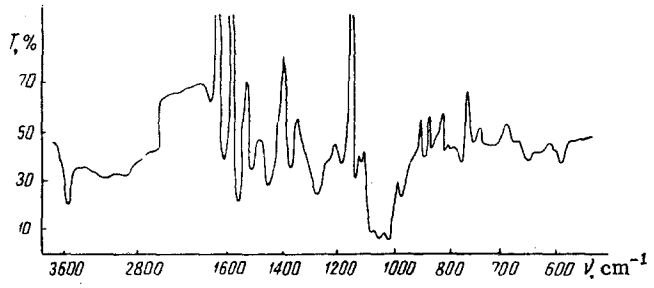

Fig. 3. Differential IR spectrum of gossypetin 7-β-D-glucopyranoside.

It can be seen from the NMR spectrum of the cotton flavonol that the signal of the C-6 proton is shifted upfield by 0.15 ppm as compared with the analogous protons in the aglycone (gossypetin), while the protons at C-2', C-5', and C-6' have not changed their chemical shifts. This fact shows that the glucose is attached at position 5 or 7. However, an acetyl group at C-5 appears in the spectrum of the glucoside, and the ratio of the integral intensities of the signals of the acetyl groups is 1 : 1 : 3.

Thus, in contrast to gossypin (gossypetin 8-glucoside), the glucose is attached to the aglycone in position 7 of ring A. Because of the inadequate solubility of the flavonol acetate it is impossible to deduce the conformation of the sugar part of the molecule. One can only point out the almost equivalent positions of the signals of the acetyl groups of the glucoside in the 2.0 ppm region and the fact that the H-1 proton of glucose gives a signal at 5.21 ppm.

In the 1100-1010 cm^{-1} region, the differential IR spectrum of the flavonol (Fig. 3) has three peaks: 1018 cm^{-1}, 1055 cm^{-1}, and 1080 cm^{-1}, which shows the pyranose form of the glucose. The hydrolysis of the flavonol with the enzyme of Aspergillus oryzae shows the β-glucosidic linkage of the aglycone to the sugar moiety.

All the properties mentioned permit the structure of gossypetin 7-β-D-glucopyranoside to be put forward for the flavonol.

EXPERIMENTAL

The combined flavonols were isolated by the usual extraction with 70% methanol from cotton flowers previously defatted with chloroform.

After 15-20 days, the water-diluted concentrated methanolic extract deposited yellow-green crystals which, on paper chromatography in the butan-1-ol–acetic acid–water (4 : 1 : 5) system, showed the presence of two flavonoids with R_f 0.22 and 0.32.

They were separated by repeated fractional crystallization from 50% aqueous ethanol or acetone. The flavonol with R_f 0.32 (quercimeritrin) was obtained first, and that with R_f 0.22 was isolated from the mother liquor. Subsequent purification was performed by recrystallization from aqueous pyridine and from 80% acetone. The flavonoid with R_f 0.22 crystallizes in two forms: as bright yellow needles and as dark green plates, with mp 228-229° C and 202-203° C, respectively.

Found %: C 52.65; H 4.45. $C_{21}H_{20}O_{13}$. Calc. %: C 52.50; H 4.16.

Nonaacetyl derivative: white crystals, mp 233-234° C (from 80% aqueous acetone).

Tetramethyl Ether. A mixture of 0.3 g of the flavonol, 50 ml of dry acetone, 3 ml of dimethyl sulfate, and 3.5 g of calcined potassium acetate was heated in a flask with a reflux condenser for 6 h. Then the mixture was filtered, the acetone was distilled off, and the residue was diluted with water. Cream-colored rhombic crystals with mp 191-192° C were obtained from 80% acetone; with ferric chloride they gave a green coloration, which shows the presence of a phenolic hydroxyl.

Pentamethyl Ether of Gossypetin. A mixture of 0.3 g of the flavonol, 50 ml of dry acetone, 4 ml of dimethyl sulfate, and 3 g of calcined potassium carbonate was heated for 30 h (until the reaction with ferric chloride was negative). From dilute ethanol, crystals deposited in the form of bright yellow needles with mp 245-246° C. The sugar had been split off.

Monoacetyl derivative of the pentamethyl ether of gossypetin: crystals in the form of cream-colored needles with mp 169° C (from 80% acetone).

Acid Hydrolysis. A mixture of 0.5 g of the flavonol and 3 ml of conc. HCl was heated in a flask placed in a boiling water bath for 15 min. The yellow-green microcrystalline aglycone with mp 304-306° C (decomp.) was obtained from 80% ethanol; R_f 0.34 (BAW).

Hexaacetyl derivative of gossypetin: Crystals in the form of cream-colored needles (80% acetone, mp 229° C).

Hexamethyl ether of gossypetin: Cream-colored crystals (80% ethanol), mp 169° C, giving no coloration with ferric chloride.

Enzymatic Hydrolysis. The flavonol (0.1 g) was hydrolyzed by the enzyme of Aspergillus oryzae in a thermostat at 36-38° C for three days. After recrystallization from 80% acetone, the yellow-green aglycone had mp 306-308° C (decomp.). The sugar was determined by paper chromatography in the ethyl acetate–pyridine–water (2 : 1 : 2) system using o-toluidine salicylate as the revealing agent. One spot was found, identical with that of glucose.

Quantitative Determination of Gossypetin and Glucose. A mixture of 0.0988 g of the flavonol and 3 ml of conc. HCl was heated in the boiling water bath for 12 min and was then diluted with water, and the aglycone that had deposited was transferred quantitatively to a weighed Schott No. 3 filter, dried to constant weight at 130-140° C, and weighed.

Found: 0.0647 g $C_{15}H_{11}O_8$. Calc.: 0.0655 g.

The acid mother liquor after the separation of the aglycone was transferred quantitatively into a 10-ml measuring flask. Two 1-ml samples were taken; these were neutralized with sodium acetate and the glucose was determined by Bjerri's method.

Found: 35 mg $C_6H_{12}O_6$. Calc.: 37 mg.

SUMMARY

The flowers of the thin-leaved cotton plant of variety 5904-I (Gossypium barbadense) contained more than 5% of combined flavonols consisting of quercimeritrin, hirsutrin, quercetin 3'-glucoside, and a flavonol with mp 228-229° C. The latter has the structure of gossypetin 7-β-D-glucopyranoside.

LITERATURE CITED

1. K. Neelakantam, R. Rao, and T. R. Seshadri, Proc. Ind. Acad. Sci., 12A, 887 (1935).
2. S. Yu. Yunusov, Izv. Akad. Nauk UzSSR, No. 4, 11 (1948).
3. A. R. Guseva and M. N. Nestyuk, Biokhimiya, 11, No. 4, 480 (1953).
4. B. Makhsudova, Z. P. Pakudina, and A. S. Sadykov, "The chemistry of plant substances," Trudy TashGU, Tashkent, III, No. 341, 77 (1968).
5. Z. P. Pakudina and A. S. Sadykov, Khim. Prirodn. Soedin., 3, 98 (1967).
6. B. Makhsudova, Z. P. Pakudina, and A. S. Sadykov, Khim. Prirodn. Soedin., 5, 322 (1969).
7. P. K. Denliev, Z. P. Pakudina, and A. S. Sadykov, Dokl. Akad. Nauk UzSSR, No. 8, 34 (1962).
8. Z. P. Pakudina, A. S. Sadykov, and P. K. Denliev, Khim. Prirodn. Soedin., 1, 52 (1965).
9. Z. P. Pakudina, A. A. Rakhimov, and A. S. Sadykov, Khim. Prirodn. Soedin., 5, 126 (1969).
10. K. V. Rao and T. R. Seshadri, Proc. Ind. Acad. Sci., 24A, 375 (1946); 25A, 397 (1947); 26A, 292 (1947).
11. K. V. Rao and T. R. Seshadri, Proc. Ind. Acad. Sci., 24A, 352 (1946).
12. T. J. Mabry, J. Kagan, and H. Rosler, Phytochemistry, 4, 177 (1965).
13. J. Massicot and J. P. Marthe, Bull. Soc. Chem. France, 1962 (1962).