STRUCTURE AND PMR SPECTRA OF ISOQUERCITRIN AND HIRSUTRIN

Z. P. Pakudina, V. B. Leont'ev,
F. G. Kamaev, and A. S. Sadykov

UDC 547.972

Isoquercitrin (quercetin 3-glucoside) is widely distributed in the vegetable kingdom [1]. Since the properties of this compound as reported in various papers differ somewhat [2-5], the authors were obviously dealing with isomers.

From the flowers of the cotton plant of variety 108-F (Gossypium hirsutum) we have previously isolated quercetin 3-glucoside and called it "hirsutrin" [6, 7]. When the total flavonols from the leaves of the cotton plant of variety 108-F were separated on a column of Capron powder, after elution with 50% ethanol a glucoside with similar properties to those of hirsutrin (Table 1) and having an identical IR spectrum (Fig. 1) was isolated.

Hirsutrin is more readily soluble in dilute acetone and crystallizes in the form of light-yellow "warts." The glucoside obtained from the leaves, which we previously described as "isoquercitrin," is readily soluble in dilute ethanol and crystallizes in the form of bright-yellow "stars." These two forms are present simultaneously in the flowers and in the leaves, but one of them is present in a greater amount in the flowers, and the other in the leaves.

The glycosides differ in the melting points of their acetyl derivatives and in their specific rotations. The IR spectrum of isoquercitrin differs from that of hirsutrin [8, 9] in the 1100-1010 cm^{-1} region. Absorption in this region of the spectrum is characteristic for the sugar moiety of glycosides [10].

The results obtained show that isoquercitrin and hirsutrin obviously differ from one another only in the structure of the glucose. To solve this question, we used the proton resonance spectra.

Since a majority of the flavonoids found in nature dissolve poorly in the organic solvents used in NMR spectroscopy, the PMR spectra of soluble derivatives of the flavonoids, acetates and methyl and trimethylsilyl ethers, are usually studied instead [11-14].

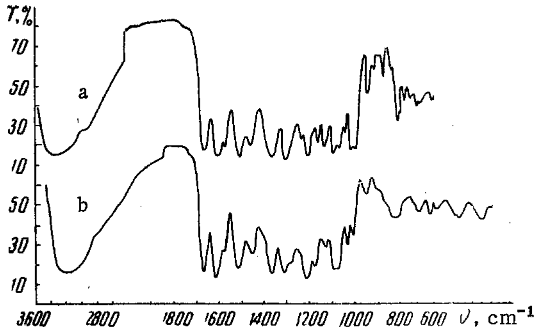

Fig. 1. IR spectra of: a) hirsutrin; b) isoquercitrin.

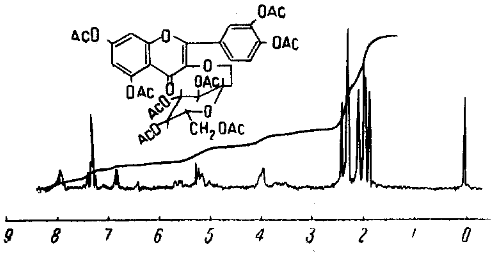

Fig. 2. NMR spectrum of acetylated hirsutrin.

Scientific-Research Institute for the Chemistry and Technology of Cotton Cellulose, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 555-559, September-October, 1970. Original article submitted May 4, 1970.

TABLE 1. Physicochemical Properties of Hirsutrin and Isoquercitrin

Properties of the flavonol	Hirsutrin	Isoquercitrin
Empirical formula	$C_{21}H_{20}O_{12}$	$C_{21}H_{20}O_{12}$
mp, °C $[\alpha]_D^{28}$, deg	221-222; 72.4 (pyridine)	218-220; −120.3 (pyridine)
Acetate of the glucoside	$C_{37}H_{36}O_{20}$	$C_{37}H_{36}O_{20}$
mp, °C	156-157	168-170
Aglycone	Quercetin	Quercetin
Sugar	Glucose	Glucose
Position of attachment of the sugar	C_3	C_3
Butan-1-ol—acetic acid—water (4 : 1 : 5)	0.69	0.69
60% Acetic acid	0.72	0.73
15% Acetic acid	0.42	0.43
UV spectra with additives, nm:		
C_2H_5OH	355, 255	360, 255
$+CH_3COONa$	365, 275	370, 265
$+H_3BO_3$	340, 265	380, 255
$+AlCl_3$	405, 270	405, 270
Qualitative reactions (coloration)		
Zn + HCl	Crimson-red	
$FeCl_3$	Dark green	
$ZrOCl_2$	Colorless	
Enzyme hydrolysis		
Aspergillus oryzae	Hydrolyzes	

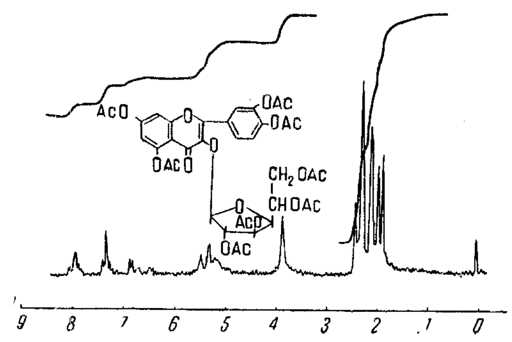

Fig. 3. NMR spectrum of acetylated isoquercitrin.

We have studied acetylated derivatives of isoquercitrin and hirsutrin in deuterochloroform solution. The samples were prepared at maximum concentration (8-10%). Hexamethyldisiloxane was used as the internal standard, its chemical shift in the δ scale being taken as 0.05 ppm.

The spectra were taken on a Hitachi H-60 spectrometer, the accuracy of the measurement of the chemical shift being ±0.01 ppm. An analysis of the PMR spectra of hirsutrin (Fig. 2) and of isoquercitrin (Fig. 3) in the weak-field region confirms the identity of their aglycone moieties. The parameters of the spectra (chemical shifts and spin–spin coupling constants) coincide with great accuracy. The assignment of the signals of the aromatic protons has been made and confirmed for numerous flavonoid derivatives [11-14].

The protons of ring B resonate in weaker fields than those of ring A, and the signal of the proton at C_6 is located in weaker fields than that of the proton at C_8.

The multiplet in the 7.8-8.1 ppm region represents a superposition of the signals of the protons at C_2' and C_6'; the signals of the protons at C_5' and C_8 and the doublet at 6.84 ppm characteristic for the proton at C_6 are located in the region of the chloroform signal. The spin–spin coupling constant, $J_{6,8} = 2$ Hz, corresponds to the usual value of the meta constant in benzene systems.

The signals of the acetyl groups appear in the 1.8-2.5 ppm region. A comparative study of the PMR spectra of the acetyl derivatives of flavonoids and their aglycones [14, 15] has established that the acetyl groups of an aglycone resonate in a weaker field than those of the sugar moiety. The acetyl groups of the aglycones of hirsutrin and isoquercitrin give identical signals in the 2.2-2.5 ppm region, the acetyl group at C_5 giving the signal in the weakest field (2.41 ppm) [14].

The protons of the acetyl groups of the sugar moiety resonate in stronger fields, at 1.8-2.2 ppm, and the shapes of the spectra in this region are different for hirsutrin and isoquercitrin, which confirms the hypothesis that these compounds differ only in the sugar moiety. If the sugar is not attached to the aglycone in position 3, the acetyl groups of the sugar moiety would be almost equivalent in the PMR spectra, which is not true in the spectra of hirsutrin and isoquercitrin. This can be explained by the anisotropy of the neighboring phenyl radical, which extends the spectrum of the acetyl groups. The value of the chemical shifts of the signals of the acetyl groups in the PMR spectrum of hirsutrin are 2.08, 1.96, and 1.86 ppm with integral intensities of 1 : 2 : 1, respectively, and for isoquercitrin they are 2 : 1 : 1, i.e., one of the acetyl groups has changed the value of its chemical shift. This information can be interpreted by assuming that the sugar residues consist of the pyranose and furanose forms of glucose. In the acetate of the furanose form, two of the acetyl groups are exocyclic, and in the acetylated pyranose form only one of them is exocyclic, which facilitates the identification of the structures of these forms in the compounds under study. A comparison of the spectra of hirsutrin and acetylated β-D-glucopyranose [15] in the region of the methylene and methine protons confirms this hypothesis. In spite of the coincidence of the spectra of acetylated hirsutrin and β-D-glucopyranose (with the exception of small changes in the chemical shifts), the parameters of the spectrum of acetylated isoquercitrin clearly differ. In the spectrum of acetylated isoquercitrin the signal in the 4.9-5.7 ppm region gives an integral intensity of 4 and the signal in the 3.82 ppm region, one of 3. Such values of the chemical shifts relate to the protons of the glucose. The combination of the signals of the protons at C_1, C_2, C_3, and C_4 corresponds to the chemical shift in the 4.9-5.7 ppm region and the signals of the protons at C_5 and C_6 to the shift at 3.82 ppm.

On the basis of their chemical properties and IR and NMR spectra, the structure of quercetin 3-β-D-glucopyranoside may be proposed for hirsutrin, and that of quercetin 3-β-D-glucofuranoside for isoquercitrin.

Consequently, the isoquercitrin widely found in nature consists either of a mixture of such isomers or of one of these forms, which have been described by various authors under the same name.

CONCLUSIONS

1. Two isomers of quercetin 3-glucoside are present in the flowers and leaves of the cotton plant of variety 108-F: hirsutrin, quercetin 3-β-D-glucopyranoside; and isoquercitrin, quercetin 3-β-D-glucofuranoside.

2. The quercetin 3-glucoside described by various workers under the single name "isoquercitrin" is a mixture of the pyranose and furanose forms, or is one of these two forms.

LITERATURE CITED

1. T. A. Geissman, ed., The Chemistry of Flavonoid Compounds, Pergamon Press (1962).
2. F. W. Heyl, J. Am. Chem. Soc., 41, 1289 (1919).
3. L. Attree and A. G. Perkin, J. Chem. Soc., 234 (1927).
4. B. H. Koeppen, Biochem. J., 83, 507 (1962).
5. V. S. Batyuk and L. F. Kol'tsova, KhPS [Chemistry of Natural Compounds], 381 (1968).
6. Z. P. Pakudina and A. S. Sadykov, KhPS [Chemistry of Natural Compounds], 98 (1967).
7. Z. P. Pakudina and A. S. Sadykov, Phenolic Compounds and Their Biological Functions [in Russian], Moscow (1968), p. 50.
8. Z. P. Pakudina, A. S. Sadykov, and P. K. Denliev, KhPS [Chemistry of Natural Compounds], 67 (1965).
9. B. Maksudova, Z. P. Pakudina, and A. S. Sadykov, KhPS [Chemistry of Natural Compounds], 11 (1967); 322 (1969).
10. I. P. Kovalev and V. I. Litvinenko, KhPS [Chemistry of Natural Compounds], 233 (1965).
11. T. J. Mabry, J. Kagan, and H. Roster, Phytochemistry, 4, 178 (1965).
12. A. C. Waiss, R. E. Lundin, and D. J. Stern, Tetrah. Let., No. 10, 513 (1964).
13. J. Missicot and J. P. Marthe, Bull. Soc. Chim. France, 1962 (1962).
14. C. A. Henrick and P. R. Jefferiss, Austr. J. Chem., 17, 934 (1964).
15. R. U. Lemieux and J. D. Stevens, Canad. J. Chem., 43, 2059 (1965).