INVESTIGATION OF FLAVONOID GLYCOSIDES

I. Monoglycosides

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Khimiya Prirodnykh Soedinenii, Vol. 1, No. 4, pp. 233-241, 1965

The flavonoids are one of the most widely distributed groups of natural compounds. The majority of them occur in plants in the form of glycosides the large variety of which is due not only to the position and wide choice of the sugars, but to the difference in the size of the oxide rings and also to the configuration of the glycoside bonds for one and the same carbohydrate constituent. The investigation of the structure of these compounds is carried out by various methods, To characterize the carbohydrate part of the glycosides it is necessary to carry out exhaustive methylation with subsequent hydrolysis and identification of the methylated sugars. These methods require considerable amounts of glycosides and are laborious.

Table 1

Characteristic Frequencies of the Pyranose and Furanose Rings in Hydrocarbons

Type	Nature of the Vibrations	Frequency cm^{-1}	References
1	Asymmetric vibrations of the pyranose ring	917 ± 13	[5]
2	Symmetrical vibrations of the pyranose ring	770 ± 14	[5]
2a	Deformation vibrations of an equatorial C_1–H group (α-anomer)	844 ± 8	[5]
2b	Deformation vibrations of an axial C_1–H group (β-anomer)	891 ± 7*	[5]
2c	Deformation vibrations of other equatorial C–H groups	880 ± 8	[6]
-	Deformation vibrations of the CH_2 groups in deoxypyranosides	867 ± 2	[7]
-	Deformation vibrations of the CH_3 groups in deoxypyranosides	967 ± 6	[7]
A	Symmetrical vibrations of the furanose ring	924 ± 13	[8]
B and C	Skeletal and deformation vibrations of the pyranose ring	879 ± 7 858 ± 7	[8]
D	Deformation vibrations of a C–H group connected with an O heteroatom	799 ± 17	[8]

* According to recent results [20], it is considered that the band at 895 cm^{-1} characterizes not the deformation vibrations of an axial C_1–H group, but the vibrations of a $-O-\underset{\displaystyle C}{\overset{\displaystyle O}{\underset{|}{\overset{|}{C_1}}}}-$ grouping in the chain.

The aim of the present work was to study the carbohydrate part of flavonoid glycosides by means of physicochemical methods (IR spectroscopy and polarimetry).

Some authors, studying the IR spectra of cyclohexane and tetrahydropyrane [1], cyclopentane [2], tetrahydrofuran [3], hydropyranols and hydrofuranols [4], and also the spectra of free sugars [5-9], have found characteristics permitting the pyranose and furanose forms of the carbohydrates and their α- and β-anomers to be distinguished, and equatorial C_2–H and C_4–H groups in the pyranosides, CH_2 and CH_3 groups in the deoxysugars, and the other features shown by the frequencies given in Table 1, to be detected.

However, the data of Table 1 have not been used up to now in the investigation of complex natural heterosides, since the absorption of the carbohydrate part in their spectra is masked by the absorption bands of the aglycones.

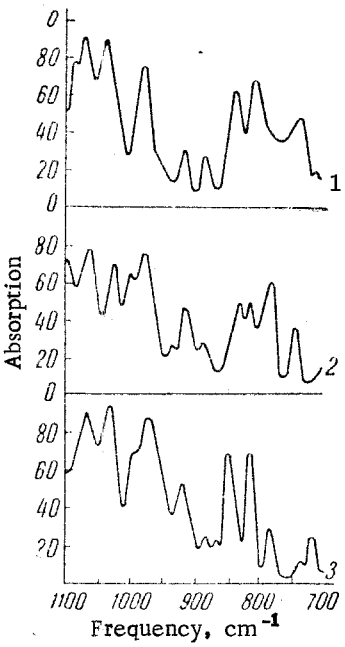
To study glycosides, in addition to IR spectroscopy it is possible to use the polarimetric method. Using the method of molecular rotation differences developed by Klyne [10] on the basis of the cardiac glycosides, it is possible to find the rotary contribution of the carbohydrate component of a heteroside (ΔC) from the equation

$$\Delta C = [M]_D \text{ of the glycoside} - [M]_D \text{ of the aglycone} \quad (A)$$

For glycosides with an optically inactive aglycone, the subtrahend is zero and Equation A assumes the form B:

$$\Delta C = [M]_D \text{ of the glycoside} \quad (B)$$

i.e., in this case the method of comparing molecular rotations is used [11]. It has been established from a considerable amount of experimental material that ΔC and $[M]_D$ for comparable glycosides with the same type of glycoside bond and size of the oxide ring can differ fron one another to the extent of ±100°, while α- and β-anomers differ by 350-500°[10].



IR spectrum of α-L-rhamnopyranose (1) and differential spectrograms of the carbohydrate part of franguloemodin 6-α-L-rhamnopyranoside (2) and kaempferol 7-α-L-rhamnofuranoside (3).

The first attempt to use the method of comparing molecular rotations for the analysis of flavonoid glycosides was made by Bredenberg and Hietala [12], who had employed it successfully for comparing the molecular rotations of the corresponding phenyl glycosides. This choice was apparently made in connection with the influence of an aromatic aglycone on the glycosidic center of a carbohydrate [13].

We have tested the possibility of using this method on flavonoid glycosides and also on some glycosides of anthraquinone, furocoumarins, and cardenolides (Table 2) and have found that more accurate results are obtained when differences in the molecular weights of the flavonoids and the glycosides investigated are taken into account. Consequently, to introduce a correction into the value of the molecular rotations of the glycosides we have proposed a coefficient K_p, equal to the quotient obtained by dividing the molecular weight of the phenyl glycoside by the molecular weight of the glycoside concerned. For comparison, the products of the molecular rotation and the coefficient K_p found have been used.

Assuming that the IR spectra of the heterosides are composed to some approximation additively from the spectra of the aglycone and the monosaccharide, in the spectral study of the flavonoid glycosides we attempted to use the method of differential analysis. To exclude the contribution of the aglycone in the spectra of the glycoside, an equivalent amount of the corresponding aglycone compressed into potassium bromide tablets was placed in the comparison channel. The IR spectra were obtained on an IR-10 spectrometer in the range from 1100 to 700 cm^{-1} using 1-2 mg of glycoside. Results of the study of 19 monoglycosides are given in the present paper (see Tables 2 and 3).

As is clear from Table 2, the presence of three strong absorption bands in the region from 1100 to 1010 cm^{-1} connected with the vibrations of the ring and with C—O stretching vibrations is probably characteristic for the pyranosides [4]. These bands are present both in the spectra of the monosaccharides (β-D-glucopyranose and α-L-rhamnopyranose) used as comparison samples and in the spectra of the carbohydrate part of the monoglycosides. The bands at 1000 ±10 cm^{-1} (strong and medium intensities) apparently relate to the stretching vibrations of C—O in the —O—C—O— grouping of the glycoside linkage [9]. In the case of the deoxysugars (L-rhamnosides) there is a band at 970 cm^{-1} relating to the terminal CH_3 group [7]. Barker and coworkers [5, 6] assigned the band at 917 ±13 cm^{-1} to the asymmetric vibrations of the pyranose ring. This band is weak and is not present in all the spectra of the monosaccharides and the carbohydrate parts of the glycosides. The band at 890 cm^{-1} is a reliable indication of the β-configuration of the glycosidic linkage; it is observed in all β-glycosides and is absent from α-glycosides. The α-configuration of the glycosidic linkage can be determined from the band at 840 ±10 cm^{-1} [5], together with the simultaneous absence of the band at 890 cm^{-1}. Equatorial C_2—H and C_4—H groups in the rhamnosides and galactosides give a low-intensity band at 876 ±9 cm^{-1}. One of the bands at 805 and 770 cm^{-1} relates to the symmetrical vibrations of a pyranose ring [5, 6, 14].

On investigating the furanosides (cf. Table 3), it was found that, in contrast to the pyranosides, they each have only two absorption bands in the 1100-1010 cm^{-1} region. Since the furanoside ring has less well-defined differences in the conformations, it is probably impossible to expect large differences in the appearance of the absorption bands of the C—H groups as a function of their spatial arrangement. This may make the detection of α- and β-anomers difficult, even with the known classification of the bands in the 930-800 cm^{-1} region into A, B, C, and D types, which should characterize the furanosides [8] (cf. Table 1). Other groupings such as CH_3— and —O—C—O— of the glycosidic linkage are located from absorption bands in the same region as in the pyranosides. As an example, the Figure shows the IR spectrum of α-L-rhamnopyranose and the differential spectrograms of the carbohydrate part of some rhamnosides.

Thus, it is possible to determine the pyranose and furanose forms of the carbohydrate substituent and also the configuration of the glycosidic bonds in pyranosides on the basis of the IR spectra. However, the question of the configuration

Table 2

Absorption Bands of the Carbohydrate Part of Flavonoid Glycosides (Pyranosides), cm^{-1}

No.	Compound	Ring vibrations and CO vibrations			—O—C—O of the glycosidic linkage	Ring —CH_3	Asymmetric vibrations of the ring	β-Anomer	Equatorial vibrations of C_2—H and C_4—H groups	α-Anomer	Symmetrical vibrations of the ring	Other bands
1	α-L-rhamnopyranose	1082 s *	1069 s	1033 s	—	974 s	909 m	—	877 m	832 m	733 w	715 w
2	Isorhamnetin 4'-L-rhamnoside	1110 s	1065 s	1015 s	993 m	972 s	932 m 918 m	—	882 w	846 m	814 s 770 m	728 w 714 m
3	Franguloemodin 6-1-rhamnoside	1096 m	1064 s	1022 m	1000 m	974 s	932 w 915 m	—	883 w	830 m	814 m 778 s	742 m 700 w
4	Xanthotoxol 8-L-rhamnoside	1080 m	1058 s	1036 m	1007 s	948 s	917 s	—	875 w	840 m	816 m 764 m	730 w 701 w
5	Kaempferol 7-L-rhamnoside	1088 s.	1055 m	1016 m	1001 m	940 m	930 m	898 w	870 w	—	822 m 788 m	737 w 707 w
6	β-D-Glucopyranose	1070 m	1048 s	1017 s	992 s	—	912 s	885 w	—	—	775 m	737 w
7	Isoliquiritigenin 4-D-glucoside	1079 s	1043 s	1027 s	997 s	—	—	888 w	—	—	805 m 765 w	—
8	Liquiritigenin 7-D-glucoside	1087 s	1045 s	1025 m	998 m	—	905 w	897 m	—	—	847 m 765 m	736 w 717 w 702 w
9	Liquiritigenin 4'-D-glucoside	1085 s	1047 s	1027 m	999 s	—	—	897 m	—	—	805 w 757 w	842 m 735 w
10	Luteolin 7-D-glucoside	1090 s	1051 s	1028 m	994 m 981 m	—	920 w	898 m	—	—	805 w 791 w	831 m 714 w 700 w

Table 2 Continued

11	Luteolin 3'-D-glucoside	1085 s	1055 s	1025 m	993 m 982 m	—	921 w	898 m	—	—	803 w 757 w	831 m 736 w 793 w 715 w
12	Quercetin 3-D-glucoside	1081 s	1055 s	1027 m	996 m	—	920 w	890 m	—	—	804 w 771 m	936 m 838 815 m 790 w
13	Strophanthidin 3-D-glucoside	1104 s	1068 s	1022 s	990 m	—	914 m	899 w	—	—	805 w 763 w	834 m 794 w 735 w 715 w
14	Quercetin 3-D-galactoside	1090 s	1065 m 1050 m	1022 s	998 m 991 m	—	925 m	895 w	870 w	—	802 w 767 s	854 m 817 w 757 s 732 w 712 w —

*Similar concentrations of the compounds under investigation were used, in the main, and the relative intensity characteristics of the bands are therefore given in the following form: strong (c.) – absorption greater than 70%; medium (cp.) – greater than 30%; and weak (c) – up to 30%.

Table 3

Absorption Bands of the Carbohydrate Part of Flavonoid Glycosides (Furanosides), cm^{-1}

No.	Compound	Ring vibrations and C–O vibrations			–O–C–O– of the glycosidic linkage	Terminal $-CH_3$	*A*	*B*	*C*	*D*	Other bands		
15	Quercetin 3-L-arabinoside	1073 m	1047 m *	—	1007 m 997 m	—	927 m	882 w	849 w	805 m	953 s 770 w	831 m	771 w
16	Kaempferol 3-L-arabinoside	1071 m	1044 s	—	1006 s 976 s	—	925 m	873 w	850 s	807 s	944 s 718 m	825 m	737 m
17	Isorhamnetin 3-D-glucoside	1080 s	1036 s	—	1004 s 970 m	—	925 w	885 w	840 w	796 w	819 w	717 w	
18	Kaempferol 3-D-glucoside	1064 s	1032 s	—	993 m 972 s.	—	918 m	881 w	857 w	841 s 716 w	780 w	731 w	
19	Quercetin 3-L-rhamnoside	1082 m	1062 m	—	1007 m 1000 m	964 s	921 m	884 w	850 w	789 w	824 m	742 w	715 w
20	Kaempferol 3-L-rhamnoside	1098 m	1062 s	—	1005 m 997 m	977 m	920 m	880 m	860 w	814 m	948 s 786 m	842 m 713 m	831 m
21	Kaempferol 7-L-rhamnoside	1064 m	1033 s	—	993 m	973 s.	918 m	880 w	857 w	810 s	840 s 717 w	778 m	731 w

* Note as for Table 2.

Table 4

Analysis of the Configuration of the Glycosidic Linkages and the Size of the Oxide Rings in the Carbohydrate Part of Flavonoid Glycosides

Nos.*	Glycoside	$[M]_D$	K_p	$[M]\cdot K_p$	Configuration of the glycosidic linkages		Size of the oxide ring in the carbohydrate part of the glycosides	
					A	B	A	B
1/15	Quercetin 3-L-arabinoside	−731.0	0.52	−380.0	α	α	Furanoside	Furanoside [15]
2—	Isorhamnetin 3-L-arabinoside	−797.0	0.50	−399.0	α	—	Furanoside	—
3/16	Kaempferol 3-L-arabinoside	−705.0	0.54	−381.0	α	α	Furanoside	Furanoside [15]
4—	Quercetin 3-L-arabinoside	−112.0	0.52	− 59.0	β	—	Furanoside	—
	Phenyl α-L-arabinopyranoside	+ 13.5	1.00	+ 13.5				
	Phenyl β-L-arabinopyranoside	+540.0	1.00	+540.0				
	Phenyl α-L-arabinofuranoside	−359.0	1.00	−359.0				
	Phenyl β-L-arabinofuranoside	− 59.0	1.00	−59.0				
5—	Quercetin 3-L-rhamnoside	−166.0	0.54	− 90.0	β	—	Furanoside	—
6/19	Quercetin 3-L-rhamnoside	−748.0	0.54	−404.0	α	—	Furanoside	—
7/20	Kaempferol 3-L-rhamnoside	−765.0	0.55	−421.0	α	—	Furanoside	—
8/21	Kaempferol 7-L-rhamnoside	−864.0	0.55	−475.0	α	—	Furanoside	—
9/3	Franguloemodin 6-L-rhamnoside	−395.0	0.57	−225.0	α	—	Pyranoside	—
10/4	Xanthotoxol 8-L-rhamnoside	−355.0	0.77	−249.0	α	α	Pyranoside	Pyranoside [16]
	Phenyl α-L-rhamnopyranoside	−254.0	1.00	−254.0				
	Phenyl β-L-rhamnopyranoside	+210.0	1.00	+210.0				
	Phenyl α-L-rhamnofuranoside	−410.0	1.00	−410.0				
	Phenyl β-L-rhamnofuranoside	− 90.0	1.00	− 90.0				
11/—	Isoliquiritigenin 4'-glucoside	−254.0	0.61	−155.0	β	β	Pyranoside	Pyranoside [17]
12/7	Isoliquiritigenin 4-glucoside	−257.0	0.61	−157.0	β	—	Pyranoside	—
13/8	Liquiritigenin 7-D-glucoside	−252.0	0.61	−154.0	β	—	Pyranoside	—
14/10	Luteolin 7-glucoside	−448.0	0.57	−255.0	β	β	Pyranoside	Pyranoside [18]
15/11	Luteolin 3'-D-glucoside	−448.0	0.57	−255.0	β	—	Pyranoside	—
16/12	Quercetin 3-D-glucoside	−371.0	0.55	−204.0	β	—	Pyranoside	—
17/17	Isorhamnetin 3-D-glucoside	−646.0	0.53	−342.0	β	—	Furanoside	—
	Phenyl α-D-glucopyranoside	+402.0	1.00	+402.0				
	Phenyl β-D-glucopyranoside	−182.0	1.00	−182.0				
	Phenyl α-D-glucofuranoside	+155.0	1.00	±155.0				
	Phenyl β-D-glucofuranoside	−364.0	1.00	−364.0				
18/14	Quercetin 3-D-galactoside	−274.0	0.55	−151.0	β	β	Pyranoside	Pyranoside [19]
19/18	Kaempferol 3-D-galactoside	−538.0	0.57	−307.0	β	—	Furanoside	—
	Phenyl α-D-galactopyranoside	+555.0	1.00	+555.0				
	Phenyl β-D-galactopyranoside	−110.0	1.00	−110.0				
	Phenyl α-D-galactofuranoside	+403.0	1.00	+403.0				
	Phenyl β-D-galactofuranoside	−379.0	1.00	−379.0				

*For comparison the second numbers are taken from Tables 2 and 3;
A — our results, B — literature data.

of the glycosidic bond in the furanosides remains unresolved. In order to determine the configuration of these bonds, and also to confirm the spectral data obtained from the investigation of the pyranosides and their glycosidic linkages, we used the modified method of comparing the molecular rotations of the flavonoid glycosides (Table 4).

It can be seen from Table 4 that the pyranose and furanose forms of the carbohydrate part and also the configuration of the glycosidic linkage in the glycosides can be determined from the magnitude of the molecular rotation. The data of Table 4 agree fairly well with the spectral data.

Summary

1. The method of differential analysis in the IR region of the spectrum has been used in an investigation of the structure of the carbohydrate part of flavonoid glycosides. This method permits the pyranose and furanose forms of the carbohydrate substituents, the α- and β-anomers of the pyranosides and other structural features characterizing the nature of the sugars in the glycosides to be determined. The method can also be used to study other glycosides – for example, hydroxyanthraquinone glycosides, furocoumarin glycosides, and cardiac and other glycosides.

2. A modified method of comparing molecular rotation has been proposed for determining the configuration of the glycosidic linkages and the size of the oxide rings in the carbohydrate part of the flavonoid glycosides.

REFERENCES

1. S. Burket and R. M. Badger, J. Am. Chem. Soc., 72, 4397, 1950.
2. J. E. Kilpatrik, K. S. Pitzer, and S. Spitzer, J. Am. Chem. Soc., 69, 2483, 1947.
3. G. M. Barrow and S. Searles, J. Am. Chem. Soc., 75, 1175, 1953.
4. N. Bagget et al., J. Chem. Soc., 4565, 1960,
5. S. A. Barker et al., J. Chem. Soc., 171, 1954.
6. S. A. Barker, et al., J. Chem. Soc., 3468, 1954.
7. S. A. Barker et al., J. Chem. Soc., 4211, 1954.
8. S. A. Barker and R. Stephens, J. Chem. Soc., 4550, 1954.
9. T. Urbanski, W. Hofman, and M. Witakowski, Bull. Akad. Polon. Sci. Ser. Sci. Chim., geol. et geogr., 7, 619, 1959.
10. W. Klyne, "Optical Rotation," in: Determination of Organical Structures by Physical Methods, Acad. Press., N. Y., 98, 1955.
11. A. P. Terent'ev and V. M. Potapov, Fundamentals of Stereochemistry [in Russian], Moscow and Leningrad, 253, 1964.
12. J. B. Bredenberg and K. P. Hietala, Acta. Chem. Scand., 15, 936, 1961.
13. L. A. Chugaev, Selected Works [in Russian] vol. 2, Moscow, 1955.
14. L. V. Solozhenikina, V. D. Shcherbukhin, and B. N. Stepanenko, DAN SSSR, 153, 960, 1963.
15. V. N. Spiridonov, A. P. Prokopenko, and D. G. Kolesnikov, ZhOKh, 34, 4126, 1964.
16. N. F. Komissarenko and V. T. Chernobai, ZhOKh, 34, 4126, 1964.
17. H. Hishida, J. Chem. Soc. Japan, 79, 714, 1958.
18. L. I. Dranik, V. T. Chernobai, and D. G. Kolesnikov, Med. prom. SSSR, no. 5, 23, 1964.
19. W. Karrer, Konstitution und Vorkommen der organischen Pilanzenstoffe, Basel, Birkhauser, 1958.
20. C. Y. Liang and R. H. Marchessault, J. Polymer Sci., 39, 339, 1959.

23 March 1965

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