A COMPARATIVE INVESTIGTION OF THE IR SPECTRA OF A CARBOHYDRATE SERIES

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ABSTRACT

The IR-Fourier spectra of a series of carbohydrates whose molecules contain pyranose rings interconnected by α-1.4-glycosidic chemical bonds are recorded and compared. The compounds studied include oligosaccharides from maltose to maltoheptaose, amylose, amylopectin and several samples of starch. The main difference found in the spectra studied relates to the dominant lines in the range of 960 cm^{-1} - 1060 cm^{-1} referring to two Gaussian components. Most probably, one of them corresponds to the stretching vibrations of α-glycosidic bonds, while the other relates to the complex of C-O, C-C, and C-O-H vibrations. The ratio of the two components values depends on the glycosidic bonds and pyranose rings number in the sugar molecules and can therefore be used to characterize the degree of carbohydrates polymerization. The comparison of the IR-Fourier spectra of starch, amylose, and amylopectin in the range investigated shows that the former is much closer in its character to amylose than to amylopectin. There are spectral lines which are almost indistinguishable from those of amylose, which suggests that they refer to weakly branched forms with a prevalence of identical glycosidic bonds.

Keywords: IR-Fourier spectrum, carbohydrate, oligosaccharide, glucose, starch, α-1,4-glycosidic bond, pyranose ring.

INTRODUCTION

A carbohydrate is any representative of a large group of compounds that contain carbon, hydrogen, and oxygen and have the general formula $C_x(H_{20})_y$. Carbohydrates (monosaccharides, oligosaccharides, polysaccharides) are an important source of energy: they are produced by plants and entering the body of animals and humans through food being one of the three main components of food. All carbohydrates are ultimately broken down in the body to simple glucose sugar, which then takes part in the metabolic processes with the release of energy. The excess carbohydrates, not required by the body for an immediate use, accumulate in the liver and the muscles as glycogen. Vegetable carbohydrates are an important building material (for example, cellulose), as well as a depot of finished products (mainly in the form of starch) [1 - 3]. Carbohydrates, despite the extremely deep elaboration of the structure and properties problems, remain an object of keen scientific interest. Recently, thanks to new research tools, additional opportunities have appeared for analyzing individual sugars.

The article presents the results of a series of carbohydrates studied by IR-Fourier transform spectroscopy, starting with maltose disaccharide and ending with high-polymer amylose, whose molecules are built from pyranose glucose rings interconnected by identical α-1.4-glycosidic C-O-C bonds, also denoted as α-(1→4).

EXPERIMENTAL

Substances of brief characteristics given in Tables 1 and 2 were objects of research. The IR-Fourier spectra of

Table 1. Characteristics of carbohydrates used for the research.

No	Trivial (common) name of carbohydrate	Content of the main sustance, %, additional characteristics	The ratio of the number of bonds α (1-4) to the number of pyranose rings in the sugar molecule	Supplier company, serial number
1	Glucose	≥ 99.5 % D- (+) - glucose BIOXTRA	0.0	Sigma No G8270
2	Maltose	≥ 98 % D - (+) - maltose monohydrate	0.50	Sigma-Aldrich No M5885
3	Maltotriose	98 %	0.67	Sigma-Aldrich No M8378, Lot 017K0679
4	Maltotetraose	96 %	0.75	Sigma-Aldrich No M8253, Lot 109K1271
5	Maltopentaose	96 %	0.80	Sigma-Aldrich No M8128, Lot 040M1774
6	Maltohexaose	≥ 90 %	0.83	Sigma-Aldrich No M9153
7	Maltoheptaose	94 %	0.86	Sigma-Aldrich No M7753, Lot 079K0987
8	Amylose	98 %	1.00	City Chemical LLC, Lot 01M54
9	Amylopectin	≥ 95 %	1.00	Sigma-Aldrich, 10118, Lot 1422493

Table 2. Samples of starches studied.

No	Manufacturer	Producing country
1	Birkamidon	Poland
2	Windmile	Holland
3	KMC Brander	Denmark
4	Birkamidon GmbH	Germany
5	Merille	France

these substances were recorded on FT-IR Nicolet 6700 spectrometer of Thermo Electron Corporation in the mode of disturbed total internal reflection. A horizontal prefix ZnSe 45° with a 12-fold reflection of the infrared laser beam at a depth of penetration into the sample of ~ 2 μm was used. The spectra were recorded at a room temperature with a resolution of 4 cm^{-1}, and a measurement accuracy of ± 0.5 cm^{-1}. The number of scans referred to 32. The structure of the individual spectral bands was analyzed using Origin Pro 8 program resources.

RESULTS AND DISCUSSION

The infrared spectrum of glucose monosaccharide (Fig. 1), as an ancestor of the studied series of compounds, is identical to those described in the literature [1 - 5], and also presented in the NIST database. According to the authors' interpretations the valence vibrations of the C-O (νCO), C-C (νCC) and C-C-H (νCOH) chemical bonds are the most pronounced in the spectrum of the substance. A similar type of vibrations of O-H and C-H bonds is found in the form of relatively weak bands. The deformation vibrations of C-C-O and C-H bonds are somewhat more intense when compared to those of O-C-H (δOCH), C-O-H (δCOOH) and C-C-H (δCCH) bonds. The second representative of the disaccharide series, maltose, can be considered as a condensation

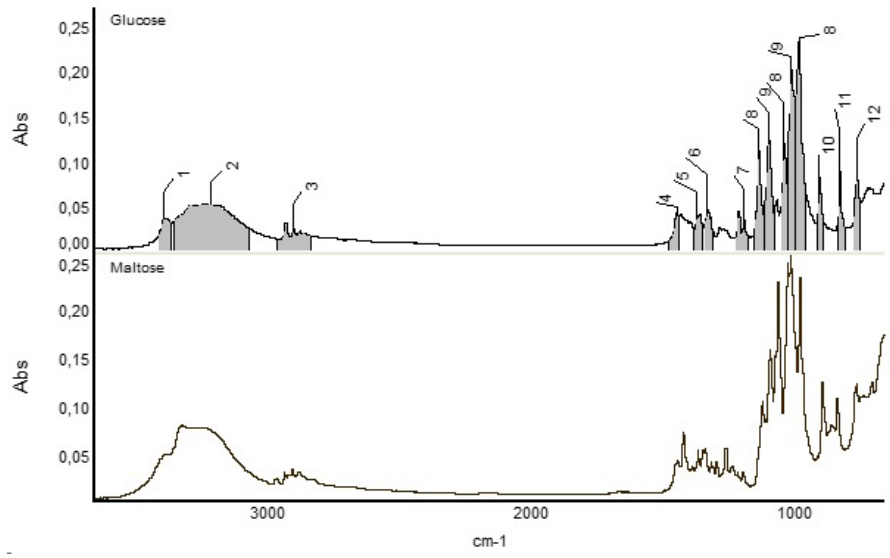

Fig. 1. IR spectra of glucose (a) and maltose (b). Areas of manifestation of bonds: 1 - νOH (hydroxyl), 2 - νOH (H_2O), 3 - ν_sCH and ν_{as}CH, 4 - δCH_2+δOCH+δCCH, 5 - δCH_2+δCOH+δCCH, 6 - δOCH+δCCH, 7 - δCH+δOH, 8 - νCO+νCC, 9 - νCO, 10 - νCO+νCCH, 11 - δCH, 12 - δCCO+δCCH.

product of glucose, one of the molecules of which has lost the hydroxyl group associated with the anomeric carbon atom, and the other a similar group at the fourth carbon atom, and the fourth carbon atom. Due to its molecular structure, the infrared spectrum of maltose should be similar in nature to that of glucose, but, at the same time, it should have significant differences. Indeed, some of the lines, characteristic of the glucose spectrum, namely those of νOH, νCO, δCOH, δOCH and δCCO, are significantly weakened or disappear altogether (Fig. 1). And this is natural, since it is these links that should be most changed as a result of the formation of the-1,4-glycosidic bridge. In fact, the spectral manifestation of most of maltose chemical bonds is well known [2, 3, 6 - 8]. But this is not valid for α(1→4) bonds. Thus, a number of publications [4, 5, 9 - 12] report that the absorption bands recorded in the ranges of 750 cm^{-1} - 950 cm^{-1} , 930 cm^{-1} - 940 cm^{-1} , and 1140 cm^{-1} - 1175 cm^{-1} could be assigned to these bonds. According to refs. [11 - 14], the presence of C–O–C bond appears as maxima at 1105 cm^{-1} and 1150 cm^{-1}, while according to refs. [3, 15] it corresponds to a band in the range of 966 cm^{-1} - 993 cm^{-1}. In terms of glycosidic bond identification in the carbohydrates IR spectra, a useful information may be obtained by comparing the spectra of glucose homologs and those of several pyranose rings containing more than two carbon atoms.

Indeed, a gradual change of the proportion of α(1→4)

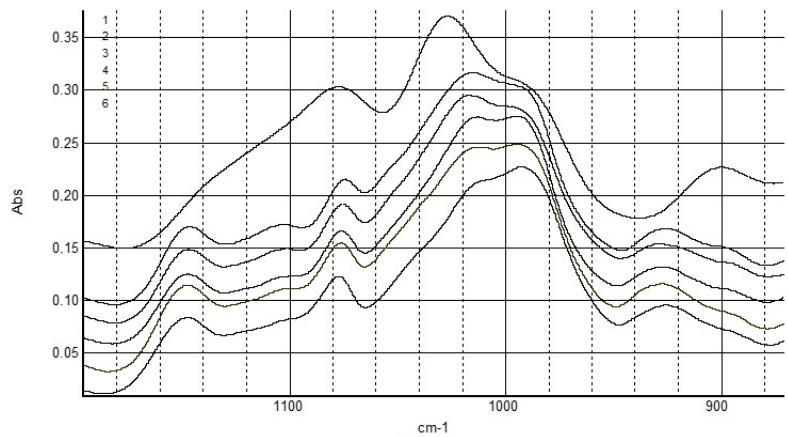

Fig. 2. IR spectrum: maltose (1), maltotriose (2), maltotetraose (3), maltohexaose (4), maltgeptaose (5), amylose (6). Spectrum line 1 is strongly maleficiated.

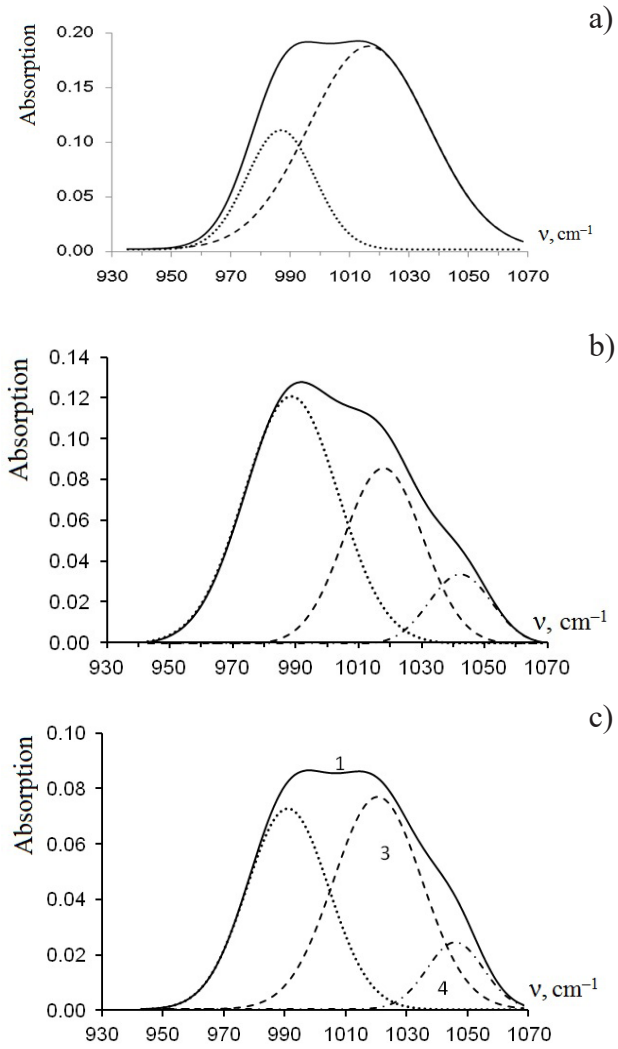

Fig. 3. The structure of the dominant lines in the IR spectra of maltotetraose (a), amylose (b) and amylopectin (c): 1 - the profile of the experimental spectral line, 2-4 - its constituent components.

bridges due to an increase or a decrease of the length of the molecular chains (a degree of polymerization) can appear in the IR spectra in the form of a smooth and distinct redistribution of the position and the magnitude of the individual lines referring to the bond type. Such a practical approach provides to follow more accurately the evolution of the desired chemical bonds, since it eliminates the jump-like factor and the hardly predicted changes of the corresponding spectral lines. The infrared spectra of oligosaccharides differ from those of glucose and maltose by the significantly lower resolution of the bands recorded in the range of 960 cm^{-1} - 1060 cm^{-1}

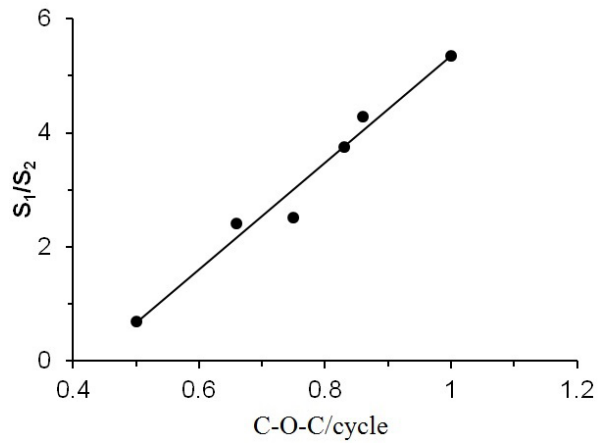

Fig. 4. Relationship between the relative area S_1 and S_2 of the calculated spectral peaks with maxima of 986 cm^{-1} - 988 cm^{-1} and 1,013 cm^{-1} - 1,017 cm^{-1} and the share of glycosidic bonds in molecules of oligosaccharides.

(Fig. 2), whose profile differs somewhat. This is especially valid for the most intensive of them. The spectral bands outside the range specified are identical for all carbohydrates. The spectrum of maltose in this range is almost identical to that of oligosaccharides, but only after a significant smoothing.

The results of the graphical analysis show that the bands of all oligosaccharides studied in the range of 960 cm^{-1} - 1060 cm^{-1} consist of low and high frequency components, as shown in Fig. 3 using the maltotetraose spectrum as an example. The position of each component in the spectrum is almost independent of the oligosaccharide type: in fact, their peaks are not recorded beyond 986 cm^{-1} - 988 cm^{-1} and 1013 cm^{-1} - 1017 cm^{-1}. The situation referring to the size of the calculated components is different: the increase of the chain length of the carbohydrate and the glycosidic bonds proportion leads to an increase of the lower frequency, and a higher frequency decrease. This is illustrated by the linear increase of the ratio of the magnitude of the components in the form of areas S_1 and S_2 (Fig. 4).

If the spectral lines comparison considered in refs. [3, 16] is accepted, then it can be assumed that the low-frequency component exhibits $\alpha(1 \rightarrow 4)$ bonds vibrations. In this case, the intensity of the calculated peak could be used as a criterion of the content of C-O-C bridging bonds or as a measure of the degree of polymerization of the carbohydrates studied.

The estimation does not require the peak itself, but

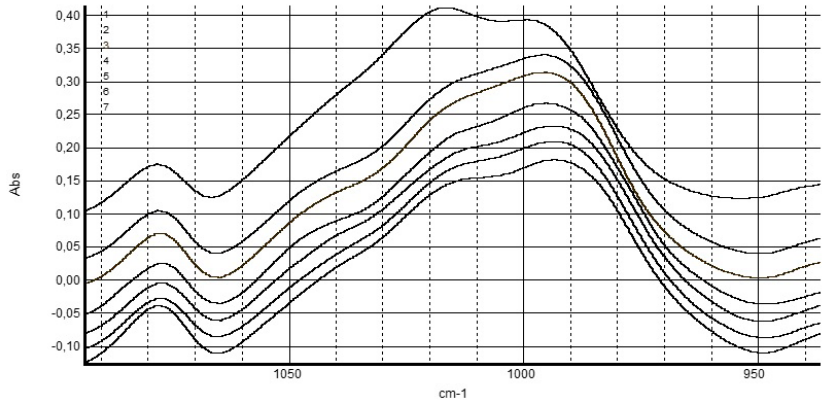

Fig. 5. The low-frequency region of the IR spectra of amylopectin (1), samples of starch listed in Table 2 (No 6) and amylose (7).

its relation to the spectral band, which depends little on the length of the sugar chain. This can be a line close to that of $\alpha(1\rightarrow4)$ in respect to its intensity and directly adjacent to it, i.e. it refers to the high frequency component mentioned above. It is assumed that the complex of νCO, νCC and δCOH vibrations [3, 15-17] is manifested in the IR spectra.

Then, a linear correlation between the ratio of the low- to the high-frequency component and that of the number of α1.4-glycosidic bonds to the number of pyranose cycles in the sugar molecules can be used to estimate the average degree of polymerization. The structural unit of the polymer is referred here to a pyranose ring.

The calculations can be done using the simple formula:

$n = 9.37/(5.335\text{-}S_1 / S_2)$,

where S_1 and S_2 are the areas of the low- and the high-frequency component of the spectral band in the range of 960 cm^{-1} - 1060 cm^{-1}. Thus, the problem of estimating the molecular composition of oligosaccharides by IR spectra, which is noted in refs. [11, 14], can be partially solved.

It is interesting to compare the spectrum of amylose, as an extreme member of a series of linear carbohydrates with $\alpha(1\rightarrow4)$bonds, with the spectrum of amylopectin, which is the second highly polymerized macrocomponent of natural starch, but with a different structure. As it turns out, the spectra of both polysaccharides are very close in magnitude, frequency, and profile in respect to most of the lines. This does not apply to the lines dominant in the spectra of both amylose and amylopectin, which have a different profile with an equal relative value (Fig. 3). The bands under consideration in both polysaccharides' spectra can be graphically divided not into two, as in oligomers, but into three components, the areas of which are of ratios of 56/33/11 in amylose and of 42/48/10 in amylopectin. The frequency of the peaks in these ratios increases from left to right. The highest frequency component provides an almost identical but not very large contribution to the dominant maximum, whereas the other two determine in fact the spectral line shape. The amylose/amylopectin ratio in the substances contained by such polysaccharides, for example starch, can be estimated on the ground of these two design bands.

Fig. 5 shows that the dominant lines in the spectra of starch provided by different manufacturers are intermediate between the maxima of amylose and amylopectin. Contrary to the expectations, they resemble much more the spectrum of a linear polysaccharide, while much less that of amylopectin.

It should be noted that there are samples among the starches studied in this work, whose spectra in the range of 960 cm^{-1} - 1060 cm^{-1} are very close to that of amylose. None of these samples shows a spectrum close to that of amylopectin.

CONCLUSIONS

It is concluded that the individual oligosaccharides from maltotriose to malteptaose have IR Fourier spectra of a similar nature. Maltose and amylose can be considered a natural continuation of this series, where they have a different location. The main differences in the spectra of the polysaccharides studied refer to the dominant maxima lying in the range of 960 cm^{-1} - 1060 cm^{-1}. The graphical analysis carried out shows that such maxima

consist of two components. That of a lower frequency is most probably attributed to the stretching vibrations of α-1.4-glycosidic bonds, while the second one is determined by the complex of νCO, νCC and δCOH oscillations. The ratio of these two components depends on the $\alpha(1\rightarrow4)$ bridges content of the individual sugars molecules and can therefore be used to characterize the degree of polymerization of these substances.

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