Carbohydrate Research, 51 (1976) 1–8

STUDY OF THE INFRARED SPECTRA OF OLIGOSACCHARIDES IN THE REGION 1,000–40 cm^{-1}

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(Received March 5th, 1976; accepted for publication, June 7th, 1976)

ABSTRACT

The i.r. spectra of disaccharides differing in monosaccharide composition and in the position and configuration of the glycosidic linkage, and also those of raffinose and model saccharides, were studied in the region 1,000–40 cm^{-1}. Two ranges may be of interest for structural analysis. The first, called "the anomeric region", is suitable for the determination of the configuration of the glycosidic linkage. The spectra of the oligosaccharides in the second region, called "the region of crystallinity", depend upon the packing of the molecules in the solid. The reasons for the present impossibility of using the far-infrared region of the i.r. spectra of lower oligosaccharides for the determination of the position of the glycosidic linkage are considered.

INTRODUCTION

The search for new physical methods for rapid and reliable identification of partial-hydrolysis products of polysaccharides and for the investigation of their structure has led to an extensive study of the spectra of carbohydrates in the far-infrared region[1–5]. Below 1000 cm^{-1}, the infrared (i.r.) spectra of carbohydrates are individual, and a number of bands are seen that increase at low temperature[5].

In the present paper, the interrelation between the monosaccharide composition of oligosaccharides, the configuration and the position of the glycosidic linkage, the values of the conformational parameters[6] ϕ and ψ, and the spectral characteristics in the region 1,000–40 cm^{-1} are considered. The compounds listed in Table I were studied.

X-Ray data indicate that the pyranose residues of compounds **1–3**, **6**, and **13** adopt the $^4C_1$(D) conformation[7–11]. The same conformation is postulated for the pyranose residues of disaccharides **4** and **5**.

EXPERIMENTAL

Compounds **1–4**, **6**, **7**, and **9–13** (Chemapol, Czechoslovakia, and E. Merck, West Germany) were further purified by recrystallization from suitable solvents,

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TABLE I

THE STRUCTURE OF THE COMPOUNDS INVESTIGATED

No.	*Compound*	*Glycosidic linkage in reducing disaccharides*		ϕ *(degrees)*	ψ *(degrees)*
		Configuration	*Position*		
1	β-Maltose monohydrate	α	1→4	181	193
2	α-Lactose monohydrate	β	1→4	−92.6	94.6
3	β-Cellobiose	β	1→4	−77.8	106
4	β-Gentiobiose	β	1→6		
5	3-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-glucose	β	1→3		
6	Sucrose				
7	α-D-Glucose				
8	β-D-Glucose				
9	Methyl α-D-glucopyranoside				
10	Methyl β-D-glucopyranoside				
11	Methyl β-D-galactopyranoside				
12	β-D-Fructose				
13	Raffinose pentahydrate				

and were then dried *in vacuo* over phosphorus pentaoxide at 25°. Compounds **1**, **2**, and **13** were hydrates. β-D-Glucose was obtained by crystallization, by addition of alcohol to a hot, aqueous solution of D-glucose. Disaccharide **5** was synthesized as described[12]. Melting points and optical rotations of the compounds were in good agreement with the data in the literature. Amorphous samples of D-glucose and disaccharides **1**–**3** were obtained by dissolution of the appropriate, crystalline compound in water, and lyophilization when mutarotation was complete.

I.r. spectra were recorded on Perkin–Elmer Model 257 (1,000–625 cm^{-1}), UR-20 (700–400 cm^{-1}), or FIS-21 (500–40 cm^{-1}) i.r. spectrophotometers for potassium bromide pellets and for Nujol mulls. Frequencies were measured to an accuracy of within $\pm 2\ cm^{-1}$. The instruments were calibrated by use of the i.r. spectra recorded for polystyrene and for water vapor.

RESULTS AND DISCUSSION

Before our analysis of the spectra is discussed, it is noted that a reducing disaccharide consists of two monomeric units, (*a*) the glycosyl group, resembling a glycoside (residue A), and (*b*) the glycose residue, resembling a free reducing sugar

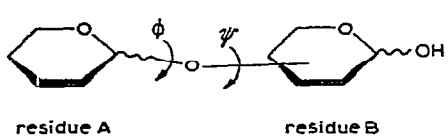

Fig. 1. Skeleton formula of a reducing disaccharide.

(residue B) (see Fig. 1). Compounds **9–11** served as models of residues A, and **7**, **8**, and **12**, of B.

The i.r. spectra of compounds **1–13** in the region 1,000–40 cm^{-1} are reproduced in Fig. 2. For convenience, the spectra are divided into three regions.

The region 1,000–800 cm^{-1}. It has been shown[13] that the band at 915 cm^{-1} in the i.r. spectra of cyclic sugars is due to the vibration of the pyranose ring. The bands in the regions 845–810 and 900–890 cm^{-1} were assigned[13] to the vibrations of the equatorial (α anomer) and axial (β anomer) C-1–H group, respectively, and

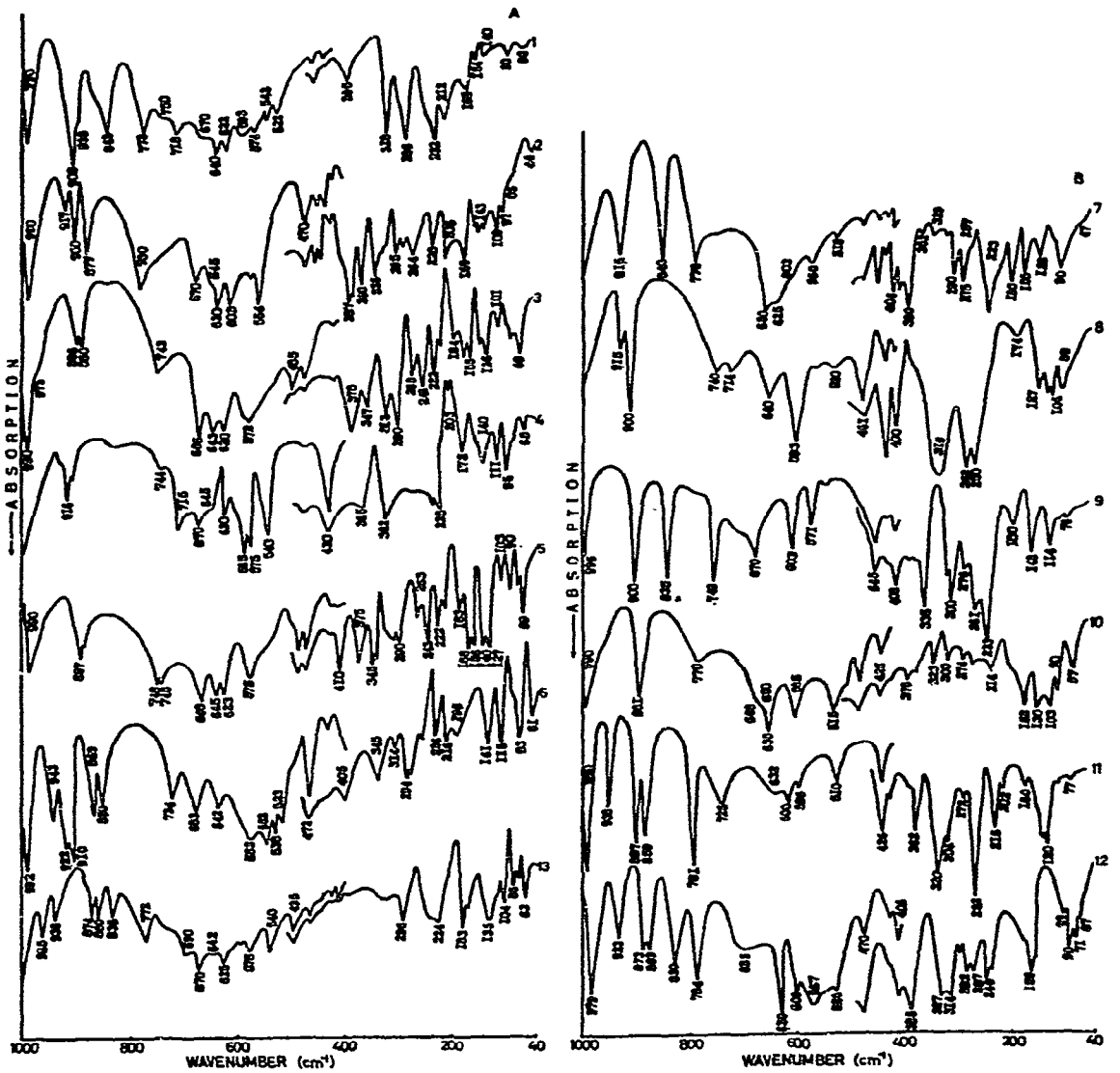

Fig. 2. The i.r. spectra of compounds **1–13**.

so this region (1,000–800 cm^{-1}) was called "the anomeric region". Later, vibrations in the 920–800-cm^{-1} range were considered to be complex[14–17], the number of "anomeric" bands being assumed to depend upon the combination of configurations of CH groups at the adjacent, asymmetric carbon atoms[14]. However, as analysis of the i.r. spectra of model compounds shows, there is no strict correlation between the number of bands in the anomeric region and the combination of configurations of CH groups. For example, one and two bands respectively are observed in the i.r. spectra of methyl β-D-glucopyranoside and β-D-glucose. We assumed that the vibration of the pyranose ring in the spectrum of methyl β-D-glucopyranoside is shifted to 881 cm^{-1} and overlaps with the anomeric vibration. Thus, the pyranose-ring vibrations in the compounds investigated may arise in the 920–881-cm^{-1} range. The bands below 870 cm^{-1} were not detected in the i.r. spectra of β anomers, irrespective of the combination of CH group configurations, whereas, in the spectra of α anomers, these bands were present in the 867–815-cm^{-1} range.

Three bands are observed in the spectra of β-maltose and α-lactose, at 908, 898, and 843 cm^{-1}, and 917, 900, and 877 cm^{-1}, respectively. It follows from comparison of the i.r. spectra of the disaccharides with the spectra of model compounds that the bands at 908, 898 cm^{-1} and 917, 900 cm^{-1} can arise from vibrations of pyranose rings. Nevertheless, it is not excluded that the band at 898 cm^{-1} is the anomeric band of residue B. The remaining bands, at 843 and 877 cm^{-1}, were assigned to

TABLE II

THE FREQUENCIES OF VIBRATIONS OF OLIGOSACCHARIDES AND MODEL COMPOUNDS IN THE ANOMERIC REGION

No.	*Compound*	*Frequencies of vibrations (cm^{-1})*	*References*
7	α-D-Glucose	915, 840	see also, ref. 18
9	Methyl α-D-glucopyranoside	900, 845	see also, ref. 19
11	Methyl α-D-galactopyranoside	920, 867, 820	4, 19
	Methyl α-D-mannopyranoside	915, 890, 846, 815	4, 19
8	β-D-Glucose	915, 900	see also, ref. 18
10	Methyl β-D-glucopyranoside	881	see also, ref. 19
11	Methyl β-D-galactopyranoside	887, 870	see also, ref. 19
	Methyl β-D-xylopyranoside	900	4, 19
12	β-D-Fructose[a]	873, 869	see also, refs. 18, 20, 21
1	β-Maltose monohydrate	908, 898, 843	
2	α-Lactose monohydrate	917, 900, 877	
3	β-Cellobiose	896, 890	
4	β-Gentiobiose	916, 908	
5	3-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-glucose	897, 890	
6	Sucrose	910, 869, 850	
13	Raffinose pentahydrate	874, 860, 835	

[a]The i.r. spectrum of β-D-fructose is given only for comparison with that of sucrose.

vibrations at the anomeric centers of the α and β anomers, respectively, as they were located in the aforementioned regions. We assigned them to residues A, as the bands at 845 and 870 cm^{-1} are present in the spectra of compounds **9** and **11** (see Table II).

Only two bands are observed in the i.r. spectra of β-cellobiose, β-gentiobiose, and disaccharide **5**. One of these bands was assigned to the pyranose-ring vibrations, and the other to the vibrations at β-anomeric centers of residues A and B. It should be noted that no anomeric bands arising from B residues of disaccharides **2** and **5** were observed in the i.r. spectra at 840 cm^{-1} (see Table III).

TABLE III

COMPARISON OF FREQUENCIES OF VIBRATIONS (cm^{-1}) IN THE ANOMERIC REGION OF RESIDUES A AND B OF OLIGOSACCHARIDES

Residue A (model compound)	*Oligosaccharide*	*Bands of the oligosaccharide*	*Residue B (model compound)*
900, *845*	β-Maltose	908, 898, *843*	915, 900
887, *870*	α-Lactose	917, 900, *877*	915, *840*
881	β-Cellobiose	896, 890	915, 900
881	β-Gentiobiose	916, 908	915, 900
881	3-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-glucose	897, 890	915, *840*
881	Sucrose	910, 869, *850*	873, 869
900, *845*	Raffinose	874, 860, 835	

From these data, it may be assumed that the i.r. spectrum in the anomeric region is suitable for the determination of the configuration of the glycosidic bond in disaccharides.

The bands in the i.r. spectrum of sucrose were assigned as follows: the band at 910 cm^{-1}, to the vibration of the pyranose ring; that at 869 cm^{-1}, to the vibration of the furanose ring; and that at 850 cm^{-1}, to the α-anomeric band of residue A. It is difficult to find in the i.r. spectrum of raffinose the band arising from pyranose-ring vibrations, as they are shifted outside this region. Of the two bands, at 874 and 860 cm^{-1}, one may be attributed to the vibration of the furanose ring, but the possibility that the band at 860 cm^{-1} is due to the libration modes of the water of hydration[16] cannot be excluded. The presence of the band at 835 cm^{-1} indicates the α-configuration of at least one of the glycosidic bonds.

The region 700–500 cm^{-1}. This region may be called "the region of crystallinity", because, as our data show, a number of the bands located in this region disappear from the i.r. spectra of amorphous mono- and di-saccharides (see Fig. 3; see also, ref. 22).

As, in the 700–500-cm^{-1} range, there are bands caused by nonplanar, bending modes of hydroxyl groups[4] participating in hydrogen-bond formation, i.r. spectra ought to be dependent on the packing of such molecules in the crystalline lattice. Intercomparison of the i.r. spectra of disaccharides (composed of identical, or differ-

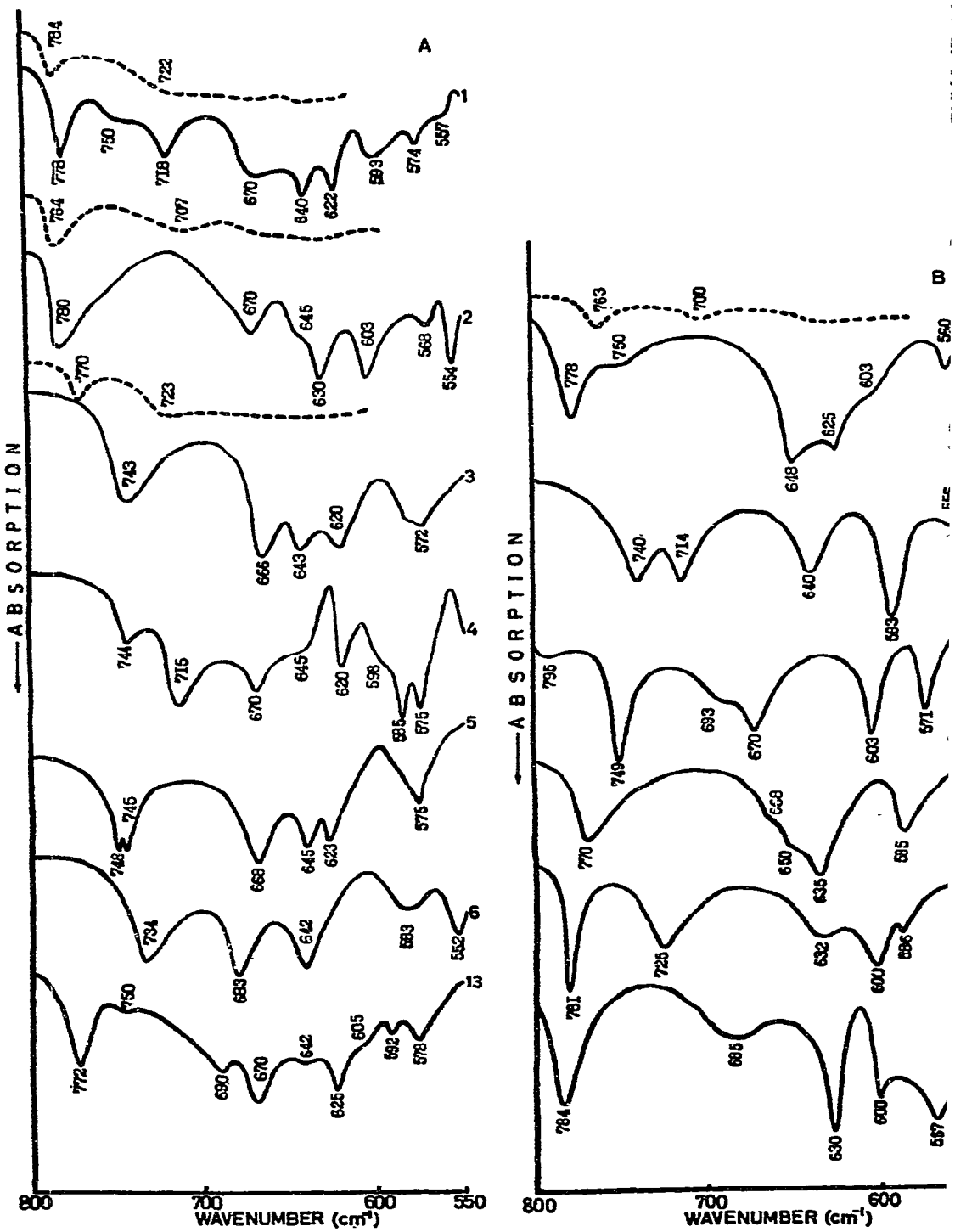

Fig. 3. The i.r. spectra of compounds 1–13 in "the region of crystallinity". (The spectra of amorphous samples are shown by dotted lines.)

ent, monosaccharide units) with each other or with the spectra of model compounds (see Table IV), shows marked differences which may be explained by the formation of inadequate sets of hydrogen bonds. For example, the hydroxyl group located at the same asymmetric carbon atom can form hydrogen bonds of differing strength (*i.e.*, the length and the angle of the hydrogen bond is changed, or the hydroxyl group participates in the formation of either a donor or a donor–acceptor hydrogen-bond[4]). On the other hand, X-ray data indicate[7–11,23,24] that the mono- and di-saccharides compared have hydrogen bonds of similar type and similar length. Apparently, this results in the appearance in the i.r. spectra of bands having the same frequency.

TABLE IV

THE FREQUENCIES OF VIBRATIONS (cm^{-1}) IN THE CRYSTALLINITY RANGE

Residue A (model compound)	*Disaccharide*	*Bands of the disaccharide*	*Residue B (model compound)*
670, 603	β-Maltose	670, 640, 622, 593	640, 593
632, 600	α-Lactose	670, 645, 630, 603	648, 625, 603
668, 650, 635	β-Cellobiose	666, 643, 620	640, 593
668, 650, 635	β-Gentiobiose	670, 645, 623, 598	640, 593
668, 650, 635	3-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-glucose	668, 645, 623	648, 625, 603
670, 603	Sucrose	683, 642	685, 630, 600

Despite some differences, there is a definite similarity between the i.r. spectra of disaccharides **1–5**. Apparently, the reason is that residues A and B represent pyranose rings adopting the same [$^4C_1$(D)] conformation. The replacement of one pyranose ring by a furanose ring (as in sucrose) influences the spectrum significantly; a band at 683 cm^{-1} appears that is observed only in the spectra of D-fructose, sucrose, and raffinose (see Fig. 3). Recently, it was shown[16] that, in the i.r. spectrum of a branched (1→3)-α-D-dextran, there is a band at 790 cm^{-1} which is absent from the spectra of branched (1→4)-α-D-dextrans. It is not surprising that similar correlations are not observed for the i.r. spectra of disaccharides **1–5** in the region 800–600 cm^{-1}. Actually, it is to be expected that, for lower oligosaccharides having differing structures, the frequencies of vibration in the far-infrared region will undergo complex changes on passing from one oligosaccharide to another (see next).

The region 500–40 cm^{-1}. Below 500 cm^{-1}, the i.r. spectra of disaccharides **1–5** differ greatly from each other. The presence of a large number of absorption bands is characteristic for this region (see Fig. 2). At the same time, comparison with the spectra of model compounds possessing the same structural features does not allow a correlation to be found between the structure (the monosaccharide composition, the position and the configuration of the glycosidic linkage, the packing of molecules in the solid state, the conformational parameters ϕ and ψ, etc.) and the i.r.-spectral characteristics in the far-infrared region. Even the strong absorption at 320–250 cm^{-1}

present in the spectrum of β-D-glucose is not observed in the spectrum of disaccharides containing a β-D-glucopyranosyl group or a β-D-glucopyranose residue. The interaction of vibrations[15] and the high sensitivity of bending skeletal and twisting vibrations to small changes in the structure of the molecule, together with the fact that, on passing from one oligosaccharide to another, at least two structural factors are changed, make the search for any correlations and the ascribing of vibrations impossible. Apparently, that is why Hineno and Yoshinaga, in their later papers[2,3,5] did not assign the bands observed in the 100–40-cm^{-1} range to inter-ring modes. Nevertheless, it is to be expected that, for higher oligosaccharides and polysaccharides, the far-infrared region may be as informative about the structure of carbohydrates as it is for regular oligo- and poly-peptides[25].

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