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Characterisation of the glycosidic linkage by infrared and Raman spectroscopy in the C–H stretching region: α,α-trehalose and α,α-trehalose-*2,3,4,6,6-*d_{10}

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ABSTRACT

I.r. and Raman spectra of solid α,α-trehalose and α,α-trehalose-*2,3,4,6,6-*d_{10} in the C–H and C–D stretching regions are recorded. The experimental data are reproduced satisfactorily by normal co-ordinate and i.r. absorption intensity calculations which take into account the specific interactions of each C–H hydrogen with nearby oxygen lone-pairs. The relevance of the region 2800–3000 cm^{-1} in α,α-trehalose-*2,3,4,6,6-*d_{10} for the study of the conformational properties of the inter-glycosidic linkage is discussed.

INTRODUCTION

The conformations of the rings of individual sugar residues in oligo- and polysaccharides are usually predictable, so that the overall shape of the polysaccharide chain can be determined if the torsion angles φ and ψ for the glycosidic linkage are known. There are only a few experimental methods for measuring these angles in solution or in the solid state. In solution, these angles have been determined in special circumstances by ^{1}H- and ^{13}C-n.m.r. spectroscopy[1] and by optical rotation[2]. In the solid state, X-ray and neutron diffraction are usually employed. However, the position of hydrogen atoms is not provided precisely by X-ray diffraction and only few compounds have been treated by neutron diffraction[3].

I.r. and Raman spectroscopy were used in the past[4] to distinguish between anomers. Most i.r. studies have dealt with deformation modes, but the interpretation of the spectra is difficult due to the enormous number of bands. The C–H stretching region, on the other hand, has not been considered because it contains few signals and, in relation to conformation, was thought to be of little interest[5]. Thus, i.r. and Raman spectroscopy were neglected until McKean[6] showed isolated C–H stretching modes to be sensitive to conformation.

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The C–H stretching diagonal force constant (K_{CH}) depends on the relative position of the C–H bond and the lone pairs of electrons on the nearest oxygen atom. This dependence was first proposed for ethers[6,7a,8] and alcohols[6,7b,8], and applied to α- and β-D-glucose in the solid state[8]. The empirical equation *1* allows K_{CH} [*i.e.*, $K(\varphi)$] to be determined from the known conformation of an adjacent oxygen atom[9].

$$K(\varphi) = \frac{K_G - K_T}{60^\circ} \varphi + K_T \qquad 1$$

Here, φ is the dihedral angle between the major lobe of the lone pairs on oxygen and the C–H bond, and K_T and K_G are values for $K(\varphi)$ at 0° and 60°, respectively, when the C–H bond is *trans* or *gauche* to the lone pairs. Equation *1* has been used to characterise inositols and hexopyranosides, and should be applicable to disaccharides.

In order to test this applicability, we have considered a disaccharide whose conformation of the glycosidic linkage is known from X-ray studies. The disaccharide selected was α,α-trehalose together with the *2,3,4,6,6-d*$_{10}$ derivative prepared by the reported catalytic exchange procedure[10].

EXPERIMENTAL AND RESULTS

α,α-*Trehalose*-2,3,4,6,6-d$_{10}$. — To a solution of α,α-trehalose (0.5 g) in D_2O (40 mL) was added deuterated Raney nickel[10] (5 mL). The mixture was boiled under reflux for 24 h, filtered, and passed through a chelating resin (Chelex). The residue was purified by recrystallisation and lyophilised.

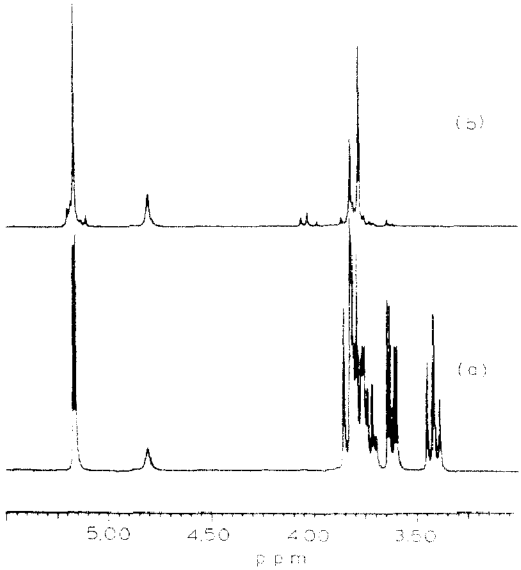

Fig. 1. ^{1}H-N.m.r. spectra of (*a*) α,α-trehalose and (*b*) α,α-trehalose dissolved in D_2O after deuterium exchange.

N.m.r. spectroscopy (Bruker CXP-300 spectrometer) was used to check the deuteration process on 2% and 5% solutions of samples in D_2O for ^{1}H- and ^{13}C-n.m.r. spectra, respectively. The ^{1}H-n.m.r. spectra shown in Fig. 1 are referenced to the H–O–D signal at 4.8 p.p.m.

Fig. 1b reveals only partial deuteration at position 3, and the ratio of intensities of the signals for H-1 and H-3 indicated that 38 ±2% of H-3 remained. An additional uncertainty of this percentage is due to the presence of other signals (~5%) due to interfering H residues and/or epimers. The ^{13}C-n.m.r. spectrum indicated that ~30% of H-3 was not exchanged. The latter value is considered to be more reliable and is used in the calculations presented below. Although the deuterium exchange was incomplete, for convenience, the product is referred to as α,α-trehalose-*2,3,4,6,6-d*$_{10}$.

I.r. and Raman spectra. — The i.r. spectra were recorded on KBr pellets, using a Perkin–Elmer FT-IR Model 1800 spectrometer. The resolution was 1 cm^{-1} and the number of scans was 256. The spectra shown in Figs. 2 and 3 were analysed in detail in the C–H stretching region (2800–3100 cm^{-1}) and in the C–D stretching region (2300–2000 cm^{-1}) for the deuterated compound. Raman spectra were obtained for powdered

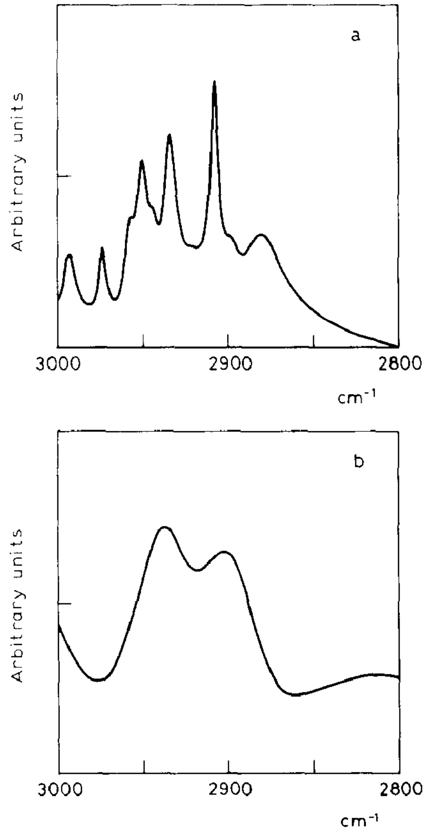

Fig. 2. I.r. spectra in the C–H stretching region of (*a*) α,α-trehalose and (*b*) α,α-trehalose-*2,3,4,6,6-d*$_{10}$.

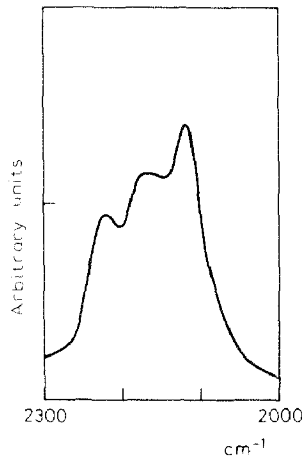

Fig. 3. I.r. spectrum in the C–D stretching region of α,α-trehalose-*2,3,4,6,6*-d_{10}.

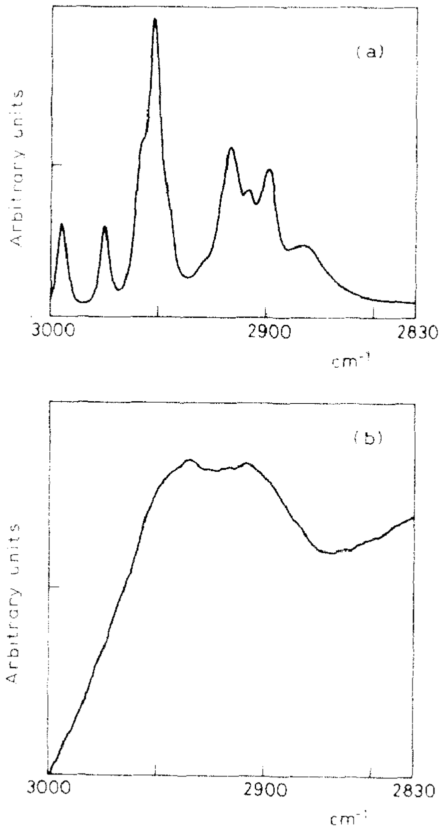

Fig. 4. Raman spectra in the C–H stretching region of (*a*) α,α-trehalose and (*b*) α,α-trehalose-*2,3,4,6,6*-d_{10}.

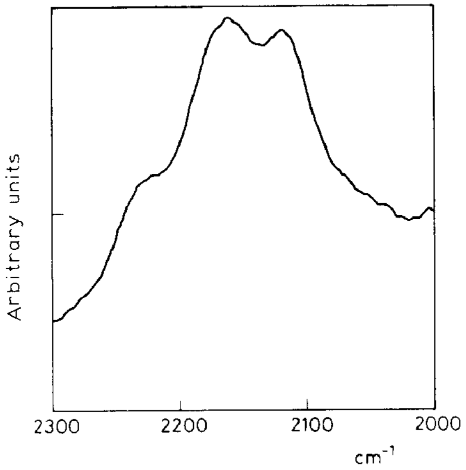

Fig. 5. Raman spectrum in the C–D stretching region of α,α-trehalose-*2,3,4,6,6-d*$_{10}$.

samples contained in capillary tubes with a SPEX 1401 Raman spectrometer (at the University of California at Berkeley). The exciting line was the 514.5-nm line of an Ar$^+$ Spectra Physics laser. The scattered radiation was viewed at 90° to the incident beam. The slit widths were set for a minimum resolution of 2 cm^{-1} and the spectra were recorded under the following conditions: increment points, 1; increment time, 1 s; laser power on the sample, 150 mW; 5 scans for α,α-trehalose and 128 for the d_{10} derivative. The spectrum of the d_{10} compound was recorded after storage of the sample overnight in the laser beam in order to reduce a strong fluorescence probably due to residual traces of catalyst. The Raman spectra are given in Figs. 4 and 5. The observed i.r. and Raman frequencies in the C–H and C–D stretching region are given in Table I.

TABLE I

Observed frequencies (cm^{-1}) in the C–H and C–D stretching region for α,α-trehalose and α,α-trehalose-*2,3,4,6,6-d*$_{10}$

α,α-Trehalose		*α,α-Trehalose*-2,3,4,6,6-d$_{10}$	
I.r. *C–H*	*Raman*	*I.r.* *C–H*	*Raman*
2993 m	2995 m	2938 m,br	2940
2074 m	2975 m	2908 m,b	2908
2957 sh	2957 sh		
2950 m	2952 s		
2944 sh	2945 sh		
2934 m	2930 sh	*C–D*	
	2916 m		
2908 s	2908 w	2230 m,w	2227 sh
2897 sh	2898 m	2175 m,w	2164 m,br
2880 m,br	2882 w,br	2120 m	2123 m

Normal co-ordinate calculations. — The vibrational kinetic energy matrices B and G[11] of α,α-trehalose were calculated using the structure determined by Brown *et al.*[12] by X-ray diffraction.

Because of the large experimental uncertainties in the X-ray co-ordinates of hydrogen atoms, the O–H and C–H bond lengths were set at their nominal values of 0.97 and 1.1 Å, respectively, retaining experimental orientation.

The force field reported by Vasko *et al.*[5] was used for all internal co-ordinates except for C–H stretching, the force constants of which were assigned using equation *1*.

The torsional angles φ and ψ were obtained from the crystal structure[12].

Figure 6 shows Newman projections for the HOCH groups relative to all positions except 1, 1′, 5, and 5′, and Fig. 7 those for C-1-H and C-1′-H with respect to O-1 and O-5 (or O-5′). For the C–H bonds at positions 1–5, the values used in equation *1* for K_T and K_G, 4.540 and 4.805 mdyne/Å, respectively, were derived from experimental studies on 1,4-dioxane[7a] and used on glucose[8] and inositols[9]. In order to reproduce the experimental frequencies of α,α-trehalose around 2990 and 2880 cm^{-1}, somewhat different values of K_T and K_G for C-6-H and C-6′-H were used in equation *1*. The values were 4.594 and 4.784 mdyne/Å, respectively, and had been derived from ethanol[7b]. The difference between the values of K_T and K_G for positions 1–5 and 6 suggests different

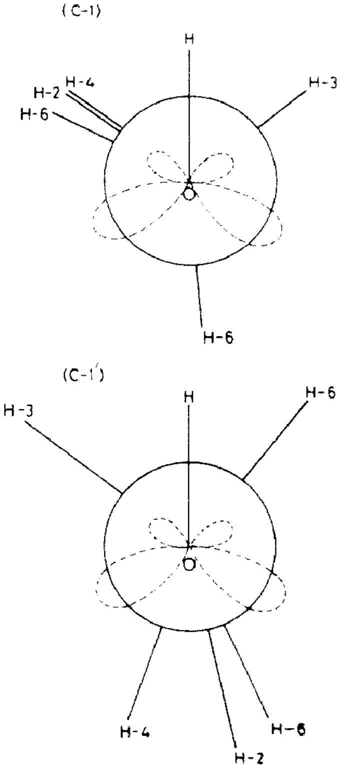

Fig. 6. Newman projections of the HOCH fragment relative to all positions except 1, 1′, 5, and 5′ (see text).

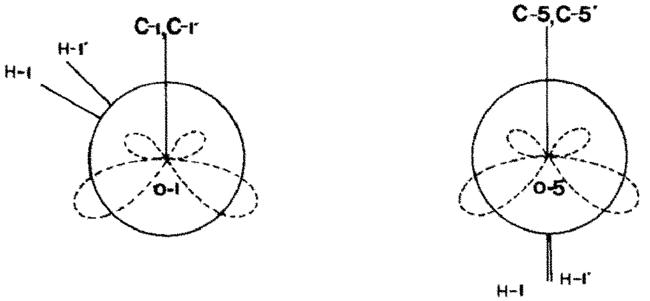

Fig. 7. Newman projections of H-C-1 and H-C-1' with respect to O-1 and O-5 (O-5') (see text).

interactions of the C–H bond and the vicinal O atoms in H–C–O–H and $-CH_2-O-H$ groups. The stretching force constants for C-1-H and C-1'-H were calculated as the average between the values obtained by applying equation *1* to C-1-H and C-1-H' in interaction with both oxygens 1 and 5 (or 5') (Figs. 6 and 7).

Table II contains the values of the dihedral angles H–C–O–H (θ), the force constants (K_{CH}), and the charges on hydrogens bonded to the carbons ($q^0{}_H$), the behaviour of which follows rules similar to those[13] for (K_{CH}) as discussed below. Tables III–V contain the calculated i.r. frequencies, eigenvectors (the contributions of C–H stretchings in the normal modes corresponding to each vibrational frequency), and absolute i.r. absorption intensities calculated from the data in Table II for α,α-trehalose, and the *2,3,4,6,6-*d_{10}, and *2,4,6,6-*d_8 derivatives.

TABLE II

The H–C–O–H dihedral angles (Θ), force constants (K_{CH}) and charges [$q^o{}_H(e)$] on hydrogen in α,α-trehalose (see Figs. 6 and 7)

Unit	Θ(°)	K_{CH} (*mdyne/Å*)	$q^o{}_H$(e)
Unit 1			
C-1-H	314.8	4.702	0.045
C-2-H	305.7	4.562	0.07
C-3-H	49.7	4.583	0.07
C-4-H	306.7	4.566	0.07
C-5-H	290.4	4.574	0.07
C-6-H	296.7	4.604	0.07
C-6'-H	175.8	4.771	0.02
Unit 1'			
C-1-H	301.6	4.675	0.045
C-2-H	168.2	4.725	0.045
C-3-H	307.4	4.568	0.07
C-4-H	198.8	4.698	0.045
C-5-H	294.4	4.555	0.07
C-6-H	156.4	4.709	0.045
C-6'-H	38.4	4.662	0.045

TABLE III

Calculated frequencies, eigenvectors, and absolute i.r. intensities for α,α-trehalose

Unit	*Frequencies* (*cm*$^{-1}$)	*Eigenvectors* (*a.m.u.*$^{-1/2}$)	*Intensities* (*esu*^{2}*cm*^{2} × 10^{36})
I	2983.4	0.52 C-6-H −0.91 C-6′-H	5.39
I′	2972.8	−0.77 C-6-H +0.66 C-6′-H −0.23 C-4-H	4.85
I′	2955.9	−0.85 C-2-H +0.50 C-4-H −0.22 C-1-H	0.27
I	2943.0	1.02 C-1-H +0.15 C-2-H	4.65
I′	2937.0	0.76 C-4-H −0.58 C-1-H −0.34 C-2-H	8.19
I′	2930.0	0.83 C-1-H −0.47 C-2-H 0.39 C-4-H	2.92
I	2913.5	−0.73 C-3-H +0.68 C-5-H	0.21
I	2907.1	0.79 C-4-H −0.62 C-2-H −0.22 C-3-H	0.46
I′	2906.8	0.79 C-3-H −0.63 C-5-H	0.25
I	2896.0	0.62 C-5-H +0.61 C-3-H −0.38 C-2-H +0.33 C-6-H	9.61
I′	2895.2	−0.76 C-6′-H −0.61 C-6-H −0.23 C-3-H	2.50
I	2885.6	0.69 C-2-H +0.64 C-4-H +0.27 C-3-H +0.24 C-6-H	1.70
I′	2885.4	−0.80 C-5-H −0.62 C-3-H	5.31
I	2878.3	0.78 C-6-H −0.44 C-5-H +0.44 C-6′-H	1.05

TABLE IV

Calculated frequencies, eigenvectors, and absolute i.r. intensities for α,α-trehalose *2,3,4,6,6-d*$_{10}$

Unit	*Frequencies* (*cm*$^{-1}$)	*Eigenvectors* (*a.m.u.*$^{-1/2}$)	*Intensities* (*esu*^{2}*cm*^{2} × 10^{36})
C-H stretching region			
I	2942.1	1.036 C-1-H	4.42
I′	2933.4	−1.036 C-1-H′	3.91
I	2900.3	1.036 C-5-3	2.77
I′	2894.6	−1.036 C-5-H′	3.01
C-D stretching region			
I	2229.7	0.63 C-6′-D −0.46 C-6-D	4.62
I′	2206.2	0.63 C-6-D −0.4 C-6′-D −0.18 C-4-D	3.85
I′	2193.3	−0.62 C-2-D +0.41 C-4-D −0.14 C-6-D	0.02
I′	2182.8	0.58 C-4-D +0.41 C-2-D +0.22 C-3-D	3.63
I	2160.3	−0.65 C-3-D −0.34 C-4-D −0.18 C-2-D	0.08
I	2156.5	−0.53 C-4-D +0.52 C-2-D +0.13 C-3-D	0.13
I′	2147.2	0.72 C-3-D −0.16 C-4-D −0.16 C-2-D	4.84
I	2137.4	−0.52 C-2-D −0.42 C-4-D +0.37 C-3-D	7.76
I	2107.0	−0.61 C-6-D −0.42 C-6′-D	1.56
I′	2102.2	−0.62 C-6′-D −0.38 C-6-D	2.64

Calculation of i.r. intensities. — The "equilibrium charges + charge-flow" (ECCF) model[13] was used. That model depicts a molecule as a collection of point charges localised on atoms that can vary during the vibrations through fluxes along bonds. The absorption intensity of the *i*th vibrational normal mode (Q_i) is proportional

TABLE V

Calculated frequencies, eigenvectors, and absolute i.r. intensities for α,α-trehalose-*2,4,6,6-d_8*

Unit	*Frequencies* (cm^{-1})	*Eigenvectors* $(a.m.u.^{1/2})$	*Intensities* $(esu^2cm^2 \times 10^{39})$
C–H stretching region			
1	2942.4	1.036 C-1-H	4.42
1′	2933.7	−1.036 C-1-H	3.93
1	2912.3	0.79 C-3-H −0.67 C-5-H	0.09
1′	2907.0	0.80 C-3-H −0.66 C-5-H	0.05
1	2892.0	0.79 C-5-H +0.67 C-3-H	5.49
1′	2886.6	0.80 C-5-H −0.66 C-3-H	5.58
C–D stretching region			
1	2229.9	−0.63 C-6′-D +0.46 C-6-D	4.62
1′	2219.7	0.56 C-6-D −0.53 C-6′-D	3.98
1′	2194.8	0.60 C-2-D −0.45 C-4-D	0.04
1′	2180.6	−0.59 C-4-D +0.46 C-2-D	6.08
1	2158.1	−0.59 C-4-D +0.46 C-2-D	0.12
1	2143.7	−0.59 C-2-D −0.46 C-4-D	5.18
1′	2126.5	−0.54 C-6′-D −0.49 C-6-D	2.37
1	2107.1	0.60 C-6-D +0.42 C-6′-D	1.58

to $\left[\frac{\partial \vec{M}_\alpha}{\partial Q_i}\right]_0^2$ where $\vec{M}$ is the molecular electric dipole moment. The αth Cartesian component of this vector[13] is given in equation *2*,

$$\left(\frac{\partial M_\alpha}{\partial Q_i}\right)_0 = \sum_{I=1}^{N} q_I^0 L_{I\alpha,i}^X + \sum_{j=1}^{M} \sum_{k=1}^{B} \left(\frac{\partial q_k}{\partial R_j}\right)_0 (\mathrm{e}_\alpha^0)_k l_k^0 L_{j,i}^R \qquad 2$$

where the first sum extends over the N atoms in the molecule (index I) and the second extends formally over all B bonds (index k) and over all M internal co-ordinates[11] (stretching, bendings, *etc.*; index *j*); $L^X_{I\alpha,i}$ and $L^R_{j,i}$ are the vibrational eigenvectors written in terms of atomic displacements and internal co-ordinates, respectively (see Tables III–V), q_I^0 is the equilibrium charge (EC) localised on atom I, and $\left[\frac{\partial q_k}{\partial R_j}\right]_0$ is the charge flux (CF) along the bond k due to the internal co-ordinate R_j [the direction cosines of bond k are $(e^0_\alpha)_k$, and its length is l^0_k]. The charge flow along C–H bonds due to C–H stretchings is fairly constant[13] ($\sim -0.200e/\text{Å}$), whereas q^0_H is sensitive to its environment. Therefore, different values were used for the charges for different conformations.

The values 0.02*e* and 0.07*e* were used for the charge on the hydrogen of C–H *gauche* and *trans* to an oxygen lone-pair, respectively, and the value 0.045*e* was used for those orientations intermediate between *gauche* and *trans**. This usage conflicts with the

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*The values for the other charge fluxes and equilibrium charges used in the calculations are immaterial to the results, since the only atoms that execute appreciable motions are the hydrogens of the C–H bonds. The equilibrium charges for carbons, oxygens, and hydrogens of O–H bonds have been assigned according to ref. 8. The detailed list will be supplied upon request.

conclusion[13] that back-donation of electronic charge occures from the oxygen to the antibonding orbital of the hydrogens *trans* to the oxygen, thereby reducing their positive charges. On the other hand, *gauche* hydrogens do not "receive" any electronic charge and have the same charge as hydrogens in *n*-paraffins. The same choice was made[8] for D-glucose. No explanation is available at present as to why the experimental results are reproduced by the above choices (see Tables III and V, and ref. 8). The results for α-D-glucose and α-trehalose are in accord. Figures 8 and 9 show the calculated spectra for the C–H stretching region obtained using a Lorentzian band-shape with 5 cm^{-1} half-height full band-width both for α,α-trehalose, and for a 3:7 mixture of the d_8 and d_{10} compounds.

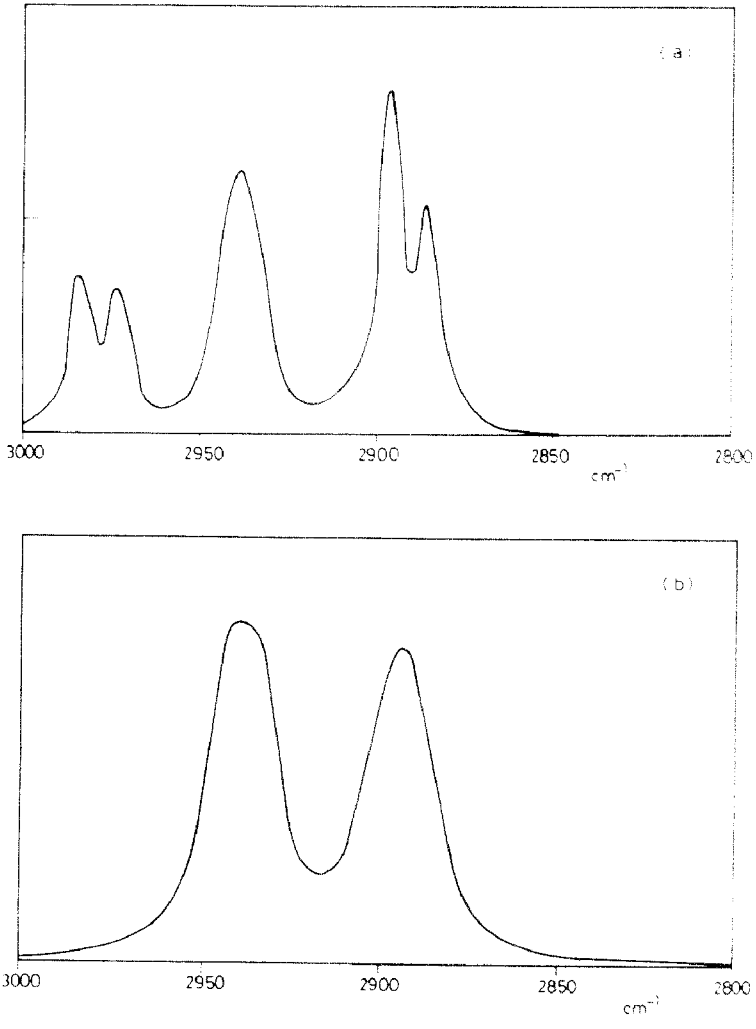

Fig. 8. Calculated i.r. spectra in the C–H stretching region of (*a*) α,α-trehalose, (*b*) a 7:3 mixture of α,α-trehalose-*2,3,4,6,6-d*$_{10}$ and α,α-trehalose-*2,4,6,6-d*$_8$.

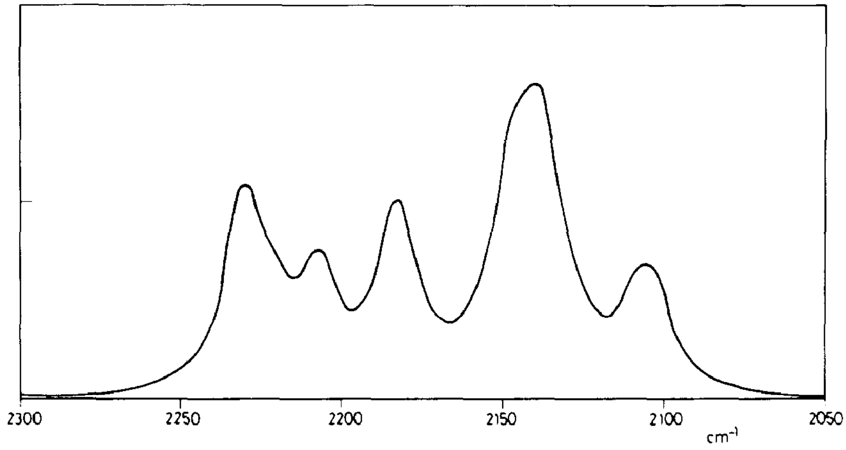

Fig. 9. Calculated i.r. spectra in the C–D stretching region of a 7:3 mixture of α,α-trehalose-*2,3,4,6,6*-d_{10} and α,α-trehalose-*2,4,6,6*-d_8.

DISCUSSION

A comparison of the calculations presented in Tables III–V and in Figs. 8 and 9 with the experimental results in Table I and Figs. 2–5 indicates an excellent correlation of i.r. and Raman spectra. The model describes different C–H bonds as having different dynamic and electrical properties, depending upon their position in the sugar molecule. Thus, i.r. and Raman signals for different C–H bonds have different frequencies and intensities. These signals are identifiable through the calculations presented in Tables III and IV. It has been verified that the i.r. and Raman frequencies are fairly well accounted for by equation *1*, in which the interaction effects for each C–H bond and nearby oxygens are accounted for. It is concluded that, for both sugar units, the bands can be assigned as follows: above 2975 cm^{-1} and below 2880 cm^{-1} to C-6-H, at ~2940 cm^{-1} to C-1-H, at 2955 and 2885 cm^{-1} to C-2-H, at ~2910 cm^{-1} to C-3-H, and at 2895 and 2885 cm^{-1} to C-5-H. The bands for C-4-H are at 2936 cm^{-1} for unit 1′ and at 2907 cm^{-1} for unit 1. Table III also shows that, due to the closeness in frequency of some C–H bands, many of those of the perhydro species contain mixed contributions. Table IV and Fig. 8, on the other hand, allow the conclusion that a direct and unequivocal interpretation of the spectra of the deuterated species in the C–H stretching region is achieved. The band at ~2940 cm^{-1} is assigned to C-1-H (C-1′-H) and that at ~2910^{-1} to C-5-H (C-5′-H). The correct i.r. intensity ratio is also predicted. As can be seen from Figs. 2 and 9, the only datum not accounted for is the abnormally large bandwidth.

This assignment for the C–H stretching region of α,α-trehalose-*2,3,4,6,6*-d_{10} means that the ratio of the intensities (I_{2940}/I_{2910}) of the bands at 2940 and 2910 cm^{-1} is sensitive to the values of φ and ψ. Therefore, by measuring this ratio for solutions or in the solid state as a function of temperature, information on the mobility of the glycosidic linkage of α,α-trehalose can be obtained, and work is in progress on this aspect.

Furthermore, since equation *1* has proved to be successful in interpreting spectra of compounds of known conformation, in the future the same equation will be used in the opposite sense, namely, to infer the conformation of the glycosidic linkage from vibrational spectra.

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REFERENCES

1 B. Casu, M. Reggiani, G. G. Gallo, and A. Vigevani, *Tetrahedron*, 22 (1966) 3061–3883; *Carbohydr. Res.*, 12 (1970) 157–170.
2 D. A. Rees, *J. Chem. Soc., B*, (1970) 877–884.
3 G. A. Jeffrey and A. D. French, *Chem. Soc. Spec. Publ.*, 6 (1978) 183–223.
4 B. Casu and M. Reggiani, *J. Polym. Sci., Part C*, 7 (1963) 171–185.
5 P. D. Vasko, J. Blackwell, and J. L. Koenig, *Carbohydr. Res.*, 19 (1971) 297–310; 23 (1972) 407–416.
6 D. C. McKean, *Chem. Soc. Rev.*, 7 (1978) 399–422.
7 (*a*) J. Caillod, O. Saur, and J. C. Lavalley, *Spectrochim. Acta, Part A*, 36 (1979) 185–191; (*b*) J. Travert, O. Saur, A. Janin, and J. C. Lavalley, *J. Mol. Struct.*, 33 (1976) 265–272.
8 G. Longhi, G. Zerbi, G. Paterlini, L. Ricard, and S. Abbate, *Carbohydr. Res.*, 161 (1987) 1–22.
9 S. Pizzini, G. Bajo, S. Abbate, G. Conti, and R. H. Atalla, *Carbohydr. Res.*, 184 (1988) 1–11.
10 H. J. Koch and R. S. Stuart, *Carbohydr. Res.*, 59 (1977) c1–c6.
11 E. B. Wilson, Jr., D. C. Decius, and P. C. Cross, *Molecular Vibrations* McGraw-Hill, New York, 1955.
12 G. M. Brown, D. C. Rohrer, B. Berking, C. A. Beevers, R. O. Gould, and R. Simpson, *Acta Crystallogr., Sect. B*, 28 (1972) 3145–3158.
13 M. Gussoni, *J. Mol. Struct.*, 141 (1986) 63–92.