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Vibrational Raman optical activity of carbohydrates

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ABSTRACT

Vibrational Raman optical activity (R.o.a.) spectra of a range of carbohydrates in aqueous solution, measured in back-scattering between 700 and 1500 cm^{-1}, are presented. Features were revealed that appear to be characteristic of details of the stereochemistry. Effects associated with the glycosidic linkage in di- and oligo-saccharides are prominent.

INTRODUCTION

Measurements of vibrational optical activity on chiral molecules can provide new information on stereochemistry because a vibrational spectrum contains bands associated with every part of the molecule[1]. Vibrational optical activity in typical chiral molecules in the disordered phase was first observed using the Raman optical activity (R.o.a.) technique, which measures small differences in the Raman scattered intensities in right- and left-circularly polarised incident light[2,3]. Until recently, lack of sensitivity has restricted R.o.a. studies to favourable samples such as neat liquids[4,5], with the complementary technique of vibrational circular dichroism (v.c.d.) finding more application in studies of stereochemistry[6]. However, a recent major advance in R.o.a. instrumentation, based on the use of a back-scattering geometry[7,8] (in place of the usual 90°-scattering arrangement) together with a cooled charge-coupled device as detector[9], has now rendered a much wider range of samples accessible to study. We now report R.o.a. spectra of a range of carbohydrates in aqueous solution, which indicate that this technique has potential for studies of the structure and stereochemistry of carbohydrates.

Most carbohydrates are not amenable to conventional electronic circular dichroism (e.c.d.) studies because they absorb below the short-wavelength limit of ~ 190 nm of most commercial instruments. Therefore, studies have been restricted to the long-wavelength tails of the first e.c.d. bands[10], or elaborate procedures, such as the dibenzoate exciton chirality method[11], have been employed. Several v.c.d. studies of carbohydrates have been reported that covered both the C–H stretching region[6,12,13] and the mid-i.r[14,15], and, although they demonstrated the potential value of vibrational optical activity, they also exposed problems associated with the complexity of the molecular vibrations, together with low sensitivity. Moreover, water is not a useful solvent for i.r. spectroscopy. Although water is an excellent solvent for Raman spectros-

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copy, prior to the present studies, the auspices for R.o.a. studies of carbohydrates in aqueous solution did not appear to be favourable because conventional Raman spectroscopy has not been used widely on account of difficulties in the interpretation of spectra because of the delocalisation of many of the normal modes over the many C–C and C–O linkages[16]. Also, the superiority of conventional F.t.-i.r spectroscopy over conventional Raman spectroscopy for following structural changes in the structures of carbohydrates in solutions in D_2O has been demonstrated[17] (but the new technique of F.t.-Raman spectroscopy gives excellent spectra of crystalline mono- and poly-saccharides[18]). However, the delocalisation of normal modes over chiral arrangements of nuclei is a prerequisite for large vibrational optical activity. In the event, because of the greater spectral range accessible to R.o.a. compared with v.c.d., the characteristic optical activity "fingerprints" associated with these delocalised vibrations are discernible immediately. Also, since the mechanisms of R.o.a. and v.c.d are quite different[1,19], weak v.c.d. intensities in carbohydrates do not necessarily mean that the R.o.a. intensities will also be weak. Indeed, the presence of C–O–C linkages has been found to be particularly favourable for large R.o.a. effects[20–22].

EXPERIMENTAL

The R.o.a. spectra were recorded using the Glasgow multi-channel instrument[23]. The original intensified diode array detector on this instrument has now been replaced by a cooled (unintensified) charge-coupled device as detector (Wright Instruments Ltd., Model AT1), which has significant advantages[9] that include increased quantum efficiency and low read-out noise. The optical system employed for the back-scattered R.o.a. measurements was similar to that described by Hug[8].

The carbohydrate samples were studied as near-saturated aqueous solutions contained in quartz microfluorescence cells that were allowed to equilibrate for several days. The R.o.a. measurements were made using a focused 600-mW argon-ion laser beam at 488.0 nm and a spectral resolution of ~ 8 cm^{-1}. All the R.o.a. spectra were acquired for 2 h.

Although R.o.a. spectra have been measured previously down to 80 cm^{-1} in the 90°-scattering configuration, back-scattering places severe demands on the capability of the spectrometer to reject stray light because laser light reflected back from the cell surfaces enters the spectrometer. For this reason, reliable R.o.a. measurements could not be made on aqueous solutions of carbohydrates below ~ 700 cm^{-1} using our existing instrument. Sharp changes in some of the R.o.a. spectra at the join between the two segments at 1060 cm^{-1} should be treated with circumspection.

RESULTS AND DISCUSSION

D-*Glucose and* D-*xylose.* — The back-scattered R.o.a. spectrum of D-glucose is shown in Fig. 1. This molecule exists preponderantly in the $^4C_1$ pyranose conformation in aqueous solution[24] with an α:β ratio of $\sim$ 1:2. For comparison, the R.o.a. spectrum of D-xylose is shown in Fig. 2, since this molecule has the same conformation in solution as D-glucose with a similar α:β ratio[24] but lacks the CH_2OH group. One difference is the presence of a broad couplet centred at 1325 cm^{-1}, negative at low and positive at high frequency, only in the former spectrum. Bands in the conventional Raman spectrum of glucose in this region have been assigned[25,26] to CH_2, C–O–H, and C–H deformations, and Cael *et al.*[27] have described a complex calculated mode of α-D-glucose at 1335 cm^{-1} in terms of CH_2 twisting, several C–O–H bends, and a high degree of C–C–H bending. Although there are small differences in the puckering of the pyranoid rings in α- and β-D-glucose with associated differences in the conformation of the pendant side groups[27], β-D-glucose would be expected to have a similar mode of vibration, perhaps shifted slightly in frequency. Therefore, it seems likely that this couplet in the solution of D-glucose originates in deformations of the CH_2OH group together with deformations of adjacent parts of the pyranoid structure, and is broadened a little due to contributions

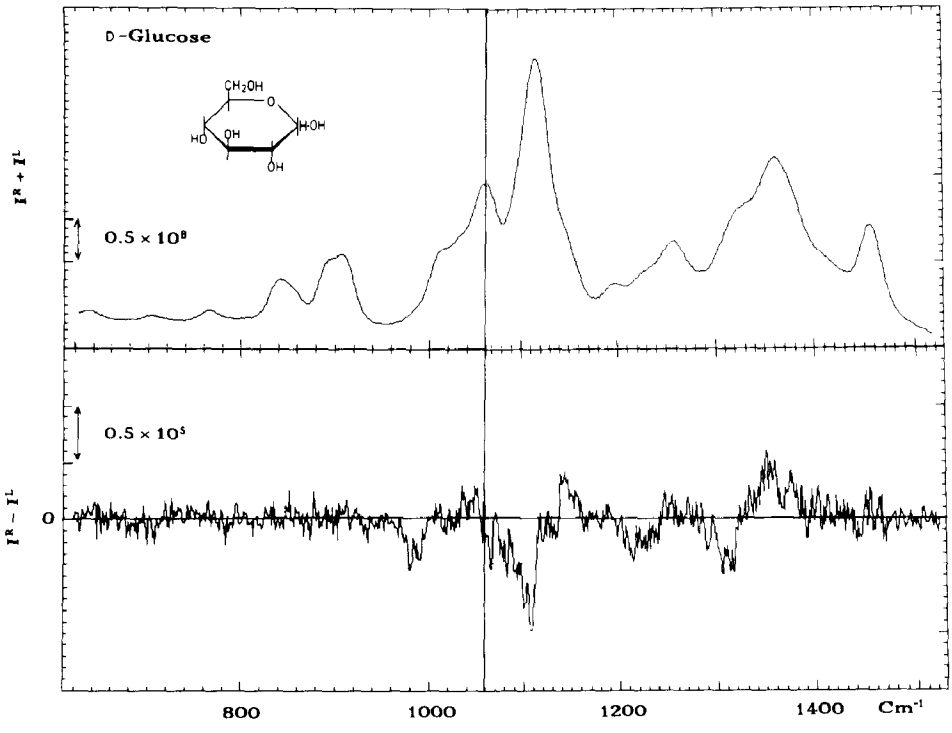

Fig. 1. The back-scattered Raman ($I^R + I^L$) and R.o.a. ($I^R - I^L$) spectra of D-glucose in aqueous solution. The intensity scales (in electron counts) are arbitrary.

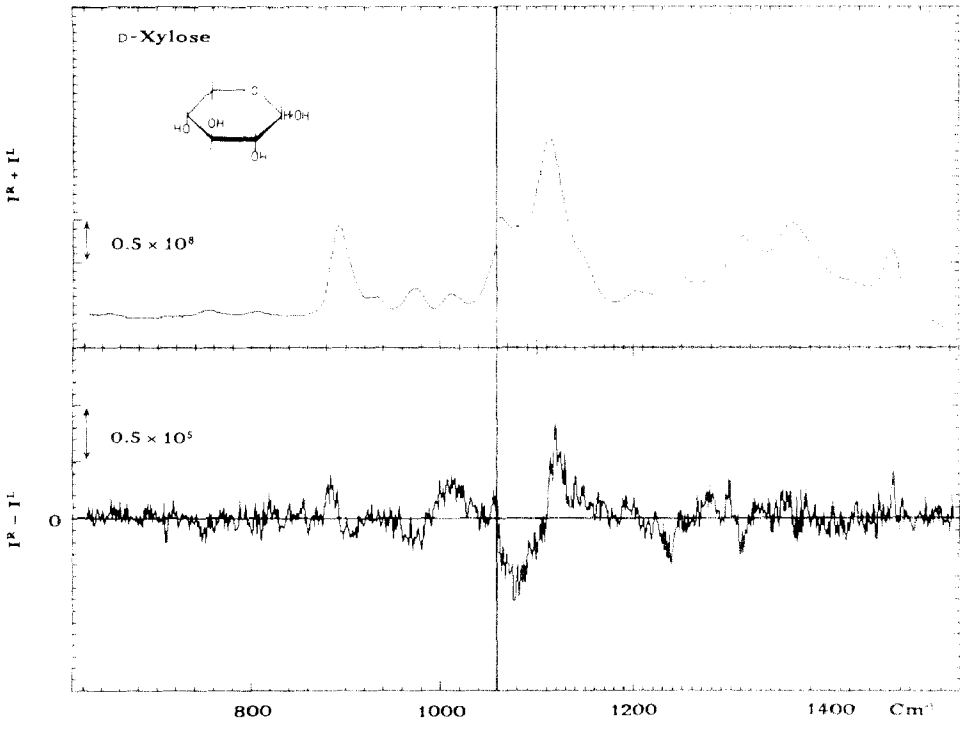

Fig. 2. The back-scattered Raman and R.o.a. spectra of D-xylose in aqueous solution.

from both anomers, a conclusion that is reinforced by the appearance of a similar couplet in the R.o.a. spectra of maltose, maltotriose, and α-cyclodextrin (see below).

A second broad, but weaker, couplet appears in the R.o.a. spectrum of D-glucose centred at ~1235 cm^{-1}, and is negative on the low- and positive on the high-frequency side. A similar couplet appears in the spectrum of D-xylose centred at ~1260 cm^{-1}. Again, bands in this region for D-glucose have been assigned[26,27] to deformations of the CH_2OH group together with contributions from all the C–O–H deformations as well as several C–C–H deformations, so that the shift to higher frequency of the D-xylose couplet can be attributed, amongst other things, to the lack of participation of the CH_2OH deformations in the normal modes.

The v.c.d. spectra of D-glucose and D-xylose were recorded also in the region of the above R.o.a. spectra and, in contrast to the R.o.a. spectrum, the v.c.d. spectrum of D-xylose was richer than that of D-glucose[15].

Both D-glucose and D-xylose show similar R.o.a. "fingerprints" between ~960 and 1170 cm^{-1}, with that of D-xylose shifted by ~20 cm^{-1} to lower frequency. The positive R.o.a. feature at ~1150 cm^{-1} for D-glucose can be assigned to a mode described by Cael *et al.*[27] as a complex coupling of ring C–O and C–C stretching together with C–O–H and C–C–H bending. The curious sharp drop to a small negative "step" might be due to contributions of opposite sign from the α and β anomers, for which this mode is

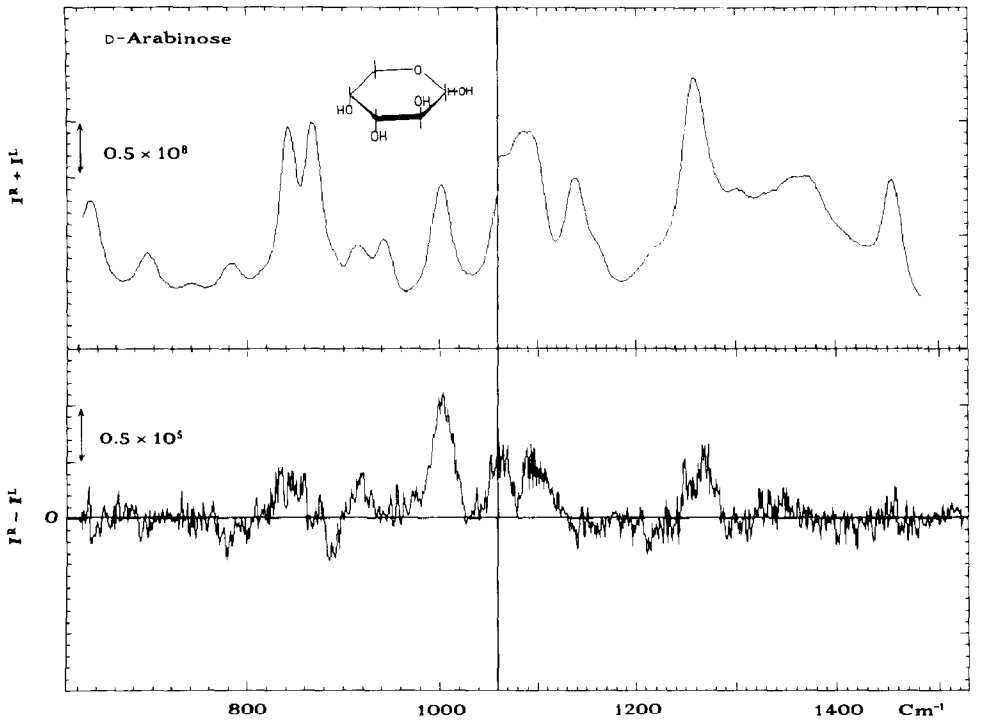

Fig. 3. The back-scattered Raman and R.o.a. spectra of D-arabinose in aqueous solution.

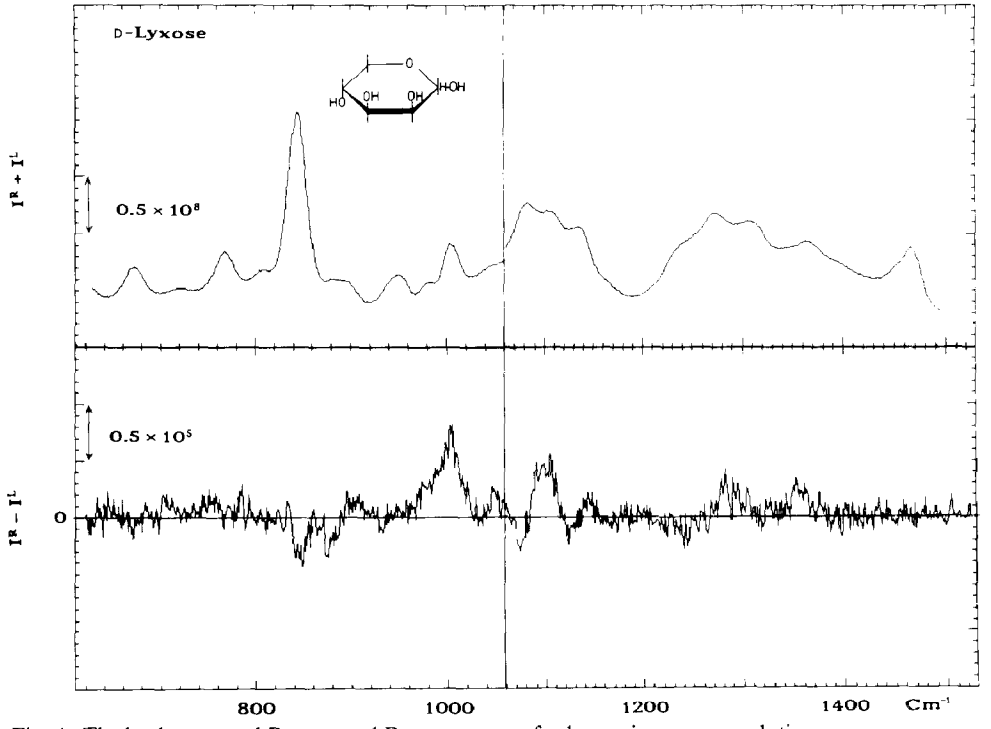

Fig. 4. The back-scattered Raman and R.o.a. spectra of D-lyxose in aqueous solution.

predicted[27] to occur at 1155 and 1150 cm^{-1}, respectively. The positive feature at slightly lower frequency for D-xylose presumably arises from a similar mode. A v.c.d. feature was observed also in this vibration and was correlated with the absolute stereochemistry and conformation of several simple pyranoses, including D-glucose and D-xylose[15]. In fact, all of the Raman bands in the region covered by this particular R.o.a. fingerprint for D-glucose have been assigned to modes that contain significant contributions from the same internal vibrational co-ordinates as those listed above for the $\sim 1150\ cm^{-1}$ mode[25-27], and since these co-ordinates are mainly from the pyranoid ring structure rather than from the CH_2OH group, it is gratifying that the R.o.a. fingerprints of D-glucose and D-xylose are so similar in this region.

D-Arabinose and D-lyxose. — The back-scattered R.o.a. spectra of D-arabinose and D-lyxose are shown in Figs. 3 and 4. Each sugar exists mainly in the pyranose conformation in aqueous solution[24]. Although a detailed analysis is not given here, it is pointed out that, whereas some similarities can be discerned (especially the large positive R.o.a. at $\sim 1000\ cm^{-1}$ in each spectrum), overall the fingerprints are distinct from each other and from those of D-xylose and D-glucose, and clearly reflect the different stereochemistry.

Comparison of the R.o.a. spectrum of D-altrose (not shown), which has the same pyranoid structure at C-2,3,4 as D-arabinose, was complicated by the presence of a significant amount of the furanose structure in aqueous solution[24]. However, although the R.o.a. features are broader generally than those of D-arabinose, there are several similar features and it may prove possible to separate the superimposed spectra of the pyranose and furanose forms.

Maltose and cellobiose. — Comparison of the back-scattered R.o.a. spectra of maltose and cellobiose (Figs. 5 and 6, respectively) reveals marked differences.

The spectrum of maltose is similar to that of D-glucose. The couplet centred at $\sim 1325\ cm^{-1}$ for D-glucose and associated with deformations of the CH_2OH group is also given by maltose, except that it is spread over a larger region and is more intense with the appearance of distinct structure. This difference could reflect less conformational possibilities for the two CH_2OH groups in maltose with similar but not identical chiral environments. The smaller couplet centred at 1235 cm^{-1} for D-glucose is given also by maltose, and the fingerprint for D-glucose between ~ 960 and 1170 cm^{-1} is reproduced closely with maltose. On the other hand, most of the equivalent features in cellobiose are generally weaker and less structured, which could be associated with a different hydrogen-bonding network and, perhaps, greater conformational flexibility around the glycosidic linkage (see below).

The most important feature in this pair of R.o.a. spectra is the couplet for maltose centred at $\sim 910\ cm^{-1}$, positive on the low- and negative on the high-frequency side. No significant feature appears in the spectrum of D-glucose in this region, so that this couplet for maltose may be associated with the glycosidic linkage. Raman bands for α-D-glucose at 845 and 914 cm^{-1} have been assigned[26,27] to modes that involve a significant contribution from C-1′–H deformations. The C–O–C stretch that involves the α-(1→4) linkage in maltose appears[28,29] in the region 920–960 cm^{-1}, and this couplet

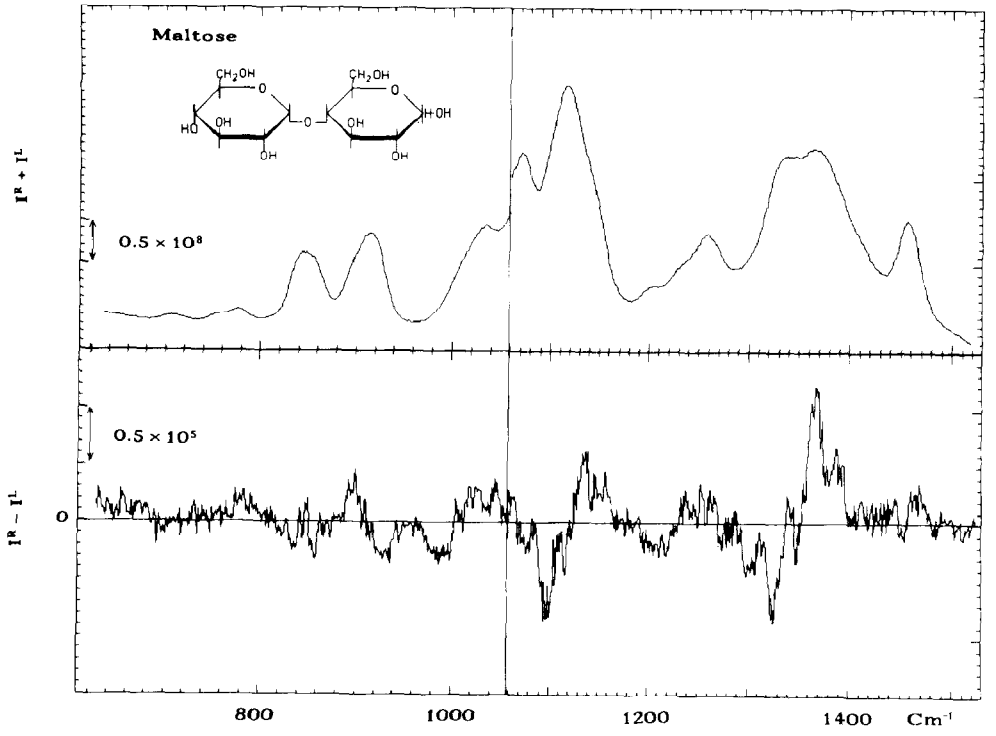

Fig. 5. The back-scattered Raman and R.o.a. spectra of maltose in aqueous solution.

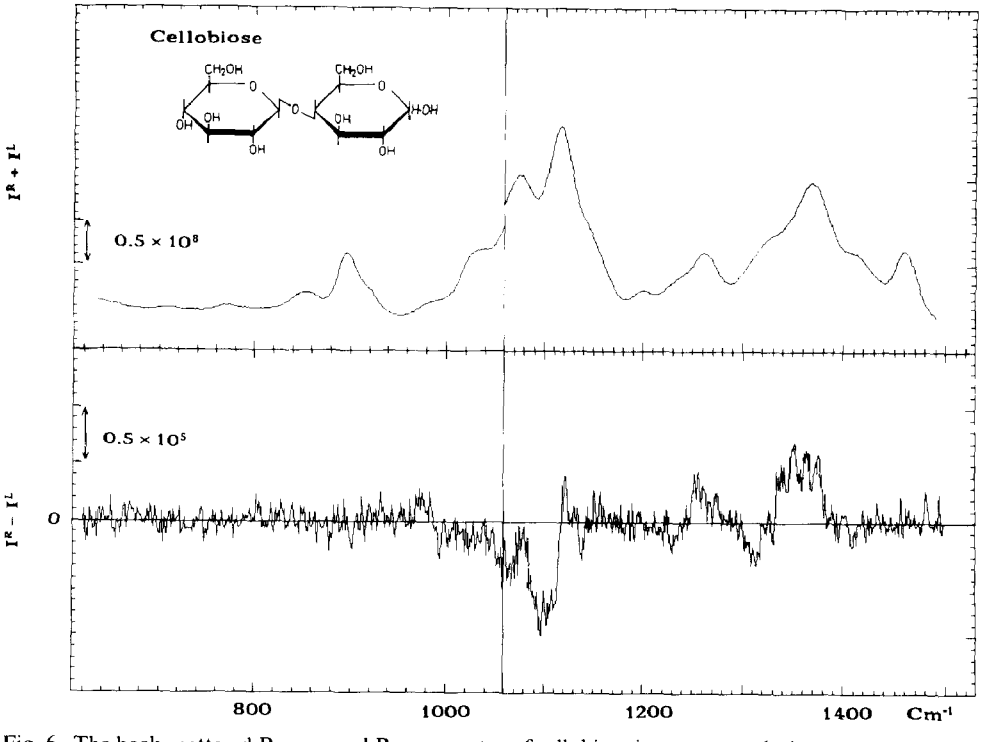

Fig. 6. The back-scattered Raman and R.o.a. spectra of cellobiose in aqueous solution.

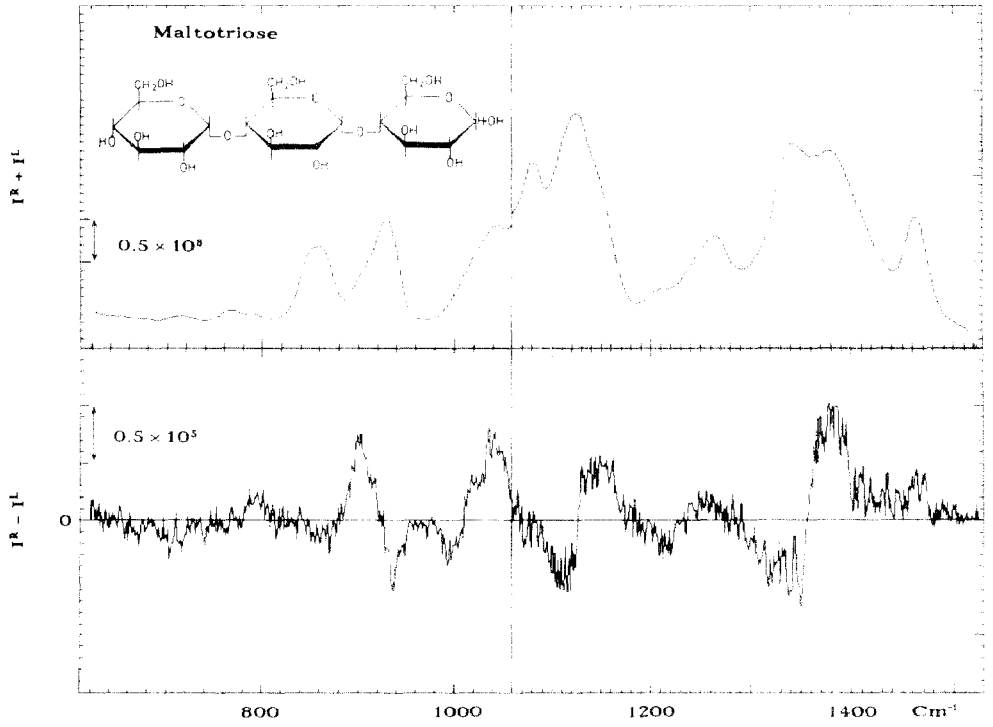

Fig. 7. The back-scattered Raman and R.o.a. spectra of maltotriose in aqueous solution.

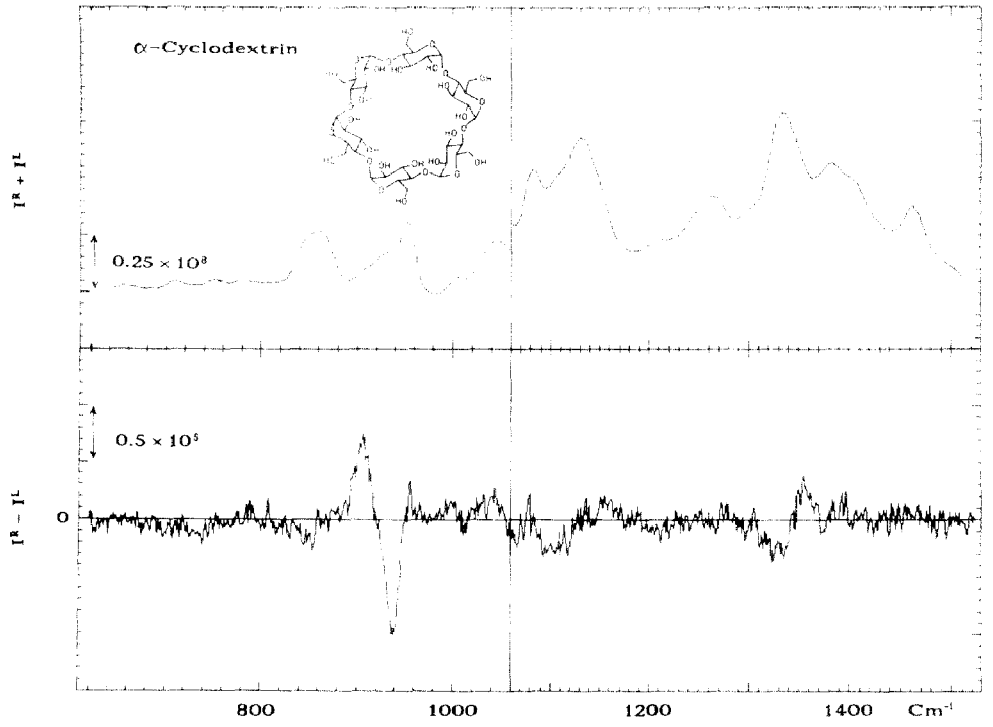

Fig. 8. The back-scattered Raman and R.o.a. spectra of α-cyclodextrin in aqueous solution.

is attributed to interactions of the C-1′–H deformations and the glycosidic C–O–C stretch, perhaps with some involvement of the C-4–H deformations. The R.o.a. spectrum of cellobiose contains no significant features in the region in which this couplet occurs for maltose, even though there are conventional Raman bands in the same place. One possible explanation is that the C-1′–H and C-4–H bonds are more nearly coplanar in cellobiose than in maltose, which is supported by the X-ray crystal data for cellobiose[30] and maltose[31]. Also, molecular dynamics calculations indicated there to be greater freedom of motion of the *eq,eq* linkage of cellobiose compared with the *ax,eq* linkage of maltose[32], so that cellobiose can adopt more conformations in solution, which would tend to remove the R.o.a.

Maltotriose and α-cyclodextrin (αCD, cyclomaltohexaose). — The back-scattered R.o.a. spectra of maltotriose and αCD are shown in Figs. 7 and 8, respectively. Maltotriose shows basically the same features as maltose, but most are broadened, which is consistent with the increased conformational possibilities. The couplet centred at $\sim 910\ cm^{-1}$ has grown relative to the other features, which reinforces its assignment to the glycosidic linkage and indicates that the conformation around the two glycosidic linkages in this molecule are similar to each other and to that in maltose.

The glycosidic feature for αCD is enormous with a Δ-value $[(I^R - I^L)/(I^R + I^L)]$ of several parts in 10^2, which is an order of magnitude larger than the largest dimensionless R.o.a. intensities usually encountered (it is not possible to provide a better estimate because the associated Raman bands are weak and overlap). A new, weak, positive feature has appeared also on the high-frequency side, which is connected with the fact that the associated Raman bands now constitute a triplet rather than the doublet for maltose and maltotriose. It has been suggested that this multiplicity indicates that not all of the glycosidic linkages in αCD are equivalent[29]. Most of the other features shown by maltose and maltotriose are discernible.

Maltose, maltotriose, and αCD each has a small but significant negative feature at $\sim 850\ cm^{-1}$ that, presumably, involves the second mode mentioned above, which contains a significant contribution from C-1′–H deformations.

1,6-Anhydro-β-D-glucopyranose. — The back-scattered R.o.a. spectrum of 1,6-anhydro-β-D-glucopyranose is shown in Fig. 9. Generally, the R.o.a. bands are more intense and sharper than for the other carbohydrates, and reflect the more rigid structure. The large bands in the region 820–950 cm^{-1} probably originate in modes similar to those involved in the glycosidic linkage; the fingerprint in the region 980–1160 cm^{-1} is reminiscent of those for D-glucose and D-xylose, and the two large positive features at ~ 1190 and $1225\ cm^{-1}$ together with the broad negative feature at 1250–1450 cm^{-1} might involve deformations of the CH_2 bridge.

Thus, the R.o.a. results re-affirm the considerable delocalisation of the vibrational modes of pyranosides, which renders experimental assignment of the majority of frequencies problematic, even in the most recent work[33], but gives readily discernible fingerprints characteristic of structural units, which obviates the need to assign the associated Raman bands in detail. The presence of detailed structure in some spectra and the contrasting broadening in others has implications for studies of conformational

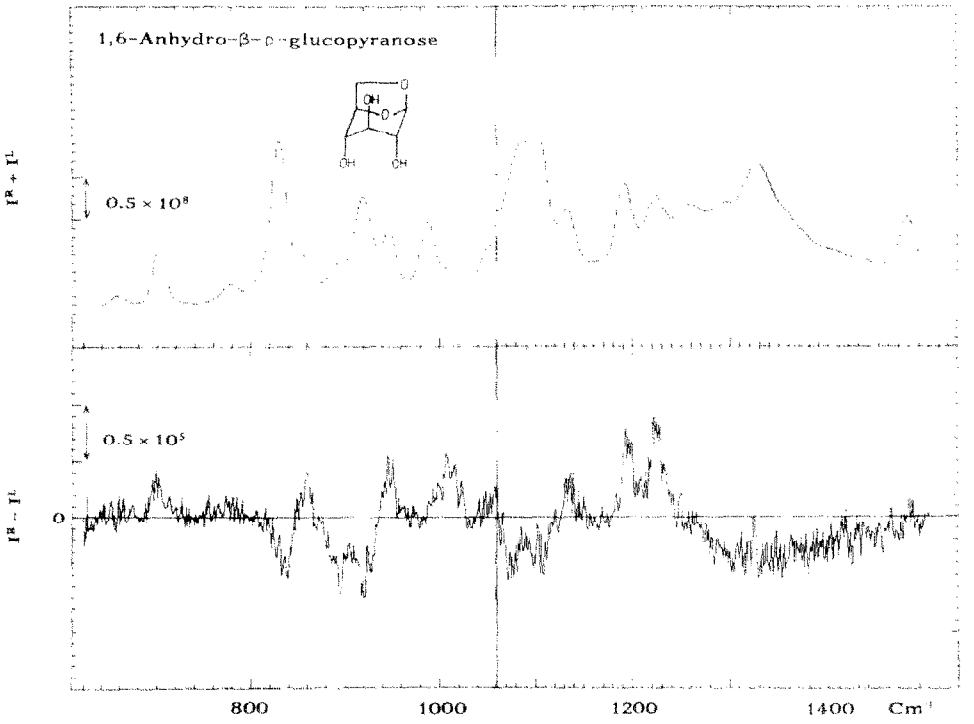

Fig. 9. The back-scattered Raman and R.o.a. spectra of 1,6-anhydro-β-D-glucopyranose in aqueous solution.

equilibria and hydrogen bonding. The potentially most valuable result is the identification of clear R.o.a. features associated with the glycosidic linkage, which appear to be sensitive to the conformation.

The use of near-saturated solutions with a rather long acquisition time (2 h) are temporary limitations which will be removed soon by current developments in instrumentation.

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