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Vibrational Raman optical activity study of D-glucose

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Abstract

Vibrational Raman optical activity (ROA) spectra in back-scattering from 600 to 1600 cm^{-1} of D-glucose, D-glucose-*1-d*, D-glucose-*6,6-d_2*, D-glucose-*O-d_5*, 5-thio-D-glucose, and D-glucosamine hydrochloride are reported. Prominent signals for D-glucose appear in the region above ~ 950 cm^{-1} and can be subdivided into a fingerprint region from ~ 950 to ~ 1200 cm^{-1}, and a CH_2 and C–O–H deformations region above ~ 1200 cm^{-1}. In the fingerprint region, ROA signals which reflect the relative orientation of substituents originate primarily in skeletal C–C and C–O stretch coordinates: C–O–H deformation coordinates also contribute to the parent Raman band intensity but not to the ROA intensity. In the CH_2 and C–O–H deformations region, negative and positive ROA bands appear at ~ 1220 and ~ 1260 cm^{-1}, which may be attributed to the *gauche–gauche* and *gauche–trans* rotamers of the exocyclic hydroxymethyl group, respectively. The ~ 1260 cm^{-1} ROA signal also reflects the anomeric configuration since only the β anomer generates an ROA signal at this wavenumber.

1. Introduction

Raman optical activity (ROA) is defined as a small differential scattering of right and left circularly polarized light from chiral molecules [1]. Recent advances in instrument technology [2] have increased the sensitivity beyond the level necessary to record ROA spectra of aqueous solutions of biological molecules. Among these, carbohydrates are particularly favourable for ROA studies, the utility of which lies in the fact that it may provide new stereochemical information [3] not accessible to other techniques: not only are the signs of ROA bands related to absolute configuration but their intensity also displays a great sensitivity to molecular conformation.

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Other chiroptical techniques such as electronic circular dichroism (ECD) have been applied to carbohydrates [4,5]. The main problem encountered in ECD studies of unsubstituted carbohydrates is that the main structural features absorb in the vacuum UV region [4]; consequently, only the long wavelength tails [5] of ECD bands are accessible to commercial instruments. This situation is somewhat improved for carbohydrates substituted with chromophoric groups, but then the optical activity is not being sampled directly [6]. In contrast, each of the 3N-6 normal modes contributes accessible bands in the ROA spectrum. Vibrational circular dichroism (VCD), the extension of circular dichroism into the IR, is a form of vibrational optical activity complementary to ROA which has also been applied to the study of carbohydrates [7,8]: however, VCD suffers from low instrument sensitivity and from the strong IR absorption of water, the natural solvent for biological activity.

NMR studies [9,10] provide the majority of spectroscopic data concerning the solution stereochemistry of carbohydrates. However, despite the fact that fewer resonances appear in carbohydrate spectra than in those of either proteins or nucleic acids, band assignment can pose a problem as a large number of these resonances fall within an extremely narrow chemical shift range. Furthermore, only an average conformation is revealed directly by NMR as a result of the relatively long timescale of the experiment. Usually, NMR data must be supplemented by theoretical calculations of potential energy surfaces [10], which serve to limit the amount of available conformational space and give meaning to data collected from NOE and spin-coupling experiments. Finally, it is worth noting that NMR can only distinguish between enantiomers through the use of chiral auxillaries [11].

The previous paper in this series [12] provided a general comparative survey of the ROA spectra of 15 monosaccharides. One important conclusion was that the ROA spectra of epimeric pairs of sugars display large differences, whereas those of homomorphic pairs are closely related. Therefore, to improve the understanding of the origin of ROA effects in carbohydrates, a detailed comparison of molecules from the same homomorphic series, including selected isotopomers, is necessary. D-Glucose and its homomorphs were chosen as the focus of this study because of the large number of papers relating to vibrational assignments, normal coordinate analyses, and force fields; and also because D-glucose is of interest as a component of many important di-, oligo-, and poly-saccharides which are the subject of current ROA studies in Glasgow [13,14].

2. Experimental

All the carbohydrate samples were dissolved in distilled H_2O to a concentration of 4.5 M and then allowed to equilibrate at room temperature for at least 24 h. The equilibrated samples were treated with charcoal to reduce fluorescence, filtered through 0.45-μm Millipore membrane filters into quartz microfluorescence cells, then centrifuged for at least 15 min to remove any dust particles which may cause spurious light-scattering. Samples of D-glucose-*1-d* and D-glucose-*6,6-d_2*

were supplied by Aldrich, 5-thio-D-glucose by Fluka, D-glucosamine hydrochloride by Sigma, and D-glucose by BDH. A sample of D-glucose-*O*-d_5 was prepared by lyophilising D-glucose from D_2O solution twice to ensure at least 90% exchange.

The major features of the Glasgow ROA instrument GUROAS1, which is described in detail elsewhere [2], are the back-scattering geometry to maximize the signal-to-noise ratio, the cooled backthinned CCD detector with its high quantum efficiency and low readout noise, the holographic notch filter which suppresses stray light from the Rayleigh line, and the Lyot depolarizer without which the spectra would be swamped with artifacts. During spectral acquisition, the laser power was ~ 700 mW at the sample, and the slit width was set for 120 μm, giving a spectral band pass of ~ 12 cm^{-1}. The spectra were recorded over 2 to 4 h, depending on the strength of the ROA signals, at constant ambient temperature.

3. Results and discussion

The ROA spectra of D-glucose, D-glucose-*1-d*, D-glucose-*6,6*-d_2, D-glucose-*O*-d_5, 5-thio-D-glucose, and D-glucosamine hydrochloride in the range 600–1600 cm^{-1} are shown in Figs. 1–6, respectively. Each can be conveniently subdivided into three distinct regions: the anomeric region from ~ 600 to 950 cm^{-1}, the fingerprint region from ~ 950 to 1200 cm^{-1}, and the CH_2 and C–O–H deformations region from ~ 1200 to 1500 cm^{-1}. Each of these subdivisions will be discussed separately, although some correlations will be drawn from the complete spectra. In this study, an empirical approach will be used as glucose is at this time still too large to be treated by *ab initio* ROA intensity calculations [15] and the normal modes are too complex for the ROA intensities to be explained by simple models such as the two-group model or the bond polarizability model [1] for the generation of ROA intensities.

The molecules discussed in this paper all belong to the *gluco* series, which share the same absolute configuration at carbon atoms 2, 3, and 4. The deuterated samples were investigated to provide a basis for band assignment. Previous conventional vibrational spectroscopy of glucose isotopomers [16–19] relied on a mixture of solution Raman spectra for the *O*-deuterated samples and solid-state IR and Raman spectra for the *C*-deuterated samples, but no solution Raman studies of the *C*-deuterated samples have been reported previously.

Anomeric region (600–950 cm^{-1}).—Unlike the other monosaccharides investigated [12] the ROA spectrum of D-glucose (Fig. 1) has few bands in this region, with only two weak positive features appearing at ~ 913 and ~ 633 cm^{-1}. D-Glucose in H_2O exists as an ~ 2:1 equilibrium mixture of β- and α-anomeric forms. From examination of the ROA spectra of methyl α- and β-D-glucopyranosides [12], it is clear that the β-anomeric form of D-glucose yields almost no ROA signal in this region. Thus, it is possible that the weakness of the signal is simply the result of the predominance of the β-anomeric form. Since β-D-glucose has no axial hydroxyl groups, we may conclude that ROA in this region somehow depends on the ability of hydroxyl groups in axial positions to interact with the exocyclic

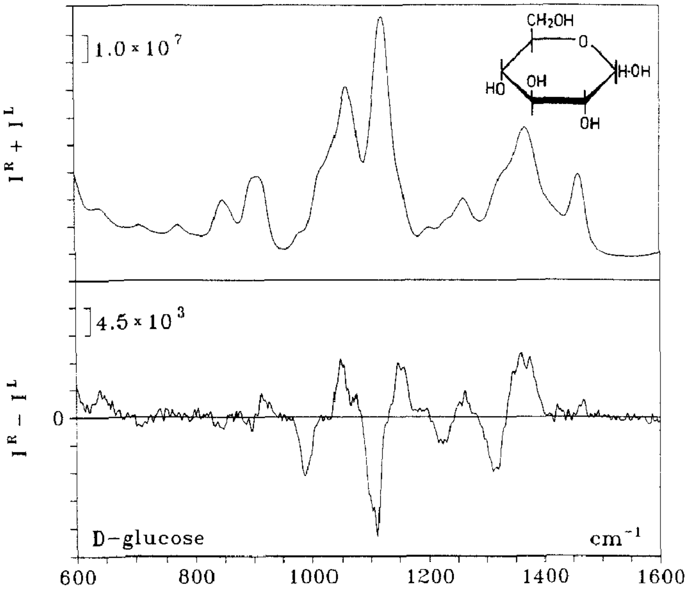


Fig. 1. The back-scattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of D-glucose in H_2O.

hydroxymethyl groups and the ring oxygen which are involved in the majority of modes found in this region: indeed, other monosaccharides such as galactose, mannose, and lyxose [12] containing axial hydroxyl groups show large ROA bands

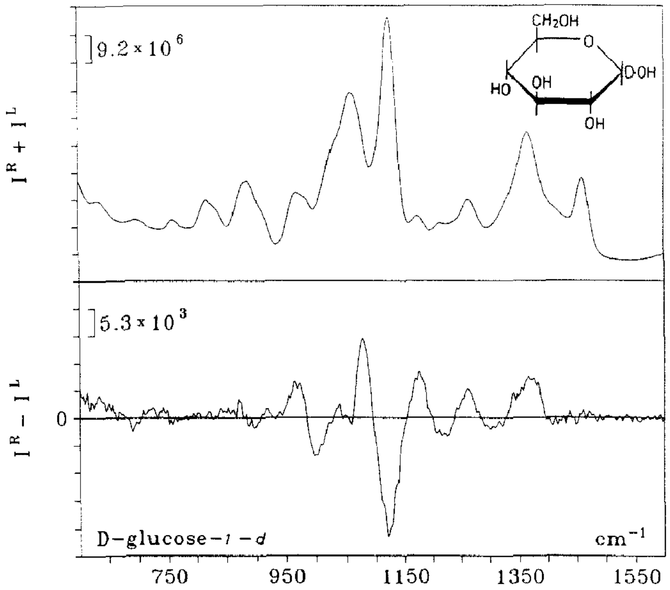


Fig. 2. The back-scattered Raman and ROA spectra of D-glucose-1-d in H_2O.

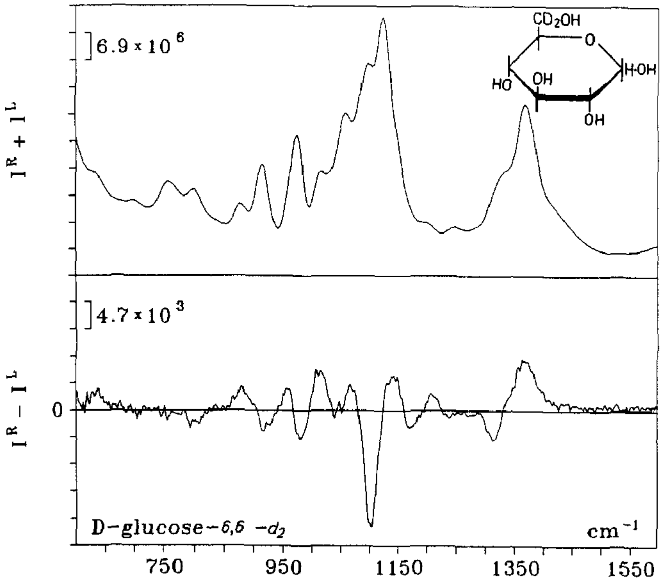


Fig. 3. The back-scattered Raman and ROA spectra of D-glucose-*6,6-d*$_2$ in H_2O.

here. The relatively low density of bands has simplified the assignment of the conventional vibrational spectrum: patterns have been found that can identify anomeric configuration [20] and ring type [21], and can distinguish between certain

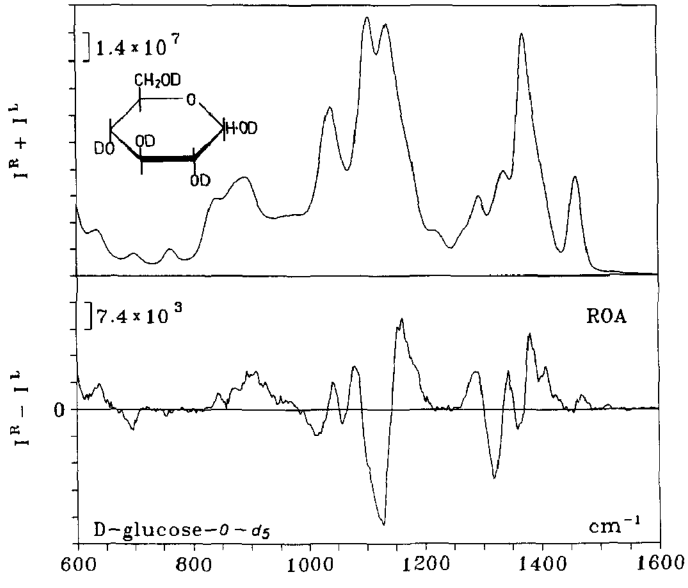


Fig. 4. The back-scattered Raman and ROA spectra of D-glucose-*O-d*$_5$ in D_2O.

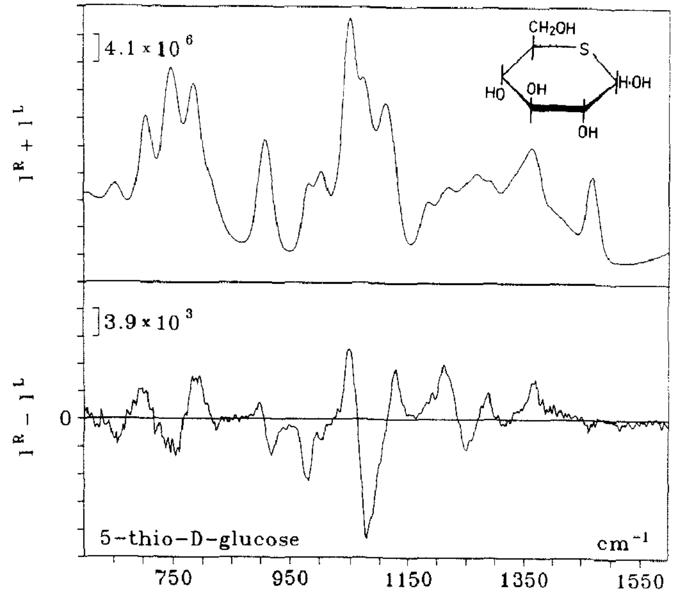


Fig. 5. The back-scattered Raman and ROA spectra of 5-thio-D-glucose in H_2O.

linkage types [20] in di- and poly-saccharides. Furthermore, the sensitivity of the ROA in this region to anomeric configuration also makes it important for studying the glycosidic link conformation in di-, oligo-, and poly-saccharides [13,14].

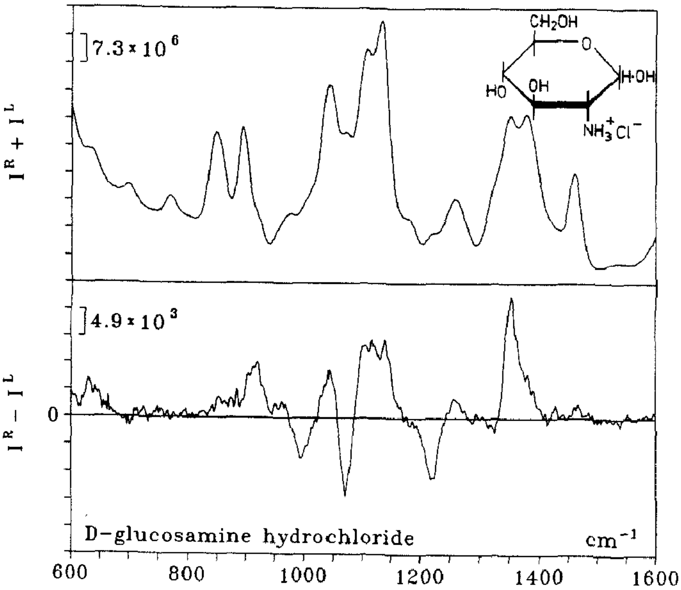


Fig. 6. The back-scattered Raman and ROA spectra of D-glucosamine hydrochloride in H_2O.

The Raman band of weak-to-medium intensity at ~ 910 cm^{-1} in D-glucose (Fig. 1) can in fact be resolved into two components, with the higher wavenumber constituent giving rise to a positive ROA feature at ~ 913 cm^{-1}. This band corresponds to the feature reported at ~ 920 cm^{-1} in the previous paper on monosaccharide ROA [12] (the disparity between the two wavenumbers can be attributed to noise fluctuations on some bands, which result in a greater uncertainty of the peak positions in the ROA bands than in the parent Raman bands). Comparison of the conventional Raman spectra of the two methyl D-glucopyranosides [12] suggests that this band is characteristic of the α-anomeric form, in accord with Barker et al. [20] who attributed this mode to a ring vibration of the α anomer. This band vanishes upon deuteration of the C-1–H position (Fig. 2) and gives rise to a negative feature when the C-6–H_2 positions are deuterated (Fig. 3), thus exposing the role that motions of these groups play in the generation of the associated ROA. Finally, a C–O–H bending contribution to the normal mode can be identified from the wavenumber shift and decrease in intensity of the Raman band in D-glucose-*O*-d_5 (Fig. 4); however, it is not clear whether the associated ROA band is also shifted, as other ROA signals shifted into this region lead to a broad ROA feature at this wavenumber. Normal coordinate analyses [19,22–24] assign this mode to a highly coupled vibration involving the C-1–O stretching mode, endocyclic stretching modes, and deformation modes around the anomeric carbon. Combining all these data, it is possible to assign this vibrational mode to a ring vibration of the α-anomeric form coupled to C–O stretches around the ring with contributions from C-1–H and CH_2 motions.

Two Raman bands of diagnostic importance are found at ~ 840 and ~ 890 cm^{-1}, and are considered indicative of the presence of the α and β anomers, respectively. Although neither gives any ROA signal in the glucose homomorphic series, these bands give rise to interesting ROA bands in α-linked disaccharides and were also highlighted in the previous paper of this series [12] as being important signals in other monosaccharides. Early work [20] assigned these bands to vibrations involving C-1–H bending, but only a small change is evident in the Raman spectrum of D-glucose-*1-d* (Fig. 2) and a much larger shift is observed in the spectrum of D-glucose-*6,6*-d_2 (Fig. 3). For D-xylose [13], which has no exocyclic hydroxymethyl group, the band at ~ 840 cm^{-1} disappears; in the spectrum of 5-thio-D-glucose (Fig. 5), which has the ring oxygen replaced by a sulfur atom, the band is shifted to lower frequency. From these results, it would appear that this vibration involves motions of the CH_2, C–O-5 and a small contribution from the C-1–H group, which is almost identical to the mode predicted by Cael et al. [23] and concurs with the work of Korolvich et al. [24] who suggested that the C-1–H contribution was minor with the majority of the potential energy distribution arising from motions of the O-1–C-1–O-5–C-5–C-6 chain.

The replacement of oxygen by sulfur in 5-thio-D-glucose leads to a dramatic increase in the intensity of Raman and ROA bands between ~ 700 and 800 cm^{-1}, leading to the conclusion that C–O–C bending motions about the ring oxygen are intimately involved in these normal modes in D-glucose, a view which is supported by normal coordinate analyses [19,22–24]. The ROA spectrum of D-glucosamine hydrochloride (Fig. 6) is very similar to that of D-glucose in this region with only the two positive bands appearing at ~ 913 and ~ 633 cm^{-1}. It would appear then that the substituent at C-2 has no effect on the ROA in this region.

Fingerprint region (950–1200 cm^{-1}).—This region of the Raman spectrum is dominated by exo- and endo-cyclic C–O and C–C stretches coupled with significant contributions from C–O–H deformations [22–24]. It was noted in a review of the vibrational spectra of carbohydrates [25] that this region is difficult to interpret on account of the widespread coupling of C–O and C–C stretches, the poor discrimination between endo- and exo-cyclic contributions, and the small differences between the configurational positions of each C–O group. This is not the case for ROA where the extensive coupling is a prerequisite for strong ROA signals, and configurational changes have a comprehensive and characteristic effect on the ROA pattern in this region [12].

For D-glucose (Fig. 1), the characteristic sign pattern is, starting from low wavenumber, negative at ~ 988 cm^{-1}, positive at ~ 1048 cm^{-1}, negative at ~ 1111 cm^{-1}, and positive at ~ 1150 cm^{-1}. Previous ROA studies [12,13] compared D-glucose with D-xylose in this region and the sign pattern was found to be the same, although wavenumber shifts were evident. D-Xylose differs from D-glucose only in that it has no exocyclic hydroxymethyl group; thus, it is clear that the ROA signals in this region are not dependent on motions of this group. The effect of endocyclic C–O stretches in this region can be gauged by examining the ROA spectrum of 5-thio-D-glucose (Fig. 5) where it is noted that the features at ~ 1150 and ~ 1111 cm^{-1} in D-glucose are shifted to ~ 1128 and ~ 1078 cm^{-1}, respectively, although they retain the same intensity, and that the two other features are unaffected. From these observations, it can be deduced that the bands at ~ 1150 and ~ 1111 cm^{-1} involve some endocyclic C–O stretch contribution, which is in accord with a normal coordinate analysis [24] that placed a 41% potential energy contribution to the mode at 1116 cm^{-1} from the C-O-5 stretch. The ROA of D-glucosamine hydrochloride (Fig. 6), which has the hydroxyl group at C-2 replaced by an NH_3^+ group while retaining the same configuration, displays profound changes in this region. The negative and positive features at lower wavenumber are retained; however, at higher wavenumber, the pattern changes quite dramatically: this is probably due to the C-2–N stretch coordinate, which is expected [26] to contribute to normal modes between ~ 1020 and ~ 1220 cm^{-1}.

Examining the ROA spectrum of D-glucose in D_2O (Fig. 4), it is clear that it displays the same sign pattern in the fingerprint region as found for D-glucose in H_2O (Fig. 1). The conventional Raman spectrum, however, does show changes, namely, a new band at ~ 1108 cm^{-1} and a shift from ~ 1060 to ~ 1042 cm^{-1} of another. From these bandshifts, it may be concluded that C–O–H deformations make a significant contribution to the conventional Raman band frequencies and intensities in this region but little contribution to the associated ROA band intensities, which are generated mainly by the exo- and endo-cyclic C–O and ring C–C stretch coordinates in the normal modes. The ROA of D-glucose-*1-d* (Fig. 2) shows only one minor change, namely, a frequency shift of the positive feature at ~ 1150 cm^{-1} in D-glucose to ~ 1175 cm^{-1}, indicating that C-1–H bending motions are involved in this normal mode and that deuterating this position decouples the vibration. However, the ROA sign and intensity are unaffected which indicates that other motions must be largely responsible for generating the ROA. Some changes are evident in the D-glucose-*6,6-d_2* spectra (Fig. 3), but the positive, negative, and positive sign pattern remains intact. The origin of these changes is difficult to explain because many of the bands in the region above ~ 1200 cm^{-1} have been shifted into this region. However, the new Raman band appearing at ~ 975cm^{-1} has previously been assigned to a CD_2 motion [16], and the new Raman band at ~ 1098 cm^{-1} can also probably be associated with CD_2 motions shifted from the ~ 1460 cm^{-1} mode in D-glucose, which has been shown [23] to be an almost pure CH_2 vibration (this fits with the ratio of corresponding wavenumbers in D-glucose and D-glucose-*6,6-d_2*, which is close to the theoretical maximum of $\sqrt{2}$ for an isotopic wavenumber shift [27]).

CH_2 and C-O-H Deformations region (1200-1500 cm^{-1}).—The effects of deuteration in this region are far more pronounced than at lower wavenumber because there is no added complication of overlap from bands shifted from higher wavenumber. Previous assignments for *C*-deuterated glucoses were based on work carried out by conventional IR spectroscopy in the solid state [16] or FT-IR in D_2O [17], but such results are not necessarily transferable to Raman spectroscopy in H_2O for two reasons: first, IR and Raman spectroscopy are complementary techniques governed by different selection rules and giving different intensities, and second, the solid-state conformation will not necessarily be the same as the solution conformation. The major contributions to normal modes in this region come from CH_2, C-O-H, and C-H deformations [12], so that samples with these groups deuterated, as we discuss here, should be of particular interest. Earlier ROA studies [12,13] concentrated on the influence of the exocyclic hydroxymethyl group in this region.

D-Glucose (Fig. 1) shows an ROA couplet, negative at low wavenumber and positive at high, centred at ~ 1240 cm^{-1}, which is unaffected by C-1-H (Fig. 2) deuteration but is shifted to lower wavenumber in D-glucose-*6,6-d_2* (Fig. 3) and is absent in D-glucose-*O-d_5* (Fig. 4). For D-glucose-*6,6-d_2*, the couplet remains intact and is centred at ~ 1190 cm^{-1}, which implies that this feature originates in a mode involving coupling between CH_2 and C-O-H vibrations. In fact, on the basis of *O*-deuteration of dextran, an α-(1 → 6)-linked polysaccharide, which exhibits no change in the ~ 1260 cm^{-1} Raman band, Vasko et al. [16] assigned this band to a C-6-O-H bending mode. Support for this assignment was provided by a normal coordinate analysis [23] which described a mode at ~ 1259 cm^{-1} involving motions of the CH_2, C-6-O-H and C-O-H groups all around the ring (Fig. 7). Further, recent conventional solid-state Raman work on glucose isotopomers assigned bands at 1267 and 1206 cm^{-1} to CH_2 modes [19].

Although both methyl α- and β-D-glucopyranosides are known from NMR studies [28] to have very similar distributions of *gauche-gauche* (*gg*) and *gauche-trans* (*gt*) rotamers of the exocyclic hydroxymethyl group (Fig. 8), only methyl β-D-glucopyranoside shows both the positive ~ 1260 and negative ~ 1220 cm^{-1} constituents of the ~ 1240 cm^{-1} ROA couplet shown by D-glucose itself. The

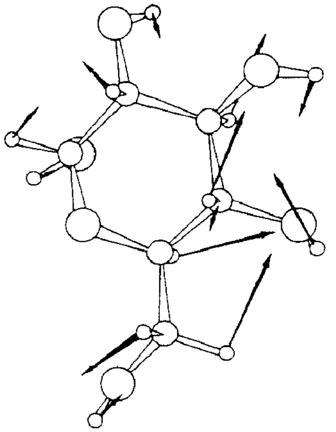
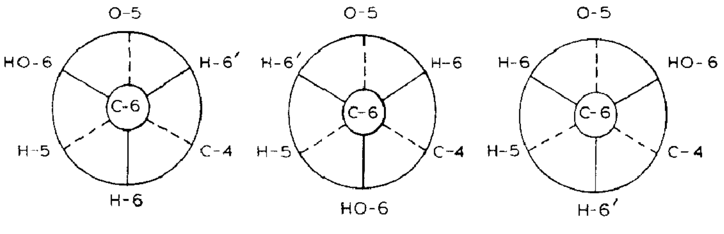


Fig. 7. The normal mode calculated at 1259 cm^{-1} adapted from ref 23 (with permission from the publisher).

positive feature at ~ 1260 cm^{-1} is absent from the ROA spectrum of methyl α-D-glucopyranoside [12], and is also absent in the disaccharide trehalose [29] and in α-cyclodextrin [14], both of which exist 100% in the α-anomeric form. On the other hand, these last three molecules all show the negative ROA band at ~ 1220 cm^{-1}. Since our deuteration studies (*vide supra*) indicate that similar vibrational coordinates contribute to the ~ 1220 and ~ 1260 cm^{-1} ROA bands, it is tempting to attribute these two features to the corresponding normal vibrations of two distinct conformers; but these cannot be simply α and β anomers since, as mentioned above, methyl β-D-glucopyranoside shows both bands. One possibility is that the positive ~ 1260 cm^{-1} ROA band arises from the *gt* rotamer of the β anomer in the D-glucose homomorphic series, and that the negative ~ 1220 cm^{-1} ROA band arises from the *gg* rotamer and is independent of the anomeric configuration. Supporting evidence for this suggestion includes the observations on the D-galactose homomorphic series discussed below.

It was noted in the previous paper in this series [12] that D-galactose and both the methyl D-galactosides exhibit a strong positive ROA feature at ~ 1260 cm^{-1},



gauche-trans (GT) *trans-gauche (TG)* *gauche-gauche (GG)*

Fig. 8. Representation of the *gg*, *gt*, and *tg* rotameric forms in D-glucose.

which recent normal coordinate analysis results [30] have attributed to a normal mode involving C-4–O–H and C-6–O–H motions. In D-galactose, the hydroxymethyl group exists in either the *gauche–trans* (*gt*) or *trans–gauche* (*tg*) rotameric forms with the *gauche–gauche* (*gg*) form being strongly disfavoured as a result of the syn-diaxial relationship of the C-4–OH and C-6–OH groups [28] (Fig. 8). The ROA band at ~ 1260 cm^{-1} in D-galactose [12] is very intense and this may be due to the proximity of the C-6–OH and C-4-OH groups, especially in the *tg* rotameric form. This is an example of the local nature of many ROA features and could explain why this band is not sensitive to the C-1 configuration in the galactose homomorphic series since the effect from C-4 overwhelms that from C-1. None of the spectra in the D-galactose series exhibits a negative feature at ~ 1220 cm^{-1}, which may provide further evidence for an assignment to the *gg* rotamer, because, as mentioned above, the gg rotamer is strongly disfavoured in molecules with an axial C-4–OH group so we would not expect any associated ROA signal in D-galactose.

Also of interest is the ROA spectrum of D-mannose [12] which exists predominantly in the α-anomeric form [9]. D-Mannose is considered to have approximately the same rotameric distribution as D-glucose [31] despite having a different anomeric population. The appearance of the D-mannose spectrum is similar to that of methyl α-D-glucopyranoside [12] in this region with the ~ 1260 cm^{-1} Raman band not yielding any ROA signal. This provides evidence that the configuration at C-2 does not significantly influence the CH_2OH group conformation. Thus, the couplet at ~ 1240 cm^{-1} in the ROA spectrum of D-glucosamine hydrochloride (Fig. 6), which differs from D-glucose only at C-2, can be explained in terms of the anomeric ratio, which is ~ 2:1 in favour of the α-anomeric form [32]. These facts provide evidence that only the β-anomeric form contributes to the positive band at ~ 1260 cm^{-1}, so we would expect it to be considerably weaker than the negative band at ~ 1220 cm^{-1} as is indeed the case for D-glucosamine hydrochloride.

The negative ROA feature at ~ 1316 cm^{-1} in D-glucose (Fig. 1) is shifted and weakened in the spectrum of D-glucose-*1*-d_1 (Fig. 2) but remains unchanged in the spectra of the other deuterated analogues (Figs. 3 and 4). A conventional IR survey [33] of a variety of monosaccharides selectively deuterated at C-1 revealed that a C-1–H bending mode appears between 1275 and 1340 cm^{-1}. In particular, a band at 1312 cm^{-1} in the spectrum of β-D-glucose was found to shift to 972 cm^{-1} upon deuteration. Thus, the positive band at ~ 970 cm^{-1} in the ROA spectrum of D-glucose-*1-d*, which is not present in D-glucose, may be attributed to a C-1–D bending coordinate that has been shifted from ~ 1316 cm^{-1}. It may be noted that the sign of the ROA band has changed and this probably reflects the different coupling opportunities of the C-1–D bending coordinate. The experimental data imply that the ~ 1316 cm^{-1} mode contains no C–O–H or CH_2 bending contribution and consists instead of an almost pure C-1–H bending motion. Further evidence is available from the ROA spectrum of D-xylose [13], which exhibits this band although it lacks an exocyclic hydroxymethyl group. FT-IR studies [17] attributed this mode to a coupled motion of C-1–H and C-5–H bends and it is predicted by normal coordinate analysis to contain contributions from all the C–H bends around the ring [19,22]. The deformations of the C-1–H bond would appear then to play an important role in the generation of the negative ROA band at ~ 1316 cm^{-1}.

The positive ROA band centred at ~ 1360 cm^{-1} in D-glucose (Fig. 1) displays distinct structure and would appear to consist of three components. Upon deuteration of the hydroxyl groups (Fig. 4), the central component is shifted from ~ 1364 to ~ 1288 cm^{-1} and can thus be assigned to C–O–H deformation. Assignment of the other two components is more difficult, as they are unaffected in the deuterated analogues, but normal coordinate analyses seem to indicate that these are C–H-related modes.

4. Concluding remarks

The ROA spectra reported and discussed in this paper highlight a number of important features which pertain not only to carbohydrate examples but also to the fundamental nature of ROA generation. In particular, as we saw in the case of the ROA bands in the fingerprint region, not all of the internal stretching and bending coordinates that make up the normal mode and affect the intensity and wavenumber of the parent Raman band are necessarily involved in the generation of the ROA signals, which often seem to be largely determined by the vibrational coordinates of the skeleton. Similar behaviour has been observed for the ROA associated with skeletal modes in other molecules, including tartaric acid and its *O*-deuterated analogue [35], and illustrates how the sensitivity of ROA to chirality renders it a more effective probe of molecular conformation than conventional vibrational spectroscopy.

For carbohydrates, the three regions of the ROA spectrum have been found to give different types of stereochemical information (Table 1). The anomeric region, although not very informative for the D-glucose monomer, has been shown to be

Table 1
Assignment of ROA signals in the spectrum of D-glucose

Wavenumber in cm^{-1} (sign)	ROA assignments	Comments
~ 913 (+)	C–C and C–O stretches; C–O–H, C-1–H, and CH_2 deformations	This ring vibration of the α anomer is coupled to C–O stretches with C-1–H and CH_2 contributions
~ 950–1200 (– + – +)	C–C and C–O stretches	This sign pattern is characteristic for ring substitution. C–O–H deformations are not involved in the generation of ROA
~ 1220 (–)	CH_2 and C–O–H deformations	This signal is characteristic for the *gg* rotamer in both α- and β-anomeric forms
~ 1260 (+)	CH_2 and C–O–H deformations	This signal is characteristic for the *gt* rotamer in the β-anomeric form
~ 1316 (–)	C-1–H deformation	

sensitive to the anomeric configuration in many other monosaccharides [12] and also to contain features related to the type and conformation of the glycosidic link in di- and oligo-saccharides containing D-glucose residues [13,14]. The fingerprint region has proven to be very sensitive to the configuration at the individual chiral centres and as such can provide a direct probe of the structural framework of carbohydrates. The stereochemistry of the pendant side-groups is most important in the generation of ROA features above ~ 1200 cm^{-1} and this region could, in the future, provide accurate conformational data on such features as the rotameric populations of the exocyclic hydroxymethyl group.

The next step towards complete understanding of monosaccharide ROA spectra will probably be based on *ab initio* calculations [15], but, as mentioned above, such calculations cannot as yet deal with molecules as large as monosaccharides. Further information might be available from the ROA spectrum at lower wavenumber than studied here, where modes involving skeletal bending and twisting vibrations and C–O torsions are found with large differences evident even in the conventional Raman spectra of homomorphic sugars [25] at low wavenumber. In fact, such studies are now feasible, since improvements in filter technology have recently enabled us to extend back-scattered ROA measurements down to ~ 250 cm^{-1}, with measurements down to ~ 350 cm^{-1} achieved at an intermediate stage [36].

5. Acknowledgments

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