Carbohydrate Research, 165 (1987) 173–187
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

VIBRATIONAL ASSIGNMENTS AND ISOMERIZATION RATE-CONSTANTS FROM TIME-DEPENDENT F.T.-I.R. SPECTRA OF SUGARS*

DARLENE M. BACK AND PRASAD L. POLAVARAPU**
Department of Chemistry, Vanderbilt University, Nashville, TN 37235 (U.S.A.)
(Received October 29th, 1986; accepted for publication in revised form, February 7th, 1987)

ABSTRACT

Time-dependent F.t.-i.r. absorption spectra of α-D-galactose, α-D-fucose, α-D-glucose-*1*-d_1, α-D-glucose-*2*-d_1, and α-D-glucose-*5,6,6*-d_3 were measured. The spectral changes were interpreted in terms of anomeric isomerization, and possible vibrational origins were suggested. The intensity changes seen for α-fucose were found to follow a reversible first-order rate-equation and the rate constants obtained from different vibrational bands were found to be consistent among themselves and in reasonable agreement with those obtained by other techniques.

INTRODUCTION

When a pure anomer is dissolved in water, the tautomeric reaction proceeds until an equilibrium is achieved. The structural changes that occur include interconversion among α and β anomeric forms and pyranose and furanose ring-forms. These changes will be reflected in the spectra as new bands developing, some initial bands decreasing in intensity, and shifts in some band positions. In order to make use of this potential source for the vibrational-band assignments of different isomers in solution, which are needed for vibrational circular dichroism interpretation[2–5], we have recently initiated[1,6–8] time-dependent F.t.-i.r. investigation of simple sugars in aqueous solutions. Earlier articles reported the findings on glucose[6,7], lyxose[8], and sucrose[1]. We now present the results of studies on galactose, fucose, and isotopomers of glucose. These experimental data and the resulting assignments are needed in order to determine the scaling factors for the force constants derived recently[9] for glucose by using a molecular-orbital scheme. The time-dependent absorption data of fucose are also used to verify whether the rate constants obtained from the absorption intensities of different bands are in agreement among themselves and with those derived from other methods. This verification is important in order to explain the kinetic isotopic effects.

*Fourier-transform Infrared Spectroscopy of Sugars. Part V. For Part IV, see ref. 1.
**To whom correspondence should be addressed.

0008-6215/87/$ 03.50

EXPERIMENTAL

Samples of carbohydrates were dissolved in H_2O or D_2O at a concentration of ~20% w/v ($g.mL^{-1}$). Immediately on dissolving, the solution was injected into a variable-pathlength cell having BaF_2 windows. The time lapse between the addition of solvent and the starting of data acquisition was <60 s. For slower reactions, such as those of D-glucose in H_2O, 32 interferograms were averaged, Fourier-processed, and ratioed against the background spectrum, and the spectra were obtained every few minutes. For fucose and galactose, spectra were obtained about every 1 min. The time gap between spectra was increased to ~10 min near the end of the reaction. Spectral deconvolution[10] and the integrated intensities were obtained by using the software supplied with our F.t.-i.r. instrument. The undeuterated samples were obtained from Sigma Chemical Co. The deuterated samples were obtained from Merck and from Cambridge Isotope Labs, Inc. The percent composition of the

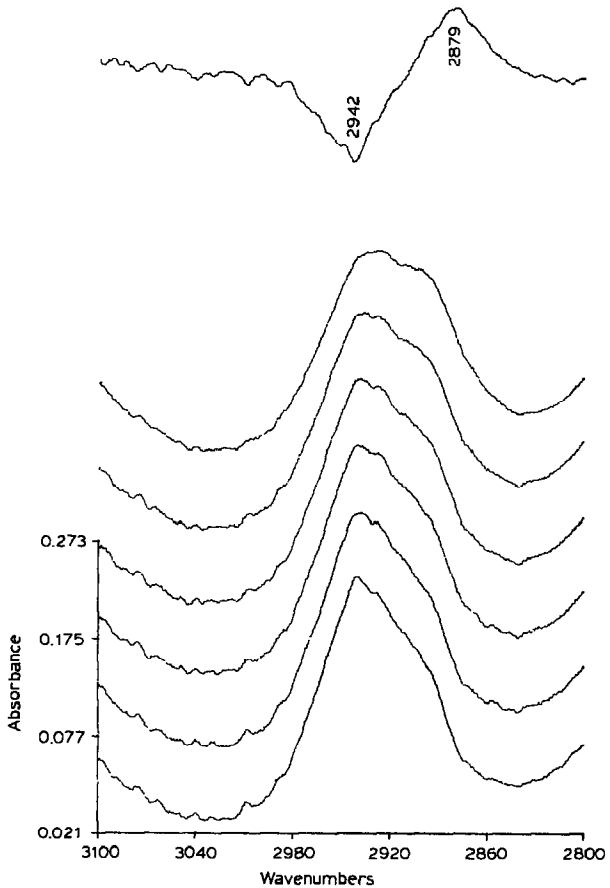

Fig. 1. Time-dependent F.t-i.r. absorption spectra of α-D-galactose in D_2O, taken at (from bottom), 0, 9, 26, 41, 71, and 169 min. [The successive spectra are moved upwards for clarity. The topmost trace is the difference between the final and initial spectra and is plotted on an arbitrary intensity scale.]

α-pyranose form in the supplied samples of glucose-*1*-d_1, glucose-*2*-d_1, glucose-*6,6*-d_2, and glucose-*5,6,6*-d_3, were, respectively, ~90, 95, 42, and 95%. These starting compositions were determined from ^{1}H-n.m.r. spectra in Me_2SO-d_6 as the solvent. Because the percentage of β anomer in the samples of glucose-*1*-d_1, glucose-*2*-d_1, and glucose-*5,6,6*-d_3 was small, these samples are labeled as α anomers in the Figure captions and in the text.

BAND ASSIGNMENTS

From the difference in time-dependent spectra (see Figs. 1 and 2) in the C–H stretching region, it is seen that, for galactose, the intensity decrease is centered at ~2942 cm^{-1}, and the intensity increase is centered at ~2879 cm^{-1} (at ~2883 cm^{-1} for fucose). The corresponding positions for glucose were found[7] to be ~2948 and ~2884 cm^{-1}. If these decreasing and increasing intensity positions are to be assigned to the isolated anomeric stretching-modes, these changes should be absent in the spectra of α-glucose-*1*-d_1. On the contrary, the time-dependent spectra of α-glucose-*1*-d_1, α-glucose-*2*-d_1 (not shown here), and α-glucose-*5,6,6*-d_3 all have (see Fig. 3) changes similar to those found for α-glucose, α-galactose, and α-fucose.

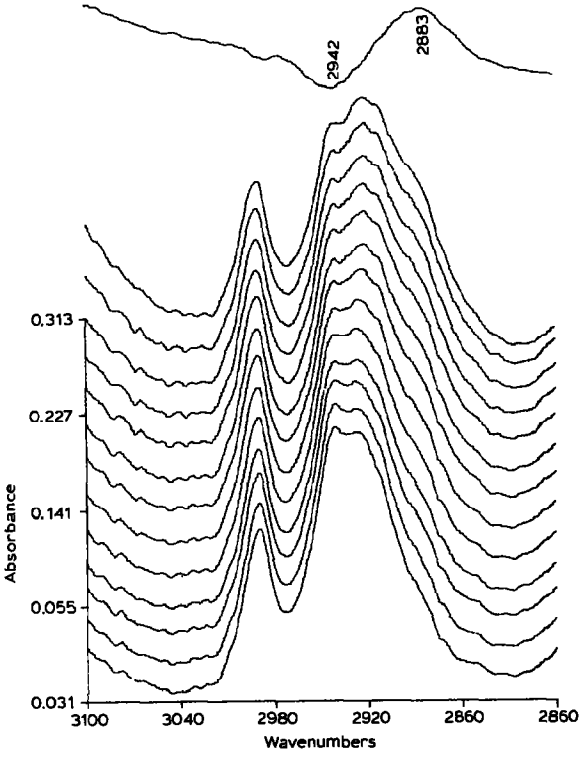

Fig. 2. Time-dependent F.t.-i.r. absorption of α-D-fucose in D_2O, taken at (from bottom) 0, 5, 11, 16, 21, 31, 41, 52, 61, 71, 84, 109, and 207 min. [See the legend to Fig. 1.]

The magnitudes of differences between the final and initial spectra of α-glucose-*1*-d_1 and of α-glucose-*5,6,6*-d_3 were about the same when identical concentrations and pathlengths were used. Consequently, the decreasing and increasing intensities at ~2940 and ~2800 cm^{-1} cannot be associated with isolated α-C–H and β-C–H stretching vibrations *(vide infra)*.

From the deconvoluted spectra (see Fig. 4) for equilibrated glucose-*1*-d_1, glucose-*6,6*-d_2 and glucose-*5,6,6*-d_3 in D_2O, the main difference between the glucose-*1*-d_1 spectrum and the other two can be seen to be the lack in the latter two spectra of intense bands at 2936 and 2891 cm^{-1}, which can be assigned to antisymmetric and symmetric CH_2 stretching modes of the CH_2OH group. Because the spectra of α-glucose, α-glucose-*1*-d_1, and α-glucose-*2*-d_1 in fluorolube (see Fig. 5) are similar, it seems reasonable to conclude that C-1–H and C-2–H stretching modes of α anomers have very little absorption. This strongly suggests that the intensity increase at ~2880 cm^{-1} and decrease at ~2940 cm^{-1} in the time-dependent spectra (see Figs. 1–3) of α anomers might be due to the variation in the normal mode composition of the other C–H stretching vibrations during α→β isomerization. The spectrum of α-glucose-*5,6,6*-d_3 in fluorolube mull is significantly different from

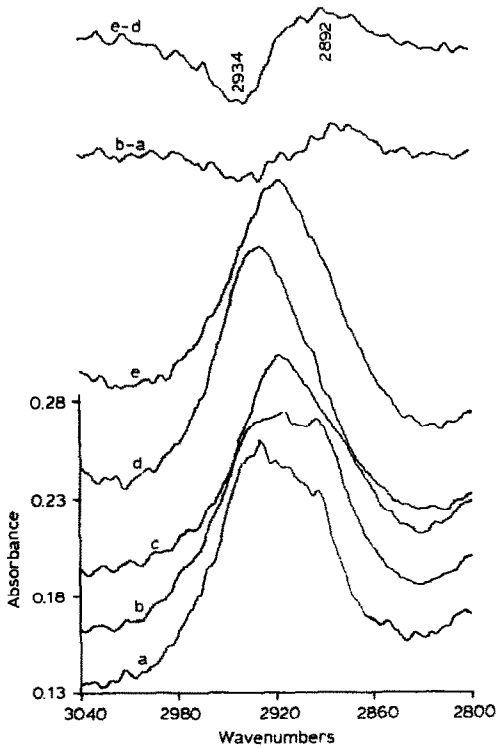

Fig. 3. F.t.-i.r. absorption spectra of isotopomers of glucose in D_2O. (a) α-D-glucose-*1*-d_1, immediately after dissolving; (b) α-D-glucose-*1*-d_1 after equilibration; (c) D-glucose-*6,6*-d_2; (d) α-D-glucose-*5,6,6*-d_3 immediately after dissolving; (e) α-D-glucose-*5,6,6*-d_3 after equilibration. [The difference spectra (top) are on arbitrary intensity scales.]

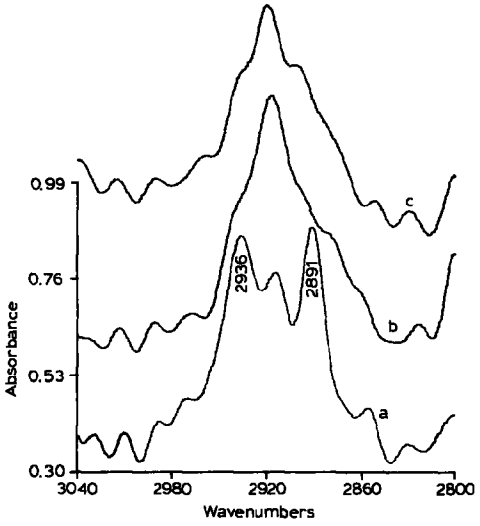

Fig. 4. Deconvoluted F.t.-i.r. absorption spectra (VF0 = 30, VF1 = 2) of (a) equilibrated D-glucose-*1*-d_1, (b) D-glucose-*6,6*-d_2, and (c) D-glucose-*5,6,6*-d_3 in D_2O.

those of α-glucose, α-glucose-*1*-d_1, and α-glucose-*2*-d_1. Since C-1–H and C-2–H stretching modes of α anomers are considered to be of weak intensity, the three intense bands seen in the spectrum of α-glucose-*5,6,6*-d_3 must be due to C-3–H and C-4–H stretching fundamentals and some combination (or overtone) band of lower frequency modes.

In the C–D stretching region, α-glucose-*1*-d_1 exhibits a band at 2181 cm^{-1} (at 2185 cm^{-1} in Me_2SO-d_6 solution); α-glucose-*5,6,6*-d_3 exhibits bands at 2100, 2132, 2154, and 2222 cm^{-1} in fluorolube mull (in Me_2SO-d_6 solution, broad bands at 2212 and 2105 cm^{-1} are present).

In the 1600–1300 cm^{-1} region, the changes in time-dependent spectra (see Figs. 6 and 7) of α-galactose consist of a weak band developing at 1320 and a band decreasing at 1339 cm^{-1}; corresponding bands in the spectra of α-fucose are at 1314 and 1340 cm^{-1}. These changes are identical to those found[7] for α-glucose at 1320 and 1338 cm^{-1}, and also for α-glucose-*1*-d_1 (see Fig. 8). These bands are not apparent in the spectra of α-glucose-*5,6,6*-d_3, but are present in the spectrum of equilibrated glucose-*6,6*-d_2, and this traces their origin to the C-5–H deformations, probably coupled to C-1–H.

The intensity increase at 1414 cm^{-1} and decrease at 1384 cm^{-1}, noted in the time-dependent spectra of α-glucose[7], are not present in the spectra of α-glucose-*1*-d_1 (see Fig. 8). Since they do appear in the spectra of α-glucose-*5,6,6*-d_3, they can be associated with the anomeric C–H deformation of the α and β forms. The spectra in Me_2SO-d_6 (see Fig. 9), although more complicated due to the presence of C–O–H deformations, do show some indication of C-1–H contribution. The 1403 cm^{-1}-band of α-glucose is not present in the spectra of β-glucose or glucose-*1*-d_1.

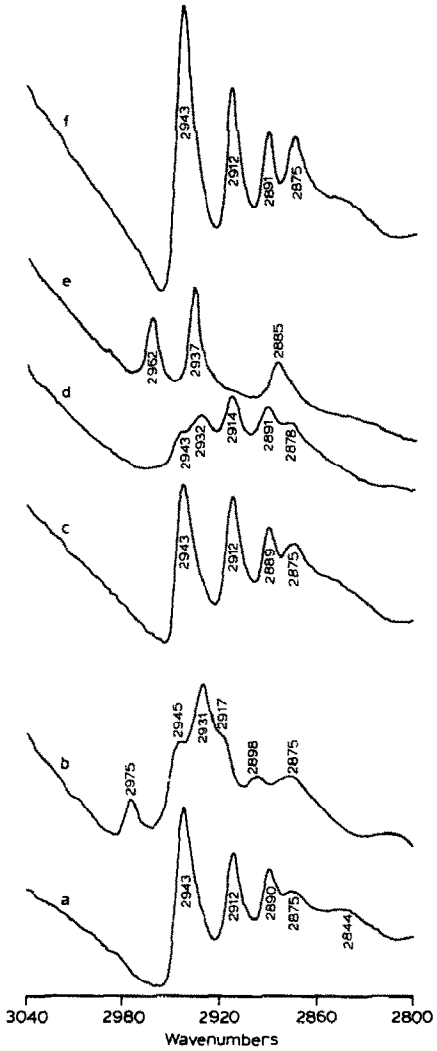

Fig. 5. F.t.-i.r. absorption spectra in fluorolube mull of (a) α-D-glucose, (b) β-D-glucose, (c) α-D-glucose-*1*-d_1, (d) D-glucose-*6,6*-d_2, (e) α-D-glucose-*5,6,6*-d_3, and (f) α-D-glucose-*2*-d_1.

The most definite assignment that can be made is for the 1451-cm^{-1} band (in Me_2SO-d_6), which is present in the spectra of α-glucose, β-glucose, and α-glucose-*1*-d_1, but not in the spectra of glucose-*6,6*-d_2 and glucose-*5,6,6*-d_3; this band is assignable to CH_2 deformation of the CH_2OH group.

In the spectra of α-fucose (see Fig. 7), there is also a band, at 1391 cm^{-1}, which is not present in the spectra of α-galactose and does not change its intensity during anomeric isomerization. This band is probably due to CH_3 symmetric deformation.

In the 1200–900-cm^{-1} region, in D_2O solution (see Figs. 10 and 11), the major intensity changes for α-galactose occur in only two bands: intensity decrease at

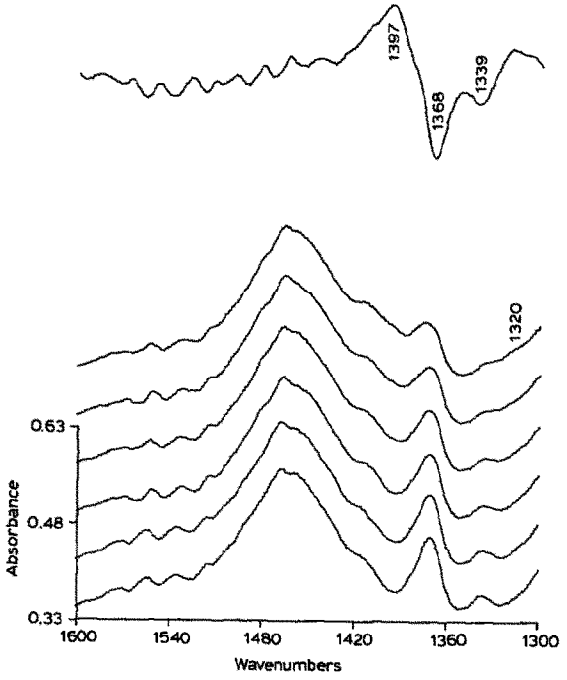

Fig. 6. Time-dependent F.t.-i.r. absorption spectra of α-D-galactose in D_2O. [See the legend to Fig. 1.]

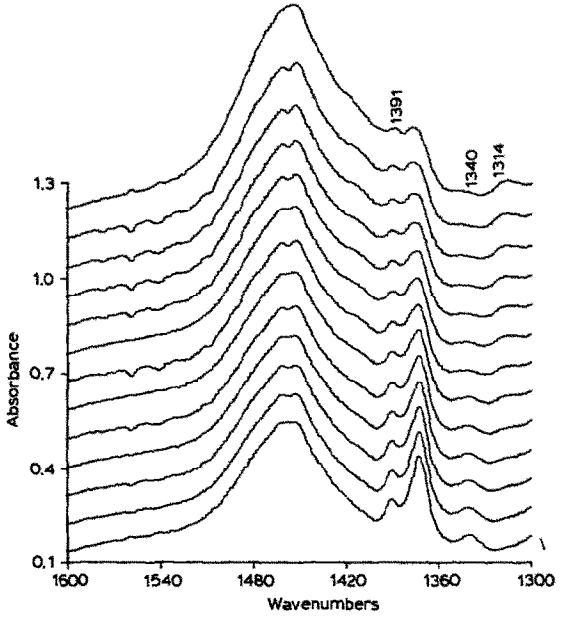

Fig. 7. Time-dependent F.t.-i.r. absorption spectra of α-D-fucose in D_2O, taken at time intervals as indicated for Fig. 2.

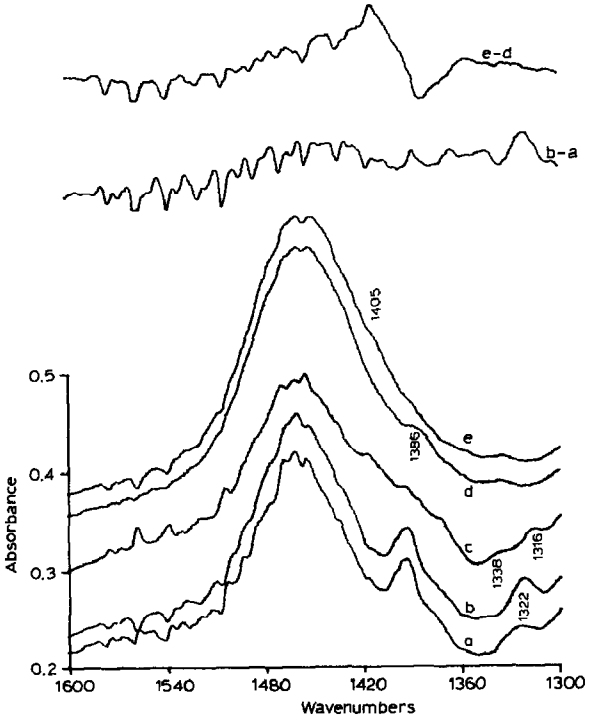

Fig. 8. F.t.-i.r. absorption spectra of isotopomers of glucose in D_2O. [The labels have the same meaning as in Fig. 3.]

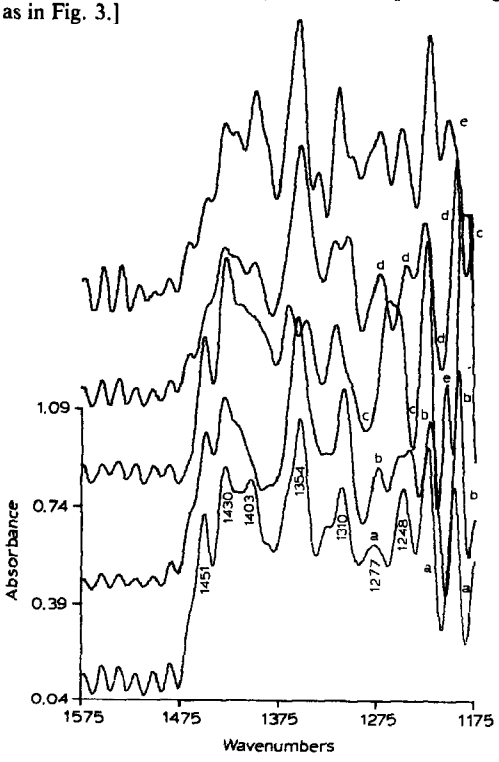

Fig. 9. Deconvoluted F.t.-i.r. absorption spectra (VF0 = 25, VF1 = 2) for isotopomers of glucose in Me_2SO-d_6. [The labels have the same meaning as in Fig. 5.]

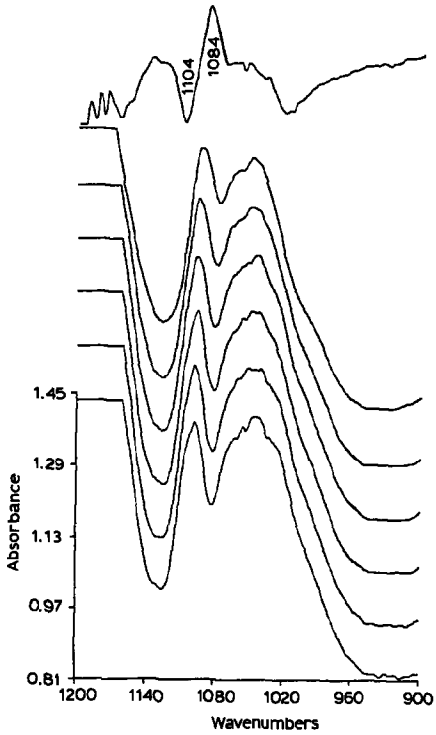

Fig. 10. Time-dependent F.t.-i.r. absorption spectra of α-D-galactose in D_2O. [See the legend to Fig. 1.]

1104 cm^{-1} and increase at 1084 cm^{-1}. Corresponding positions in α-fucose spectra are at 1102 and 1076 cm^{-1}. Although it would be unwarranted to associate intensity decrease to an isolated α-C–O stretch and increase to isolated β-C–O stretch, the influence of α and β anomers is clearly revealed. In the spectra of α-glucose[7], however, the intensity decrease was at 1055 and the increase at 1082 cm^{-1}. This clearly shows that frequency order of influence for α and β anomers is reversed from galactose and fucose to glucose. The change in orientation at C-4 for galactose and fucose (OH axial, H equatorial) to glucose (OH equatorial, H axial) is probably responsible for this reverse in the frequency order.

The influence of orientation of substituents at C-4 is also evident when the spectra (see Figs. 12 and 13) in H_2O are compared with those in D_2O. The difference spectra for glucose[7] in the 1200–900-cm^{-1} region are very similar in H_2O and D_2O, indicating that there are only minor influences from the COH deformation in this region. For galactose (and fucose), however, the difference spectra in H_2O are significantly different from those in D_2O, indicating a strong influence of COH deformations in the 1200–900-cm^{-1} region. To understand the various intensity changes in H_2O for galactose and fucose, the following points can be made. If developing and decreasing bands are due to isolated anomeric C–O stretches, we would normally expect the developing and decreasing bands to show different

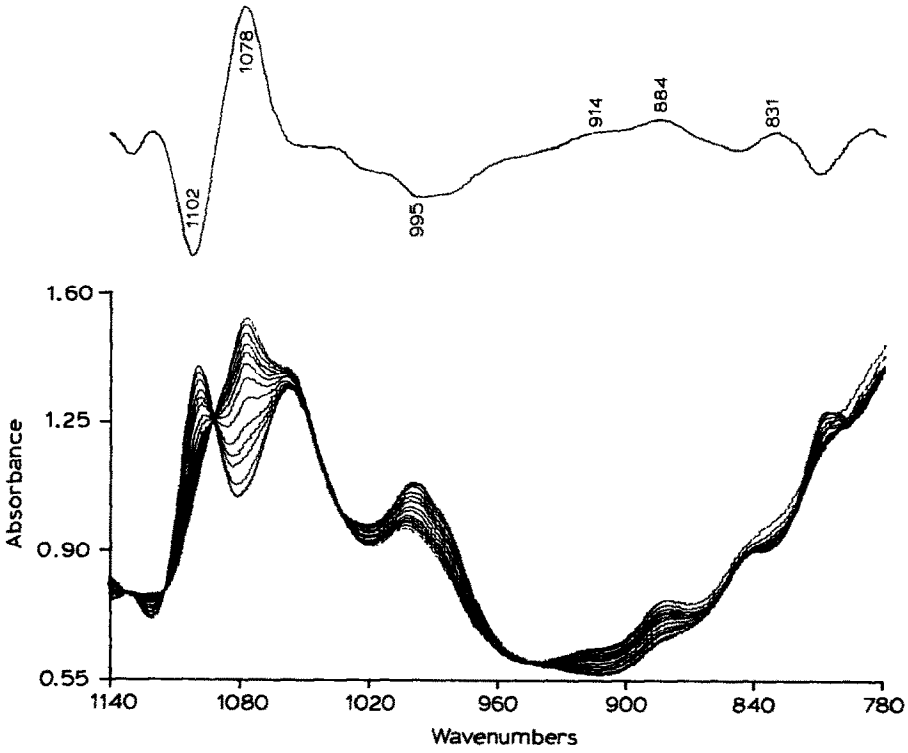

Fig. 11. Time-dependent F.t.-i.r. absorption spectra of α-D-fucose in D_2O, taken at 14 different times within a 200-min period. [All spectra were plotted on the same scale. The difference between final and initial spectra is displayed at the top with an arbitrary intensity scale.]

magnitudes in the difference spectra. On the other hand, if during anomeric isomerization, a band is shifting due to variation in the normal mode composition of a vibration and its frequency but not its intensity, is influenced, the difference spectrum should show a bisignate couplet with equal magnitudes for positive and negative halves; if the intensities are also affected, it is difficult to distinguish this effect from the one already mentioned for isolated anomeric stretches.

The increase at 1178 cm^{-1} and decrease at 1167 cm^{-1} in the α-fucose spectra (see Fig. 13) are most probably due to frequency shift on going from the α to the β anomer. The same reasoning appears valid for the 1152-cm^{-1} band's shifting to ~1167 cm^{-1} in α-galactose spectra (Fig. 12) and for the 1148 cm^{-1} band's shifting to 1165 cm^{-1} in α-glucose[7] spectra. These bands (all at ~1150 cm^{-1}) are of particular interest for correlation of vibrational circular dichroism in sugars[2,3]. Because the intensity of the 1165-cm^{-1} band for glucose relative to that of other bands in the 1200–900-cm^{-1} region, is quite different from that for the ~1167-cm^{-1} band for galactose, the influence of the orientation at C-4 is indicated. The corresponding band for fucose at ~1178 cm^{-1} has a relative intensity similar to that for galactose, but the frequency has shifted; therefore the influence of C-5 is also revealed. The

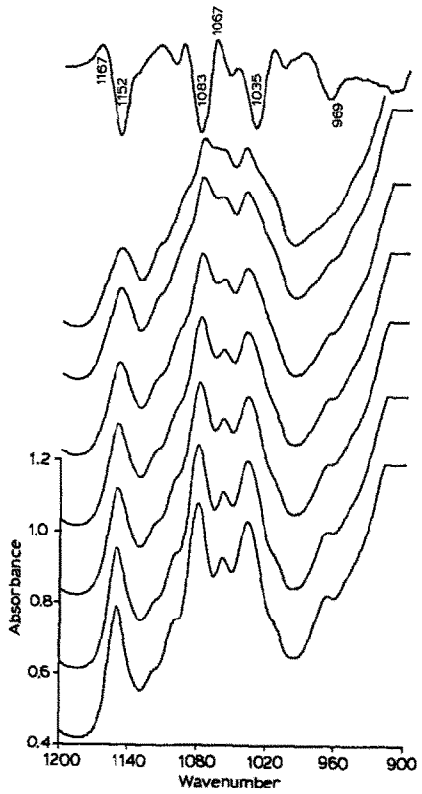

Fig. 12. Time-dependent F.t.-i.r. absorption spectra of α-D-galactose in H_2O, taken at 0, 10, 17, 27, 42, and 64 min, and 16 h. [The difference between the final and the initial spectra is displayed at the top with an arbitrary intensity scale.]

spectra of isotopomers of glucose in Me_2SO-d_6 indicate (see Fig. 14) that the band at ~1150 cm^{-1} is present in the same vicinity in the α-glucose, β-glucose, α-glucose-*5,6,6-d*$_3$, and glucose-*6,6-d*$_2$ spectra, but not in that of α-glucose-*1-d*$_1$, where, instead, a band at ~1185 cm^{-1} with significant intensity is present. From all these experimental data, the 1150 cm^{-1} band appears to originate from a delocalized vibration, probably of C–O and C–C stretches, with a significant influence from α-C-1–H.

KINETICS

From the rate of change in the intensities of the sensitive bands, as discussed in the previous section, the rate constants for isomerization can be obtained. There is one major difference in the infrared investigation of reaction kinetics from such other techniques as polarimetry. In polarimetry, one monitors a single property (optical rotation) which is a cumulative effect from all possible structural changes. On the contrary, in the time-dependent F.t.-i.r. spectra, one monitors all vibra-

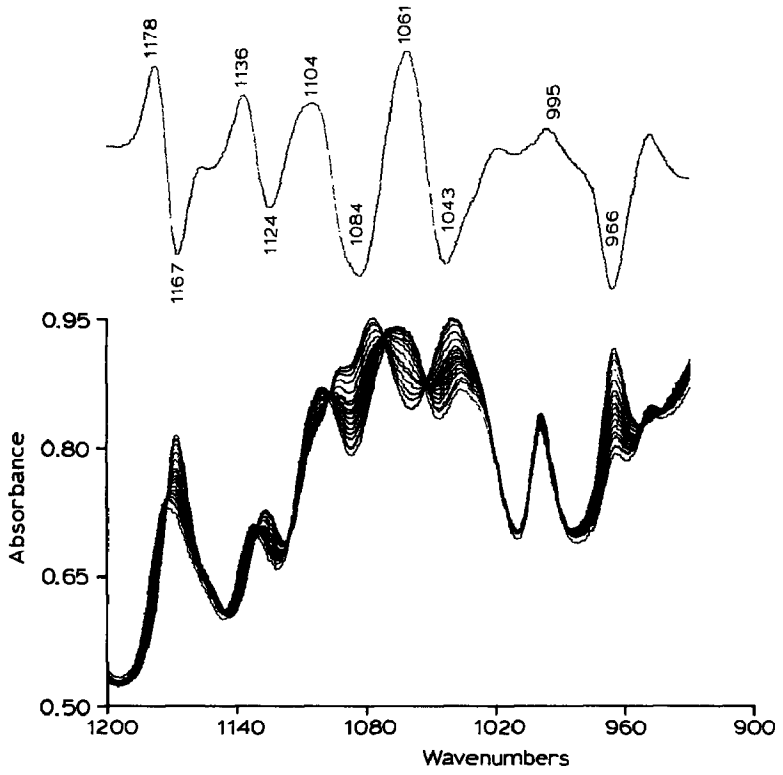

Fig. 13. Time-dependent F.t.-i.r. absorption spectra of α-D-fucose in H_2O, taken at 18 different times within a 75-min period. [All spectra were plotted on the same scale. The difference between the final and the initial spectra is displayed at the top with an arbitrary intensity scale.]

tional intensities at the same time, and because some of the vibrational bands can have contributions from the intermediates, the kinetics of different vibrational bands do not have to be the same if the overall reaction is a result of several intermediate reactions and if the intermediates have a long enough lifetime. For this reason, we found it necessary to verify the rate constants obtained from the intensity changes associated with different vibrational bands. For a reversible first-order reaction, the rate constant is given as $K = (k_1 + k_2) = (1/t)\log_{10}[(A_0 - A_\infty)/(A_t - A_\infty)]$, where k_1 and k_2 are rate constants for forward and backward reactions, and A_0, and A_∞ are integrated absorbances at time, $t = 0$ and ∞. Examination of the reversible first-order plots will reveal whether or not the intermediates are contributing to the changes in vibrational band intensities. These plots for some of the bands of α-fucose in H_2O, as well as D_2O, in the 1200–900-cm^{-1} region, are shown in Fig. 15, along with the rate constants. Only those bands having significant intensity changes, and away from solvent bands, are considered. It may be noted that all bands considered gave a good fit to the reversible first-order equation. The rate constants obtained from different bands are also consistent among themselves,

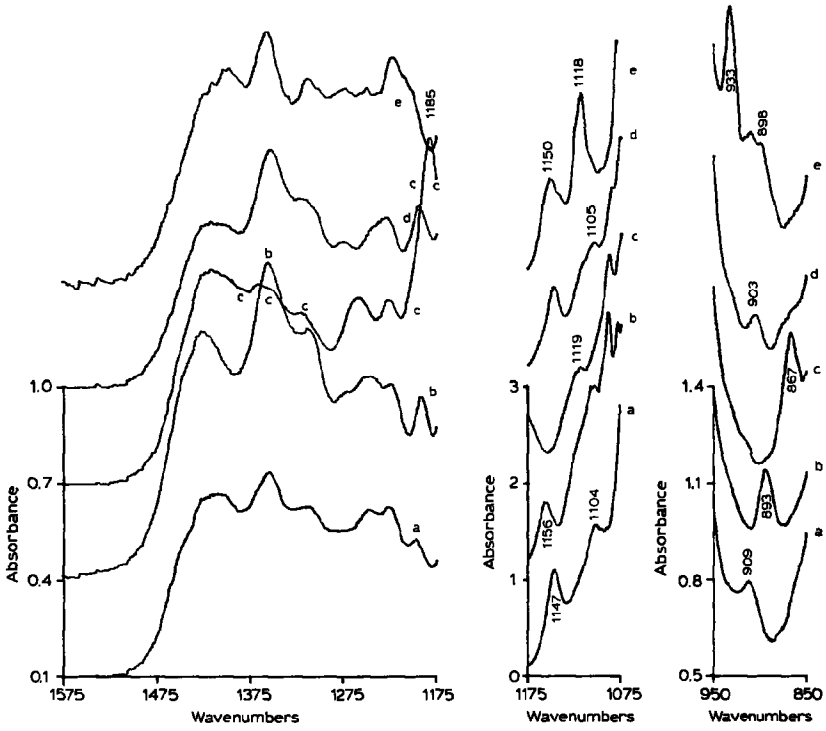

Fig. 14. F.t.-i.r. absorption spectra of the isotopomers of glucose in Me_2SO-d_6. [Labels have the same meaning as in Fig. 5.]

except for a slightly higher value (0.0494) found for the 1061-cm^{-1} band in H_2O. The average value of the rate constant for α-fucose in H_2O is 0.035 min^{-1}, which is slightly higher than the 0.0227 min^{-1} that was reported[11] in the literature for a 4% solution; this difference may be attributed to the difference in concentration employed in the present study. In D_2O, the average rate-constant is found to be 0.011 min^{-1}; no data are yet available in the literature for comparison. Because an equilibrium solution of fucose in H_2O also contains a small proportion of the furanose form, deviations from the reversible first-order reaction-rate may be anticipated. However, such small concentrations of furanose form are hard to identify in the i.r. spectra, and, moreover, about 30–60 seconds pass before recording of the F.t.-i.r. spectra can be started, during which time, the bulk of the fast pyranose–furanose reaction would have been completed.

From these kinetic plots, it appears that reliable rate-constants can be obtained from the time-dependent, F.t.-i.r. spectra of such relatively fast reactions as α-pyranose to β-pyranose isomerization of fucose, where the effects of intermediates were not obvious (except for small changes in the slopes of kinetics plots for different bands). If the reactions are much slower, the effects due to inter-

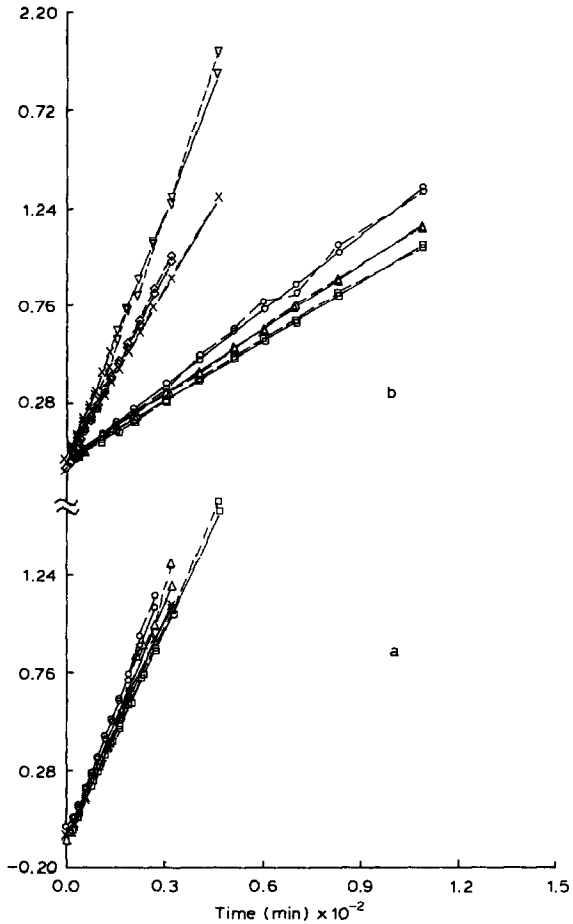

Fig. 15. Plot of $\log_{10}[(A_0 - A_\infty)/A_t - A_\infty)]$ *vs.* time for various bands of α-D-fucose in H_2O and D_2O. [Experimental points are connected by dashed line. Solid line indicates least-squares fit of the data. The rate constants K and the regions integrated are as follows: (a) □-□-□, K = 0.0336, 1171–1162 cm^{-1}; ○-○-○, K = 0.0414, 1140–1130 cm^{-1}; △-△-△, K = 0.0385, 1130–1118 cm^{-1}; ×-×-×, K = 0.0343, 1095–1086 cm^{-1}; (b) ×-×-×, K = 0.0276, 1052–1021 cm^{-1}; ◇-◇-◇, K = 0.0309, 971–958 cm^{-1}; ▽-▽-▽, K = 0.0413, 956–943 cm^{-1}; □-□-□, K = 0.0096, 1110–1093 cm^{-1}; ○-○-○, K = 0.0123, 1083–1069 cm^{-1}; △-△-△, K = 0.0104, 1015–963 cm^{-1}. The last three are for D_2O solutions and the remaining are for H_2O solutions. The regions for integration are determined from the bandwidths in deconvoluted spectra.]

mediates (in terms of curvature in plots) may be identifiable, in which case, different vibrational bands may yield different rate-constants.

ACKNOWLEDGMENTS

This work was supported by grants from NIH (GM29375) and Vanderbilt University. We thank C. M. Tummalapalli for recording the spectra of compounds in fluorolube mull, and for the computer plots of kinetic data.

REFERENCES

1 P. L. POLAVARAPU, S. R. CHATTERJEE, AND D. F. MICHALSKA, *Carbohydr. Res.*, 137 (1985) 253–258.
2 D. M. BACK AND P. L. POLAVARAPU, *Carbohydr. Res.*, 133 (1984) 163–167.
3 T. M. CHANDRAMOULY AND P. L. POLAVARAPU, unpublished results.
4 C. MARCOTT, H. A. HAVEL, J. OVEREND, AND A. MOSCOWITZ, *J. Am. Chem. Soc.*, 100 (1978) 7088–7089.
5 M. G. PATERLINI, T. B. FREEDMAN, AND L. A. NAFIE, *J. Am. Chem. Soc.*, 108 (1986) 1389–1397.
6 D. M. BACK AND P. L. POLAVARAPU, *Carbohydr. Res.*, 121 (1983) 308–311.
7 D. M. BACK, D. F. MICHALSKA, AND P. L. POLAVARAPU, *Appl. Spectrosc.*, 38 (1984) 173–180.
8 D. F. MICHALSKA, D. M. BACK, AND P. L. POLAVARAPU, *Carbohydr. Res.*, 131 (1984) 29–38.
9 D. M. BACK AND P. L. POLAVARAPU, *J. Comp. Chem.*, (in press).
10 J. K. KAUPPINEN, D. J. MOFFATT, H. H. MANTSCH, AND D. G. CAMERON, *Anal. Chem.*, 53 (1981) 1454–1455.
11 W. PIGMAN AND H. S. ISBELL, *Adv. Carbohydr. Chem.*, 23 (1968) 11–57.