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IDENTIFICATION OF THE CHARACTERISTIC, ANOMERIC BANDS IN THE INFRARED SPECTRUM OF LYXOSE IN AQUEOUS SOLUTION*

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ABSTRACT

Time-dependent, Fourier-transform infrared spectra of solutions of lyxose in H_2O and D_2O were measured, in order to identify the vibrational bands characteristic of each anomer. The anomeric C–H stretching and deformation bands, and also the anomeric C–O stretching bands, were identified.

INTRODUCTION

The structural aspects of sugars in aqueous solution are usually studied by using polarimetry and nuclear magnetic resonance (n.m.r.) techniques. In principle, vibrational spectroscopy should also be sensitive to the structural changes of sugars in solution. Infrared spectroscopy has been employed[2] in the past for investigations of sugars, but those studies were limited to solid samples; in other words, infrared studies were mainly conducted for samples in either the pellet or mull form. The spectra obtained for samples in the solid state will differ significantly from those for samples in solution, especially when several conformers are possible in solution. This is, in fact, true for sugars, as they are known to exist as different structural isomers in aqueous solutions[3].

Thus, infrared-spectral examination of sugars in aqueous solution acquires a special significance and is important for identifying the vibrational bands characteristic of different conformers. However, such investigations have not been undertaken, perhaps due to the belief that the strong, absorption bands of water might submerge those of the sample being examined. Although two articles[4] reported the feasibility of making infrared investigations on sugars in aqueous solution, no systematic studies have been undertaken, owing to the relatively poor spectral quality achievable with dispersive, infrared spectrometers.

Recently, we reported[1,5] that Fourier-transform, infrared (F.-t.i.r.) spectroscopy provides spectra of excellent quality for sugars in aqueous solution. Despite strong absorption of the H_2O and D_2O solvents, infrared spectra of superior

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quality in the 1500–900-cm^{-1} region can be obtained with H_2O as the solvent, and in the C–H stretching, 1800–1300 and 1175–700-cm^{-1} regions, with D_2O as the solvent. In two earlier reports[1,5], we showed that the time-dependent, F.-t.i.r. spectra can be used to identify the vibrational bands characteristic of the anomers of D-glucose, and two different types of structural change of β-D-fructose, in aqueous solution.

Although several studies[6] in the literature refer to lyxose, the main emphasis was related to electronic circular dichroism, optical rotation, and conformational-energy calculations. We are aware of only one infrared study[7] of D-lyxose, wherein spectra were obtained for both pyranose anomers and for a freeze-dried sample in the solid state. The present study is, then, believed to be the first infrared investigation of solutions of L-lyxose in H_2O and D_2O. The equilibrium composition of the lyxopyranose anomers in water solution is known to be 75% of α and 25% of β. Furthermore, α-D-lyxopyranose exists in the $^4C_1$ as well as the $^1C_4$ conformation, as each contains an equal number[3] of hydroxyl groups in the axial and equatorial positions. On the other hand, β-D-lyxopyranose is known to exist only in the $^4C_1$ form, which contains one axial and three equatorial hydroxyl groups. The present F.-t.i.r. investigation was intended to find the vibrational bands characteristic of these different structural isomers. Lyxose is also known[8] to exist in the furanose forms. The percentage composition of the furanose forms is, however, extremely small compared to those of the pyranose forms, and, therefore, the detection of the i.r. absorption bands characteristic of furanose forms was not possible with the instrumentation available at present.

EXPERIMENTAL

Samples (0 1 g) of L-lyxose (Sigma Chemical Company) in weighing bottles were magnetically stirred during the addition of distilled water (0.5 mL). The solutions were stirred for a few seconds, and aliquots were quickly drawn into syringes and transferred to a cell having barium fluoride windows. The cells were placed in the sample compartment of a Nicolet 6000-C F.-t.i.r. spectrometer, and data collection was started immediately. The interferograms were collected continuously in block averages of 16 or 32, and stored in different memory segments. The Fourier transformation was conducted after the data collection was complete. The spectral resolution was 4 cm^{-1}, and the detector employed was either a HgCdTe detector having appropriate optical filters (to prevent saturation), or a TGS detector.

RESULTS

Time-dependent, F.-t.i.r. spectra of L-lyxose having a starting composition of 95% of the α and 5% of the β anomer are shown in Fig. 1 for the C–H stretching region. As the band assignments were made from the increase or decrease of the absorption intensities with time, the presence of a small percentage of the β

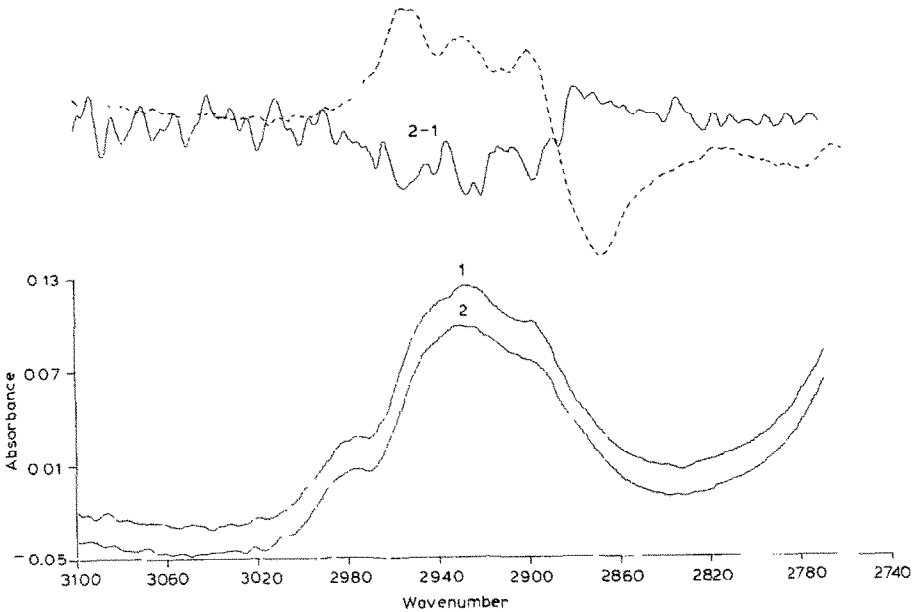

Fig. 1. F.-t.i.r. spectra of L-lyxose in D_2O, with a starting composition of 95% of the α anomer. [Trace 1 was obtained immediately after dissolving, and that numbered 2, after 1 h. The difference between the two is shown at the top. The dashed curve represents a similar difference for L-lyxose having a starting composition of 27% of the α anomer].

anomer was not considered to alter the conclusions drawn here; this is a valid assumption, as the presence of a minor proportion of the β anomer is not readily detectable in infrared spectra (see later). From the time-dependent spectra shown in Fig. 1, the intensity of a band at ~2875 cm^{-1} is seen to grow, while that of that near 2897 cm^{-1} decreases. Because, in solution, the bands are generally broad, the exact positions of these bands is somewhat uncertain.

The difference spectra are more helpful for the location of the band positions. For this reason, the difference between initial and final spectra is also displayed in Fig. 1. In this difference spectrum, the increase in intensity is centered at 2870 cm^{-1}, whereas the intensity decrease is centered at three positions, namely, 2897, 2927, and 2950 cm^{-1}. It may be noted that conversion of some of the α into the β form can cause two noticeable effects in the spectra. One is that, if the anomeric C–H stretching mode of the β is different from that of the α form, the intensity of the band corresponding to the β form increases, whereas that of the α form decreases. Secondly, as the α form decreases, the other C–H stretching modes that are coupled to the anomeric, C–H stretching might also show variations in intensity. However, it is reasonable to assume that the major changes in intensity will be associated with the anomer-characteristic modes, whereas the minor intensity changes will be associated with the modes that are coupled to the anomer-characteristic modes. The band at 2870 cm^{-1} can, therefore, be clearly as-

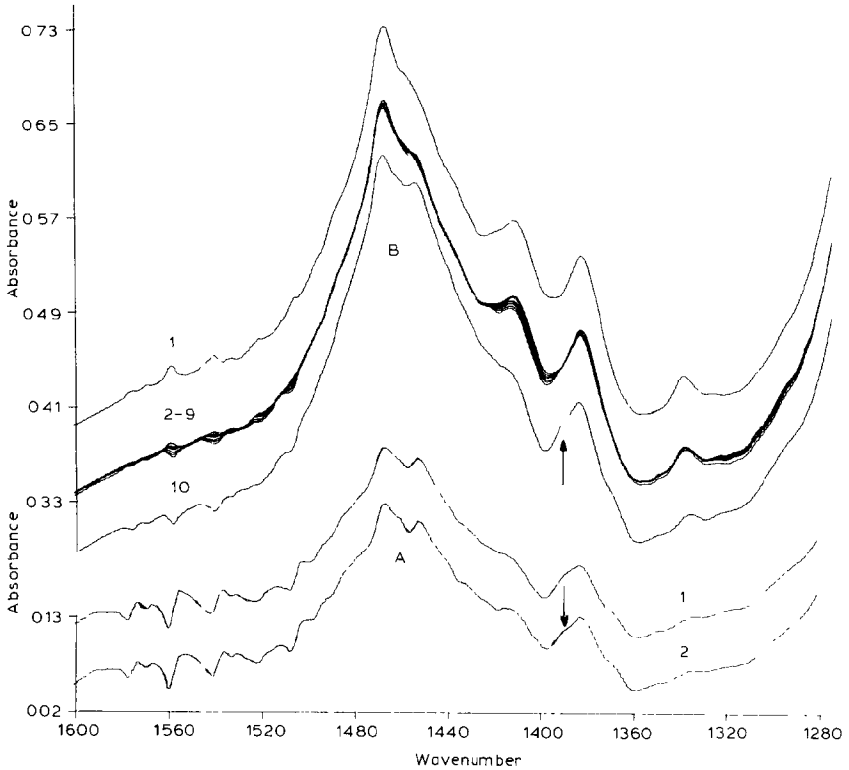

Fig. 2. F.-t.i.r. spectra of L-lyxose in D_2O.[(A) Trace 1 was obtained immediately after dissolving L-lyxose (95% α) in D_2O; trace 2, after 1 h. (B) Spectra for L-lyxose having a starting composition of 27% of α. Trace 1, immediately after dissolving, and those numbered 2–9 are successive in time, each averaged over 34-s data collection. Trace 10, after 24 h. The arrows indicate the bands discussed in the text.]

sociated with the anomeric, C–H stretching mode of the β form. However, the corresponding mode of the α form remains uncertain, as there are three possible band centers for decrease in intensity. Reverse trends in the intensities were found for L-lyxose having a starting composition of 73% of the β and 27% of the α anomer.

In our study[1] of D-glucose, the anomeric, C–H deformation modes of the α and β forms were found to be located at 1338 and 1320 cm^{-1}, respectively, with a very weak intensity. In the case of L-lyxose, also, these modes appear to be very weak, and, unfortunately, are overlapped by neighboring bands. In Fig. 2a, the high-frequency shoulder on the 1384-cm^{-1} band is seen to decrease with time; this shoulder is not present in the initial spectrum of L-lyxose having a starting composition of 73% of β anomer, and was seen to develop with time (see Fig. 2b). Therefore, the anomeric, C–H deformation mode of the α form may be associated with the band located at ~1390 cm^{-1}. The position of the corresponding band for the β form is not clear, and it may be overlapped by the 1380 cm^{-1} band. There are several changes in intensity in the 1500–1300-cm^{-1} region, but these are most probably

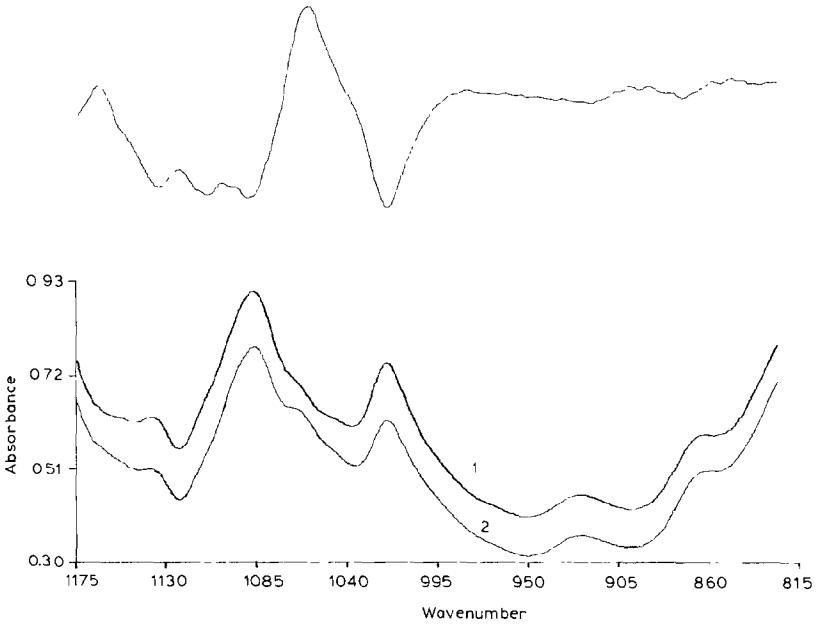

Fig. 3. F.-t.i.r. spectra of L-lyxose having a starting composition of 95% of the α anomer, in D_2O. [The numbers 1 and 2 and the top trace have the same meanings as in Fig. 1.]

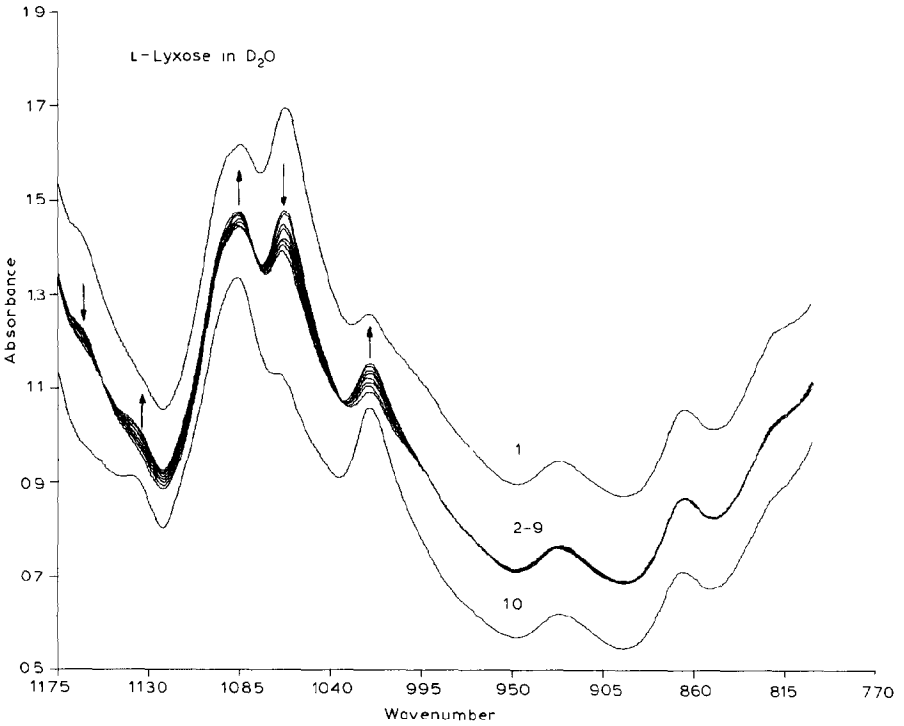

Fig. 4. F.-t.i.r. spectra of L-lyxose having a starting composition of 27% of the α anomer, in D_2O. [Numbers 1–10 have the same meaning as in Fig. 2B. Arrows pointing up indicate increase in intensity with time, and those pointing down, decrease in intensity with time.]

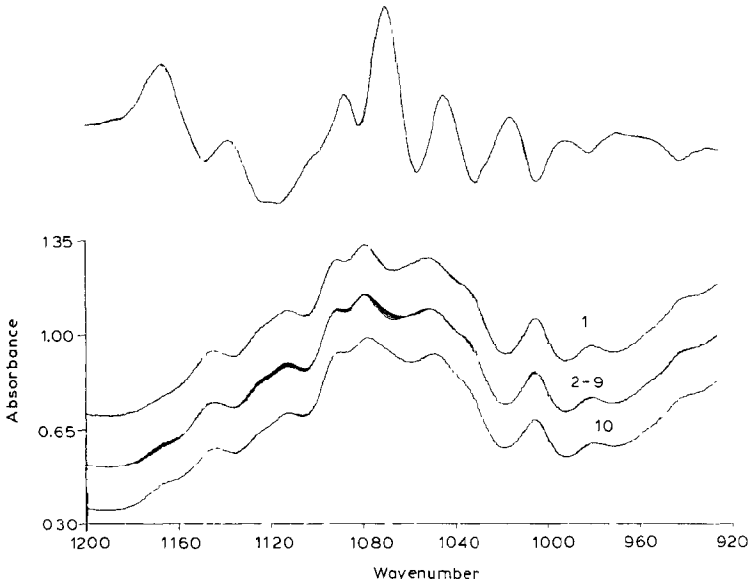

Fig. 5. F.-t.i.r. spectra of L-lyxose (95% α) in H_2O. [Trace 1 was obtained immediately after dissolving; traces 2–9, successively, each averaged over 34-s data collection; and trace 10, after 30 min. The difference between the final and initial spectra is displayed at the top.]

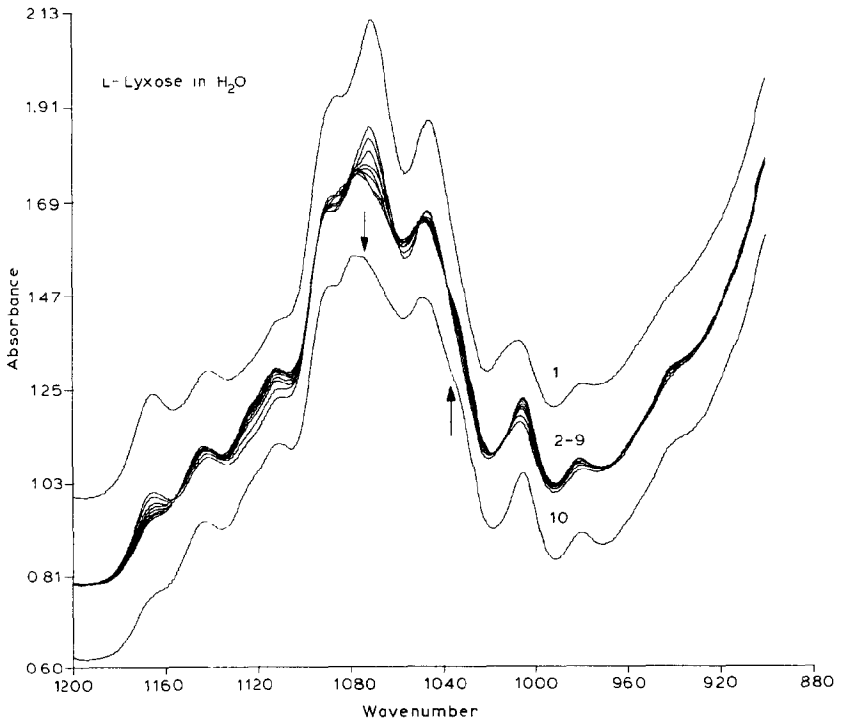

Fig. 6. F.-t.i.r. spectra of L-lyxose having a starting composition of 27% α, in H_2O. [The numbers have the same meaning as in Fig. 2B].

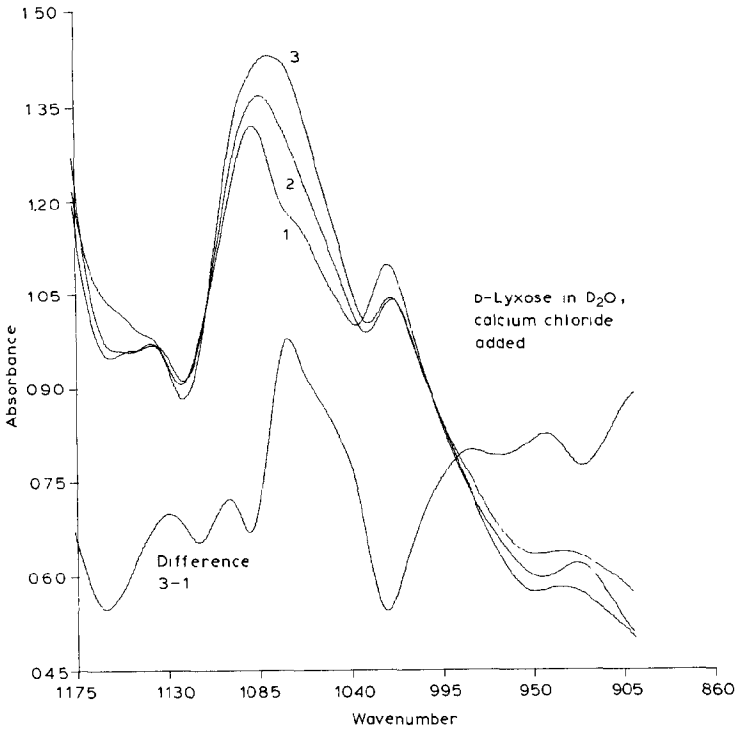

Fig. 7. F.-t.i.r. spectra of D-lyxose in the presence of $CaCl_2$ in D_2O solution; see text for explanation.

due to the remaining C–H deformation modes that are coupled to the anomeric, C–H deformation modes.

The most significant changes in intensity are seen in the 1200–1000-cm^{-1} region (see Fig. 3), where the anomeric C–O stretching modes are expected. In D_2O solution, a band at ~1060 cm^{-1} develops with time, and a band at 1020 cm^{-1} significantly decreases in intensity. In order to make sure that these bands are associated with the β and α form, respectively, we also obtained the time-dependent, F.-t.i.r. spectra of L-lyxose having a starting composition of 73% of the β anomer. Here, the band at 1060 cm^{-1} decreased (see Fig. 4) and that at 1020 cm^{-1} increased significantly with time. This and further observations (see later) suggested that the bands at 1020 and 1060 cm^{-1} may be associated with the anomeric, C–O stretching of the α and β forms, respectively.

Because α-L-lyxose is known to exist in both the $^4C_1$ and $^1C_4$ form, there should be one more anomeric C–O stretching band corresponding to α-L-lyxose. The intensity changes at 1085 cm^{-1} are parallel to those at 1020 cm^{-1}, and are of similar magnitude; this suggests the possibility for associating the 1085-cm^{-1} band with the anomeric, C–O stretching of the second α form. One difference is, however, that the band at 1085 cm^{-1} appears to be the origin of the remaining C–O stretching modes as well, because this band is stronger in intensity, and does not disappear, even for L-lyxose that is preponderantly the β anomer.

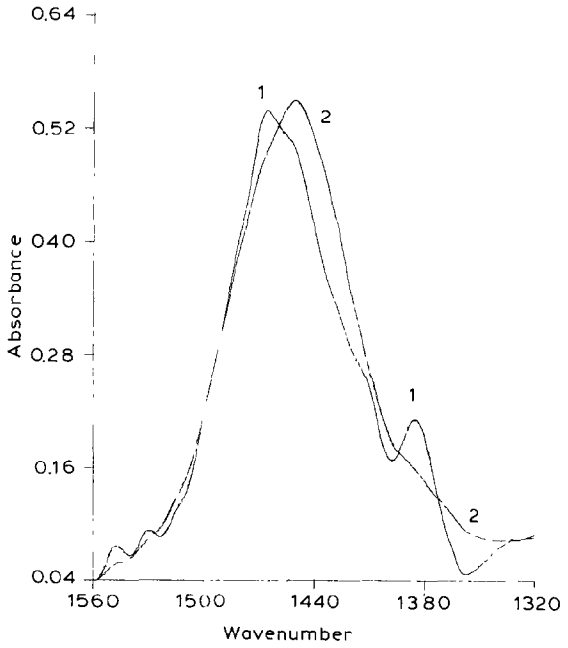

Fig. 8. F.-t.i.r. spectra of D-lyxose in the presence of $CaCl_2$ in D_2O solution. [Trace 1 is for D-lyxose equilibrated in D_2O; trace 2 was recorded 1 h after adding $CaCl_2$.]

For solutions in H_2O the identification of anomeric, C–O stretching modes is somewhat difficult, owing to the presence of C–O–H deformation modes in the same region. In Fig. 5, a band at 1070 cm^{-1} developed with time, and the band at 1030 cm^{-1} decreased. These effects were reversed in the time-dependent spectra for L-lyxose containing 73% of the β anomer; *i.e.*, the band at 1070 cm^{-1} decreased with time, while that at 1030 cm^{-1} increased (see Fig. 6). Thus, the bands at 1030 and 1070 cm^{-1} can be respectively associated with the α and β forms, and they are shifted from those found at 1020 and 1060 cm^{-1} in D_2O solvent. These shifts in frequencies with solvent are attributed to the decoupling of C–O stretching and C–O–H deformation modes, due to the deuteration of the hydroxyl groups in the D_2O solvent. Owing to the presence of C–O–H deformation modes and their overlap with the C–O stretching modes in water solution, the anomeric, C–O stretch due to the second α form of L-lyxose is not apparent in Figs. 5 and 6.

As stated earlier, it is known that the α anomer of D- or L-lyxopyranose exists in both the $^4C_1$ and the $^1C_4$ form, whereas the β anomer exists in only one conformation, *i.e.*, $^4C_1$ for β-D-lyxose and $^1C_4$ for β-L-lyxose. From n.m.r. studies[9], it was found that, in the presence of $CaCl_2$, the $^1C_4$ form of β-D-lyxose also exists in solution, because the axial–equatorial–axial sequence of the hydroxyl groups favors complexation with calcium ions. We have also recorded the n.m.r. spectra of lyxose in $CaCl_2$ solution, and found them to be in agreement with those reported[9]. The essence of these n.m.r. results is that, in $CaCl_2$ solution, the proportion of the

β form increases, and β-D-lyxose exists entirely in the $^1C_4$ conformation. Our infrared spectra, shown in Fig. 7, are in agreement with these observations. The spectrum labeled 1 in Fig. 7 represents D-lyxose at equilibrium in D_2O; that labeled 2 was taken immediately after adding $CaCl_2$ to the equilibrated solution. Spectrum 3 was obtained after 1 h, and no further spectral changes were noted. The difference between the final and initial spectra is also displayed in Fig. 7. The intensity of the bands at 1020 and 1085 cm^{-1} can be seen to have decreased, indicating decrease in the proportion of the α form; this is in agreement with the aforementioned, n.m.r. observation, and also supports the earlier assignments of these absorption bands to the α form.

At 1060 cm^{-1}, where the anomeric, C–O stretching of β-D-lyxose-$^4C_1$ should have been present, there are now two bands having increased intensity, and these are situated at 1070 and 1050 cm^{-1}. This indicates, in agreement with the n.m.r. observations, that the proportion of the β form has increased. The change in frequency of the C–O stretching bands of the β form is probably attributable to change from the $^4C_1$ to the $^1C_4$ form and, to some extent, to complexation with the calcium ions. Thus, the bands at 1070 and 1050 cm^{-1} are associated with the stretching modes of C–O bonds in an axial–equatorial–axial sequence (and coordinated with calcium ions), and of the remaining, free, axial C–O bond. The C–O stretching band positions of free β-D-lyxose-$^1C_4$ can be considered to differ slightly from those in the presence of calcium ions.

With our earlier suggestion that the anomeric, C–H deformation modes of the α and β forms could lie under the band envelope at 1380 cm^{-1}, the 1380-cm^{-1} band would be expected to decrease considerably in intensity after addition of $CaCl_2$. This is because, in the presence of $CaCl_2$, the proportion of the α form decreases, and the anomeric, C–H deformation mode of β-D-lyxose-$^1C_4$ shifts from that in the $^4C_1$ form. As may be seen in Fig. 8, the intensity of the band at 1380 cm^{-1} decreased considerably, supporting this hypothesis; however, we could not locate the anomeric deformation mode of the "new" β form, probably owing to its weak intensity.

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