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Note

Laser-Raman spectra of lactose

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The laser-Raman spectra of D-glucose, cellobiose, maltose, and dextran in solution in H_2O and in D_2O have recently been investigated in some detail by Vasko *et al.*[1]; assignments for the bands observed have been proposed on the basis of deuterium substitution[1] and a normal coordinate analysis of α-D-glucose[2]. It was found by these authors that the solution spectra of the four carbohydrates in the region 1500–700 cm^{-1} are similar, whereas, below 700 cm^{-1}, distinct features are observed for each sugar. The Raman spectrum of crystalline α-D-glucose was found to be similar to that of D-glucose in aqueous solution[2].

We have now examined the Raman spectra of α-lactose monohydrate and β-lactose in the crystalline state, and of α-lactose, β-lactose, and equilibrated lactose in aqueous solution. All five spectra are different over the entire wavelength range from 1500 to ~50 cm^{-1}. It thus appears that laser-Raman spectroscopy can be a useful tool for identifying and investigating the different anomeric forms of the same sugar. By the same token, this diversity of the spectra of the same sugar in different forms makes it more difficult to assign the observed Raman lines to specific group frequencies or valence vibrations.

Figures 1A and 1B show the spectra of crystalline α-lactose monohydrate and β-lactose. The pronounced differences observed over the entire wavelength range from 1500 to 50 cm^{-1} suggest a difference in conformation. There are 16 specific hydrogen-bonds fixing the position of an α-lactose molecule in the crystal[3]. A change in this network would influence all COH vibrations and, through coupling, the skeletal ring-vibrations also, resulting in an entirely different spectrum. The crystal structure of α-lactose monohydrate is known[3,4]. Both sugar moieties adopt a strainless, chair conformation. The molecule exhibits an almost symmetrical twist about the glycosidic bond[4]. General considerations[3–6] suggest that, in β-lactose, the chair conformation of the pyranoid moieties is retained, but the twist around the COC bridge could well be quite different[4]. Rotational isomerism around the bridge COC bonds would most influence the bridge CO stretching frequencies. The largest differences between the two spectra are, in fact, observed at ~1100 and ~350 cm^{-1}, the characteristic frequency regions for stretching and bending vibrations of the COC grouping[7]. Similar differences have been found in the infrared spectra of α-lactose monohydrate and β-lactose[8]. However, the infrared spectra are, by far, not so well resolved, and show fewer distinct peaks. The relative intensities are also different, as would be expected[2].

Figure 1C shows the Raman spectrum of an aqueous solution of lactose after equilibration (~60% β, ~40% α). Instead of relatively sharp lines, only broad, ill-defined clusters are observed. In addition to the α,β-isomerism, rotational isomerism is now possible around the COC bridge, as well as around each C–OH bond. The specificity of the 16 distinct, external hydrogen-bonds[3] is lost; they are replaced by solute–solvent interactions of a much less specific nature. The overall result is a spectrum, containing broad, unresolved clusters of lines, that resembles neither of the crystal spectra, and most probably reflects the presence of a high number of rotational isomers (somewhat analogously to the ill-defined solution spectra of hydrocarbons for which rotation is possible around each C–C bond[7]). Fig. 2 shows the spectra of α-lactose and β-lactose in water, obtained immediately after dissolution at 0°. These spectra also show ill-resolved clusters of bands characteristic of a mixture of a large number of rotational isomers.

A detailed interpretation of any of the observed spectra is not, at present, possible. The molecule has $3 \times 45 - 6 = 129$ vibrational modes, all of them Raman-active. Twenty-two CH and OH vibrations are expected at frequencies above 1500 cm^{-1}, and the remaining 107 in the frequency range studied. Furthermore, most vibrations are "mixed", as evidenced by the results of deuteration studies of other sugars[1], calculations on D-glucose[2], and general experience with molecules containing COH groupings[7]. A few qualitative statements may be made, based on the "averaged" spectra of the isomers in aqueous solution. Most compounds containing COH groupings exhibit[7] coupled CO stretching and COH bending modes at ~1100–1000 and 1400–1300 cm^{-1}. The two clusters of lines close to 1100 and 1360 cm^{-1} should correspond to these motions. The bridge COC stretching vibrations are also expected in the 1100–1000 cm^{-1} region[7,9]. The relatively sharp line at 1461 cm^{-1} is essentially a CH_2 bending mode[1,2]. The strong line close to 350 cm^{-1}, which is missing from the laser-Raman spectrum of D-glucose[1], may be associated with COC bending[9,10], and the cluster of lines between 500 and 400 cm^{-1}, with various CCO and OCO bending modes[2]. Skeletal stretching and stretch–bend modes are probably distributed[11] over the entire range from 1400 to ~600 cm^{-1}; they are very strongly coupled, and are sensitive to even small structural changes.

Although the laser-Raman spectra of lactose isomers are at present difficult to interpret in detail, the results indicate that the spectra are very sensitive to small structural changes, and that the technique should be useful for studying and identifying closely related isomers, as well as derivatives of carbohydrates in general.

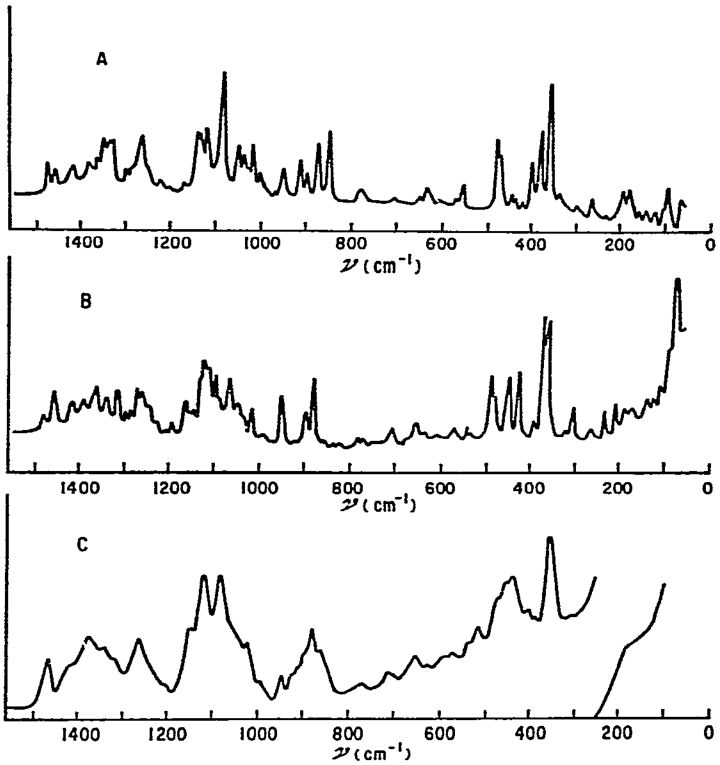

Fig. 1. Laser-Raman spectra of lactose, 488-nm excitation, 250 mW, and 3 cm^{-1} slit-width. [A, Crystalline α-lactose·H_2O, B, crystalline β-lactose, and C, equilibrated solution of lactose in water.]

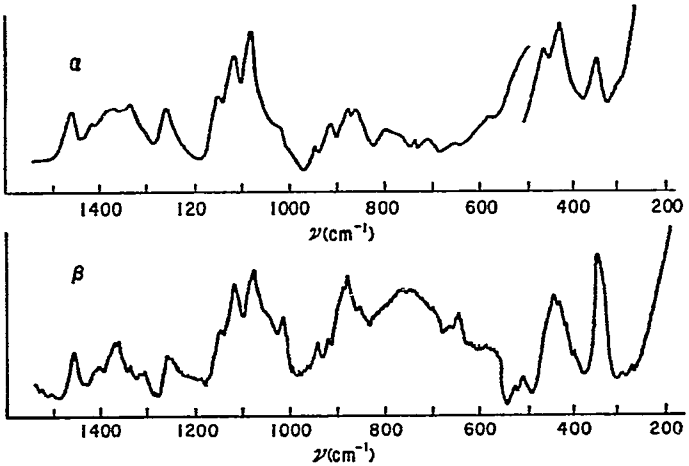

Fig. 2. Laser-Raman spectra of α-lactose and β-lactose in aqueous solution, obtained immediately after dissolution at 0°.

EXPERIMENTAL

Laser-Raman spectra were recorded with a Spex Ramalog System* equipped with a Model 1401 spectrometer, sample optics for 90° excitation, and a Carson Argon Ion laser. The 488-nm line was used for excitation, laser power at the sample was 250 mW, and the spectral slit-width was 3 cm^{-1}. Crystalline α-lactose monohydrate and β-lactose were obtained from Eastman Kodak Company (White Label). One solution of lactose in water was equilibrated overnight at 80°. The spectra of almost saturated solutions were recorded.

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