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A CROSS-POLARIZATION–MAGIC-ANGLE SAMPLE SPINNING N.M.R. STUDY OF SEVERAL CRYSTAL FORMS OF LACTOSE

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ABSTRACT

Five different crystalline forms of lactose were investigated by cross-polarization–magic-angle sample spinning (CP–MAS) ^{13}C-n.m.r. spectroscopy. Both the anhydrous β-lactose and the α-lactose monohydrate structures are known, and the CP–MAS n.m.r. data are in agreement with those structures. The structure of the stable, anhydrous α-lactose has not been reported. The CP–MAS n.m.r. results indicate that the crystal must have two or more lactose molecules per unit cell. The chemical shifts measured for two mixed crystals having $\alpha:\beta$ ratios of 5:3 and 4:1 are a direct observation of the fact that both materials are real mixed crystals rather than glasses or physical mixtures of crystals of pure α- and β-lactose. The chemical shifts also indicate that the lactose molecules in both mixed crystals are in environments similar to the crystalline environment of the stable, anhydrous α-lactose.

INTRODUCTION

The combined use of ^{13}C- and ^{1}H-n.m.r. spectroscopy has proved useful for chemical investigations of carbohydrates in solution. A cursory survey of this journal will illustrate the application of n.m.r. to structural studies and dynamics as well as straightforward analyses in chemical synthesis and isolation. To date, most of the interest has revolved around high-resolution n.m.r. in solution, but recent developments in solid-state n.m.r.[1–6] yield spectra of resolution approaching that available in solution. This has sparked a large number of publications in which high resolution n.m.r. in solids has been applied to a diversity of materials, ranging from crystals of small molecules[7] to coal and shale[8]. In the carbohydrate field, several studies of cellulose have shown the applicability of the technique for study of structure and morphology[9–11]. A recent report has suggested the use of solid-state n.m.r. for

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determination of the primary structure of polysaccharides[12]. Pfeffer *et al.* applied high-resolution n.m.r. in solids, in combination with solution ^{1}H-n.m.r., to obtain information about the tautomeric forms of lactulose in the solid. In the same study, they found that, although maltulose exists as a single tautomer in the solid state, the solid-state n.m.r. data show evidence of at least three "forms"[13].

This work is a solid-state investigation of lactose, which has been known for years to exist in several crystalline forms[14–19]. In particular, we have obtained the solid-state ^{13}C spectra of α-lactose monohydrate, anhydrous α-lactose, β-lactose, and two mixed crystals of lactose having $\alpha:\beta$ ratios of 5:3 and 4:1. Several investigations of the mixed crystals of lactose have suggested that they were actually mixed complexes having both anomers co-crystallized rather than simply a mixture of crystals of pure α and pure β anomers[14–17]. In this study, the n.m.r. spectra show chemical shifts and even new peaks that cannot be attributed to a simple mixture of pure crystals. In addition, we repeat Pfeffer's cautionary remark that the solid-state high-resolution n.m.r spectra reflect details of the crystal arrangement beyond the primary structure[13].

EXPERIMENTAL

Samples. — The preparation of the various forms of lactose examined in this investigation and the analytical procedures employed in the determination of purity and anomeric composition (X-ray powder spectra and m.p.) have been described previously[18]. Moisture determinations were made with a Photovolt Aquatest II instrument.* Densities of all samples of lactose were measured in density-gradient columns made with heptane, carbon tetrachloride, and bromoform, according to the procedure of Orr *et al.*[20]. The moisture determinations demonstrated that only the α-lactose monohydrate contained water. The density determinations showed that the samples were homogeneous and crystalline by virtue of the fact that only a single narrow band settled in the column and the fact that the densities of the mixed complexes cannot be obtained by the properly weighted average of the densities of α-lactose and β-lactose. Table I reports the densities obtained.

Spectra. — Most of the solid-state, cross-polarization, magic-angle sample spinning (CP–MAS) ^{13}C-n.m.r. experiments reported here were performed with a "home built" spectrometer described previously[22]. The spectrometer operates at an applied magnetic field of 1.4 T or a ^{13}C resonance frequency of 15 MHz. The probe uses a rotor–stator design originally described by Henriot and Huguenard[22] and developed for n.m.r by Andrew[3]. The spinning speeds were 2–2.2 kHz The ^{13}C magnetization was generated by spin-lock cross-polarization from the protons[1] with the Hartmann-Hahn[23] matching condition satisfied and proton decoupling with $\gamma B_1/2\pi$ of 60 kHz. Cross-polarization contact-times were 1 ms with a repetition time between successive cross polarizations of 10 s. Free-induction decays were generally 2 K data points, zero filled to 8 K prior to Fourier transformation, with no

TABLE I

DENSITY DATA

Form of lactose	*Density (g/mL)*
α-Lactose monohydrate	1.537
Anhydrous α-lactose	1.565
Lactose (α:β = 4:1)	1.573
Lactose (α:β = 5:3)	1.584
β-lactose	1.587

digital filtering applied. Between 7000 and 10,000 transients were taken for each spectrum (total data-acquisition times between 20 and 28 h per spectrum).

Several spectra were also obtained with a Bruker* CXP-200 instrument, operating at an applied field-strength of 4.7 T. These spectra were obtained with the same experimental conditions as described except that only 2 K transients were taken and the r.f. field strengths were only ~45 kHz.

RESULTS AND DISCUSSION

The X-ray powder patterns obtained for all five forms of lactose studied gave sharp bands, characteristic of crystalline materials. The spacings in the diffraction patterns agree with previously reported spacings for both the pure anomers and the mixed crystals[16,19]. The density measurements gave a single, sharp band for each material studied. In general, the single band indicates that the materials are homogeneous and the sharpness of the band indicates that the materials are crystalline. Amorphous or glassy materials give a spread of densities and generally have much lower densities.

For a complete discussion of the details of high-resolution n.m.r. of solids, the reader is referred to a series of review articles on the subject[24–27]. We only point out that the ^{13}C magnetization is generated by cross polarization from the proton magnetization. Optimal use of this technique requires a knowledge of the proton relaxation-times, especially T_1. In crystalline sugars, proton T_1 values may range from a few seconds to hundreds of seconds[13,28]. The choice of repetition times of 10 s in this work was made as a compromise that gave an adequate signal-to-noise ratio – no attempt was made to measure proton T_1 values and optimize the experiment.

There is a common misconception that chemical shifts measured in CP–MAS experiments in solids are the same as those measured for corresponding atoms in

*Certain commercial companies are named in order to specify the experimental procedure adequately. Such identification does not imply recommendation by the National Bureau of Standards, the U.S. Department of Commerce, or the U.S. Department of Agriculture, nor does it imply that the equipment is the best available for the purpose.

TABLE II

ASSIGNMENT OF ANHYDROUS CHEMICAL SHIFTS FOR α-LACTOSE IN FIG. 1C

Carbon atom	*Chemical shift*[a]	*Approximate integral*[b]
C-1′ (Gal)	106.3	2
C-1 (Glc)	93.7	2
C-4 (Glc)	87.4	1
C-4 (Glc)	84.1	1
C-2′–C-5′ (Gal) and C-2, C-3, C-5 (Glc)	67–80	14
C-6 and C-6′	64.1	3
C-6 or C-6′	59.4	1

[a]Chemical shifts are expressed in p.p.m., with positive values measured downfield from tetramethylsilane. The uncertainty in chemical-shift measurement is primarily due to the finite linewidths and is ~0.2 p.p.m. or less for the individual resonances. [b]Integrals are normalized to the smallest peak.

solution n.m.r. In fact, the chemical shifts measured by CP–MAS reflect conformations that are frozen out in the solid, as well as hydrogen-bonding and crystal-packing effects[7,20]. Usually, these effects are no larger than ~10 p.p.m. and so it is possible to make limited assignments of resonances by analogy to solution n.m.r. assignments. Fig. 1 shows CP–MAS spectra of anhydrous α-lactose, at two different applied field-strengths, together with those of α-D-glucose and β-D-galactose, the constituent monosaccharides. (The peak at 33.6 p.p.m. arises from a small chip of linear polyethylene used as a chemical-shift reference[21].) There are several general features of ^{13}C n.m.r. of sugars in solution[29] that are also true of these spectra. The highest-field peak in the spectra of glucose and galactose is that of C-6 and the lowest-field one that of C-1. The C-2–C-5 resonances fall between ~65 and 80 p.p.m. and have not been specifically assigned for the solid. The configuration of the anomeric carbon atom affects its chemical shift, C-1β being to low field of C-1α. A downfield shift is experienced upon *O*-glycosylation. Thus, the resonances of anhydrous α-lactose (Fig. 1C) are assigned as in Table II.

There is no reported X-ray crystal structure of anhydrous α-lactose, and so we have no structural point of comparison for the lactose n.m.r. spectra in Fig. 1. The splitting of the C-4 resonance of glucose into two peaks of equal amplitude implies that there are at least two nonequivalent glucose molecules per unit cell. This observation is corroborated by the splitting of the C-6 peak into two peaks having the intensity ratio of 3:1 (Fig. 1C). Fig. 1D is the spectrum of the same sample of anhydrous α-lactose taken at the higher applied-field of 4.7 T. As noted in earlier work[11], the higher applied-field gives somewhat better resolution for carbohydrates. In this case, it is sufficient to split the broad peak at 64.1 p.p.m. in Fig. 1C into a poorly resolved triplet in Fig. 1D. The resolution is still not good enough to give accurate integrals, but qualitatively this triplet could be the expected 1·1·1 triplet.

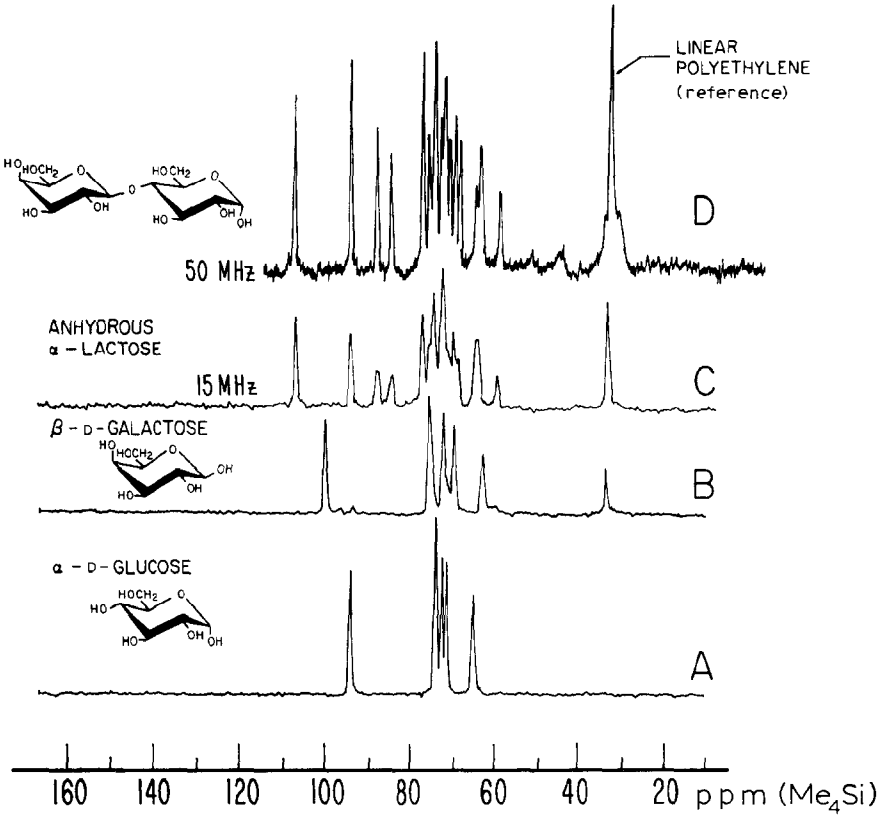

Fig. 1. Cross-polarization–magic-angle sample spinning ^{13}C-n.m.r. spectra and chemical formulas of: (*A*) α-D-glucose, (*B*) β-D-galactose, (*C*) stable anhydrous α-lactose at a ^{13}C resonance frequency of 15 MHz, (*D*) stable anhydrous α-lactose at 50 MHz. The peak at 33.6 p.p.m. arises from a chip of linear polyethylene used as a chemical-shift reference. The 50-MHz spectrum shows two broad resonances between 40 and 55 p.p.m. arising from residual signal from the perdeuteriopoly(methylmethacrylate) rotor.

The two C-4 peaks at 84.1 and 87.4 p.p.m. imply that there are at least two lactose molecules per unit cell, or a total of four hexose rings per unit cell. The observed splitting of the C-6 and C-6′ resonances further indicates that one of the pendant C-6 or C-6′ atoms is constrained into an orientation very different from the others.

The CP–MAS ^{13}C-n.m.r. spectrum of α-lactose monohydrate is shown in Fig. 2 together with the spectra of anhydrous α- and β-lactose. The monohydrate exhibits a relatively simple spectrum for which the peaks are assigned in Table III. The crystal structure of the monohydrate has been determined from X-ray data[30] and is relatively simple. There are two molecules per unit cell which are symmetry related, and thus both molecules have the same bond angles and hydrogen bonding, so that they will have identical chemical shifts for equivalent carbon atoms. The single molecule of water of hydration is involved in four hydrogen bonds, but all lactose molecules in the crystal are identically hydrogen bonded to the water molecules

TABLE III

ASSIGNMENT OF CHEMICAL SHIFTS FOR LACTOSE FROM FIG. 2

Carbon atom	*Chemical shift*[a]	*Approximate integral*[b]
α-Lactose monohydrate		
C-1′ (Gal)	107.6	1
C-1 (Glc)	93.4	1
C-4 (Glc)	87.5	1
C-2′–C-5′ (Gal)		
and	68–78	7
C-2, C-3, C-5 (Glc)		
C-6 and C-6′	62.8	2
Anhydrous β-lactose		
C-1′ (Gal)	103.4	1
C-1 (Glc)	98.7	1
C-4 (Glc)	81.5	1
??	79.2	1
C-2′–C-5′ (Gal)		
and	71–78	5
C-2, C-3, C-6 (Glc)		
??	68.0	1
C-6 and C-6′	62.9	1.7

[a] [b] See footnotes in Table II

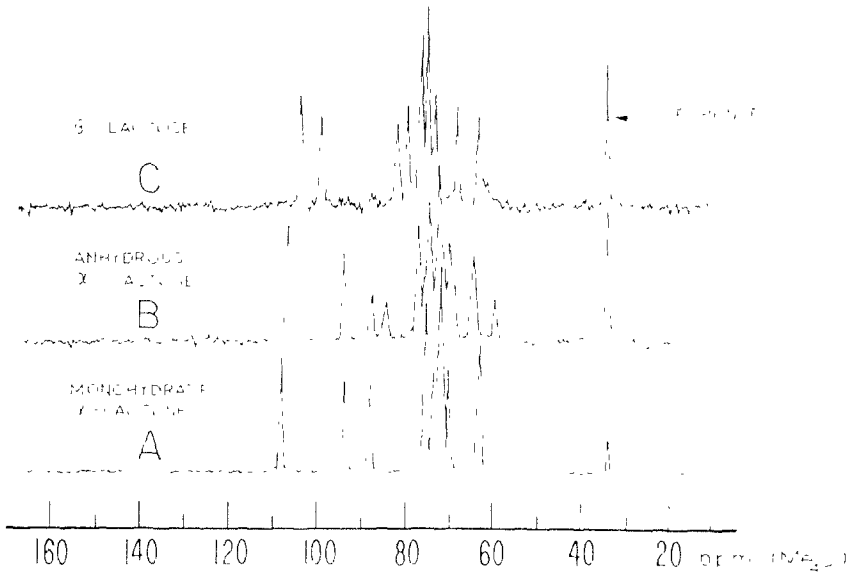

Fig. 2. CP-MAS spectra of three simple crystalline forms of lactose: (*A*) α-lactose monohydrate, (*B*) stable anhydrous α-lactose, and (*C*) β-lactose, obtained at a ^{13}C resonance frequency of 15 MHz.

and thus no splitting of the resonances would be expected from hydrogen bonding. The crystal structure of β-lactose has also been determined from X-ray data[31] and, like α-lactose monohydrate, there are two lactose molecules per unit cell and these are symmetry related. Thus, the basic pattern of the n.m.r. spectrum should reflect only one magnetically inequivalent lactose molecule, which is the case in Fig. 2.

There are two chemical shifts that are noteworthy. The resonances at 68.0 and 79.2 p.p.m. are somewhat separated from the other, similar carbon resonances having shifts between 71 and 78 p.p.m. The shifts noted are not outside the range of shifts measured for similar carbon atoms in other sugars[29,32]. Additionally, the peak assigned to C-4 is somewhat upfield of the C-4 resonances in either of the α-lactose spectra. Presumably, these shifts reflect the bond lengths or extensive hydrogen-bonding in the crystal (which also may explain the higher density observed for β-lactose). At present, our understanding of the effects of crystal packing, bond distortions, and hydrogen bonding on the ^{13}C chemical shifts in solids is insufficient to draw any conclusions about the origins of these small shifts.

The peak assigned to C-6 and C-6′ (62.9 p.p.m.) is somewhat shortened because it is broad and has an unexplained, upfield tail. The area of this peak is only slightly less than twice the area of the C-1, C-1′, or C-4 peaks (13:15.5). High-field (50 MHz) spectra of β-lactose show no improved resolution over the spectrum in Fig. 2 and so it is doubtful that the broadness is because of unresolved splitting of resonances from different, well defined molecules in the unit cell. The width is probably due to chemical-shift dispersion arising from heterogeneities in the exact rotational orientation of C-6 and C-6′.

The original impetus for this n.m.r. study of various crystalline forms of lactose was an attempt to determine what could be learned about the structure of the 5:3, α:β form of lactose originally described by Hockett and Hudson[14]. The spectrum of that material is shown at the bottom of Fig. 3. The upper plot is a spectrum synthesized by taking the spectra of anhydrous α-lactose (Fig. 2B) and β-lactose (Fig. 2C), normalizing them, and then adding them in the ratio of 5:3. Thus, Fig. 3B is the spectrum expected if the (5:3)-lactose were simply a mixture of pure α- and pure β-lactose. There are obvious differences between the two spectra plotted in Fig. 3, which can be explained by the fact that the sample examined is a mixed crystal and not a simple mixture of α- and β-lactose. Most of the differences are only qualitatively interpretable. The anomeric carbon region (90–110 p.p.m.) of the "real" spectrum contains 5 peaks, whereas Fig. 3B has only 4 peaks. In principle, the simplest unit-cell should contain 8 lactose monomers and as many as 16 peaks might be expected in this region. The breadth of the peaks in the "real" spectrum is probably because of dispersions of chemical shifts due to imperfections in the crystal or to relaxation (homogeneous) broadening, because the peak at 33.6 p.p.m. (linear polyethylene) has a width of <10 Hz, indicating that there is little instrumental broadening except for a possible inhomogeneity in B_0 of ~ 1 Hz. The region of the spectrum assigned to C-4 carbon atoms in glycosidic bonds (80–90 p.p.m.) shows severe differences between Fig. 3A and 3B. These differences cannot be interpreted

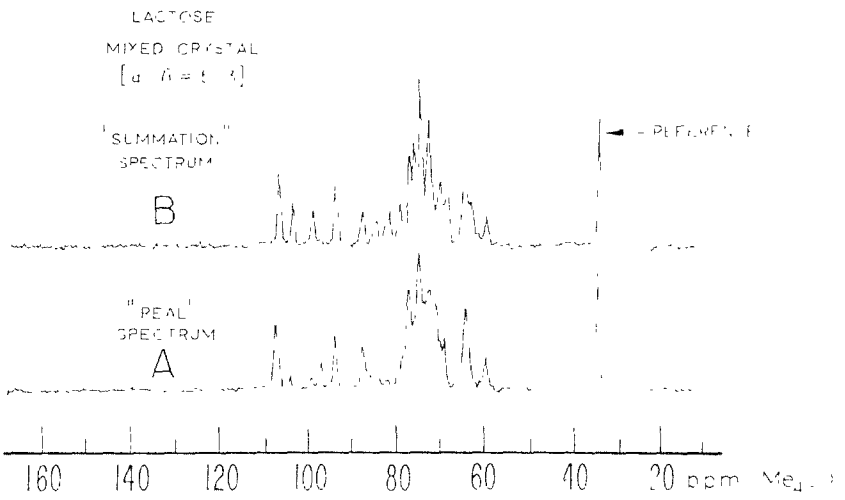

Fig. 3. (*A*) The spectrum of mixed-crystal lactose having a molar ratio of $\alpha:\beta$ of 5.3. (*B*) A spectrum obtained by computer addition of the normalized spectra of anhydrous α- and β-lactose added in the ratio of 5:3. Both spectra were obtained at a ^{13}C resonance frequency of 15 MHz. They are plotted with the same integral intensities for ease in comparison

quantitatively, but again they indicate that the (5:3)-lactose is a true mixed-crystal. There are no good data assigning chemical shifts of sugars in the central region of the spectrum (between ~68 and 80 p.p.m.). In this region, the chemical differences between the assigned carbon atoms are smaller than the physical differences expected from hydrogen bonding and crystal packing. We only note that there are differences between the two spectra in Fig. 3.

Interestingly, the chemical shift noted for one of the C-6 or C-6′ atoms in anhydrous α-lactose (59.4 p.p.m.) is preserved in the mixed crystal. This indicates that whichever conformation or crystal-packing effect produced that shift in the pure α-lactose remains in the mixed crystal. The fact that the C-6 peak at ~63.5 p.p.m. is broad and shows no splitting is probably rationalized by C-6 being a pendant CH_2OH group that can exist in several rotational orientations. The chemical shifts for the different rotamers are probably very small and cannot be resolved.

Fig. 4 shows the spectrum of crystalline lactose with the molar ratio of $\alpha:\beta$ = 4:1. The lower spectrum is the one obtained from the material itself, whereas the upper was generated by computer addition of the spectra of anhydrous α- and β-lactose spectra in the ratio of 4:1. The conclusion reached from these data is the same as for the (5:3)-lactose already noted, namely, the (4:1)-lactose is a true mixed crystal rather than a physical mixture of crystals of α- and β-lactose. Several other interesting points can be made relative to Fig. 4. First, the line-widths in Fig. 4A are quite narrow, indicating that the material is very crystalline or at least that there is little physical inhomogeneity in the solid state. In the anomeric region of the spectrum (90–110 p.p.m.), the peaks due to the α anomer are not significantly shifted from the pure α-lactose, but the peaks due to the β anomer are substantially shifted.

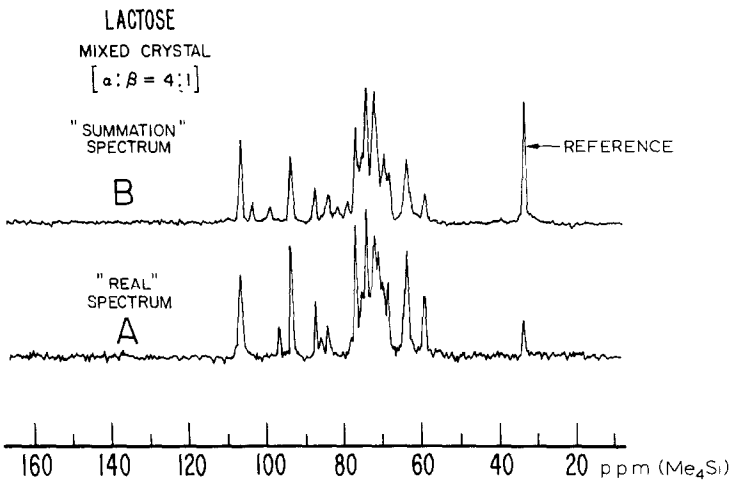

Fig. 4. (*A*) The CP–MAS spectrum of a mixed crystalline lactose having an $\alpha:\beta$ ratio of 4:1. (*B*) A spectrum obtained by addition of the spectra of α- and β-lactose in the ratio of 4:1. Both spectra were obtained at a ^{13}C resonance frequency of 15 MHz and are plotted with the same integral areas.

The same observation can be made for the C-4 peaks of the glucose residue in the shift-range between ~80 and 90 p.p.m. Logically, this implies that the crystal structure of the (4:1)-lactose must be relatively similar to the crystal structure of anhydrous α-lactose to the extent that bond lengths, bond angles, and hydrogen bonding about the C-1, C-1′ and C-4 carbon atoms are not vastly different. This conclusion is further substantiated by the fact that the region of the spectrum assigned to C-6 carbon atoms (55–68 p.p.m.) looks very similar to the same region in pure α-lactose. The peak at ~59.5 p.p.m. is still present in the (4:1)-lactose, although this is a rather high-field shift for C-6 in pyranoid sugars[31] and indicates an orientation for one of the C-6 atoms that is not significantly populated in solution.

CONCLUSIONS

Although high-resolution n.m.r. in solids will never replace X-ray and neutron-diffraction crystal structures for obtaining detailed information about atomic positions in solids, it is sensitive to chemical and physical interactions in a way that can provide useful structural data. We have demonstrated that crystalline anhydrous α-lactose exists in a crystal form with at least two lactose molecules per unit cell, and that the anhydrous form is more complicated in the solid than is the monohydrate. The chemical shifts and intensity patterns in the ^{13}C-n.m.r. spectra are molecular-level evidence demonstrating that the mixed-crystal lactoses having $\alpha:\beta$ molar ratios of 5:3 and 4:1 are true mixed crystals and not simple physical mixtures. In both of these mixed crystals, the ^{13}C chemical shifts indicate that the lactose molecules have bond lengths and angles similar to those found in anhydrous α-

lactose, although none of the crystal structures have been published. Crystalline carbohydrates give very high-resolution n.m.r. spectra under CP–MAS conditions, affording qualitative structural information beyond the simple primary structure. We stress that the CP–MAS n.m.r. technique is sensitive to molecular environments, and the observed shifts in compounds where the chemical differences are as small as they are in sugars may be more dependent upon bond distortions than on primary structure.

REFERENCES

1 A. PINES, M. G. GIBBY, AND J. S. WAUGH, *J. Chem. Phys.*, 59 (1973) 569–590.
2 I. J. LOWE, *Phys. Rev. Lett.*, 2 (1959) 285.
3 E. R. ANDREW, *Int. Rev. Phys. Chem.*, 1 (1981) 195–224, and references therein.
4 J. SCHAEFER, E. O. STEJSKAL, AND R. BUCHDAHL, *Macromolecules*, 8 (1975) 291–296.
5 E. LIPPMAA, M. ALLA, AND T. TUHERM, *Proc. XIX Congress Ampère, Heidelberg*, (1976) 113–118.
6 A. N. GARROWAY, W. B. MONIZ, AND H. A. RESING, *Preprints Am. Chem. Soc. Div. Org. Coatings Plastics Chem.*, 36 (1976) 133–138.
7 E. T. LIPPMAA, M. A. ALLA, T. J. PEHK, AND G. ENGELHARDT, *J. Am. Chem. Soc.*, 100 (1978) 1929–1931.
8 K. W. ZILM, R. J. PUGMIRE, D. M. GRANT, R. E. WOOD, AND W. H. WISER, *Fuel*, 58 (1979) 11–16.
9 R. H. ATALLA, J. C. GAST, D. W. SINDORF, V. J. BARTUSKA, AND G. E. MACIEL, *J. Am. Chem. Soc.*, 102 (1980) 3249–3251.
10 W. L. EARL AND D. L. VANDERHART, *J. Am. Chem. Soc.*, 102 (1980) 3251–3252.
11 W. L. EARL AND D. L. VANDERHART, *Macromolecules*, 14 (1981) 570–574.
12 L. D. HALL AND M. YALPANI, *Carbohydr. Res.*, 91 (1981) C1–C4.
13 P. E. PFEFFER, K. B. HICKS, AND W. L. EARL, *Carbohydr. Res.*, 111 (1983) 181–194.
14 R. C. HOCKETT AND C. S. HUDSON, *J. Am. Chem. Soc.*, 53 (1931) 4455–4456.
15 A. OLAND, R. A. BERNHARD, AND T. A. NICKERSON, *J. Food Sci.*, 42 (1966) 1066–1068.
16 J. H. BUSHILL, W. B. WRIGHT, C. H. F. FULLER, AND A. V. BELL, *J. Sci. Food Agric.*, 16 (1965) 622–628.
17 T. J. BUMA, *Neth. Milk Dairy J.*, 32 (1978) 258–261.
18 F. W. PARRISH, K. D. ROSS, AND T. D. SIMPSON, *Carbohydr. Res.*, 71 (1979) 322–326.
19 T. D. SIMPSON, F. W. PARRISH, AND M. L. NELSON, *J. Food Sci.*, 47 (1982) 1948–1951, 1954.
20 R. S. ORR, L. C. WEISS, H. B. MOORE, AND J. N. GRANT, *Textile Res. J.*, 25 (1955) 592–600.
21 W. L. EARL AND D. L. VANDERHART, *J. Magn. Reson.*, 48 (1982) 35–54.
22 E. HENRIOT AND E. HUGUENARD, *C. R. Acad. Sci.*, 180 (1925) 1389–1392.
23 S. R. HARTMANN AND L. L. HAHN, *Phys. Rev.*, 128 (1962) 2042–2053.
24 C. P. SLICHTER, *Principles of Magnetic Resonance*, 2nd edn., Springer-Verlag, Berlin, 1978.
25 M. MEHRING, *High Resolution NMR in Solids*, in P. DIEHL, E. FLUCK, AND R. KOSFELD (Eds.), *NMR, Basic Principles and Progress*, Vol. 11, Springer-Verlag, Berlin, 1976.
26 J. SCHAEFER AND E. O. STEJSKAL, in G. C. LEVY (Ed.), *Topics in Carbon-13 NMR Spectroscopy*, Vol. 3, Wiley-Interscience, New York, 1979, pp. 283–324.
27 J. R. LYERLA, in M. SHEN (Ed.), *Contemporary Topics in Polymer Science*, Vol. 3, Plenum Publishing Corp., New York, 1979, pp. 143–213.
28 W. L. EARL, unpublished data.
29 B. COXON, in C. K. LEE (Ed.), *Developments in Food Carbohydrate-2*, Applied Science Publishers, London, 1980, pp. 351–390.
30 D. C. FRIES, S. T. RAO, AND M. SUNDARALINGAM, *Acta Crystallogr. Sect. B*, 27 (1971) 994–1005.
31 K. HIROTSU AND A. SHIMADA, *Bull. Chem. Soc. Jpn.*, 47 (1974) 1872–1879.
32 P. E. PFEFFER, K. M. VALENTINE, AND F. W. PARRISH, *J. Am. Chem. Soc.*, 101 (1979) 1265–1274.