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Carbohydrate Research 264 (1994) 135–140

CARBOHYDRATE
RESEARCH

Note

# High resolution solid state $^{13}$C NMR study of crystalline methyl D-xylopyranoside anomeric mixtures

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Received 4 January 1994; accepted in final form 17 May 1994

*Keywords:* Methyl D-xylopyranosides; $^{13}$C solid state NMR; Anomeric crystalline mixture; Cocrystallization

High resolution solid state $^{13}$C cross polarization with magic angle spinning NMR spectroscopy ($^{13}$C CP/MAS NMR) can provide interesting and rapid indications concerning the isomeric composition of sugars in the solid state. This technique may be used as a screening tool, before undertaking a more complex X-ray diffraction single crystal structural study. Solid state $^{13}$C NMR is especially sensitive to the anomeric equilibrium which can result from the crystallization of solutions of reducing sugars. It is also well adapted to the observation of conformational changes.

When two isomers crystallize simultaneously from their solutions, it is to be expected that the less soluble component will crystallize preferentially. Therefore, the existence of anomers of reducing sugars in the same unit cell is considered as unusual. 2-Acetamido-2-deoxy-D-glucose [1] and 6-deoxy-$\alpha$-L-sorbofuranose [2] have, however, been reported to present a cocrystallization of the two anomers with a small proportion of the $\beta$ anomeric form. The cocrystallization of different isomers is more common in the case of reducing disaccharides, such as, for example, lactulose [3] and lactose [4,5]. A crystalline mixture of glycoside anomers has been reported in a 1:1 ratio for *n*-octyl $\alpha$- and $\beta$-D-glucopyranoside [6].

The solid state $^{13}$C NMR spectra of methyl $\alpha$- and $\beta$-D-xylopyranoside have already been published [7]. The $\alpha$ anomer crystallizes with two nonequivalent molecules per asymetric unit of the monoclinic cell and gives two signals for each type of carbon atom in the NMR spectrum of the solid. The $\beta$ anomer crystallizes with only one molecule per asymetric unit,

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*SSDI* 0008-6215(94)00173-D

Table 1
Recrystallization experiments for $\alpha$- and $\beta$-D-xylopyranoside mixtures

| Experiment no. | Ratio of the starting compounds $\alpha:\beta$ (%) | Yield of crystallization (%) | Relative composition of the crystals $\alpha:\beta$ (%) | Solid state $^{13}$C NMR (number of lines) |
|---|---|---|---|---|
| 1 | 100% $\alpha$ | 73 | pure $\alpha$ | 11 |
| 2 | | | 98:2 | 17 |
| 3 | 91:9 | 75 | 93.4:6.6 | 22 |
| 4 | 80:20 | 76 | 78:22 | 27 |
| 5 | 68:32 | 53 | 57:43 | 16 |
| 6 | 50:50 | 39 | pure $\beta$ | 6 |

and thus yields one signal for each carbon atom in the solid state $^{13}$C NMR spectrum. We now report the solid state $^{13}$C NMR spectra of cocrystallized mixtures of methyl $\alpha$- and $\beta$-D-xylopyranoside, obtained from solutions having different anomeric ratios.

## 1. Experimental

*Materials and methods.*—Pure methyl $\alpha$- and $\beta$-D-xylopyranoside were commercial samples obtained from Sigma.

The crystallization in various ratios of the two anomers was done by weighing the pure compounds in different proportions (Experiments 1–6 in Table 1). Typically, a total amount of 1 g was dissolved in 2 mL of hot aq EtOH (90%). To the cold solution, was added ether (5 mL) and the crystallization was allowed to proceed overnight at 4°C. The crystalline powder was filtered and dried at room temperature. The yield of crystallization is given in Table 1.

The analyses of the anomeric composition of the resulting crystalline powder were carried out on a small number of crystals by using $^{13}$C NMR on solutions in $D_2O$ (10 mg/0.5 mL) under quantitative conditions given by the inverse gated decoupling $^{13}$C NMR technique with an AC 200 Bruker spectrometer.

*Solid state $^{13}$C CP/MAS NMR.*—The spectra were obtained with a Bruker MSL200 spectrometer operating at 50.3 MHz. A sample (250 mg) was placed in a double-bearing rotor made of zirconia. The spinning rate was set at 3 kHz. The $^1$H frequency was set to give a 90° pulse value of 4.5 $\mu$s. The contact time and the recycle delay were 2 ms and 2 s, respectively. For each spectrum, 500 transients were collected. The chemical shift values were obtained via the glycine carbonyl signal, set at 176 ppm relative to tetramethylsilane.

## 2. Results

The cocrystallization of methyl $\alpha$- and $\beta$-D-xylopyranoside in various ratios (0 to 50% of the $\beta$ anomer) in 30:70 aqueous ethanol–ether gave the results in Table 1.

The crystals of the two anomeric mixtures containing from 2 to 22% of the $\beta$ anomer, show complex high resolution solid-state CP/MAS $^{13}$C NMR spectra, with more than 17

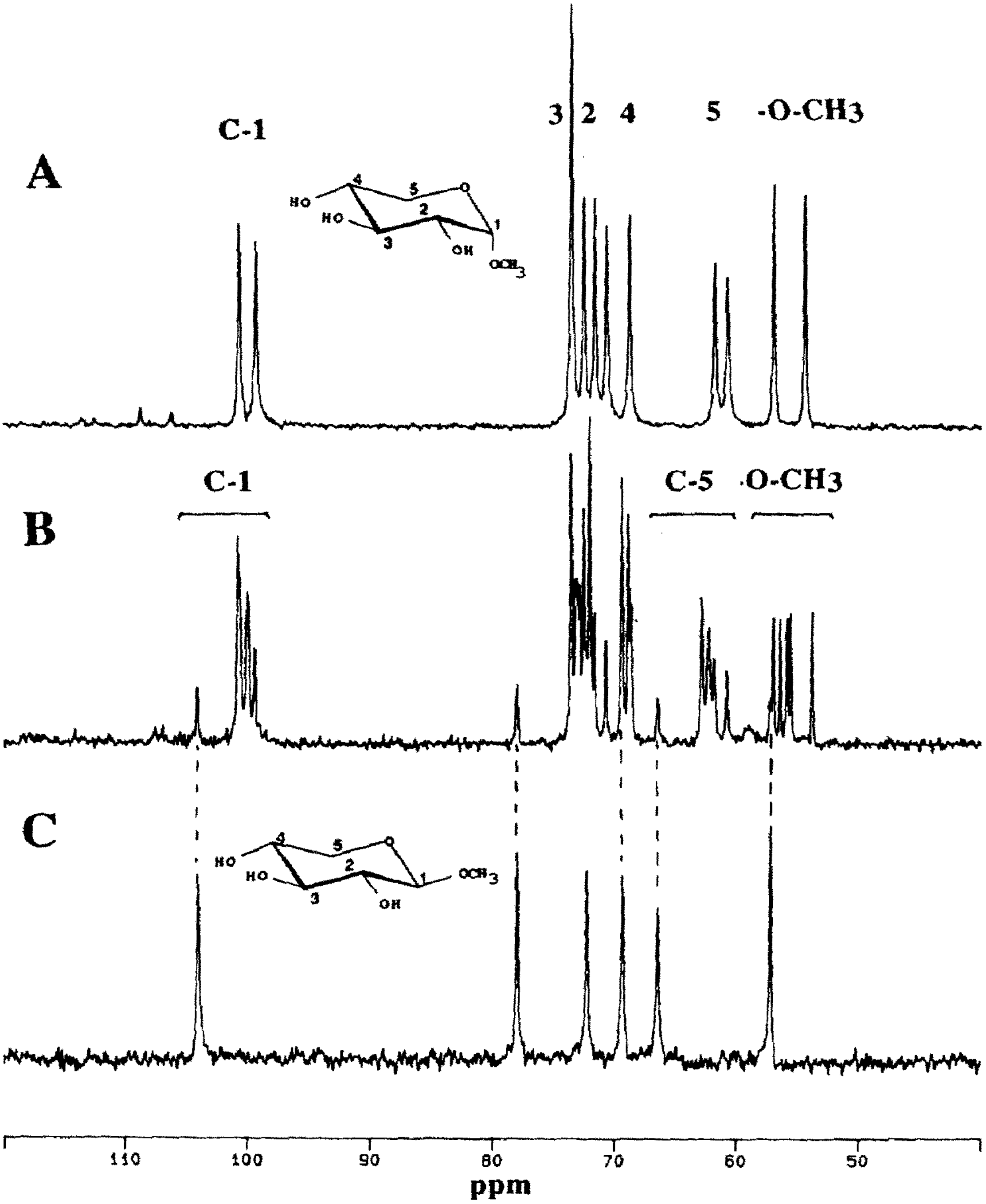

Fig. 1. 50-MHz high resolution solid state CP/MAS $^{13}$C NMR spectra of: A, methyl $\alpha$-D-xylopyranoside; B, crystalline powder of methyl $\alpha$- and $\beta$-D-xylopyranoside mixture (78:22); C, methyl $\beta$-D-xylopyranoside.

signals (11 signals for the $\alpha$ and 6 signals for the $\beta$ anomer) which is to be expected for a mixture of pure crystalline compounds (Figs. 1A and C).

The spectrum of crystals containing 22% of the β anomer is shown as an example in Fig. 1B. From the spectrum of the mixture, the subspectrum of the α anomer (Fig. 1A) can be inferred directly (doublets at similar chemical shift values). The subspectrum of the β anomer corresponds to the six single signals of lower intensity. Among the 27 signals of the spectrum in Fig. 1B, all the carbon atoms appear under five lines (see for example C-1, C-5, and OMe signals). These five lines can be deconvolved into the α doublet plus another doublet of different intensity and splitting, and one single line due to the β anomer. The two doublets arise from the α anomer which indicates the existence of two types of crystals, both having two different molecules per asymetric unit. Several possibilities have then to be considered to explain this fact; either the existence of a hydrated form, or a complex, or the coexistence of polymorphic crystals. The existence of a hydrate, originating from the crystallization in the presence of aqueous ethanol can be ruled out, since the recrystallization of the pure α anomer under the same experimental conditions yielded a crystalline powder with a solid state $^{13}$C NMR spectrum identical with that of the starting compound (11 lines). The existence of two phases for the α anomer must then be proposed. The first gives precisely the solid state $^{13}$C NMR spectrum of the pure α compound, while the second α′ gives doublet signals of different intensity, splitting, and chemical shift values. This complex spectrum is then in favour of "true mixed-crystals" [5], as was noticed in the α and β lactose crystals mixture study using solid state $^{13}$C NMR.

Crystals having different α:β ratios varying from 2 to 43% of the β anomer were prepared as indicated in Table 1. The spectrum shown in Fig. 2B was obtained from a crystalline powder containing up to 43% of the β anomer. This spectrum represents the addition of the two pure anomers subspectra. It is an indication of the existence of a mixture of the two independent types of crystals in the crystalline powder. It should be noted here that the β anomer signals are always weaker than their expected intensity obtained from the analytical determination.

In Fig. 2A is shown the solid state $^{13}$C NMR spectrum of the α anomer, which has been recrystallized from the mother liquors of Experiment 5, resulting in crystals containing 2% of the β anomer. Signals of the β anomer are not seen, due to the low concentration of the β form. Nevertheless, in this spectrum 17 signals are observed, instead of the 11 signals expected for the α anomer; this is in favour of the existence of a new α′ crystal. Thus, the recrystallization of pure α anomer yields the α type of crystal (Fig. 1A), while its recrystallization in the presence of traces of the β anomer yields a second type of crystal (Fig. 2A).

The powder X-ray-diffraction diagrams of the pure α and β anomers and that of the mixture of α- and β-D-xylopyranoside (93.4:6.6) have been achieved. Analysis of the X-ray diagram of the mixture shows no characteristic pattern of the β form, because of its low proportion (6.6%), and the presence of additional ring patterns that were not observed in the diagram of the α anomer. This result is also in favour of the existence of a new type of crystal for methyl α-D-xylopyranoside when the recrystallization occurs from an anomeric mixture

In this study, we have shown that cocrystallization of two methyl D-xylopyranoside anomers can occur over a wide range of the two components (2 to 22% of the β form). The cocrystallization of the α and β isomers yields a new type of crystal called α′. The new

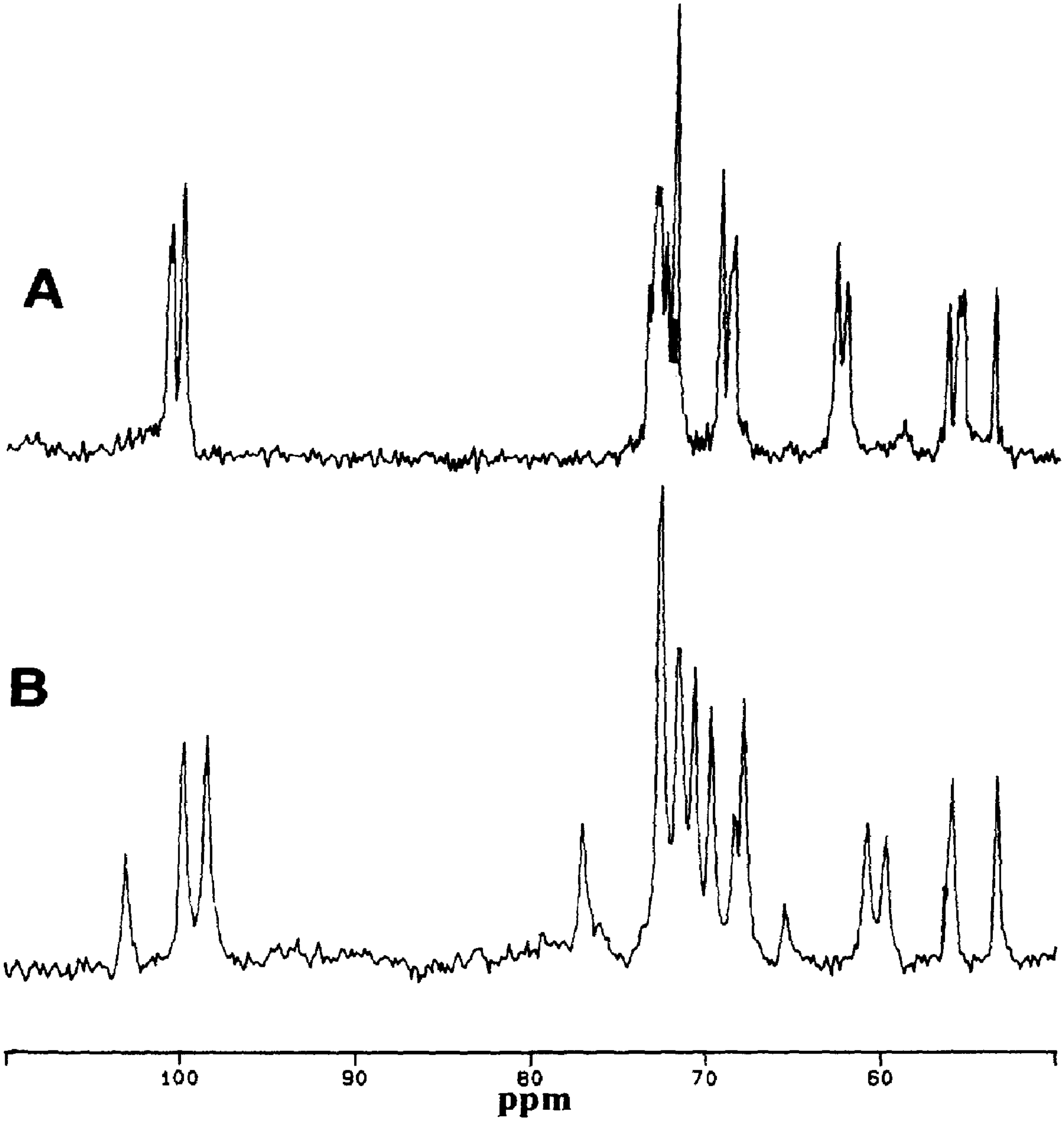

Fig. 2. Solid state $^{13}$C NMR spectra of $\alpha$- and $\beta$-D-xylopyranoside mixtures having different ratio of anomers: A, $\alpha$:$\beta$ 98:2; B, $\alpha$:$\beta$ 57:43.

$\alpha'$ crystal, as revealed by solid-state $^{13}$C NMR, contains two independant molecules per asymetric unit. The $\beta$ isomer probably crystallizes in the $\alpha'$ lattice.

When the composition of the solid is greater than 22% of the $\beta$ anomer, the two isomers crystallize as a mixture of two independent crystalline forms, as observed for pure compounds.

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