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Note

Solution and solid-state ^{1}H- and ^{13}C-n.m.r. spectroscopy of sodium α-L-guluronate dihydrate

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α-L-Guluronic acid (α-L-GulA) is the most important constituent in the brown algal polysaccharide alginate as far as the physico-chemical properties of the polymer are concerned. The fractional contents and the distribution of this residue along the polymer chain profoundly affect the ion binding and gelation properties of alginate[1–3].

A recent X-ray study[4] of the crystal structure of sodium α-L-GulA dihydrate indicated partial anomeric disorder with α:β ~9:1. The objective of the present work was to confirm, by an independent method, the interpretation of the X-ray data.

Fig. 1 shows the ^{1}H-n.m.r. spectrum of a solution of sodium α-L-GulA dihydrate in D_2O at 24°. The chemical shifts and spin–spin coupling patterns of the strong peaks are similar to those reported for methyl β-D-gulopyranosiduronic acid[5]. A large value (8.3 Hz) of $J_{1,2}$ and a collapse of the H-3 and H-4 resonances was observed in each spectrum. The $J_{1,2}$ value indicates H-1,2 to be *trans*-diaxial and implies that, in the L configuration, the guluronate molecule exists in the $^1C_4$ conformation, mainly as the β-pyranose form in aqueous solution. The less shielded equatorial H-1 of the α-pyranose form resonates at 5.2 p.p.m. ($J_{1,2}$ 3.6 Hz). The tautomeric equilibrium for α-L-GulA in aqueous solution also shows one furanose form. Its H-1 resonance at 5.3 p.p.m. ($J_{1,2}$ 2.9 Hz) is attributable to a β-furanose ring[6]. Stereochemical effects favor this 1,2-*trans* anomer strongly over the corresponding 1,2-*cis* α anomer[7]. Integration of the peaks for anomeric protons yielded the percentages of pyranose and furanose anomers shown in Table I. The distribution of anomers in solution in D_2O is similar to that found for L-gulose[7] (Table I).

The ^{1}H-n.m.r. spectra of sodium α-L-GulA in solution in dimethyl sulfoxide (Me_2SO) (Fig. 2), a solvent that inhibits mutarotation[8], clearly demonstrate that, when the restrictions of the crystal lattice are removed, the fraction of α anomer is considerably higher than at equilibrium. Due to residual water in the solvent and water from the crystals, ring opening occurs also in solution in Me_2SO, thereby allowing a conformational equilibration to take place, which leads to a pyranose α:β ratio similar to that

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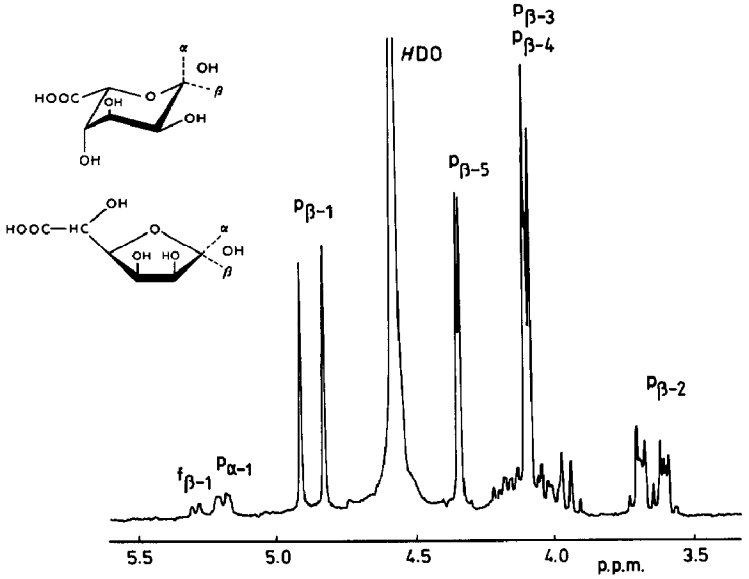

Fig. 1. 100 MHz ^{1}H-n.m.r. spectrum of sodium α-L-GulA in D_2O (10 mg/0.4 mL) at pD 7 and 45°.

in aqueous solution (Table I). A pronounced increase in the proportion of the furanoid anomer was noted in Me_2SO relative to that in D_2O. Figure 3 shows the time-dependent decrease of the proportion of the α anomer as measured by ^{1}H-n.m.r. spectroscopy after dissolution of the crystals in Me_2SO. A linear plot was obtained which, when extrapolated to zero time, points to a considerably lower fraction (0.55) of the α anomer than found in the crystal. However, this value is uncertain since the low solubility of uronates in Me_2SO limits the accuracy. Nevertheless, it is clear that the α anomer preponderates in the crystal, in accord with the X-ray result[4].

Complementary information on the crystal structures of mono- and oligo-saccharides may be obtained from c.p.-m.a.s. ^{13}C-n.m.r. spectroscopy[9]. The ^{13}C c.p.-m.a.s. spectrum of crystalline sodium α-L-GulA dihydrate and the proton-decoupled 25-MHz

TABLE I

Tautomeric composition of sodium α-L-GulA dihydrate

State	*Composition (%)*		
	β-p	α-p	β-f
Solution (D_2O)[a]	77	17	6
Solution (Me_2SO)[a]	65	15	20
Crystalline[b]	10	90	
L-Gulose in solution (D_2O)[c]	81	16	3

[a] Values obtained from integration of the H-1 resonances for mutarotation at equilibrium at 24°. [b] Values obtained from integration of the C-1 resonances. [c] Values obtained from ref. 7.

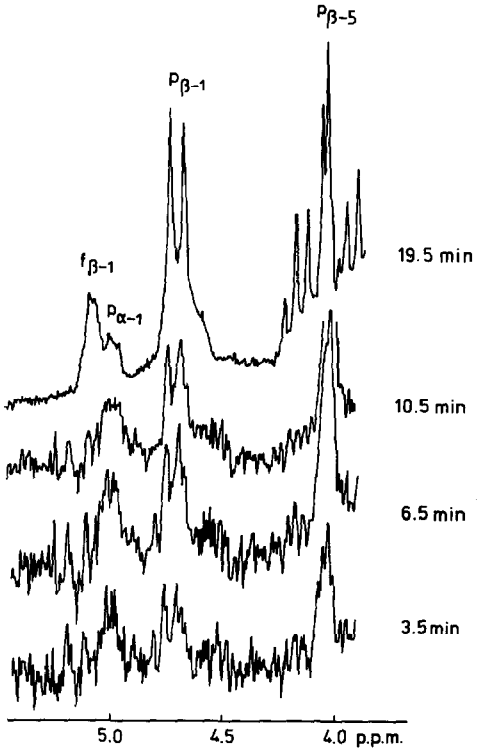

Fig. 2. The time dependence of the region for anomeric protons in the 100 MHz ^{1}H-n.m.r. spectrum of a solution (2 mg/0.4 mL) of sodium α-L-GulA dihydrate in Me_2SO at 24°. Zero time was taken as the addition of solvent.

^{13}C-n.m.r. spectrum of a solution of these crystals in D_2O are shown in Fig. 4. Each spectrum contains one strong and one weak set of resonances corresponding to the pyranose anomers. The spectral region of the C-1 resonance is well documented. The C-2 and C-5 peaks of the β-pyranose in solution were identified by selective proton spin decoupling (data not shown). In the solid-state spectrum, individual resonances corre-

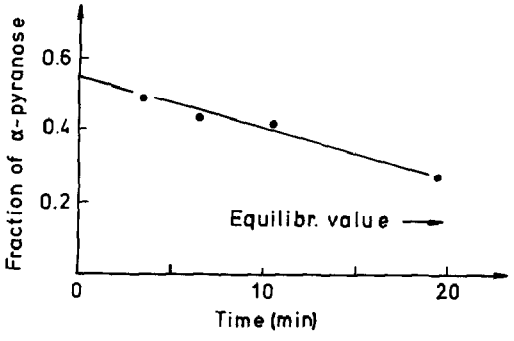

Fig. 3. ^{1}H-N.m.r. time-course of the fraction of α-pyranose for a solution of sodium α-L-GulA dihydrate in Me_2SO at 24°. The material was dissolved at zero time.

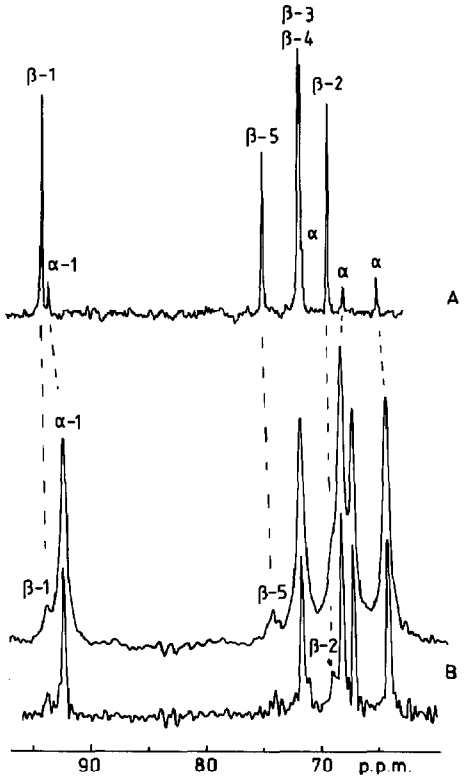

Fig. 4. ^{13}C-N.m.r. spectra of sodium α-L-GulA dihydrate at 24°: *A*, aqueous solution (25 mg/0.4 mL); and *B*, solid state. The bottom spectrum has been resolution enhanced by Lorentz-Gauss transformation. The signals from the carboxyl groups are not shown.

sponding to carbon atoms in each anomer could be assigned by comparison with data for an aqueous solution.

A semi-quantitative picture of the anomeric composition in the crystalline state was obtained from the integrated intensities of the resolved resonances in Fig. 4B. An α:β ratio of 9:1 was obtained, which agreed with the value estimated from the X-ray study. This finding strongly supports the interpretation of the c.p.-m.a.s. ^{13}C-n.m.r. spectrum. The patterns of the chemical shifts of the two anomers in solution and in the solid state agree to within ± 2 p.p.m. (Table II). The differences in chemical shifts may be accounted for by changes in nuclear shielding induced by neighboring molecules in the crystal.

Some important information can be drawn from Fig. 4B: (*a*) the line widths are narrow ($\sim$35 Hz), as expected from crystalline material[12]; (*b*) the main lines show no multiplicities, which implies that all molecules of the α anomer are equivalent in structure and environment; and (*c*) the similarities between the solid-state and solution spectra confirm that the guluronate ring has the $^1C_4$(L) conformation in the crystalline state.

This picture is reasonable in view of the true mixed crystal as indicated in the

TABLE II

^{13}C Chemical shifts (p.p.m.) in solution[a] (D_2O) and in the crystalline state[b] for sodium α-L-GulA dihydrate

	C-1	*C-2*	*C-5*	*C-6*
Sodium α-L-GulA (D_2O)	93.8	65.4		177.5
Sodium α-L-GulA (solid)	92.3	64.2		179.2
$\Delta\delta$ (solid − D_2O)	−1.5	−1.2		1.7
Sodium β-L-GulA (D_2O)	94.4	69.7	75.3	176.8
Sodium β-L-GulA (solid)	93.7		74.0	
$\Delta\delta$(solid − D_2O)	−0.7		−1.3	

[a] Chemical shifts are expressed relative to that of Me_4Si, but were calculated with reference to the C-1 resonances[10] of α- and β-D-glucopyranose at 92.9 and 96.7 p.p.m., respectively. The reference spectrum of D-glucose in solution in D_2O at 24° was obtained separately. [b] Chemical shifts relative to that[11] of the ^{13}C resonance of C=O in solid glycine at 176 p.p.m.

X-ray structure. The β anomer fits into the crystal matrix in a manner analogous to that of the α anomer. Its occurrence in the crystal was indicated by a peak in the electron density in the region near the anomeric carbon atom. This peak was diffuse, but its position and intensity required the presence of some β anomer. Relative occupancies of the two forms were determined in the crystallographic refinement.

Solid-state ^{13}C-n.m.r. spectroscopy is superior to X-ray diffraction in providing information on the anomeric composition in the crystal.

EXPERIMENTAL

Crystalline sodium α-L-guluronate dihydrate was prepared[13] after acid hydrolysis of L-guluronic acid block-polymer.

N.m.r. spectroscopy. — Solution ^1H- and proton-decoupled ^{13}C-n.m.r. spectra were obtained by using a 5-mm C/H probe on a JEOL FX-100 NMR spectrometer (8 k data points, spectral widths of 5 and 1 kHz, a 75° pulse, and pulse repetition times of 3 and 5 s for ^{13}C and ^1H, respectively). An internal deuterium field-frequency lock was used. The ^1H-n.m.r. spectrum in D_2O was referenced against internal sodium 3-(trimethylsilyl)propionate-d_4. For solutions in Me_2SO, the ^1H chemical shifts are reported relative to the solvent peak at 2.62 p.p.m.

The high-resolution solid-state ^{13}C-n.m.r. spectrum was obtained using a Bruker MSL 200 spectrometer. C.p.-m.a.s. (50.33 MHz) was used with a recovery time between acquisitions of 4 s and a contact time of 1 ms. The spinning rate was 4.1 kHz. The spectrum was obtained with 4 k data points, zero filled to 8 k prior to Fourier transformation with 5-Hz digital filtering. Chemical shifts are expressed relative to that of Me_4Si. The referencing material used was glycine run separately in the rotor prior to running the sample alone. The low-field peak of glycine[11] was set to 176 p.p.m.

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