Solid state NMR and X-ray diffraction studies of α-D-galacturonic acid monohydrate

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Abstract

Crystalline α-D-galacturonic acid monohydrate has been studied by ^{13}C CPMAS NMR and X-ray crystallography. The molecular dynamics were investigated by evaluating ^{13}C spin-lattice relaxation in the rotating frame ($T_{1\rho}$) and chemical-shift-anisotropy properties of each carbon. Only limited molecular motions can be detected in the low frequency ($< 10^4$ Hz) range by ^{13}C relaxation time measurements ($T_{1\rho}$) and changes of chemical shift anisotropy properties as a function of temperature. X-ray analysis (at both ambient temperature and 150 K) shows that the acid has the usual chair-shaped, pyranose ring conformation, and that the acid and water molecules are linked, through all their O–H groups, in an extensively hydrogen-bonded lattice. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: α-D-Galacturonic acid; X-ray structure; Molecular dynamics; Chemical shift anisotropy; Spin-lattice relaxation; CPMAS

1. Introduction

α-D-Galacturonic acid (GA), is the monomer of polygalacturonic acid which forms the backbone of pectin in the plant cell walls.[1] The molecular details of the structure and dynamics of pectin are of vital importance to the understanding of plant cell wall properties. However, little is known even about these aspects of its monomer, galacturonic acid. Crystal structures of its sodium–calcium and –strontium salts[2,3] and of methyl galacturonic acid methyl ester[4] have been determined, but not of the parent acid.

The ^{1}H relaxation behaviour of α-D-galacturonic acid showed[5,6] that the motions of the exchangeable protons, i.e., those of the sugar hydroxyl groups and water of crystallisation, were the major relaxation mechanisms for the spin-lattice relaxation. The sugar ring does not have substantial motion in the solid state. However, the ^{1}H relaxation behaviour of both plant cell walls[6–8] and pectin itself[9] showed that there were some other motions present in addition to those of exchangeable protons in both the high frequency (10^8 Hz at ~ 360 K) and low frequency (10^5 Hz at ~ 240 K) regions. [13]C MAS studies showed that there were substantial differences in the dynamics of pectin and cellulose in plant cell wall materials,[6,10–13] and in mixtures of cellulose and pectin.[14] In addition, hydration effects on cellulose and pectin were also different.[6,8–14] With such a complex system, solid state NMR (high resolution and proton relaxation studies) has, so far, resulted only in the identification of fractions usually labelled 'mobile' or

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'rigid'.[6,7,10–13] Quantification and, thus, precise testable descriptions have been lacking.

As a part of our systematic investigation into molecular structure and dynamics of plant cell walls and its model systems, we report in this paper on the crystal structure and molecular dynamics of α-D-galacturonic acid monohydrate.

2. Results and discussion

Crystal structure analyses. — The analysis of the first commercial sample of 'α-D-galacturonic acid monohydrate' showed that this preparation was of the mixed salt, Na,Ca-tri-α-D-galacturonate hexahydrate, whose structure has been reported and described by Gould et al.[2] and Thanomkul et al.[3]

A second preparation of the sample showed the expected structure of the parent acid to-
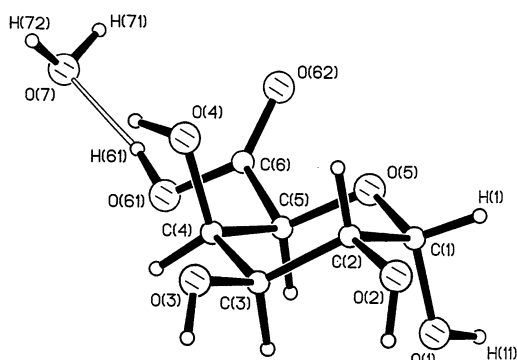

Fig. 1. View of the structure of α-D-galacturonic acid mono-hydrate, indicating the atom numbering scheme. O(7), H(71) and H(72) form the water of crystallisation molecule.
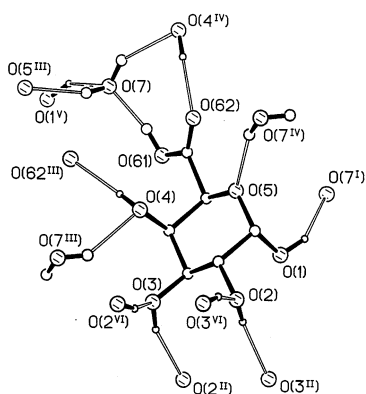

Fig. 2. Molecules of α-D-galacturonic acid and water, showing the hydrogen bond links to adjacent molecules. Roman superscripts, denoting symmetry operations, are defined in Table 1.
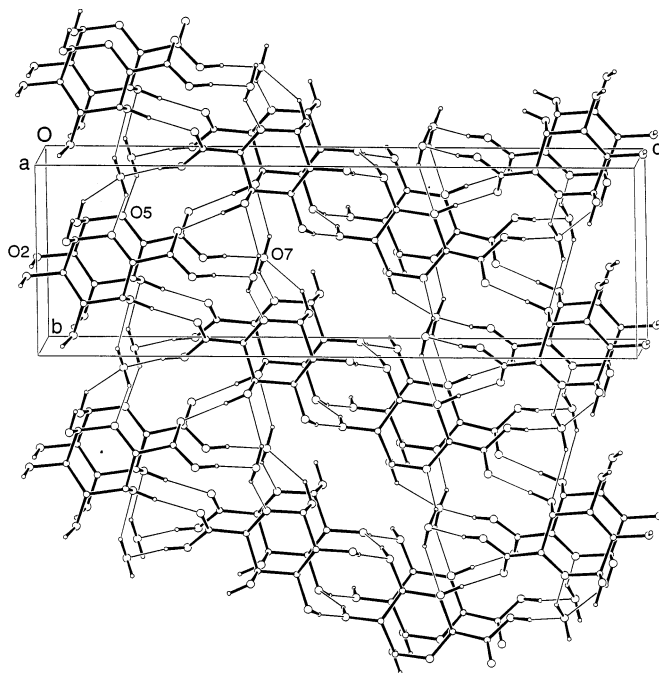

Fig. 3. The packing and extensive, three-dimensional hydrogen bonding scheme in crystals of α-D-galacturonic acid monohydrate. The atoms labels indicate the GA and water molecules that comprise the asymmetric unit of the crystal. The finer lines represent H···O hydrogen bonds.

gether with a water molecule of crystallisation as shown in Fig. 1. Its structure was determined at both 293 and 150 K. The acid molecule adopts the normal pyranose chair conformation with the substituent groups at the C-2, -3 and -5 positions in the equatorial sites, and the hydroxyl groups at C-1 and C-4 mutually trans in the axial positions. All the hydrogen atoms in the structure were identified and refined (with C–H and O–H distance constraints). All the O–H groups are donor groups in hydrogen bonds which link acid and water molecules in an extensive hydrogen bonding lattice (Figs. 2 and 3); except for O(1) and O(61), all the oxygen atoms are also acceptor atoms of hydrogen bonds (Fig. 2). The dimensions in the hydrogen bonds are included in Table 1, which lists bond lengths and angles from X-ray data measured at 150 K.

The ring chair conformation is similar to that found in other galactopyranose structures.[2–4] The similarities extend out to the C(6) carboxylate group which normally shows an O(5)–C(5)–C(6)–O(62) torsion angle close to 0°, i.e., O(62) is essentially cis to O(5) about

the C(5)–C(6) bond; in our structure, the torsion angle is − 7.0(3)°, in the Na,Ca-salt angles of − 16.5 and − 18.3° have been calculated,[2,3] and for the methyl ester,[4] the angle is 2.4(3)°. Identification of the carbonyl O-atom is clear in the acid (our structure) and the methyl ester-both are cis to O(5) and have short C–O bonds; in the Na,Ca-salt, however, the alignment is less clear with the two C(6)–O distances less distinct, perhaps resulting from delocalisation. The involvement of all the oxygen atoms in hydrogen bonds is also a common feature in these compounds; some oxygen atoms in the Na,Ca-salt are also involved in coordinating the metal ions. Consequently, all these structures have extensive, three-dimensional intermolecular bonding networks.

Although the atoms' thermal ellipsoids from the low-temperature data-set are much smaller than those from the room temperature study, the bond lengths and angles differ insignificantly. However, intermolecular distances for those bonds involving non-hydrogen atoms are all ca. 0.02–0.03 Å (i.e., ca. 8σ), shorter in the low-temperature results; the O···O hydrogen bond distances from the room temperature data are included in Table 1. This is consistent with the ^{1}H NMR results, which showed that the motions

Table 1
Molecular dimensions in a-D-galacturonic acid monohydrate at 150 K (unless otherwise indicated) [a]

Principal dimensions

C(1)–O(1)	1.400(3)	C(4)–O(4)	1.435(3)
C(1)–C(2)	1.523(4)	C(4)–C(5)	1.540(3)
C(1)–O(5)	1.425(3)	C(5)–O(5)	1.424(3)
C(2)–O(2)	1.423(3)	C(5)–C(6)	1.518(3)
C(2)–C(3)	1.516(3)	C(6)–O(61)	1.313(3)
C(3)–O(3)	1.435(3)	C(6)–O(62)	1.212(3)
C(3)–C(4)	1.524(3)		
O(1)–C(1)–C(2)	108.1(2)	C(3)–C(4)–C(5)	109.6(2)
O(1)–C(1)–O(5)	112.1(2)	O(4)–C(4)–C(5)	110.9(2)
C(2)–C(1)–O(5)	109.2(2)	C(4)–C(5)–O(5)	112.3(2)
C(1)–C(2)–O(2)	110.4(2)	C(4)–C(5)–C(6)	108.9(2)
C(1)–C(2)–C(3)	109.8(2)	O(5)–C(5)–C(6)	106.7(2)
O(2)–C(2)–C(3)	113.5(2)	C(1)–O(5)–C(5)	114.9(2)
C(2)–C(3)–O(3)	111.2(2)	C(5)–C(6)–O(61)	110.9(2)
C(2)–C(3)–C(4)	109.9(2)	C(5)–C(6)–O(62)	123.4(2)
O(3)–C(3)–C(4)	109.3(2)	O(61)–C(6)–O(62)	125.6(2)
C(3)–C(4)–O(4)	107.8(2)		

Torsion angles

O(5)–C(1)–C(2)–C(3)	59.1(2)
C(1)–C(2)–C(3)–C(4)	− 58.0(2)
C(2)–C(3)–C(4)–C(5)	53.2(2)
C(3)–C(4)–C(5)–O(5)	− 51.4(2)
C(4)–C(5)–O(5)–C(1)	55.8(2)
C(5)–O(5)–C(1)–C(2)	− 58.9(2)

Hydrogen bond dimensions

	O···O	H···O	O–H···O	O···O at 293 K
O(1)–H(11)···O(7[I])	2.883(3)	2.19(3)	134(3)	2.916(3)
O(2)–H(21)···O(3[II])	2.796(3)	1.93(4)	158(4)	2.822(3)
O(3)–H(31)···O(2[II])	2.698(3)	1.87(4)	159(4)	2.719(3)
O(4)–H(41)···O(62[III])	2.769(2)	1.89(3)	166(4)	2.790(3)
O(61)–H(61)···O(7)	2.585(2)	1.70(3)	173(4)	2.601(3)
O(7)–H(71)···O(4[IV])	2.866(3)	2.06(3)	152(3)	2.896(3)
O(7)–H(72)···O(5[III])	2.745(3)	1.95(3)	149(3)	2.763(3)

[a] Bond lengths are in Å, angles in °; e.s.ds are in parentheses; symmetry operations: I, $1-x$, $y-1/2$, $1/2-z$; II, $1/2+x$, $1.5-y$, $-z$; III: $-x$, $1/2+y$, $1/2-z$; IV: $-x$, $y-1/2$, $1/2-z$.
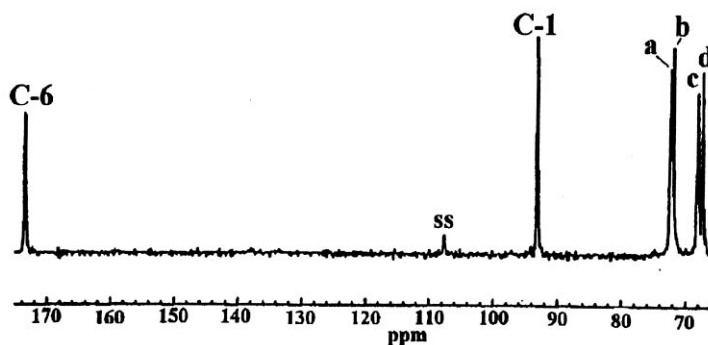
Fig. 4. ^{13}C CPMAS spectrum of α-D-galacturonic acid monohydrate.

Table 2
^{13}C NMR data for α-D-galacturonic acid and a methylated derivative

	C-1	C-2	C-3	C-4	C-5	C-6
α-D-Galacturonic acid (pyranosidic form)						
In the solution state [a] (pH 4), δ_L (ppm)	93.76	69.46	70.67	72.17	72.63	176.84
	C-1	d	c	b	a	C-6
In the solid state, δ_S (ppm)	93.12	67.30 [c]	68.06 [c]	71.96 [c]	72.33 [c]	173.42
$\Delta = \delta_L - \delta_S$	+0.64	+2.16	+2.61	−0.21	+0.30	+3.42
Methyl (methyl α-D-galactopyranosid) uronate						
In the solution state [b], δ_L (ppm)	100.43	68.46	69.62	70.88	71.34	172.02
In the solid state, δ_S (ppm)	99.84	68.80 [c]	69.95 [c]	72.00 [c]	72.03 [c]	170.24
$\Delta = \delta_L - \delta_S$	+0.59	−0.34 [c]	−0.33 [c]	−1.12 [c]	−0.69 [c]	+2.78

[a] See Ref. 15.
[b] See Ref. 16.
[c] Tentative assignments according to solution state chemical shifts.

of the exchangeable protons were on the time scale of 10^{-5}–10^{-4} s at 150–360 K and that the motions of ring protons were even slower.[5]

To investigate further on the motions of each carbon, measurements on ^{13}C relaxation times and the parameters of chemical shift anisotropy were carried out.

Solid state NMR spectroscopy.—The ^{13}C CPMAS spectrum of α-D-galacturonic acid monohydrate (GA) (Fig. 4) has been reported previously[5] and the resonances have been assigned by comparison with its solution state spectrum.[15,16] However, unambiguous assignments of CPMAS spectra are not always reliable when guided only by the chemical shifts in the solution state. This is particularly so for carbohydrates, since they all have large numbers of hydroxyl groups and the hydrogen bond structure in the solution may differ from that in the crystalline solid. Nevertheless, in our case both C-1 and C-6 (carboxyl) can be

assigned unambiguously because they are well separated from the other resonances. For C-2, C-3, C4 and C-5, reliable assignments cannot be made unambiguously. We label them as 'a, b, c, d' as shown in Fig. 4 and Table 2. This is suitable for the purpose of comparing ring carbons and non-ring carbons since C-1–C-5 represent ring carbons and C-6 is the exocyclic carbon.

The chemical shifts of these carbons in the solid[5] and solution[15,16] states (Table 2) clearly showed some differences. The difference, $\Delta = \delta_{liquid} - \delta_{solid}$, reflects changes of intermolecular interactions[17] such as hydrogen bonding involving hydroxyl groups of the acid and the water of crystallisation. It can also provide information about the conformational flexibility of fragments of the molecule.[17] As can be seen from Table 2, Δ of C-6 appeared to be largest amongst all carbons while smaller values of Δ are apparent for all ring carbons. As

discussed in the previous section, all hydroxyl groups in the molecule are involved in hydrogen bonding with other oxygen centres. The H···O hydrogen bond distance between the carboxylic acid group and the water molecule (1.70 Å, Table 1) is smaller and the bond is stronger, as expected, than any other H···O hydrogen bond.

^{13}C spin-lattice relaxation.—The ^{13}C spin lattice relaxation time, T_1, which is sensitive to motions with frequency comparable to its Larmor frequency ($\sim 10^8$ Hz), was slow on a time scale of 100 s for all carbons in GA. This indicated that T_1 was much longer than 100 s. It also suggests the lack of high frequency ($\sim 10^8$ Hz) motions for all carbons. In contrast, ^{13}C spin-lattice relaxation time in the rotating frame, $T_{1\rho}$, which is more sensitive to low frequency ($\sim 10^5$ Hz) motions, was quite short (Table 3). Since the relaxation mechanism is a dipolar interaction of ^{13}C with protons, a carbonyl carbon is expected to relax more slowly than carbons with a proton attached on them. However, $T_{1\rho}$ of the exocyclic carbon (C-6) was substantially shorter than for the ring carbons at ambient temperature. In addition, $T_{1\rho}$ of all carbons showed a decreasing trend with an increase of the sample temperature, implying that motion was on the low temperature side of the $T_{1\rho}$ minimum. This indicates that the motional correlation time is longer than 10^{-5} s. Our studies[5] on GA and its methyl derivative indicated that motions of ring protons were slow on a time scale of 10^{-5} s and that H_2O and OH protons were moving at this rate. Therefore, it is postulated that the principal relaxation mechanism is by interaction of carbon with the water and hydroxyl protons. This explains the rapid relaxation of the exocyclic carbons (C-6) which is due to its close proximity to water protons.

Unlike in the solution state, where averaged chemical shifts resulting from fast isotropic molecular motions are used as finger-prints of chemical bonds, chemical shift anisotropy (CSA) in the solid state is only partially averaged. This is not always undesirable since CSA bears important structural and dynamic information. The CSA of a powdered sample describes the three-dimensional chemical shielding of the nucleus by the surrounding electrons and is conveniently parameterised with three principal shielding components.[18] For the sake of clarity, in the following discussion, we use a deshielding convention for CSA. Therefore, the principal components are denoted σ_{11}, σ_{22}, σ_{33} and ordered by convention so that

$$|\sigma_{33} - \sigma_{AV}| \geq |\sigma_{11} - \sigma_{AV}| \geq |\sigma_{22} - \sigma_{AV}|$$

where σ_{AV} is the isotropic motion averaged chemical shift and defined as:

$$\sigma_{AV} = \frac{1}{3}(\sigma_{11} + \sigma_{22} + \sigma_{33}) \qquad (1)$$

From these components, the shielding anisotropy $\Delta\sigma$ can be defined as:

$$\Delta\sigma = \sigma_{33} - \frac{(\sigma_{11} + \sigma_{22})}{2} \qquad (2)$$

The $\Delta\sigma$ is an interesting quantity since it is related to the environment of the nucleus in the molecule. For ^{13}C, the size of CSA is normally dependent on the molecular structure and the motion in the carbon site.[18] The occurrence of a molecular motion often leads to partial or complete averaging of the CSA pattern. When the frequency of a molecular motion is comparable to $\Delta\sigma$ or higher, the CSA parameters will be greatly affected provided that sufficient range of angles with respect to the static magnetic field is experienced during the motion.[18] If the motion reaches the limit of fast isotropic rotation, only the isotropic chemical shift can be observed as in the case of solution state.[18]

Table 3
Solid state ^{13}C–$T_{1\rho}$ relaxation data for α-D-galacturonic acid monohydrate

	303 K	320 K	365 K
C-1	0.182 ± 0.001	0.152 ± 0.012	0.090 ± 0.011
C-2 [a] (d)	0.255 ± 0.012	0.205 ± 0.016	0.137 ± 0.029
C-3 [a] (c)	0.169 ± 0.002	0.144 ± 0.013	0.086 ± 0.015
C-4 [a] (b)	0.113 ± 0.007	0.090 ± 0.005	0.050 ± 0.006
C-5 [a] (a)	0.154 ± 0.010	0.133 ± 0.006	0.088 ± 0.009
C-6	0.045 ± 0.001	0.026 ± 0.001	0.015 ± 0.001

[a] Tentative assignments according to solution state chemical shifts, letter in parenthesis indicates labelling as described in the text.

Table 4
Solid state ^{13}C NMR data for α-D-galacturonic acid [a]

	C-1	d	b and c	a	C-6
303 K					
σ_{11}	114	94	93	55	244
σ_{22}	96	68	69	70	185
σ_{33}	70	40	42	91	92
$\|\sigma_{33}-\sigma_{11}\|$	44	54	51	36	152
$\|\Delta\sigma\|$ [b]	35	41	39	29	123
320 K					
σ_{11}	114	92	49	92	242
σ_{22}	96	72	63	74	184
σ_{33}	70	39	93	50	94
$\|\sigma_{33}-\sigma_{11}\|$	44	53	44	42	148
$\|\Delta\sigma\|$	35	43	37	33	119
355 K					
σ_{11}	115	46	49	52	239
σ_{22}	97	63	63	72	182
σ_{33}	68	93	93	93	99
$\|\sigma_{33}-\sigma_{11}\|$	47	47	44	41	140
$\|\Delta\sigma\|$	38	39	37	31	112
365 K					
σ_{11}	71	44	48	92	234
σ_{22}	90	66	65	72	181
σ_{33}	119	92	92	52	106
$\|\sigma_{33}-\sigma_{11}\|$	48	48	44	40	128
$\|\Delta\sigma\|$	39	37	36	30	102
Me–Gal [c]	C1	C2	C3	C4	C5
σ_{11}	86	89	51	54	56
σ_{22}	97	68	72	68	69
σ_{33}	119	46	95	88	94
$\|\sigma_{33}-\sigma_{11}\|$	33	43	44	34	38
$\|\Delta\sigma\|$	28	33	34	27	32

[a] The CSA parameters are all in ppm, experimental uncertainty is about 10%.

[b] $\|\Delta\sigma\|$ is used to avoid confusion to sign changes caused by adherence to the ordering convention for σ described in the text.

[c] Data for methyl-α-D-galactopyranoside at room temperature were from Ref. 19.

Measured values for the principal components of CSA for GA at various temperatures are tabulated in Table 4. As far as we are aware, there are no reported CSA data for this compound. The only relevant data available were for methyl glycosides of α-D-galactopyranose,[19] α-D-glucopyranose[19,20] and α-D-mannopyranose[19] and for sucrose.[21] Although these data cannot be used to validate data for galacturonic acid, they do provide comparative information. It is particularly interesting to note that, at room temperature, the absolute values for $\Delta\sigma$ for all the ring carbons in methyl α-D-galactopyranose are 27–34 ppm[19] and those in methyl α-D-glucopyranose are 28–32 ppm.[19,20] Our data for the ring carbons at 303 K (29–41 ppm) are comparable within the margin of error. The breadth of CSA powder pattern, $\|\sigma_{33}-\sigma_{11}\|$, for ring carbons (36–54 ppm) in GA is also broadly comparable with the data for methyl α-D-galactopyranose (33–44 ppm).[19]

The magnitude of the $\Delta\sigma$ values, $\|\Delta\sigma\|$, showed slightly different temperature dependences for the exocyclic carbon, C-6, and the ring carbons. Whilst $\|\Delta\sigma\|$ for C-6 showed a steady decrease as the temperature increased, that for the ring carbons showed little change over the temperature range we used. Chemical shift anisotropy is a second rank tensor interaction and therefore the effects of motions on its magnitude depend both on the frequency and the amplitude of the motion. Motions with frequency components less than the width of the CSA will not affect it. The observation of a temperature effect on $\|\Delta\sigma\|$ for C-6 indicates that there must be motions present with frequency components of the order of 10^4 Hz (the width of the CSA in frequency units). However, since the reduction in CSA is small the indication is that the amplitude of the motion in angular terms is small.

3. Conclusions

The X-ray structure of this sample showed that every hydroxyl group in the acid molecule and the water molecule is involved in an extensive three-dimensional hydrogen-bonding scheme (Fig. 3). ^{13}C CPMAS NMR results showed that carbon atoms of α-D-galacturonic acid monohydrate in the crystalline form had limited low frequency motions ($\tau > 10^{-5}$ s). Smaller thermal ellipsoids for all the carbon and oxygen atoms at 150 K are consistent with the notion that motions of these atoms are reduced as the temperature is decreased.

4. Experimental

Sample preparation.—The first sample of 'α-D-galacturonic acid monohydrate' was taken directly from a supply from Sigma and recrystallised from aq EtOH. Crystal structure analysis showed that the crystal selected (typical, we believe, of the whole sample) was Na,Ca-tri-α-D-galacturonate hexahydrate, whose structure has already been reported.[2,3]

Our second sample, a new batch purchased from Sigma (lot no. 97H0674), was recrystallised from aq EtOH by letting EtOH diffuse into the aq solution of the substrate in a closed desiccator. Colourless fine needles were furnished and used for X-ray crystallography and for ^{13}C CPMAS studies. X-ray diffraction confirmed this sample to be α-D-galacturonic acid monohydrate.

Solid state NMR spectroscopy.—All ^{13}C CPMAS experiments were carried out on a Bruker MSL-300 spectrometer operating at 300.13 and 75.46 MHz for proton and carbon, respectively. A standard Bruker double-bearing magic-angle spinning probe (BL-7 type) was used to spin the sample at the desired rate. Typically, a 200-mg sample was packed into a 7 mm Zirconia rotor with a Kel-F cap. A single contact cross-polarisation (CP) pulse sequence[22] was employed with a pulse to flip back[23] the remaining ^{1}H magnetisation after the acquisition time. The Hartmann–Hahn condition[24] was set with glycine. The other parameters were: ^{1}H 90° pulse length, 4 μs; contact time, 1 ms; recycle delay, 10–100 s. The chemical shift was referenced externally with glycine (carbonyl peak at 176.03 ppm).

For the measurements of the principal values of chemical shift anisotropy (CSA), the sample was spun at about 1 kHz to enable spinning side bands (SSB) to be recorded. The values were extracted from the intensities of SSB graphically following the method of Herzfeld and Berger.[25] The experimental uncertainty was estimated below 10%.

For carbon relaxation measurements, the sample was spun at about 4 kHz. Spin-lattice relaxation times of carbons (^{13}C–T_1) were measured using the method described by Torchia.[26] Typically 12 relaxation delays were used varying from 0.5 to 100 s with 16–32 transients accumulated for each experiment. The measurement of $T_{1\rho}$ (^{13}C) was carried out by spin-locking carbon magnetisation without proton decoupling following CP process. The ^{13}C signal intensities, acquired with high power proton decoupling, as a function of spin-locking time gave $T_{1\rho}$ relaxation profiles. Typically 20 spin-locking delays were used varying from 10 μs to 200 ms.

X-ray crystal structure determination

Crystal data for α-D-galacturonic acid monohydrate. $C_6H_{10}O_7 \cdot H_2O$, $M_w = 212.2$. Orthorhombic, space group $P2_12_12_1$ (no. 19), $a = 4.7923(5)$, $b = 7.4426(9)$, $c = 23.148(3)$ Å, $V = 825.6(2)$ Å^3. $Z = 4$, $D_{calc} = 1.707$ g cm^{-3}, $F(000) = 448$, $T = 293$ K, μ(Mo K$_\alpha$) = 1.6 cm^{-1}, λ(Mo K$_\alpha$) = 0.71069 Å.

Crystals of the acid are clear, colourless rectangular prisms. One crystal, ca. $0.10 \times 0.14 \times 0.45$ mm, was mounted on a glass fibre. After preliminary photographic examination, this was transferred to an Enraf–Nonius CAD4 diffractometer (with monochromatic radiation) for determination of accurate cell parameters (from the settings of 25 reflections, $\theta = 9–11°$, each centred in four orientations) and for measurement of diffraction intensities (1088 unique reflections to $\theta_{max} = 27°$; 1000 were 'observed' with $I > 2\sigma_1$).

During processing, corrections were applied for Lorentz-polarisation effects and to eliminate negative net intensities (by Bayesian statistical methods). No absorption or deterioration corrections were necessary. The structure was determined by direct methods routines in the SHELXS program[27] and refined by full-matrix least-squares methods, on F^2 values, in SHELXL.[28] Hydrogen atoms were located in difference maps and refined with constrained C–H or O–H distances; their isotropic temperature factors were refined freely. All the non-hydrogen atoms were refined with anisotropic thermal parameters. At the conclusion of the refinement, $wR_2 = 0.098$ and $R_1 = 0.039$[28] for all 1088 reflections weighted $w = [\sigma^2(F_o^2) + (0.050P)^2 + 0.26P]^{-1}$ with $P = (F_o^2 + 2F_c^2)/3$; for the 'observed' data only, $R_1 = 0.037$. The Flack x value for this configuration was 1.0(19); refinement of the inverted structure gave the same R-values and a Flack x value of $-0.3(20)$. We were there-

fore unable to distinguish the absolute configuration from the refinement; the D configuration results are assumed knowing that our sample has been prepared from a natural pectin sample which consists of galacturonic acid having only the D configuration and that the manufacturer's reported optical rotation measurements are in agreement with the D configuration.

In the final difference map, the highest peaks (ca. 0.36 e $Å^{-3}$) were all near the mid-points of the C–C and C–O bonds.

In a following, low-temperature study of the same crystal, we obtained the crystal data for α-D-galacturonic acid monohydrate at 150 K.

$C_6H_{10}O_7 \cdot H_2O$, $M_w = 212.2$. Orthorhombic, space group $P2_12_12_1$ (no. 19), $a = 4.7624(12)$, $b = 7.4030(15)$, $c = 23.068(4)$ Å, $V = 813.3(3)$ $Å^3$. $Z = 4$, $D_{calc} = 1.733$ g cm^{-3}, $T = 150$ K.

Of the 1073 unique reflections measured to $\theta_{max} = 27°$, 1009 were 'observed'. Corresponding refinement gave convergence with $wR_2 = 0.096$ and $R_1 = 0.038$ for all 1073 reflections weighted $w = [\sigma^2(F_o^2) + (0.054P)^2 + 0.29P]^{-1}$; for the 'observed' data only, $R_1 = 0.035$. Again, the calculation of the Flack parameters [$-1.7(17)$ and $2.5(17)$] and comparison of the final R-factors (for the enantiomer reported and the inverted structure) did not distinguish the absolute configuration clearly.

Scattering factors for neutral atoms, in both refinements, were taken from Ref. 29. Computer programs used in this analysis have been noted above or in Table 4 of Ref. 30, and were run on a DEC-AlphaStation 200 4/100 in the Nitrogen Fixation Laboratory at the John Innes Centre.

5. Supplementary material

Atomic coordinates, thermal parameters and bond lengths and angles for both analyses have been deposited at the Cambridge Crystallographic Data Centre (CCDC). Copies can be obtained free of charge from The Director, 12 Union Road, Cambridge CB2 1EZ, UK (Tel.: +44-1223-336408; fax +44-1223-336033; e-mail deposit@ccdc. cam.ac.uk or www: http://www.ccdc.cam. ac.uk). Any request to the CCDC for this material should quote the full literature citation and the reference number CCDC 151377 and 151378.

Acknowledgements

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