Note

¹H-N.m.r. spectroscopy of *O*-carboxymethyl derivatives of D-glucose

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Carboxymethylation is a common method used to modify the properties of cellulose and starch but, as a rule, not all of the hydroxyl groups are substituted. The products can be regarded as copolymers of unsubstituted, mono- (2-, 3-, and 6-), di- (2,3-, 2,6-, and 3,6-), and tri-substituted (2,3,6-) D-glucose moieties. Characterisation of the structures of the monomers in a hydrolysate of carboxymethylcellulose, using ¹³C-n.m.r. spectroscopy[1,2] and g.l.c.–(e.i.)-m.s.[3], has been reported. ¹H-N.m.r. spectroscopy (90 MHz) of hydrolysates of carboxymethylcellulose has been used to determine the distribution of the carboxymethyl groups and the degree of substitution[4]. However, the low resolution of the ¹H-n.m.r. spectra precluded the assignment of all of the signals. A new procedure for the determination of the distribution of the substituents in carboxymethylcellulose based on the analysis of hydrolysates by high-pH anion-exchange chromatography on CarboPac PA-1 with pulsed amperometric detection (h.p.a.e.c.-p.a.d.)[5] allowed the isolation of all of the monomers.

We now report the complete assignment of the ¹H-n.m.r. spectra of the carboxymethylated derivatives of D-glucopyranose with substituents at O-2, O-3, O-6, O-2,3, O-2,6, O-3,6, and O-2,3,6. Each of the above derivatives was an α,β-mixture with various α,β-ratios. Most of the resonances of the skeleton protons were assigned by combining information from 2D homonuclear Hartmann–Hahn (HOHAHA) and 1D spectra (Tables I and II). By choosing a suitable mixing time for the 2D HOHAHA spectra, the number of cross-peaks on the individual skeleton proton tracks was limited to one or two. Most of the assignments were deduced from these spectra, using additional information such as intensity differences and coupling patterns. When necessary, 2D HOHAHA spectra were recorded with longer mixing times which showed partial or complete subspectra on the H-1 track of each anomer, or 2D double-quantum filtered ¹H–¹H correlation (DQF-¹H–¹H COSY) spectra were taken. The resonances of the skeleton protons that overlapped in the 2D spectra were assigned on the basis of iterative simulation and matching.

The signals for the CH_2COO groups appeared in a region (δ 3.9–4.3) where,

TABLE I

^{1}H-N.m.r. chemical shift data $(\delta)^a$ for D-glucose and its mono-carboxymethylated derivatives, and $\Delta\delta$ values^b

	Compound							
	Glc		Position of CH_2COO group					
			O-2		O-3		O-6	
Proton	α	β	α	β	α	β	α	β
H-1	5.224^c	4.637^c	5.406	4.710	5.237	4.656	5.229	4.641
			(0.182)	(0.073)	(0.013)	(0.019)	(0.005)	(0.004)
H-2	3.525	3.236	3.367	3.112	3.626	3.332	3.543	3.253
			(−0.158)	(−0.124)	(0.101)	(0.096)	(0.018)	(0.017)
H-3	3.707	3.481	3.819	3.601	3.560	3.381	3.704	3.494
			(0.112)	(0.120)	(−0.147)	(−0.100)	(−0.003)	(0.013)
H-4	3.402	3.393^c	3.440^d	3.424^d	3.511^e	3.492^e	3.470	3.452
			(0.038)	(0.031)	(0.109)	(0.099)	(0.068)	(0.059)
H-5	3.828	3.459	3.812^f	3.452^f	3.824^f	3.464	3.932	3.575
			(−0.016)	(−0.007)	(−0.004)	(0.005)	(0.104)	(0.116)
H-6a	3.835	3.891^c	3.836	3.885	3.826^f	3.883	3.770	3.822
			(0.001)	(−0.006)	(−0.009)	(−0.008)	(−0.065)	(−0.069)
H-6b	3.754^c	3.714	3.753^d	3.712	3.750	3.709	3.724	3.691
			(−0.001)	(−0.002)	(−0.004)	(−0.005)	(−0.030)	(−0.023)
$CH_AH_BCOO^g$			4.032	4.146	4.218	4.227	3.928	3.942
$CH_AH_BCOO^g$			4.150	4.266	4.218	4.227	3.974	3.982

^{a} Chemical shifts (δ) are relative to the signal of sodium 4,4-dimethyl-4-silapentane-1-sulphonate (using internal acetone at δ 2.225) in D_2O, pD $\geq$ 7. ^{b} Substituent effects $(\Delta\delta)$ are given in parentheses, a negative value indicates an upfield shift. ^{c} These values differ by > 0.01 p.p.m. from the reported value^6. ^{d} Determined on the basis of HOHAHA measurements. ^{e} Assignments may have to be interchanged. ^{f} Obtained by spectrum simulation with an accuracy of 0.005 p.p.m.. ^{g} The resonance of H_A has the lower chemical shift.

except for the resonance of H-5α in 6-O-carboxymethyl-D-glucose, no signals for skeleton protons were observed. Unequivocal assignment of these signals for the monosubstituted derivatives was possible using differences in intensities to differentiate between the α and β anomers.

The resonances of the CH_2COO groups at O-2 and O-3 in the 2- and 3-monosubstituted derivatives, respectively, had chemical shifts in the range δ 4.032–4.266, and those of the CH_2COO group at O-6 in the 6-monosubstituted derivatives were in the range δ 3.928–3.982. Assuming that the chemical shift of the resonances of the CH_2COO group at O-6 is not influenced significantly by 2- or 3-carboxymethylation, then the signals of the group at O-6 for the 2,6- and 3,6-disubstituted derivatives and the 2,3,6-trisubstituted derivatives can be assigned (Table II). The assignments of the signals of the CH_2COO groups at O-2 and O-3 of the 2,3-di- and 2,3,6-trisubstituted derivatives were deduced from rotating frame n.O.e. spectroscopy (ROESY) measurements. Cross-peaks between the signals for the H-1 atoms and those for the CH_2COO groups were used to identify the signals of the CH_2COO group at O-2 in the 2,3-di- and 2,3,6-tri-substituted derivatives. The remaining CH_2COO signals for the

TABLE II

Experimental[a] and calculated[b] ^{1}H-n.m.r. chemical shift data (δ) for di- and tri-carboxymethylated glucose residues

| | Positions of CH_2COO groups | | | | | | | |
| | O-2,3 | | O-2,6 | | O-3,6 | | O-2,3,6 | |
Proton	α	β	α	β	α	β	α	β
H-1	5.425	4.719	5.406	4.710	5.242	4.661	5.420	4.718
	(5.419)	(4.729)	(5.411)	(4.714)	(5.242)	(4.660)	(5.424)	(4.733)
H-2	3.482	3.260	3.385	3.126	3.642	3.346	3.502	3.268
	(3.468)	(3.208)	(3.385)	(3.129)	(3.644)	(3.349)	(3.486)	(3.225)
H-3	3.665	3.519[c]	3.821	3.607	3.55[c,d]	3.388	3.671[c]	3.522[c]
	(3.672)	(3.501)	(3.816)	(3.614)	(3.557)	(3.394)	(3.669)	(3.514)
H-4	3.531	3.480[c]	3.518	3.495	3.564[d]	3.543	3.548[c]	3.597[c]
	(3.549)	(3.523)	(3.508)	(3.483)	(3.579)	(3.551)	(3.617)	(3.582)
H-5	3.824[c]	3.462[c]	3.927[c]	3.568	3.965[c]	3.598[c]	3.962[c]	3.598
	(3.808)	(3.457)	(3.916)	(3.568)	(3.928)	(3.580)	(3.912)	(3.573)
H-6a	3.828[c]	3.884	3.776	3.825	3.791	3.840	3.800	3.842
	(3.827)	(3.877)	(3.771)	(3.816)	(3.761)	(3.814)	(3.762)	(3.808)
H-6b	3.746	3.704	3.733	3.697	3.707	3.673	3.686[c]	3.663[c]
	(3.749)	(3.707)	(3.723)	(3.689)	(3.720)	(3.686)	(3.719)	(3.684)
2-CH_AH$_B$COO[f]	4.117	4.173	4.028	4.139			4.113	4.177
2-CH$_A$$H_B$COO[f]	4.168	4.322	4.151	4.271			4.158	4.304
3-CH_AH$_B$COO[f]	4.146	4.214			4.195	4.204	4.156	4.210
3-CH$_A$$H_B$COO[f]	4.316	4.268			4.245	4.253	4.299	4.253
6-CH_AH$_B$COO[f]			3.928	3.943	3.955	3.967	3.972	3.959
6-CH$_A$$H_B$COO[f]			3.978	3.985	3.955	3.967	3.972	3.959

[a] Chemical shifts (δ) are relative to the signal of sodium 4,4-dimethyl-4-silapentane-1-sulphonate (using internal acetone at δ 2.225) in D_2O, pD $\geq$ 7. [b] Calculated chemical shifts (δ) relative to those for D-glucose resonances are in parentheses. [c] Determined on the basis of HOHAHA measurements. [d] Assignments may have to be interchanged. [e] Obtained by spectrum simulation with an accuracy of 0.005 p.p.m. [f] The resonance of H_A has the lower chemical shift.

2,3-disubstituted derivatives were assigned using differences in intensities to distinguish the signals from the α and β anomers and taking into account the ROESY data for the 2,3,6-trisubstituted derivatives. Likewise, the signals for the remaining CH_2COO groups at O-2 and O-3 in the 2,3,6-trisubstituted derivatives were assigned using differences in intensities in order to identify the signals from the α and β anomers, and taking into account the ROESY data for the 2,3-disubstituted derivatives.

The value of J_{gem} for the CH_2COO groups varied between -15.7 and -16.3 Hz, and their coupling patterns depended on the position and the presence and location of other CH_2COO groups. Most of the methylene protons are non-equivalent and the signals were pairs of doublets. The CH_2COO signals of both anomers of the 3-monosubstituted derivative and those of the substituent at O-6 of both anomers of the 3,6-di- and 2,3,6-tri-substituted derivatives were singlets.

The CH_2COO group at O-2 and O-3 in the monosubstituted derivatives caused an upfield shift ($\Delta\delta$ 0.096–0.158) of the resonance for H-2 and H-3, respectively, and a downfield shift ($\Delta\delta$ 0.073–0.182) of the resonances of H-1,3 and H-2,4, respectively. For the 6-monosubstituted derivatives, the signals for H-4 and H-5 were shifted downfield by 0.06 and 0.11 p.p.m., respectively, and those of H-6a and H-6b were shifted upfield by 0.07 and 0.03 p.p.m., respectively.

The effect of carboxymethylation on the J values was small. Only the $J_{H-6a,6b}$ value changed from -12.2 Hz in D-glucose to -11.3 Hz in the glucose residues bearing a CH_2COO group at O-6. The ^3J values between the skeleton protons were within 0.5 Hz of those for unsubstituted D-glucose, indicating that the time-averaged ring conformation does not change significantly upon carboxymethylation. Therefore, additivity of the chemical shift effects upon carboxymethylation might be expected and, hence, the chemical shifts for the skeleton protons of the di- and tri-substituted derivatives were calculated using the substituent effects observed for the monosubstituted derivatives. The results, shown in parentheses in Table II, indicate that, with certain exceptions, the deviation between calculated and experimental chemical shifts was not more than 0.03 p.p.m. The exceptions involve H-5α ($\Delta\delta$ 0.037) in the 3,6-disubstituted derivative, H-2β ($\Delta\delta$ 0.052) and H-4β ($\Delta\delta$ 0.043) in the 2,3-disubstituted derivative, and H-2β ($\Delta\delta$ 0.043), H-4α ($\Delta\delta$ 0.069), H-6α ($\Delta\delta$ 0.038), and H-5α ($\Delta\delta$ 0.050) in the 2,3,6-trisubstituted derivative.

The ^{1}H-n.m.r. data presented here should be useful in the assignment of ^{1}H-n.m.r. spectra of carboxymethylated gluco-oligosaccharides.

EXPERIMENTAL

Prior to ^{1}H-n.m.r. spectroscopy, the desalted samples were treated repeatedly with D_2O (99.9 atom % D, MSD Isotopes), finally using 99.96 atom % D at pD ≥ 7. Resolution-enhanced 500-MHz ^{1}H-n.m.r. spectra were recorded using a Bruker AM-500 spectrometer (Bijvoet Center, Department of NMR Spectroscopy) at a probe temperature of 27°. Chemical shifts (δ) are expressed in p.p.m. downfield from the signal for internal sodium 4,4-dimethyl-4-silapentane-1-sulphonate, but were measured by reference of the signal of internal acetone (δ 2.225 in D_2O at 27°)[7] with an accuracy of 0.002 p.p.m.

HOHAHA spectra were recorded using the pulse sequence 90°–t_1–SL–acq[8,9], where SL stands for a multiple of the MLEV-17 sequence. The spin-lock field strength ranged from 7.7 to 10.4 kHz, and the total spin-lock mixing time from 18 to 80 ms. The time-proportional phase-increment (TPPI) method[10] was used for modulation of the t_1 amplitude, and 200–640 free induction decays (fid's) of 2048 data points, 8–40 scans each, were collected. The spectral width was 1500, 2000, or 3000 Hz in each dimension.

ROESY involved the pulse sequence 90$_\theta$°–t_1–SL–acq[11], where SL stands for a continuous spin-lock pulse of 200 ms at a field strength of 2.5 kHz. The carrier frequency was placed at the left side of the spectrum at 5.6 p.p.m. in order to minimise HOHAHA-type magnetisation transfer. The HOD signal was suppressed by presaturation during

1.0 s. The TPPI method was used for modulation of the t_1 amplitude, and 512 fid's of 2048 data points of 40 scans each were collected. The spectral width was 3000 Hz in each dimension.

DQF-^{1}H–^{1}H COSY was carried out using the pulse sequence $90°–t_1–90°–90°$–acq, where phase-cycling of the receiver and detector allowed for coherence transfer through a double quantum filter[12]. The TPPI method was used for modulation of the t_1 amplitude, and 900 fid's of 2048 data points, 32 scans each, were collected. The spectral width was 3000 Hz.

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