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Note

Spin–lattice relaxation-times for two isomers of *N*-acetylneuraminyl-lactose*

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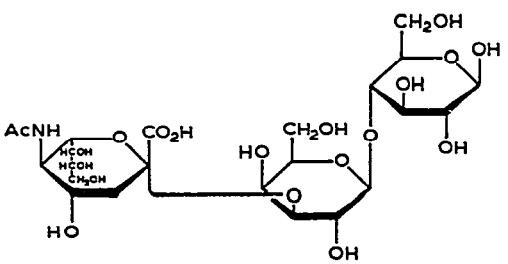
Cell-surface carbohydrate-chains often terminate in the extracellular medium with a sialic acid group, that of *N*-acetylneuraminic acid. This relatively strong acid is largely ionized at physiological pH, and its charge has been implicated in the deterring of cell and platelet agglutination[1]. Nonreducing, terminal sialyl groups are absolutely necessary for the interaction of influenza virus with erythrocytes[2], and their absence from plasma glycoproteins leads to rapid catabolism by the liver[3]. It is also known that, among the membrane oligosaccharides that have been sequenced[4], there recurs a terminal trisaccharide involving *N*-acetylneuraminic acid, D-galactopyranose, and either 2-acetamido-2-deoxy-D-glucopyranose or 2-acetamido-2-deoxy-D-galacto-pyranose. It was therefore, of interest to learn more about the properties of these unique trisaccharide units.

Synthesis, or cleavage from cell membranes, of quantities of these particular saccharides sufficient for n.m.r. studies is difficult; however, a closely related tri-saccharide, *N*-acetylneuraminyl-lactose, is readily available. Two isomeric forms occur in bovine colostrum, specifically *O*-(*N*-acetyl-α-neuraminic acid)-(2→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose (**1**) and *O*-(*N*-acetyl-α-neuraminic



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acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose, distinguished by the α-(2→3) and α-(2→6) glycosidic bonding between the neuraminic acid group and the D-galactopyranosyl residue. The α-(2→3)-linked isomer is the more abundant in colostrum by a factor of ~6, and it has been observed, by ^{13}C-n.m.r. spectroscopy, by Eschenfelder *et al.*[7] and, more recently, by Czarniecki and Thornton[8]. The latter authors also measured the spin–lattice relaxation-time (T_1) of the α-(2→3) isomer. Here, we report the assignment and T_1 values of the α-(2→6) isomer, and also some reassessment of the assignment for the α-(2→3) isomer. The α-(2→6)-linked *N*-acetylneuraminic acid appears to occur in blood-plasma glycoproteins and red-blood, cell-surface oligosaccharides at least as frequently as the α-(2→3) isomer.

The α anomeric form of *N*-acetylneuraminic acid would not be expected to complex with Ca^{2+} in the exceptional way in which the β anomer does[6], as the glycerol-1-yl side chain in the former is on the opposite side of the ring from the carboxyl group. However, in a trisaccharide, the other pyranose rings might participate in such complexing. It was, therefore, of interest to ascertain whether either, or both, of the sialyllactose isomers would complex with Ca^{2+}.

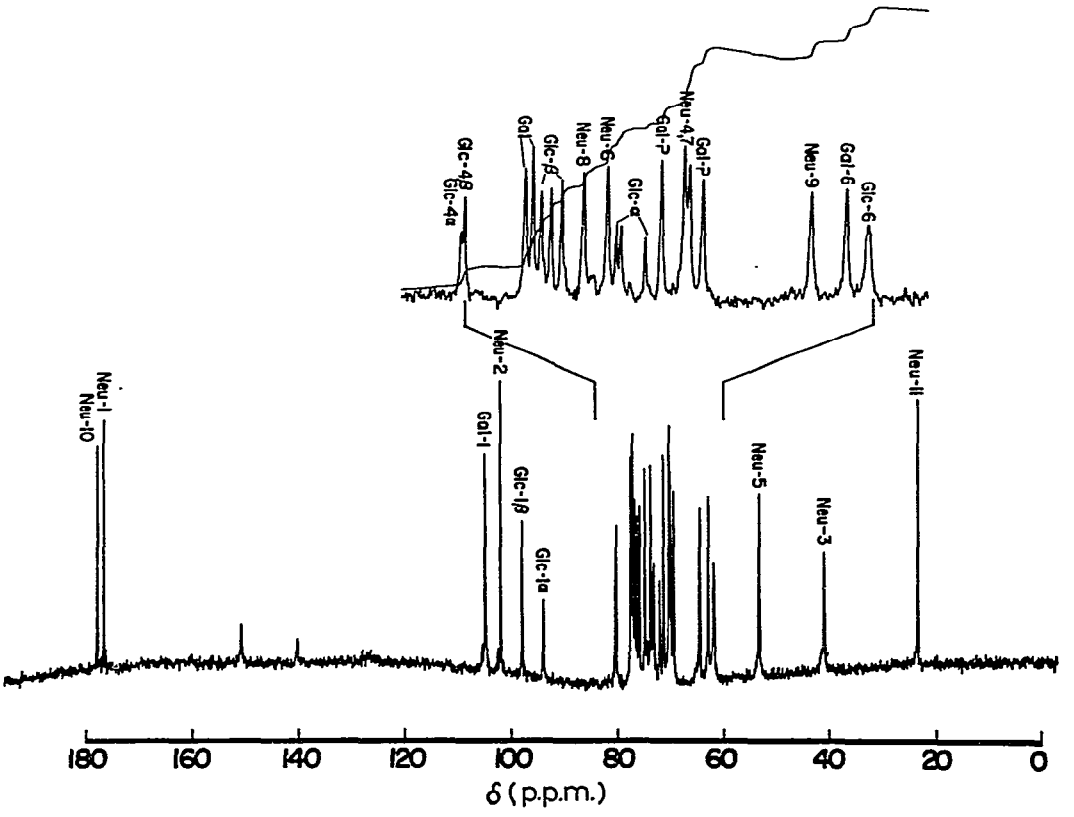

Fig. 1. Proton-decoupled, ^{13}C-n.m.r. spectrum of an aqueous solution of *O*-(*N*-acetyl-α-neuraminic acid)-(2→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose at pD 7.0.

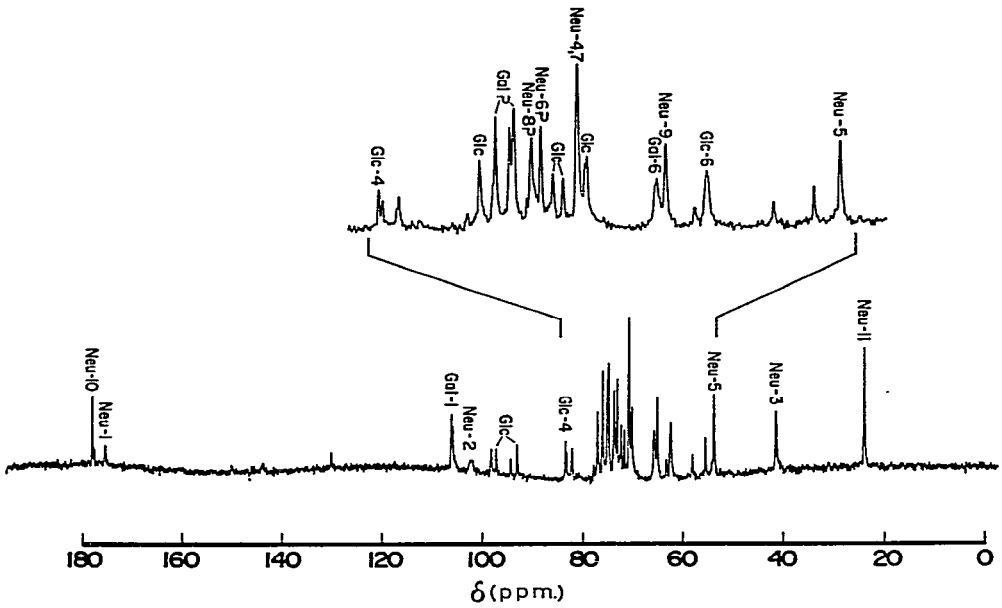

Fig. 2. Proton-decoupled, ^{13}C-n.m.r. spectrum of an aqueous solution of *O*-(*N*-acetyl-α-neuraminic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose adjusted to pD 7.0 with NaOH.

The ^{13}C-n.m.r. spectra of the α-(2→3) and α-(2→6) isomers are shown in Figs. 1 and 2. The unassigned lines are attributed to impurities identified by their decrease in intensity with successive purification steps. The positive identification of the spectrum in Fig. 2 as that of the α-(2→6) isomer is demonstrated by the C-6 resonance of galactopyranose, which is well defined at 64.6 p.p.m., compared to 61.2 p.p.m. for the α-(2→3) isomer.

Table I gives a comparison of the T_1 values measured for the two isomers, the α-(2→3) data being of those Czarniecki and Thornton[8]. It should be particularly noted that the T_1 value for C-6 of the galactopyranosyl residue decreased from 0.14 s for the α-(2→3) isomer to 0.08 s for the α-(2→6) isomer, as would be expected for the more restricted motion of the glycosidically linked carbon atom in the latter.

The T_1 values are, in general, very similar for the two isomers, indicating that, in solution, there is, in their conformations, no great difference that might affect their internal motions. As observed for the α-(2→3) isomer[8], the *N*-acetylneuraminic acid ring appears to be the least mobile, presumably because of the anchoring character of the charged carboxyl group. The observation of α and β anomers permits the assignment of some of the ^{13}C signals in the 65–80-p.p.m. range. [The assignment[8] of the 78.5-p.p.m. line in the α-(2→3) spectrum to the D-galactopyranosyl residue appears to be incorrect, as our spectrum for it definitely shows the glucopyranose α- and β-anomeric signals to be separated by 0.12 p.p.m.]. Inability to resolve, and assign, all of the galactopyranose and glucopyranose peaks makes any decision that the motion increases along the carbohydrate chains towards glucopyranose difficult.

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS (δ_C), ASSIGNMENTS, AND T_1 VALUES FOR TWO ISOMERS OF *N*-ACETYL-NEURAMINYL-LACTOSE

Carbon atom	*α-(2→3) Isomer*[a]		*α-(2→6) Isomer*	
	δ_C *(p.p.m.)*[b]	T_1 *(s)*	δ_C *(p.p.m.)*[b]	T_1 *(s)*
Neu-10	175.2	[c]	176.16	[c]
Neu-1	174.0	[c]	174.73	[c]
Gal-1	102.8	0.16	104.70	0.16
Neu-2	100.0	[c]	101.52	[c]
Glc-1β	95.9	0.24	96.85	0.18
Glc-1α	92.0	0.11	93.01	0.11
Gal	78.5[d]	0.17		
Glc-α			76.44	0.23
Glc-β			75.81	0.18
Glc	75.6	0.20		
Gal	75.3	0.18		
			74.91	0.14
Glc	74.9	0.20		
Glc	74.5	0.24		
Glc	74.0			
			73.74[d]	0.14
Gal	73.0	0.18		
			72.95	0.13
Glc			72.17	—[e]
			71.97	0.14
Neu	71.9	0.16		
Glc	71.4	0.27	71.21	0.15
Glc	70.2	0.23	70.54	0.21
	69.5[f]	0.19	69.62[g]	0.14
Neu	68.4[f]	0.12		
Neu	67.7	0.14		
Neu-9	63.0	0.13	63.88	0.13
Gal-6	61.2	0.14	64.57	0.08
Glc-6[h]	60.4	0.09	61.40	0.10
Neu-5	52.0	0.13	53.10	0.14
Neu-3	39.8	0.05	41.28	0.08
Neu-11	22.4	0.75	23.28	0.81

[a]From ref. 8, Table III. [b]Relative to Me_4Si. [c]T_1 too long to be measured. [d]This assignment is dubious, see text. [e]Could not resolve. [f]Integrates to two carbon atoms. [g]Integrates to three carbon atoms. [h]Unresolved anomers.

but it does appear that the glucopyranose T_1 values are generally higher than the remaining resonances, attributed to the galactopyranosyl residue. As for the α-(2→3) isomer, the glucopyranose C-1β atom has a longer T_1 value than the C-1α atom, suggesting segmental, anisotropic motion along the molecular length.

Only small, induced chemical-shifts were observed when Ca^{2+} was added to either of these two trisaccharide solutions, indicating that no exceptional complexing occurred *via* changes in tertiary structure.

EXPERIMENTAL

Preparation of *N*-acetylneuraminyl-lactose, and separation of the two isomers, followed the procedures of Schneir and Rafelson[9]. Total, bound, and free *N*-acetylneuraminic acid were determined by the Ehrlich reaction[10], by the periodate–resorcinol method of Jourdian *et al.*[11], and by the thiobarbituric acid method[12], respectively. ^{13}C-N.m.r. spectra and spin–lattice relaxation-times were measured with a Varian XL-100 n.m.r. spectrometer by the usual procedures[6,8].

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