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INVESTIGATION OF THE CONFORMATION IN SOLUTION OF SOME DERIVATIVES OF *N*-ACETYLNEURAMINO-1,4-LACTONE DIETHYL DITHIOACETAL BY ONE- AND TWO-DIMENSIONAL N.M.R. TECHNIQUES

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ABSTRACT

A series of derivatives of *N*-acetylneuramino-1,4-lactone diethyl dithioacetal has been investigated by n.m.r. spectroscopic techniques. The complete assignment of the ^{1}H and ^{13}C resonances was achieved by measurements of relaxation times, selective decoupling experiments, and two-dimensional shift-correlation spectroscopy. The conformational analysis of the acetylated derivative was based on $J_{H,H}$ values and nuclear Overhauser effects using the assignments of all the acetate groups. The influence of different substituents on the conformational behaviour is discussed.

INTRODUCTION

Structural transformations on *N*-acetylneuraminic acid (Neu5Ac) are of biological importance[1,2]; although many regio- and stereo-selective variations (especially for pyranoid derivatives[3,4]) have been reported, no detailed studies of furanoid derivatives[5,6] have been described. The *N*-acetylneuramino-1,4-lactone diethyl dithioacetal derivatives[7] that we used in our investigation[8] show a significant structural change, which may lead to new strategies of transformations of sialic acids.

N.m.r. spectroscopy has been widely used in carbohydrate chemistry[9–11], including two-dimensional (2D) techniques and measurements of relaxation times[12–16]. We have used homonuclear decoupling experiments, measurements of relaxation times for ^{1}H and ^{13}C, and 2D-shift correlations to assign ^{1}H and ^{13}C signals.

DISCUSSION

The derivatives **1–6** of *N*-acetylneuramino-1,4-lactone diethyl dithioacetal were investigated, and the assignments of ^{1}H and ^{13}C resonances are given in Tables I and II.

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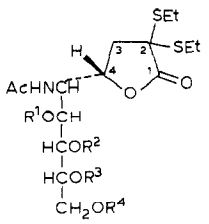


Compound	R^1	R^2	R^3	R^4
1	H	H	H	H
2	H	H	H	^{t}BuMe$_2$Si
3	Ac	Ac	Ac	Ac
4	Ac	Ac	Ac	^{t}BuMe$_2$Si
5	Ac	Ac	^{t}BuMe$_2$Si	^{t}BuMe$_2$Si
6	—CO—		Ac	^{t}BuMe$_2$Si

The ^{1}H signals for the tetra-acetate **3** were well resolved. The assignment of the resonances started with the low-field signal for NH. Homonuclear decoupling experiments allowed localisation of all the resonances for the side chain and the lactone ring; H-8,9,9′ gave rise to a typical ABX-pattern, as did H-3,3′,4. The $J_{4,5}$ value was rather small, due to a dihedral angle of ~90°, which led to broadening of the resonances. The ethylthio groups gave resonances at 2.50–2.89 (CH_2) and 1.23–1.25 p.p.m. (t, CH_3). The differences in the chemical shifts of the signals of the four OAc groups were rather small and the signal at highest field was assigned to NAc. In order to assign the signals for the OAc groups, a T_1-experiment was performed and the data are compiled in Table I. The differences between the T_1 values are rather small, which complicates the interpretation of the results; therefore, the ^{13}C relaxation times (see Table II) were determined, showing also a certain sequence. The preliminary assignments based on the two sets of T_1 data were inter-related by a 2D-^{13}C-^{1}H-shift correlation experiment as shown in Fig. 1. The sequence of the proton relaxation times correlates well with the data taken from the ^{13}C series. The shift correlation spectrum in Fig. 2 gives the complete assignment of all the protonated carbon atoms.

The relaxation data for the side-chain carbon atoms showed increasing values on going from C-5 to C-9 and confirmed the conclusions regarding the assignment of the acetates. The side chain of *N*-acetylneuraminic acid has the same configuration as mannose. Comparison of the literature data[17] for acetylated mannose diethyl dithioacetal with the proton shifts for **3** showed a good agreement for H-7,8,9,9′. Differences in chemical shifts were observed for the other hydrogen atoms, which can be understood in terms of the influence of substituents. The coupling constants for **3** also agreed well with those of acetylated mannose diethyl dithioacetal in the same region. Deviations were observed for H-4,5,6 (see Table III).

The small value of $J_{4,5}$ reflects the strong influence of the γ-lactone ring on the conformation. Additionally, i.r. data indicated that the amide moiety formed

TABLE I

^{1}H-N.M.R. DATA (CHEMICAL SHIFTS, p.p.m.) FOR SOLUTIONS IN $CDCl_3$ (INTERNAL Me_4Si) FOR **3–6**[a]

Atom/group	**3**		**4**		**5**		**6**	
H-3	2.36/2.43	(0.29)	2.35/2.43	(0.32)	2.33/2.46	(0.33)	2.44/2.49	(0.35)
H-4	4.59	(0.69)	4.61	(0.78)	4.71	(0.75)	5.01	(0.74)
H-5	4.36	(0.62)	4.36	(0.73)	4.46	(0.67)	4.35	(0.66)
H-6	5.43	(0.61)	5.41	(0.74)	5.33	(0.61)	4.95	(0.82)
H-7	5.38	(0.60)	5.38	(0.68)	5.17	(0.61)	4.64	(0.73)
H-8	5.06	(0.87)	4.96	(0.92)	3.94	(0.61)	5.00	—
H-9	4.00/4.23	(0.33)	3.69/3.57	(0.36)	3.56/3.69	(0.32)	3.79/3.99	(0.33)
NH	5.88	(0.43)	5.72	(0.52)	5.90	(0.53)	6.07	(0.53)
NAc	2.00	(0.63)	1.99	(0.70)	1.97	(0.68)	2.10	(0.62)
OAc	2.11 Ac-6	(0.70)	2.14 Ac-6,7	(0.80)	2.10 Ac-6,7	(0.72)	2.12	(0.75)
	2.15 Ac-7	(0.72)	2.09 Ac-6,7	(0.79)	2.09 Ac-6,7	(0.71)		
	2.07 Ac-8	(0.75)	2.04 Ac-8	(0.82)				
	2.05 Ac-9	(0.87)						
Silyl ^{t}Bu			0.87	(0.94)	0.90 C-9	(0.87)	0.93	(0.74)
					0.87 C-8	(0.64)		
Me			0.02	(1.80)	0.09/0.06	C-8 (1.18,1.13)	0.13/0.17	(1.24,1.30)
					0.09/0.06	C-9 (1.59,1.49)		
S-Et CH_2	2.50–2.89		2.52–2.90	(0.92)	2.56–2.94		2.56–2.94	(0.80–0.82)
CH_3	1.25/1.23	(1.16)	1.24/1.23	(1.35)	1.22/1.23	(1.35,1.32)	1.25/1.27	(1.13,1.10)

[a]The values in parenthesis are the corresponding relaxation times (s). For the acetates, T_1-values of the broader methyl-group component are given. Line splitting was observed only during a 40–50-ms period (for details, see ref. 18).

TABLE II

^{13}C-N M R DATA (CHEMICAL SHIFTS, p.p.m.) FOR SOLUTIONS IN $CDCl_3$ (INTERNAL Me_4Si) FOR **1–6**

Atom/group	**1**	**2**	**3**	**4**	**5**	**6**	*^{13}C-T_1-values (s) of* **3**
C-2	58.32	58.36	58.00	58.00	57.76	57.95	—
C-3	41.25	41.48	41.32	41.40	41.82	41.30	0.21
C-4	76.40	76.27	74.86	74.89	74.98	74.84	0.34
C-5	51.49	51.44	49.10	49.08	50.52	51.69	0.31
C-6	71.39*	70.42*	69.32	69.32	69.84	75.06	0.33
C-7	69.21*	69.63	68.44	68.64	71.11	78.36	0.35
C-8	70.36*	69.02*	68.54	70.91	72.78	72.37	0.38
C-9	64.41	64.18	62.02	61.69	63.77	60.67	0.26
NHAc (Me)	22.76	22.63	22.80	22.88	23.03	22.82	1.07
S-Et CH_3	13.85/13.85	13.67/13.84	13.58/13.33	13.58/13.32	13.63/13.42	13.67/13.38	—
CH_2	24.92/24.36	24.85/24.25	24.70/23.83	24.68/23.79	24.71/23.93	24.78/23.97	0.64/0.71
OAc (Me)			20.48 (C-6)	20.59 (C-6,7)	20.92 (C-6,7)	20.62	1.03
			20.44 (C-7)	20.63 (C-6,7)	20.78 (C-6,7)		1.07
			20.58 (C-8)	20.80 (C-8)			1.11
			20.36 (C-9)				1.37
Carbonyl	173.41	173.37	171.58	171.56	171.36	171.49	
	173.24	172.82	170.96	170.83	170.33	171.38	
			170.28	169.73	169.95	169.53	
			169.73	169.60	169.79	153.19 (carbonate)	
			169.68	169.52			
			169.50				
Silyl ^{t}Bu C_q		18.28		18.00	18.15/18.06	18.21	
Me		25.92		25.56	25.82/25.72	25.70	
Me		−5.34/−5.42		−5.73	−4.75/−4.91	−5.54/−5.62	
					−5.54/−5.61		

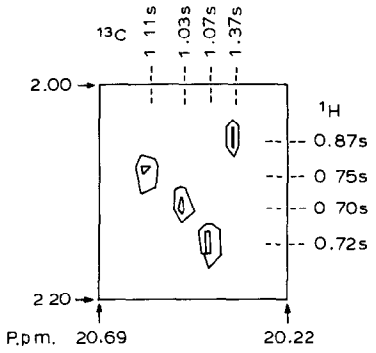


Fig. 1. 2D-^{13}C-^{1}H-shift correlation spectrum of **3**, showing the region for OAc groups; ^{1}H and ^{13}C relaxation times are given and the assignment of the acetate groups.

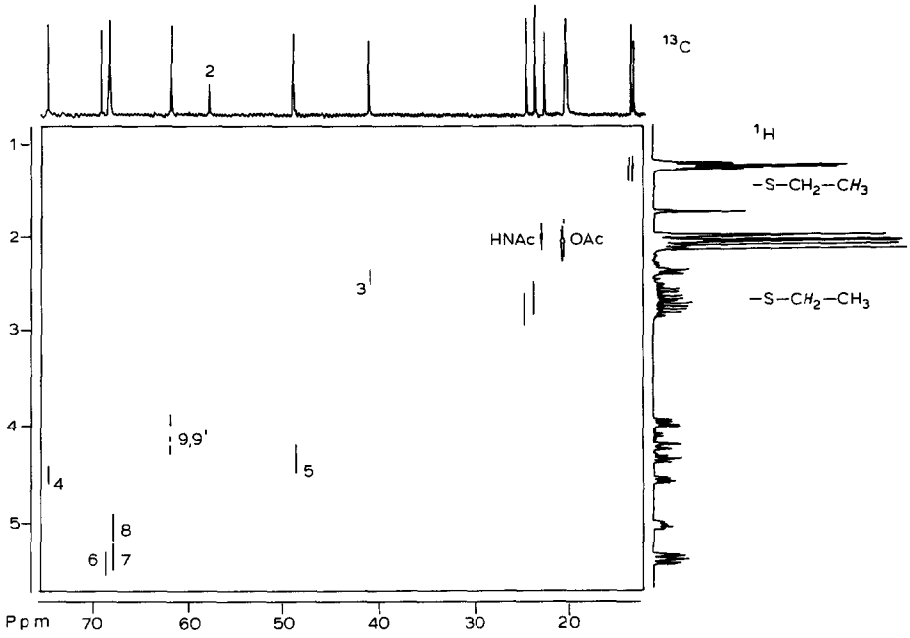


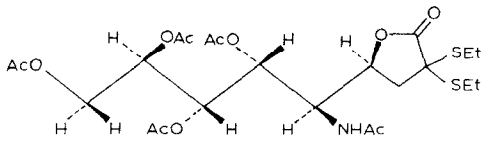
Fig. 2. 2D-^{13}C-^{1}H-shift correlation spectrum of **3**.

intermolecular hydrogen-bonds at higher concentrations. Conformational analysis of the acetylated mannose diethyl dithioacetal was based mainly on application of the Karplus equation and avoidance of parallel 1,3-interactions[17]. N.O.e. difference experiments were performed on the acetates in order to obtain further insight into the conformational behaviour of **3** in chloroform solution. Starting from the resonance for AcO-6, that for H-5 can be found; from AcO-7, difference signals at the positions of the resonances of H-4, H-6, and H-8 were observed, and AcO-8 gave rise to a n.O.e. at H-6 and H-8. These data can be explained by a zigzag

TABLE III

$J_{H,H}$ VALUES (Hz, ±0.2 Hz) FOR **3–6**

J	3	4	5	6
3,3′	−13.7	−14.0	−14.0	—
3,4	9.0	9.3	9.7	—
3′,4	7 0	6.7	6.3	—
4,5	<0.5	<0.5	0.9	<0.5
5,NH	10.1	10.0	10.0	9.8
5,6	9.7	9.5	7.2	10.2
6,7	2.1	—	2.8	4.2
7,8	8.7	9.0	5.2	2.4
8,9	5.5	5.5	6.6	5.3
8,9′	3.0	4.0	5.0	3 0
9,9′	−12.5	−11.3	−10.7	−11 9



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conformation of the backbone (**1**), as described for the acetylated mannose diethyl dithioacetal, assuming a greater rotameric flexibility around the C-6–C-7 bond. This assumption accords with the observed $J_{6,7}$ value of 1.7 Hz.

Selective silylation at position 9 gives **4**, and the remaining proton signals are nearly unchanged with only the signals of both H-9,9′ shifted to higher field, as expected. Differentiation of AcO-6 and AcO-7 could not be achieved. Further silylation gives **5** with the silyl groups at positions 8 and 9. This can also be proved by ^{1}H-n.m.r. data. Comparing **3–5**, a change of the vicinal coupling constants involving H-8 is shown, revealing the influence of the bulky substituents on the conformation of the side chain. The structure of the cyclic 6,7-carbonate **6** was also deduced from the ^{1}H-n.m.r. data, the couplings involving H-7 reflecting the effect of ring closure. The ^{13}C resonances of **4–6** were assigned on the basis of selective decoupling experiments, using the known proton assignment and comparison with the data for **3**. The values of the chemical shifts of the signals of the carbon atoms of the lactone ring remain nearly unchanged throughout the whole series, except for that of C-4, which shows the influence of the free hydroxyl groups in **1** and **2**. The resonance of C-4 in **3–5** is readily identified because of its characteristic shift range at lowest field within the sp^3-region. The signals for C-5 and C-9 can be assigned on the basis of their multiplicities as given from *J*-modulated ^{13}C-spectra. The signals of C-6/C-9 reveal the influence of the substituent silyl groups and the cyclic carbonate, respectively, confirming the conclusions drawn from the ^{1}H-n.m.r. data. The ring closure in **6** shifts the signals for C-6 and C-7 strongly downfield (5.7

and 9.7 p.p.m., respectively, in comparison with **4**). The ^{13}C-data for **1** and **2** are also given in Table II, in order to support the assignments for **3–6**. The ^{1}H spectrum of a solution of **1** in $CDCl_3$ shows broad lines for all hydrogen atoms. After the addition of 20% of CD_3OD, the resonances became sharp, which supports the i.r. data on hydrogen bonds. The same effect can be observed in the ^{13}C-n.m.r. spectrum.

EXPERIMENTAL

The preparation and purification of the compounds used in this study have been described[8]. N.m.r. spectra were recorded with a Bruker WM-250 spectrometer equipped with an 80K ASPECT-2000 computer running the DISNMRP programme. The deuterium resonance of the solvent provided the field-frequency lock. All measurements were performed using 5-mm tubes. Typical parameters were as follows.

^{1}H: SF, 250.13 MHz; SI 16k; SW, 2500 Hz; PW, 1 μs (15°); AQ, 3.27 s; NS, 80–240; temperature, 297 K; concentration, 4–5 mg/mL, $CDCl_3$. N.O.e. and relaxation-time measurements were performed on degassed samples, using freeze–pump–thaw cycles. Relaxation-time measurements: recycle delay, 15 s; PW(90°), 5.9 μs. The inversion–recovery pulse sequence was used. N.O.e. measurements: D_1, 3 s; DP, 45L; direct accumulation of difference signals using an appropriate microprogramme.

^{13}C, *J*-modulated spectra were recorded using the pulse sequence $D_1(S_1,BB)$–$D_2(S_2,DO)$–90°(^{13}C)–D_3–180°(^{13}C,BB)–D_3–acquisition: SF, 62.9 MHz; SI, 32k; SW, 16,000 Hz; AQ 1.0 s; PW, 17 μs(90°); DP, 6H/12H(2W,0.5W); D_3, 7.1 ms (= $1/J$ for J = 141 Hz); NS, 1000–6000; recycle delay, 3.5 s; temperature, 303 K; concentration, 30–80 mg/mL, $CDCl_3$.

2D-^{1}H-^{13}C-shift correlation: the pulse sequence used was D_1–90°(^{1}H)–$t_1/2$–180°(^{13}C)–$t_1/2$–D_3–90°(^{1}H),90°(^{13}C)–D_4–acquisition(BB); SF, 62.9 MHz; SI, 4k × 128; SW_1, 1250 Hz, SW_2, 4000 Hz; PW(90°,^{1}H), 17.0 μs; PW(90°,^{13}C), 16.6 μs; AQ, 0.51 s; NS, 240; recycle delay, 4 s; tmperature, 303 K; concentration, 70 mg/mL, $CDCl_3$; D_3 3.5 ms (= $1/2J$); D_4, 1.75 ms (= $1/4J$ for J = 143 Hz, refocusing of all multiplicities). In both dimensions, quadrature detection was applied by appropriate phase cycling. Fourier transformation was done after zero-filling to 4k × 256.

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