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Note

Application of ^{13}C-n.m.r. spectroscopy in the structural study of complex hetero-oligosaccharides

ALEXANDER S. SHASHKOV, NIKOLAY P. ARBATSKY, VARVARA A. DEREVITSKAYA, AND NIKOLAY K. KOCHETKOV

N. D. Zelinsky Institute of Organic Chemistry, Academy of Sciences of U.S.S.R., Leninsky Prospect 47 117334, Moscow (U.S.S.R.)

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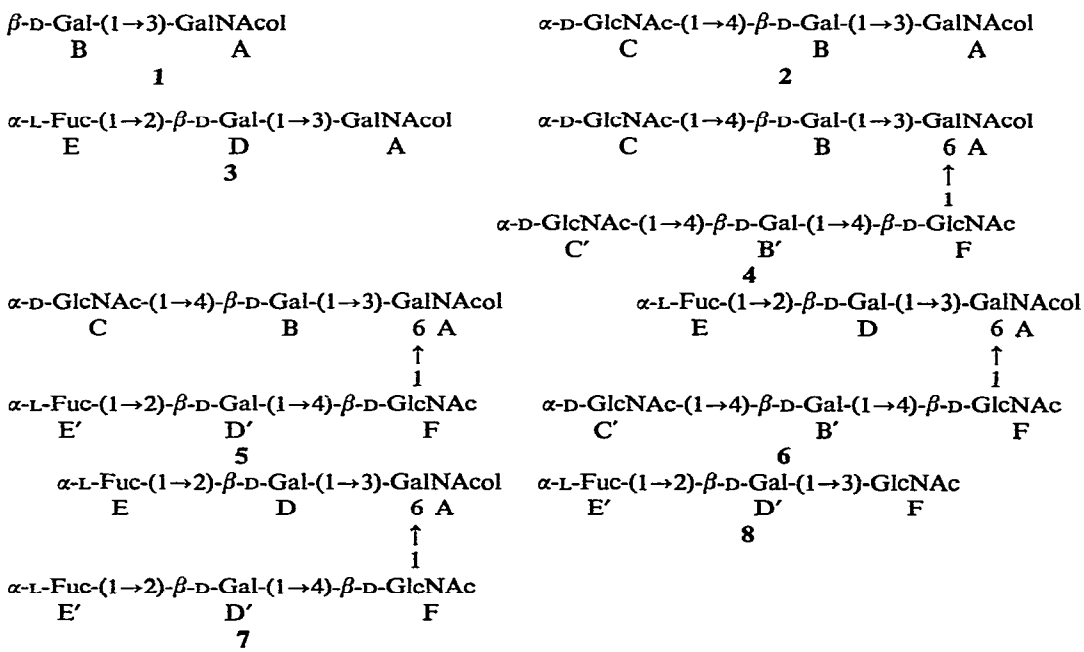
^{13}C-N.m.r. spectroscopy has been applied successfully to homo-oligosaccharides and homo-polysaccharides[1]. For complex hetero-oligosaccharides, the method is limited by the difficulties in obtaining suitable compounds and in the interpretation of the spectra.

We now report a ^{13}C-n.m.r. study of the series of hetero-oligosaccharides **1–7** (all sugar units are pyranoid) isolated from blood-group substance H, the structures of which have been established[2,3]. The ^{13}C-n.m.r. spectra of the synthetic trisaccharide[4] **8** and 2-acetamido-2-deoxy-D-galactitol (**9**) have also been recorded.



The structures of **2–8** are formed by different combinations of the fragments C-B-A, E-D-A, C′-B′-F, and E′-D′-F (see formulae). Therefore, the basis for interpretation of their spectra was reciprocal analysis, together with the use of the known spectroscopic data for the corresponding model methyl glycosides[5,6] and oligosaccharides[7].

For example, the structure of **2** (C-B-A) was elucidated completely from its spectrum. The presence of 22 resonances indicated three monomeric residues, two

TABLE I

THE CHEMICAL SHIFTS[a] FOR THE CARBON ATOMS OF THE OLIGOSACCHARIDES **1–9**

Compound	*Unit*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CO*	*CH_3CO*
9	A	62.3	52.25	69.25	70.6	70.2	63.9	175.0	22.45
1	A	61.2	52.0	77.0	69.9	69.8	63.5	175.0	22.45
	B	104.4	71.7	73.1	69.1	75.6	61.6		
2	A	61.25	52.1	77.1	70.2	69.8	63.4	175.0	22.6
	B	104.9	70.4	72.65	77.6	76.1	61.05		
	C	98.6	54.75	71.6	71.0	72.65	60.8	175.4	22.6
3	A	61.05	52.2	75.3	70.1	69.6	63.4	175.1	22.9
	D	102.9	79.9	72.5	69.3	75.6	61.6		
	E	101.9	70.1	70.3	72.9	69.1	16.2		
4	A	61.0	52.1	77.0	71.1	69.6	68.2	174.8	22.6
	B	105.1	70.4	72.7	77.75	76.1	61.0		
	C	98.6	54.7	71.5	71.2	72.7	60.9	175.3	22.7
	F	101.9	55.95	73.3	79.5	75.5	60.9	175.3	23.05
	B′	104.0	70.4	72.7	77.3	76.35	61.0		
	C′	98.9	54.7	71.5	71.2	72.7	60.9	175.0	22.7
5	A	61.1	52.1	77.0	71.5	69.5	68.2	174.8	22.6
	B	105.0	70.35	72.65	77.75	76.0	61.1		
	C	98.5	54.7	71.5	71.0	72.65	60.9	175.3	22.6
	F	102.0	55.9	74.2	77.0	76.0	60.9	175.3	23.0
	D′	101.0	77.0	72.35	68.9	76.0	61.8		
	E′	100.1	69.8	70.35	73.1	67.85	16.0		
6	A	61.05	52.25	75.1	71.6	69.7	68.5	175.0	22.5
	D	102.95	80.1	72.7	69.2	75.6	61.7		
	E	101.9	70.0	70.3	73.0	69.1	16.1		
	F	102.2	56.0	73.3	79.6	75.5	60.75	175.0	23.05
	B′	104.0	70.3	72.7	77.4	76.4	61.05		
	C′	98.9	54.7	71.4	71.2	72.7	60.75	175.0	23.05
7	A	60.9	52.1	75.1	71.6	69.7	68.5	175.1	22.7
	D	102.8	79.9	72.5	69.0	75.6	61.5		
	E	101.8	70.0	70.3	73.0	69.1	16.1		
	F	102.2	55.9	74.2	77.1	75.6	60.9	175.1	22.9
	D′	101.0	77.1	72.35	69.0	75.9	61.5		
	E′	100.0	69.7	70.3	73.0	67.5	15.9		
8	F[b]	91.9	54.2	72.5	69.5	75.9	61.2	175.2	22.6
	D′	101.4	77.3	72.45	69.1	75.9	61.8		
	E′	100.1	70.0	70.35	73.3	67.3	15.9		

[a]For solutions in D_2O (internal Me_2SO; 39.45 p.p.m. from Me_4Si). [b]The chemical shifts are given for the α anomer of the reducing end.

of which are cyclic (two anomeric signals), one being 2-acetamido-2-deoxyglucose (signals at 22.6, 54.8, and 175.4 p.p.m.). The presence of four signals in the region 61–64 p.p.m. corresponding to the carbon atoms of CH_2OH groups[8], as well as the signal at 52.1 p.p.m., indicates the third residue to be a 2-acetamido-2-deoxyhexitol. The downfield shift (~8 p.p.m.) of the signals for C-3 and C-4 of the 2-acetamido-2-deoxyhexitol and galactose residues, respectively, in comparison with those in the spectra of **9** and **2**, indicates the positions of substitution in units A and B. Two signals (98.6 and 104.9 p.p.m.) in the resonance region of C-1 coincide with those of C-1 for methyl 2-acetamido-2-deoxy-α-D-glucopyranoside[6] and methyl β-D-galactopyranoside[5]. The full data are presented in Table I.

The correctness of the signal attributions is confirmed by the fact that oligosaccharides possessing identical fragments mentioned above exhibit similar sets of signals corresponding to these fragments. The chemical shifts of the signals due to fragments C-B and C′-B′ are completely or almost identical. The fragments E-D and E′-D′ differ in the position of signals of C-1,5 in units E and E′, and C-1,2 in units D and D′, respectively. The chemical shifts of the signals of fragment E-D are different from those expected on the basis of the literature data[5], possibly because these fragments are attached to units A or F which differ in their chemical and stereochemical properties.

With one exception, the ^{13}C-n.m.r. data of the oligosaccharides confirmed their structures. It was found that both of the terminal 2-acetamido-2-deoxyglucose residues (C and C′), not just one of them (C, as supposed earlier[3]), have the α configuration; the $[\alpha]_D$ values support this conclusion.

The results reported herein demonstrate the potential of ^{13}C-n.m.r. spectroscopy in structural studies of complex hetero-oligosaccharides.

EXPERIMENTAL

Oligosaccharides **1–8** were obtained as described previously[2–4]. ^{13}C-N.m.r. spectra were recorded at 15.08 MHz with a Bruker WP-60 instrument at ambient temperature and with a deuterium lock; Me_2SO was used as the internal standard for solutions of oligosaccharides in D_2O (30–120 mg/ml). The shift difference of Me_2SO versus Me_4Si (39.5 p.p.m.) was confirmed in a separate experiment. Proton-decoupled FT-spectra (100 Hz/cm) were measured by using a repetition time of 1.1 sec, a pulse width of 5.5 μsec (~45°), and an accumulation of 30,000–120,000.

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