^{13}C-NMR spectra of some aldobiouronic acid derivatives

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^{13}C-N.m.r. spectra of five aldobiouronic acid derivatives have been measured. The spectra show effects of substituents and glycosidic linkages upon chemical shifts of ^{13}C-n.m.r. signals.

Были измерены спектры ^{13}C-ЯМР 5-ти производных альдобиуроновых кислот. На основании их анализа обсуждается влияние заместителей и гликозидных связей на химический сдвиг сигналов ^{13}C-ЯМР.

^{13}C-N.m.r. studies on di- and oligosaccharides are important from the point of view of application of thus obtained results in the determination of the type and the site of linkages in polysaccharides. ^{1}H-N.m.r. spectra of di- and oligosaccharides measured with spectrometers working at up to 100 MHz are commonly very complex and complete proton-signal assignments are impossible. As far as ^{13}C-n.m.r. spectra of this class substances are concerned, since the selective proton-decoupling method can only be used to a limited extent, signal assignments are possible only in cases when data obtained with model compounds are available.

There are works in the literature [1—3] describing the use of ^{13}C-n.m.r. spectroscopy in the determination of anomeric configuration of di- and oligosaccharides, but works on aldobiouronic acids are scarce.

The assignment of ^{13}C-n.m.r. signals to individual carbon atoms in oligosaccharides has been accomplished applying rules for monosaccharides [2] the validity of which has been extended [4] also to di- and oligosaccharides.

The objective of the present work was to interpret ^{13}C-n.m.r. spectra of some β-linked aldobiouronic acid derivatives containing acetylated and methylated glucose units, with the aim to apply the obtained results in studies on oligo- and polysaccharides composed of the same basic units. Spectra of the following substances have been measured: methyl 3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D--glucopyranosyluronate)-2,4,6-tri-*O*-methyl-α-D-glucopyranoside (*I*); methyl 4--*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-2,3,6-tri-*O*-methyl--α-D-glucopyranoside (*II*); methyl 6-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-gluco-

pyranosyluronate)-2,3,4-*O*-methyl-α-D-glucopyranoside (*III*); methyl 2-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-3-*O*-methyl-α-D-glucopyranoside (*IV*); 6-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-1,2:3,4-di-*O*-isopropylidene-α-D-galactose (*V*).

Unequivocal assignments (Table 1) could be done to signals for C-1 and C-1′ and, thus, it was possible to assign anomeric configuration of reducing and

Table 1

^{13}C-NMR chemical shifts (p.p.m.) found in the spectra of aldobiouronic acid derivatives

	Compound				
	I	*II*	*III*	*IV*	*V*
C-1	97.20	97.85	97.98	99.41	97.07
C-2	82.51	82.32	82.64	81.47	71.27
C-3	78.87	82.71	84.27	82.12	71.79
C-4	80.89	79.33	80.11	72.12	71.53
C-5	70.82	71.60	72.05	70.10	71.27
C-6	72.31	70.43	70.56	61.98	68.48
Me-1	55.03	55.16	55.09	55.48	—
Me-2	58.02	58.21	58.15	—	—
Me-3	—	60.81	60.68	61.33	—
Me-4	60.55	—	60.42	—	—
Me-6	59.12	59.38	—	—	—
Me-6′	52.82	52.88	52.88	52.82	52.88
C-1′	101.61	101.35	101.68	101.81	102.26
C-2′	72.90	72.90	72.90	71.40	72.90
C-3′	70.82	71.60	72.05	70.62	71.92
C-4′	72.57	72.57	72.77	70.82	72.64
C-5′	70.69	70.62	69.91	69.19	70.56

nonreducing sugar units. Further, signals C-2—C-5 of both pyranoid rings, signal C-6 (by off-resonance decoupling) and that of O—CH_3 groups could also be assigned. ^{13}C-N.m.r. signals of C=O groups appear at the lowest magnetic field (170.23—168.21 p.p.m.) and their assignment to methoxycarbonyl and acetyl groups is impossible without having compounds synthesized using ^{13}C-enriched reagents. The same holds for CH_3 signals of acetyl groups (appearing at 20.33—20.72 p.p.m.), but these are not important for structural studies of similar type of substances.

^{13}C-N.m.r. chemical shifts observed for C-1 of the reducing units (97.20—99.41 p.p.m.) are comparable to those of monomeric methyl glycoside [5]

and confirm the α configuration of the anomeric carbon atom bearing the aglycon. A larger chemical shift was observed (Table 1) with the 1→2-linked compound *IV*. Here, the β effect resulting from the glycosidic linkage is more pronounced than with substances bearing at C-2 a methoxyl group. ^{13}C-N.m.r. chemical shifts for C-1 of the nonreducing end-unit appear at 101.35—102.26 p.p.m. These values differ from chemical shifts found for C-1 of methyl β-D-glucopyranoside (104.30 p.p.m.) [6]. However, the signal for C-1 of methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside appears at 101.6 p.p.m. [7] demonstrating the shielding β effect of the acetyl group.

Table 2

Differences (Δ p.p.m.) between ^{13}C signals in methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside and those of reducing ring involved in the glycosidic linkage (compounds *I*—*IV*)

C atom	C atom in the reducing end-unit	C atom in per-*O*-methyl-α-D-glucose	Type of glycosidic linkage	Δ p.p.m.
C-2	81.47	82.58	$\beta(1\rightarrow2)$	−1.11
C-3	78.87	84.28	$\beta(1\rightarrow3)$	−5.41
C-4	79.33	80.61	$\beta(1\rightarrow4)$	−1.28
C-5	70.56	72.41	$\beta(1\rightarrow6)$	−1.85

Table 2 shows the differences between chemical shifts of skeletal C atom signals found [5] in the spectrum of methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside and the corresponding signals in the reducing sugar unit for C atoms involved in the glycosidic linkage. These differences result from the sterical shielding effects, the magnitude of which depends upon the type and configuration of the glycosidic linkage. These phenomena should be taken into account when methylated monosaccharides are used as models in ^{13}C-n.m.r. studies of di- and oligosaccharides.

Assignment of signals for C-2′, C-3′, and C-4′ bearing acetyl groups was based on comparison with spectra of methyl tetra-*O*-acetyl-β-D-glucopyranoside [1, 7]. Unambiguous assignment of signals of methoxyl groups (Table 1) on the reducing unit was done on the basis of four types of glycosidic linkages present in the studied substances.

Experimental

^{13}C-N.m.r. spectra for solutions in chloroform-d were measured at 25°C in a noise decoupled and off-resonance decoupled mode, with a Jeol FX-60 spectrometer, using tetramethylsilane as the internal standard. The following parameters were applied: pulse

interval 0.5 s, pulse width 4 μs (45° flip angle), sweep width 4000 Hz, and 8 K real data points. The average number of accumulations was 1500 and 5000 for noise and off-resonance decoupled spectra, respectively. Chemical shifts (Table 1) are given with respect to tetramethylsilane. Compounds *I*—*V* were prepared as described [8, 9].

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