^{13}C NMR spectra of 2-*O*-β-D-glucopyranosylurono-D-manno-pyranose and 6-*O*-β-D-glucopyranosylurono-D-galactopyranose

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The structures of two aldobiouronic acids, 2-*O*-β-D-glucopyranosylurono--D-mannopyranose and 6-*O*-β-D-glucopyranosylurono-D-galactopyranose, have been confirmed by interpretation of their ^{13}C NMR spectra.

С использованием ^{13}C ЯМР спектров подтверждены структуры двух альдобиуроновых кислот, 2-*O*-β-D-глюкопиранозилуроно-D-маннопиранозы и 6-*O*-β-D-глюкопиранозилуроно-D-галактопиранозы.

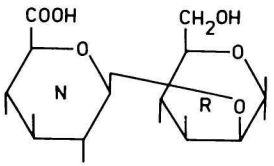
Determination of the types and configurations of glycosidic bonds between the sugar residues in oligo- and polysaccharides is a pretentious task. Elucidation of these problems is usually achieved by combination of laborious chemical methods. ^{13}C NMR spectroscopy is a powerful tool that can give the same information about the structures of the above-mentioned compounds as much more tedious chemical methods [1, 2]. ^{13}C NMR spectra of polysaccharides may be interpreted on the basis of the spectra of simple relevant model compounds, *e.g.* mono-*O*-methyl derivatives of the corresponding methyl glycosides [3, 4] or other carbohydrate alkyl ethers [5]. However, oligosaccharides structurally related to the polysaccharide are much more valuable models.

In paper [6] we described the structural features of the polysaccharide obtained from apricot-tree gum, *Prunus armeniaca* L. There we made use of information about two low-molecular fragments, 2-*O*-β-D-glucopyranosylurono-D-mannose and 6-*O*-β-D-glucopyranosylurono-D-galactose, which were isolated from partial hydrolyzate of the polysaccharide and identified by chemical and physicochemical methods available at that time [7—9]. Identification of the anomeric nature of glycosidic bonds on the basis of different absorptions of compounds with different configuration in the IR region or on the basis of their specific optical rotations is considered to be insufficient. Recently, ^{13}C NMR spectroscopy has been extensively used for elucidation of types and configurations of glycosidic bonds in oligo- and polysaccharides, therefore, it seemed reasonable to apply this method also in the case of the title compounds.

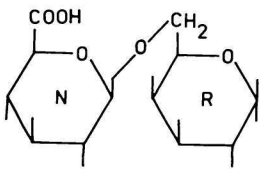
The present paper deals with interpretation of the ^{13}C NMR spectra of two aldobiouronic acids, 2-*O*-β-D-glucopyranosylurono-D-mannose and 6-*O*-β-D--glucopyranosylurono-D-galactose, not described in the literature so far.

Experimental

Aldobiouronic acids *I* and *II* (Formula 1) were isolated from partial hydrolyzate of the apricot-tree gum polysaccharide [6].



I



II

Proton-decoupled ^{13}C NMR spectra of the compounds were recorded with a Bruker-Physik WP-60 FT spectrometer at 15.08 MHz. The compounds were examined as 5 % solutions in D_2O at 30 °C, acquisition time = 1.08 s, pulse width = 4 μs (40°), Hz/PT = 0.917. Chemical shifts were measured relative to internal 1,4-dioxan ($\delta = 67.7$ ppm relative to tetramethylsilane).

Results and discussion

Assignments of ^{13}C signals of aldobiouronic acids *I* and *II* were accomplished on the basis of comparison with chemical shifts of the constituent sugars and their derivatives, *i.e.* D-mannose [10], 2-*O*-methyl-D-mannose [3], D-galactose [11], and methyl D-glucopyranosiduronic acid [12] presented in Table 1. We took into consideration further that the shifts caused by *O*-glycosylation of hydroxyl groups were similar to those caused by *O*-alkylation. The resonance of a carbon atom involved in a linkage is strongly downfield-shifted (α-carbon effect) and the resonances of carbon atoms adjacent to the linked carbon atom are usually upfield-shifted by a small, but not necessarily similar, amount (β-carbon effect). In signal assignment we made use also of empirical rules stated by *Bradbury* and *Jenkins* on the basis of analysis of the ^{13}C NMR chemical shift data on aqueous solutions of series of mono-, di-, and oligosaccharides and their methyl derivatives in the literature survey [13].

Table 1

^{13}C Chemical shifts/ppm of D-mannose [10], 2-*O*-methyl-D-mannose [3], D-galactose [11], and methyl D-glucopyranosiduronic acid [12]

Substance	C-1	C-2	C-3	C-4	C-5	C-6	OCH_3-1
α-D-Mannose	95.5	72.3	71.9	68.5	73.9	62.6	
β-D-Mannose	95.2	72.8	74.8	68.3	77.6	62.6	
2-*O*-Methyl-α-D-mannopyranose	91.8	81.6	71.0	68.3	73.3	62.1	
2-*O*-Methyl-β-D-mannopyranose	95.0	82.6	74.5	68.0	77.5	62.1	
α-D-Galactose	93.2	69.4	70.2	70.3	71.3	62.2	
β-D-Galactose	97.3	72.9	73.8	69.7	76.0	62.0	
Methyl α-D-glucopyranosiduronic acid	100.7	71.9	73.8	72.5	71.9	?	56.7
Methyl β-D-glucopyranosiduronic acid	104.3	73.8	76.5	72.3	75.6	?	58.5

Table 2

Chemical shifts/ppm for ^{13}C NMR spectra of aldobiouronic acids *I* and *II*

Substance	Residue	C-1		C-2		C-3		C-4	C-5		C-6	
		α	β	α	β	α	β	α,β	α	β	α	β
I	R	92.9	94.6	79.3	81.5	70.3	74.0	67.9	73.4	77.2	61.4	
	N	102.4	104.5	73.2		76.6		75.6	76.1		176.3	
II	R	93.2	97.3	69.1	72.6	69.8	73.5	70.2	69.8	74.5	69.1	69.6
	N	103.4		73.5		76.0		72.0	75.2		172.9	

R — reducing unit.
N — nonreducing unit.

Chemical shifts of ^{13}C NMR signals of aldobiouronic acids *I* and *II* (Formula 1) are presented in Table 2 and some characteristics of the spectra are presented in Table 3.

The most valuable information about the structures of the compounds mentioned above was obtained from chemical shifts of the signals of carbon atoms involved in the linkage, *i.e.* the anomeric C-1 of the nonreducing residue (N) and the glycosyloxylated carbon atom of the reducing moiety (R).

According to [13], for a sugar linked to a reducing sugar residue the resonance for C-1 is split into a doublet by the nearby α and β anomers and the extent of splitting depends on the linkage position (the largest one appears with the $(1\rightarrow2)$ linkage).

With the compound *I* this resonance appeared at $\delta = 102.4$ ppm and $\delta = 104.5$ ppm. The positions of these signals unambiguously confirmed the β-configuration of the glycosidic bond (in case of α-configuration the signal should have appeared at $\delta < 101$ ppm) and the splitting of the signal indicated that the anomeric carbon atom was involved in a $(1\rightarrow2)$ linkage. The $(1\rightarrow2)$ linkage was unambiguously proved by the position of the signals for C-2 of the reducing residue at $\delta = 79.3$ ppm and $\delta = 81.5$ ppm. These values represented a downfield-shift by +6.9 ppm and 8.7 ppm (α-carbon effect) against the signals of the unsubstituted D-mannopyranose unit. The shifts of signals for carbon atoms adjacent to the *O*-glycosylated carbon were for C-1 −2.6 ppm and −0.6 ppm and for C-3 −1.7 ppm and −0.8 ppm, which differed from those reported for 2-*O*-methyl-D-mannopyranose in the literature [3]. Different values of the β-carbon effects for the individual anomers are, to a certain extent, also characteristic of the $(1\rightarrow2)$ linkage [13].

The well resolved signal at $\delta = 176.3$ ppm (characteristic of carbonyl groups) was assigned to C-6 of the nonreducing residue and proved the presence of the uronic acid unit in the disaccharide.

With the compound *II* the signal for C-1 of the nonreducing moiety appeared at $\delta = 103.4$ ppm. Splitting of the signal was not observed in accordance with the conclusions in [13]. The extent of splitting in the case of $(1\rightarrow6)$ linkage is small (0.04—0.3 ppm) and at relatively low magnetic field is not visible at all. The position of the signal indicated, similarly as with the compound *I*, the β-configuration of the glycosidic bond. The signal at $\delta = 172.9$ ppm, assigned to C-6 of the nonreducing residue, proved the presence of the uronic acid unit in the disaccharide.

The signals observed in the spectrum of the compound *II* at $\delta = 69.1$ ppm and $\delta = 69.6$ ppm corresponded to signals for C-6 in the 6-*O*-substituted D-galactopyranose unit. Since the chemical shifts of the resonances for C-6 pyranoid α- and β-monosaccharides are, according to [11], similar, *O*-glycosylation at the position C-6 of the reducing residue in the compound *II* produced a pair of downfield-

Table 3

Some diagnostically important characteristics of the ^{13}C NMR spectra (δ/ppm) of aldobiouronic acids *I* and *II*

Substance	Linkage	Residue N		Residue R						
				Glycosyloxylated C-atom		α-Effect		β-Effect		
				α	β	α	β		α	β
I	β-(1→2)	102.4	104.5	79.3	81.5	+6.9	+8.7	C-1	−2.6	−0.6
								C-3	−1.7	−0.8
II	β-(1→6)	103.4		69.1	69.6	+6.9	+7.6	C-5	−1.5	−1.4

N — nonreducing unit.
R — reducing unit.

-shifted resonances (by +6.9 ppm and 7.6 ppm) that were nearly coincident and proved the (1→6) glycosidic linkage in the molecule.

The signals at $\delta = 69.8$ ppm and $\delta = 74.5$ ppm belonged to C-5 of the reducing residue and were upfield-shifted relative to the corresponding resonances in unsubstituted D-galactopyranose by almost the same values for both anomers, *i.e.* −1.5 ppm and −1.4 ppm (β-carbon effect). This is in agreement with the empirical rules, too.

The chemical shifts of the other not discussed signals of carbon atoms of both compounds were similar as the corresponding ones of the respective constituent saccharides.

It can be concluded that the structures of both aldobiouronic acids, determined by chemical methods in [7—9], were confirmed by the results of ^{13}C NMR study. However, confirmation of the published structures was not the only reason for interpretation of the ^{13}C NMR spectra of the title aldobiouronic acids. These compounds frequently occur as building units in acidic plant polysaccharides (gums, mucilages) [14], therefore, they can serve as model compounds for interpretation of the spectra of heteropolysaccharides containing similar building units in the molecule and the obtained knowledge can be utilized in structural studies of the corresponding biopolymers.

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