STRUCTURE AND ^{13}C MAR NMR SPECTRA OF VARIOUS FORMS OF ISOMERIC METHYL XYLOBIOSIDES

Jan SCHRAML[a], Eva PETRÁKOVÁ[b], Ján HIRSCH[b], Raivo TEEÄÄR[c] and Endel LIPPMAA[c]

[a] *Institute of Chemical Process Fundamentals, Czechoslovak Academy of Sciences, 165 02 Prague 6 - Suchdol, Czechoslovakia*
[b] *Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Czechoslovakia and*
[c] *Institute of Chemical Physics and Biophysics, Estonian Academy of Sciences, Tallinn 200001, U.S.S.R.*

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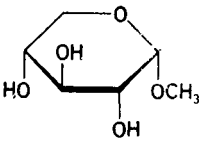
Dedicated to late Academician Eduard Hála.

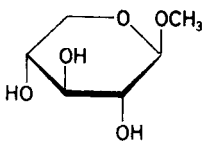
All so far known crystalline forms of all positional isomers of methyl β-D-xylopyranosyl-β-D--xylopyranoside were prepared and their high resolution ^{13}C NMR spectra measured by the magic angle rotation (MAR) method using cross-polarization (CP). According to these spectra only two modifications of methyl 4-O-(β-D-xylopyranosyl)-β-D-xylopyranoside (modifications *IVβ* and *IVγ*) contain more than one molecule in the crystal elementary cell; in all other studied disaccharide samples all molecules are equivalent. The majority of carbon-13 chemical shifts could be assigned, at least partially, by a comparison with solution spectra. Some of the glycosidic carbons in the units with methyl aglycone (ring A) exhibit surprisingly large paramagnetic shifts with respect to the solution chemical shifts. Apparently, molecular structures at these sites in solids differ most from the average structures the molecules assume in the liquid phase. Assuming that solid state chemical shifts are affected also by similar factors as the shifts in solution, the methyl aglycone carbon chemical shifts indicate that the frozen conformation of the ring A in the solid is closer either to $^1C_4$ conformation (modifications *IIIα* and *II*) or to $^4C_1$ conformation (modification *IVα*) than are the respective equilibrium conformations in solution. Thermal analysis has proven, however, that the xylobioside forms with two nonequivalent molecules in the cell similarly as some other forms are indeed hydrates. Thus it is shown that all the methyl xylobiosides are monomorphous, all the „polymorphs" are not true polymorphs but forms hydrated to a different extent.

Polymorphism is an important feature of polysaccharides which has been greatly utilized by Nature and finds wide industrial applications. Though polymorphs of cellulose have been studied in a great deal by ^{13}C NMR, polymorphism of other polysaccharides has received much less attention[1], e.g. to the best of our knowledge xylanes have not yet been studied by this method at all. As an initial step for such an investigation of complex xylanes we have undertaken the present study of model methyl xylobiosides some of which have been reported[2] to be poly-

morphous with the forms differing considerably in their melting points. For comparison we have also included both anomers of methyl D-xylopyranoside (*IA* – α-anomer, *IB* – β-anomer).

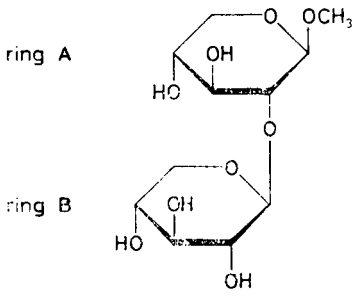


IA

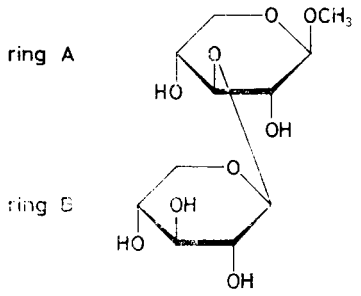


IB

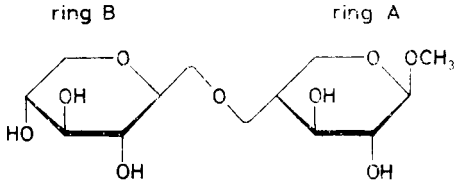
Methyl 2-O-(β-D-xylopyranosyl)-β-D-xylopyranoside (compound *II*) has been prepared only in one modification, methyl 3-O-(β-D-xylopyranosyl)-β-D-xylopyranoside (*III*) in two modifidations, *IIIα* and *IIIβ*, and methyl 4-O-(β-D-xylopyranosyl)--β-D-xylopyranoside (*IV*) in three forms, *IVα*, *IVβ*, and *IVγ* (α, β, γ denote modifications in the decreasing order of their m.p.).



II



III



IV

EXPERIMENTAL

Chemicals

Unless the compound was of commercial origin, the preparation followed exactly the procedure described in the literature[2,3]. Identity of all the compounds was checked by solution ^1H and ^{13}C NMR spectra (in ^2H_2O). Solution spectra of different forms of the same compound were identical and agreed with those of authetic samples (Table I). Polymorphs were identified by comparison of their melting points with literature data.

Since it was found during the course of this work that some of the forms easily change their moisture content together with their structure, the melting point measurements were repeated after each NMR spectrum recording.

Methyl α-D-xylopyranoside (*IA*) was crystallized from methyl ethyl keton, m.p. 84–86°C. Methyl β-D-xylopyranoside (*IB*; Lachema Brno) was purified by crystallization from absolute alcohol and then recrystallized from ethyl acetate, m.p. 154–155°C.

Recrystallization of methyl 2-O-(β-D-xylopyranosyl)-β-D-xylopyranoside (*II*) from ethanol afforded white crystalline product, m.p. 155–157°C, lit.[2] m.p. 153–154°C. Various attempts to prepare other modifications have all led to the products with the same melting point given above.

Recrystallization of methyl 3-O-(β-D-xylopyranosyl)-β-D-xylopyranoside (*III*) from ethanol : : acetone (1 : 4) gave crystals that melted at 160–162°C, lit.[2] m.p. 161–162°C (modification *IIIα*). Modification *IIIβ*, m.p. 93–95°C, was obtained using methanol : acetone (1 : 8) mixture for crystallization.

Methyl 4-O-(β-D-xylopyranosyl)-β-D-xylopyranoside (*IV*), chromatographically pure, after recrystallization from ethanol showed m.p. 170·5–171°C, lit.[2] m.p. 170–171°C (modification *IVα*). When absolute ethanol was used for crystallization and hot solution was cooled down slowly, the obtained needles melted at 148–149°C, lit.[4] m.p. 148–149°C (modification *IVβ*). Crystallization from methanol or from methanol : petroleum ether (1 : 2) yielded modification *IVγ* melting at 97–98°C, lit.[5] m.p. 103–104°C.

^{13}C MAR NMR Measurements

^{13}C NMR spectra of solid disaccharides were measured using magic angle rotation (MAR) combined with cross-polarization (CP). The experiments wre carried out on a Bruker CXP-200 spectrometer operating at 50·3 MHz. Home-built MAR accessory of double-bullet design was employed. Ceramic cylindrical rotors with 7 mm o.d. and 0·4 cm^3 sample volume machined from Macor (Corning. Corp.), were spun at about 4–4·5 kHz at the magic angle (54·7°) to the main magnetic field. R.f. excitation and decoupling fields coresponded to 5 μs $\pi/2$ pulse. CP contact time was set 1 ms and relaxation delay varied up to 30 s. The methyl carbon line of hexamethylbenzene (δ = 16·9) served as a reference. The number of scans needed to achieve good signal-to-noise ratio varied between 40 and 3 500 depending especially upon the amount of the sample available. Methyl carbon lines were assigned on the basis of selective polarization inversion[6] and delayed decoupling[7] experiments performed on a MSL-200 Bruker spectrometer.

Thermal Analysis

The analysis was performed on a Perkin–Elmer Thermobalance TGS-1. Samples (0·4–0·9 mg) were heated at the rate of 10°C/min in the air.

RESULTS AND DISCUSSION

The ^{13}C CP MAR NMR spectra of the studied xylopyranosides are shown in Figs 1–4. Obviously, the spectra of *IB*, *II*, *IIIα*, *IIIβ*, and *IVα* reflect the symmetry on the molecular level; the spectra contain as many lines as there are non-equivalent carbon atoms present in the molecule. The lines in these spectra were assigned according to the assignments published for solution spectra (Table I); with a few exceptions (denoted in Table II) the assignments seem to be relatively safe as the differences in isotropic chemical shifts are sufficiently large. Assignments of methyl lines were confirmed by special experiments[6,7]. Some unusual chemical shift values are commented below.

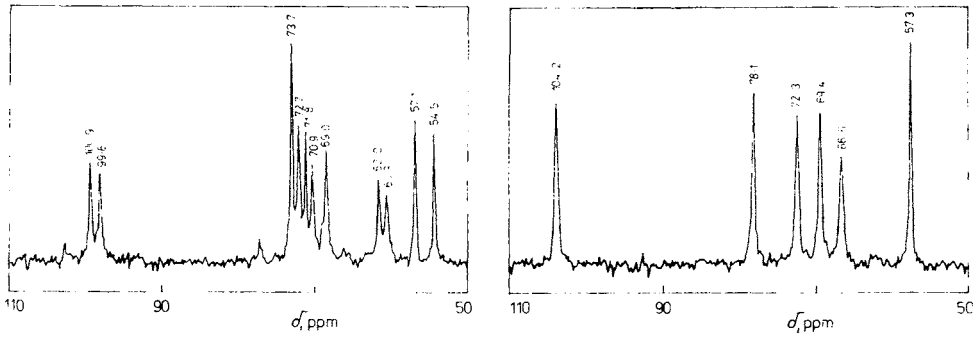
In the spectra of the remaining forms (*IA*, *IVβ*, and *IVγ*) we find more lines than expected on the basis of molecular formulas. In the course of our work Taylor et al.[11] published the results of their investigation of the spectra of *IA* and *IB*. (The chemical shifts reported by Taylor et al.[11] for *IA* and *IB* are 0·7–0·9 and 0·9 to 1·1 ppm, respectively, more paramagnetic than the values given here.) Using the X-ray diffraction data[12–14], according to which the elementary crystal cell contains two non-equivalent molecules of *IA*, they concluded that the small conformational differences along with variations in intermolecular interactions result in significantly different chemical shifts for chemically equivalent carbon sites in the two molecules.

Two possible sources for increased line multiplicity in the spectra of solids (as compared with the spectra of isotropic solutions) are[11]: (i) lower site symmetry in the crystal than is the symmetry for the isolated molecule and (ii) more than one chemically equivalent molecule in the crystal unit cell. Since the xylopyranosides *I–IV* have all their carbon atoms chemically non-equivalent, the molecular symmetry cannot be reduced any further by the crystal structure. Hence, the only possible source of the observed line multiplicity is the presence of crystallographically non-equivalent (though chemically equivalent) molecules in the forms *IVβ* and *IVγ*.

In order to estimate the number of crystallographically non-equivalent molecules in *IVβ* and *IVγ* let us inspect more closely the characteristic spectral regions of anomeric ($\delta = 100-110$) and methyl aglycone carbons ($\delta = 50-63$). In the anomeric carbon region of the spectrum of *IVγ* we see (Fig. 4*c*) one strong line (with approximate relative intensity 3–4) and three weeker lines (all with approximately the same relative intensity of 1). Such pattern can be explained by the presence of three crystallographically non-equivalent molecules in the lattice with three anomeric carbons having accidentally the same chemical shift ($\delta = 102{\cdot}7$, it is worth mentioning that this line is present in the spectrum of *IVα* as an impurity). This interpretation is confirmed by the presence of two well resolved lines and one shoulder in the methyl aglycone carbon region though their intensities are not in the expected 1 : 1 : 1 ratio. Such non-ideal intensity ratios are, however, often found for methyl carbon lines as a consequence of different relaxation times and varying effectiveness

of polarization transfer. For the sake of the subsequent discussion it should be noted here that two of the week anomeric carbon lines have practically the same chemical shifts as the lines in the spectrum of *IVα*, one of the lines has exactly the same shift (δ = 107·3), the other (δ = 106·3) differs by 0·5 ppm from the shift in *IVα*. Also, the methoxyl carbon at δ = 61·0 differs by 0·4 ppm from the shift of the corresponding line in *IVα*. Since the chemical shifts of anomeric and aglycone methyl carbons are sensitive to the conformation of a saccharide[15], the close similarity of the chemical shift values suggests that one of the three disaccharide molecules has conformation very similar to that in *IVα* modification, the other two molecules have different conformations or their carbon atoms are subject to different interactions or both.

The spectrum of *IVβ* (Fig. 4*b*) is even more complex. There are five resolved lines in the anomeric carbon region with approximate intensity ratios 2 : 1 : 1·5 : 1 : 3.



a *b*

FIG. 1

^{13}C CP MAR NMR spectra of monosaccharides *I*. *a* Monosaccharide *IA* and *b* monosaccharide *IB*

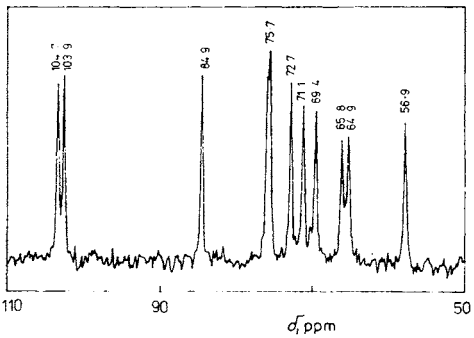
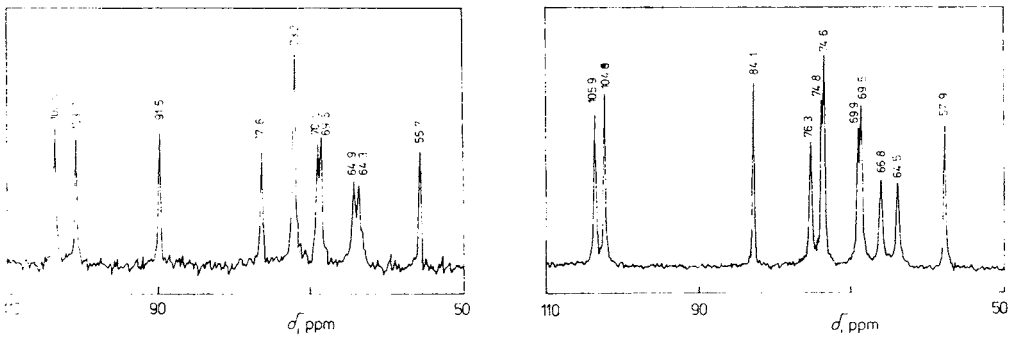


FIG. 2

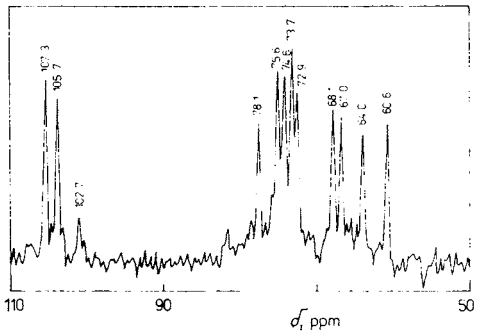
^{13}C CP MAR NMR spectrum of disaccharide *II*



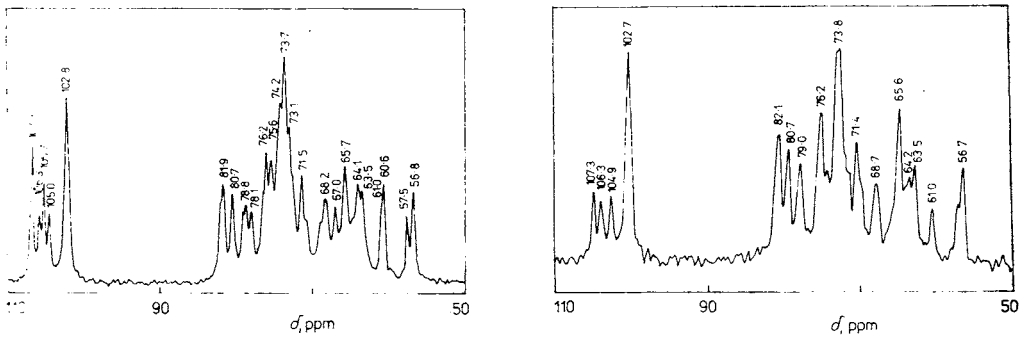
a *b*

FIG. 3

^{13}C CP MAR NMR spectra of disaccharide *III*. *a* Modification *IIIα* and *b* modification *IIIβ*



a



b *c*

FIG. 4

^{13}C CP MAR NMR spectra of disaccharide *IV*. *a* Modification *IVα*, *b* modification *IVβ*, and *c* modification *IVγ*

TABLE I

Solution ^{13}C NMR chemical shifts in xylopyranosides *I*–*IV*[a]

Compound	Ring	C-1	C-2	C-3	C-4	C-5	OCH_3
IA[b]	A	100·6	72·3	74·3	70·4	62·0	56·0
IB[b]	A	105·1	74·0	76·9	70·4	66·3	58·3
II[c]	A	104·9	81·8	76·4	70·2	65·9	58·1
	B	103·7	74·7	76·8	70·4	66·3	
III[c]	A	104·9	73·7	85·3	69·0	66·0	58·4
	B	104·8	74·6	76·9	70·4	66·4	
IV[c]	A	105·1	74·0	75·0	77·7	64·1	58·4
	B	103·1	74·0	76·9	70·4	66·5	

[a] Literature data and assignments for ^2H_2O solutions, different methods of referencing were used. [b] Data from ref.[8], external tetramethylsilane reference; values given in ref.[9] are systematically 1·1–2·3 ppm smaller. [c] Data from ref.[10] internal referencing to the line of methanol ($\delta = 50{\cdot}15$).

TABLE II

Solid state ^{13}C NMR chemical shifts in xylopyranosides *I*–*IV*[a]

Compound	Ring	C-1	C-2	C-3	C-4	C-5	OCH_3
IA	*A*[b]	100·9	72·7[c]	73·7[c]	69·0[c]	62·0	57·1
	A[b]	99·6	71·8[c]	73·7[c]	70·9[c]	60·9	54·5
IB	A	104·2	72·3	78·1	69·4	66·5	57·3
II	A	104·7	84·9	75·7[d]	71·1	64·9[d]	56·9
	B	103·9	72·7	76·0[d]	69·4	65·8[d]	
IIIα	A	106·0[d]	73·2	91·5	69·6[d]	64·3[d]	55·7
	B	103·2[d]	73·2	77·6	70·0[d]	64·9[d]	
IIIβ	A	105·9[d]	74·8[d]	84·1	69·5[d]	66·8[d]	57·9
	B	104·6[d]	74·6[d]	76·3	69·9[d]	64·5[d]	
IVα[e]	A	107·3	73·7[c]	74·6[c]	78·1[c]	64·0	60·6
	B	105·7	72·9[c]	75·6[c]	68·1	67·0	

[a] Solid state chemical shifts in δ — scale assigned according to the corresponding solution spectra. [b] Two monosaccharide molecules in the elementary crystal cell, the chemical shifts shown on the same line do not necessarily belong to the same molecular unit. [c] Assignments can be interchanged. [d] Ring assignment can be reversed. [e] Assignments of the lines in the spectra of other modifications of *IV* are discussed in the text.

The chemical shifts of two of these lines (with intensities 2 and 1·5) match fully the anomeric carbon lines in *IVα*, the position and intensity ratios of the remaining three match within 0·1 ppm three lines in the spectrum of *IVγ*. Similarly, in the aglycone methyl carbon spectral region, we see three well resolved lines and one shoulder at $\delta = 61{\cdot}0$. The chemical shifts of these lines also match within 0·1 ppm the chemical shifts of methoxyl carbon lines in *IVα* and *IVγ*, the position of the shoulder is the same as the position of the remaining line in the spectrum of *IVγ*. These findings suggest the presence of four crystallographically non-equivalent disaccharide *IV* molecules in the sample. In view of intensity ratios and on the basis of comparison of the modification *IVγ* and *IVβ* it seems most likely that the modification *IVβ* is a mixture of the other two modifications of the compound *IV*.

Of all the remaining lines in the spectra of *IVβ* and *IVγ* only the paramagnetically shifted lines ($\delta = 78{\cdot}5-83$) and the lines in the region $\delta = 63-66$ can be partially assigned with some confidence. Since any other assignment would require assumption of much larger solid state effects (with the possible exception of assigning some of the lines in the former region to C-3 in the B rings), we assign these lines to C-4 and C-5 carbons of the A rings, respectively, in different molecules. With this assignment the numbers of the lines in the former region support our conclusions about the numbers of crystallographically non-equivalent molecules in the lattices of these modifications. The appearance of the spectra in the latter region indicate that either some other carbon nuclei resonate in this region or that line intensty of C-5 carbons varies similarly as mentioned above for methyl carbons. At present, all the remaining lines ($\delta = 66-77$) cannot be reliably assigned either to carbon atoms, or rings, or non-equivalent molecules.

Similar large paramagnetic shifts as just discussed for C-4 carbons in A rings of of *IVβ* and *IVγ* are also found for C-3 of A ring in *IIIα* and for C-2 carbon in A ring of *II* which modifications contain each only one molecule in the crystal elementary cell. However, no such paramagnetic shifts (relative to solution spectra) were found in the spectra of monosacchardies *IA* and *IB* and disaccharide modifications *IIIβ* and *IVα*. It is characteristic that all the large (>3 ppm) paramagnetic shifts are observed for carbon atoms of the ring A bearing xylopyranosyl residue. In order to understand why the effect of crystal lattice is the largest at these sites and why in *III* the highest melting modification is involved while such shifts are not found in *IVα* we have subjected the compounds to thermal analysis suspecting that the modifications might differ by hydration in the lattice (no elemental analysis has been published for these modifications).

According to thermal analysis, the disaccharides *II*, *IIIβ*, *IVβ*, and *IVγ* contain 6·28, 6·48, 5·23, and 6·84% (by weight) of water, respectively. Disaccharides *IIIα* and *IVα* are anhydrous.

These results show that the alleged polymorphism is not a true polymorphism, the modifications of each of the compounds differ in their water contents. The water

content of 5·73% corresponds to 1 : 1 hydration of a methyl xylobioside. Accordingly, modifications that have higher water content (*II*, *IIIβ*, and *IVγ*) contain some additional water above 1 : 1 hydration in the crystal lattice. Modification *IVβ* is, in agreement with the above discussed NMR results, a mixture of anhydrous modification *IVα* and 1 : 1 hydrated form *IVγ*.

Though these result clarify the question of polymorphism of methyl xylopyranosides, they do not explain why carbons bearing xylopyranosyl residue experience large paramagnetic shifts in some hydrated form (in *II* and *IVγ*) while such effects are not observed in other hydrated form (*IIIβ*) and vice versa in anhydrous modifications.

Possible clue is provided by methyl aglycone carbon chemical shifts if we assume that these shifts are affected by similar factors in solids as in solutions. In solution the shift depends on conformation of the ring A, the methoxyl is shielded some 2 ppm more in axial than in equatorial position on C-1 (ref.[15]). Accordingly, the large range of methyl carbon chemical shifts in solid disaccharides (4·9 ppm as compared with 0·3 ppm in solution) indicates that the ring A assumes different conformations in solid disaccharides *II*–*IV*. Moreover, larger shielding of methyl carbons in solid modifications *IIIα* and *II* and of some methyl carbons in *IVβ* and *IVγ* than in their solutions or than in solid *IVα* suggests that the ring A takes in these solids conformations which are closer to $^1C_4$ conformation than is $^1C_4$ to equilibrium conformations taken by this ring in solutions. Similarly, modification *IVα* has conformation of the A ring closer to $^4C_1$ conformation in solid than in solution and the conformations of *IIIβ* are similar in solid and solution. This classification of the conformations of the rings A in the solids matches the paramagnetic shifts of glycosidic carbons discussed above – the paramagnetic shifts are found in the rings with conformations close to $^1C_4$.

Though quantitative estimation of glycosylation shifts according to the recently advanced theory of Shashkov et al.[16] would require detailed knowledge of the structure of the compounds in solid, qualitative considerations along the lines of the theory[16] provide additional support to the above hypothesis about the conformation of the rings A. In the ideal $^4C_1$ conformation of methyl β-D-xylopyranoside there is only one equatorial proton (H-5) while in $^1C_4$ conformation there are always equatorial protons on both carbons in β position to the site of glycosylation. With equatorial protons in both β positions (as in *II* and *IIIα*) there is always a proton that is close to H-1 hydrogen atom of the ring B leading through 1,4-interaction to an upfield shift of C-1 of the ring B. Such interaction is less likely when there is only one (as in *IVα*) or none (as in *III(β)*) such equatorial proton. Hence, replacing the methyl aglycone of *IB* by methyl β-D-xylopyranoside residue should produce an upfield shift of C-1 carbon of the B ring in *IIIα* and *II* as observed. The analogous shift observed in *IVα* is downfield.

More significant shielding differences that are observed on glycosylation of the ring A cannot be discussed without detailed knowledge of the structure of the compounds in solid. Exact knowledge is also needed to test the hypothesis used above in interpretation of methyl aglycone chemical shifts. The required X-ray diffraction study is under way[17].

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REFERENCES

1. Lindberg J. J., Hortling B.: Adv. Polym. Sci. *66*, 1 (1985).
2. Kováč P.: Collect. Czech. Chem. Commun. *45*, 892 (1980).
3. Hudson C. S.: Am. Chem. Soc. *47*, 265 (1925).
4. Kováč P., Hirsch J., Kováčik V.: Chem. Zvesti *32*, 514 (1978).
5. Whistler R. L., Bachrach J., Tu C. C.: J. Am. Chem. Soc. *74*, 3059 (1952).
6. Wu Xiaoling, Zhan Shanmin, Wu Xuewen: J. Magn. Reson. *77*, 343 (1988).
7. Opella S. J., Frey M. H., Cross T. A.: J. Am. Chem. Soc. *101*, 5856 (1979).
8. Gorin P. A. J., Mazurek M.: Can. J. Chem. *53*, 1212 (1975).
9. Breitmaier E., Voelter W.: Tetrahedron *29*, 227 (1973).
10. Kováč P., Hirsch J., Shashkov A. S., Usov A. I., Yarotsky S. V.: Carbohydr. Res. *85*, 177 (1980).
11. Taylor M. G., Marrchessault R. H., Perez S., Stephenson P. J., Fyfe C. A.: Can. J. Chem. *63*, 270 (1985).
12. Brown C. J., Cox G., Llewellyn F. J.: J. Chem. Soc., A *1966*, 922.
13. Takagi S., Jeffrey G. A.: Acta. Crystallogr., B *33*, 3033 (1977).
14. Takagi S., Jeffrey G. A.: Acta Crystallogr., B *34*, 3103 (1978).
15. Shashkov A. S., Chizhov O. S.: Bioorg. Khim. *2*, 437 (1976).
16. Shashkov S. S., Lipkind G. M., Knirel Y. A., Kochetkov N. K.: Mag. Reson. Chem. *26*, 735 (1988).
17. Bauer J., Kratochvíl B., Petráková E., Hirsch J., Schraml J.: Unpublished results.

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