^{13}C MAS NMR AND ^{1}H–^{29}Si AND ^{1}H–^{13}C HETERONUCLEAR CORRELATION STUDY OF MODEL XYLOOLIGOSACCHARIDES

Jan SCHRAML[a], Eva PETRÁKOVÁ[b], Ján HIRSCH[b], Jan ČERMÁK[a], Václav CHVALOVSKÝ[a], 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 of the Estonian Academy of Sciences, Tallinn 200001, U.S.S.R.*

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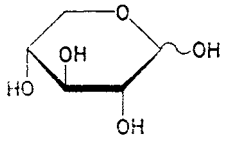
Anomer structures of crystalline D-xylopyranose, all positional isomers of β-D-xylopyranosyl-D--xylopyranose and 2,4-di-β-D-xylopyranosyl-D-xylopyranose were determined by ^{13}C MAS NMR spectroscopy. The saccharides were pertrimethylsilylated by different methods yielding different ratios of pertrimethylsilylated anomers. The NMR spectra (^{1}H, ^{13}C, and ^{29}Si) were assigned by two-dimensional chemical shift correlations. In the anomeric mixtures complete overlap in parts of proton spectra is frequent. In such a case, samples with different anomer ratios considerably facilitate assignment of both ^{13}C and ^{29}Si NMR lines. In analytical applications, however, that method of trimethylsilylation must be chosen which does not allow anomerization prior to silylation. ^{29}Si NMR spectra furnish correct number of OH groups present in the parent compound prior to silylation. Assigned silicon chemical shifts can be used for determination of the sites of glycosidation in oligosaccharides. Glycosidic carbon atoms are considerably shielded after pertrimethylsilylation.

Recently, we encountered[1] difficulties in applying ^{29}Si NMR to saccharides. Though the origin of the difficulties – anomerization – was obvious, it could lead to incorrect analysis of samples with unknown structure. In order to set guidelines for ^{29}Si NMR analysis of unknown saccharides we have undertaken a study of trimethylsilylation of model oligosaccharides derived from D-xylopyranose compounds *I* – *V*. Since the structure of solid saccharides *I* – *V* has not yet been determined, we have attempted to establish the population of anomers by high resolution solid state ^{13}C NMR spectroscopy. Anomerization through trimethylsilylation was followed by ^{1}H and ^{13}C NMR spectroscopy of pertrimethylsilylated products in solution. The spectra of trimethylsilylated anomeric mixtures were assigned by a combination of two-dimensional methods.

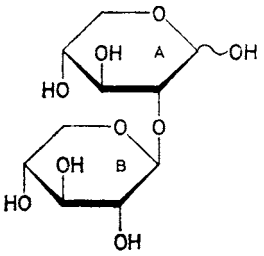
RESULTS AND DISCUSSION

^{13}C MAS NMR *Determination of the Anomers Present*

The high-resolution solid state ^{13}C NMR spectra of compounds *I*–*V* are shown in Figs 1–3. The chemical shifts of characteristic lines (C-1 and C-5 atoms) are indicated in the figures; all the chemical shifts are given in Table I. It is obvious from the spectra that the compounds *I*–*IV* crystallize essentially as one anomer only and that all the molecules are equivalent in the crystal lattices. (The spectra of compounds *III*, *IV*, and *V* indicate the presence of minor amounts of the other anomer.)



I



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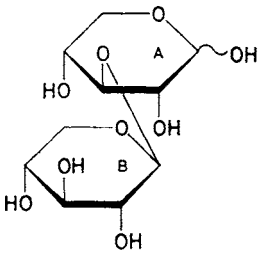

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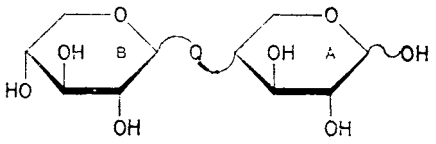
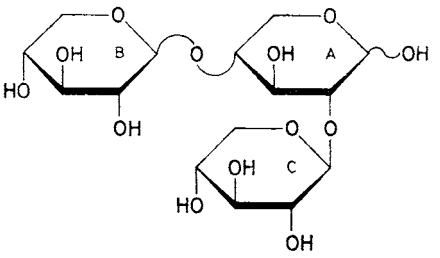


IV



V

The lines are assigned[2] by comparison with the assigned solution spectra of anomeric mixtures (Table II). The chemical shifts of C-1 atoms permit differentiation between the C-1 atoms in rings A and B (α-effect of glycosidic bonds ranging from +7 to +10 ppm, ref.[5]). Also the difference between the chemical shifts of C-1 atoms in the two anomers (ring A) is sufficient ($\delta = 93$ in α and $\delta = 97$ in β anomer) in most cases to distinguish both of them. If the observed C-1 chemical shift has an intermediate value the anomers can be differentiated according to the other characteristic line, *i.e.* acording to C-5 carbon atom chemical shift ($\delta = 60-62{\cdot}5$ in α and $\delta = 65{\cdot}5-66{\cdot}5$ in β anomer).

The anomer structure (Table I) of solid compounds *I*–*IV* was determined according to the above rules unambiguously.

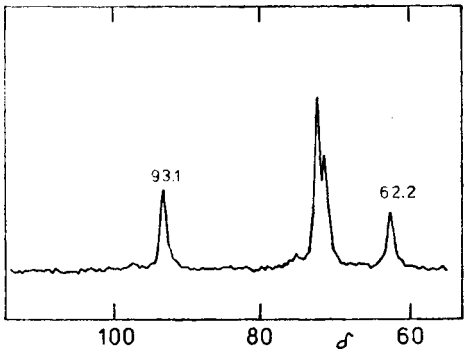


FIG. 1

^{13}C MAS NMR spectrum of monosaccharide *I*. Characteristic chemical shifts are given in the spectrum

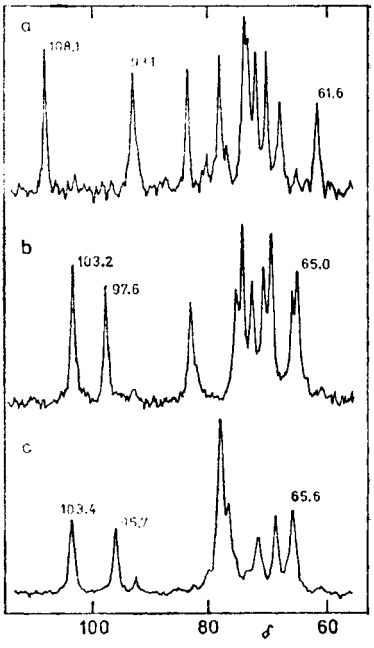


FIG. 2

^{13}C MAS NMR spectra of disaccharides *II*–*IV*. a Disaccharide *II*, b disaccharide *III*, and c disaccharide *IV*. Characteristic chemical shifts are given in the spectrum

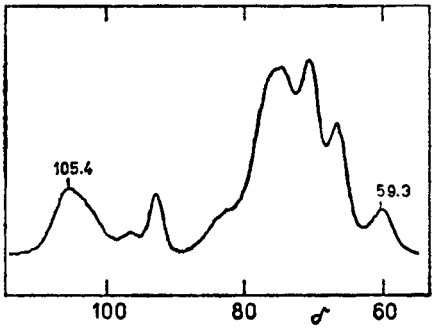


FIG. 3

^{13}C MAS NMR spectrum of trisaccharide *V*. Indicated are the chemical shifts corresponding to the positions of the markers

TABLE I

Solid state ^{13}C chemical shifts in saccharides *I*— *V*

Compound	Anomer	Ring	C-1	C-2	C-3	C-4	C-5
I	α	A	93·1	71·9[a]	71·9[a]	71·0[a]	62·2
II	α	A	93·1	83·8	73·2	72·0	61·6
		B	108·1	73·8	78·2	70·1	67·8
III	β	A	97·6	72·8[a]	82·9	69·4	65·0
		B	103·2	74·3[a]	75·4	70·7	65·7
IV	β	A	95·7	71·5[a]	76·5[a]	77·6	65·6
		B	103·4	77·6	77·6	68·5	65·6
V	α	A	92·6	74·0	75·0	70·0	59·3
		B	105·0	74·0	75·0	70·0	66·0
		C	105·0	74·0	75·0	70·0	66·0

[a] Tentative assignment.

TABLE II

^{13}C Chemical shifts of saccharides *I*— *V* in $^{2}H_2O$ solution

Compound	Anomer	Ring	C-1	C-2	C-3	C-4	C-5
I[a]	α	A	93·3	72·5	73·9	70·4	62·1
	β	A	97·6	75·1	76·9	70·3	66·3
II[a]	α	A	93·1	81·9	73·0	70·4	61·7
	β	A	96·5	82·9	74·5	70·4	66·2
	α, β	B	105·9	74·3	76·7	70·4	66·2
			104·9				
III[a]	α	A	93·3	72·1	82·9	68·9	62·1
	β	A	97·6	74·9	85·3	68·9	65·5
	α, β	B	104·7	74·6	76·8	70·4	66·3
IV[a]	α	A	93·2	72·1[b]	72·6[b]	77·7	60·0
	β	A	97·7	75·1	75·1	77·6	64·1
	α, β	B	103·0	74·0	76·8	70·4	66·4
V[c]	α	A	92·8	81·6	71·1	77·5	59·7
	β	A	96·4	82·9	73·9	77·5	63·9
	α, β	B	103·0	73·9	76·8	70·4	66·3
	α, β	C	105·8	74·3	76·8	70·4	66·3
			105·0				

[a] Data taken from ref.[3]. [b] The assignment may be reversed. [c] Data taken from ref.[4].

Repeated crystallization has not improved the quality of the spectrum of compound *V*, the lines have remained broad. The broad lines are apparently due to overlap of lines and, perhaps, to the presence of several crystallographically nonequivalent xylopyranose units in the lattice. Despite that, the relative ratio of lines at $\delta = 96{\cdot}3$ and 92·6 as well as of those at $\delta = 66$ and 59·3 clearly demonstrate that the compound is present dominantly as α anomer in the solid state.

Trimethylsilylation

Using trimethylsilylation by N,O-bis(trimethylsilyl)acetamide (BSA), anomer ratio of the pertrimethylsilylated saccharides can be varied and thus the analysis and spectral assignment facilitated. The anomer ratio is simply controlled by the time the free saccharide is allowed to anomerize in pyridine prior to addition of BSA to the reaction mixture. In general, one can vary the anomer ratio by this procedure from the equilibrium value to that in the solid state. Using this method we varied the anomer ratio for *II* and *III* from 6 : 1 to 2 : 1.

The pertrimethylsilylated compounds are denoted here by letter S following the number of the parent saccharide and by α and β denoting the anomer. Thus, for example *III*,Sα means pertrimethylsilylated anomer α of the compound *III*. The xylopyranose rings are labelled A, B, C as in the parent compounds *I* – *V*.

Spectral Assignments

Since the 2 D NMR assignment experiments are time-consuming, we have adopted a time saving strategy and measured the 2 D spectra in solutions with anomer ratio as close to 1 : 1 as possible. In this way one 2 D experiment of each type suffices for line assignment in the spectra of both anomers. Problems caused by multiplet overlap were solved by one-dimensional spectra of mixtures with different anomer ratios. Using a spectrometer operating at 200 MHz this approach is, however, not feasible for trisaccharides. Therefore, the spectra of *V*, S were assigned only by comparison with those of other pertrimethylsilylated saccharides[6,7] which is not very reliable procedure for ^{29}Si chemical shifts assignment.

^{1}H *and* ^{13}C NMR *Spectra*

The starting points for the assignments are the characteristic ^{13}C NMR lines mentioned above and the values of vicinal proton–proton (H-1, H-2) coupling constants which are close to 3·5 and 7·2 Hz in the α and β anomers, respectively[8]. (The hydrogen as well as silicon atoms are labelled by the number of the nearest skeletal carbon atom.) From the assigned CH-1 and CH_2-5 multiplets (Figs 4 and 5) we proceed to assign all the remaining multiplets using the synergetic combination of homonuclear and heteronuclear chemical shift correlations[9]. In addition to generally used as-

signment procedure[10] we take advantage of the observations[9] that (i) the cross peaks exhibit reproducibly a characteristic pattern when the related compounds are measured under the same conditions and that (ii) the heteronuclear correlations when measured with the same digital resolution as homonuclear correlations, reproduce along the ^1H axis the multiplet structure of proton–proton homonuclear correlations (unless excessive line-broadening is used).

The details of assignment procedure are given below for the spectra of trimethylsilylated anomer mixture of saccharide *I*. Application of the procedure to di- and

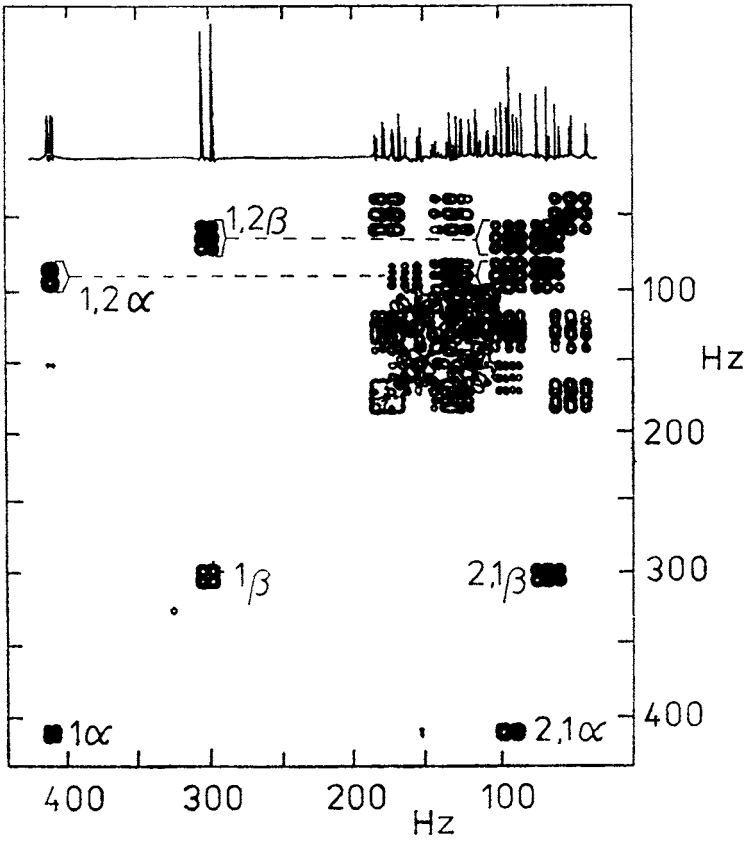


FIG. 4

^1H NMR spectrum and its homonuclear chemical shift correlation contour map (mixture of trimethylsilylated anomers *I*, Sα and *I*, Sβ), diagonal peaks are labelled by proton number followed by anomer designation, cross peaks are marked by the numbers of connected protons separated by comma and by anomer label

trisaccharides is only complicated by a larger number of lines that require more careful evaluation and more detailed presentation of the spectra.

From the CH-1 diagonal peaks (Fig. 4) we are led through 1, 2 cross peaks to diagonal peaks of H-2 protons of α and β anomers. The H-2 β multiplet is only partially overlapped with another multiplet (that will be identified as H-5′ β multiplet later), it exhibits, besides the mentioned 1, 2 cross peak (Fig. 4), only cross peak 2, 3 β which brings us to H-3 β diagonal multiplet (Fig. 6). This multiplet is completely overlapped with the already assigned H-2 α diagonal multiplet. The overlap and insignificant difference in the structure of the corresponding peaks in the heteronuclear 2 D spectra (Fig. 7) complicate the assignment of ^{13}C NMR lines to C-2 α

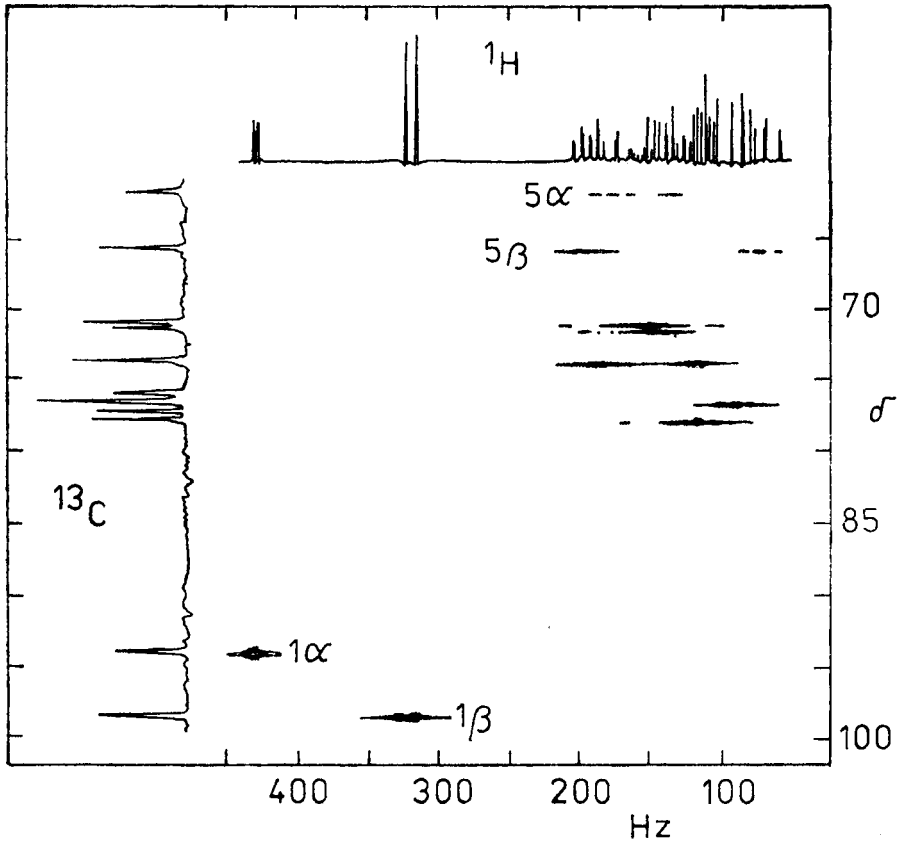


FIG. 5

^{1}H and ^{13}C NMR spectra and their heteronuclear chemical shift correlation contour map (the same sample as in Fig. 4

and C-3 β carbon atoms in α and β anomers, resp. In this case the ambiguity is removed by ^{13}C NMR spectrum of another anomer mixture. There are two types of not yet assigned cross peaks correlating the H-2 α and H-3 β overlapping multiplets with other parts of the ^{1}H NMR spectrum. One of them has the typical pattern of 3, 4 cross peaks found in methyl β-D-xylopyranosides[6,7]. It correlates the well resolved H-3 β proton multiplet with broad low-intensity H-4 β proton multiplet; the other cross peak correlates the well resolved quadruplet of H-2 α and triplet of H-3 α. These two cross peaks bring us to H-4 β (not marked in Fig. 6) and H-3 α diagonal peaks. There are two ^{13}C NMR lines with heteronuclear cross peaks corresponding approximately to the same proton multiplets as exhibited by H-4 β proton. They are again differentiated by the spectrum of another mixture. More cross peaks

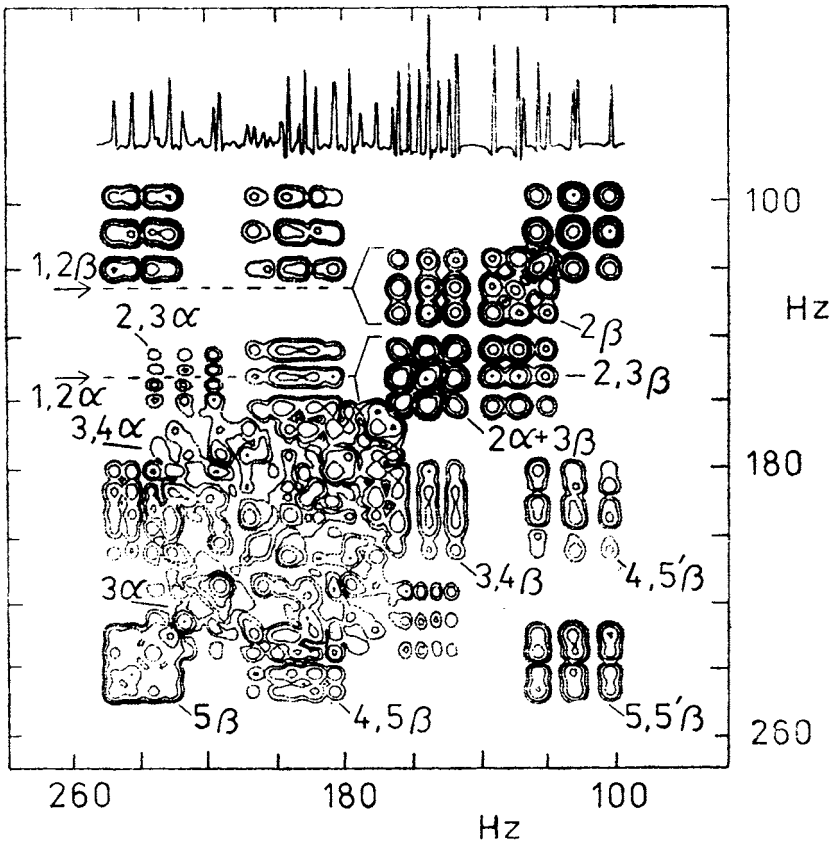


FIG. 6
Expanded parts of the spectra shown in Fig. 4

of H-4 β proton are found in the f_1 and f_2 frequency ranges occupied by the symmetrical pair of 3,4 β and 4,3 β cross peaks. These are 4,5 β and 4,5′ β (with symmetrical 5,4 β and 5′,4 β) cross peaks that identify H-5 β and H-5′ β multiplets. Their position in the spectrum is in agreement with the C-5 cross peaks in the heteronuclear correlation (Fig. 7). Thus the assignment is completed for the β anomer. The already assigned H-3 α multiplet shows (besides the assigned 2,3 β cross peaks) only broad cross peaks in the frequency range 160 to 210 Hz which overlaps with H-4 β multiplet. According to the heteronuclear correlation H-5 and H-5′ protons of α anomer resonate in the same region. Therefore, the protons H-4, H-5, and H-5′ form a strongly coupled spin system. The remaining, not yet assigned ^{13}C line must belong to C-4 α with the corresponding H-4 α protons resonating in

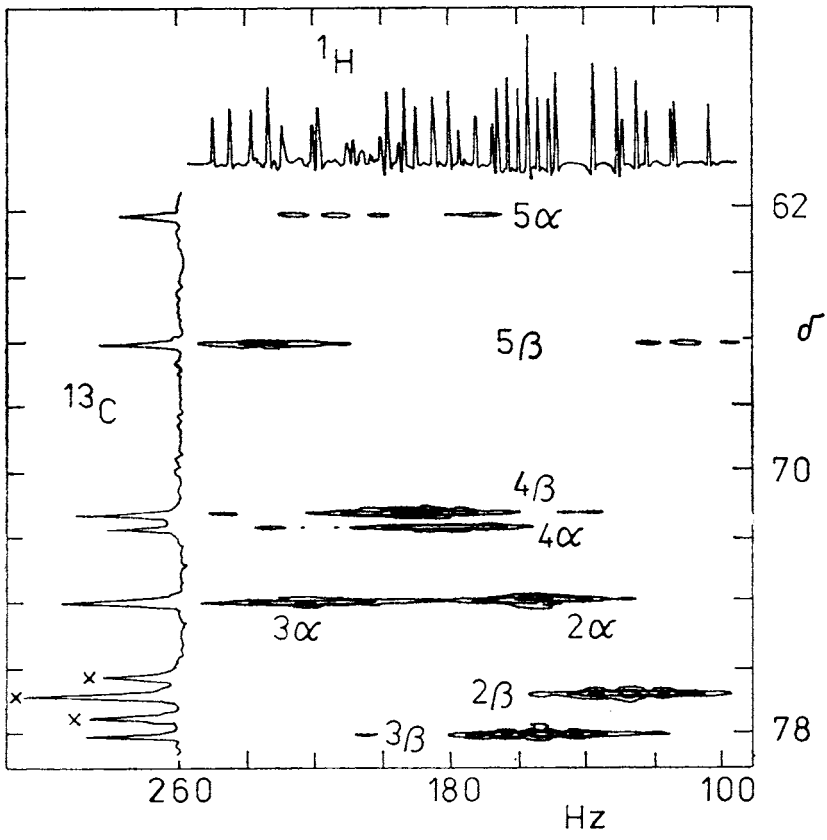


FIG. 7

Expanded parts of the spectra shown in Fig. 5 (the lines of the solvent denoted by asterisks)

the mentioned frequency range and overlapping completely with H-4 β multiplet. That completes the assignment of ^{1}H and ^{13}C NMR spectra.

Since the ^{13}C NMR spectra are recorded with proton decoupling, the assigned spectra yield directly ^{13}C chemical shifts. In the case of ^{1}H NMR spectra exact spectral analysis would be required in order to provide the values of the chemical shifts, these values are not needed for the ^{13}C and ^{29}Si line assignment and so no attempts were made to analyse the spectra here.

^{29}Si NMR *Spectra*

When the heteronuclear chemical shift correlated ^{1}H–^{29}Si NMR spectra are recorded with a good signal/noise ratio and without decoupling in the ^{1}H direction, assignment is usually no problem. The spectra are well spread out (Fig. 8) and the cross peaks repeat the splitting pattern of homonuclear ^{1}H–^{1}H and heteronuclear ^{1}H–^{13}C correlated 2 D spectra.

Chemical Shifts

^{13}C *Chemical shifts.* Comparison of the corresponding values in Tables II and III reveals that the overall effect of pertrimethylsilylation is an increase of chemical shifts of the carbon atoms bearing trimethylsiloxy group by 1–3 ppm. In contrast, the carbon atoms engaged in glycosidic bonds (*i.e.* those carbon atoms which cannot bear a trimethylsiloxy group like C-2 of ring A in *II*,S or C-1 atoms of rings B in all disaccharides) are considerably shielded by pertrimethylsilylation. The effects on C-5 atoms are of varying magnitude and sign (in most of the compounds the effects are small and in the deshielding direction).

The observed overall effects of trimethylsilylation are not true substituent effects as the comparison unavoidably involves in addition to substituent effects also a drastic change in the solvent. Despite that, the noted different effects of pertrimethylsilylation on glycosidic and trimethylsilylated carbon atoms can be useful in structural analysis.

^{29}Si *Chemical shifts.* The continued search for trends in ^{29}Si chemical shifts has revealed only one regularity in the data of Table III: The Si-1 silicon chemical shifts in the β anomers are always the largest chemical shifts observed in any of the compounds, $\delta > 19{\cdot}8$. The same holds true only for some of the measured α anomers. No other rules for empirical assignment were found. Despite that the silicon lines are well separated, the differences in their chemical shifts are small and concentration dependent.

From the analytical point of view it is worth mentioning that the number of silicon lines was in all cases in agreement with the number of OH groups present originally in the saccharide. However, 1 : 1 anomer mixtures should be avoided when simple analytical applications are intended. The assigned ^{29}Si chemical shifts lead to the determination of the site of glycosidation similarly as in methyl glycosides.

CONCLUSION

Anomer structure of crystalline model xylooligosaccharides was determined by ^{13}C MAS NMR spectroscopy using solution data for line assignment.

Trimethylsilylation combined with ^{29}Si NMR spectroscopy is a useful method for establishing the number of hydroxyl groups in free saccharides providing the proper method is used for trimethylsilylation. The method of derivatization should exclude the possibility of anomerization and partial silylation. In order to gain further information from ^{29}Si NMR spectra it is necessary to use some of experimental methods of line assignment. The assigned ^{29}Si chemical shifts identify the sites of glycosidation which can be verified by comparison of ^{13}C chemical shifts in the parent and pertrimethylsilylated compounds.

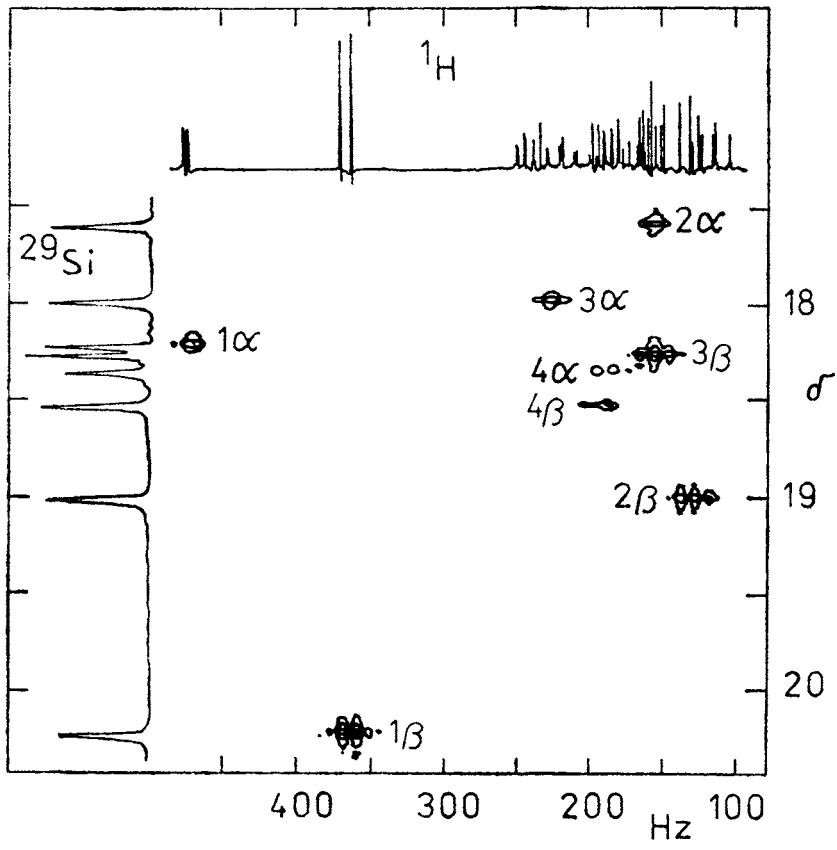


FIG. 8

^{1}H and ^{29}Si NMR spectra and their heteronuclear chemical shift correlation contour map (the same sample as in Fig. 4)

TABLE III

NMR parameter of saccharides *I*, S – *V*, S

Compound	Anomer	Ring	Coupling[a] ^3J(1, 2)	^{29}Si Chemical shifts[b]				^{13}C Chemical shifts[b]				
				Si-1	Si-2	Si-3	Si-4	C-1	C-2	C-3	C-4	C-5
I, S	α	A	3·11	18·25	17·61	18·01	18·39	94·25	74·12[c]	74·12[c]	71·90	62·33
	β	A	7·13	20·26	19·05	18·29	18·56	98·69	77·01	78·23	71·48	66·26
II, S	α	A	3·53	19·41	—	17·94	17·72[d]	93·68	76·10	75·70	73·04[d]	61·50
		B	7·44	—	19·01	18·43	18·40[d]	102·86	75·37	78·17	71·72[d]	66·23
	β	A	6·87	19·94	—	18·45[d]	18·24[d]	96·60	78·17	79·41	72·03[d]	66·13[d]
		B	7·40	—	18·26	18·43[d]	18·17[d]	101·64	75·51	78·33	71·87[d]	65·86[d]
III, S	α	A	3·20	19·02	17·06	—	18·77	94·06	75·01	76·70	69·40	62·18
		B	7·30	—	18·10	18·28	18·00	102·34	75·37	78·67	71·89	66·16
	β	A	7·14	20·20	19·02	—	19·08	98·85	77·90	80·08	69·15	66·48
		B	7·30	—	18·28	18·43	18·18	102·21	75·37	78·52	71·89	66·16
IV, S	α	A	3·09	18·28[e]	17·63[e]	18·35[e]	—	93·85	74·12[e]	72·52[e]	75·42[e]	58·92
		B	7·23	—	18·60[e]	19·08[e]	18·39[e]	101·58	77·58[d]	78·75	71·49	66·10
	β	A	7·00	19·98	19·25[d]	18·77	—	98·85	74·99[d]	76·35	75·09	62·92
		B	7·23	—	19·03[d]	18·52	18·39	101·58	77·58[d]	78·75	71·49	66·10
V, S[f]	α	A	3·27	19·20	—	19·17	—	93·65	75·46	75·46	73·45	58·32
		B	7·17	—	19·14	19·08	18·56	100·96	76·03	78·88	71·46	65·96
		C	7·45	—	18·52	18·48	18·41	102·67	75·25	78·10	71·66	66·20
	β	A	7·00	19·99	—	18·63	—	96·87	77·14	78·88	74·91	62·60
		B	7·10	—	19·08	18·52	18·45	101·28	77·19	78·28	71·83	65·96
		C	7·28	—	19·39	18·35	18·38	101·58	75·25	78·28	71·46	66·07

[a] Proton–proton coupling constants in Hz, approximate error ±0·04 Hz. [b] In δ scale, approximate error ±0·04 ppm, silicon atoms numbered as the nearest skeletal carbon atoms. [c] Not resolved at low concentration. [d] Shift assignments to a particular ring are tentative. [e] Less abundant anomer, lines not assigned by experiment. [f] Lines assigned by comparison only.

EXPERIMENTAL

Saccharides *II*—*V* were prepared previously[3,4,11,12]. Saccharide *I* was a commercial product (Lachema Brno) which was recrystallized from aqueous methanol solution similarly as was saccharide *V* for solid state measurements.

Trimethylsilylation

The first attempts at trimethylsilylation of saccharides *I*—*V* were carried out according to the described procedure[13,14] using trimethylchlorosilane (TMCS) as a silylating reagent in pyridine solution or in a mixture of pyridine and formamide. While this procedure offered fully silylated products of saccharide *I*, silylation of *II* or *III* led to a mixture of products silylated to a different extent and with a variable anomer ratio. Similar were the results when mixture of TMCS and hexamethyldisilazane (HMDS) was used. Since silylation by N,O-bis(trimethylsilyl)acetamide (BSA) gave reproducible results with satisfactory control of anomer ratio no other silylating method was sought. The adopted procedure was a modification of the procedure proposed by Klebe *et al.*[15]. Approximately 200 mg of the saccharide were placed into a dry small reaction vessel equipped with a teflon septum seal. A 200% stoichiometric excess of BSA was added through syringe. The reaction mixture was vibrationally stirred and heated at 60—70°C for about 1 h. Excess reagents were distilled off *in vacuo* under stream of dry nitrogen. In this way anomeric mixtures were obtained in which the solid state anomer was dominating. Mixtures with different anomer ratios were obtained by placing dry pyridine into the reaction vessel for a specific period of time (24 h) prior to introduction of BSA.

^{13}C CP MAS experiments were carried out at 50·3 MHz on a Bruker CXP-200 spectrometer using home-built MAS accessory of double-bullet design. 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°). RF excitation and decoupling fields corresponded to 5 μs $\pi/2$ pulse. 1 ms contact and various delay times till 30 s were used. Hexamethylbenzene CH_3 line ($\sigma = 16{\cdot}9$) served as the reference.

The NMR spectra of solutions were measured as described previously[6,7]. In short the compounds were measured in deuteriochloroform solutions using hexamethyldisilane as a reference. The spectra were measured on a Varian XL-200 spectrometer using H-2Z standard software version. The opeating frequencies were 200, 50·3, and 39·7 MHz for ^1H, ^{13}C, and ^{29}Si NMR, respectively.

IUPAC Names of the Studied Compounds

1,2,3,4-tetrakis(O-trimethylsilyl)-α-D-xylopyranose (*I*, Sα), 1,2,3,4-tetrakis(O-trimethylsilyl)-β-D-xylopyranose (*I*, Sβ), 1,3,4-tris(O-trimethylsilyl)-2-O-(2,3,4-tris(O-trimethylsilyl)-β-D-xylopyranosyl)-α-D-xylopyranose (*II*, Sα), 1,3,4-tris(O-trimethylsilyl)-2-O-(2,3,4-tris(O-trimethylsilyl)-β-D-xylopyranosyl)-β-D-xylopyranose (*II*, Sβ), 1,2,4-tris(O-trimethylsilyl)-3-O-(2,3,4-tris(O-trimethylsilyl)-β-D-xylopyranosyl)-α-D-xylopyranose (*III*, Sα), 1,2,4-tris(O-trimethylsilyl)-3-O-(2,3,4-tris(O-trimethylsilyl)-β-D-xylopyranosyl)-β-D-xylopyranose (*III*, Sβ), 1,2,3-tris(O-trimethylsilyl)-4-O-(2,3,4-tris(O-trimethylsilyl)-β-D-xylopyranosyl)-α-D-xylopyranose (*IV*, Sα), 1,2,3-tris(O-trimethylsilyl)-4-O-(2,3,4(tris-O-trimethylsilyl)-β-D-xylopyranosyl)-β-D-xylopyranose (*IV*, Sβ), 1,3-bis(O-trimethylsilyl)-2,4-bis-(O-2,3,4-tris(O-trimethylsilyl)-β-D-xylopyranosyl)-α-D-xylopyranose (*V*, Sα), 1,3-bis(O-trimethylsilyl)-2,4-bis(O-2,3,4-tris(O-trimethylsilyl)-β-D-xylopyranosyl)-β-D-xylopyranose (*V*, Sβ).

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