Carbohydrate Research, 177 (1988) 69–80
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHESIS OF BRANCHED D-XYLOFURANAN BY SELECTIVE, RING-OPENING POLYMERIZATION OF SILYLATED 1,5-ANHYDRO-β-D-XYLOFURANOSE, AND ITS CONVERSION INTO A BLOOD ANTICOAGULANT

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(Received September 18th, 1987; accepted for publication in revised form, December 5th, 1987)

ABSTRACT

Selective ring-opening polymerization and copolymerization of 1,5-anhydro-D-xylofuranose derivatives were studied. Stereoregular (1→5)-α-D-xylofuranan was synthesized from a new monomer, 1,5-anhydro-2,3-di-*O*-(*tert*-butyldimethylsilyl)-β-D-xylofuranose (**3**), with phosphorus pentafluoride and antimony pentachloride as catalysts in dichloromethane. Copolymerization of **3** with 1,5-anhydro-2,3-di-*O*-benzyl-β-D-xylofuranose, and desilylation of the copolymer with tetrabutylammonium fluoride gave a partially benzylated stereoregular (1→5)-α-D-xylofuranan which was glycosylated with 3,4,6-tri-*O*-acetyl-β-D-mannose 1,2-(methyl orthoacetate) to give a branched polymer. Deprotection of the benzylated polymer having D-mannosyl branches with sodium in liquid NH_3 gave (1→5)-α-D-xylofuranans having 2- or 3-*O*-α-D-mannopyranosyl branches. Sulfation of the free D-xylofuranans was achieved with piperidine sulfate. The sulfated D-xylofuranan having branches showed high blood-anticoagulant activity.

INTRODUCTION

Sulfated polysaccharides are of interest because of their blood-anticoagulant activity[1] and because, at least with synthetic D-ribofuranan and D-xylofuranan, activity has been shown against an acquired immune deficiency syndrome (AIDS)-associated retrovirus[2].

Ring-opening polymerization of a 1,5-anhydropentofuranose (≡ 1,4-anhydropentopyranose) can, in principle, lead to the formation of polymers containing four different monomeric units, α- or β-furanose and α- or β-pyranose. Stereoselectivity is influenced by the configuration of the monomer, the substituent groups, the choice of catalyst, and the reaction conditions. Most commonly, stereoselective polymerization (as in the case of 1,5-anhydro-D-xylofuranose[3] and 1,5-anhydro-L-arabinofuranose[4] derivatives) favors α-furanan formation, although alkylidene[5,6]

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0008-6215/88/$ 03.50

and *tert*-butyldimethylsilyl[7] derivatives of 1,5-anhydro-D-ribofuranose yielded (1→4)-β-D-ribopyranan. The β-(1→4)-pyranan structure is characteristic of naturally occurring cellulose and D-xylan, but has not yet been prepared synthetically from D-glucose or D-xylose, respectively.

We have successfully synthesized stereoregular polysaccharides that have biological activities by ring-opening polymerization of anhydro sugars, and we now report the stereoselective ring-opening polymerization and copolymerization of a new monomer, namely, 1,5-anhydro-2,3-di-*O*-(*tert*-butyldimethylsilyl)-β-D-xylofuranose (**3**), its glycosylation with D-mannosyl units to form a D-mannosyl-branched D-xylofuranan, the deprotection and sulfation of these products, and preliminary, *in vitro* studies of their blood-anticoagulant activity.

RESULTS AND DISCUSSION

*Polymerization of 1,5-anhydro-2,3-di-*O-(tert-*butyldimethylsilyl)-β-*D-*xylofuranose* (**3**). — The starting monomer, **3** was synthesized by pyrolysis of D-xylose[8] (**1**) and subsequent protection of the hydroxyl groups of the product (**2**) to give **3** or **4**. Polymerization of **3** with cationic catalysts at low temperature gave high-molecular-weight polymers (**5**). The results of some polymerizations are summarized in Table I. Because there are two possible ring-opening modes of scission, of the 1,4- and the 1,5-ring, and two steric structures, four possible monomeric units (*i.e.*, 1,5-α-furanose, 1,5-β-furanose, 1,4-α-pyranose, and 1,4-β-pyranose), may be formed in the polymer backbone by polymerization. However, as is shown later, in the Figures, 1,5-α- and 1,5-β-furanose units were mainly formed.

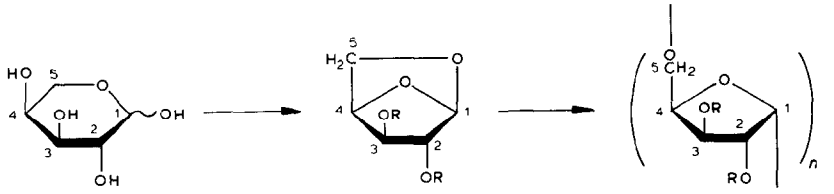
Phosphorus pentafluoride and antimony pentachloride as catalysts at −78 and −60° gave from **3** a completely stereoregular 2,3-di-*O*-(*tert*-butyldimethylsilyl)-(1→5)-α-D-xylofuranan (**5**) in 86.8 and 44.1% yield, respectively (Nos. 2 and 3 in

TABLE I

RING-OPENING POLYMERIZATION OF 1,5-ANHYDRO-2,3-DI*O*-(*tert*-BUTYLDIMETHYLSILYL)-β-D-XYLOFURANOSE

No.[a]	*Catalyst*		*Temperature (°)*	*Time (h)*	*Yield (%)*	$[\alpha]_D^{25}$[b] *(degrees)*	$\overline{M}_n$[c] $\times 10^4$	*(1→5)-α-f*[d] *(%)*
	Formula	*mol% to monomer*						
1	PF_5	3	−60	2	78.4	+107.5	1.6	91.5
2	PF_5	2	−78	0.5	86.8	+113.6	2.4	100.0
3	$SbCl_5$	3	−60	5	44.1	+110.2	1.4	100.0
4	$SbCl_5$	3	−20	5	37.1	+23.5	0.7	49.6
5	$BF_3 \cdot OEt_2$	3	−20	24	9.5	+103.7	1.8	91.6

[a]Monomer concentration: 40–50% (w/v); solvent: CH_2Cl_2. [b]Measured in $CHCl_3$ (*c* 1). [c]Determined by g.p.c. [d]Calculated from the ^{13}C-n.m.r. spectrum.



1 D-xylose

2 R = H
3 R = $(Me_3C)Me_2Si$
4 R = $PhCH_2$

5 R = $(Me_3C)Me_2Si$
6 R = $PhCH_2$
7 R = H
8 R = SO_3Na or H

Table I). The stereoregularity of the polymer obtained decreased with increasing polymerization temperature (Nos. 1 and 4 in Table I). With boron trifluoride etherate as the catalyst, polymerization of the benzylated monomer **4** gave a stereoregular polymer (**6**) in high yield[2]. However, polymerization of the silylated monomer **3** under the same conditions afforded a slightly less stereoregular polymer (α-content 91.6%) in a very low yield (9.5%, No. 5 in Table I). The stereoregular polymers had high positive values of specific rotation of +113.6° and +110.2°, indicating that the polymerization was achieved with α-specificity. The number-average molecular weight ($\overline{M}_n$) of the polymers ranged from 0.7×10^4 to 2.4×10^4.

Fig. 1 shows the 67.8-MHz, ^{13}C-n.m.r. spectra of the silylated polymers prepared by use of (A) $SbCl_5$ at −20°, (B) PF_5 at −60°, and (C) PF_5 at −78°. In the spectrum of a highly stereoregular, silylated D-xylofuranan (see Fig. 2C), there were five peaks (C-1, 101.9; C-2, 77.9; C-3, 76.0; C-4, 76.5; and C-5, 68.0 p.p.m.) in the range of 68 to 102 p.p.m. However, in spectrum 2A, a number of overlapping absorptions appeared, indicating nonstereoregularity of the polymer backbone. The C-1 atom in spectrum 2B showed two peaks, at 101.9 and 109.0 p.p.m., due to the (1→5)-α and (1→5)-β configurations. The formation of stereoregular polymer suggests that the polymerization proceeds *via* a trialkyloxonium ion intermediate which reacts stereospecifically to lead to α-linkages[2].

Copolymerization of **3** *and* **4**. — Because the homopolymerization of each monomer, **3** and **4**, with phosphorus pentafluoride as the catalyst gave a stereoregular (1→5)-α-D-xylofuranan, copolymerization of **3** and **4** was carried out with 3 mol% phosphorus pentafluoride at −60°. Copolymers were obtained in high yield. Table II summarizes the results of copolymerization.

High positive specific rotations ranging from +102° to +152° were observed for all copolymers obtained at various mole fractions of **3** and **4**. The mole fractions of **3** and **4** units in the copolymers were almost the same as the mole fractions of the monomers in the feed, which were calculated from the integrated area of the proton resonances of the benzyl (**4**) and dimethyl (**3**) groups which appear at 7.22 and 0.86 p.p.m., respectively. Antimony pentachloride as catalyst gave a copolymer with low stereoregularity ($[\alpha]_D^{25}$ +102°) in 67% yield (No. 5 in Table II).

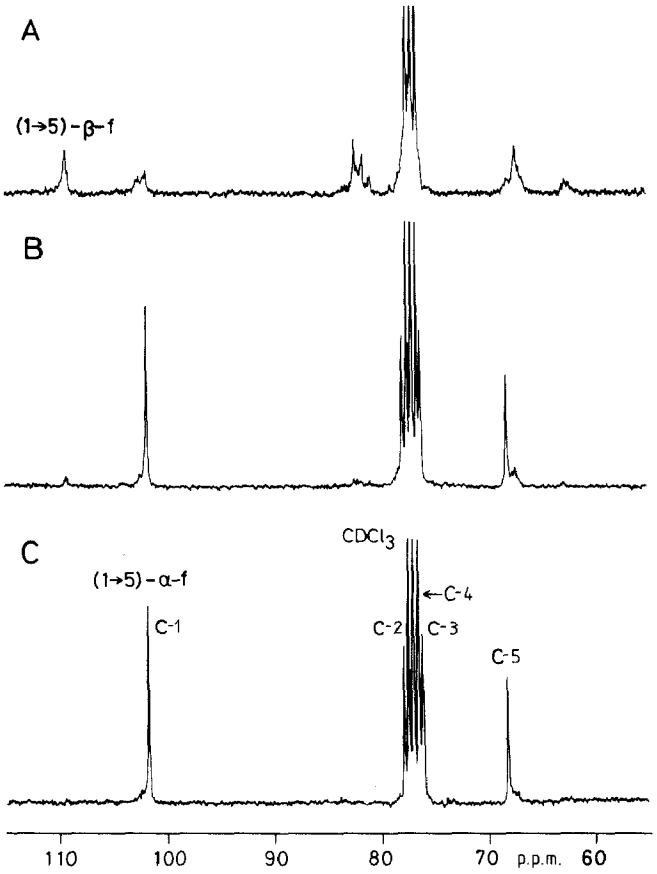


Fig. 1. ^{13}C-N.m.r. spectra of (A) poly[1,5-anhydro-2,3-di-*O*-(*tert*-butyldimethylsilyl)-β-D-xylofuranose] prepared by use of $SbCl_5$ at −20° and (B) prepared by use of PF_5 at −60°, and (C) 2,3-di-*O*-(*tert*-dimethylbutylsilyl)-(1→5)-α-D-xylofuranan.

TABLE II

RING-OPENING COPOLYMERIZATION OF **3** AND **4**

No.[a]	**3** *Feed (g)*	**4** *Feed*		*Catalyst*	*Yield (%)*	$[\alpha]_D^{25}$[b] *(degrees)*	$\overline{M}_n$[c] $\times 10^4$	*(1→5)-α-f*[d] *(%)*	*Mole fraction units of* **4** *in copolymer*
		g	*mole fraction*						
1	0.35	0.15	0.33	PF_5	93.5	+126.0	2.2	100	0.37
2	0.25	0.25	0.47	PF_5	87.5	+132.8	3.6	100	0.51
3	0.15	0.35	0.73	PF_5	89.3	+152.9	2.7	100	0.73
4	0.10	0.40	0.82	PF_5	92.1	+138.2	3.9	100	0.78
5	0.15	0.35	0.73	$SbCl_5$	67.0	+102.1	1.5	87	0.76

[a]Monomer concentration: 50% (w/v); solvent: CH_2Cl_2; catalyst: PF_5, 3 mole%, $SbCl_5$, 2 mol%; time: 0.3–0.5 h; temp.: −60°. [b]Measured in $CHCl_3$ (*c* 1). [c]Determined by g.p.c. using THF as the solvent. [d]Calculated from the ^{13}C-n.m.r. spectrum.

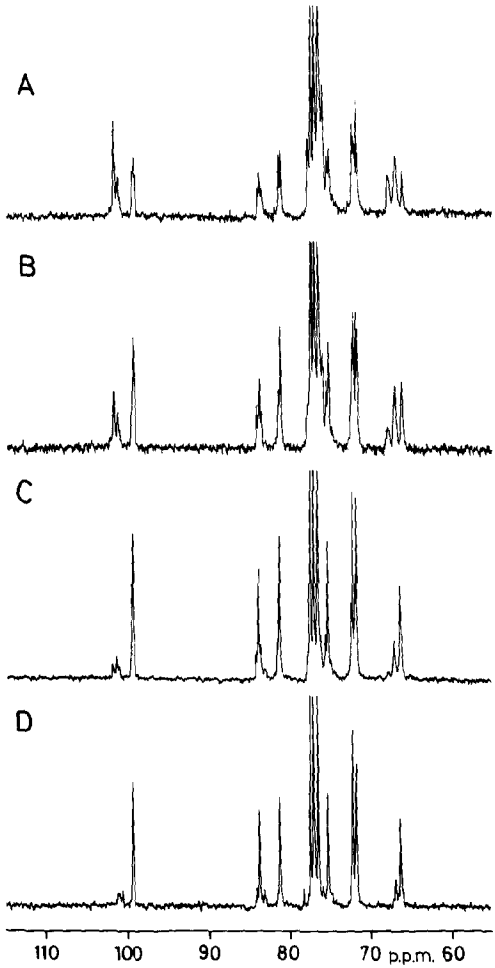


Fig. 2. ^{13}N.m.r. spectra of the copolymer prepared by PF_5 at −60°. [(A) **3**:**4** = 7:3, (B) = 1:1, (C) = 3:7, and (D) 1:4.]

Fig. 2 shows the ^{13}C-n.m.r. spectra of the copolymers. No absorption due to (1→5)-β-furanose units appeared at ~110 p.p.m. The (1→5)-α-linked C-1 atoms due to the units from **3** and **4** in the polymer backbone appeared at 99 and 101 p.p.m., respectively. These copolymers were composed only of the (1→5)-α-linked structure.

Deprotection. — Desilylation of the homopolymer and the copolymer gave the corresponding free D-xylofuranan and partially benzylated xylofuranan (Nos. 2 and 9 in Table III), respectively. The specific rotation of free (1→5)-α-D-xylofuranan had a very high positive value (+156.4°). The 2D n.m.r. spectra of the polymer are shown in Fig. 3.

TABLE III

DEPROTECTION OF SYNTHETIC SUBSTITUTED POLYSACCHARIDE TO AFFORD FREE POLYSACCHARIDE

Polymer				*Free polysaccharide*					
No.	$[\alpha]_D^{25}$[b] *(degrees)*	$\overline{M}_n$ $\times 10^4$	$\overline{d.p.}$	*No.*	*Yield (%)*	$[\alpha]_D^{25}$[c] *(degrees)*	$\overline{M}_n$[d] $\times 10^4$	$\overline{d.p.}$	*(1→5)-α-f (%)*
1[a]	+113.0	2.4	66	4	99	+156.4	1.0	73	100
2[a]	+138.2	3.9		5	90	[e]	[e]		100
3[f]	+131.6	1.5	42	6	82	+143.9	0.7	42	100

No. 1: 2,3-di-O-*(tert*-butyldimethylsilyl)-(1→5)-α-D-xylofuranan.
No. 2: Stereoregular copoly [**3** (2): **4** (8)].
No. 3: Benzylated D-xylofuranan with D-mannopyranosyl branches.
[a]With Bu_4NF in THF; time: 1.5 h; temp.: reflux. [b]Measured in $CHCl_3$ (*c* 1). [c]Measured in H_2O (*c* 1). [d]Determined by g.p.c. using phosphate buffer as solvent. [e]Not determined, because of insolubility in H_2O. [f]With Na/liq. NH_3; time: 30 min., temp.: −78°.

As the H-1 atom is an acetal proton, the H-1 absorption appears at the most downfield position (4.65 p.p.m.) of all of the polymer backbone absorptions. Taking the H-1 resonance as the reference, it was concluded that the resonance of H-2 must be located at 3.65 p.p.m. (by observation of the cross peaks with H-1). Proceeding in the same manner, all proton absorptions of (1→5)-α-D-xylofuranan were completely assigned. The assignment of ^{13}C absorptions was easily determined from the C–H COSY spectrum (see Fig. 3B).

Glycosylation of partially benzylated D-*xylofuranan.* — Previously glycosylation of 2,4-di-*O*-benzylated dextran by the orthoester method had given (1→6)-α-D-glucopyranan having 3-*O*-α-D-mannopyranosyl branches[9], and so the same method for glycosylation was used in this work. Fig. 4 shows the scheme of glycosylation and some physical properties of the branched polymers. Desilylation of the copolymer (**3**:**4** = 1:4 in the feed) gave, in 90% yield, a partially benzylated D-xylofuranan which was glycosylated with 3,4,6-tri-*O*-acetyl-β-D-mannose 1,2-(methyl orthoacetate), using 2,6-lutidinium perchlorate as catalyst in 1,2-dichlorobenzene; this gave an α-D-mannosylated xylofuranan having $[\alpha]_D^{25}$ +131.6°.

Debenzylation and deacetylation were performed with sodium in liquid NH_3, to give free polysaccharide having α-D-mannosyl branches, $[\alpha]_D^{25}$ +143.9°, in 82% yield (No. 3 in Table III).

Fig. 5 shows the ^{13}C-n.m.r. spectra of solutions in D_2O of (A) (1→5)-α-D-xylofuranan with 2(or 3)-*O*-α-D-mannosyl branches, and (B) stereoregular (1→5)-α-D-xylofuranan. In spectrum 5A, small absorptions due to D-mannopyranosyl side-chains appeared at 63.5, 72–84, and 100 p.p.m. By comparing the integrated areas of the C-1 absorptions at 104 p.p.m. (C-1 of D-xylose unit) and 100 p.p.m., it was possible to calculate the mole fraction of branches. The degree of branching was estimated to be 10% in the main chain of the polymer.

Sulfation of free polysaccharides, and blood-anticoagulant activity. — The sul-

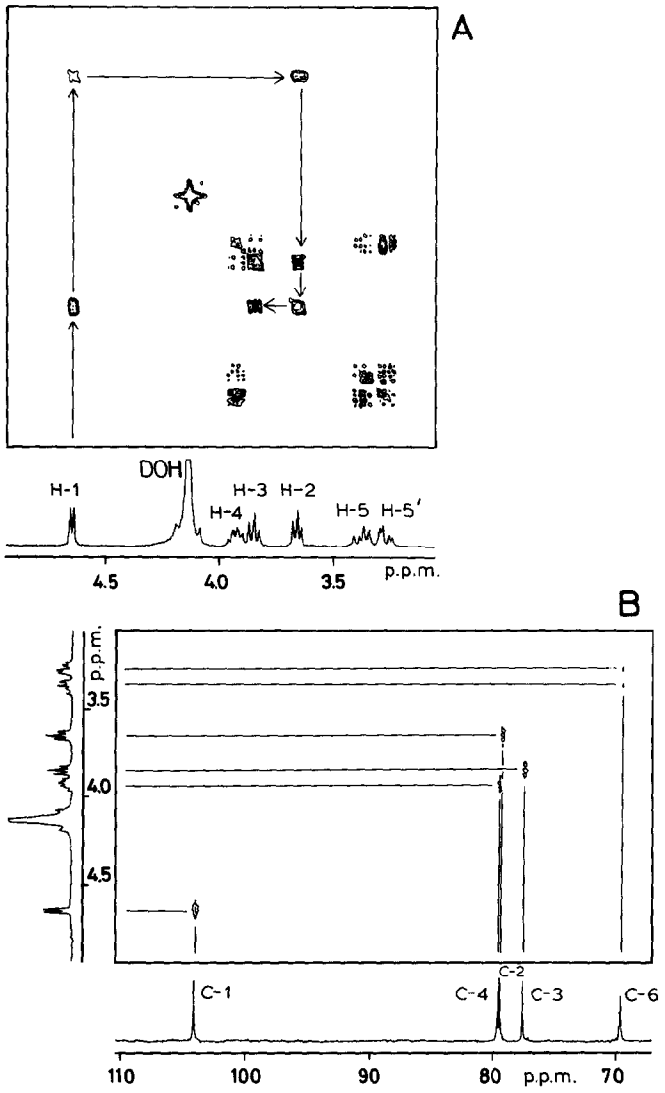
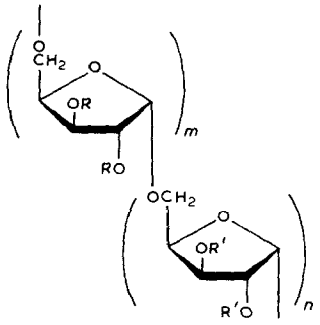


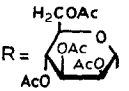
Fig. 3. Contour plot of 2D (A) H–H COSY and (B) C–H COSY spectra of stereoregular (1→5)-α-D-xylofuranan.

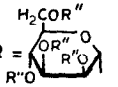
fation of (1→5)-α-D-xylofuranan and D-mannosylated D-xylofuranan was conducted with piperidine sulfate in dry Me_2SO. For the D-xylofuranan and branched D-xylofuranan, the number of sulfate groups per sugar residue was 1.0 and 1.4, respectively, based on elemental analysis for sulfur.

Fig. 6 shows the ^{13}C-n.m.r. spectra of (A) sulfated (1→5)-α-D-xylofuranan and (B) sulfated D-xylofuranan having D-mannosyl branches. Absorptions due to the branching (see Fig. 6B) were not explicit in the spectrum, because of the low



9 R = $(Me_3C)Me_2Si$ 10 R = H
R' = $PhCH_2$ R' = $PhCH_2$

11 R =  or H $[\alpha]_D = +131.6°$ $\bar{M}n = 1.5 \times 10^4$
R' = $PhCH_2$

12 R =  or H $[\alpha]_D = +143.9°$ $\bar{M}n = 0.7 \times 10^4$
R' = R'' = H

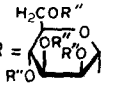
13 R =  or H $[\alpha]_D = +77.0°$ $\bar{M}n = 0.7 \times 10^4$
R' = R'' = SO_3Na or H

Fig. 4. Steps in the synthesis of sulfated D-xylofuranan having 3-*O*-α-D-mannopyranosyl branches.

degree of branching (~10%). However, it was decided that the absorptions due to D-mannosyl branches appeared in the range of 72 to 76 p.p.m. and 97 to 100.5 p.p.m., by comparison with Fig. 6A.

The blood-anticoagulant activity *in vitro* of the sulfated D-xylofuranans was determined. It is well known that heparin is a sulfated glucosaminoglycuronan having very high blood-anticoagulant activity (169 units/mg), and the active pentasaccharide moiety has[10] a high affinity to antithrombin III (AT III). On the other hand, dextran [(1→6)-α-D-glucopyranan] sulfate does not have an affinity to AT III, but binds strongly to thrombin[11].

Previously, it was reported[1] that a sulfated D-xylofuranan ($\overline{M}_n$ 12,000) with high molecular weight had high anticoagulant activity (69 units/mg). Such a high-molecular-weight polymer might have cytotoxicity. Thus, it might be desirable to synthesize sulfated polysaccharides with high activity but low molecular weight, *i.e.*, <10,000.

The blood-anticoagulant activity test *in vitro* was performed by using bovine

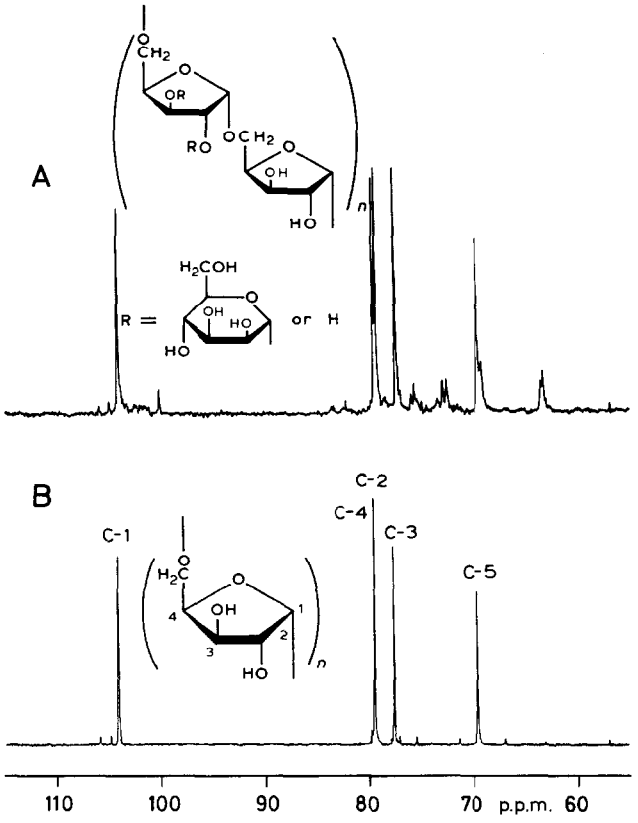


Fig. 5. ^{13}C-N.m.r. spectra of (A) (1→5)-α-D-xylofuranan having α-D-mannpyranosyl branches and (B) stereoregular (1→5)-α-D-xylofuranan.

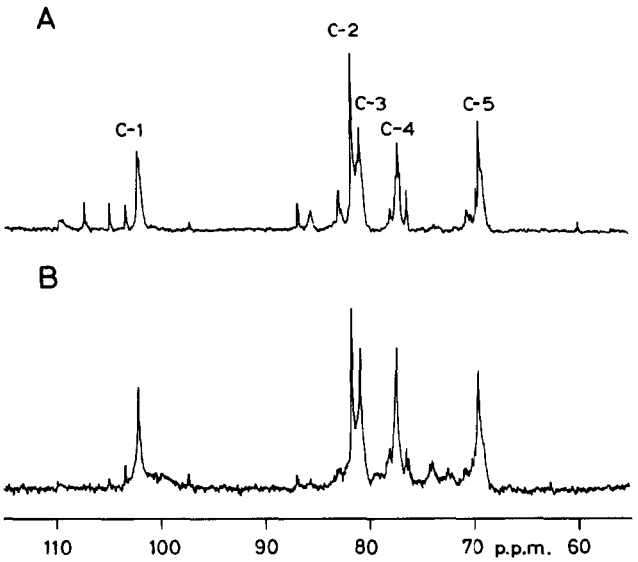


Fig. 6. ^{13}C-N.m.r. spectra of (A) sulfated (1→5)-α-D-xylofuranan and (B) sulfated α-D-xylofuranan having α-D-mannopyranosyl branches.

TABLE IV

ANTICOAGULANT ACTIVITY (A.A) *in vitro* OF SULFATED POLYSACCHARIDES

No.	*Backbone structure*	*Sulfur content (%)*	*Number of sulfated groups per sugar unit*[b]	$[\alpha]_D^{25}$[a] *(degrees)*	$\overline{M}_n \times 10^4$	*A.A. (units/mg)*
1	(1→5)-α-D-xylofuranan	14.22	1.0	+93.1	0.7	28
2	(1→5)-α-D-xylofuranan [(1→3)-α-D-mannopyranosyl, 0.1 branch/sugar unit]	16.33	1.4[c]	+77.0	0.7	50

Commercial dextran sulfate NC-1032: A.A. = 20.6 units/mg.
[a]Measured in H_2O (*c* 1). [b]Calculated from percentage of sulfur. [c]Taking into account the ratio of sulfur to carbon, the degree of substitution is 1.4.

plasma according to a modification of a procedure described in the United States Pharmacopoeia[12]. Table IV summarizes the results of the anticoagulant activity compared with that of commercial dextran sulfate (Meito Sangyo, NC-1032, 20.6 units/mg). It was found that the sulfated D-xylofuranan with D-mannosyl branches shows an activity (50 units/mg) higher than that of the stereoregular sulfated D-xylofuranan (28 units/mg). It has been found that the anticoagulant activity of the sulfated polysaccharides depends on the stereoregularity of the polymer backbone and the degree of the sulfation of the polymer. The sulfation of natural, branched dextran (B-742) and 3-*O*-D-mannosyldextran gave polysaccharides with very high anticoagulant activity, 74 and 100 units/mg, respectively. These results suggest that the branched structure of polysaccharides is also an important factor as regards the blood-anticoagulant activity of heparinoids.

EXPERIMENTAL

General methods. — Specific rotations were measured for solutions in $CHCl_3$ by means of a Perkin–Elmer 241 polarimeter. ^1H- (270 MHz), ^{13}C- (67.8 MHz), and 2D n.m.r. spectra were recorded with a JEOL JMN GX-270 spectrometer. Molecular weights were determined by gel-permeation chromatography (g.p.c.), and calculated by using polystyrene standards and dextran standards as references for THF-soluble and water-soluble compounds, respectively.

*1,5-Anhydro-2,3-di-*O*-(*tert*-butyldimethylsilyl)-β-D-xylofuranose* (**3**). — This compound was prepared by silylating 1,5-anhydro-β-D-xylofuranose (**2**) according to the method of Hakimelahi and co-workers[13]. 1,5-Anhydro-β-D-xylofuranose (**2**; 10 g, 76 mmol) was added to a solution in dry THF (115 mL) of dry pyridine (35 mL), and silver nitrate (40 g, 0.24 mol). Then, (*tert*-butyldimethyl)chlorosilane (34.8 g, 0.23 mol) was added, and the mixture was stirred for 20 h at room temperature. After filtration, the filtrate was poured into 5% aqueous $NaHCO_3$ solution, the mixture extracted with $CHCl_3$, and the extract dried (anhydrous Na_2SO_4), and

evaporated. The residue was purified by chromatography on a column of silica gel with 12:1 hexane–ethyl acetate, to give pure **3** (10.2 g) in 37.1% yield; $[\alpha]_D^{25}$ +15.7° (*c* 1, $CHCl_3$).

Polymerization. — The polymerization was conducted at 1.33–13.33 mPa in sealed glass ampoules as described previously[14]. Polymers were purified by reprecipitation using a chloroform–methanol system, and then freeze-dried from benzene.

Deprotection. — The deprotection of the silylated and benzylated polymers was carried out by means of tetrabutylammonium fluoride in THF, or sodium in liquid ammonia, as previously described[3].

*Glycosylation of partially benzylated (1→5)-α-*D*-xylofuranan* (**10**) *with 3,4,6-tri-O-acetyl-β-*D*-mannose 1,2-(methyl orthoacetate).* — To **10** (0.35 g) in chlorobenzene (50 mL) was added 3,4,6-tri-*O*-acetyl-β-D-mannose 1,2-(methyl orthoacetate) (1.02 g). After the mixture had been stirred for 30 min at the boiling temperature, and partly distilled to remove small amounts of water with the chlorobenzene azeotrope, 2,6-lutidinium perchlorate (3 mg) was added. The stirring was continued for another 20 min. under reflux, and then the solution was poured into water. The organic layer was combined with a chloroform extract of the water, washed with water, dried (anhydrous sodium sulfate), and evaporated, and the residue dissolved in chloroform (10 mL). The polymer was purified by precipitation from chloroform solution with methanol, followed by freeze-drying from benzene; yield of **11**, 0.36 g, $[\alpha]_D^{25}$ +131.6° (*c* 1, $CHCl_3$); $\overline{M}$ 1.5 × 10^4.

Sulfation. — The sulfation was carried out by using piperidine sulfate according to the method of Nagasawa and Yoshidome[15]. A mixture of (1→5)-α-D-xylofuranan (0.1 g), piperidine sulfate (0.8 g, 5 mmol), and dry Me_2SO (40 mL) was stirred for 30 min at 65–70°. After cooling, saturated $NaHCO_3$ solution (50 mL) was added, and the mixture was dialyzed with running saturated $NaHCO_3$ solution (18 L) for 6 h, and then with running ion-exchanged water for 3 days in a cellulose tube. After the aqueous solution had been concentrated to 5–10 mL under diminished pressure below 35°, a solution of the residue in water was freeze dried, to give sulfated D-xylofuran, yield 0.09 g, $[\alpha]_D^{25}$ +93.1°, $\overline{M}_n$ 0.5 × 10^4. Sulfation of D-xylofuranan with D-mannosyl branches was performed by the same method.

Anticoagulant activity test. — The blood-anticoagulant activity test *in vitro* was carried out at 37° by using bovine plasma according to a modification of the procedure given in the United States Pharmacopoeia[12]. To 9 clean, 15 × 150-mm test-tubes were added graded amounts of 0.016% sulfated D-xylofuranan solution in physiological saline solution (0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mL, respectively). Physiological saline solution was added to each tube to make the total volume to 0.8 mL. Then 1.0 mL of bovine plasma which had been pretreated with sodium citrate was added to each tube, and 0.2 mL of 2% (w/v) aqueous calcium chloride was immediately added. In the same manner, standard dextran sulfate (Meito Sangyo, NC-1032, 20.6 units/mg) was set up in a series. After addition of the calcium chloride, the extent of clotting in each tube was determined by

recognizing three grades (0.25, 0.50, and 0.75) between zero and full clotting (1.0). The anticoagulant activity (P) of the sulfated D-xylofuranan synthesized was calculated from the following equations.

$$x_s = x_i + (y_i - 0.5)(x_{i+1} - x_i)/(y_1 - y_{i+1}) \quad (1)$$

(or x_u)

$$M = x_s - x_u + \log R \quad (2)$$

$$P = \text{antilog } M \quad (3)$$

where x_s and x_u are the concentration of the sulfated standard and the sample when the grade of clotting is 0.5, x_i and x_{i+1} are the average log values of the concentration of the sulfated sample or standard dextran sulfate in the 3 successive tubes, y_i and y_{i+1} are the average log values of the clotting of the sulfated sample or standard dextran sulfate of the 3 successive tubes, M is the log potency of the sulfated sample, and R is a value of the anticoagulant activity of the standard dextran sulfate (20.6 units/mg), respectively.

ACKNOWLEDGMENTS

We are grateful to Messrs. K. Iwata, H. Hokkoku, and T. Ogawa of Meito Sangyo Co., Ltd., for valuable discussions and for gifts of bovine plasma and standard dextran sulfate NC-1032.

REFERENCES

1 K. HATANAKA, T. YOSHIDA, S. MIYAHARA, T. SATO, F. ONO, T. URYU, AND H. KUZUHARA, *J. Med. Chem.*, 30 (1987) 810–814.
2 H. NAKASHIMA, O. YOSHIDA, T. S. TOCHIKURA, T. YOSHIDA, T. MIMURA, Y. KIDO, Y. MOTOKI, Y. KANEKO, T. URYU, AND N. YAMAMOTO, *Lancet, Jpn. J. Cancer Res.*, 78 (1987) 1164–1168.
3 T. URYU, J. YAMANOUCHI, S. HAYASHI, H. TAMAKI, AND K. MATSUZAKI, *Macromolecules*, 16 (1983) 320–326.
4 T. YOSHIDA, M. KIDA, AND T. URYU, *Polym. J.*, 19 (1987) 923–931.
5 T. URYU, K. KITANO, K. ITO, J. YAMANOUCHI, AND K. MATSUZAKI, *Macromolecules*, 14 (1981) 1–90.
6 T. URYU, J. YAMANOUCHI, T. KATO, S. HIGUCHI, AND K. MATSUZAKI, *J. Am. Chem. Soc.*, 105 (1983) 6865–6871.
7 T. URYU, M. YAMANAKA, M. DATE, AND M. OGAWA, *Macromolecules*, in press.
8 P. KÖLL, S. DEYHIM, AND K. HEYNS, *Chem. Ber.*, 106 (1973) 3565–3570.
9 T. URYU, M. YAMANAKA, M. HENMI, K. HATANAKA, AND K. MATSUZAKI, *Carbohydr. Res.*, 157 (1986) 157–169.
10 I. BJÖRK AND U. LINDAHL, *Mol. Cell. Biochem.*, 48 (1982) 161–182.
11 G. OSHIMA, T. NAGAI, AND K. NAGASAWA, *Thromb. Res.*, 35 (1984) 601–611.
12 U.S. Pharmacopeia National Formulary, USP XXI, 1985.
13 G. H. HAKIMELAHI, Z. A. PROBA, AND K. K. OGILVIE, *Tetrahedron Lett.*, (1981) 4775–4778.
14 T. URYU, H. TACHIKAWA, K. OHAKU, K. TERUI, AND K. MATSUZAKI, *Makromol. Chem.*, 178 (1977) 1929–1940.
15 K. NAGASAWA AND H. YOSHIDOME, *Chem. Pharm. Bull.*, 17 (1969) 1316–1323.