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Anti-HIV activity of sulfonated arabinofuranan and xylofuranan

T. Yoshida[a,*], B.W. Kang[a], K. Hattori[a], T. Mimura[b], Y. Kaneko[b], H. Nakashima[c], M. Premanathan[c], R. Aragaki[c], N. Yamamoto[d], T. Uryu[e]

[a]*Polymer Science Department, Graduate School of Science, Hokkaido University, Kita-10 Nishi-8, Kita-ku, Sapporo 060-0180, Japan*
[b]*Ajinomoto Company Inc., Chuo-ku, Tokyo 108-0031, Japan*
[c]*Department of Microbiology and Immunology, Kagoshima University Dental School, Kagoshima 890-0075, Japan*
[d]*Tokyo Medical and Dental University School of Medicine, Bunkyo-ku, Tokyo 113-0034, Japan*
[e]*Department of Environmental and Material Engineering, Teikyo University of Science and Technology, Uenohara, Yamanashi 409-0193, Japan*

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Abstract

In order to elucidate the relationship between the structure and anti-HIV activity of sulfonated polysaccharides, two anhydro-pentose monomers having different configurations of substituents at C2 and C3 positions from D-ribose, 1,4-anhydro-2,3-di-*O-tert*-butyldimethylsilyl-α-L-arabinofuranose and -D-xylofuranose, were polymerized, respectively, with PF_5 catalyst to give (1 → 5)-α-L-arabinofuranan and (1 → 5)-α-D-xylofuranan derivatives. After deprotection and subsequent sulfonation, sulfonated arabinofuranan and xylofuranan having various molecular weights and degree of sulfonation were obtained. Commercially available xylan ((1 → 4)-β-D-xylopyranan) was also sulfonated to give sulfonated xylan. These sulfonated pentosans having higher degrees of sulfonation of 1.4–1.9 (maximum, 2) were found to have potent anti-HIV activities in the EC_{50} of 0.1–0.6 μg/ml, which were as high as those of sulfonated ribofuranans and ribopyranans reported previously, suggesting that the sulfonated pentofuranan-type polysaccharides having a high degree of sulfonation had potent anti-HIV activity. In addition, the sulfonated arabinofuranan and xylofuranan had higher blood anticoagulant activities, 36 and 28 unit/mg, respectively, than those of sulfonated xylan with six-membered pentopyranosidic unit, 14–15 unit/mg, suggesting that the flexible furanan-type backbone structure worked effectively in the high activity.

Keywords: Sulfonated arabinofuranan; Xylofuranan; Xylopyranan; Ring-opening polymerization; Anti-HIV activity; Blood anticoagulant activity

1. Introduction

Acquired immunodeficiency syndrome (AIDS) is a disease of the immune system and caused by a retrovirus, human immunodeficiency virus (HIV) (Broder, 1987; Dalgleish & Weiss, 1990). Sulfonated polysaccharides are one of the potent anti-HIV compounds (Uryu, 1993). In particular, curdlan sulfonate synthesized by sulfonation of naturally occurring linear 1,3-β-D-glucan, curdlan, has the potent inhibitory effect on HIV multiplication without serious side-effects and toxicity in vitro (Yoshida et al., 1990) and in vivo (Kaneko et al., 1990). In 1994, the phase I/II test of curdlan sulfonate to patients indicated that the number of CD4 positive T4 lymphocytes in the blood increased (Gordon, Guralnik, Kaneko, Mimura, Baker & Lang, 1994). The inhibitory mechanism of sulfonated polysaccharides has been assumed to inhibit the binding of HIV to CD4 positive cells (Mitsuya, Yarchoan & Broder, 1990), that is, by the ionic interactions of negatively charged sulfonate groups in the polysaccharide with the V3 loop region (Este et al., 1997; Jagodzinski et al., 1994, 1996) and/or with positively charged amino acid residues of no. 506 through 518 in gp120 (Uryu et al., 1992), an envelope glycoprotein of HIV, the conformation of gp120 changed to reduce the infectivity to the CD4 receptor (Jeon, Katsuraya, Kaneko, Mimura & Uryu, 1997). These electrostatic mechanisms are similar to those of a blood anticoagulant heparin with an anticoagulant factor antithrombin III (Oscarsson, Pejler & Lindahl, 1990; Villanueva, 1984).

It is important for the basic elucidation of inhibitory mechanisms and for the developments of effective HIV drugs to know the relationship between structure and biological activity of sulfonated polysaccharides. The biological activities of sulfonated polysaccharides originated strongly from both degree of sulfonation and molecular weights, and decreased slightly with a decrease of the stereoregularity of polysaccharide main chain (Choi et al., 1997; Hatanaka et al., 1987). For sulfonated ribofuranans

* Corresponding author. Tel.: + 81-11-706-2986; fax: + 81-11-706-2986.
E-mail address: yoshida@sci.hokudai.ac.jp (T. Yoshida).

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Table 1
Ring-opening polymerization of 1,4-anhydro-2,3-di-*O*-*tert*-butyldimethylsilyl-α-L-arabinopyranose (ADSA) and -α-D-xylopyranose (ADSX)

No	Monomer[a]	Catalyst (mol%)		Time (h)	Yield (%)	$\bar{M}_n \times 10^{3}$ [b]	$[\alpha]_D^{25}$ (degree)[c]	α-Content (%)[d]
1	ADSA	PF_5	2	2	84	50.0	−20.4	95
2	ADSX	PF_5	3	1	87	16.0	+114.1	98
3	ADSX	PF_5	3	1.5	79	14.0	+107.5	97
4	ADSX	PF_5	3	2	73	13.3	+102.8	94

[a] Monomer, 0.5 g; solvent, CH_2Cl_2; 1.0 ml; temp., −60°C.
[b] Determined by GPC.
[c] Measured in $CHCl_3$ (c 1%).
[d] From ^{13}C NMR spectrum.

which have *cis*-substituents in every sugar unit, higher degrees of sulfonation caused higher activities (Hatanaka et al., 1991). Although sulfonated 2- or 3-deoxyribofuranan having degree of sulfonation of 1.0 had no anti-HIV activity, the activity increased with a decrease of the deoxy unit in the copoly(deoxyribose-ribose) main chain (Choi et al., 1997), suggesting that the number of sulfonate group plays an important role in the anti-HIV activity. Sulfonated ribofuranans having number average molecular weights less than 6×10^3 had low anti-HIV activity (Choi et al., 1996).

In this study, (1 → 5)-α-L-arabinofuranan, (1 → 5)-α-D-xylofuranan, and (1 → 4)-β-D-xylopyranans (xylans) having different configurations of substituents from both (1 → 5)-α-D-ribofuranan (Yoshida, Katayama, Inoue & Uryu, 1992) and (1 → 4)-β-D-ribopyranan (Yoshida et al., 1994) previously reported, were sulfonated and then their biological activities such as anti-HIV and blood anticoagulant activities were discussed to elucidate the relationship between their structures and biological activities. In addition, ^{13}C NMR spectra of pentosan derivatives were measured in detail.

2. Materials and method

2.1. Measurement

^{13}C NMR spectra (100 and 67.8 MHz) were recorded at 21°C on a JEOL α-400 and GX-270 spectrometers, respectively, in $CDCl_3$ or D_2O solution. Tetramethylsilane (TMS) or 3-(trimethylsilyl)-propanesulfonic acid sodium salt (DSS) was used as an internal standard. Molecular weights were determined by an organic phase GPC in THF (column: TOSOH TSK-gel, G3000H_{XL}, G4000H_{XL}, and G5000H_{XL}, 7.6 mm × 600 mm × 3) by the use of polystyrene (Shodex standard SM-105) as a reference or an aqueous phase GPC in phosphate buffer (column: TOSOH TSK-gel, G2500PW, G3000PW, G4000PW, 7.6 mm × 600 mm × 3) by the use of standard pullulan (Shodex standard P-82) at 40°C. Specific rotations were measured on a JASCO DIP-140 polarimeter at 25°C.

Table 2
Deprotection of 2,3-di-*O*-*tert*-butyldimethylsilyl-(1 → 5)-α-L-arabinofuranan and -α-D-xylopyranan

No	Polymer		Free polysaccharide		
	$\bar{M}_n \times 10^{3}$ [a]	$[\alpha]_D^{25}$ (degree)[b]	Yield (%)[c]	$\bar{M}_n \times 10^{3}$ [a]	$[\alpha]_D^{25}$ (degree)[b]
Poly(ADSA)					
5	50.0	−20.4	95	30.0	−18.4
Poly(ADSX)					
6	13.3	+102.8	76	9.2	+101.7
7	14.0	+107.5	73	5.6	+96.3
8	16.0	+114.1	83	6.2	+102.1

[a] Determined by GPC eluted with THF.
[b] Measured in CHCl3 (c 1%).
[c] From ^{13}C NMR spectrum.

2.2. Materials

Piperidine *N*-sulfonic acid was prepared from piperidine and chlorosulfonic acid as outlined by Nagasawa and Yoshidome (1969). The commercially available xylan ($\bar{M}_n = 30.0 \times 10^3$, Fluka Biochemika, Buchs, Switzerland) was used without any further purification.

2.3. Polymerization and sulfonation

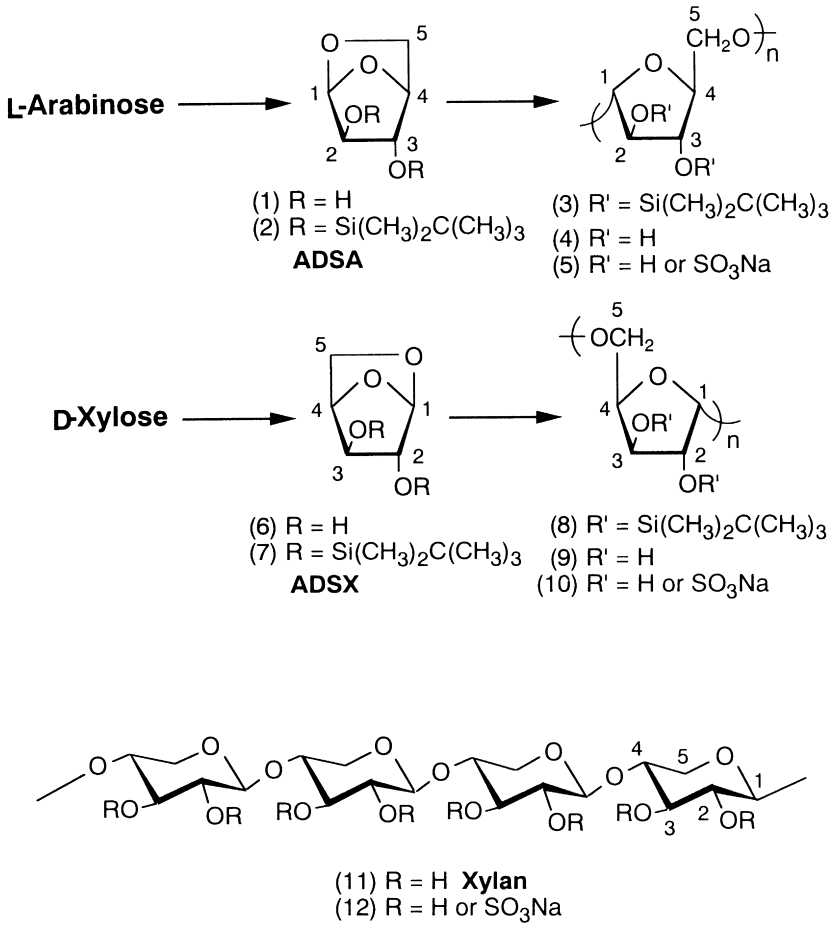
1,4-Anhydro-2,3-di-*O*-*tert*-butyldimethylsilyl-α-L-arabinofuranose (ADSA) (0.5 g, mmol) was polymerized with PF_5 (2 mol% to monomer) as a catalyst in methylene chloride (1.0 ml) at −60°C to give (1 → 5)-α-L-arabinofuranan after deprotection with *tetra-n*-butylammonium fluoride. The arabinofuranan (74 mg, 0.56 mmol) in dry DMSO (20 ml) was sulfonated with piperidine *N*-sulfonic acid (0.3 g, 1.6 mmol) for 1 h at 80°C. After the reaction mixture was cooled in an ice bath and then neutralized with saturated $NaHCO_3$ solution, acetone (200 ml) was poured onto the reaction mixture to form a white precipitate. The precipitate was isolated with centrifugation, washed with acetone several times, dissolved in deionized water and then dialyzed for one day against deionized water. The dialyzate was freeze-dried to afford a powdery sulfonated arabinofuranan (106.8 mg). The degree of sulfonation was calculated to be 1.6 by the results of elementary analysis.

Sulfonated xylofuranan was synthesized from 1,4-anhydro-2,3-di-*O*-*tert*-butyldimethylsilyl-α-D-xylofuranose (ADSX) by the same procedures as above. The degree of

Table 3
Sulfation of arabinofuranan, xylofuranan, and xylan by piperidine-*N*-sulfonic acid

No.	Free polysaccharide			PSA[a]	Time (h)	Sulphate polysaccharide							DS[c]
	g (mmol to sugar unit)	$\bar{M}_n \times 10^{3}$ [b]	$[\alpha]_D^{25}$ (degree)[c]	*g* (mmol)		Yield (mg)	$\bar{M}_n \times 10^{3}$ [b]	$[\alpha]_D^{25}$ (degree)[c]	Elemental analysis (%)				
									C	H	S	N	
Arabinofuranan													
9	74 (0.56)	30.1	−20.4	0.30(1.6)	1.0	107	24.2	+5.1	18.2	2.9	15.7	0	1.6
Xylofuranan													
10	200 (1.52)	9.2	+101.7	2.0(12.0)	1.0	86	15.9	+9.1	33.4	5.6	12.8	0.4	0.7
11	100 (0.76)	6.2	+102.1	0.75(4.5)	1.3	75	17.6	+1.0	19.2	2.9	15.8	0	1.5
12	100 (0.76)	9.2	+101.7	0.50(3.1)	1.2	84	13.1	+0.5	23.9	3.5	13.1	0	1.0
13	100 (0.76)	6.2	102.1	0.38(2.3)	1.0	66	17.1	+0.9	26.6	3.8	10.2	0	0.7
Xylan													
14	200 (1.52)	15.0	−15.2	2.0(11.4)	1.0	163	58.5	−1.1	21.9	3.4	16.0	0.5	1.6
15	200 (1.52)		−15.2	1.5(9.1)	1.1	180	42.0	−0.4	19.3	3.1	16.1	0	1.6
16	200 (1.52)		−15.2	1.5(9.1)	1.1	174	47.4	−0.4	21.5	3.2	14.4	0	1.3
17	200 (1.52)		−15.2	1.5(9.1)	1.3	178	43.1	−0.4	19.5	3.5	15.8	0.5	1.6
18	200 (1.52)		−15.2	1.0(6.0)	1.0	200	74.9	0.8	37.7	3.9	4.6	0	0.2

[a] Piperidine-*N*-sulfonic acid.
[b] Determined by GPC.
[c] Degree of sulfation (DC).



Scheme 1.

sulfonation ranged from 0.7 to 1.5. Commercially available xylan was sulfonated with piperidine *N*-sulfonic acid in DMSO to give sulfonated xylans having a degree of sulfonation between 0.2 and 1.6. The results are summarized in Tables 1–3.

2.4. Anti-HIV activity

Anti-HIV activity assay of sulfonated arabinofuranan was carried out with the inhibition of HIV-induced cytopathic effects and expression of virus antigen in MT-4 cell (Nakashima, Kido, Kobayashi, Motoki, Neushul & Yamamoto, 1987), which is a HIV-sensitive cell. Curdlan (CS) and dextran sulfonates (DS) were used as reference polysaccharides. The anti-HIV activity was determined by the number of viable cells and the percentage of virus antigen positive cells on the 3- and 5-day incubations after HIV infection. The highest value of complete inhibition (EC_{100}) for sulfonated polysaccharides against HIV infection, 3.3 μg/ml, is nearly equal to the EC_{50} of 0.13 μg/ml for curdlan sulfonate.

The anti-HIV activity was also assayed by the MTT method using $HIV_{HTLV\text{-}IIIB}$ virus and MT-4 cell (Pauwels et al., 1988), which is a more convenient method in the first stage of anti-HIV assay for test compounds. The MT-4 cell was infected with HIV at multiplicity of 0.01 and then MT-4 cell (1.5×10^5 cells/ml) and HIV-infected MT-4 cells were co-incubated in the presence of various concentrations of sulfonated polysaccharides for five days in a CO_2 incubator at 37°C. The viability of both HIV infected and uninfected MT-4 cells was measured spectrophotometrically by the changing color of the test solutions from yellow to blue according to the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The 50% inhibitory concentration of test compounds against infection of HIV to MT-4 cell was represented as EC_{50}. For the cytotoxicity of the pentosan sulfonates, uninfected MT-4 cell was cultured with pentosan sulfonates in various concentrations. The cytotoxicity was defined as CC_{50} which means, a 50% preventative concentration for the replication of MT-4 cell.

2.5. Blood anticoagulant activity

Anticoagulant activity of sulfonated pentosans was determined by using whole bovine plasma according to a modified method of the US Pharmacopoeia National Formulary (1985). The coagulation time of bovine plasma containing different concentrations of sulfonated pentosans was

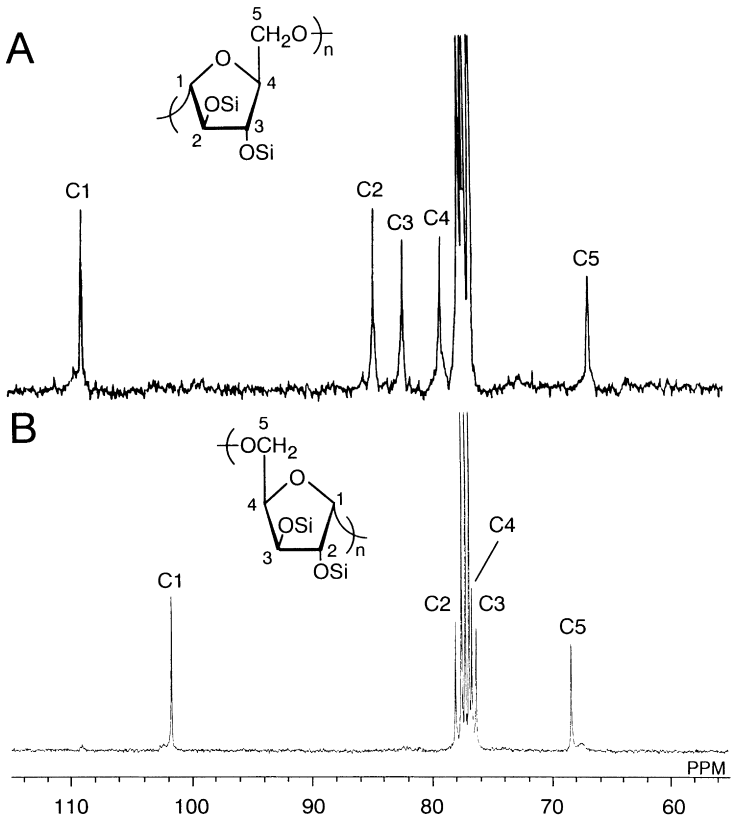


Fig. 1. 270 MHz ^{13}C NMR spectrum of (A) 2,3-di-*O*-*tert*-butyldimethylsilyl-(1 → 5)-α-L-arabinofuranan and 400 MHz spectrum of (B) 2,3-di-*O*-*tert*-butyldimethylsilyl-(1 → 5)-α-D-xylofuranan ($CDCl_3$ as solvent). Number-average molecular weights and specific rotations of (A) $\bar{M}_n = 50.0 \times 10^3$ and $[\alpha]_D^{25} = -20.4°$, and (B) $\bar{M}_n = 16.0 \times 10^3$ and $[\alpha]_D^{25} = +114.1°$, respectively.

compared with that of standard dextran sulfonate (H-039, 22.7 unit/mg).

3. Results and discussion

3.1. Synthesis of arabinofuranan and xylofuranan

(1 → 5)-α-L-Arabinofuranan and -α-D-xylofuranan were prepared by ring-opening polymerization (Uryu, 1990) of *tert*-butyldimethylsilylated 1,4-anhydro-α-L-arabinofuranose (ADSA) and -D-xylofuranose (ADSX) in good yields, respectively, according to our previous reports (Koyama, Harima, Matsuzaki & Uryu, 1985; Uryu et al., 1983; Yoshida, Kida & Uryu, 1987; Yoshida, Arai, Mukai & Uryu, 1988). The synthesis of sulfonated (1 → 5)-α-L-arabinofuranan and -α-D-xylofuranan, and the structure of sulfonated xylan is shown in Scheme 1.

The results of polymerization are summarized in Table 1. The silylated arabinose monomer, ADSA, was polymerized at −60°C with PF_5 as a catalyst under high vacuum to give 2,3-di-*O*-*tert*-butyldimethylsilyl-(1 → 5)-α-L-arabinofuranan in a good yield. The proportion of 1,5-α furanosidic unit in polymer backbone was calculated to be more than 95% by the intensity of the C1 region in the ^{13}C NMR spectrum (Fig. 1A). All signals were assigned by using 2D NMR measurements. The number-average molecular weight and specific rotation were 50.0×10^3 and −20.4°, respectively. Large negative specific rotation and the chemical shift of C1 signal at 109 ppm suggest the 1,5-α furanosidic linkage for L-type of sugar unit. The ring-opening polymerization of 1,4-anhydro-xylose monomer, ADSX, gave (1 → 5)-α-D-xylofuranan derivatives (nos. 2–4) having number-average molecular weights of 13.3×10^3–16.0×10^3 and large positive specific rotations.

As summarized in Table 2, after desilylation of polymers with fluoride ion, (1 → 5)-α-L-arabinofuranan and -α-D-xylofuranan having free hydroxyl groups were obtained in good yields. The number-average molecular weights and specific rotations were 30.0×10^3 and 5.6×10^3–9.2×10^3, and −18.4° and around +100°, for arabinofuranan and xylofuranan, respectively. Fig. 2 represents the ^{13}C NMR spectra of arabinofuranan (A) and xylofuranan (B) in D_2O. Signals having small intensities appeared in both spectra A and B, probably because of the partial cleavage at the C1–O–C4 linkage in the arabinofuranan (Yoshida et al., 1987) and the stereoregularity of main chain in xylofuranan, respectively.

Arabinofuranan and xylofuranan were sulfonated with piperidine *N*-sulfonic acid to give the corresponding sulfonated pentofuranans. Commercially available xylan having 1,4-β pyranosidic structure was also sulfonated (see Scheme 1). The results of sulfonation are given in Table 3. Specific

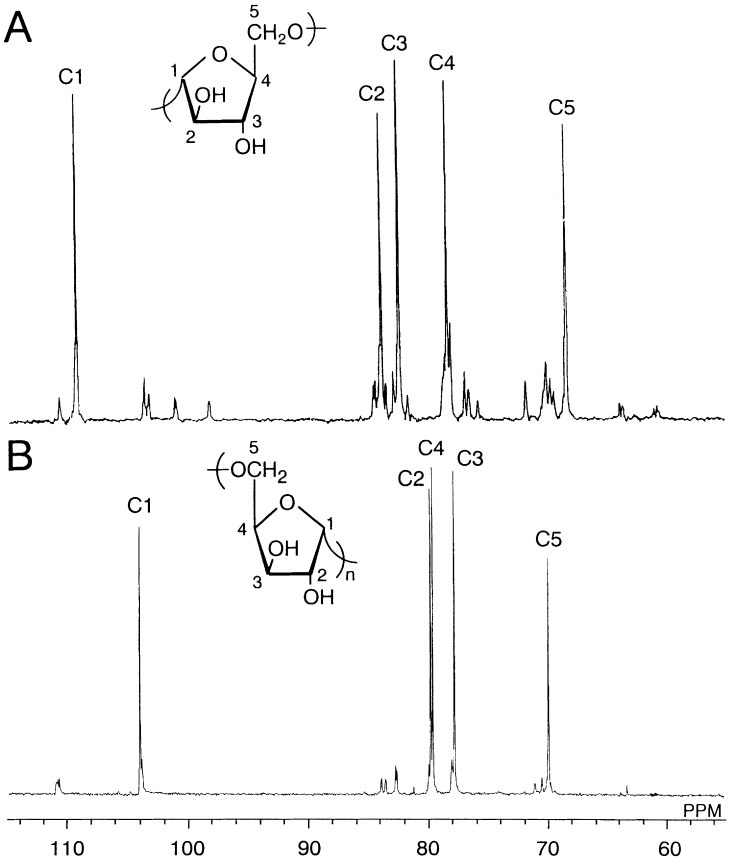


Fig. 2. 270 MHz ^{13}C NMR spectrum of (A) (1 → 5)-α-L-arabinofuranan and 400 MHz spectrum of (B) (1 → 5)-α-D-xylofuranan (D_2O as solvent). Number-average molecular weights and specific rotations of (A) $\bar{M}_n = 30.0 \times 10^3$ and $[\alpha]_D^{25} = -18.4°$, and (B) $\bar{M}_n = 9.2 \times 10^3$ and $[\alpha]_D^{25} = +102.1°$, respectively.

rotations changed to around $[\alpha]_D^{25} = 0°$ (c1, H_2O) after sulfonation. The number-average molecular weights were larger than those before sulfonation without arabinofuranan (no. 9), suggesting that no cleavage of polymer main chain occurred during sulfonation procedures. The degree of sulfonation was calculated to be from 0.2 to 1.6 (maximum, 2.0) by the results of elementary analysis.

In the ^{13}C NMR spectra of sulfonated arabinofuranan and xylofuranan having degrees of sulfonation of 1.5 and 1.6 (Fig. 3); after sulfonation, each carbon signal was sifted to the lower or upper magnetic field and was broadened compared to those before sulfonation.

The ^{13}C NMR spectra of naturally occurring xylan (A) before and (B) after sulfonation are shown in Fig. 4. In Fig. 4A, five signals having strong intensities were assigned to C1–C5 carbons because of the 1,4-β xylopyranosidic main chain by using 2D NMR measurements. The content of 1,4-β xylopyranosidic unit was calculated to be about 90% by the intensity of C1 signals. Many small signals were observed, which was probably because other sugar units such as galactose and arabinose appeared in the spectrum before sulfonation. After sulfonation, sulfonated xylan having a degree of sulfonation of 1.6 gave a complex spectrum (B). A similar complex NMR spectrum was obtained by the sulfonation of branched ribofuranan and ribopyranan (Yoshida et al., 1992, 1994). The small components and branches affected the ^{13}C NMR spectra of sulfonated polysaccharides.

3.2. Anti-HIV activity

In order to investigate the relationship between the structure and biological activities of sulfonated polysaccharides, anti-HIV and blood anticoagulant activities of the sulfonated pentosans having different configurations and conformations from ribose polysaccharides were examined by the MTT method using a HIV-sensitive MT-4 cell and by a slightly modified method of the US Pharmacopoeia, respectively. The anti-HIV activity of sulfonated polysaccharides was based on the prevention of HIV-induced cytopathic effects and expression of virus-specific antigens (Mitsuya et al., 1990). It was reported previously that sulfonated ribofuranan and ribopyranan inhibited completely the replication of HIV (EC_{100}) in vitro at the concentration as high as 3.3 μg/ml which was almost equal to the 50% inhibitory concentration (EC_{50}) below 1.0 μg/ml (Yoshida et al., 1992, 1994). Thus, the anti-HIV activity of sulfonated arabinofuranan, xylofuranan, and xylan was assayed to examine the structure–activity relationships. The results are represented in Table 4. It was found that sulfonated

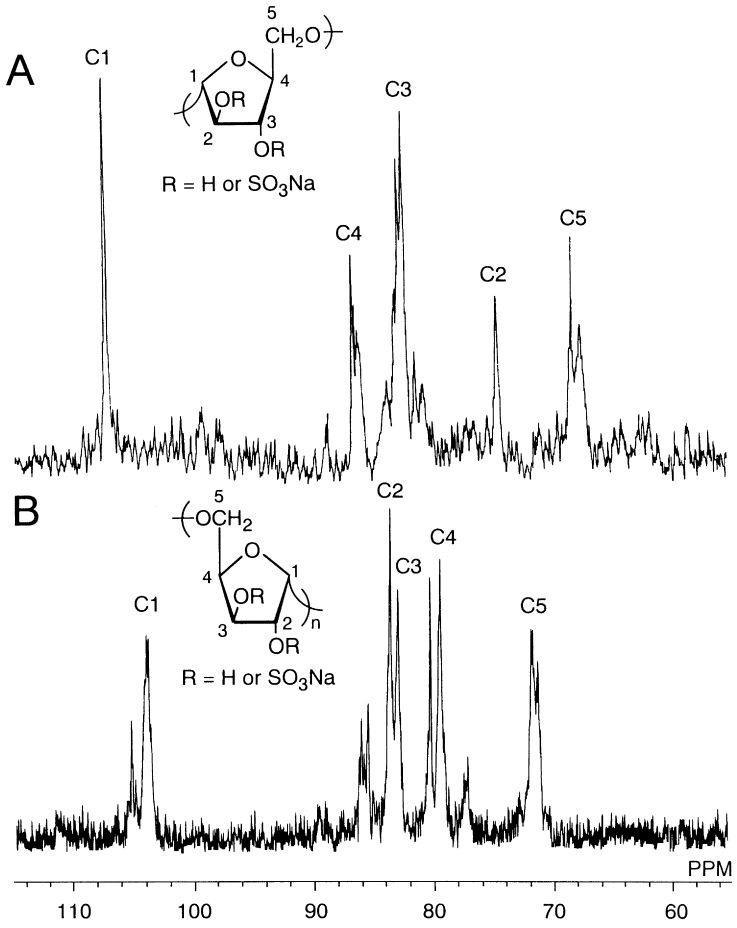


Fig. 3. 270 MHz ^{13}C NMR spectrum of (A) sulfonated (1 → 5)-α-L-arabinofuranan and 400 MHz spectrum of (B) sulfonated (1 → 5)-α-D-xylofuranan (D_2O as solvent). Number-average molecular weights and specific rotations of (A) $\bar{M}_n = 24.2 \times 10^3$ and $[\alpha]_D^{25} = +5.1°$, and (B) $\bar{M}_n = 17.6 \times 10^3$ and $[\alpha]_D^{25} = +1.0°$, respectively.

arabinofuranan having a number-average molecular weight of 24.3×10^3 and degree of sulfonation of 1.6 (maximum, 2.0) had potent anti-HIV activity on complete inhibition of HIV infection to MT-4 cell as high as 3.3 μg/ml. Sulfonated xylofuranan having high DS also had potent anti-HIV activity at EC_{50} of 0.2 μg/ml (no. 21). However, sulfonated xylofuranan having low degree of sulfonation (no. 20) had low anti-HIV activity. Further, pyranan-type sulfonated xylans (nos. 22 and 23) having degrees of sulfonation of 1.6 and 1.4 were found to have strong anti-HIV activity at the EC_{50} of 0.1 μg/ml. In no. 24, anti-HIV activity of sulfonated xylan decreased slightly to 0.6 μg/ml, though having a high molecular weight and degree of sulfonation. This sulfonated xylan seemed to contain partially pyridinium salts of sulfonate groups by the results of elementary analysis as shown in Table 3 (no. 17). The pyridinium salts as the counter cation of sulfonate groups reduced the anti-HIV activity of sulfonated lentinan (Hatanaka & Uryu, 1989). Thus, it was found that sulfonated pentosans synthesized here exhibited high anti-HIV activity that was independent of both the configuration and the conformation in the sugar unit. These sulfonated pentosans had a low cytotoxicity at the CC_{50}, i.e. more than 1000 μg/ml without sulfonated xylan (no. 24).

3.3. Blood anticoagulant activity

Another important biological activity of sulfonated polysaccharides, blood anticoagulant activity (Boeckel & Petitou, 1993), was assayed by using whole bovine plasma according to the US Pharmacopoeia as shown in Table 4. The blood anticoagulant activity of sulfonated arabinofuranan and xylofuranan (nos. 19 and 21) was 36 and 28 unit/mg, respectively, compared with that of standard dextran sulfonate (H-039) of 22.7 unit/mg. It was found that the anticoagulant activity of the five-membered furanan-type polysaccharides was about 2.0 times higher than that of sulfonated xylan having a six-membered pyranosidic structure (14 and 15 unit/mg). Also, sulfonated ribofuranan had a higher activity of 56 unit/mg than that of the sulfonated ribopyranan (36 unit/mg). Although these sulfonated arabinofuranan and xylofuranan had a similar degree of sulfonation to that of sulfonated xylan, the molecular weight of sulfonated xylan was approximately 2 times as high as

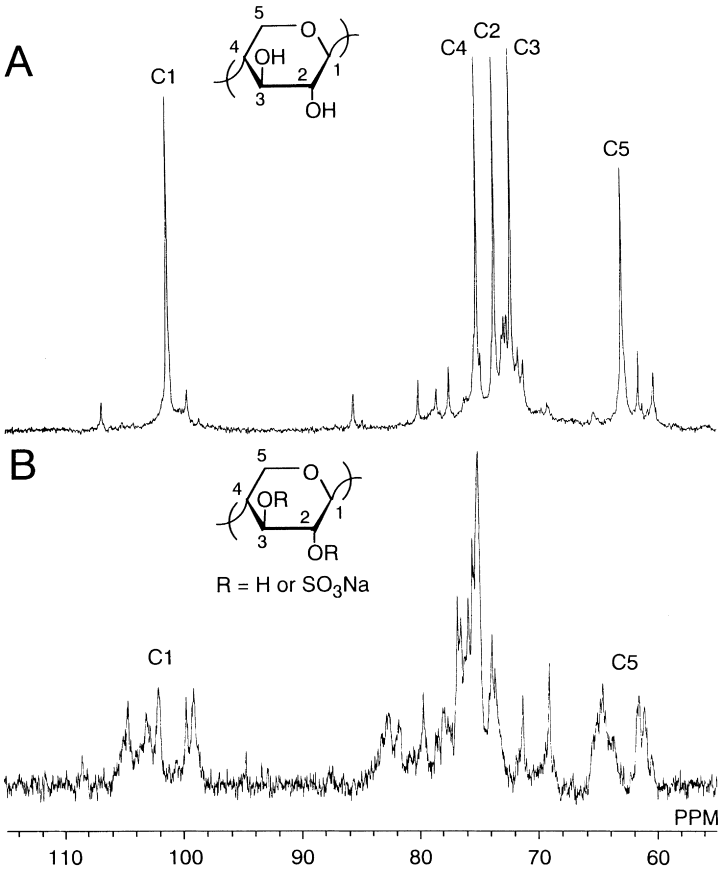


Fig. 4. 400 MHz ^{13}C NMR spectra of (A) commercially available xylan and (B) sulfonated xylan (D_2O as solvent). Number-average molecular weights and specific rotations of (A) $\bar{M}_n = 30.0 \times 10^3$ and $[\alpha]_D^{25} = -15.2°$, and (B) $\bar{M}_n = 47.1 \times 10^3$ and $[\alpha]_D^{25} = -0.4°$, respectively.

Table 4
Anti-HIV and anticoagulant activities of sulfonated pentosans

No.		Sulfonated polysaccharide				EC_{50} (μg/ml)[a]	CC_{50} (μg/ml)[b]	Anticoagulant activity (unit/mg)[c]
		$\bar{M}_n \times 10^3$	$[\alpha]_D^{25}$ (degree)[d]	S content (%)	DS[e]			
19	Arabinofuranan	24.3	+5.1	15.7	1.6	3.3[f]	> 1000	36
20	Xylofuranan	17.1	+0.9	10.2	0.7	108	> 1000	~0
21	Xylofuranan	17.6	+1.0	15.8	1.6	0.2	> 1000	28
22	Xylan	42.2	−0.4	16.1	1.6	0.1	> 1000	14
23	Xylan	47.1	−0.4	14.4	1.4	0.1	> 1000	n.d.
24	Xylan	42.8	−0.4	15.8	1.6	0.6	567	15
	RFS[g]	1.7	+83.0	17.6	1.9	3.3[g]	> 1000	56
	RPS[h]	1.2	+7.8	17.9	1.9	0.1	> 1000	36
	DS[i]	8.5	+92.1	18.4	1.8	0.86	> 1000	22.7
	CS[j]	79.0	+3.0	14.1	1.6	0.13	> 1000	< 10

[a] 50% Effective concentration.
[b] 50% Cytotoxic concentration.
[c] Dextran sulfonate H-039, 22.7 unit/mg.
[d] Measured in water at 25°C (c, 1%).
[e] Degree of sulfonation per sugar unit.
[f] Sulfonated ribofuranan.
[g] Minimum effective concentration for 100% inhibition of AIDS virus infection.
[h] Sulfonated ribofuranan.
[i] Standard dextran sulfate, H-039.
[j] Standard curdlan sulfonate.

that of the sulfonated arabinofuranan and xylofuranan. The polysaccharide backbone having a five-membered furanosidic structure is more flexible than that of the pyranan-type polysaccharides (Schuerch, 1972, 1981). Thus, the flexible backbone structure of sulfonated pentofuranans plays an important role in enhancing the blood anticoagulant activity. Dextan (DS) and curdlan sulfonates (CS) having a rigid six-membered backbone structure provided a relatively low blood anticoagulant activity.

In conclusion, sulfonated arabinofuranan, xylofuranan, and xylopyranan had potent anti-HIV activity, which was dependent on the degree of sulfonation. For the blood anticoagulant activity, sulfonated arabino- and xylofuranans having a flexible backbone structure were found to have a higher activity than that of sulfonated xylopyranans, suggesting that the conformation of the polymer backbone plays an important role in the high blood anticoagulant activity. However, the configuration of substituents might be independent. Further detailed investigations on the interactions of sulfonated polysaccharides with HIV and blood coagulant factors are now in progress.

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