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SYNTHESIS OF WATER-SOLUBLE, BRANCHED POLYSACCHARIDES HAVING D-MANNOPYRANOSE, D-ARABINOFURANOSE, OR OLIGO-D-ARABINOFURANOSE SIDE-CHAINS AND THEIR ANTITUMOR ACTIVITY*

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ABSTRACT

Branched polysaccharides having D-mannopyranose, D-arabinofuranose, or oligo-D-arabinofuranose side-chains were synthesized by the reaction of 3,4,6-tri-*O*-acetyl-(1,2-*O*-ethylorthoacetyl)-β-D-mannopyranose, 3,5-di-*O*-benzoyl-(1,2-*O*-ethylorthobenzoyl)-β-D-arabinofuranose, or 3-*O*-benzoyl-(1,2,5-*O*-orthobenzoyl)-β-D-arabinofuranose with cellulose acetate or curdlan acetate, followed by de-esterification. The structure and antitumor activity of the water-soluble portion of the polysaccharides thus obtained were investigated. Polysaccharides synthesized from (1→3)-β-D-glucan as the main chain with oligo-D-arabinofuranose side-chains exhibited high antitumor activity.

INTRODUCTION

Many natural polysaccharides, for example, lentinan[1], schizophyllan[1], pestalotan[2] and other fungal (1→6)-branched (1→3)-β-D-glucans[3,4] exhibit high inhibition of the growth of implanted tumors in mice. All of them are branched polysaccharides. Kochetkov *et al.*[5,6] first synthesized branched polysaccharides, by the orthoester method from cellulose acetate. Pfannemüller *et al.*[7–9] prepared water-soluble, branched polysaccharides by reaction of orthoester or bromo derivatives of glucose, maltose, and malto-oligosaccharides at O-6 of 2,3-di-*O*-phenylcarbamoylcellulose and -amylose. Ito and Schuerch[10] synthesized (1→6)-α-glucan branched at C-3 of a synthetic linear dextran. We have synthesized several branched poly-

*Part 4 of a series: "Synthesis of Water-soluble Branched Polysaccharides and Their Antitumor Activity".

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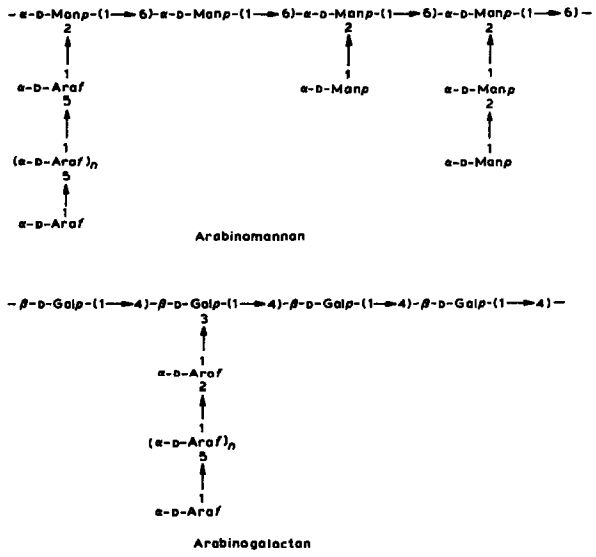

Fig. 1. Structures of the cell-wall polysaccharide of *Mycobacterium tuberculosis*.

saccharides to study the relationship between their structure and their antitumor activity. Branched polysaccharides were synthesized having β-D-glucosyl branches attached to a main chain of cellulose[11], curdlan[12], ivory nut mannan, or konjak glucomannan[13]. Some of those polymers had antitumor activities as high as natural ones. This paper reports syntheses of water-soluble branched polysaccharides by attaching D-mannopyranose, D-arabinofuranose, or oligo-D-arabinofuranose to curdlan acetate and cellulose acetate.

Mildly deleterious tubercle bacilli (BCG), and their cell-wall polysaccharides, have been used for immunotherapy[1]. Misaki and Azuma[14] proposed that the cell-wall polysaccharides of *Mycobacterium tuberculosis* have two components (Fig. 1); one is (1→6)-α-D-mannopyranan with side chains of D-mannopyranose and oligo-D-arabinofuranose and the other is (1→4)-β-D-galactan* with oligo-D-arabinofuranose side-chains. We have synthesized analogues of those polysaccharides and found some of them have high antitumor activity.

EXPERIMENTAL

Orthoesters. — 3,4,6-Tri-*O*-acetyl-(1,2-*O*-ethylorthoacetyl)-β-D-mannopyranose[15], 3,5-di-*O*-benzoyl-(1,2-*O*-ethylorthobenzoyl)-β-D-arabinofuranose (Ara-

*The presence of (1→6)-linked D-galactofuranose residues was also indicated by a recent investigation (A. Misaki, A. Yamaguchi, Y. Satsuma, and H. Kobatake, *Abstr. XII Int. Carbohydr. Symp.*, Utrecht, The Netherlands, 1984, p. 298.

bicyclic orthoester), and 3-*O*-benzoyl-(1,2,5-*O*-orthobenzoyl)-β-D-arabinofuranose(Ara-tricyclic orthoester)[16] were prepared by procedures reported in the literature. Ara-bicyclic orthoester was used as a monomer to synthesize branched polysaccharides having monosaccharide branches and Ara-tricyclic orthoester for polysaccharides having oligosaccharide branches.

Materials. — Curdlan acetates (d.s. 1.75 and 1.92) and cellulose acetates (d.s. 1.75, 2.06, and 2.15) were used as starting polymers. Curdlan acetates were prepared by acetylation of curdlan [a linear (1→3)-β-D-glucan, purchased from Wako Pure Chemical Industry, Osaka] with dry pyridine and acetic anhydride[12]. The cellulose acetates were prepared by hydrolysis of cellulose triacetate without acid catalyst to give polymers of high d.p.[11].

Activation of the starting polymers by solvent exchange. — It was necessary to activate curdlan acetates and cellulose acetates by immersing them in an ethanol–water system before the condensation reaction. The procedure was described in our previous paper[11].

Condensation of 3,4,6-tri-O-acetyl-(1,2-O-ethylorthoacetyl)-β-D-mannopyranose with activated curdlan acetate [expt. no. Man-2(3)]. — To a suspension of 1.0 g of activated curdlan acetate (d.s. 1.75) in chlorobenzene (30 mL) was added 2.1 g of 3,4,6-tri-*O*-acetyl-(1,2-*O*-ethylorthoacetyl)-β-D-mannopyranose, and the suspension was heated. When 5–6 mL of the solvent had distilled off, 0.15 g of 2,6-dimethylpyridinium perchlorate was added and the mixture was boiled under reflux for 120 min. After the reaction, the suspension was cooled to 70–80°, poured into a large excess of methanol, and the precipitate was filtered with a glass filter. The product was washed with methanol and ether, and dried *in vacuo*; yield 1.1 g. The condensation was repeated by the same procedure; yield 1.2 g.

Condensation of Ara-bicyclic orthoester with activated curdlan acetate [expt. no. Ara-1(2)]. — Curdlan acetate (d.s. 1.92, 0.7 g) was swollen by heating it in chlorobenzene (30 mL) for 60 min at 70°. Ara-bicyclic orthoester (2.8 g) in chlorobenzene (10 mL) was then added to the solution, which was heated. When 10 mL of the solvent had distilled off, 30 mg of 2,6-dimethylpyridinium perchlorate was added and the solution was boiled under reflux for 120 min. The product was then poured into methanol, and the precipitate was washed with methanol and ether, and dried *in vacuo;* yield 0.8 g. The condensation reaction was repeated by the same procedure; yield 0.8 g.

Condensation of Ara-tricyclic orthoester with activated curdlan acetate [expt. no. Ara-2(2)]. — Activated curdlan acetate (d.s. 1.92, 0.57 g) was swollen by heating it in chlorobenzene (32 mL) for 30 min at 55°. Ara-tricyclic orthoester (1.0 g) in chlorobenzene (30 mL) was then added to the solution, which was heated. When 16 mL of the solvent had distilled off, 20 mg of 2,6-dimethylpyridinium perchlorate was added and the solution was boiled under reflux for 120 min. The mixture was poured into methanol, and the product was washed with methanol and ether, and dried *in vacuo*; yield 0.64 g. The condensation reaction was repeated by the same procedure; yield 0.70 g.

Condensation of Ara-tricyclic orthoester with activated cellulose acetate [*expt. no. Ara-4(2)*]. — Activated cellulose acetate (d.s. 2.15, 0.62 g) was swollen by heating in chlorobenzene (60 mL) for 30 min at 60°. Ara-tricyclic orthoester (0.85 g) was added to the solution, which was heated. When 13 mL of the solvent had distilled off, 20 mg of 2,6-dimethylpyridinium perchlorate was added, and the solution was boiled under reflux for 120 min. The solution was poured into methanol and the precipitated product was washed with methanol and ether, and dried *in vacuo*; yield 0.82 g. The condensation reaction was repeated by the same procedure; yield 0.80 g.

Synthesis of water-soluble polysaccharides and analysis for content of acetyl groups. — The branched polysaccharides were converted into water-soluble polymers by deacylation with 0.5M aqueous NaOH or M methanolic sodium methoxide.

A. Deesterification by aqueous NaOH and analysis of the degree of branching. This method was applied to branched polysaccharides having D-mannopyranose side-chains. An aliquot sample (0.1 g) was suspended in 5 mL of 0.5M aqueous NaOH and kept for 17 h. The solution was titrated with 0.1M HCl and the degree of branching (N_t, the number of side-chain mannose groups per 100 main-chain glucose residues) was calculated by the following equation.

$$N_t(\%) = \frac{a(162.2 + 42.0 \times b) - b}{4 - 330.3 \times a} \times 100 \qquad (1)$$

where a = moles of acetyl groups per g of branched polysaccharide, and b is the d.s. of the starting curdlan acetate or cellulose acetate. The values ($162.2 + 42.0 \times b$) and 330.3 are respectively the molecular weight of the partially acetylated glucose residues in the main chain and that of the peracetylated mannosyl groups in the side chain. The equations $a = (4N_t + 100b)/[100(162.2 + 42.0\ b) + 330.3\ N_t]$, and thence Eq. *1* are thus derived.

The saponified solution was then diluted with water and dialyzed against water for 2–3 days. The insoluble residue was removed, and the solution was concentrated and freeze-dried. The theoretical total yields (water-soluble plus water-insoluble portions) were 80–90%.

B. Deesterification by methanolic sodium methoxide. An aliquot sample (~0.5 g) was suspended in M sodium methoxide in methanol (150 mL) and kept for 17–24 h. After the reaction, water (100 mL) was added and methanol was distilled off at low pressure. The aqueous solution was then stirred for 1–2 h at room temperature, and made neutral with 1–3M HCl. The solution was then diluted with water and dialyzed against water for 3–4 days. The insoluble portion was removed by centrifugation and the solution was concentrated. The product was precipitated by acetone, washed with ether, and dried *in vacuo* or freeze-dried from aqueous solution. The theoretical yields were 90–95%.

Analysis of the constitution of water-soluble polysaccharides. — The water-soluble polysaccharide (~1 mg) was heated in 90% formic acid (~1 mL) for 2 h at

100°. The acid was distilled off, and the residue was heated in 1 mL of 2M trifluoroacetic acid for 6 h at 100°. The acid was removed by evaporation and sugars in the hydrolyzate were analyzed as their alditol acetates by a Hitachi gas chromatograph NO-163 (column, 3% ECNSS-M on Chromosorb-W, at 190° the molar response of arabinose and glucose was 0.88:1 after formic acid and CF_3CO_2H treatment). The ratio (R_c) of arabinose to glucose residues was calculated.

Methylation analysis. — The method described in our previous paper was used[11].

Determination of molecular weight. — Molecular weights were determined by gel-permeation chromatography (g.p.c.) for protected branched polysaccharides obtained from cellulose acetate and for saponified products obtained from curdlan acetate.

A. Polysaccharides obtained from cellulose acetate. For the partially acetylated samples, g.p.c. was performed with a Toyo Soda HLC-802A high-performance liquid chromatograph equipped with a refractive index detector. TSK-GEL H columns were used at 37° with tetrahydrofuran as eluent. The molecular weight was calculated from a calibration curve that expresses the relationship between elution volume and the molecular weight of standard polystyrenes.

B. Polysaccharides obtained from curdlan acetate. For the saponified samples, g.p.c. was performed with a Toyo Soda HLC-803D high-performance liquid chromatograph equipped with an RI-8 detector. TSK-GEL G4000SW, G3000SW, and G2000SW columns were used at 25° with 0.1M phosphate buffer as eluent. Molecular weights were calculated from a calibration curve that expresses the relationship between elution volume and the molecular weights of standard dextrans.

Optical rotations. — Optical rotations of oligosaccharide-branched polysaccharides were measured with a Perkin–Elmer Model 241 polarimeter with a 1-dm cell. Polymer Ara-4(2) had $[\alpha]_D^{25}$ +9.7° (*c* 0.134, H_2O) and polymer Ara-2(2) had $[\alpha]_D^{25}$ +16.5° (*c* 0.115, H_2O).

Antitumor activity. — Ascites Sarcoma 180 (10^6 cells) used in the present assay were supplied by Dr. T. Sasaki, The National Cancer Institute, Tokyo. The water-soluble polysaccharides were suspended in phosphate-buffered saline at concentration of 25 mg per 10 mL. The suspensions were stirred overnight and then autoclaved for 10 min at 120°. The polysaccharide solution (100 μL) was injected intraperitoneally for ten days, starting 24 h after implantation of ascites tumor (the dose of each injection was 1–10 mg/kg ICR-JCL mouse). All mice were kept under observation for 5 weeks and then sacrificed for evaluation of the effect of treatment on tumor growth. The inhibition ratios were calculated by the following equation.

$$\text{Inhibition ratio}(\%) = (A - B)/\text{A} \times 100 \qquad (2)$$

where A is the average tumor weight of the control groups, and B is that of the treated groups.

RESULTS AND DISCUSSION

Activation of the main-chain polysaccharides. — The starting polymers were activated by solvent exchange with ethanol–water. Both curdlan and cellulose have high molecular weights, and it was necessary to pre-swell the polymers in chlorobenzene at 50–70° for ~1 h in order to effect the condensation reaction.

Synthesis, structure, and antitumor activity of branched polysaccharides having D-*mannopyranose side-chains.* — 3,4,6-Tri-*O*-acetyl-(1,2-*O*-ethylorthoacetyl)-β-D-mannopyranose was condensed with activated curdlan acetate (d.s. ~2) in chlorobenzene with 2,6-dimethylpyridinium perchlorate as catalyst. The reaction time was 120 min. By repeating this procedure two to three times, the degree of branching (N_t, Eq. *1*) was 12 and 37% (Table I). When unsubstituted curdlan was used as the starting polymer under similar conditions, the attempt to introduce side chains was unsuccessful.

The free polysaccharide Man-1(2), with a degree of branching (N_t) of 12%, was 87% soluble in water, and the other [Man-2(3)], with a degree of branching of 37%, dissolved in water completely.

When cellulose acetate was used as the starting polymer, a branched polysaccharide [Man-3(2)] with a degree of branching of 52%, which dissolved in water completely, was obtained. The results indicate that the deesterified products become completely soluble in water when the degree of branching exceeds 30%.

The branched polysaccharides had molecular weights of 275,000–340,000, indicating that no degradation of the main chain occurred during the condensation reactions.

The structures of the products were determined by methylation analysis of the water-soluble portions of Man-1(2) and Man-3(2) (Table II). The degrees of branching (N_c and N_b) were obtained respectively from the constitution and the branching units of the branched polysaccharides in the methylation analysis, and were calculated by the following equations (e.g., for a branched polysaccharide synthesized from cellulose acetate as the main chain).

(*a*) From the constituent sugars:

TABLE I

SYNTHESIS OF BRANCHED POLYSACCHARIDES HAVING D-MANNOPYRANOSE SIDE-CHAINS

Expt. No.	*Starting polymer*	*D.s.*	*Reaction time (min)*	*Degree of branching (%)*[a]	*Solubility in water (%)*
Man-1(2)	Curdlan acetate	1.92	120+120	11.9	87
Man-2(3)	Curdlan acetate	1.75	120+120+120	36.5	100
Man-3(2)	Cellulose acetate	2.15	120+120	51.6	100
Man-4(2)	Cellulose acetate	2.06	120+120	30.3	100

[a]Determined by titration analysis.

TABLE II

THE RESULTS OF METHYLATION ANALYSIS OF POLYSACCHARIDES HAVING D-MANNOPYRANOSE SIDE-CHAINS

Methyl sugar[a]	*Retention time (min)*	*Relative mole ratio*	
		Man-1(2)	*Man-3(2)*
2,3,4,6-Man	5.54	1.00	1.00
2,4,6-Glc	6.87	4.12	
2,3,6-Glc	7.02		1.27
2,6-Glc	7.95	0.20	0.10
3,6-Glc	8.19		0.15
2,3-Glc	8.71		0.50
2,4-Glc	8.80	0.29	
3-Glc	10.01		0.14

[a]Man, mannitol acetate; Glc, glucitol acetate.

$$N_c(\%) = \frac{(2,3,4,6\text{-M})}{(2,3,6\text{-G}) + (2,3\text{-G}) + (2,6\text{-G}) + (3,6\text{-G}) + (3\text{-G})} \times 100 \qquad (3)$$

where the numbers in parentheses indicate the positions of MeO groups, M is mannitol acetate, and G is glucitol acetate.

N_c values of Man-1(2) and -3(2) were 22 and 46%, respectively.

(*b*) From the ratio of sugar residues at the branching point to those in the main chain.

$$N_b(\%) = \frac{(2,3\text{-G}) + (2,6\text{-G}) + (3,6\text{-G}) + (3\text{-G}) \times 2}{(2,3,6\text{-G}) + (2,3\text{-G}) + (2,6\text{-G}) + (3,6\text{-G}) + (3\text{-G})} \times 100 \qquad (4)$$

The N_b values of Man-1(2) and -3(2) were 11 and 48%, respectively.

Table II indicates that the ratio of monosaccharide branching at C-6 to that at C-4 is 58:42 for Man-1(2) and the ratio of the branches at C-6,C-3, and C-2 is 67:13:20 for Man-3(2). It is noteworthy that Man-1(2) has no branching at C-2 and no double-branching per glucose unit in the main chain.

Free polysaccharides obtained from Man-1(2) and Man-3(2) were tested for antitumor activity. Man-1(2) has the curdlan main chain and Man-3(2) the cellulose main chain. The results (Table III) show that Man-1(2) and Man-3(2) had inhibition ratios of 82.7 and 32.0%, respectively. The (1→3)-β-D-glucan is thus more effective than (1→4)-β-D-glucan as the main chain of branched polysaccharides having antitumor activity.

Synthesis of water-soluble polysaccharides having D-*arabinofuranose or oligo-*D-*arabinofuranose side-chains. — A. Branched polysaccharides having* D-*arabinofuranose side-chains.* Monosaccharide-branched polysaccharides were synthesized from unsubstituted curdlan, curdlan acetate (d.s. 1.92), and cellulose acetate (d.s. 2.15) as starting polymers. 3,5-Di-*O*-benzoyl-(1,2-*O*-ethylorthobenzoyl)-β-D-

TABLE III

ANTITUMOR ACTIVITY OF BRANCHED POLYSACCHARIDES

Branched polysaccharides[a] *Expt. no.*	*Dose (mg/kg)*	*Tumor weight (g)*[b]	*Tumor inhibition (%)*	*Complete regression*[c]
Man-1(2) (curd-S)	10	1.39 ±0.52	82.7	1/7
Man-3(2) (cell-S)	10	5.46 ±1.03	32.0	0/7
Ara-1(2) (curd-S)	10	1.99 ±1.07	75.2	3/7
Ara-2(2) (curd-L)*	1	0.03 ±0.03	99.7	6/7
Ara-2(2) (curd-L)	10	0.00 ±0.00	100.0	6/6
Ara-3(3) (curd-L)*	5	0.00 ±0.00	100.0	7/7
Ara-3(3) (curd-L)*	10	0.00 ±0.00	100.0	7/7
Ara-4(2) (cell-L)	10	5.67 ±0.38	29.4	0/7
control		8.03 ±0.96		0/7
*control		10.45 ±1.09		0/7

[a]Curd-S and curd-L indicate curdlan with mono- and oligo-saccharide side-chains, respectively. Cell-S and cell-L indicate cellulose with mono- and oligo-saccharide side-chains, respectively. Branched polysaccharides identified with an asterisk indicate the same series of experiments including a control, and those without the asterisk show another series. [b]Tumor weight: average ± standard error. [c]Complete regression indicates the ratio of the number of mice, for which the tumor disappeared completely after the administration of polysaccharide solution, to the number of mice tested.

arabinofuranose was less reactive than 3,4,5-tri-*O*-acetyl-(1,2-*O*-ethylorthoacetyl)-*α*-D-glucopyranose (acetylated glucose orthoacetate). Consequently unsubstituted curdlan underwent negligible branching by arabinofuranosyl groups, in contrast to glycosylation with acetylated glucose orthoacetate.

The degree of branching was greater when acetylated polymers were used as starting material but the degree of branching was still low, even after repeating the condensation. the free polysaccharide Ara-1(2), having a degree of branching of 9.3%, was 57% soluble in water (Table IV). In order to obtain completely water-soluble, monosaccharide-branched polysaccharides, it was necessary to repeat the condensation reaction several times.

*B. Branched polysaccharides having oligo-*D*-arabinofuranose side-chains.* The Ara-tricyclic orthoester was more reactive than the Ara-bicyclic orthoester. Repetition of the condensation gave completely water-soluble products from both curdlan acetate and cellulose acetate. The oligosaccharide-branched polysaccharide from curdlan acetate was completely soluble, even when the degree of branching was low.

Acetylated and benzoylated branched polysaccharides were only partially deesterified with 0.5M NaOH, but were completely deesterified by M methanolic sodium methoxide; dialysis was used for purification to remove compounds of low molecular weight.

The structures of branched polysaccharides having D*-arabinofuranose or oligo-*D*-arabinofuranose side-chains.* — The structures of polysaccharides having D-arabinofuranose or oligo-D-arabinofuranose branches were determined by con-

TABLE IV

SYNTHESIS OF BRANCHED POLYSACCHARIDES HAVING D-ARABINOFURANOSE SIDE-CHAINS

Expt. no.[a]	*D.s.*[b]	*Reaction time (min)*	*Ratio* (R_c)[c] *Ara/Glc*	*Length* (L_b)[d] *of branches*	*Solubility in water (%)*
Ara-1(2) (curd-S)	1.92	120+120	9.3	1.00	57
Ara-2(2) (curd-L)	1.92	120+120	27.0	1.71	100
Ara-3(3) (curd-L)	1.75	120+120+120	[e]	[e]	100
Ara-4(2) (cell-L)	2.15	120+120	66.7	2.28	100

[a]Curd-S and curd-L indicate that the starting polymer is curdlan acetate with mono- and oligo-saccharide branches, respectively. [b]D.s. of the starting polymers. [c]Determined by constitutional analysis of water-soluble portions of the free polysaccharides. [d]Determined for water-soluble portions of the free polysaccharides. [e]Not determined.

stitution and methylation analyses. The ratio (R_c) of arabinose to glucose residues was calculated from the constitution. The results of methylation analysis are shown in Table V. The ratio (R_m) of arabinose to glucose residues and the degree of branching (N_b) are calculated by Eqs. *5* and *6* (e.g., for a polysaccharide synthesized from curdlan as the main chain) from the results of methylation analysis.

$$R_m(\%) = \frac{(2,3,5\text{-A}) + (2,3\text{-A}) + (3,5\text{-A})}{(2,4,6\text{-G}) + (2,4\text{-G}) + (2,6\text{-G})} \times 100 \qquad (5)$$

$$N_b(\%) = \frac{(2,4\text{-G}) + (2,6\text{-G})}{(2,4,6\text{-G}) + (2,4\text{-G}) + (2,6\text{-G})} \times 100 \qquad (6)$$

Numbers in parentheses indicate the positions of CH_3O groups, A denotes arabinitol acetate, and G glucitol acetate.

The length (L_b) of branching in oligosaccharide-branched polysaccharides is calculated by Eq. 7.

$$L_b = [(2,3,5\text{-A}) + (2,3\text{-A}) + (3,5\text{-A})]/(2,3,5\text{-A}) \qquad (7)$$

A. Monosaccharide-branched polysaccharides synthesized from curdlan acetate [Ara-1(2)]. The R_c value was 9.3% from analysis of the sugar composition and R_m = 9.8% from the methylation analysis. The degree of branching (N_b) calculated from Eq. *6* was 4.1%. The value of R must agree with N_b, because the branch was a monosaccharide. The N_b value obtained from the methylation analysis may be erroneously low. The ratio of branching at C-6 and C-4 was 1.1:1, and monosubstitution at C-2 was negligible. The result was similar to that of a previously synthesized branched curdlan having single glucosyl groups[11]. Double substitution was not detected, presumably because of the low degree of branching. The possible structure of the monosaccharide-branched polysaccharide obtained from curdlan acetate is indicated in Fig. 2(A).

TABLE V

THE RESULTS OF METHYLATION ANALYSIS OF BRANCHED POLYSACCHARIDES WITH D-ARABINOFURANOSE SIDE-CHAINS

Methyl sugar[a]	*Retention time (min)*	*Relative mole ratio*		
		Ara-1(2)	*Ara-2(2)*	*Ara-4(2)*
2,3,5-Ara	3.30	1.00	1.00	1.00
3,5-Ara	3.58		0.09	0.14
2,3-Ara	4.84		0.62	1.14
2,4,6-Glc	6.83	9.80	5.37	
2,3,6-Glc	7.02			2.16
2,6-Glc	7.95	0.20	0.13	0.04
3,6-Glc	8.19			0.08
2,3-Glc	8.71			0.90
2,4-Glc	8.79	0.22	0.57	

[a]Ara, arabinitol acetate; Glc, glucitol acetate.

B. Oligosaccharide-branched polysaccharide synthesized from curdlan acetate [*Ara-2(2)*]. The ratio (R_c) of arabinose to glucose was 27.0% from the composition analysis and R_m = 28.2% and N_b = 11.5% from the methylation analysis. The length of branches (L_b) was 1.71. The value R obtained by multiplication of N_b with L_b was 32.4%, which is near the R_m value obtained from the methylation analysis. The ratio of branching position at C-6 to that at C-4 was 4.4:1. In this case also, there was little single substitution at C-2. Doubly branched units were not detected, presumably because of steric effects or because of the low degree of branching. 1,2,4-Tri-*O*-acetyl-3,5-di-*O*-methyl-D-arabinitol was detected on methylation analysis. This alditol acetate results from internal arabinose residues in the chain branches, indicating the existence of (1→2) linkages together with (1→5) linkages. The (1→2) linkage must have been formed by the rearrangement of an intermediate acyloxonium cation from the tricyclic orthoester[17]. However, the content of (1→2) linkages was low. The possible structure of the oligosaccharide-branched polysaccharide prepared from curdlan acetate is indicated in Fig. 2(B).

C. Oligosaccharide-branched polysaccharide synthesized from cellulose acetate- [*Ara-4(2)*]. The R_c-value was 66.7% from the composition analysis and R_m = 71.6% and N_b = 32.1% from the methylation analysis. The length of branches (L_b) was 2.28. In this instance, the value of $N_b \times L_b$ is 73.2%, which is near the value from the composition analysis. The ratio of branching at C-6, C-3, and C-2 was 25:1:2, indicating that the branching occurred preferentially at C-6. Double branching was negligible, presumably because of steric effects. 3,5-Di-*O*-methyl-D-arabinitol tetraacetate was detected in the methylation analysis. The possible structure of the oligosaccharide-branched polysaccharide synthesized from cellulose acetate is shown in Fig. 2(C).

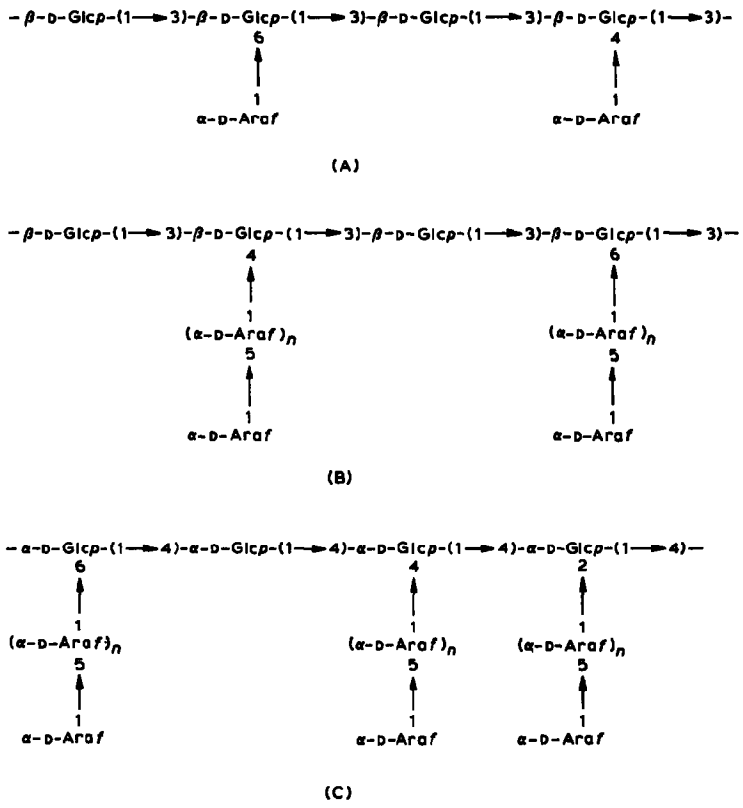

Fig. 2. Possible structures of branched polysaccharides synthesized from (A) curdlan having D-arabinofuranose side-chains, (B) curdlan with oligo-D-arabinofuranose side-chains, and (C) cellulose having oligo-D-arabinofuranose side-chains. (The intervals between branches are statistical.)

D. Configuration of the glycosidic linkages in the branches. — It is known that glycosidation with β-orthoesters gives α-glycosides, and vice versa[18,19]. In our previous paper[20], we reported ^{13}C-n.m.r. investigations on branched (1→3)-β-D-glucan and (1→4)-β-D-glucan bearing glucose side-chains, synthesized by the reaction of 3,4,5-tri-*O*-acetyl-(1,2-*O*-ethylorthoacetyl)-α-D-glucopyranose with the polymer main-chains. The spectra showed that a considerable proportion (20%) of side-chain glucosyl groups are α-glycosidically linked to the main chain. The optical rotations given in the Experimental section suggest that the branches are largely α-linked, but more studies are necessary on the ^{13}C-n.m.r. spectra of the branched polysaccharides, to obtain quantitative data.

Molecular weight of branched polysaccharides having D-*arabinofuranose side-chains.* — Molecular weights were determined by g.p.c. The free polysaccharide Ara-2(2) having oligosaccharide-branches obtained from curdlan acetate showed a wide distribution of molecular weight (230,000–660,000) with three broad peaks at 230,000–310,000, 340,000–390,000, and 470,000–590,000.

The polymer Ara-4(2), a protected oligosaccharide-branched polysaccharide obtained from cellulose acetate, showed single peak at 320,000 of rather narrow distribution, and the deesterified product showed a single peak at 175,000–200,000, also of narrow distribution. Taking into consideration the decrease of molecular weight upon deesterification (~50%), it was deduced that deesterification did not cause degradation.

Antitumor activity of water-soluble branched polysaccharides having D-*arabinofuranose side-chains.* — The antitumor activities of the branched polysaccharides are listed in Table III. The antitumor activity of water-soluble, branched polysaccharides is evidently influenced by the main-chain polymer; the polymer with (1→3)-β-D-glucan as the main chain showed higher activity than that with (1→4)-β-D-glucan as the main chain [compare Ara-2(2) and Ara-3(3) with Ara-4(2)]. The oligosaccharide-branched polysaccharides Ara-2(2) and Ara-3(3) obtained from curdlan acetate showed strong antitumor activity, almost 100% inhibition for Sarcoma 180 even at a dose level of 1 mg/kg mouse. These polysaccharides are the most active of those we have synthesized thus far.

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