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A TYPICAL DERMATAN SULFATE ISOLATED FROM WHALE INTESTINE

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ABSTRACT

Alkaline extraction of whale intestine, followed by pronase digestion and precipitation of heparin (ω-heparin) with dodecyltrimethylammonium chloride gave a supernatant fraction containing dermatan sulfate. Ethanol at 20% concentration precipitated dermatan sulfate from the supernatant fraction. The crude dermatan sulfate was further fractionated by ion-exchange column chromatography on Dowex-1 (Cl^- form), eluting stepwise with aqueous sodium chloride. The fractions eluted with 1.5M and 1.75M sodium chloride contained a typical dermatan sulfate. Chemical and enzymic studies of these preparations revealed that the sulfate groups were located solely at O-4 of the 2-acetamido-2-deoxy-D-galactose residues. L-Iduronic acid was assumed to be distributed uniformly in the backbone of the polysaccharide chain, with D-glucuronic acid being located in the linkage region to the protein core. A new method for determining the ratio of D-glucuronic acid to L-iduronic acid is also described.

INTRODUCTION

Whale heparin (ω-heparin) has been isolated from lung and intestinal mucosa by precipitation with dodecyltrimethylammonium chloride, after initial alkaline extraction and subsequent pronase digestion[1,2]. The supernatant fraction has previously been suggested to contain a typical dermatan sulfate[3] (**1**). In this paper we report the isolation and characterization of this dermatan sulfate from the supernatant fraction.

The work of Fransson and his co-workers has indicated microheterogeneity of **1** from such tissues as human umbilical cord[4], pig skin[5], and hog intestinal mucosa[6]. However, it has proved difficult to perform quantitative analyses of this type of copolymer structure composed of two types of disaccharide residues, one containing L-iduronic acid and the other D-glucuronic acid, because direct acid hydrolysis of **1** leads to decomposition of the uronic acids.

Taylor *et al.*[7] estimated the content of L-iduronic acid and D-glucuronic acid in heparin by radiochromatography of an acid hydrolyzate of the deamination products obtained after prior carboxyl reduction. To determine the ratio of D-glucuronic acid to L-iduronic acid, we devised a new method employing gas–liquid chromatography in place of radiochromatography, after the carboxyl reduction according to the procedure of Taylor *et al.*[7].

EXPERIMENTAL

Materials. — The supernatant fraction of heparin precipitates was supplied by Dr. T. Shibata. This fraction was the supernatant from the heparin precipitate obtained with 40% dodecyltrimethylammonium chloride in 1.2% sodium chloride solution after alkaline extraction of whale intestine followed by digestion with pronase[8]. The preparation showed 22 units (British Pharmacopoeia) per mg of anticoagulant activity. A standard specimen of **1** from hog mucosa was kindly supplied by Dr. M. B. Mathews. 3-(Dimethylaminopropyl)-1-ethyl-carbodiimide (EDC) was purchased from Nakarai Chemical Co., Kyoto. Chondroitinase ABC and chondroitinase AC-II were obtained from Seikagaku Kogyo Co. Ltd., Tokyo. Chondroitinase B was prepared from chondroitin 6-sulfate-adapted *Flavobacterium heparinum*, and then purified by column chromatography on hydroxyapatite, eluting with a linear gradient of sodium chloride. The details will be published elsewhere. Standard specimens of L-idose and 1,6-anhydro-L-idopyranose used in g.l.c. were prepared by the methods of Vargha[9] and Stoffyn and Jeanloz[10]. Other materials were commercial products.

Determination of constituents. — Uronic acid was estimated by the carbazole–sulfuric acid methods of Dische[11] and Bitter and Muir[12], and also by the orcinol–hydrochloric acid method of Brown[13]. Total hexosamine was assessed by the Elson–Morgan procedure of Gardell[14] after hydrolysis with 3M hydrochloric acid for 15 h at 100°. 2-Amino-2-deoxy-glucose and -galactose were determined in the same hydrolyzate with a Hitachi 034 liquid chromatograph. Sulfate was assayed by the method of Dodgson and Price[15]. Hexose was determined by the anthrone–sulfuric acid[16], and the phenol–sulfuric acid reactions[17].

Optical rotation. — Optical rotations were measured with a Jasco Model ORD/UV-5 spectropolarimeter for an aqueous solution (20 mg in 2 ml).

Estimation of molecular weight. — Intrinsic viscosity was estimated with a Ubbelohde viscometer, using a solution in 0.066M phosphate buffer (pH 7.0) containing 0.35M sodium chloride. The molecular weight was calculated from the intrinsic viscosity by using the equation of Mathews and Dorfman[18].

Purification of dermatan sulfate (**1**). — A portion (25 g, as the sodium salt) of the supernatant fraction was dissolved in 1250 ml of 0.5M acetic acid containing 5% of calcium acetate. Ethanol was added to the solution to a final concentration of 20%, with stirring in an ice-bath. The mixture was refrigerated (4°) overnight and then centrifuged. The supernatant was further fractionated with ethanol at successive concentrations of 30 and 80%, by the same procedure. The specimens thus obtained at each concentration of ethanol were washed once with sodium chloride-saturated ethanol, once with ethanol and then with ether, and dried *in vacuo* over sulfuric acid. Of these fractions, the 20%-ethanol precipitate was refractionated by the same procedures as just described. As the refractionated sample was shown by electrophoresis on a cellulose acetate membrane to contain a small amount of heparin, this sample was fractionated by ion-exchange chromatography as follows. A portion (1 g, as the calcium salt) was dissolved in 5 ml of water, and the solution was applied to a column (1.0 × 42.5 cm) of Dowex-1 X2 (Cl^- form, 200–400 mesh). Successive elution with water (200 ml), and 500 ml each of 1.25, 1.5, 1.75, and 2.0M sodium chloride, gave eluates that were dialyzed exhaustively, and the non-dialyzable fractions were concentrated to low volume at 40° *in vacuo* and then lyophilized.

Determination of L*-iduronic acid and* D*-glucuronic acid.* — A portion (6 mg, as the sodium salt) of **1** was treated with 20 mg of EDC in 2 ml of water at pH 4.75 for 1 h at room temperature. Subsequently, two volumes of 3M sodium borohydride were added, and the reduction was conducted for 3 h at 50°. The mixture was cooled and the excess of reductant was decomposed by addition of 3 ml of 1.5M sulfuric acid. The mixture was then dialyzed exhaustively against distilled water, and the non-dialyzable fraction was evaporated to dryness at 40° *in vacuo*. The carboxyl-reduced **1** thus obtained was hydrolyzed with 2 ml of 0.05M hydrochloric acid in the presence of 100 mg of Dowex-50W X2 (H^+ form, 200–400 mesh) for 24 h at 100°. The mixture was then cooled and filtered. The resin was washed 3 times with 2-ml portions of water. The filtrate and washings were combined and then concentrated to low volume at 40° *in vacuo*. Thereafter, the hydrolyzate was treated with 1 ml of 1% sodium borohydride for 3 h at room temperature. To the mixture was added 1 ml of Dowex-50W X2 (H^+ form) to decompose the excess of reductant. The mixture was filtered and the resin was washed 3 times with 2-ml portions of water. The filtrate and washings were combined and 2-ml portions of methanol were evaporated repeatedly from the product to remove borate, and then the residue was dried over calcium chloride in an evacuated desiccator. 1,6-Anhydro-L-idose and D-glucitol thus obtained from the carboxyl-reduced **1**, and the reference specimens, were trifluoroacetylated by the method of Imanari *et al.*[19]. G.l.c. of the trifluoroacetyl derivatives was carried out in a Hitachi Type 163 gas–liquid chromatograph, by using a 2-m glass column packed with 2% of OV-105 coated on Gas-Chrom P (80–100 mesh), at 130°, with nitrogen as the carrier gas at a flow rate of 30 ml/min. The trifluoroacetylated 1,6-anhydro-L-idose and D-glucitol derived from carboxyl-reduced **1** were quantitatively determined from the chromatograms by using a Sic intelligent integrator Model 500 A; a known amount of xylitol was used as the internal standard.

Electrophoresis. — Electrophoresis on a cellulose acetate membrane (Separax) was performed under the following conditions: (*a*) pyridine–formic acid (pH 3.0) at 1 mA/cm for 30 min[20]; (*b*) 0.1M hydrochloric acid at 2.5 V/cm for 3 h[21]; and (*c*) 0.3M calcium acetate at 1 mA/cm for[22] 3 h. After electrophoresis, substances were stained with Alcian Blue (0.1% in 80% ethanol).

Enzymic digestion with chondroitinases. — *A.* Digestion of the purified **1** with chondroitinase ABC from *Proteus vulgaris* was performed by the procedure of Suzuki *et al.*[23,24]. Aliquots of the incubation mixtures, taken at zero time and at 3 h, were mixed with 0.1M hydrochloric acid, and the absorbance at 235 nm was measured. In addition, an incubation mixture of the same composition was analyzed for **2**, **3**, and **4** by paper chromatography, by using 5:3 (v/v) *n*-butanoic acid–0.5M ammonia as solvent, according to the procedure of Suzuki *et al.*[24].

B. Digestion with chondroitinase AC-II from *Arthrobacter aurescence* was carried out by the procedure of Hiyama and Okada[25]. To the incubation mixture was added 0.1M hydrochloric acid, and the absorbance at 235 nm was read. Gel filtration before and after enzymic digestion was carried out as follows. Either 3 mg of an intact sample or 1 mg of its product of digestion with 6 units of enzyme was dissolved in a small volume of 0.025M sodium chloride, and the solution was filtered through a column (1.6 × 77 cm) of Sephadex G-200 gel, pre-equilibrated with the same concentration of salt. The column was then eluted with the same salt solution at a flow rate of 10 ml/h. Fractions (3.5 ml) were collected and the content of uronic acid and the absorbances at 235 nm of each fraction were measured.

C. Digestion with chondroitinase B from *Flavobacterium heparinum* was performed as follows. An aliquot sample (100 μg) was dissolved in 100 μl of 0.25M Tris–hydrochloric acid buffer (pH 7.0). Chondroitinase B (1 unit) in 0.5 ml of water was added and the solution was incubated for 3 h at 30°. Hydrochloric acid (0.1M, 2 ml) was added to aliquots (200 μl each), taken at zero time and 3 h, and the absorbances at 235 nm of these solutions were measured.

RESULTS

Purification of dermatan sulfate (**1**). — The supernatant fraction from the heparin precipitate obtained from whale intestine was fractionated with ethanol in the presence of calcium acetate as described in the Experimental section. The yields of the precipitates at 20, 30, and 80% concentration of ethanol were 19.0, 2.0, and 4.9 g, respectively (as calcium salts), from 25 g (as sodium salt) of the supernatant fraction. The recovery on reprecipitation at 20% ethanol concentration was 90%. The second 20% ethanol precipitate contained dermatan sulfate plus a small proportion of heparin, as judged from electrophoresis on a membrane of cellulose acetate. Although the fraction precipitated at 30% ethanol concentration contained a certain proportion of **1**, this fraction contained chondroitin 4- and/or 6-sulfate. Moreover, the 80% ethanol precipitate did not contain any **1**. Therefore, these two fractions were not studied further.

As the second 20% ethanol precipitate contained a small proportion of heparin, this fraction was fractionated by ion-exchange column chromatography on Dowex-1 X2 (Cl^- form) as already described. By this method, three fractions were prepared, and these were eluted at 1.25, 1.5, and 1.75M concentration of salt. Hexosamine analysis of acid hydrolyzates of these fractions showed that the 1.25M fraction contained only 2-amino-2-deoxy-glucose and the other two fractions contained 2-amino-2-deoxy-galactose. These fractions gave single bands in electrophoresis on a membrane of cellulose acetate. The electrophoretic mobility of the 1.25M fraction was the same as that of the 1.25M fraction of heparin (ω-heparin) reported by Kotoku

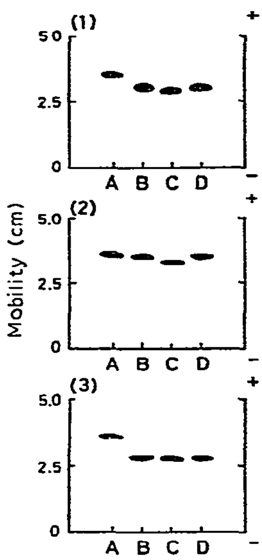


Fig. 1. Tracing of electrophoretograms on cellulose acetate membrane of **1a** and **1b** in pyridine–formic acid (pH 3.0) at 1 mA/cm for[20] 30 min (*1*), in 0.1M hydrochloric acid at 2.5 V/cm for[21] 3 h (*2*), and in 0.3M calcium acetate at 1 mA/cm for[22] 3 h and (*3*), staining with alcian blue (0.1% in 80% ethanol). A, chondroitin 6-sulfate from shark cartilage; B, dermatan sulfate from hog gastric mucosa; C, **1a**; and D, **1b**.

TABLE I

ANALYTICAL DATA[a] FOR **1a** AND **1b**

Sample	*Uronic acid*				*Hexosamine*		*Sulfate*[h]	[α]$_D^{20}$ *(in water)*	*Molecular weight*[i]
	b	*c*	*d*	*e*	*Total*[f]	*GlcN/GalN*[g]			
1a	10.7	26.8 (1.11)[j]	36.5	0.29	26.8 (1.00)[j]	0.01 >	14.2 (1.21)[j]	−65°	11,300
1b	11.3	28.2 (1.15)[j]	38.9	0.30	27.3 (1.00)[j]	0.01 >	16.5 (1.36)[j]	−62°	13,000

[a]Expressed as mg per 100 mg of sample. [b]Determined by the carbazole–sulfuric acid method of Dische[11], and expressed as D-glucuronic acid. [c]Determined by the carbazole–sulfuric acid method of Bitter and Muir[12], and expressed as D-glucuronic acid. [d]Determined by the orcinol–sulfuric acid method of Brown[13], and expressed as D-glucuronic acid. [e]The ratio of [b] to [d]. [f]Determined by the Elson–Morgan reaction of Gardell[14], and expressed as 2-amino-2-deoxygalactose. [g]Molar ratio of 2-amino-2-deoxy-glucose to -galactose, determined with a liquid chromatograph. [h]Assayed by the method of Dodgson and Price[15]. [i]Calculated from the intrinsic viscosity according to the method of Mathews and Dorfman[18]. [j]Molar ratio to total hexosamine.

et al.[2]. Also, the high dextrorotation (+70° in water) of the 1.25M fraction indicated its heparin nature. On the other hand, the 1.5 and 1.75M fractions showed mobilities on electrophoretograms similar to that of a standard specimen of **1** obtained from hog-gastric mucosa, although the former migrated slightly more slowly than the latter, and more slowly than the standard **1**, in both pyridine–formic acid (pH 3.0) and in 0.1M hydrochloric acid (Fig. 1). These findings indicate that these two fractions were dermatan sulfate (**1**). The 1.5 and 1.75M fractions were therefore designated as **1a** and **1b**, respectively. The recoveries (as sodium salt) of **1a** and **1b** were 375 and 510 mg, respectively, from 1.0 g (as the calcium salt) of the second 20% ethanol precipitate.

Characterization of **1a** *and* **1b**. — Analytical data for **1a** and **1b** are shown in Table I. As may be seen in this Table, the C/O ratios of **1a** and **1b** were 0.29 and 0.30, respectively. These low values are similar to those reported previously[6] for **1**. Moreover, the uronic acid values obtained by the carbazole–sulfuric acid methods of Dische[11] and Bitter and Muir[12] also agree well with those reported for this glycosaminoglycan[12]. The content of sulfate in **1a** was slightly lower than that in **1b**, a result was also confirmed by enzymic digestion with chondroitinase ABC to be described later. The optical rotations of **1a** (−65° in water) and **1b** (−62° in water) were quite similar to those reported previously[2-5]. The foregoing observations indicated that **1a** and **1b** were both dermatan sulfate. The intrinsic viscosities of **1a** and **1b** were 0.308 and 0.345, respectively, in 0.01M sodium phosphate buffer (pH 7.0) containing 0.38M sodium chloride. The molecular weights, calculated by the equation of Mathews and Dorfman[18] from the intrinsic viscosities, were 11,300 and 13,000, respectively.

Determination of ratio of D-*glucuronic acid to* L-*iduronic acid*. — The carboxyl groups of **1a** and **1b** were reduced with sodium borohydride after activation with EDC as described in the Experimental section. Conditions for the reduction were optimal at 50°, as shown by Taylor and Conrad[27]. Analytical data for the reduction products of **1a** and **1b** are shown in Table II. These preparations contained negligible uronic acid, indicating that reduction of the carboxyl groups was quantitative. The ratios of the hexose values obtained by the phenol–sulfuric acid reaction to those by the anthrone–sulfuric acid reaction of **1a** and **1b** were 0.58 and 0.56, respectively. On the other hand, the ratios for authentic D-glucose and 1,6-anhydro-L-idose were 0.98 and 0.40, respectively. These findings indicated that the carboxyl-reduced **1a** and **1b** contain mainly L-idose. Acid hydrolysis of the carboxyl-reduced **1a** and **1b** by Dowex-50 (H^+ form) in 0.05M hydrochloric acid gave the best yield of neutral monosaccharides when it was conducted by heating for 24 h at 100°, as reported by Lee and Cherniak[28]. Under the same conditions of acid hydrolysis, 1,2-*O*-isopropylidene-L-idose gave 1,6-anhydro-L-idopyranose as the sole product, by way of the intermediate sugar (L-idose). Therefore, the gas-chromatographic analysis of 1,6-anhydro-L-idose was conducted with standard specimens of D-glucose, D-xylose, and D-galactose under various conditions. The best result was obtained when the trifluoroacetyl derivatives of these sugars, after reduction with sodium borohydride, were applied to a column of OV-105 under conditions described in the Experimental section. Gas chromatograms of the *O*-trifluoroacetyl derivatives of the authentic 1,6-anhydro-L-idose, xylitol, D-glucitol, and galactitol are shown in Fig. 2, and those of the reduction products of the acid hydrolyzates of the carboxyl-reduced **1a** and **1b** are shown in Figs. 3A and 3B, respectively. As may be seen from Figs. 3A and

TABLE II

ANALYTICAL DATA[a] FOR CARBOXYL-REDUCED **1a** AND **1b**

Sample	*Hexose*			*Uronic*[e] *acid*	*2-Amino-2-deoxy-galactose*[f]	*Sulfate*[g]
	Anthrone[b]	*Phenol*[c]	*Average*[d]			
Reduced **1a**	39.9	23.0	31.5 (1.03)[h]	1>	30.5 (1.00)[h]	13.5 (1.00)[h]
Reduced **1b**	40.5	22.6	31.1 (1.13)[h]	1>	27.4 (1.00)[h]	15.5 (1.27)[h]

[a] Expressed as mg per 100 mg of sample. [b] Determined by the anthrone–sulfuric acid reaction[16], and expressed as D-glucose. [c] Determined by the phenol–sulfuric acid reaction[17], and expressed as D-glucose. [d] Average value of [b] and [c]. [e] Determined by the carbazole–sulfuric acid method of Bitter and Muir[12], and expressed as D-glucuronic acid. [f] Determined by the Elson–Morgan reaction of Gardell[14]. [g] Assayed by the method of Dodgson and Price[15]. [h] Molar ratio to 2-amino-2-deoxy-galactose.

3B, the latter compounds contained 1,6-anhydro-L-idose and D-glucitol. The ratios of D-glucitol to 1,6-anhydro-L-idose were calculated as 8.0:100 in **1a** and 7.5:100 in **1b** from the peak areas shown in the figures. These values should correspond to the ratios of D-glucuronic acid to L-iduronic acid in the original samples. Recoveries of 1,6-anhydro-L-idose and D-glucitol from the carboxyl-reduced dermatan sulfate were almost quantitative (>95%).

Enzymic digestion of **1a** *and* **1b**. — Chondroitinase ABC from *Proteus vulgaris*

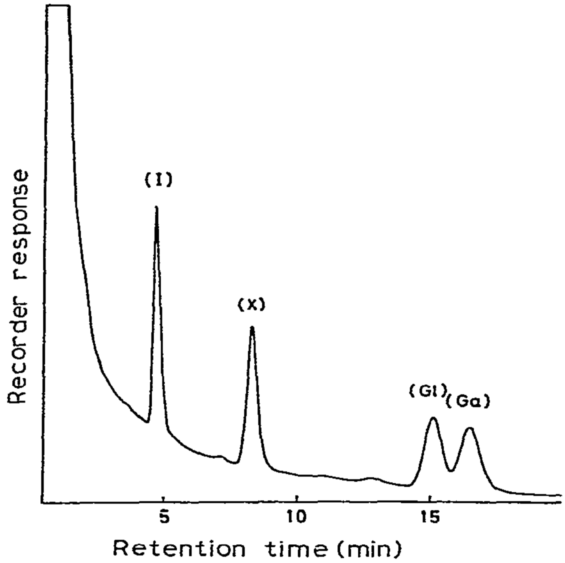


Fig. 2. Gas chromatograms of the trifluoroacetyl derivatives of the authentic 1,6-anhydro-L-idopyranose (I), xylitol (X), glucitol (Gl), and galactitol (Ga). Gas chromatography was performed as described in the text.

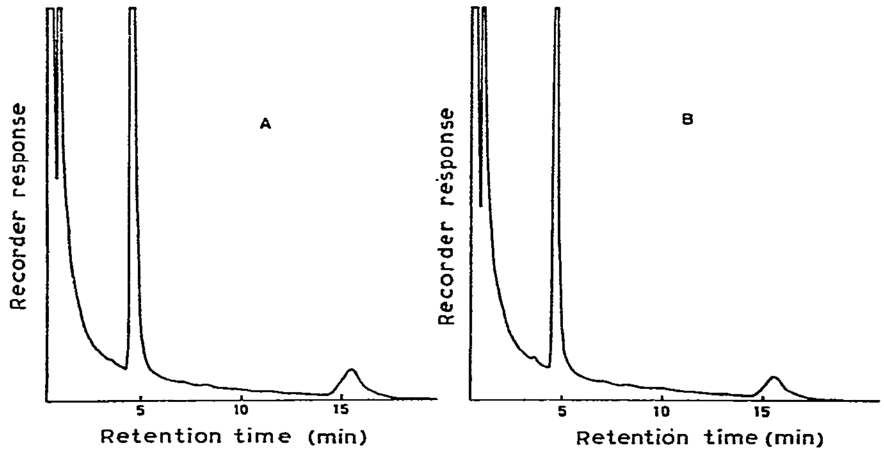


Fig. 3. Gas chromatograms of the trifluoroacetyl derivatives of the reduction products of the acid hydrolyzates of the carboxyl-reduced **1a** (A) and **1b** (B). Gas chromatography was performed as described in the text.

TABLE III

ANALYTICAL DATA[a] FOR THE DIGESTION PRODUCTS OF **1a** AND **1b** WITH CHONDROITINASE ABC

	2	**3**	**4**	**5**
Digestion product of **1a**	13.2	86.8	T[b]	ND[c]
Digestion product of **1b**	9.0	91.0	T[b]	ND[c]

[a] Expressed as per cent in the total unsaturated oligosaccharides. [b] Trace. [c] Not detected.

quantitatively cleaved the 2-acetamido-2-deoxy-D-galactosidic linkages of **1a** and **1b** to give unsaturated disaccharides (Fig. 4). The ratios of **2**, **3**, and **4** are shown in Table III. As may be seen from Table III, although **2** was detected, **4** and **5** were not. Approximately 90% of the unsaturated disaccharides was **3**, suggesting that the sulfate groups might be distributed uniformly at position 4 of the 2-acetamido-2-deoxy-D-galactose residues throughout the backbone structure of the dermatan sulfate. On the other hand, digestion of **1a** and **1b** with chondroitinase AC-II produced only small proportions of unsaturated oligosaccharides. The ratios of the absorbances at 235 nm of the digests after incubation for 3 h with chondroitinase AC-II and chondroitinase ABC were 7.4:100 for **1a** and 7.0:100 for **1b**, on the basis of the same amounts of the sample. These values are similar to the ratios of D-glucuronic acid to L-iduronic acid in these samples. Moreover, chondroitinase B from *Flavobacterium heparinum* cleaved approximately 93% of the 2-acetamido-2-deoxygalactosidic linkages of **1a** and **1b**. These data support the results of the determination

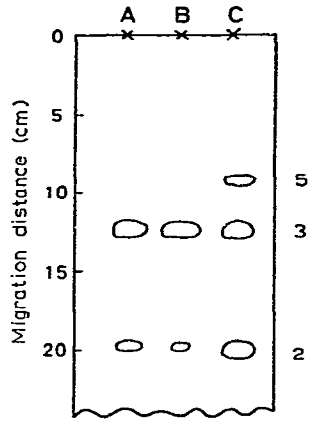
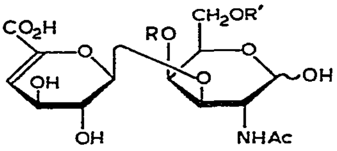


Fig. 4. Tracing of a paper chromatogram of the digestion products of **1a** (A) and **1b** (B) with chondroitinase ABC. C, references of **2**, **3**, and **5**. Paper chromatography was carried out as described in the text.

of the D-glucuronic acid to L-iduronic acid ratios in these two specimens by the new method described here.



2 R = R' = H
3 R = SO_3H, R' = H
4 R = H, R' = SO_3H
5 R = SO_3H, R' = H, plus one additional sulfate group

The gel-filtration patterns of **1a** and **1b** before and after digestion with chondroitinase AC-II are shown in Figs. 5A and 5B, respectively. Although a small peak of retarded material absorbing at 235 nm was observed after the digestion, the elution patterns of the main peaks were similar before and after digestion. The data showed that the positions of the peaks absorbing at 235 nm corresponded to those of the unsaturated oligosaccharides of lower molecular weights. Moreover, the main peak-substances did not show any absorption at 235 nm after digestion with the enzyme. Based on these findings, the possibility that the location of the linkages in **1a** and **1b** susceptible to chondroitinase AC-II are in the middle and in the non-reducing region of the polysaccharide chains could be excluded. The elution pattern of the main peak of the digestion products of **1b** showed a slight shoulder, but that of **1a** showed a symmetrical profile.

DISCUSSION

The molecular weights calculated from the intrinsic viscosities of the present

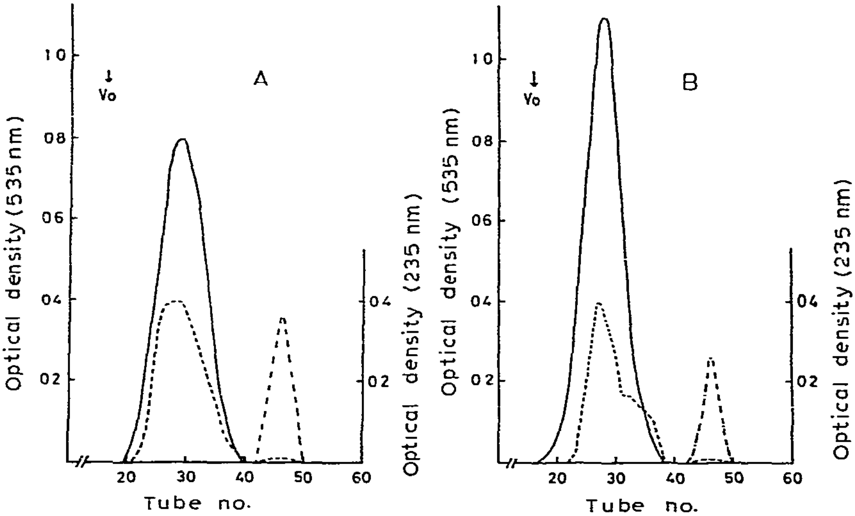


Fig. 5. Gel-filtration patterns of **1a** (A) and **1b** (B) before and after the digestion with chondroitinase AC-II. —— and ------, optical densities obtained by the carbazole–sulfuric acid reaction of Bitter and Muir[12] for uronic acid before and after the enzymic digestion, respectively; -·-·-, optical density at 235 nm after the enzymic digestion; Vo, void volume. Gel filtration was performed as described in the text, using 3 mg each of the intact samples and 1 mg each of their digestion products.

specimens of dermatan sulfate (**1**) were different from that shown to be 27,000 according to Mathews *et al.*[29] of the standard **1** from hog-gastric mucosa, but were comparable with those from pig skin, obtained by Fransson and Rodén[5]. The difference cannot be evaluated, because the methods of preparation differed.

Inoue and Miyawaki[30] reported that the content of D-glucuronic acid in **1** obtained from whale intestine was negligible compared with the large content of L-iduronic acid. However, the present data obtained by our new analytical method showed that the ratios of D-glucuronic acid to L-iduronic acid in **1a** and **1b** were 8.0:100 and 7.5:100, respectively; these values were also supported by the results of enzymic digestion. The discrepancy between previous results[30] and those reported here concerning the contents of D-glucuronic acid and L-iduronic acid in specimens of **1** might arise from differences in the analytical methods used. Although it has been reported that acid hydrolysis of glycosaminoglycans resulted in significant decomposition of uronic acid, the degradation of D-glucose and L-idose in the carboxyl-reduced **1** was shown to be very small under the present conditions of hydrolysis, as mentioned in the Results section.

Judging from the molecular weights and the content of D-glucuronic acid in the present specimens of **1**, two D-glucuronic acid residues might be present in each polysaccharide chain. The results of gel filtration of **1a** and **1b**, before and after digestion with chondroitinase AC-II, might exclude the possibility of the location of D-glucuronic acid residues in the middle and the non-reducing portion of the polysaccharide chains, as described in the Results section. It has been shown already[31] that at least one D-glucuronic acid residue is generally present in the linkage region of **1**. Although the work of Fransson[6] indicated the presence of a D-glucuronic acid residue in the non-reducing portion of the dermatan sulfate obtained from hog-intestinal mucosa (which contained 5% of D-glucuronic acid in the total uronic acid), the location of the D-glucuronic acid residues in the present two specimens of **1** might be in the linkage region, as indicated by the results of the enzymic digestion already mentioned.

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