exhibited by the common carotenoids, as shown by Figs. 2 and 3.

Many of the infrared absorption maxima of eschscholtzxanthin can be correlated with the maxima of the common carotenoids and in this way with particular structural groups.[15] Maxima at 2960 and 2870 cm.$^{-1}$ are due to methyl groups; that at 3030 to —CH=CH—; that at 2926 to —CH_2—; those at 1450 and 1375 to CH_3C; those at 1380 and 1365 to $(CH_3)_2C$; that at 1030 to C—O.

Chromatoyraphy of eschscholtzxanthin and eschscholtzxanthin diacetate. In columns of activated magnesia, which attracts double bonds as well as hydroxyl groups,[3,16,17] and with petroleum ether (b.p. 20–40°)-25% acetone as solvent, eschscholtzxanthin formed a purple-orange zone above zeaxanthin, neoxanthin, and lycopene. In sugar columns, which do not attract double bonds,[16,17] and with petroleum ether plus benzene, the eschscholtzxanthin was sorbed with violaxanthin below neoxanthin and above zeaxanthin plus lutein. In sugar columns with petroleum ether plus 0.5% *n*-propyl alcohol, the eschscholtzxanthin was sorbed below violaxanthin, above zeaxanthin plus lutein, and just above chlorophyll *b*.[16]

In magnesia columns and with petroleum ether plus 25% acetone, eschscholtzxanthin acetate was sorbed with zeaxanthin and lycopene, far above lutein acetate. In sugar columns with petroleum ether plus 0.5% *n*-propyl alcohol, the acetate was weakly sorbed forming a pink-yellow zone, not separated from lutein acetate and separated incompletely below cryptoxanthin.

(15) L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, 2nd Ed., Methuen, London; Wiley, New York, 1958.

(16) H. H. Strain, *Chloroplast Pigments and Chromatographic Analysis*, 32nd Annual Priestly Lectures, Pennsylvania State University, University Park, 1958.

(17) H. H. Strain, *J. Am. Chem. Soc.*, **70**, 588 (1948).

DISCUSSION

The visible spectra and the chromatographic sequences show that eschscholtzxanthin contains twelve conjugated double bonds. Most of the infrared absorption maxima of eschscholtzxanthin are similar to those of the common carotenoids and have been related to absorption by particular structural units characteristic of these substances. The most striking differences between the infrared spectra of eschscholtzxanthin and those of the common carotenoids are the two bands of the former, at 955 cm.$^{-1}$ and 977 cm.$^{-1}$, and the single band of the latter, at 965 cm.$^{-1}$ This indicates that eschscholtzxanthin is a derivative of *sym*-dehydro-β-carotene (dehydroretrocarotene)[4,5] with a single band at the center of the molecule. It shows that this xanthophyll cannot be a derivative of γ-carotene.[6]

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ARGONNE, ILL.

[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINE, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY AND THE EDWARD DANIELS FAULKNER ARTHRITIS CLINIC, PRESBYTERIAN HOSPITAL]

The Structure of Keratosulfate of Bovine Cornea[1]

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The structure of keratosulfate from bovine cornea has been studied by methylation techniques. Hydrolysis products from methylated, sulfated, and desulfated polymers establish the disaccharide, *O*-β-D-galactopyranosyl-(1 → 4)-(2-acetamido-2-deoxy-D-glucose 6-sulfate) linked (1 → 3) to the D-galactose of the next recurrent disaccharide unit, as the repeating sequence in keratosulfate.

Keratosulfate, initially isolated from cornea[2] and recently reported from diverse sources,[3] is distinguished from the other connective tissue mucopolysaccharides by the absence of uronic acid and the presence of galactose. Its atypical nature is further characterized by its resemblance to blood group substances, exemplified by: 1) the presence of small amounts of easily hydrolyzable methylpentose, 2) its cleavage by blood group substance-cleaving enzymes in contrast to its inertness toward hyaluronidases and chondrosulfatases, and 3) the cross reaction of the desulfated polysaccharide with antiblood group sera.[4]

(1) This work was supported in part by grants from the National Science Foundation and the U. S. Public Health Service.

(2) K. Meyer, A. Linker, E. A. Davidson, and B. Weissmann, *J. Biol. Chem.*, **205**, 611 (1953).

(3) K. Meyer, P. Hoffman, and A. Linker, *Science*, **128**, 896 (1958).

(4) O. Rosen, P. Hoffman, and K. Meyer, *Federation Proc.*, **19**, No. 1, 147 (1960).

Keratosulfate is readily hydrolyzed to monosaccharides whereas the glucuronic acid–containing mucopolysaccharides, due to the resistant glucuronidic linkage, are cleaved hydrolytically to disaccharides. Sufficiently vigorous conditions to cleave the disaccharides cause extensive degradation of the glucuronic acid. This resistance to hydrolysis increases the difficulty of performing structural studies on acidic mucopolysaccharides by methylation techniques. In fact, the only apparently successful study involved chondroitin sulfate B,[5] in which D-glucuronic acid is replaced by the more readily hydrolyzable L-iduronic acid. It was the absence of this major obstacle which made a methylation study of keratosulfate appear feasible. As in the study on chondroitin sulfate B, the only difference in monosaccharide products from methylated keratosulfate and its desulfated polymer would be due to methylation at the site of sulfate ester linkage, the remaining unmethylated sites from both polymers being due to glycosidic linkages. The reasonable assumption that both the glucosamine and galactose components are present in pyranose forms is borne out by their normal rates of hydrolysis.

Desulfation of keratosulfate by the methods used with the uronic acid–containing mucopolysaccharides[6] caused extensive degradation, and the nondialyzable product, recovered in low yield, proved to be intractable. A better yield of a more tractable product could be obtained by decreasing the acidity, which could be achieved most readily by the addition of dry cation-exchange resin and solid sodium chloride to a suspension of keratosulfate in anhydrous methanol. However, as described below, desulfation of methylated keratosulfate was the method of choice.

Keratosulfate could be repeatedly methylated with Haworth's reagents[7] with essentially no loss of sulfate. Treatment of the methylated product with Dowex 50(H^+) to remove cations and effect solution in organic solvents caused the ready loss of sulfate ester. Hence, methylation without desulfation could not be continued in organic medium as is necessary with Purdie's reagents[8]; on the other hand, a mild method of desulfation to a soluble product was achieved which kept the glycosidic degradation within reasonable bounds and permitted permethylation of the desulfated polysaccharide.

Maximum methylation of both the sulfated and desulfated polysaccharides occurred after several treatments with Haworth's reagents, followed by Purdie's reagents for the latter compound. None of the methods outlined in the experimental section succeeded in increasing the methoxyl content. In both cases the methoxyl content was about 80% of the theoretical for a permethylated product of alternating anhydro units of D-galactose and 2-acetamido-2-deoxy-D-glucose. However, several considerations lead to the conclusion that undermethylation was not significant and, in particular, that there was not a resistant hydroxyl which would necessitate an ambiguous choice of sulfate ester and glycosidic linkages. Firstly, in isolated keratosulfate the sum of the analytical values as anhydro units for 2-acetamido-2-deoxy-D-glucose, D-galactose, and sulfate never total 100%; small amounts of amino acids can generally be detected chromatographically after acid hydrolysis; hence, a lower methoxyl value due to the presence of nonhydroxylated impurities cannot be excluded. Secondly, the methoxyl values of the permethylated polysaccharides, based on an anhydro-disaccharide repeating unit, indicate that up to 75% of one hydroxyl per disaccharide unit has remained unsubstituted; this amount of hydroxyl would cause easily detectable absorption in the 3400–3600-cm.$^{-1}$ region of the infrared spectra, whereas no significant absorption was recorded in this region. Thirdly, analysis of the relative amounts of methylated monosaccharides (see below) indicated that the major glucosamine and galactose derivatives in each case were the products from the permethylation of a straight chain polysaccharide.

Maximal hydrolysis of the methylated polysaccharides to monosaccharide derivatives was determined reductimetrically and polarimetrically. The monosaccharide products were separated by ion exchange and chromatography, were crystallized or converted to crystalline derivatives, and their identities were established by comparison with authentic samples. The relative amounts of methylated monosaccharides were determined reductimetrically after chromatographic separation by the method of Hirst *et al.*[9] and are listed in Table I. Densitometer readings of the developed chromatograms gave essentially the same results. It can be

TABLE I

RELATIVE AMOUNTS OF METHYLATED MONOSACCHARIDES[a]

	I[b]	II[c]
2-Amino-2-deoxy-D-glucose:		
3-*O*-Methyl	43	20
3,6-Di-*O*-methyl	12	39
D-Galactose:		
2,4-Di-*O*-methyl	15	9
2,4,6-Tri-*O*-methyl	30	27
2,3,4,6-Tetra-*O*-methyl	0	5

[a] See Experimental for details. [b] From sulfated polysaccharide. [c] From desulfated polysaccharide.

(5) R. W. Jeanloz and P. J. Stoffyn, *Federation Proc.*, **17**, 249 (1958).

(6) T. G. Kantor and M. Schubert, *J. Am. Chem. Soc.*, **79**, 152 (1956).

(7) W. N. Haworth, *J. Chem. Soc.*, **107**, 13 (1915).

(8) T. Purdie and J. C. Irvine, *J. Chem. Soc.*, **83**, 1021 (1903).

(9) E. L. Hirst, L. Hough, and J. K. N. Jones, *J. Chem. Soc.*, 928 (1949).

seen that the major change occurring with desulfation is the decrease in the yield of 2-amino-2-deoxy-3-*O*-methyl-D-glucose and the concurrent increase in the yield of 2-amino-2-deoxy-3,6-di-*O*-methyl-D-glucose, establishing conclusively that the loss of sulfate ester was from the 6- position of 2-amino-2-deoxy-D-glucose. The small amount of 2-amino-2-deoxy-3,6-di-*O*-methyl-D-glucose from the keratosulfate can be accounted for by the slight deficiency in sulfate in the polymer, which increased somewhat during methylation. The 2-amino-2-deoxy-3-*O*-methyl-D-glucose from the desulfated polysaccharide was due to the 10% residual sulfate, which we did not attempt to remove since extended desulfation also caused significant glycosidic cleavage resulting in too great a loss of nondialyzable polysaccharide.

As can also be seen from Table I, 2,4,6-tri-*O*-methyl-D-galactose was the major galactose product from both sulfated and desulfated polysaccharides, with virtually no change in the relative amounts of methylated galactose derivatives, certainly insufficient to accommodate a significant amount of sulfate ester. The small amount of dimethylgalactose from both polymers is probably due to undermethylation. If it were due to branching, a comparable amount of trimethylglucosamine or tetramethylgalactose should have been detected. The small amount of tetramethylgalactose from the desulfated polymer is undoubtedly due to the shortened chain lengths due to the methanolysis occurring concomitant with desulfation.

The isolated 2-acetamido-2-deoxymono-*O*-methyl-D-glucose derivative, even after repeated recrystallizations, did not correspond in physical properties to any of the reported monomethyl derivatives. Its color yield in the Morgan and Elson reaction[10] eliminated the possibility of its being the 4-*O*-methyl derivative. Its infrared spectrum differed markedly from that of the 6-*O*-methyl derivative and resembled that of the other possible derivative, the 3-*O*-methyl, differing slightly in the 700–1300-cm.$^{-1}$ region, as outlined in Table II. The major difference was due to an 830–855-cm.$^{-1}$ and an 888-cm.$^{-1}$ peak in the spectra of the isolated monomethyl and authentic 2-acetamido-2-deoxy-3-*O*-methyl-D-glucose, respectively. These peaks appeared to correspond to the 850-cm.$^{-1}$ absorption indicative of α-D-glucopyranose linkages and the 884–900-cm.$^{-1}$ absorption for β-D-glucopyranosides, which, as Stacey *et al.*[11] have shown, also appear in the spectra of the 2-acetamido-2-deoxy-α- and β-D-glucoses. This indicated that the difference was due to the configurations about the anomeric carbon. Verification was obtained from the spectra of the two compounds, which became identical after equilibration of the samples in water, as would be required for anomeric compounds. Further evidence came from mutarotation studies, which defined the isolated monomethylglucosamine as a crystalline α-D-glycose and the authentic sample as a β-D-glycose, both giving essentially identical equilibrium values. This established the crystalline derivative from keratosulfate as 2-acetamido-2-deoxy-3-*O*-methyl-α-D-glucose.

TABLE II

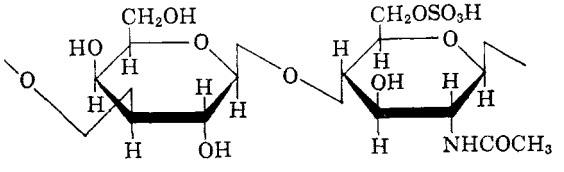
INFRARED ABSORPTION PEAKS OF 2-ACETAMIDO-2-DEOXY-MONO-*O*-METHYL-D-GLUCOSES

A Mono-*O*-methyl, Cm.$^{-1}$	B Authentic 3-*O*-Methyl, Cm.$^{-1}$	A and B after Equilibration, Cm.$^{-1}$
772 (w)		770 (w)
	858 (w)	
830–855 (w)		
	888 (w)	
915 (w)	915 (w)	
955 (w)	955 (w)	955 (w)
975 (m)	975 (w)	975 (w)
1025–1050 (s)	1025–1050 (s)	1040–1050 (s)
	1060 (s)	
1065–1070 (s)	1075 (s)	1070–1080 (s)
1115 (s)	1110 (s)	1115 (s)
	1150 (m)	
1185 (w)	1185 (w)	1185 (w)
1245 (w)	1245 (w)	1245 (w)

The 2-acetamido-2-deoxy-3,6-di-*O*-methyl-D-glucose was identified by infrared spectra and mixed melting point. The color yield in the Morgan and Elson reaction precluded substitution in the 4-position. If any galactosidic linkages in the desulfated polymer were to positions other than the 4- position of 2-amino-2-deoxy-D-glucose, then methylated 2-acetamido-2-deoxy-D-glucose derivatives should have been present with greatly reduced Morgan and Elson color values; these were not detected.

The 2,4,6-tri-*O*-methyl-D-galactose obtained as the major galactose derivative from both keratosulfate and the desulfated polysaccharide and characterized by infrared spectra and mixed melting point established the glucosaminidic linkage to the 3- position of D-galactose.

The β-D nature of both linkages has been reported previously[4]; hence, keratosulfate contains the repeating unit (1 → 3)-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranosyl 6-sulfate:



Treatment of keratosulfate and its desulfated polymer with periodic acid resulted in the very slow consumption of periodate in both cases with the same end point being reached of one mole of

(10) D. Aminoff, W. T. J. Morgan, and W. M. Watkins, *Biochem. J.*, 51, 379 (1952).

(11) S. A. Barker, A. B. Foster, M. Stacey, and J. M. Webber, *J. Chem. Soc.*, 2218 (1958).

TABLE III

CONSUMPTION OF PERIODIC ACID (MOLES/ANHYDRODISACCHARIDE UNIT)

Time (hr.)	4	23	43.5	67.5	141	189	223
Keratosulfate	0.045	—	0.15	0.17	0.21	0.20	0.20
Desulfated polymer		— 0.15	0.15	0.16	0.20	0.20	0.20

periodate consumed for a chain length of ten monosaccharide units (see Table III). The lack of activity of both polymers is consistent with the proposed structure for keratosulfate.

EXPERIMENTAL

References to most of the analytical methods have been cited previously.[12] A modified Morgan-Elson method[10] was used for the determination of free 2-acetamido-2-deoxy-D-glucose color value. Hexose was determined by the anthrone method,[13] using galactose as a standard.

Melting points were obtained on a microscope hotstage and are uncorrected.

Infrared spectra were obtained with potassium bromide pellets in a Perkin-Elmer Model 21 spectrophotometer with sodium chloride prism.

Paper chromatograms were developed by the descending flow of *n*-butyl alcohol–ethanol-water (4:1:1) on Whatman No. 1 paper. Whatman No. 3MM paper was used for the elution of larger quantities of sugars. Ninhydrin and aniline trichloroacetate spray reagents were utilized for the detection of the sugars. R_g values are the ratios of the distances traveled by the methylated sugars to 2,3,4,6-tetra-*O*-methyl-D-glucose.

Keratosulfate. Bovine cornea was the source of all samples of keratosulfate used in this study.[1] Since the other mucopolysaccharides of bovine cornea contain glucuronic acid and galactosamine, the keratosulfate was refractionated by alcohol precipitation until their virtual absence was verified by the absence of carbazole color,[14] and by the ninhydrin oxidation method.[15]

Methylation of keratosulfate. Keratosulfate calcium salt (2.0 g., *anal.:* hexosamine, 28.9; galactose, 21.0; SO_4, 16.8) was dissolved in 20 ml. of water and maintained in an ice bath under nitrogen. Dimethyl sulfate (6 ml.) and 6 ml. of carbon tetrachloride were added, and then 18 ml. of 30% sodium hydroxide was added dropwise over a period of 6 hr. to the vigorously stirred mixture. The reaction mixture was slowly warmed to room temperature, and the stirring was continued overnight. The mixture was again cooled in an ice bath, and 30 ml. of dimethyl sulfate and 90 ml. of 30% sodium hydroxide were alternately added in small portions for 3 hr.; stirring was continued for 4 hr. at ice bath temperature and overnight at room temperature. After neutralizing with acetic acid to *p*H 7, the mixture was dialyzed for 2 days, concentrated under reduced pressure to about 40 ml., filtered, and lyophilized, yielding 1.84 g. of a white powder. Repeated treatment with 30 ml. of dimethyl sulfate and 90 ml. of 30% sodium hydroxide yielded 1.38 g. of a white powder;

Anal.: OCH_3, 19.1.

Three further methylations under the same conditions did not increase the methoxyl content significantly; yield, 1.10 g.

Anal. Calcd. for $C_{14}H_{18}O_9NSCa_{1/2}(CH_3O)_4$: SO_4, 18.30; OCH_3, 23.83. Found: SO_4, 14.2; OCH_3, 19.7; $[\alpha]_D^{24}$ +3° (*c* 1.5, water).

The product was soluble in water and insoluble in all organic solvents tested. Treatment with Dowex 50 (H^+) to obtain the free acid caused partial desulfation. The cetylpyridinium complex was soluble in methanol. However, treatment of the complex with methyl iodide and silver oxide in methanol did not increase the methoxyl content.

Preparation of desulfated and methylated keratosulfate. Keratosulfate (2.0 g., *anal.:* hexosamine, 26.1; galactose, 30.8; SO_4, 12.1) was treated twice with dimethyl sulfate and 30% sodium hydroxide as above. The product (1.37 g.) was suspended in 100 ml. of absolute methanol with 7 g. of dried Dowex 50 (H^+), and the mixture was shaken for 2 days, resulting in the solubilization of the white powder. The solution was filtered from the resin and combined with 50 ml. of a water wash. After dialysis for 2 days, the solution was concentrated under reduced pressure and lyophilized.

Anal.: SO_4, 2.7.

One further treatment with Dowex 50 (H^+) as above yielded 0.72 g.

Anal., SO_4, 1.4.

The desulfated product was treated twice more with dimethyl sulfate and 30% sodium hydroxide and was then refluxed twice with 25 ml. of methyl iodide and 1 g. of silver oxide in a small volume of absolute methanol for 6 hr. each time. After filtration the product was precipitated by the addition of diethyl ether, yielding 0.41 g. of a white powder.

Anal. Calcd. for $C_{14}H_{18}O_5N(CH_3O)_5$: OCH_3, 35.63. Found: OCH_3, 31.1; $[\alpha]_D^{24}$ +6° (*c* 1.0, water).

Further methylation with methyl iodide and silver oxide in *N,N*-dimethylformamide did not increase the methoxyl content. Refluxing in *N,N*-dimethylformamide caused extensive degradation. Treatment with a solution of diazomethane in ether did not change the methoxyl value.

Hydrolysis of the methylated polysaccharides. The methylated products from keratosulfate and the desulfated polymer were heated in *N* sulfuric acid at 100° (*c*, 1.4%), and aliquots were withdrawn periodically for the determination of optical rotation and reducing value. The resulting data indicated that maximum hydrolysis occurred after 17 hr. with the sulfated product and after 10 hr. with the desulfated product.

The hydrolyzed solutions were neutralized with barium carbonate, and the mixtures were filtered, washed with water and then with methanol. The filtrates were concentrated under reduced pressure at 30–35°, yielding 750 mg. of a sirup from 1.06 g. of the methylated sulfated polymer and 615 mg. of a sirup from 0.81 g. of the methylated desulfated polymer.

Paper chromatographic examination indicated that the hydrolyzed products from both polymers contained monomethyl-glucosamine (R_g 0.22), dimethylglucosamine (R_g 0.35), dimethylgalactose (R_g 0.51), trimethylgalactose (R_g 0.71); and in addition, tetramethylgalactose (R_g 0.93) was detected in the hydrolyzed product from the desulfated polymer.

Relative amounts of methylated monosaccharides. The hydrolyzed sirups (20 mg. of each) were applied to thick paper, chromatographed, and, after spray detection on guide strips, each component was eluted from the appropriate section with water. The solutions were concentrated under reduced pressure, diluted to 10 ml., and the concentration of reducing sugar in each extract was determined by the hypoiodite method.[9] The relative amounts are recorded in Table I.

Isolation of methylated monosaccharides. The sirup (750 mg.) from the hydrolysis of the methylated sulfated polymer was dissolved in 100 ml. of water and passed through a Dowex 50 (H^+) column which was washed with 300 ml. of water. The combined effluents were evaporated under reduced pressure to a sirupy mixture of methylated galactose monosaccharides (206 mg.). The absence of hexosamine derivatives was indicated by the negative ninhydrin reaction. The column was eluted with 600 ml. of 0.3% sulfuric acid, and the eluate was neutralized with barium carbonate,

(12) M. M. Rapport, K. Meyer, and A. Linker, *J. Am. Chem. Soc.*, **73**, 2416 (1951).

(13) W. E. Trevelyan and J. S. Harris, *Biochem. J.*, **50**, 298 (1952).

(14) Z. Dische, *J. Biol. Chem.*, **167**, 189 (1947).

(15) P. J. Stoffyn and R. W. Jeanloz, *Arch. Biochem. Biophys.*, **52**, 373 (1954).

filtered, and concentrated to a sirup (154 mg.) of methylated 2-amino-2-deoxy-D-glucose derivatives. Similarly, the sirup (615 mg.) from the hydrolysis of the methylated desulfated polymer yielded a galactose fraction (210 mg.) and a glucosamine fraction (181 mg.).

Each fraction was separated into its components on Whatman No. 3MM paper, and the individual components were eluted from the paper with water and concentrated to sirups under reduced pressure; four fractions being obtained from the sulfated polymer and five fractions from the desulfated polymer.

2-Acetamido-2-deoxy-3-O-methyl-α-D-glucose. The monomethylglucosamine fraction (R_g 0.22; 49.2 mg.) from the sulfated polymer was dissolved in methanol, decolorized with active carbon, and concentrated to a sirup. The sirup was dissolved in 4 ml. of anhydrous methanol containing hydrochloric acid and, as the hydrochloride did not crystallize, 20.0 mg. of silver carbonate was added. The mixture was shaken for 1 hr., filtered, and washed with 2 ml. of methanol. Acetic anhydride (0.003 ml.) was added, and the solution was maintained in the refrigerator overnight, for 12 hr. at room temperature, and then concentrated to a sirup after the addition of water. The sirup was dissolved in 30 ml. of water, passed through a Dowex 50 (H^+) column (50 × 14 mm.) which was washed with 60 ml. of water, and the combined effluents were concentrated to a sirup, dissolved in methanol, decolorized with carbon, and again concentrated to a sirup. The sirup gave approximately the same color value as authentic 2-acetamido-2-deoxy-3-*O*-methyl-D-glucose in the Morgan and Elson reaction[10] and had the same R_f value. The sirup was dissolved in absolute methanol, and ether was added to incipient turbidity, yielding 6.3 mg. of crystals, m.p. 140–145°, which rose after two more recrystallizations to 162–163°.

Anal. Calcd. for $C_9H_{17}O_6N$: OCH_3, 13.2. Found: OCH_3, 15.2. (The methoxyl determination suffered from the lack of sufficient material. However, the value conclusively establishes the compound as a monomethyl derivative); $[\alpha]_D^{23}$ +86° (initial) to +33° (final) (*c* 0.2, water).[16]

The infrared spectrum differed from that of the authentic 3-*O*-methyl derivative as tabulated in Table II. Both crystalline samples, after solution in water and equilibration for 20 hr., followed by lyophilization with potassium bromide, yielded identical spectra (Table II). The mutarotation of the authentic 2-acetamido-2-deoxy-3-*O*-methyl-D-glucose: −40° (initial) to +30° (final) (*c* 0.1, water) defined it as the β-D anomer.

The corresponding monomethylglucosamine fraction (R_g 0.22; 12.6 mg.) from the desulfated polymer was treated in the same manner and yielded crystals, m.p. 142–145° (R_f 0.55), which gave a positive Morgan and Elson reaction and an identical infrared spectrum, establishing the identity of the monomethylglucosamine derivatives from both polymers.

2-Acetamido-2-deoxy-3,6-di-O-methyl-α-D-glucose. The dimethylglucosamine derivative (R_g 0.35; 33.1 mg.) from the desulfated polymer was *N*-acetylated in the same manner as the monomethyl derivative described above and was crystallized twice from methanol-ether, m.p. 188–191°; mixed m.p. 187–197°; $[\alpha]_D^{25}$ +50° (20 min.) to +41° (final); (*c* 0.3, water). The authentic sample of 2-acetamido-2-deoxy-3,6-di-*O*-methyl-α-D-glucose had m.p. 205–207° (reported[17]: m.p. 232–233°; $[\alpha]_D$ +90° to +37°).

Anal. Calcd. for $C_{10}H_{19}O_6N$: OCH_3, 24.9. Found: OCH_3, 25.8.

Both the isolated and authentic samples gave identical infrared spectra, equivalent Morgan and Elson color values, and identical R_f values of 0.72.

The dimethylglucosamine derivative (R_g 0.35; 17.1 mg.) from the sulfated polymer was converted to the *N*-acetyl derivative, which had a m.p. of 188–190° and was identical with the authentic 2-acetamido-2-deoxy-3,6-di-*O*-methyl-α-D-glucose by the criteria described above.

2,4-Di-O-methyl-N-phenyl-D-galactosylamine. The dimethylgalactose fraction (R_g 0.51; 30.9 mg.) was decolorized with active carbon in methanol and was then refluxed with 0.06 ml. of aniline in 6 ml. of absolute alcohol for 3 hr. Concentration to 3 ml. and refrigeration yielded 11.6 mg. of crystals which were twice recrystallized from methanol-ether; m.p. 216–219°, mixed melting point with authentic 2,4-di-*O*-methyl-*N*-phenyl-D-galactosylamine, 215–219°.

Anal. Calcd. for $C_{14}H_{21}O_5N$: OCH_3, 21.9. Found: OCH_3, 22.6.

The product similarly derived from 20.1 mg. of sirup (R_g 0.51) isolated from the desulfated product had a m.p. of 219–221°; mixed m.p. 217–220° and $[\alpha]_D^{28}$ −189° (*c* 0.8, pyridine). Reported[18] m.p., 216°; $[\alpha]_D$ −180° (pyridine). The derivatives from both sources and the authentic sample gave identical infrared spectra.

2,4,6-Tri-O-methyl-D-galactose. The trimethylgalactose fractions from both polymers (R_g 0.71) were decolorized with active carbon in methanol, crystallized from chloroform–petroleum ether (b.p. 30–60°), and recrystallized twice from ethyl acetate–petroleum ether. From 66.2 mg. of the sirup from the desulfated polymer, 21.2 mg. of crystalline material was obtained, m.p. 89–91°; mixed m.p. 90–95°; $[\alpha]_D^{19}$ +100° (initial) to +91° (final) (*c* 0.2, water) reported[19] m.p., 95–96°.

Anal. Calcd. for $C_9H_{18}O_6$: OCH_3, 41.9. Found: OCH_3, 42.5.

From 64.1 mg. of the sirup from the sulfated polymer, a comparable yield of crystals was obtained with essentially similar physical constants. The infrared spectra of the crystalline products and the authentic 2,4,6-tri-*O*-methyl-D-galactose were identical.

2,3,4,6-Tetra-O-methyl-N-phenyl-D-galactosylamine. From the desulfated polymer, 17.7 mg. of a sirup was obtained with the same R_g value of 0.93 as authentic 2,3,4,6-tetra-*O*-methyl-D-galactose. The sirup was refluxed with 0.01 ml. of aniline in 3 ml. of anhydrous ethanol for 3 hr. The addition of ether–petroleum ether followed by refrigeration yielded 6.1 mg. of needles, m.p. 185–187°; reported[20] m.p., 186–188°.

Anal. Calcd. for $C_{16}H_{25}O_5N$: OCH_3, 39.9. Found: OCH_3, 39.6.

Desulfated keratosulfate. Keratosulfate calcium salt (70.0 mg.; *anal.*, hexosamine, 26.1; galactose, 30.8; SO_4, 12.1) was shaken in 30 ml. of anhydrous methanol containing 2 g. of dried Dowex 50 (H^+) and 1.00 g. of sodium chloride for 2 days at room temperature. The suspension was filtered, washed with water, and the combined filtrates were dialyzed and lyophilized. The procedure was repeated once more on the product, yielding 35.0 mg. of a white lyophilized powder; *anal.*, SO_4, 1.3.

Periodate oxidation. The polysaccharides at approximately 0.4% in 0.02*M* periodic acid solution were maintained at 4° in the dark. Aliquots were removed at intervals for titration by the arsenite method.[21] The results are recorded in Table III.

(16) Authentic m.p. 195–198°, [α] +33° (water); ref. R. W. Jeanloz, *Advances in Carbohydrate Chem.* **13**, 189 (1958). This compound was first reported m.p. 183–185°, ref. R. Kuhn, A. Gauhe, and H. H. Baer, *Chem. Ber.*, **87**, 1138 (1954).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE OHIO STATE UNIVERSITY]

Synthesis of Amino Sugars by Reduction of Hydrazine Derivatives. 2-Amino-2-deoxy-L-lyxose (L-Lyxosamine) Hydrochloride[1-3]

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2-Amino-2-deoxy-L-lyxose (L-lyxosamine) has been synthesized as the crystalline hydrochloride by reduction and hydrolysis of the derivatives obtained by the action of hydrazine on methyl 3,5-*O*-isopropylidene-2-*O*-*p*-tolylsulfonyl-α,β-L-xylofuranoside. The mechanism of the displacement of the *p*-tolylsulfonyloxy group by hydrazine is discussed.

It is now well established[3-7] that the nucleophilic displacement of secondary *p*-tolylsulfonyloxy groups in sugar rings by hydrazine, amines, and probably ammonia takes place with Walden inversion when no suitably placed participating group is available. Hydrazinolysis of secondary *p*-toluenesulfonate esters proceeds under milder conditions than ammonolysis, and reduction of the resulting hydrazino sugars gives better yields of amino sugars. This synthetic route has been successfully used in this Laboratory for the preparation of the 2-amino-2-deoxy derivatives of the pentoses D-ribose,[3] L-ribose,[8] and D-lyxose.[3]

Although hydrazinolysis of 1,2:5,6-di-*O*-isopropylidene-3-*O*-*p*-tolylsulfonyl-α-D-glucofuranose[9] gives the 3-deoxy-3-hydrazino-D-allose derivative[5,6,8] as the major product, a side reaction leads to the formation of an unsaturated sugar, believed to be a 3,4-glycosene derivative.[9] This alternative reaction mechanism may be partly responsible for the relatively low yields of 2-amino-2-deoxy pentoses prepared by hydrazinolysis of *p*-toluenesulfonate esters.[3,8,10]

The present work is concerned with the synthesis of 2-amino-2-deoxy-L-lyxose, the enantiomorph of the previously described 2-amino-2-deoxy-D-lyxose.[3,10] Seven of the eight possible 2-amino-2-deoxypentoses have now been reported[3,6,8,10-12]; 2-amino-2-deoxy-L-xylose has not yet been described. The occurrence of rare amino sugars in a number of antibiotics is noteworthy,[13] and it is interesting that the sugar moiety of the antibiotic novobiocin, 3-*O*-carbamoylnoviose,[14] has the L-lyxose configuration. L-Lyxose has been reported[15] as a degradation product of an antibiotic.

The starting material for the synthesis, L-xylose, was prepared by the procedure described by Hamamura and co-workers,[16] and was converted, through methyl α,β-L-xylofuranoside, to methyl 3,5-*O*-isopropylidene-α,β-L-xylofuranoside, essentially by the procedure of Baker and co-workers[17] for the D-analogs. The two anomers were partially separated as distilled sirups and were converted into crystalline 2-*p*-nitrobenzenesulfonate esters. Sulfonylation of methyl 3,5-*O*-isopropylidene-α,β-L-xylofuranoside with *p*-toluenesulfonyl chloride gave crystalline methyl 3,5-*O*-isopropylidene-2-*O*-*p*-tolylsulfonyl-α-L-xylofuranoside (I) and the β-L anomer (II), the latter in two dimorphous forms. The physical constants of I and II were in good agreement with those reported by Anderson

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(2) Reported in part in *Abstracts Papers Am. Chem. Soc.*, 139, 2D (1961).

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