theless, it is clear that the materials in the various peaks represent oligosaccharides of increasing molecular weight.

TABLE I

GLUCOSAMINE CONTENT AND END-GROUP ANALYSIS OF THE PEAKS

Peak	Glucosamine content,[a] g.	% of material placed on column	N/reducing value[b] mol./mol.
I	1.37	27.5	1.03
II	0.822	16.5	1.90
III	.614	12.2	2.90
IV	.434	8.7	4.30
V	.244	4.8	5.40
VI-end	.443	8.9	

[a] After acid hydrolysis (4 N HCl for 30 hours at 100°).
[b] Reducing value by ferricyanide method[8] with glucosamine as standard.

The material in peak I was characterized as glucosamine hydrochloride by the following criteria: (a) isolation of the crystalline hydrochloride, (b) demonstration by paper chromatography and ionophoresis that the compound was homogeneous, (c) conversion to a crystalline derivative, the α-1,3,4,6-tetraacetyl-N-(*p*-methoxybenzylidene)-D-glucosamine.[19] The derivative melted at 185–186° (uncor.) and did not depress the m.p. of an authentic sample.

An aliquot of the material in peak II was isolated and acetylated by methods to be described elsewhere. The octaacetate so obtained melted at 285–288° (uncor., dec.) and did not depress the dec. point of octaacetyl chitobiose obtained by the acetolysis of chitin.[4] The characterization of the material in peak II is preliminary, however, due in part to the high decomposition point of the acetylated derivative.

From the data offered above it would appear that it is now possible to separate glucosamine oligosaccharides. Although acidic oligosaccharides have been obtained[20] which contain N-acetylglucosamine and glucuronic acid, this is apparently the first report of the separation of some members of a series of positively charge hexosamine oligosaccharides.

Acknowledgment.—It is a pleasure to acknowledge the expert technical assistance provided by Mr. Roy M. Seppala.

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ANN ARBOR, MICHIGAN

[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT DEPARTMENT, U. S. NAVAL POWDER FACTORY]

Studies on the Decarboxylation of Oxidized Cellulose Acetate through the Formation of the Acid Chloride from the Carboxyl Group and its Reaction with Silver Oxide and Bromine[1,2]

BY F. A. H. RICE AND ARTHUR RUSSELL JOHNSON

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It has been found that the carboxylic acid groups in oxidized cellulose acetate, oxidized cellulose acetate after reduction with sodium borohydride and oxidized cellulose acetate after reduction and reacetylation can be replaced by acid chloride groups and the acid chloride group can in turn be replaced with bromine by reaction in tetrachloroethane with a mixture of silver oxide and bromine. Treatment of the decarboxylated polymer with sodium borohydride leads to the loss of the halide without any marked decrease in the intrinsic viscosity of the product. Viscosity studies on the products indicate that very little if any depolymerization takes place when reduced reacetylated oxidized cellulose is decarboxylated. The decrease in intrinsic viscosity shown on decarboxylation of oxidized cellulose acetate, however, suggests that chain scission takes place during the treatment of oxidized cellulose acetate containing acid chloride groups with silver oxide and bromine.

In previous communications it was shown that the silver salt of penta-*O*-acetyl-D-gluconic acid would react with bromine to form silver bromide, carbon dioxide and *aldehydo*-1-bromopenta-*O*-acetyl-D-arabinose.[3] Furthermore, it was demonstrated that the silver salt of alginic acid, a polymannuronic acid,[4] would, on treatment with bromine, quantitatively evolve carbon dioxide.[5] Reduction of the product of decarboxylation of alginic acid proved to be extremely difficult and hydrolysis of the product, although it yielded the expected compounds, did so only in very small amounts.[5]

In the course of studies on the decarboxylation of silver salts, it was discovered that an acid could be quantitatively decarboxylated *via* its acid chloride[6] by treating the acid chloride with silver oxide and bromine. It was apparent that this latter reaction would be of particular value for studying acetylated polysaccharides which contain carboxylic acid groups, insofar as it should be possible to transform the carboxylic acid group of the acetylated polysaccharide into the acid chloride and hence substitute by reaction with silver oxide and a halide the carboxylic acid groups in the polysaccharide with the halide. If the halide could be replaced by hydrogen, the over-all reaction would have substituted a pentose for the original uronic

[1] Published with permission of the Bureau of Ordnance, Navy Department. The opinions and conclusions are those of the authors.

[2] Presented in part at the 131st Meeting of the American Chemical Society, Miami, Florida, April, 1957.

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acid. It should then be possible to study the hydrolytic products of the polysaccharide with greater ease, since the uronic acid constituents which are notably susceptible to degradation by acid would have been replaced by a pentose.

Initially attempts were made to apply the reaction to a study of a commercial oxycellulose prepared by treating cellulose with dinitrogen tetroxide.[7] Attempts were made to acetylate the oxycellulose after the manner of Godman, Haworth and Peat[8] with a mixture of acetic anhydride, acetic acid and thionyl chloride. However, the oxycellulose at our disposal was not acetylatable by this procedure.

It was decided, therefore, to use oxidized cellulose acetate[9] which also contains carboxylic acid groups for our preliminary investigation of the feasibility of decarboxylating polysaccharide acetates through the reaction of acid chloride groups with silver oxide and bromine.

Our initial studies were undertaken to ascertain whether or not a small percentage of carboxylic acid groups in a relatively high molecular weight polymer could be substituted by an acid chloride group, and consequently the polymer decarboxylated through reaction with silver oxide and bromine. It seemed also advisable to investigate the extent, if any, to which depolymerization could be expected at each step of the reaction. In order to follow any degradation of the polymer, resort was had to the usual standard methods based on viscosimetry[10] for determining the degree of polymerization.

Results and Discussion

The oxidized cellulose acetate[9] was prepared by oxidizing acetone-soluble cellulose acetate with chromic acid.[11] A small amount of material was found to be insoluble in *sym*-tetrachloroethane and this was removed by dissolving the oxidized cellulose acetate in *sym*-tetrachloroethane, filtering and precipitating with petroleum ether. The resulting polymer was found to have an intrinsic viscosity very close to that of a fraction of cellulose acetate of average molecular weight of 29000.[12] The optical rotation, however, was $[\alpha]^{24}\text{D}$ $-5°$ (dioxane, c 1) compared to $[\alpha]^{24}\text{D}$ $-8°$ (dioxane, c 1) for the cellulose acetate of molecular weight 29000.

The carboxyl groups in the oxidized cellulose acetate were transformed into acid chloride groups by dissolving the oxidized cellulose acetate in thionyl chloride and, after warming at 40–50° for several hours, filtering and precipitating the polymer by adding petroleum ether (b.p. 30–60°). The product, after thorough washing with petroleum ether and drying contained between 1–2% chlorine.

The oxidized cellulose acetate was very slowly degraded when subjected to the action of thionyl chloride. Figure 1 shows how the intrinsic viscosity of a sample of oxidized cellulose acetate dissolved in thionyl chloride decreased at intervals over a period of 100 hours. The viscosity of the final product dissolved in *sym*-tetrachloroethane is shown for comparison. Table I shows how the reduced viscosity (η_{sp}/c) of a 1% solution of oxidized cellulose acetate dissolved in thionyl chloride decreased over a period of 100 hours. It will be seen that although there is a continuous decrease in the reduced and also intrinsic viscosity of a thionyl chloride solution of oxycellulose acetate, the rate of decrease is comparatively slow. If the values for specific viscosity shown in Table I are plotted as a function of time there is a definite indication that the specific viscosity is approaching a limiting value at the end of 100 hours.

Table I

Change in Specific Viscosity with Time of Oxycellulose Acetate Dissolved in Thionyl Chloride

Temperature 25 ± 0.1°

Time, hr.	5	8	24	32	50	100
η_{sp}	0.7788	0.7744	0.6665	0.6432	0.5725	0.4820
η_{sp}/c	0.7714	0.7671	0.6602	0.6372	0.5671	0.4775

The most satisfactory method of substituting the carboxyl group by the acid chloride without a consequent marked decrease in viscosity was found to be through the action of limited quantities of thionyl chloride on the oxycellulose acetate dissolved in dioxane at 40–50°. The oxidized cellulose acetate with its carboxylic acid groups transformed into acid chlorides by this latter method could be isolated by precipitation with petroleum ether. The final product contained between 1 and 2% chlorine. Figure 2 shows the reduced viscosity (η_{sp}/c) of the oxidized cellulose acetate after treatment with thionyl chloride plotted as a function of concentration (c). Cellulose acetate of average molecular weight 29000 (broken line) and oxidized cellulose acetate regenerated from the acid chloride by treatment with water are shown for comparison. It is apparent that the carboxyl groups in oxidized cellulose acetate can be substituted by acid chloride groups with very little decrease in molecular weight as indicated by the small change in intrinsic viscosity. Indeed, one might be tempted to attribute the small change in intrinsic viscosity to the fact that the two polymeric materials are not strictly homologous insofar as one contains carboxyl groups and the other acid chloride groups and hence need not have the same solubility[13] were it not that the oxidized cellulose acetate, regenerated from the material containing acid chloride groups by reaction with water, shows an intrinsic viscosity which is less than that of the original oxidized cellulose acetate (Fig. 2). The slight change in the slope of η_{sp}/c *vs.* c for the regenerated oxidized cellulose acetate as compared to the original oxidized cellulose acetate may possibly indicate that small changes in the shape[14] or

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(7) E. C. Yackel and W. O. Kenyon, This Journal, **64**, 121 (1942); C. C. Unruh and W. O. Kenyon, *ibid.*, **64**, 127 (1942).

(8) G. L. Godman, W. N. Haworth and S. Peat, *J. Chem. Soc.*, 1908 (1939).

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(10) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 266; M. L. Huggins in "Cellulose and Cellulose Derivatives," ed. E. Ott, Interscience Publishers, Inc., New York, N. Y., 1946, p. 943.

(11) Personal communication, Dr. C. H. Penning, Eastman Products, Inc., Kingsport, Tenn.

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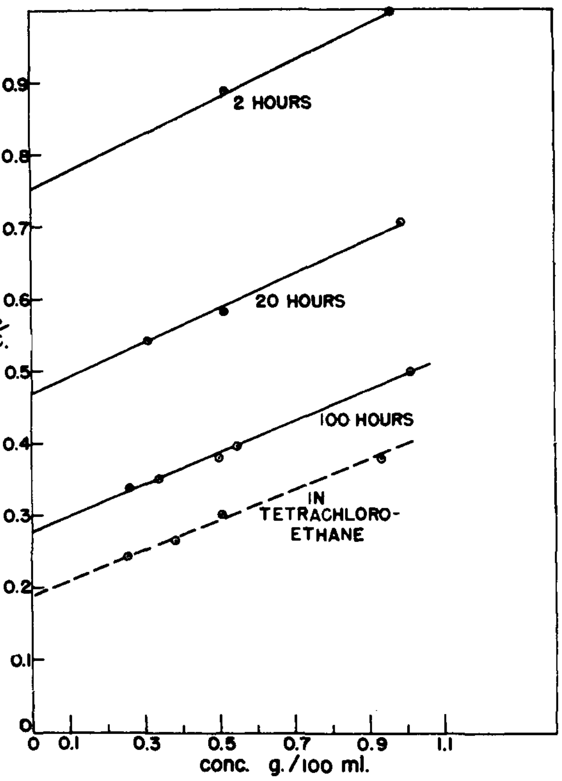

Fig. 1.—Plot of η_{sp}/c against c of a solution of oxidized cellulose acetate in thionyl chloride after the intervals of time indicated in the figure; temperature 25 ± 0.1°.

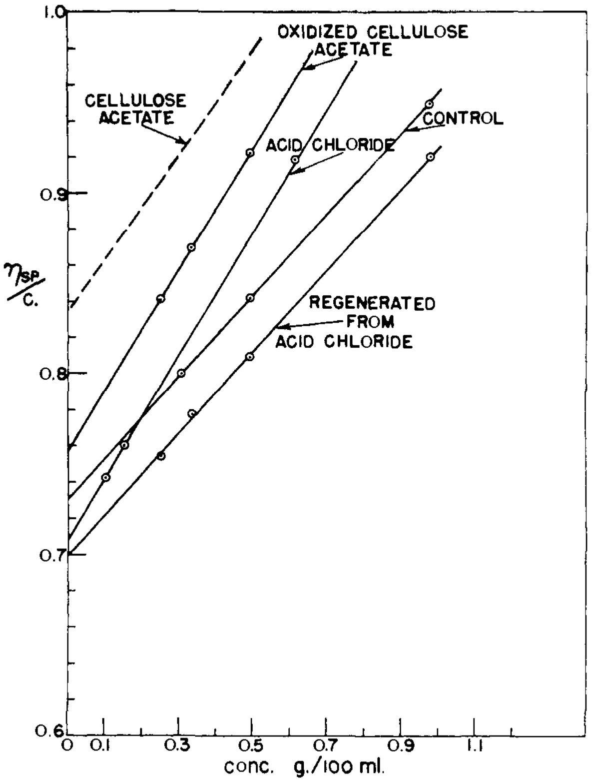

Fig. 2.—Plot of η_{sp}/c against c of cellulose acetate of molecular weight 29000, oxidized cellulose acetate and several of its derivatives, as indicated in the figure. Control represents oxidized cellulose acetate that has been treated in *sym*-tetrachloroethane solution with silver oxide and bromine.

structure of the polymer apart from the formation of the acid chloride take place on treatment with thionyl chloride.[14]

Since decarboxylation of the oxidized cellulose acetate would necessitate the treatment of the acid chloride with a mixture of silver oxide and bromine, the action of this mixture on the viscosity of oxidized cellulose acetate was investigated. Figure 2 shows the effect of treating oxidized cellulose acetate in *sym*-tetrachloroethane with silver oxide and bromine. There is only a slight decrease in intrinsic viscosity. Again the changed slope of the η_{sp}/c *vs.* c relationship may indicate a slight change in the shape or fine structure of the oxidized cellulose acetate.[14]

When oxidized cellulose acetate in which the carboxyl groups had been replaced by acid chloride groups was dissolved in *sym*-tetrachloroethane and treated with a mixture of silver oxide and bromine, carbon dioxide was evolved. The resulting decarboxylated oxidized cellulose acetate was isolated, after filtration, by precipitating it from solution with petroleum ether. Further purification was achieved by dissolving the precipitate in dioxane and precipitating with ether. Table II gives a comparison between the observed and calculated values for carbon dioxide evolution based on the chlorine content of the acid chloride and the bromine content of the decarboxylated polymer. Analytical values for halide in the same sample as well as in individual samples showed considerable variation. When samples of oxidized cellulose acetate were treated with thionyl chloride, the product did not contain any sulfur. Sulfur should be present if part of the chlorine were due to the adsorption of thionyl chloride. We believe that the variation in the analytical values for halogen is due to the difficulty of analyzing for a halide in a polymeric material. A portion of the bromine in the decarboxylated material may possibly be due to adsorbed bromine. Analyses on material obtained after only one precipitation with petroleum ether showed values as high as 12%. One additional precipitation of the polymer from dioxane, by the addition of ether, dropped the bromine content to approximately 5%. Additional purification did not change the bromine content beyond the individual variation obtained on analyses of the same sample. In general (Table II), it would seem that mixtures of silver oxide and bromine react quantitatively with the acid chloride of oxycellulose acetate with a substitution of bromine for the acid chloride group. Figure 3 shows the effect of decarboxylation on the viscosity of oxidized cellulose acetate.

TABLE II

COMPARISON OF OBSERVED AND CALCULATED VALUES FOR CO_2 AND Br OBTAINED ON DECARBOXYLATION OF OXYCELLULOSE ACETATE

Cl	CO_2, equiv. in ml. 0.1 *N* HCl/g.		Br	
Found	Found	Calcd.	Found	Calcd.
1.6	10.0	9.2	4.0	4.5
2.0	6.3	11.5	5.38 5.25	5.6

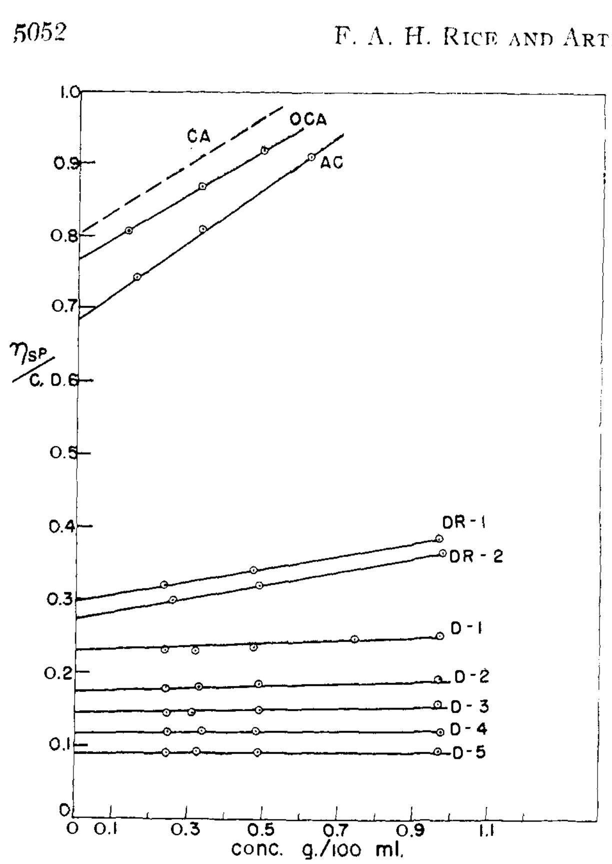

Fig. 3.—Plot of η_{sp}/c against c of oxidized cellulose acetate and several of its derivatives: OCA, oxidized cellulose acetate; AC, oxidized cellulose acetate in which the carboxylic acid groups have been substituted by acid chloride groups; D1, D2, D3, D4 and D5 are several preparations of decarboxylated oxidized cellulose acetate; DR1 and DR2, decarboxylated oxidized cellulose acetate after reduction with sodium borohydride; cellulose acetate (CA broken line) of molecular weight 29000 is shown for comparison.

Although acid chloride groups can be substituted for carboxylic acid groups with little change in the intrinsic viscosity and the oxidized cellulose acetate also can be treated with silver oxide and bromine without any great decrease in intrinsic viscosity, oxidized cellulose acetate on decarboxylation *via* the acid chloride shows a marked loss in intrinsic viscosity. D1, D2, D3, D4 and D5 (Fig. 3) represent the reduced viscosity–concentration relationships of a number of decarboxylated oxidized cellulose acetate samples. Although the intrinsic viscosity varies somewhat within the group there is a notable decrease in intrinsic viscosity compared to that of the original oxidized cellulose acetate. Some change in specific viscosity due to a change in solubility[13-15] might be expected as a consequence of the loss of carboxyl groups and the consequent change in solubility. The magnitude of η_{sp}/c, however, if due to changed solubility should be related to the amount of non-solvent which must be added to cause the formation of the new phase.[15] Although no attempt was made to determine accurately the amount of non-solvent (petroleum ether) necessary to cause a separation of the phases of oxidized cellulose acetate or the decarboxylated product, rough measurements indicated that any difference was too small to account for the observed decrease in specific viscosity.

Various investigators have suggested that cellulose and cellulose derivatives exist in dispersing agents as preformed micelles or particles,[16] perhaps associated through weak hemiacetal linkages.[17] The loss of carboxylic acid groups conceivably could, if the polymer chains in the oxidized cellulose acetate were associated through these groups, cause dissociation and hence a decrease in reduced and intrinsic viscosity. If this were the explanation for the decrease in viscosity on decarboxylation, however, the treatment of the oxidized cellulose acetate before decarboxylation, especially the formation of the acid chloride, might be expected to cause a much greater loss in reduced viscosity than was observed.

Degradation of a hydrolytic type would account for the observed decrease in reduced or intrinsic viscosity observed for the decarboxylated oxidized cellulose acetate. The lack of any appreciable degradation due to reaction of silver oxide and bromine with oxidized cellulose acetate, however, indicates that a scission of the polymer chain takes place only after decarboxylation, and hence is probably related to the substitution of carboxyl by halide on carbon-5. It has been suggested[18] that oxycellulose contains α-alkoxy ketone groups. It is at least possible that oxidation of acetone-soluble cellulose acetate gives rise to ketonic groups on the same D-glucose monomer on which the primary alcohol is oxidized and that the juxtaposition of these groups forms an unstable link in the polymer chain, particularly after or during decarboxylation. In line with this suggestion it was found (following) that oxidized cellulose acetate which had been reduced with sodium borohydride and reacetylated could be decarboxylated without a similar decrease in reduced or intrinsic viscosity.

Oxidized cellulose acetate after reduction with sodium borohydride showed an increased intrinsic viscosity (Fig. 4). The effect of concentration on the reduced viscosity (η_{sp}/c) is also more marked. We consider this to be due to greater interaction between the polymer chains[19] due to formation of free hydroxyl groups by reduction of keto or aldehydo groups. The reacetylated product behaved as might be expected and showed the intrinsic viscosity as well as the variation of η_{sp}/c with concentration similar to that shown by cellulose acetate of 29000 average molecular weight or our preparation of oxidized cellulose acetate. Substitution of acid chloride groups for carboxyl yielded a product with essentially unchanged intrinsic viscosity. We suggest that the change in η_{sp}/c *vs.* c relationship is due to a change in inter- or intramolecular interaction due to the replacement of the carboxyl groups by acid chlorides.

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Decarboxylation of the reduced reacetylated oxidized cellulose did not yield a product with the greatly changed intrinsic viscosity found in the case of oxidized cellulose acetate.

The products obtained by reducing either the decarboxylated oxidized cellulose or the reduced reacetylated oxidized cellulose with sodium borohydride showed an increase in intrinsic viscosity over that of the decarboxylated material (Figs. 3 and 4). If the increase in intrinsic viscosity and change in the reduced viscosity *vs.* concentration relationship observed when oxidized cellulose acetate is reduced is due to the formation of free hydroxyl groups it is likely that the same explanation accounts for the change observed in the reduction of decarboxylated oxidized cellulose acetate. That the formation of hydroxyl groups is not the complete story, however, is indicated by the fact that after reduction of the decarboxylated, reduced, reacetylated, oxidized cellulose acetate, the reduced viscosity–concentration relationship as well as the intrinsic viscosity is essentially that of the reduced reacetylated oxycellulose acetate. One is tempted to consider that the intrinsic viscosity of the decarboxylated product obtained from reduced, reacetylated oxidized cellulose acetate reflects more nearly the true average molecular weight of the polymer and that the increased intrinsic viscosity of the other derivatives (Fig. 4) follows as a consequence of the modification of the shape of the polymer chain or interaction between chains due to the presence of the hydroxyl, carboxyl or acid chloride groups.

It is possible that the change in reduced and intrinsic viscosity observed on reduction of the decarboxylated product is due to added inorganic salts.[20] We do not believe this to be true since we were unable to find any appreciable changes in viscosity after additional purification of the product by washing it with water and precipitating it after filtration of a dioxane solution by the addition of ether.

It is of interest to note the influence of carboxyl groups on the specific optical rotation. Although the optical rotation of the oxidized cellulose acetate is essentially the same in the two solvents (dioxane and *sym*-tetrachloroethane) that would not be expected to interact to any great extent with acidic groups, a solution of the polymer in thionyl chloride shows a greatly increased rotation. It is likely that the increased rotation is due to the formation of the acid chloride since the acid chloride when it is formed and isolated shows essentially the same rotation in dioxane, *sym*-tetrachloroethane and thionyl chloride. Decarboxylated products, in addition, show almost the same optical rotation in the three solvents. The observation suggests the possibility of determining the number of carboxylic acid groups in a cellulose derivative through the measurement of the optical rotation in an inert solvent and a solvent with which the acidic group can react.

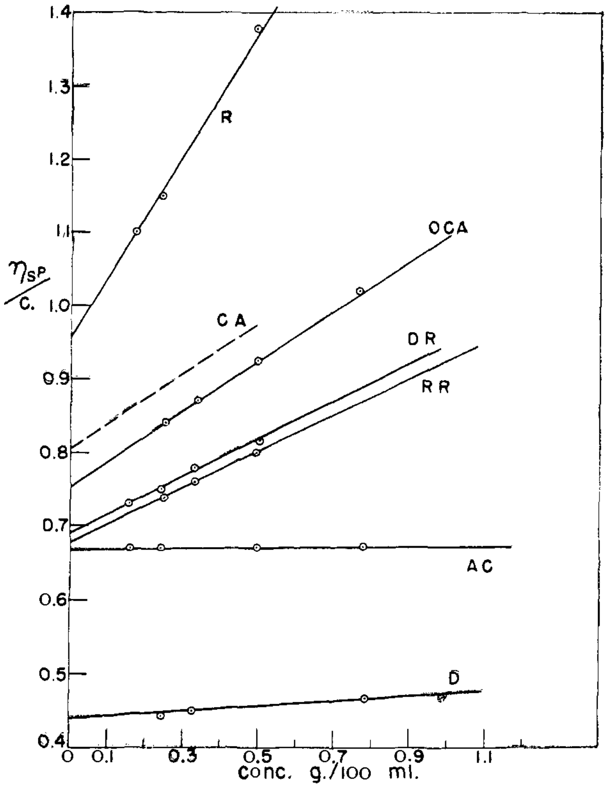

Fig. 4.—Plot of η_{sp}/c against c of oxidized cellulose acetate and several of its derivatives: OCA, oxidized cellulose acetate; R, oxidized cellulose acetate after reduction with sodium borohydride; RR, oxidized cellulose acetate, reduced and reacetylated; AC, the reduced and reacetylated oxidized cellulose acetate after replacement of its carboxylic acid groups with acid chloride groups; D, the decarboxylated product from reduced, reacetylated oxidized cellulose; DR, the decarboxylated product after reduction with sodium borohydride; CA, cellulose acetate (mol. wt. 29000) shown for comparison.

Experimental

Viscosities were measured with a modified Ubbelohde type viscometer[21] with a flow time of 118.6 sec. for dioxane. Solutions of approximately 1% (g./ml.) concentration were made by dissolving the accurately weighed sample in an accurately weighed quantity (approximately 25 ml.) of solvent. Dilutions were made directly in the viscometer by adding weighed quantities of solvent. The temperature was maintained at 25 ± 0.1°.

Cellulose Acetate of Average Molecular Weight 29000.[12]—Cellulose acetate of average molecular weight of 29000 had $[\alpha]^{24}\text{D}$ −4.7° (dioxane, c 1); Hess and Schultze[22] reported $[\alpha]$ −22° ($CHCl_3$) for unfractionated cellulose acetate; Bazhenov and Vol'kenshtein[23] report triacetylcellulose to have $[\alpha]\text{D}$ −15.5° to −11.5° at temperatures from −10 to 80°.

Oxidized Cellulose Acetate.[9]—The oxidized cellulose acetate was purified by dissolving a quantity (10 g.) at 50° in redistilled *sym*-tetrachloroethane (500 ml.), removing a small amount of insoluble material by filtration through Celite[24] on a coarse fritted glass büchner funnel, and pre-

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(22) K. Hess and G. Schultze, *Ann.*, **455**, 81 (1927).

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(24) No. 535 obtained from the Johns-Manville Co., New York, N. Y.

cipitating the oxidized cellulose acetate by adding five volumes of petroleum ether (b.p. 30–60°) to the filtrate. The precipitate was collected by filtration on a fritted glass büchner funnel, washed several times with *anhydrous* ether and dried under a high vacuum at room temperature. The optical rotation was determined in several solvents and was found to be $[\alpha]^{21}_{\mathrm{D}} + 4.5°$ (glacial HOAc, *c* 1.5), $[\alpha]^{21}_{\mathrm{D}} -13°$ ($SOCl_2$, *c* 3), $[\alpha]^{25}_{\mathrm{D}} -10°$ ($C_2H_2Cl_4$, *c* 4), $[\alpha]^{22}_{\mathrm{D}} -10°$ (dioxane, *c* 1).

Anal. Calcd.[25] for $C_{140}H_{184}O_{95}$: C, 49.64; H, 5.48; OAc, 43.17. Found: C, 48.01; H, 5.33; OAc,[26] 43.69.

Substitution of an Acid Chloride for the Carboxylic Acid Groups in Oxidized Cellulose Acetate. Method 1.—The oxidized cellulose acetate (10 g.) was dissolved in 500 ml. of freshly redistilled thionyl chloride and the solution warmed at 40–50° for 2 hours. The solution was then filtered through a layer of Celite[24] on a coarse fritted glass büchner funnel, and poured into 3 l. of petroleum ether (b.p. 30–60°). The petroleum ether had been previously dried over sodium metal. The precipitate was collected by filtration on a dry fritted glass funnel, washed with petroleum ether (5 × 500 ml.) and dried over sodium hydroxide under reduced pressure in a vacuum desiccator at room temperature. The optical rotation was found to be $[\alpha]^{21}_{\mathrm{D}} +1°$ (glacial HOAc, *c* 3), $[\alpha]^{25}_{\mathrm{D}} -22°$ ($SOCl_2$, *c* 3), $[\alpha]^{22}_{\mathrm{D}} -15°$ ($C_2H_2Cl_4$, *c* 2) $[\alpha]^{22}_{\mathrm{D}} -9°$ (dioxane, *c* 1),

Anal. Calcd.[25] for $C_{140}H_{183}O_{94}Cl$: C, 49.37; H, 5.42; Cl, 1.04; OAc, 42.93. Found: C, 48.53; H, 5.22; Cl, 1.44; OAc,[26] 43.07.

Method 2.—The oxidized cellulose acetate (10 g.) was dissolved in dioxane (500 ml.) which had been freshly distilled from sodium metal. Thionyl chloride (25 ml.) was added and the solution heated at 40–50° for 4 hours. After filtration through Celite on a fritted glass büchner funnel four volumes of petroleum ether (b.p. 30–60°) was added and the precipitated polysaccharide removed by filtration on a fritted glass funnel. The optical rotation was found to be $[\alpha]^{22}_{\mathrm{D}} -9°$ (dioxane, *c* 0.5), $[\alpha]^{21}_{\mathrm{D}} +1°$ (*glacial* HOAc, *c* 0.8), $[\alpha]^{22}_{\mathrm{D}} -22°$ ($SOCl_2$, *c* 1). Analysis was essentially the same as for method 1.

Regeneration of Oxidized Cellulose Acetate from the Acid Chloride of Oxidized Cellulose Acetate.—The acid chloride of oxidized cellulose acetate (5 g.) was dissolved in acetone (200 ml.), 10 ml. of distilled water was added and the solution concentrated to dryness under reduced pressure at room temperature. The dry residue was dissolved in dioxane (200 ml.) filtered and precipitated by the addition of ether (1 liter). The precipitate was washed with ether and dried under reduced pressure at room temperature; $[\alpha]^{15}_{\mathrm{D}} -10°$ ($C_2H_2Cl_4$, *c* 0.5), $[\alpha]^{23}_{\mathrm{D}} +1°$ (glacial HOAc, *c* 0.7), $[\alpha]^{20}_{\mathrm{D}} -20°$ ($SOCl_2$, *c* 1), $[\alpha]^{24}_{\mathrm{D}} -10°$ (dioxane, *c* 1).

Anal. Calcd.[25] for $C_{140}H_{184}O_{95}$: C, 49.64; H, 5.48; OAc, 43.17. Found: C, 47.29; H, 5.82; Cl, none; OAc,[26] 43.36.

Decarboxylation of Oxidized Cellulose Acetate *via* the Acid Chloride.—Oxidized cellulose acetate that had been treated with thionyl chloride as in method 1 (5 g.) was suspended in 500 ml. of *sym*-tetrachloroethane (previously redistilled and dried over phosphorus pentoxide) and stirred at 40–50° for 15–20 minutes to dissolve the sample. Silver oxide (1.5 g.) together with 20 ml. of a solution of dry bromine in carbon tetrachloride[6] (1:10 by volume) was added and the mixture warmed at 40–50° for 5 hours. The carbon dioxide evolved was determined by titration.[6] The mixture was filtered through a layer of Celite[24] on a coarse fritted glass filter and the filtrate poured into 4 liters of petroleum ether (b.p. 30–60°, dried over sodium). The precipitate was collected by filtration on a fritted glass büchner funnel. The polymer was further purified by dissolving a quantity (8 g.) in dioxane (80 ml. distilled from sodium metal) filtering and reprecipitating the polysaccharide by the addition of *anhydrous* ether (800 ml.). After washing several times with *anhydrous* ether the material had $[\alpha]^{22}_{\mathrm{D}} -10°$ ($C_2H_2Cl_4$, *c* 2), $[\alpha]^{25}_{\mathrm{D}} -15.6°$ ($SOCl_2$, *c* 2), $[\alpha]^{25}_{\mathrm{D}} +3°$ (glacial HOAc, *c* 2), $[\alpha]^{22}_{\mathrm{D}} -10°$ (dioxane, *c* 1).

A sample (10 g.) of oxidized cellulose acetate treated as in method 2 was dissolved in dry *sym*-tetrachloroethane (750 ml.) and treated with silver oxide (2 g.) and a solution (20 ml.) of bromine in dry *sym*-tetrachloroethane (1:10 by volume) and warmed at 40–60° for 6 hours, at the end of which time no additional carbon dioxide was evolved. The mixture was filtered, and the polysaccharide precipitated with petroleum ether and reprecipitated from dioxane with *anhydrous* ether as above; $[\alpha]^{23}_{\mathrm{D}} -16.1°$ (dioxane, *c* 1), $[\alpha]^{21}_{\mathrm{D}} -17.5°$ ($SOCl_2$, *c* 4), $[\alpha]^{23}_{\mathrm{D}} +3°$ (glacial HOAc, *c* 2), $[\alpha]^{23}_{\mathrm{D}} -18°$ ($C_2H_2Cl_4$, *c* 2.5).

Anal. Calcd.[25] for $C_{139}H_{183}O_{93}Br$: C, 48.79; H, 5.39; Br, 2.34; OAc, 42.74. Found: C, 48.47; H, 5.22; Br, 2.5–4.0; OAc,[26] 43.36; CO_2 evolution equiv. to 4.5–5.7 ml. of 0.1 *N* HCl per g.

Treatment of Oxidized Cellulose Acetate with Silver Oxide and Bromine.—Oxidized cellulose acetate (5 g.) was dissolved in 500 ml. of *sym*-tetrachloroethane, treated with 2.0 g. of silver oxide and 25 ml. of a solution of bromine in *sym*-tetrachloroethane (1:10 by volume). The mixture was warmed at 40–50° for 8 hours. No carbon dioxide was evolved. The mixture was filtered and the polysaccharide isolated as above; $[\alpha]^{21}_{\mathrm{D}} -14°$ ($C_2H_2Cl_4$, *c* 1.2), $[\alpha]^{21}_{\mathrm{D}} +3.2°$ (*glacial* HOAc, *c* 2), $[\alpha]^{21}_{\mathrm{D}} -21°$ ($SOCl_2$, *c* 1.5), $[\alpha]^{22}_{\mathrm{D}} -15°$ (dioxane, *c* 1).

Anal. Calcd.[25] for $C_{140}H_{184}O_{95}$: C, 49.64; H, 5.48; OAc, 43.17. Found: C, 47.29; H, 5.82; Br, 0.0; OAc,[26] 43.36; CO_2 evolution, none.

Reduction of Decarboxylated Oxidized Cellulose Acetate with Sodium Borohydride.—An amount of 5.0 g. of decarboxylated oxidized cellulose acetate was dissolved in 100 ml. of dioxane (distilled from sodium metal) and treated with 2.0 g. of powdered sodium borohydride. The mixture was stirred at 40° for 2 hours and then filtered through a layer of Celite[24] on a fritted glass büchner funnel. The filtrate was added to 400 ml. of *anhydrous* ether and the precipitate collected by filtration, and further purified by dissolving it in dioxane, filtering and precipitating with ether; $[\alpha]^{16}_{\mathrm{D}} +4.6°$ (glacial HOAc, *c* 0.8), $[\alpha]^{17}_{\mathrm{D}} -7.4°$ ($C_2H_2Cl_4$, *c* 0.5), $[\alpha]^{16}_{\mathrm{D}} -8°$ ($SOCl_2$, *c* 0.5), $[\alpha]^{17}_{\mathrm{D}} -8°$ (dioxane, *c* 1).

Anal. Calcd.[25] for $C_{139}H_{184}O_{93}$: C, 49.94; H, 5.55; OAc, 43.73. Found: C, 49.85; H, 5.55; OAc,[26] 42.56.

Reduction of Oxidized Cellulose Acetate with Sodium Borohydride.—Oxidized cellulose acetate (10 g.) was dissolved in dioxane (500 ml. distilled from sodium metal) and stirred for 4 hours at room temperature with powdered sodium borohydride (2 g.). The mixture was then filtered and the polysaccharide precipitated with *anhydrous* ether (1 liter). The precipitate was separated by filtration on a fritted glass büchner funnel and washed with half-saturated aqueous sodium bicarbonate (50 ml.), water (50 ml.), *absolute* ethanol (200 ml.) and ether (200 ml.). After drying for 24 hours under a high vacuum at room temperature, the material was redissolved in dioxane (500 ml.), reprecipitated with ether (2 liters), washed with ether and dried under a high vacuum at room temperature; $[\alpha]^{23}_{\mathrm{D}} -7.6°$ ($C_2H_2Cl_4$, *c* 1.2), $[\alpha]^{23}_{\mathrm{D}} +1.60°$ (glacial HOAc, *c* 1.2), $[\alpha]^{23}_{\mathrm{D}} -20.5°$ ($SOCl_2$, *c* 1.6), $[\alpha]^{20}_{\mathrm{D}} -7.3°$ (dioxane, *c* 1).

Anal. Calcd.[25] for $C_{140}H_{186}O_{95}$: C, 49.61; H, 5.53; OAc, 44.18. Found: C, 48.56; H, 5.67; OAc,[26] 43.35.

Acetylation of Reduced Oxidized Cellulose Acetate.—Reduced oxidized cellulose acetate (5 g.) was dissolved in acetic anhydride (100 ml.), cooled to −10° and then 2 ml. of *concd.* sulfuric acid added to the solution. The solution was allowed to stand at 0° overnight, at room temperature for 4–8 hours, and then poured into 1 liter of ice and water buffered with sodium acetate (5 g.), and stirred for 3–4 hours. The precipitate was collected by filtration on a fritted glass büchner funnel and washed with ice-water (1 liter), absolute ethanol (200 ml.) and ether (200 ml.). After drying in a high vacuum at room temperature for several days the substance showed $[\alpha]^{21}_{\mathrm{D}} -19°$ ($C_2H_2Cl_4$, *c* 1.6), $[\alpha]^{20}_{\mathrm{D}} \pm 0.0°$ (*glacial* HOAc, *c* 1.3), $[\alpha]^{19}_{\mathrm{D}} -35°$ ($SOCl_2$, *c* 1.5), $[\alpha]^{21}_{\mathrm{D}} -17.5°$ (dioxane, *c* 1).

Anal. Calcd.[25] for $C_{142}H_{188}O_{96}$: C, 49.71; H, 5.52; OAc, 43.87. Found: C, 48.01; H, 5.33; OAc,[26] 43.69.

Treatment of Reacetylated Reduced Oxidized Cellulose Acetate with Thionyl Chloride.—The reacetylated reduced oxidized cellulose acetate was treated with thionyl chloride by the same procedure (method 2), as was used for oxidized cellulose acetate. The product had $[\alpha]^{21}_{\mathrm{D}} -22.0°$ (C_2H_2-

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[25] Calculated on the basis of one carboxylic acid and one reducing group per twelve glucose units. These ratios, found by trial and error, were considered to give the most consistent agreement between observed and calculated values for the entire series of derivatives.

[26] A. Chaney and M. L. Wolfrom, *Anal. Chem.*, **28**, 1614 (1956).

Cl_4, *c* 0.8), $[\alpha]^{23}\mathrm{D}$ +4° (*glacial* HOAc, *c* 0.8), $[\alpha]^{21}\mathrm{D}$ −32° ($SOCl_2$, *c* 0.6), $[\alpha]^{21}\mathrm{D}$ −22° (dioxane, *c* 1).

Anal. Calcd.[25] for $C_{142}H_{187}O_{95}Cl$: C, 49.44; H, 5.46; Cl, 1.03; OAc, 43.63. Found: C, 48.53; H, 5.22; Cl, 1.03–1.60; OAc,[26] 43.07.

Decarboxylation of Reacetylated Reduced Oxidized Cellulose Acetate.—The product formed by the reaction of reacetylated reduced oxidized cellulose acetate with thionyl chloride was treated with silver oxide and bromine following the procedure used for the product obtained from the reaction of oxidized cellulose acetate with thionyl chloride; $[\alpha]^{15}\mathrm{D}$ −12.3° ($C_2H_2Cl_4$, *c* 0.4), $[\alpha]^{17}\mathrm{D}$ ±0.0° (*glacial* HOAc, *c* 0.6), $[\alpha]^{16}\mathrm{D}$ −14° (dioxane, *c* 1), $[\alpha]^{24}\mathrm{D}$ −13.5° ($SOCl_2$, *c* 1.7).

Anal. Calcd.[25] for $C_{141}H_{187}O_{94}Br$: C, 48.86; H, 5.44; Br, 2.31; OAc, 43.42; CO_2, 5.8 ml. of 0.1 *N* NaOH per g. Found: C, 48.47; H, 5.22; Br, 2.50; OAc,[26] 43.07; CO_2, 6.1 ml. of 0.1 *N* NaOH per g.

Reduction of Decarboxylated Reacetylated Reduced Oxidized Cellulose Acetate with Sodium Borohydride.—An amount of 5. g. of the decarboxylated reacetylated reduced oxidized cellulose acetate was dissolved in 500 ml. of dioxane (distilled from sodium). Sodium borohydride (2 g.) was added and the mixture stirred for 4 hours at 40–50°. Stirring was continued overnight at room temperature, at the end of which time a thick thixotropic gel had formed. The gel was broken up, filtered and the solid triturated with water (2 × 500 ml.), filtered, and washed with aqueous half-saturated sodium bicarbonate (2 × 100 ml.), water (2 × 500 ml.) and absolute ethanol (2 × 500 ml.). The polymer was dried overnight under a high vacuum at room temperature and then dissolved in 500 ml. of dioxane and precipitated by the addition of approximately two volumes of ether; yield approximately 5 g.; $[\alpha]^{18}\mathrm{D}$ −20° ($C_2H_2Cl_4$, *c* 0.4), $[\alpha]^{20}\mathrm{D}$ +5° (*glacial* HOAc, *c* 0.8), $[\alpha]^{21}\mathrm{D}$ −21° ($SOCl_2$, *c* 0.4); $[\alpha]^{18}\mathrm{D}$ −19° (dioxane, *c* 1).

Anal. Calcd.[25] for $C_{141}H_{188}O_{94}$: C, 49.99; H, 5.60; OAc, 44.44. Found: C, 49.85; H, 5.55; OAc,[25] 42.56.

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INDIAN HEAD, MD.

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Interaction of Bacterial Polyglucosans with Concanavalin-A

BY J. A. CIFONELLI AND F. SMITH[1]

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The globulin, concanavalin-A, obtained from Jack Bean meal, which has been shown previously to give a precipitate with glycogen and not with the apparently closely related amylopectin, has been used to ascertain whether certain bacterial polyglucosans are related to glycogen or to amylopectin.

Glycogen and amylopectin have been reported to be similar in respect to properties and structures.[2–5] Some of the properties displayed by the polyglucosans obtained from *Neisseria perflava* appear to be intermediate between glycogen and amylopectin.[6,7] One preparation was found[3,13] by periodate oxidation and by methylation to have, like glycogen, an average repeating unit of about 12 residues whereas it behaved like amylopectin in giving a reddish-purple color with iodine and undergoing a 55–59% degradation upon treatment with β-amylase.

In view of the apparent relationship of the *Neisseria* polyglucosans to both glycogen and amylopectin it was decided to investigate their behavior when treated with concanavalin-A, a globulin from jackbean meal,[8–12] since it had been observed that this protein gives a precipitate with glycogen[9,11,12] but not with amylopectin.[12]

Certain of the enteric bacterial polyglucosans were likewise investigated as were various dextrans to ascertain whether they reacted with concanavalin-A.

Methods.—The preparation of concanavalin-A reagent from jackbean meal was described earlier.[11,12] The procedure for estimating "glycogen-values" is based on the absorbancy obtained upon treatment of one mg. of the material with the concanavalin-A reagent, in relation to the absorbancy obtained upon similar treatment of one mg. of a standard glycogen.[11,12]

α-Amylolysis was carried out by treating 0.2% solutions of the polysaccharides at 25° with 0.1 volume of diluted saliva (1:10) buffered to *p*H 7.0 with phosphate.

β-Amylolysis was performed at *p*H 4.5 and 25°, using 1 mg. of Wallerstein β-amylase of analytical purity with 100 mg. of polysaccharide in 50 ml. of 0.01 *M* acetate buffer solution.

Results and Discussion

It had been noted earlier[12] that neither amylopectin nor its β-limit dextrin were precipitated on treatment with concanavalin-A reagent, whereas all of the glycogens examined did so. Since *Neisseria perflava* cultures which contained sucrose were stated to produce amylopectin-like polysaccharides having in some respects properties intermediate between those of glycogen and amylopectin,[3,6] it appeared of interest to examine the *Neisseria* polysaccharides with concanavalin reagent. The results are shown in Table I.

Contrary to the results obtained with plant amylopectins, the *Neisseria* amylopectin-like sub-

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