3-methoxy-5-methylacetophenone[11] was reduced by the Clemmensen method[12] with 75 g. of amalgamated zinc in 75 ml. of concd. hydrochloric acid and 75 ml. of water, and refluxed for 8 hr. The reaction mixture was extracted with ether. After removal of the ether, the residue distilled at 90–92°/1 mm., yield, 65%.

Anal. Calcd. for $C_{10}H_{14}O_2$: C, 72.28; H, 8.43. Found: C, 72.04; H, 8.28.

The 3,5-dinitrobenzoate melted at 161–162°.

Anal. Calcd. for $C_{17}H_{16}O_7N_2$: C, 56.66; H, 4.41; N, 7.77. Found: C, 56.85; H, 4.40; N, 7.53.

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[11] M. F. Browne and R. L. Shriner, *J. Org. Chem.*, **22**, 1320 (1957).

[12] E. Clemmensen, *Ber.*, **47**, 51 (1914).

Acetylation of Alginic Acid. I. Preparation and Viscosities of Algin Acetates

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Alginic acid can be completely acetylated with acetic anhydride and catalytic amounts of perchloric acid. Partially acetylated products may be obtained by removing samples during the controlled reaction. To keep the alginic acid in an activated form, 10–20% of water has to be retained in the fiber. If the viscosity of aqueous solutions of ammonium acetyl alginates is plotted against the degree of acetylation (D.A.), a maximum of the viscosity is obtained between a D.A. of 0.5 and 0.8. In the same range the rate of acetylation increases faster than expected. The results give strong support to the assumption of hydrogen bridge bondings between the vicinal hydroxyl groups in the uronic acid units as the reason for the low reactivity of alginic acid and alginates.

The acetylation or, generally, esterification of hydroxyl groups is one of the most important chemical procedures for changing properties of polysaccharides and other carbohydrates. Products of great commercial value such as cellulose acetate, starch acetate, etc., have thus been developed. The usual methods applied are the catalytic reaction with acetic anhydride or acetic acid, the reaction with acetyl chloride in the presence of an organic base, and a few others, all of them being applied in an anhydrous medium.

In contrast to most polysaccharides alginic acid, a glycuronoglycan of presumably β-(1→4) linked D-mannuronic acid units[1,2] and in a minor percentage[3] of L-guluronic acid units[4,5] cannot be acetylated by the classical methods.[6] Attempts to acetylate alginic acid by the reaction with ketene[7] seemed to be more successful but only about one acetyl group per uronic acid unit could be introduced.

Chamberlain, Cunningham, and Speakman reported the diacetate of alginic acid for the first time in 1946 using a water swollen alginic acid fiber in their experiments.[8,9] However, the degradation during the reaction was too severe to do any viscosity studies with sufficient accuracy. Takahashi reported the diacetate also[10] obtained by treatment of alginic acid with acetic anhydride vapor.

The present work provides information on a method by which fully as well as partially acetylated alginic acid may be obtained. The degradation is reduced to a minimum as indicated by the fact that the end products retain a high viscosity. The study of the variation of viscosity with D.A. gives information on the reactivity of alginic acid.

It is essential for a successful reaction to use a wet alginic acid fiber as starting material with as low an ash content as possible. The following partial dehydration has to be carried out in a way which permits an even distribution of the residual water. For this reason drying at elevated temperature is not suitable. A complete dehydration is disadvantageous because it inactivates the alginic acid. The favorable influence of the presence of some water probably is due to the association of the water molecules with the hydroxyl groups of the uronic acid units which otherwise form hydrogen bridge bondings to a larger extent. When hydrogen bridge formation is favored a substitution of the hydroxyl groups involved becomes very difficult.

If the reaction is performed at moderate temperatures, products with degrees of acetylation of up to 1.85 are obtained. The theoretical D.A. of 2 based upon the assumption that an alginic acid of 100% purity is fully acetylated and that the presumed structure of alginic acid is correct cannot be reached

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[1] S. K. Chanda, E. L. Hirst, E. G. V. Percival, and A. G. Ross, *J. Chem. Soc.*, 1833–1837 (1952).

[2] E. L. Hirst, J. K. N. Jones, and W. O. Jones, *J. Chem. Soc.*, 1880 (1939).

[3] R. L. Whistler and K. W. Kirby, *Z. Physiol. Chem.*, **314**, 46–48 (1959).

[4] F. G. Fischer and H. Dörfel, *Z. Physiol. Chem.*, **302**, 186–203 (1955).

[5] R. L. Whistler and R. G. Schweiger, *J. Am. Chem. Soc.*, **80**, 5701–5704 (1958).

[6] V. C. Barry, T. Dillon, and P. O'Muineachain, *Proc. Roy. Soc. Dublin*, **21**, 289 (1936).

[7] A. Wassermann, *Nature*, **158**, 271 (1946).

[8] N. H. Chamberlain, G. E. Cunningham, and J. B. Speakman, *Nature*, **158**, 553 (1946); British Patent 573,591 (1945); U. S. Patent 2,403,707 (1946).

[9] L. deKeijser, *Compt. rend. 27e congr. intern. chim. ind., Brussels 1954; Industrie Chim. belge*, **20**, Spec. No., 358–362 (1955).

[10] Takeo Takahashi, Koji Kimoto, and Yoshio Takamo, *J. Chem. Soc. Japan, Ind. Chem. Sect.*, **54**, 536–538 (1951).

unless the reaction temperature is raised considerably and the material thus is degraded to a large extent and provides for additional free hydroxyl groups.

Variations of the viscosity with reaction time and D.A. of 0.052 *N* aqueous solutions are given in Fig. 1 and 2. In this series a 2.5-fold excess of acetic anhydride was used. A 0.052 *N* solution corresponds to a 1% solution of ammonium alginate, to a 1.22% solution of ammonium monoacetyl or a 1.44% solution of ammonium diacetyl alginate.

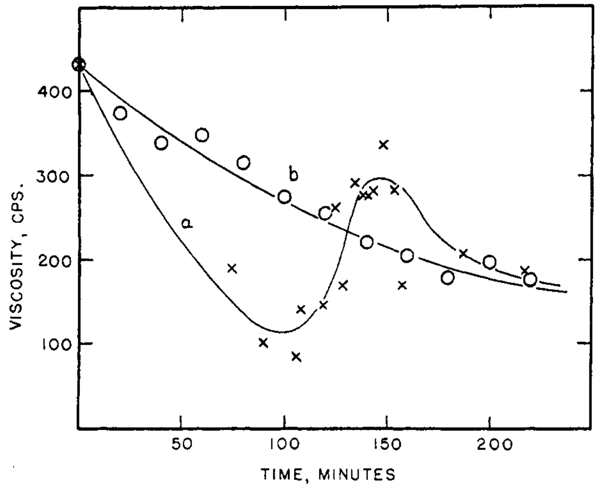

Fig. 1.—Viscosity of 0.052 *N* aqueous solutions *vs.* reaction time. (a) Sample being acetylated using perchloric acid as catalyst. (b) Sample treated with acetylating mixture but without catalyst to avoid acetylation. Under acetylation conditions this curve would be steeper since perchloric acid as a strong mineral acid contributes to a higher degree of hydrolysis

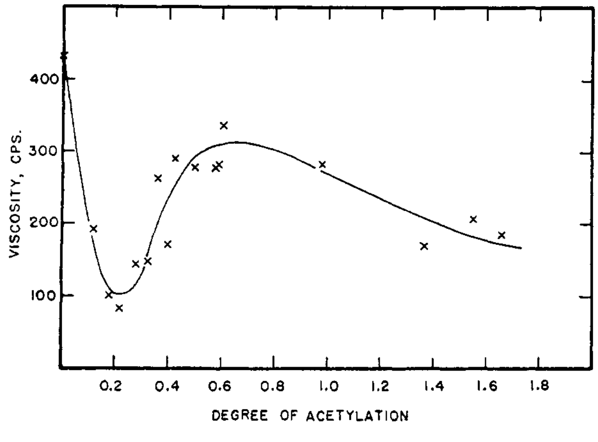

Fig. 2.—Viscosity of 0.052 *N* aqueous solutions *vs.* degree of acetylation

Another experiment in which alginic acid with a low viscosity of 135 cp. was used gave identical results. The minimum was found to be about 70 cp. the maximum about 85 cp. at a D.A. of 0.5–0.6.

A chemical reaction of a polysaccharide usually is combined with an alteration of its viscosity. This change is expected to be negative in the case of an acetylation under described conditions because of: I. Partial acid hydrolysis represented in Fig. 1 by curve (b), and II. The gradual replacement of hydroxyl groups by acetyl groups. Such substitution is a viscosity reducing step also, assuming that hydroxyl groups contribute to a higher viscosity by their ability to associate with water molecules. The addition of (I) and (II) would produce a hydrolysis type curve differing from (b) by a steeper slope only.

Experimental results, however, indicate a viscosity maximum at a D.A. of <1. As pointed out above, hydroxyl groups may contribute to a higher viscosity and thus a viscosity maximum may correspond with a maximum of the number of hydroxyl groups. This may occur, indeed, if hydrogen bridge bondings between the vicinal hydroxyl groups block them to a certain extent. Since the hydroxyl group of the monoacetate unit cannot form such bonding, the number of unblocked groups may really increase during the first phase of the reaction, until the second reaction step monoacetate → diacetate becomes significant. The viscosity change by this effect would be positive, reaching a maximum at a D.A. of ≤ 1. Then it would become negative and drop to a constant value (Fig. 3, III).

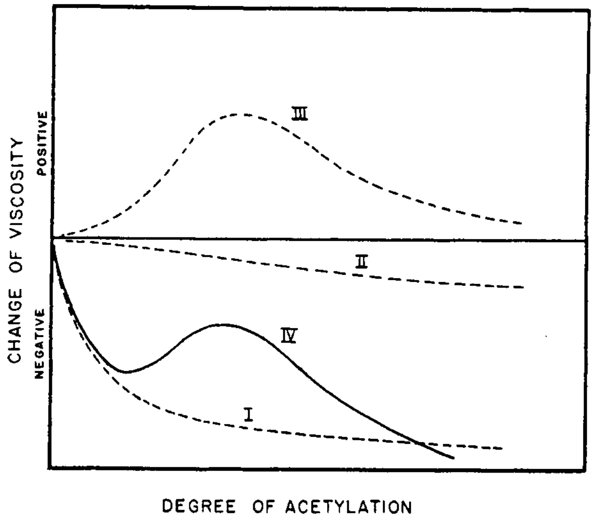

Fig. 3.—Influence on viscosity by (I) hydrolysis, (II) introduction of acetyl groups, and (III) by reducing the extent of hydrogen bridge bondings. Curve (IV) results when (I), (II), and (III) are added

Figure 3 shows all three viscosity changing effects as change of viscosity against D.A. Their addition produces a curve of the same type as obtained experimentally. The exact position of the viscosity maximum depends on the extent of hydrogen bridge bonding and on how far the two acetylation steps are overlapping.

A similar example indicating intermolecular hydrogen bridge bondings in cellulose is the water solubility of cellulose ethers, especially ethyl cellulose, at a medium D.A. while the cellulose itself, as well as highly etherified cellulose are insoluble.

The rate of reaction given in Fig. 4 for three different experiments in which about a three-fold excess of acetic anhydride was added at once is very low at the beginning of the reaction. In this period, part of the acetic anhydride is hydrolyzed by the residual water as indicated by an exothermic reaction during which the D.A. stays close to O. In the advanced part of the reaction the rate increases gradually, becomes unexpectedly fast at a D.A. of between 0.5 and 0.7 and levels out above a D.A. of 1.5–1.6.

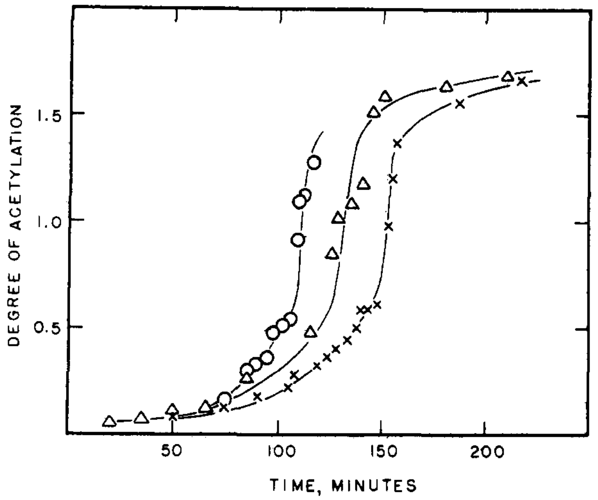

Fig. 4.—Degree of acetylation *vs.* reaction time from three different experiments

Because of the high rate of reaction between a D.A. of 0.7 and 1.5, the preparation of a product with a medium D.A. is difficult. The rate, however, can be lowered considerably by using less acetic anhydride; the acetylation then may be interrupted at any stage more conveniently.

Considering that it is a two-phase reaction and that the end product swells much better in the reaction mixture than the starting material, a gradual increase of the rate is expected. The sudden change, however, at a D.A. of between 0.5 and 0.7 may indicate an increased availability of hydroxyl groups although the reaction is too complex to draw any final conclusions from rate studies.

The results so far give strong evidence of the presence of hydrogen bridge bondings between vicinal hydroxyl groups as the reason for the low reactivity of alginic acid and alginates. Since monoacetyl alginic acid cannot form such hydrogen bridge bondings it may undergo further reaction at the residual free hydroxyl group much more easily than alginic acid. However, it has to be considered that an acetyl alginic acid with an average D.A. of 1 has nonacetylated and diacetate units also, both of which would have to be excluded.

Experimental

Preparation of Ammonium Diacetyl Alginate.—A portion of 1000 g. of wet alginic acid removed directly from the production line and containing 75–80% of water and 0.3–1.0% of ash as calcium oxide mainly was washed with 1000 ml. of glacial acetic acid by mixing it thoroughly in a Hobart mixer for about 10 min. and then removing the liquid by filtration on a Büchner funnel using a rubber dam. This was repeated twice. Another suitable method of reducing the water content was washing with 800 ml. of acetone, followed by partial freeze drying. Then a mixture of the washed material with 1500 ml. of glacial acetic acid and 1500 ml. of acetic anhydride was placed in a Hobart mixer. While mixing, 1–2 ml. of perchloric acid was added at such a rate that the temperature did not exceed 40–45°. This required about 2 hr. After a total reaction time of about 3 hr. the mixture was pressed out, mixed, and washed thoroughly with water and pressed out again. Washing with water was continued until the wash water had a *p*H of 6.

The free diacetyl alginic acid was suspended in water, and while mixing, a slight excess of ammonium hydroxide was added. When a very thick, homogeneous sirup was obtained, mixing was stopped, the sirup was poured in a thin stream into acetone and mixed thoroughly. The solvent was decanted and more acetone was added to harden the product. After a few hours the solvent was filtered off and the acetyl alginate was air-dried and finally dried in a desiccator over calcium chloride. The yield was 300–350 g.; the D.A. 1.75–1.85.

The ammonium diacetyl alginate is soluble in water, swells in lower alcohols, and is insoluble in other organic solvents. To aqueous solutions methanol up to 95% or ethanol up to 75–80% may be added without causing precipitation or gelation. It forms gels or precipitates with copper(II), tin(II), and with tri- and tetravalent ions. It does not precipitate or gelatinize with calcium, barium, magnesium, iron(II), manganese(II), or zinc.

Preparation of Partially Acetylated Ammonium Alginates.—A portion of 1000 g. of wet alginic acid was washed with glacial acetic acid as described above and then placed in a Hobart mixer with 1000 ml. of glacial acetic acid and 800 ml. of acetic anhydride. While mixing, 300–400 ml. more of acetic anhydride and the required amount of perchloric acid as catalyst were added at such a rate that the temperature did not exceed 40°. Portions of about 50 g. were removed when the desired D.A. was reached. The D.A. could be roughly estimated by removing very small samples, blotting them on filter paper, and testing their consistency when mixed with water:

Fibrous but strongly swollen	D.A. 0.2–0.4
Paste with fibrous appearance	D.A. 0.4–0.7
Heavy paste	D.A. 0.7–1.2
Insoluble, rubbery	D.A. $\geq$1.2

The material was washed three to four times with:

70% Isopropyl alcohol for D.A. 0.2–0.4
90% Isopropyl alcohol for D.A. 0.4–0.8
100% Isopropyl alcohol for D.A. 0.8–1.3
Water for D.A. $\geq$1.3

Then it was neutralized with ammonium hydroxide, precipitated from acetone, and dried as described already. The yields were nearly quantitative. The D.A. varied from 0.2 to 1.6 depending on the reaction time.

Acetyl Determination.—A small sample of ammonium acetyl alginate was dried at 80° *in vacuo* over calcium chloride and sodium hydroxide for about 1 hr. A 0.5-g. portion of the dried material was placed in a round-bottom flask connected with condenser and receiving flask. Then 10 ml. of 85% phosphoric acid and 25 ml. of water were added, and the flask was heated in an oil bath and kept at

150–155°. Under these conditions the product was hydrolyzed, and the acetic acid formed was isolated at the same time by distillation. When the reaction mixture became slightly sirupy 25 ml. more of water was added; this was repeated twice. After 3–4 hr. the distillate was titrated with 0.1 N sodium hydroxide using phenolphthalein as indicator. The sodium hydroxide consumed is indicative of the acetic acid formed. The equivalents for 1 g. of the products with a D.A. of 1 and of 2 were calculated and plotted on a curve against the D.A. The D.A. of the analyzed products was taken from this curve.

Determination of the Degree of Hydrolysis.—A portion of 1000 g. of wet alginic acid was washed three times with 1000 ml. of glacial acetic acid each time. Then it was placed in a Hobart mixer with 1500 ml. of acetic acid and 1500 ml. of acetic anhydride and stirred while the temperature was kept at about 40°. No perchloric acid was added. Samples of about 50 g. were removed every 20–30 min. They were washed with 50% methanol until neutral, mixed with water, and neutralized with a slight excess of ammonium hydroxide. The thick sirup was poured in a thin stream into acetone while mixing thoroughly, the liquid was filtered off and the fibrous precipitate was dried in a desiccator. An analysis of the last sample indicated that no acetyl groups were introduced. The viscosities of all samples as an indication of hydrolysis were determined as described below. The results are given in Figure 1 (b).

Determination of the Viscosity.—For the viscosity determinations 1-g. portions of ammonium alginates were weighed and dissolved in 100 ml. of water by stirring for about 1 hr. The weight of the acetylated samples was corrected by multiplying with a factor, M_A/M_B, in order to obtain the same molarity in all solutions. M_A is the equivalent weight of the ammonium salt of the corresponding acetylated uronic acid unit. M_B is the equivalent weight of the ammonium salt of the nonacetylated uronic acid unit. In order to exclude any influence by calcium ions which might be present in trace amounts, 1 g. of hexametaphosphate was added. The viscosity was determined with a Brookfield viscometer at 25° and 60 r.p.m. Results are given in Figures 1 and 2.

Acetylation of Alginic Acid. II. Reaction of Algin Acetates with Calcium and Other Divalent Ions

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Aqueous solutions of ammonium diacetyl alginate and of acetyl alginates with considerably lower degrees of acetylation do not change their consistency in the presence of most divalent ions. This leads to the conclusion that gelation or precipitation of alginates with calcium ions occurs through a complex involving two carboxyl groups from neighboring units and two hydroxyl groups in a unit of probably another chain. The hydroxyls have to be vicinal and thus belong to one unit. Free hydroxyl groups of monoacetate units do not take part in the formation of this complex.

It is a well known fact that solutions of the ammonium or alkali salt of some polysaccharides containing carboxyl groups gelatinize with traces of di- or trivalent ions, and, if more such ions are present, form fibrous precipitates.[1] One method for the production of alginic acid[2] is partially based on this fact. In numerous applications use is made of the insolubilization of alginates by the addition of a calcium salt.

This precipitation reaction may be explained by assuming crosslinkage through the divalent ions involving carboxyl groups of different chains and thus forming a huge molecule.[3] After sufficient growth, gelation or precipitation should be expected. Another very simple explanation is the assumption of the mere insolubility of the corresponding salt comparable with the insolubility of the silver salt where the type of crosslinkage mentioned above is out of the question.

By the acetylation of alginic acid, experimental data were obtained which gave strong support for a new explanation of the reaction path concerning gelation or precipitation of alginates with divalent ions.

The first unexpected result was the compatibility of the ammonium diacetyl alginate with calcium and other divalent ions. Thus, it became evident that the carboxyl groups are only partially responsible for the precipitation and that the assumptions above cannot be correct in the case of algin. The presence of free hydroxyl groups is required, and apparently gelation occurs by crosslinkage through chelate formation involving both carboxyl and hydroxyl groups. On the other hand the two carboxyl groups have to be in the same chain and in close proximity. Otherwise carboxyl groups alone would be sufficient for gelation or precipitation.

During the partial acetylation of alginic acid another surprising observation was made which makes possible a more accurate description of the structure of the complex. Not only the diacetyl alginates, but also products with considerably lower degree of acetylation (D.A.) showed complete compatibility with divalent ions. A product with a D.A. of about 1.4 consists exclusively of mono- and diacetate units. However, in spite of the presence of monoacetyl units and thus of free hydroxyl groups no gelation could be noticed on the

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(1) R. L. Whistler, "Industrial Gums," Academic Press Inc., New York and London, 1959, pp. 78, 416–417.

(2) F. C. Thornley and M. J. Welch, U. S. Patent 1,814,981 (1931).

(3) R. L. Whistler, "Industrial Gums," Academic Press Inc., New York and London, 1959, p. 409.