

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 acid² 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

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

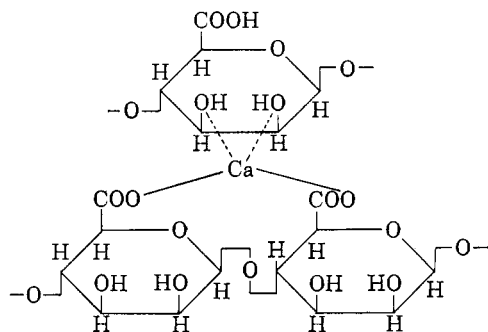
(2) F. C. Thornley and M. J. Welch, U. S. Patent 1,814,981 (1931).

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

TABLE I

D.A.	Viscosity (cp.)	Consistency and/or Viscosity (cp) after Addition of CaCl ₂			
		0.25 g.	0.50 g.	0.75 g.	1.00 g.
0.05	171	Precipitates; with increasing D.A. increasing amounts of CaCl ₂ are necessary			
0.10	134				
0.27	100				
0.48	91				
0.85	116				
0.98	61	Gelatinous, lumps	Thinning out		
1.09	119	Heavy gel	gel	~1900	Grainy, ~1300
1.18	108	~2000, thin gel	~2000	~1900	1750
1.21	34	Gel	gel	~3500	~3000
1.39	71	Soft gel, ~3800	~3800	~3200	~2800
1.42	64	700-900, thin gel	1200-1300	1200-1300	1100-1200
1.47	55	75	91	95	96
1.51	100	43	44	44	44
1.59	98	37	36	36	35
1.76	43	100	116	126	131
		54	55	54	54
		28	29	28	28

addition of calcium ions. Since in this product the nonacetylated unit is the only one missing it must be concluded that this unit with both hydroxyls free is responsible for the precipitation reaction. The chelate structure may now be completed as shown in Figure 1.



Calcium with its primary valences connects to carboxyl groups, probably on neighboring units. Coordinate bondings are extending to two vicinal hydroxyl groups of a third unit. This third unit may be in the same chain and thus keep the macromolecule in a coiled shape or it may be in another chain forming a huge molecule with a net structure. The fact that gels are obtained when complex-forming metal ions are added is evidence for the latter case. It is very likely, however, that both intra- and intermolecular complexes coexist.

According to the description above, and supposing that further acetylation to the diacetate would occur only after the formation of the monoacetate has been completed, all reaction products with D.A. ≥ 1 should be insensitive towards calcium ions. Only at D.A. < 1 should a change of the consistency be noticed. On lowering the D.A., starting from 1.0, at first higher viscosities, then light gels, heavy gels and finally precipitates should be obtained if mixed with a solution of a calcium salt in excess. The range of D.A. in which gels and viscosity increases are noticed would be expected

to be narrow since a slight difference in the number of crosslinkages would cause a great difference in the appearance of the product.

The preceding Table I shows the consistency of 1% solutions of ammonium acetyl alginates when 0.25 g., 0.50 g., 0.75 g., or 1.0 g. of calcium chloride is added. Where possible, the viscosity in cps. is given, measured with a Brookfield viscometer at 25° and 60 r.p.m.

The consistencies of the solutions when mixed with calcium chloride vary with the D.A. in the way as discussed above. However, the effects correspond with higher D.A. values and the gel range is wider than assumed. If one considers that the acetylation is not homogeneous and that the reaction monoacetate \rightarrow diacetate becomes significant when nonacetylated units still are available, this has to be expected. A product with an average D.A. of 1.0, then, contains nonacetylated units besides mono- and diacetate units. The critical D.A. where the sensitivity towards calcium ions disappears has to be above one. The discrepancy may be considered an estimate of how much the two reaction steps overlap.

Similar measurements were made with zinc, barium, manganese(II), and magnesium, and results are shown in Table II.

Principally the same observations were made. The critical D.A. obtained by different ions is very close to the same value. However, the extent of gelation or viscosity increase depends on the ion used, and this is understandable since the metal ion is the center of the complex. Manganese(II) or magnesium obviously form this complex to a very slight extent only and thus do not react with acetyl alginates unless the D.A. is very low. With ammonium alginate they cause an increase of viscosity only.

Further evidence for the conclusions above might be obtained by infrared spectrophotometry, although spectra of polysaccharides usually do not show clear and steep peaks.

TABLE II

D.A.	Viscosity (cp.)	Consistency and/or Viscosity (cp.) after Addition of								1 g. MnCl ₂ or 1 g. MgCl ₂
		ZnCl ₂				BaCl ₂				
		0.25 g.	0.50 g.	0.75 g.	1.00 g.	0.25 g.	0.50 g.	0.75 g.	1.00 g.	
0.6	46	~1420, gel, lumps	~1150	500, thinning out	310	Heavy gel	Thinning out			31
0.7	39	~1180, gel, lumps	~1130	620	380	Heavy gel	Thinning out			
0.87	47	~600, thin gel	.790	540	360	Heavy gel	Heavy gel	Thinning out		30
0.98	61	410	390	390	240	Gel, ~4850	~3300	~2400	1850	
1.21	45	99	133	107	89	Gel, ~3700	~2950	~2400	1630	24
1.39	70	117	134	121	107	Solution 1240	1160	1050	900	51
1.42	66	48	54	54	52	140-190	160-190	150	140	
1.47	55	32	31	32	31	31	30	29	29	

Experimental

Preparation of Ammonium Acetyl Alginate.—The acetyl alginates were prepared as described in the preceding paper.⁴ The diacetate was obtained by washing 1000 g. of wet alginic acid (25% solids, 0.5% ash) three times with 800 cc. of glacial acetic acid. The liquid was filtered off on a Büchner funnel each time and then the material was reacted with a mixture of 1400 to 1500 cc. of acetic anhydride, 1000 cc. of acetic acid, and 2 cc. of perchloric acid. The perchloric acid was added at such a rate that the temperature of the reaction mixture did not exceed 45°. After a reaction time of about 3 hr. the material was pressed out, mixed and washed thoroughly with water until the wash water was neutral, then it was neutralized with ammonium hydroxide. The salt was precipitated in acetone and dried at 45° in the presence of an air stream.

The lower D.A. products were prepared by starting the

reaction with 800 cc. of acetic anhydride. Then 300–400 cc. more of acetic anhydride was added during the reaction. At the desired D.A. the reaction was interrupted, the liquid removed by filtration and the material was washed with isopropyl alcohol until the odor of acetic acid had disappeared and then neutralized with ammonium hydroxide. Products with D.A. >1.3 were washed with water. The ammonium salt was precipitated in acetone and dried at 45° in the presence of an air stream.

Reactivity with Divalent Ions.—A 1-g. sample of ammonium acetyl alginate was dissolved in 100 cc. of distilled water, and its viscosity was measured with a Brookfield viscometer at 25° and 60 r.p.m. Then four 0.25-g. portions of calcium chloride as aqueous solutions were added successively while stirring vigorously. After each addition the consistency and, if possible, the viscosity were tested. The results are given in Table I.

The same measurements were made after adding barium chloride, zinc chloride, manganese dichloride, or magnesium chloride in place of the calcium chloride. Results are shown in Table II.

(4) R. G. Schweiger, *J. Org. Chem.*, **27**, 1788 (1962).

The Addition of Sodium Methoxide to Carbohydrate α -Nitroolefins. Synthesis of 2-*O*-Methyl-D-mannose and 2-*O*-Methyl-D-ribose

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The reaction of *D-arabino*-3,4,5,6-tetraacetoxy-1-nitro-1-hexene with sodium methoxide in methanol solution gives 1-deoxy-2-*O*-methyl-1-nitro-*D*-mannitol. Hydrolysis of the nitro group (Nef reaction) then gives 2-*O*-methyl-*D*-mannose. Similarly, *D-erythro*-3,4,5-triacetoxy-1-nitro-1-pentene has been employed for the synthesis of 2-*O*-methyl-*D*-ribose.

The addition of sodium methoxide to simple, aliphatic α -nitroolefins, to yield alkyl nitroalkyl ethers, has long been known.¹ In the sugar series, Fischer and Baer² postulated such an addition of sodium methoxide to an intermediate α -nitroolefin to explain the monomethylated products arising from the action of the base on 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-nitro-*D*-glucose. We

have now examined the action of sodium methoxide on two acetylated carbohydrate α -nitroolefins and find that the reaction leads to a useful method of preparation for the 2-*O*-methyl aldoses.

When a solution of *D-arabino*-3,4,5,6-tetraacetoxy-1-nitro-1-hexene (I)³ in methanol was treated at room temperature with slightly more than two equivalents of sodium methoxide, and the solution subsequently was acidified with acetic acid and

(1) J. Loevenich, J. Koch, and U. Pucknat, *Ber.*, **63**, 636 (1930); C. T. Bahner, U. S. Patent 2,391,815, Dec. 25, 1945; A. Lambert, C. W. Scaife, and A. E. Wilder-Smith, *J. Chem. Soc.*, 1474 (1947).

(2) H. O. L. Fischer and H. H. Baer, *Ann.*, **619**, 53 (1958).

(3) J. C. Sowden and H. O. L. Fischer, *J. Am. Chem. Soc.*, **69**, 1048 (1947).