POLYSACCHARIDE SULFATES. II. SULFURIC ACID ESTERS OF GLYCURONANS HAVING VARIOUS DEGREES OF SUBSTITUTION

RICHARD G. SCHWEIGER* AND THOMAS R. ANDREW
Research Laboratory of Kelco Company, San Diego, California (U. S. A.)
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ABSTRACT

By use of an *N,N*-dimethylformamide–sulfur trioxide complex in *N,N*-dimethylformamide as a solvent, and after proper activation, alginic and pectic acids are sulfated without difficulty up to a degree of substitution (d.s.) of about 1.8. Lower d.s. levels are obtained by decreasing the proportion of complex. Degradation, as indicated by viscosity measurements, is minor. All sulfated glycuronans exhibit reactivity with proteins that becomes more pronounced as the d.s. increases. Solutions of algin sulfates having d.s. >1.0 gelatinize in the presence of the heavier alkali-metal ions. Only two other polysaccharide sulfates, κ-carrageenan and cellulose sulfate, prepared recently, exhibit such properties.

Among the polysaccharides, glycuronans present the most difficulties for the substitution of their hydroxyl groups. For example, conventional methods of acetylating alginic acid [composed of β-D-(1→4)-linked D-mannuronic[1,2] and L-guluronic[3] acid residues], and pectic acid (a D-galacturonan[4]) were unsuccessful[5]. Under more rigorous conditions, highly degraded products usually result. Although the close proximity of the electronegative carboxyl group may be responsible to some extent for this lack of reactivity, it has been proposed recently that the principal reason is the extensive formation of hydrogen bonds between hydroxyl groups. It was shown that alginic acid[6] and pectic acid[7] could be acetylated without difficulty after the extent of hydrogen bonding had been decreased; that is, the acids had been properly "activated".

In sulfation of a glycuronan, not only is the lack of reactivity of the polymer a handicap but, additionally, the usual conditions for sulfation are vigorous and apt to degrade polysaccharides. Thus, Whistler and Spencer[8] could not esterify alginic acid by use of a triethylamine–sulfur trioxide complex. Apart from a few patents describing the preparation of highly degraded algin sulfates[9] and pectin sulfates[10] to be used as blood anticoagulants, no other reports of a successful method for sulfating glycuronans has appeared in the literature so far. In the preceding article of this series[11], a new method of sulfating cellulose was reported. A complex of

*To whom inquiries should be directed. Present address: Grain Processing Corporation, Muscatine, Iowa 52761, U. S. A.

N,*N*-dimethylformamide (DMF) and sulfur trioxide is used as the sulfating agent and, under certain conditions, little degradation of the cellulose molecule occurs and a product of high degree of substitution (d.s.) is obtained. When this method is applied to alginic or pectic acids[12], the starting material requires prior activation. The activation consists of fully hydrating the free acid and subsequently, removing the water by washing with a polar solvent, in this case, glacial acetic acid[6,7]. Apparently water greatly decreases intramolecular hydrogen bonding between hydroxyl groups. The activated state is maintained temporarily after replacement of the water by a polar solvent. This assumption is supported by the fact that activated glycuronans, stored over a period of hours or days, are gradually deactivated and finally become unsuitable as a starting material. *N*,*N*-Dimethylformamide, instead of glacial acetic acid, is unsuitable, since both glycuronans are highly swellable in aqueous DMF. Unlike cellulose, alginic and pectic acids are not activated by pretreating the dry materials with DMF, and no substitution occurs after such treatment. The presence of acetic acid does not noticeably influence the reaction rate or efficiency. The sulfation proceeds readily, and d.s. values of up to 1.8 are obtained. Lower d.s. values result upon decreasing the proportion of complex. This is in contrast to the sulfation of cellulose where, even with insufficient amounts of complex, product of high d.s. was obtained, explained by a so-called peeling process[11].

During the sulfation, the viscosity of the resulting products, based on 1% (w/v) solutions, is usually decreased to 10–50% of its original value. Taking into account that this viscosity decrease is caused largely by the increase in molecular weight through incorporation of the sulfate groups, degradation as indicated by viscosity changes appears to be minor.

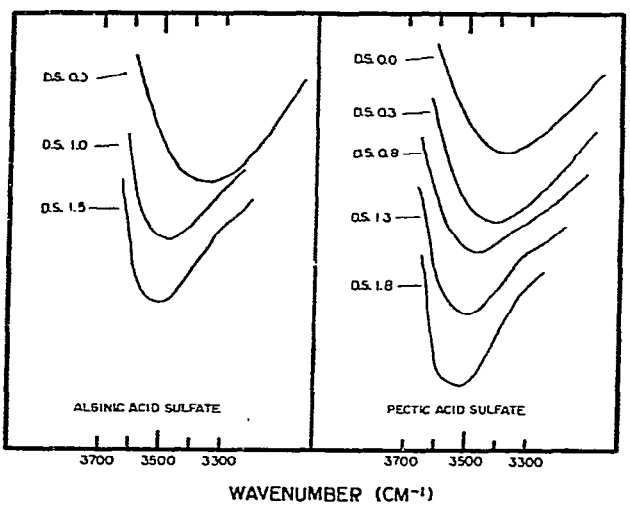

Fig. 1. Infrared spectra of the sodium salts of alginic and pectic acid sulfates having various degrees of substitution.

The i.r. spectrum of unsubstituted sodium alginate shows a strong peak at about 3350 cm^{-1}, typical of hydrogen-bonded hydroxyl groups. With increasing d.s., the peak gradually shifts to higher wavenumbers to a value of slightly greater than 3500 cm^{-1} at d.s. >1, indicative of non-bonded hydroxyl groups (Fig. 1). Previous evidence of the presence of hydrogen bonds between hydroxyl groups was obtained in a different way during acetylation studies[6]. The hydrogen bonds have been considered to be the principal reason for the lack of reactivity of the hydroxyl groups in alginic acid and alginate. The i.r. data further support this view. That the hydrogen bonds are intramolecular, that is, non-crosslinking, is shown by the fact that, during substitution, that is, with elimination of hydrogen bonds, the viscosity is decreased only moderately. In the case of intermolecular bonds, the viscosity changes would be expected to be quite large, because substitution would decrease the degree of crosslinking and, thus, decrease the apparent molecular weight. In the extreme case, such as in cellulose, there would be a transition from complete insolubility to solubility, and within this transition, compounds having extremely high viscosities at a d.s. level just sufficiently high for solubilization, and compounds having much lower viscosities at the higher d.s. levels, would be produced. Further support for the foregoing assumption, namely, prevalence of hydrogen bonds between the vicinal hydroxyl groups of the same residue, is the fact that the shift towards

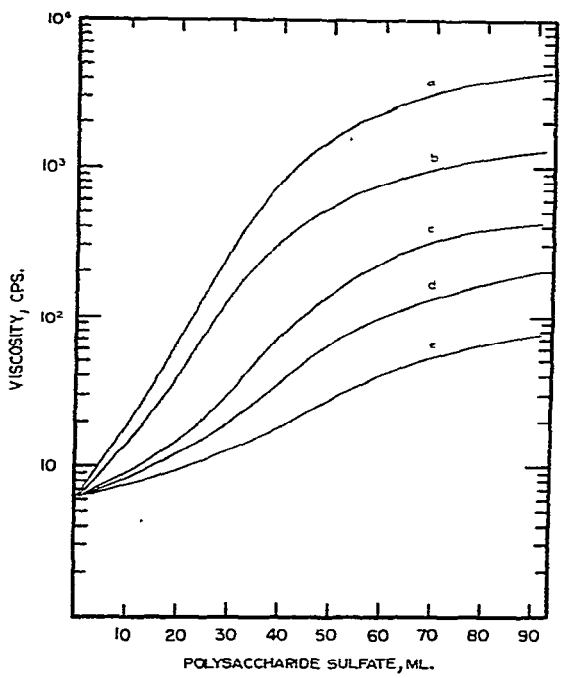

Fig. 2. Viscosity of 5% aqueous solutions of sodium caseinate after the addition of the sodium salts of alginic acid sulfates having d.s. of (*a*) 1.5 and (*b*) 1.0, and of pectic acid sulfates of (*c*) 1.8, (*d*) 1.3, and (*e*) 0.8.

higher wavenumbers occurs essentially in the d.s. range 0.0–1.0. Pectate and sulfated pectates produce similar i.r. spectra (Fig. 1), and, in combination with previous results[7], the same conclusions may be drawn for pectate as for alginate.

As expected by comparison with previous results[11,13], algin sulfate is remarkably resistant to saponification by alkali. Thus, algin sulfate (sodium salt of alginic acid sulfate) having d.s. 1.5 could not be appreciably desulfated by heating in M sodium hydroxide. Unsubstituted sodium alginate produced, after treatment under the same conditions followed by dialysis, a much smaller portion of non-dialyzable, high-molecular-weight materials than did algin sulfate. This observation indicates that substitution with sulfate groups renders the glycuronan more stable to depolymerization through alkaline degradation.

Reactivity of the glycuronan sulfates with protein, a behavior quite common with strongly anionic polymers, is demonstrated by increases in viscosity observed with aqueous solutions of sodium caseinate on the addition of the sodium salts of alginic and pectic acid sulfates (Fig. 2). The reactivity increases with increasing d.s., as would be expected by assuming that cross-linkage occurs mainly through salt-like combinations between the basic sites of the protein and the highly negative groups of the polysaccharide sulfate. The relatively large difference in the viscosity increase found between the addition of the sodium salt of pectic acid sulfate and that of algin sulfate having similar d.s. can be ascribed to a molecular-weight difference between these polymers. Considerable differences in molecular weight are indicated, indeed, by the relatively large differences in viscosity of their 1% aqueous solutions, 9–10 centipoises and about 60 centipoises, respectively.

Among the polysaccharide sulfates described in the previous literature, only carrageenan (occuring naturally in red marine algae) and cellulose sulfate (described in the preceeding article of this series) form thermoreversible gels with the heavier alkali-metal ions. The algin sulfate now described constitutes the third polysaccharide sulfate exhibiting such properties. As is shown in Table III, solutions of algin sulfate having d.s. >1 gelatinize in the presence of potassium, rubidium, and cesium ions. The gel strength increases in the order $K^+ < Cs^+ < Rb^+$. No gels are obtained if the d.s. $\lesssim 1$. None of the sodium salts of the pectic acid sulfates form gels under these conditions. This may be ascribed to the molecular weight of the polymer being too low. Similar results have been reported previously for cellulose sulfate[11], where no gels could be produced when the 1% viscosity was at or below about 10 centipoises, that is, the molecular weight was below a certain minimum.

Since a number of gellable polysaccharide sulfates of different structure and d.s. are now available, it may be possible, after further studies, either to support or to modify a hypothesis on the gelation mechanism of carrageenan advanced by Bayley[14].

EXPERIMENTAL

Sulfation of alginic and pectic acids. — The DMF–SO_3 complex was prepared and used with excess DMF as described previously[11].

Activated alginic acid (100 g dry basis), obtained by dehydrating the wet fiber with glacial acetic acid[6], was mixed with 400 ml of DMF for 15 min in a jacketed "Day Mixer" cooled with ice–water. Subsequently, in a number of separate experiments, various amounts of ice-cold DMF–SO_3 complex of between 150 and 800 g were added in three portions, and mixing was continued for 2–3 h. The sulfated alginic acid was precipitated by the addition of a mixture of acetone and methanol (9:1, v./v.), washed with acetone–methanol, dissolved in water, and neutralized by the addition of sodium hydroxide. The sodium salt was precipitated by pouring the solution slowly and with stirring into about 2 volumes of methanol, and was dried *in vacuo*. A rise in temperature during neutralization was avoided by the addition of ice. The d.s. was determined[11] by weighing the amount of $BaSO_4$ formed after hydrolysis of a dialyzed aliquot and addition of $BaCl_2$. The lowest levels of complex used gave d.s. levels of ~0.1. The d.s. increased with increasing amount of complex, up to 1.6–1.7 at 400–500 g of complex per 100 g of alginic acid. Larger proportions did not result in any further increase of the d.s. Yields were quantitative, and increased with the d.s. Viscosities varied between 40 and 150 centipoises, with the starting material having a viscosity of about 300 centipoises, and usually decreased with increasing d.s. All viscosities were measured with a Brookfield Viscometer, LVF Model, at 60 r.p.m. and 25°. The concentration of all solutions used was 1% in distilled water unless stated otherwise.

Pectic acid, obtained from sodium pectate (Sunkist Growers, Ontario, California) was activated as described in a previous article[7], and dehydrated by washing with glacial acetic acid. It was sulfated under the same conditions and at similar complex-to-glycuronan ratios as the alginic acid derivative just described. Similar results were obtained. A maximum d.s. of 1.7–1.8 was reached with 400–500 g of complex per 100 g of pectic acid. Yields were quantitative, and viscosities varied between 9 and about 30 centipoises from a starting material of viscosity 28 centipoises.

TABLE I

VISCOSITIES (CENTIPOISES) OF THE SODIUM SALTS OF A NUMBER OF ALGINIC AND PECTIC ACID SULFATES AS AQUEOUS SOLUTIONS AT VARIOUS CONCENTRATIONS

Concentration (%)	*2.0*	*1.0*	*0.5*	*0.25*	*0.1*
Algin SO_4, d.s. 1.5	303	62	22.6	14.8	9.4
Algin SO_4, d.s. 1.0	268	60.1	20.5	12.2	9.0
Pectin SO_4, d.s. 1.8	18.4	9.7	7.0	5.6	4.5
Pectin SO_4, d.s. 1.3	19.5	9.3	6.9	5.5	4.2
Pectin SO_4, d.s. 0.8	24.4	10.0	6.0	4.9	4.0

I.r. spectra were obtained with a Perkin–Elmer Model 337 spectrophotometer, and samples were used as thin films prepared from aqueous solutions.

Saponification. — Algin sulfate (d.s. 1.5, Table I) was treated with alkali as described previously[11] by heating 10-g portions as 5% solutions in water, M, 2M, and 4M sodium hydroxide for 2 h at 100°. Additionally, unsubstituted sodium alginate

(viscosity 400 centipoises) was heated in M sodium hydroxide under the same conditions. The non-dialyzable portion of each sample was isolated as described previously[11] determination of d.s., viscosity, and yield, and results are given in Table II.

TABLE II

VISCOSITY, YIELD, AND D.S. DATA

	Water	M *NaOH*	2M *NaOH*	4M *NaOH*
Algin sulfate				
D.s.	1.5	1.5	1.3	0.3
Viscosity, centipoises	45.5	3.5	3.0	3.0
Yield, g	7.52	3.42	1.44	0.3
Sodium alginate				
Viscosity, centipoises		3.5		
Yield, g		0.85		

Reaction with protein. — Sodium caseinate (10 g) was dissolved in 200 ml of distilled water, and 5 ml portions of a 2% aqueous solution of the sodium salt of the glycuronan sulfate were added with stirring. After each addition, the viscosity was measured and plotted on a curve *vs.* the total amount (ml) added. The results are shown in Fig. 2. Glycuronan sulfates used were those shown on Table 1. The reactivity with protein is indicated by the difference in viscosity between the sample and a blank in which the polysaccharide sulfate solution is added to 200 ml of water instead of to 5% sodium caseinate. Table I shows that, in the blanks, the viscosity would increase to about 30–35 centipoises for the sodium salts of the alginic acid sulfates and to about 8 centipoises for those of the pectic acid sulfates.

Gelation with alkali-metal ions. — Portions of 1.5 g of the sodium salt of each glycurunan sulfate listed in Table I was dissolved each in 50 ml of hot water by using 150-ml specimen bottles (height 80 mm, inside diameter of neck, 41 mm). In one series, a hot aqueous solution of 1 g of KCl, in another, 1.6 g of RbCl, and in a third series, 2.26 g of CsCl, was added to each sample. All samples were adjusted to 100 ml with water, cooled to 20°, kept for 2 h at this temperature, and the gel strength was measured with a Bloom Gelometer having a Lucite plunger of 1-inch diameter. The gels were melted by heating in a hot-water bath, cooled to 15°, maintained for 2 h at that temperature, and the gel strength determined again. The solutions of the sodium salts of all pectic

TABLE III

GEL-STRENGTH DATA

	KCl	*RbCl*	*CsCl*
Gel strength, g			
at 20°	21.3	41.4	23.1
at 15°	28.0	53.9	37.4

acid sulfates and that of alginic acid sulfate having a d.s. of 1 did not gelatinize at any temperature. The results from the algin sulfate having a d.s. of 1.5 are given in Table III.

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