



Fig. 3. Influence of phloroglucinol on the rate of degradation. Correlation between decrease of viscosity and thiobarbituric acid reaction.

Alginate from *L. digitata*, Espevær, 10/6. pH 9.8, 80°.

● Without addition.

○ 0.001 M phloroglucinol added.

dehyde prior to alginate preparation gives rise to alginates with values of intrinsic viscosity of the same magnitude as for *L. digitata* alginates ( $[\eta] = 25-35$  dl/g).

In the preceding note a method was described by which it is possible to detect to what extent  $\beta$ -elimination is responsible for the degradation reaction. This method was applied to a solution of alginate from *L. digitata* containing 0.001 M phloroglucinol at pH 9.8 and 80°. The rate of degradation and the correlation between the decrease in viscosity and the formation of colour with thiobarbituric acid is given in Fig. 3. For comparison, the results without phloroglucinol are also given. Addition of phloroglucinol leads to a rapid degradation during the first 5 h, and the degradation is not accompanied by the formation of unsaturated uronic acid derivatives, as shown by the thiobarbituric acid reaction. All phloroglucinol was destroyed by oxidation or polymerization after approximately 5 h, and the rate of degradation and formation of derivatives giving colour by thiobarbituric acid became normal. The mechanism of the rapid degradation in the presence of phenols is thus different from the usual mechanism of thermal degradation in the slightly alkaline medium.

We have found that a number of reducing substances, such as hydroquinone, sodium sulphite, sodium hydrogen sul-

phide, cystein, ascorbic acid, hydrazine sulphate and leuco-methylene blue have the effect of increasing the rate of degradation of the alginate. We have also observed that the presence of ascorbic acid increases the degradation of pectin and carrageenin. In a patent concerned with the use of alginate in well-drilling<sup>4</sup>, the use of hydrazine sulphate to reduce the viscosity of the alginate solution is described. Recently, ascorbic acid has been shown to degrade deoxyribonucleic acid<sup>5</sup>. Work is in progress in this laboratory to further elucidate the influence of reducing substances on the degradation of polysaccharides.

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### On the Solubility of Sulfated Galactosaminoglycans (Chondroitinsulfates)

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As shown by Scott<sup>1</sup> and Stacey and Baker<sup>2</sup> acidic polysaccharides form insoluble complexes with quaternary ammonium ions. Scott showed that these complexes are soluble in salt solutions and that different salt concentrations were required to dissolve different complexes. Several approaches for the separation of polysaccharides based on these circumstances have been published (for references see Scott<sup>3</sup>). These complexes are also soluble in organic solvents<sup>4-5</sup>.

Working with the development of methods for the separation of connective tissue polysaccharides we investigated the solubility of the complexes formed between cetylpyridinium ions and three isomeric galactosaminoglycans, chondroitin-4-sulfate (CS-4) (earlier called chondroitin-sulfate A), chondroitin-6-sulfate (CS-6) (earlier called chondroitin-sulfate C), and dermatan-sulfate (DS) (earlier called chondroitin-sulfate B or  $\beta$ -heparin). (For nomenclature see Jeanloz<sup>6</sup>). These substances differ in the following respect: The uronic acid in CS-4 and CS-6 is glucuronic acid while DS contains idouronic acid, CS-4 and DS have the sulfate group attached to carbon atom 4 in the amino sugar moiety, while CS-6 has its sulfate in position 6.

It has been shown<sup>7</sup> that the  $pK_a$  for the glucuronic acid in CS-4 is around 3 while the corresponding value for the idouronic acid in DS is 3.6. It could thus be possible that the CP-complexes of CS-4 and DS might behave different in acidic solutions<sup>8</sup>. It has been shown<sup>9</sup> that in salt solutions containing 0.1 M acetic acid the solubility of the CP-complexes of the two polysaccharides are different. An attempt to separate them was, however, unsuccessful. Later, however, it was shown that the DS preparation used<sup>10</sup> contained as an impurity CS-4 (and probably CS-6).

Differences also exist in the solubility of the CP-complexes in organic solvents. CS-6 and DS seem to behave nearly similarly while CS-4 shows different properties. After trying several systems, it was found that in a mixture of propanol 40 % v/v, methanol 20 % v/v, glacial acetic acid 1.5 % v/v, and water 38.5 % v/v and containing 0.4 % w/v of cetylpyridinium chloride (CPC) the CP-complex of CS-4 can be completely dissolved while the complexes of the other two polysaccharides are insoluble at room temperature. Based on this information the following experiment was made: 20  $\mu$ g of the different polysaccharides (CS-4 from cartilage, CS-6 and DS, the two last mentioned obtained by the courtesy of Dr. K. Meyer, Columbia University, New York) were applied separately to micro columns packed with cellulose and prepared as described earlier<sup>11,12</sup>. To a fourth column prepared in the same way a mixture of 20  $\mu$ g of each polysaccharide was added. The columns were eluted with the following solvents. (1) 1 % CPC in water. (2) 0.3 M NaCl in water. (3) propanol 40 %, methanol 20 %, glacial acetic acid 1.5 %, water 38.5 %, containing 0.4 % CPC. (4) 0.75 M MgCl<sub>2</sub> in 0.1 M acetic

Table 1.

Polysaccharide added	Amount of polysaccharide as $\mu$ g hexosamine eluted by solvent No.		
	3	4	5
CS-4	6.1	0.2	—
CS-6	0.3	5.0	—
DS	—	0.3	5.2
Mixture	6.1	7.0	6.0

Solvents 1 and 2 contained no polysaccharides.

acid. (5) 0.75 M NaCl. All salt solutions contained 0.05 % CPC. Between solvents 2 and 3, solvents 3 and 4, as well as between solvents 4 and 5, the column was eluted with 0.05 % CPC in water in order to remove any solvent left from the previous step. The fractions were analysed and the amount of polysaccharide expressed as  $\mu$ g hexosamine as described earlier<sup>9,11,12</sup>. The result is shown in Table 1. A clear cut separation is obtained. When the experiment is repeated with polysaccharides obtained from other sources similar results are obtained.

In order to test the fractionation principle on polysaccharide fractions obtained from tissues, some experiments on the macro-scale were carried out. The columns used were prepared as described by Antonopoulos *et al.*<sup>9</sup> When a galactosaminoglycan fraction obtained from human aorta from which all glucosaminoglycans had previously been removed, was used, no material which could be eluted with the mixture of organic solvent was obtained. The fraction eluted with 0.75 M MgCl<sub>2</sub> in 0.1 M acetic acid gave an infrared spectrum in agreement with that for CS-6 as reported by Matthews<sup>13</sup>. The fraction eluted with 0.75 M MgCl<sub>2</sub> in water gave an infrared spectrum consistent with DS. Analyses of uronic acids by Dische's carbazol method<sup>14</sup> and hexosamines by the Elson and Morgan procedure as modified by Blix<sup>15</sup> gave a ratio also in agreement with the polysaccharide mentioned. This finding is in agreement with earlier findings that human aorta contains CS-6 and dermatansulfate<sup>16</sup>. A similar analysis made on tracheal cartilage showed that the fraction eluted with the organic solvent consisted of CS-4 while that eluted with 0.75 M MgCl<sub>2</sub> in 0.1 M acetic consisted of CS-6 no material was eluted in the subsequent step.

The solubility of the CS-4 and CS-6 complexes in the propanol, methanol, acetic acid

mixture was also dependent on the temperature. At about 5°C both complexes are insoluble, at 20°–30°C only the CS-4 complexes are soluble and at 65°C both complexes are soluble. On cooling the CS-6 complex first precipitates and the CS-4 complex does not precipitate until the temperature is lowered below 10°C. Another factor of importance for the solubility of the CP-complexes is the presence of salt in the solution. Concentrations of salts ( $\text{Na}_2\text{SO}_4$  or  $\text{MgCl}_2$ ) necessary to dissolve the CP-hyaluronic acid complex in water are sufficient to dissolve the CP-CS-6 complex in the mixture of organic solvents used in this investigation.

A detailed description of the technique for separations on the macro- as well as on the micro-scale is to be published elsewhere.

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## The Identification of Organic Compounds

### III. Preparation of *p*-Phenylphenacyl Oxalate

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Drake and Bronitsky<sup>1</sup> introduced *p*-phenylphenacyl bromide as a reagent for the identification of carboxylic acids. The ester was prepared by refluxing the sodium salt of the acid with *p*-phenylphenacyl bromide in alcoholic solution. The ester of oxalic acid could not be prepared in this way due to the insolubility of sodium oxalate. By using the methylammonium salt, however, Drake and Bronitsky obtained the oxalate, m.p. 165.5° (decomp.).

In a previous paper<sup>2</sup> an improved method for the preparation of *p*-bromophenacyl oxalate was given. This procedure has now been used for the preparation of *p*-phenylphenacyl oxalate. Sodium oxalate and *p*-phenylphenacyl bromide were refluxed in methyl cellosolve. A derivative with m.p. 246° (decomp.) was obtained and the analytical data confirmed that it was the diester of oxalic acid. Obviously a side reaction has taken place by using the methylammonium salt. We have not been able to obtain a compound with a constant melting point of about 165° so the structure of the compound prepared by Drake and Bronitsky still remains to be elucidated.

*Experimental.* Our procedure for the preparation of *p*-bromophenacyl oxalate<sup>2, p. 641</sup> was followed. 0.25 g of oxalic acid dihydrate and 1 g of *p*-phenylphenacyl bromide were used. Yield about 45%. Usually a pure derivative was obtained; it can be recrystallised from glacial acetic acid (sparingly soluble). M.p. 246° (decomp.) (corr.). Capillary tube introduced at 225–230°, rate of heating 4°/min. (Found: C 75.08; H 4.55. Calc. for  $\text{C}_{30}\text{H}_{22}\text{O}_6$ : C 75.30; H 4.63).

Microanalysis by Mr. A. Bernhardt, Max-Planck-Institut, Mülheim, Germany.

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