ride was found to give less than 2% of *m*-isomer, further illustrating the strong *o*-*p*-directing effect of negatively substituted vinyl groups.

2. The mean value for the ratio of rate constants, *k*(cinnamic acid)/*k*(benzene), determined by the competitive nitration of cinnamic acid and benzene was found to be 0.111. This ratio is of the same order as that obtained by Bird and Ingold[10] for the competitive nitration of chlorobenzene and benzene.

3. The *o*-*p*-orienting effect of negatively substituted vinyl groups has been accounted for by the assumption that resonance stabilization in the transition state, involving the electrons of the olefinic bond, is the determining factor.

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[CONTRIBUTION FROM THE BIOCHEMISTRY DEPARTMENT, ABBOTT RESEARCH LABORATORIES]

Polysaccharide Acid Esters as Cation Exchange Media

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In the development of a method for the purification of histamine[1] there arose a need for a cation exchange medium with properties not possessed by the commercially available cation exchangers. It was found that if cotton were *partially esterified* with succinic acid the acid ester so produced would function very nicely as a cation exchanger. The preparation of acid esters by the partial esterification of cellulose and other polysaccharides was then studied further with a view toward a more general use of these materials as cation exchange media.

To this end we have partially esterified cotton with glutaric, maleic, phthalic, and succinic acids and wood cellulose and starch with succinic acid.

Experimental

Esterification of Polysaccharides.—Two methods of esterification were used: (1) The acid anhydride, 0.2 mole, was dissolved in 300 ml. of pyridine. Ten grams of polysaccharide was suspended in this solution and the temperature was maintained at 60° for eighteen to twenty-four hours. The insoluble product was washed successively with water, dilute hydrochloric acid, water and alcohol and then dried *in vacuo;* (2) the acid anhydride, 0.2 to 0.4 mole, and 10 g. of anhydrous sodium acetate were dissolved in 300 ml. of glacial acetic acid. Ten grams of polysaccharide was introduced and the temperature was maintained at 100° for forty-eight hours. The product was washed with dilute hydrochloric acid, water and alcohol and then dried. The latter method is preferable for most purposes as it produces little or no obvious changes in the gross structure of the materials esterified. Satisfactory preparations of cotton acid maleate could be made only by this method. Starch was esterified in this way. During the esterification of wood cellulose and starch the reaction mixture was stirred continuously.

Determination of Cation Binding Capacity and Degree of Esterification.—Free carboxyl groups were determined by shaking a given amount of polysaccharide acid ester with an excess of 0.05 *M* sodium carbonate for fifteen to twenty minutes and titrating an aliquot of the solution for either total sodium or carbonate sodium. In this determination the original concentration of the second sodium ion of sodium carbonate should exceed the sodium ion binding capacity of the acid ester. The values so obtained agreed closely with the values for free carboxyl groups obtained by electrometric titrations. Direct titration with sodium hydroxide in the presence o an indicator was less reliable.

The degree of esterification was calculated from the determination of free carboxyl groups on the assumption that the number of ester linkages was equal to the number of free carboxyls.

Results and Discussion

The esterification of polysaccharides with dicarboxy acids is not new. This was perhaps first claimed by Levy,[2] and since then such esters have been covered in numerous patents.

In nearly all previous reports the polysaccharides were almost completely esterified and the products were soluble in organic solvents as the free acids or in water as the sodium salts. To our knowledge there is no previous report of the partial esterification of polysaccharides with dicarboxy acids for the preparation of cation exchange media. By effecting only partial esterification of the polysaccharides under conditions which minimize changes in mechanical structure, one can obtain products with physical properties which are very desirable for many cation exchange procedures. These partially esterified products do not jel in organic solvents as the free acids or in water as the sodium salts. In appearance they resemble the original polysaccharides very closely. The fibers of cotton acid esters seem more wiry and more brittle than cotton. The starch acid esters have less affinity for water than does the original starch.

Table I shows some representative values for the degree of esterification and the sodium ion binding capacity of the polysaccharide acid esters. The degree of esterification has a rather wide range. As many as one-fourth of the available hydroxyls of cotton have been esterified without noticeable breakdown of the fibers; with starch nearly half of the available hydroxyls were esterified and the product did not jel in water or alcohol either as the sodium salt or as the free acid. The degree of esterification can be controlled within reasonable limits.

The polysaccharide acid esters participate

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[1] F. C. McIntire, L. W. Roth and J. L. Shaw, *J. Biol. Chem.*, **170**, 537 (1947).

[2] H. A. Levy, *Ind. Eng. Chem.*, **12**, 743 (1920).

Table I

Sodium Ion Binding Capacity and Degree of Esterification of Typical Polysaccharide Acid Ester Preparations

Acid ester	Sodium ion binding capacity, milliequivalents per gram	Available hydroxyls esterified, %
Cotton succinate	3.15	25
Cotton maleate	1.13	6.9
Cotton glutarate	0.80	4.8
Cotton phthalate	1.7	12.3
Starch succinate	4.9	52

rapidly in cation exchange reactions. Cations can be removed from them completely by washing with an equivalent (or slightly more) of dilute hydrochloric acid. For example: 5 ml. of a solution containing 0.4 mg. of histamine free base in 1.4×10^{-3} *M* trisodium phosphate (*p*H about 12) was filtered through 100 mg. of cotton acid succinate (sodium ion binding capacity 0.32 milliequivalent). The histamine was removed quantitatively from solution by the cotton acid succinate and then was removed completely from the cotton succinate by washing with 1 ml. of 0.4 *N* hydrochloric acid followed by 2 ml. of water.

The estimation of the acidic strength of the polysaccharide acid esters was approached in two ways:

(1) Measurements of *p*H were made on the aqueous phase at equilibrium (0.1 g. of polysaccharide acid ester in equilibrium with 10 ml. of water) after the polysaccharide acid ester had been half neutralized with sodium hydroxide. The *pKa* values so obtained are shown in Table II.

(2) The extent to which the polysaccharide acid esters would remove sodium ion from a sodium acetate solution was determined as follows: A quantity of each acid ester having 0.5 milliequivalent of free carboxyl group was suspended in 10 ml. of 0.05 *M* aqueous sodium acetate. One-half hour of shaking or mixing was allowed for the system to come to equilibrium. Duplicate 3-ml. samples of the aqueous phase were removed and the free acetic acid was determined by titration with 0.1 *N* sodium hydroxide. This titration may be considered a direct measure of the sodium ions removed from the sodium acetate solution by the polysaccharide acid ester. If it can be assumed that the acetate ion and the acid esters actually compete for sodium ion and that the distribution of sodium ion between the acetate ion and the polysaccharide acid esters will be dependent upon the strengths of the acids, these values should give an indication of the acidic strengths of the polysaccharide acid esters relative to acetic acid. In Table II the *pKa* values are shown along with the per cent. sodium ion bound by the polysaccharide acid esters at equilibrium, when the acid ester carboxyl, acetate ion, and sodium ion were present in equivalent amounts. That the polysaccharide acid esters are as weakly acidic as the *pKa* values indicate is difficult to believe. The data on competition with acetate ion are more in accord with expectations. The significance of the great quantitative differences between these two types of data is a matter for future consideration.

Table II

The Acidic Strength of Polysaccharide Acid Esters

Acid ester	*pKa*	Na p. e.[a]/Total Na, %
Cotton glutarate	8.8	12
Cotton succinate	7.8	16.8
Starch succinate	7.5	18
Cotton maleate	5.7	40.4
Cotton phthalate	5.6	40.6

[a] Na p. e. = sodium bound by the polysaccharide acid ester.

The usefulness of the polysaccharide acid ester cation exchangers as compared to the ion exchange resins[3] is yet to be determined. Cations can be eluted with dilute solutions of strong acids much more readily from the acid esters than from the sulfonic acid exchange resins. A comparison with carboxy exchange resins has not been attempted. One attractive feature of the polysaccharide acid esters is the availability of exchangers of different acidic strengths. The acid esters have a high ion-binding capacity as compared to the carboxy resins available at present, and their mechanical properties are particularly suited for semi-micro or micro operations. It is likely that other polysaccharide acid esters may be produced which are either weaker or stronger acids than those reported here. The polysaccharide acid esters have one serious limitation to their use, *i. e.*, their ease of saponification. This fact prevents their extensive use in systems where an excess of strong bases may be encountered.

Summary

A series of new cation exchange media, the polysaccharide acid esters, have been reported. Their preparation and properties have been described.

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[3] Robert J. Myers, "Advances in Colloid Science," Interscience Publishers, Inc., New York, 1942, Vol. I, pp. 312–351.