

other, and once this occurs further catalyst addition does not change the viscosity of either solution. Rapid degradation can be achieved by addition of large amounts of catalyst, whereas the most effective and rapid way of increasing the over-all solution viscosity of monomeric solutions is by successive additions of *very* small amounts of catalyst.

It must be noted that in all cases the viscosity of the entire solution is measured including possible unreacted monomer.

Acknowledgment.—We wish to thank Dr. H. S. Taylor of Princeton University for many useful discussions and suggestions.

Summary

The existence of simultaneous polymerization and degradation in toluene solutions of styrene and polystyrene has been demonstrated. Steady states resulting from the competition of these reactions have been observed.

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RECEIVED FEBRUARY 10, 1945

[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY¹]

The Acylation of Pectin

BY J. F. CARSON, JR., AND W. D. MACLAY

This paper describes the preparation of a series of esters of citrus pectin. These derivatives were prepared for a further study of the physical chemistry of pectin and as possibly useful materials for determinations of molecular weight and molecular size distributions. Because of the incomplete solubility of the esters in organic solvents, no viscosity or osmotic pressure measurements are reported. The method used, namely, esterification with an acid anhydride or acid chloride in the presence of excess pyridine, has not heretofore been applied successfully to pectin.

Schneider and co-workers,² who attempted the direct acetylation of purified citrus pectin, described their products as poorly soluble and degraded. Their reactions were carried out by swelling pectin in acetic acid, followed by esterification at 20–40° with acetic anhydride in the presence of various catalysts such as sulfuric acid, perchloric acid, zinc chloride and pyridine in 1% concentration. These investigators prepared the diformate and diacetate of pectin by the reaction of formic acid and acetic anhydride, respectively, on the previously prepared pectin dinitrate. These esters were soluble in acetone, but always contained residual nitrogen and were actually mixed nitrate-formate and nitrate-acetate esters.

The technique used for the esterification of pectin consists in preparing a pectin-water paste, partially dehydrating the pectin with acetone, and gradually replacing the water and acetone with pyridine to yield the pectin in a highly swollen gelatinous state. As an alternative method, freshly precipitated pectin can be swollen directly in pyridine. In this form, pectin is readily esterified by lower fatty acid anhydrides in excess pyridine. Of all esterification procedures, the pyridine-acid anhydride method is considered

to be the mildest and least degrading on polysaccharides, as studies with cellulose^{3a} and starch^{3b} have shown. The extent of degradation occurring in the acylation of pectin by this procedure was not determined. It has been established, however, that pyridine itself under the same conditions of temperature as used in the esterification reactions has no appreciable degrading action, as shown by viscosity measurements and methoxyl analyses on pectin recovered from its pyridine gel.

Pectin diacetate, dipropionate, and dibutyrate were obtained by the reaction of citrus pectin with the corresponding acid anhydride in pyridine at 45°. Esterification was usually 90–95% complete in three to five hours and, by esterifying a second time at room temperature, esters were obtained with acyl contents very close to the theoretical values calculated for a diester of a partially methylated polygalacturonide.⁴ Repeated esterification in pyridine failed to increase the acyl content beyond the diester stage. The reaction proceeds apparently without loss of methyl ester groups. Pectin diacetate prepared in this way is insoluble in water and has a low solubility in organic solvents. It is partially soluble in chloroform, tetrachloroethane, pyridine, and acetic acid, but is insoluble in ethanol, acetone, ether, and benzene. The dipropionate and dibutyrate

(3) (a) Staudinger and Daumiller, *Ann.*, **529**, 219 (1937); (b) Staudinger and Husemann, *ibid.*, **527**, 195 (1937); Mullen and Pacsu, *Ind. Eng. Chem.*, **34**, 1209 (1942).

(4) Analyses for methoxyl and uronic anhydride indicate that 90% of the molecule can be accounted for as polygalacturonide. The 10% of non-galacturonide material probably consists largely of araban and galactan as shown by Beaven, Hirst and Jones, *J. Chem. Soc.*, 1867 (1939). Although the presence of an araban diester would increase the acyl analyses only slightly, the presence of appreciable quantities of galactan triester should yield an acyl content significantly higher than the actual values found. Because of uncertainty as to the relative quantities of araban, galactan, and non-carbohydrate materials in the non-galacturonide fraction, the actual percentages of acyl found were compared with the theoretical values for a diester of a pure polygalacturonide as explained in Table I, footnote (a).

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Schneider and co-workers, *Ber.*, **69**, 309, 2530 (1936); **71**, 1353 (1938).

exhibit greater solubility in organic solvents, being completely soluble in pyridine, partially soluble in acetone, methyl ethyl ketone, dioxane, chloroform, and benzene, but insoluble in ethanol and aliphatic hydrocarbons. Approximately 17% of the dipropionate and 13% of the dibutyrate were insoluble in methyl ethyl ketone.

The cause of this type of fractionation has not been ascertained. Analyses of the soluble and insoluble fractions of the dipropionate gave the following results, respectively: % propionyl 38.3, 38.0 (theor. 38.4%); % methoxyl 6.96, 6.64 (theor. 6.40%); % uronic anhydride 52.8, 52.3 (theor. 52.8%); % ash 0.10, 1.5. With the exception of a difference in ash content and a small but significant difference in methoxyl content, the two fractions gave essentially the same analytical results. That the fractionation is probably not a result of salt formation follows from the fact that a dipropionate prepared from a low-ash (0.18%) pectin gave approximately the same percentage of insoluble fraction as the dipropionate from a pectin having an ash content of 0.8%. Pectin dipropionate and dibutyrate, on evaporation of their solutions, form films of moderate strength and flexibility. Films of the diacetate are very weak and fragile. The pectin esters all gradually dissolve in aqueous alkali and are hydrolyzed.

Pectin laurate, myristate, and palmitate were prepared by reaction of pectin with the corresponding acid chloride in pyridine. These esters had acyl contents indicating esterification of approximately 1.0 to 1.6 hydroxyl groups per anhydrogalacturonic acid unit. Repeated esterification with large excesses of acid chloride increased the acyl contents only slightly, and the esters usually had methoxyl contents somewhat lower than the calculated values, indicating some hydrolysis of methyl ester during the reaction. Pectin laurate, myristate, and palmitate prepared in this way were soft waxy solids, partially soluble in ether, acetone, dioxane, benzene and other fat solvents. On evaporation of their solutions, the esters yielded soft tacky films.

A benzoate of pectin was prepared by the reaction with benzoyl chloride and pyridine. This derivative contained approximately 1.6 benzoyl groups per repeating unit and was a horny resinous material almost completely insoluble in organic solvents, swelling only slightly in pyridine or *m*-cresol. Analytical data on the new pectin esters are recorded in Table I.

Experimental

The pectin employed in the acylations was a commercial product obtained from lemon peel and isolated by the aluminum hydroxide precipitation method. It was 175 grade, had an intrinsic viscosity of 3.9 (*pH* of 6 in 0.9% sodium chloride), and had the composition: moisture, 10.3%; ash, 0.8%; methoxyl, 10.3% (Zeisel); uronic anhydride, 84.7%.

Pretreatment of Pectin.—The following pretreatment is typical of all experiments and is described in detail. Forty

TABLE I
ANALYTICAL DATA ON PECTIN ESTERS

Ester	Calcd. ^a	% Acyl Found	Degree of esterification		Saponification equivalent		
			Calcd.	% Found ^b	Calcd.	% Found ^c	
Diacetate	32.0	32.3 ± 0.4 ^b	2.0	7.07	7.30	89.7	92.8
Dipropionate	38.4	38.7 ± .4 ^b	2.0	6.40	6.83	99.0	101.1
Dibutyrate	43.7	43.4 ± .5 ^b	2.0	5.85	6.13	108.4	110.2
Laurate	66.7	53.1 ^c	1.2	4.62	3.38		
Myristate	69.8	59.2 ^c	1.3	4.04	3.34		
Palmitate	72.2	65.8 ^c	1.6	3.37	3.05		
Benzoate	58.5	50.6 ^c	1.6				

^a The calculated values for acyl and for saponification equivalents were determined for diesters of a partially methylated polygalacturonide, assuming that no methyl ester is hydrolyzed in the reaction. The calculated values for methoxyl for the diacetate, dipropionate and dibutyrate were determined for diesters, and the values for the laurate, myristate and palmitate were calculated for the degree of esterification found. ^b Acetyl, propionyl, and butyryl analyses were determined by a modification of the procedure of Cramer, Gardner and Purves, *Ind. Eng. Chem., Anal. Ed.*, **15**, 319 (1943). The deviation between duplicate analyses by this method was approximately 2.5% of the determined values. ^c The lauroyl, myristoyl, palmitoyl, and benzoyl were determined by cold alcoholic-alkali saponification followed by neutralization with hydrochloric acid, extraction of the fatty acid or benzoic acid with ether or petroleum ether (30–60°), and titration of the free acid. ^d Methoxyl analyses were carried out by E. P. Clark's modification of the Vieböck-Schwappach method, *J. Assoc. Off. Agr. Chem.*, **15**, 136 (1932); **22**, 100, 622 (1939). That the methoxyl values were not influenced by alcohol adsorbed on the esters has been shown by the following experiments: A sample of pectin propionate (% methoxyl = 6.64), after humidification over water for one week followed by drying at 50° over phosphorus pentoxide *in vacuo*, had a methoxyl content of 6.60%. Similarly the propionate after humidification over acetone for a week, followed by drying *in vacuo* (50°), had a methoxyl content of 6.64%. Samples of pectin acetate after precipitation from chloroform into ether, and pectin propionate and butyrate after precipitation from methyl ethyl ketone into ether, showed no change in methoxyl content. ^e Saponification equivalents were determined by the Eberstadt method, Genung and Mallatt, *Ind. Eng. Chem., Anal. Ed.*, **13**, 369 (1941).

grams of air-dry powdered pectin (10.3% moisture) was covered with 50 ml. of 95% ethanol. Approximately 300 ml. of water was stirred in rapidly and the mass triturated to a stiff paste. Five hundred ml. of acetone was stirred into the paste, the large lumps were broken into small pieces, and the suspension was allowed to stand for thirty minutes. The pectin was recovered by pressing through cheesecloth, after which it was transferred to four 250-ml. centrifuge bottles. To each bottle there was added 150 ml. of pyridine. The mixture was stirred for a few minutes and allowed to stand for two or three hours; then excess pyridine was removed by centrifuging. The whole process of steeping in pyridine and centrifuging was repeated three times to yield pectin in a highly swollen reactive form suitable for esterification.

Pectin Dipropionate.—Preparation of pectin dipropionate will be described in detail; the procedures for the diacetate and dibutyrate varied only in the method of isolation. Forty grams of air-dry pectin, pretreated as described, reacted with 650 g. of pyridine and 260 g. (2.0 moles) of propionic anhydride for three hours at 45 ± 3°. The reaction was carried out with vigorous stirring and the anhydride was added in two portions: 100 g. at the start and the remainder at the end of the first hour. The clear viscous solution was cooled to 20° and poured in a fine stream into three liters of ice water, whereupon the ester precipitated as a white fibrous material. The product was isolated by filtration and purified by successive washings with 3%

hydrochloric acid, distilled water, 95% ethanol, and ether. After drying at 50° *in vacuo* for forty-eight hours over phosphorus pentoxide, the ester weighed 53.0 g. (94% yield) and had the composition: % methoxyl calcd., 6.40; found, 6.85; % propionyl calcd., 38.4; found, 37.3; sapon. equiv. calcd., 99.0; found, 101.7.

In a further esterification, 47 g. of ester, 1000 ml. of pyridine, and 35 g. of propionic anhydride were shaken together for forty-eight hours at 20–24°. The pectin dipropionate was isolated and purified as before and had the composition: % methoxyl, 6.85; % propionyl, 38.6; sapon. equiv., 101.1; ash, 0.33%. A third esterification in which the ester was shaken for one week with pyridine and propionic anhydride gave a product with the propionyl content essentially unchanged, % propionyl 38.7; % methoxyl, 6.83.

Pectin Diacetate and Dibutyrate.—These esters were prepared by the reaction of pectin with the corresponding acid anhydride in pyridine under conditions essentially the same as for the dipropionate with the same molar proportions of reactants at 45–50° for three to five hours. The products were precipitated as fine flocs by pouring the reaction mixture into cold 3% aqueous hydrochloric acid. These esters were isolated by centrifuging and were purified by washing successively with 3% hydrochloric acid, distilled water, 95% ethanol, and ether. To ensure maximum acylations the esters were usually acylated a second time by shaking a solution of the ester in pyridine and acid anhydride for two days at room temperature as described in the preparation of pectin dipropionate.

Pectin Laurate, Myristate and Palmitate.—These esters were prepared from acid chlorides made by the procedure of Ralston⁵ from Armour Neo-Fats. The preparation of a lauroyl ester of pectin is typical. Twenty grams of air-dry pectin, pretreated as described, reacted with 700 g. of pyridine and 200 g. of lauroyl chloride in a two-liter, three-neck flask. The acid chloride was added in small quantities throughout the course of the reaction. The reaction mixture was stirred vigorously for five hours at 55–60° and allowed to stand overnight. When the thick viscous mixture was poured into four liters of 3% hydrochloric acid, the ester and unreacted lauric acid coagulated at the surface as a brown sticky mass, which was stirred until coherent enough to be transferred into two liters of 95% ethanol. The alcohol was decanted and the partially

granular product was washed repeatedly with 95% ethanol acidified with hydrochloric acid. It was purified by dispersion in 600 ml. of hot dioxane, followed by precipitation into two liters of absolute ethanol. A second purification in this manner yielded 34 g. of a pale-amber, waxy product containing no free lauric acid and having a lauroyl content of 49.4%, equivalent to 1.0 lauroyl group per repeating unit.

A second esterification in which 30 g. of this pectin laurate, 500 g. of pyridine, and 30 g. of lauroyl chloride were shaken together for two days at room temperature yielded 34 g. of ester having a lauroyl content of 53.1%, indicating a degree of esterification of 1.2 and a methoxyl content of 3.38%. The theoretical methoxyl content for this degree of esterification is 4.62%.

Pectin Benzoate.—Pectin was benzoylated by shaking a suspension of 15 g. of pectin in 600 g. of pyridine and 90 g. of benzoyl chloride for 36 hours at 20°. The mixture was poured into two liters of 95% ethanol and the hard resinous product was recovered by filtration. It was washed repeatedly with hot distilled water, hot 95% ethanol, acetone, and finally petroleum ether (30–60°). The ester was a hard amber-colored resinous material with a benzoyl content of 50.6%, indicating a degree of esterification of approximately 1.6 benzoyl groups per repeating unit. Methoxyl determinations gave inconsistent values, probably because of the insolubility of the ester in the Zeisel reagent.

The authors wish to thank R. M. McCready for uronic anhydride determinations.

Summary

1. A technique is described for the esterification of pectin.
2. Pectin diacetate, dipropionate and dibutyrate were prepared by esterification of citrus pectin with the acid anhydride in pyridine.
3. Lauroyl, myristoyl, palmitoyl, and benzoyl esters of citrus pectin have been prepared by esterification of pectin with the corresponding acid chloride in pyridine; the degree of esterification attained varied from 1.2 to 1.6 acid groups per anhydrogalacturonic acid unit.

ALBANY, CALIFORNIA

RECEIVED FEBRUARY 12, 1945

(5) Ralston, *THIS JOURNAL*, **61**, 1019 (1939).

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

Diethylstilbestrol and its Monomethyl Ether

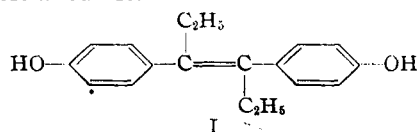
BY A. L. WILDS AND WARREN R. BIGGERSTAFF

The brilliant and fruitful investigations of Dodds and co-workers on synthetic compounds possessing the physiological action of the female sex hormone estrone were brought to a climax in 1938 by the discovery of diethylstilbestrol (I).¹ This compound was found to be of the same order of potency as estradiol, the most active of the naturally occurring hormones. Since 1938 much work has been done with the aim of improving the original synthesis of diethylstilbestrol reported by Dodds, Golberg, Lawson and Robinson and to develop other methods suitable for its commercial preparation.² Of the new approaches,

(1) Dodds, Golberg, Lawson and Robinson, *Nature*, **141**, 247 (1938); *Proc. Roy. Soc. (London)*, **B127**, 140 (1939).

(2) This work has been reviewed recently by Jones, "Annual Reports on the Progress of Chemistry," 1943, p. 137.

that of Kharasch and Kleiman³ is outstanding since it is reported to give diethylstilbestrol in 19 to 22% yield by a two-step process from readily available anethole.



In connection with the synthesis of certain related compounds, on which we hope to report at a later date, we were interested in further improvements in the original diethylstilbestrol synthesis of Dodds, *et al.*,¹ and particularly in modifications which would be suitable for preparing stilbenes

(3) Kharasch and Kleiman, *THIS JOURNAL*, **65**, 11 (1943).