at 40° for one hundred minutes, and then acidified with hydrochloric acid. After standing in the ice box overnight, the product was filtered, washed with water, and dried in air. Yield of derivative was 0.59 g., dry basis; negative Millon test; N, 4.69%. Other products prepared in similar fashion at 0° or room temperature had higher nitrogen contents (5.1–5.9%) and gave positive Millon tests. Treatment of dry I with mixtures of anhydrous pyridine and acetic anhydride had no effect.

Acetylation attempts under acidic conditions were also made. Treatment with acetic anhydride for 1.5 hours at 110°, or with a mixture of acetic acid and acetic anhydride had little effect. Addition of concentrated sulfuric acid brought about reaction as follows: I (0.34 g.) was treated with 2.5 cc. of acetic anhydride and 2 drops of concentrated sulfuric acid. The mixture warmed up, and further warming brought about complete solution. On cooling to room temperature, precipitation occurred, and the mixture was poured into water with stirring, filtered, washed thoroughly, and dried in air. The yield of the product, a yellow powder, was 0.45 g., dry basis; negative Millon test; N, 4.71%.

Attempts to Prepare other Derivatives.—Inorganic salts could be prepared by the addition of neutral salts to aqueous solutions of I at *p*H 7–8. Barium, copper, and mercury salts precipitated immediately. Numerous preparations of the barium salt ranged from 11.2 to 15.1% in barium content. A picrate was also made, but it was amorphous, and X-ray patterns indicated no crystallinity. Methylated derivatives were of no value for analytical purposes.

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Summary

1. The reaction of tyrosine with two moles of formaldehyde under alkaline conditions has been studied by determinations of (a) optical rotation and (b) the decrease in concentration of free formaldehyde of the reaction mixture.

2. Analytical data indicate that two moles of formaldehyde reacted with one mole of tyrosine, with the elimination of one mole of water.

3. None of the formaldehyde combined in the reaction product could be liberated by acid hydrolysis.

4. The behavior of the reaction product was indicative of a polymeric substance.

5. The amorphous reaction product has been characterized.

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[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY[1]]

The Acylation of Polyuronides with Formamide as a Dispersing Agent

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

In connection with investigations of derivatives of pectic materials, a technique was required for the acylation of pectic acids. Attempts to acylate pectic and alginic acids in the past have been unsuccessful, probably because of the difficulty of keeping the polysaccharide in a reactive swollen state during esterification and because the physical properties of the lower esters, particularly the acetates, make their isolation laborious. A method[2] has recently been described for the acylation of pectin, in which the pectin, after suitable swelling in water, followed by a gradual replacement of the water by pyridine, is readily esterified by an acid anhydride or an acid chloride; however, this procedure was unsatisfactory when applied to pectic or alginic acids. Barry, *et al.*,[3] have experienced difficulty in acetylating alginic acid, but were able to obtain acetates by reaction with acetic anhydride after treatment with hydriodic acid. Their products were highly degraded and the analytical results are difficult to interpret.[4] Hirst[5] and co-workers were unable to acetylate alginic acid by several conventional methods and reported that the procedure of Barry, *et al.*, gave highly degraded esters.

In the present technique, the polyuronide was dispersed in formamide to a smooth paste or a viscous sol. The quantity of formamide required varies with the polysaccharide and is governed by the viscosity of the dispersion. Esterification was performed by the addition of pyridine to the formamide paste, followed by the gradual addition of the appropriate acid anhydride.

Experimental

This method of acylation can be illustrated by the procedure for the preparation of pectic acid propionate which is typical for the acylation of pectic and alginic acids and gums tragacanth and arabic, and that for the acetylation of white potato starch.

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Pectic Acid Propionate.—Thirty-five grams of enzymic pectic acid no. 1 (dried *in vacuo* at 60° for eighteen hours) was added in small quantities to 400 g. of formamide at 50° in a 2-liter, 3-necked flask equipped with a thermometer, separatory funnel and a stainless steel stirrer. The suspension was stirred for one hour at 45–50°, during which time the pectic acid became completely dispersed to a stiff paste. Pyridine (300 g.) was added in small portions over a period of thirty minutes with vigorous stirring at 45–50° and the mixture cooled to 30°. Two hundred grams of propionic anhydride was added in portions of 50 g. each hour and the reaction mixture was stirred for five hours at 30° and allowed to stand overnight at room temperature. The thick viscous solution was poured into 5 liters of cold 2% hydrochloric acid containing 500 g. of chopped ice, the ester precipitating as a cream-colored flocculent material. (If a pyridine solution of pectic or alginic acid propionate or butyrate is poured into a neutral precipitating bath, only a partial precipitation occurs, most of the ester remaining in solution.) The precipitated ester was stirred for an hour, isolated by filtration, washed first with cold 0.5% hydrochloric acid, then four times with 250-ml. portions of distilled water, re-suspended in 3 liters of distilled water, allowed to stand overnight and filtered. Pectic acid propionate obtained as a wet pasty material was dried for forty-eight hours *in vacuo* at 35° over solid sodium hydroxide, ground in a Wiley mill to 40 mesh, and redried for analysis forty-eight hours *in vacuo* at 50° over phosphorus pentoxide. The yield was 52 g., 91%. The ester had a propionyl content of 40.2% (calculated for a polygalacturonide dipropionate, 39.6%).

For re-esterification, 40 g. of the ester was dispersed in 500 ml. of pyridine to which was added 75 g. of propionic anhydride, and the reaction solution was allowed to stand for five days at room temperature. The ester was isolated by precipitation into 2000 ml. of Skellysolve B. Pectic acid propionate was filtered, washed several times with petroleum ether, suspended in 2 liters of 1% hydrochloric acid, stirred for several hours, filtered and washed with distilled water until free of chlorides. The ester was partially dried for several days *in vacuo* over solid sodium hydroxide. To obtain the product in a better physical condition for drying, it was dispersed in 500 ml. of dioxane, then poured into 3 volumes of Skellysolve B. The precipitated ester was filtered, washed with petroleum ether, and dried *in vacuo* at 50° over phosphorus pentoxide; per cent. of propionyl, 41.2. A third esterification in the same manner for one week yielded ester essentially unchanged in propionyl content; per cent. propionyl, 41.1.

White Potato Starch Triacetate.—Here the procedure was similar to that above, except that a slightly higher temperature (65°) was required for dispersing the starch which had been previously dried *in vacuo* at 60° over phosphorus pentoxide. The precipitated ester was purified by repeated washing in ethanol to yield the acetate as a white fluffy mass. The completely esterified triacetate with the theoretical acetyl content was obtained by re-esterification in pyridine and acetic anhydride for three days at room temperature. The ester had a nitrogen content of 0.01%.

Acetylation and Propionylation of Formamide.—Several investigators[6] have shown that acid chlorides and in a few cases acid anhydrides may react with amides, resulting in acylation or dehydration to a nitrile. With acetic anhydride and formamide, the dehydration is not appreciable, since Deschamps[7] observed that at refluxing temperatures, formation of hydrogen cyanide proceeded only to the extent of one per cent. The approximate extent of acetylation or propionylation of formamide has been measured under conditions of temperature and relative concentration of reagents approximating the actual conditions of esterification. Solutions containing pyridine, acetic anhydride, and formamide were allowed to stand at 23 and 40°, and at periodic intervals aliquots were decomposed with water and the acid titrated with standard alkali to a phenolphthalein end-point. With acetic anhydride and formamide at 23° at the expiration of six, twenty-four and forty-eight hours, 28, 42, and 50%, respectively, of the original anhydride was consumed, and at 40° after four and six hours, 34 and 40%, respectively, was consumed. With propionic anhydride at 23°, after twenty-four and forty-eight hours, 23 and 31%, respectively, of the anhydride was consumed, and at 40°, after four and six hours, 17 and 23%, respectively, of the original anhydride had reacted.

Materials.—All of the polysaccharides except starch were de-ashed before using by washing with acidified ethanol or by percolation through ion exchangers. The ash contents were all less than 0.8%. **Pectin** was a commercial sample manufactured from lemon peel. **Citrus Pectic Acid no. 1** (**enzymic**) was prepared from fresh lemon peel by the procedure of Baier and Wilson.[8] **Citrus Pectic Acid no. 2** was prepared by cold alkaline de-esterification of lemon pectin. **Apple Pectic Acid** (acid de-esterified) was prepared by the general procedure of Speiser, Eddy and Hills.[9] It had the neutralization equivalent (corrected for alkalinity of ash), 192.2. **Alginic Acid** was a commercial sample originally extracted from *Macrocystis pyrifera*. **White Potato Starch** and the gums **Arabic** and **Tragacanth** were commercial samples.

Results

The various esters prepared and the conditions of esterification are recorded in Table I. Attempted acetylation of various pectic acids and alginic acid yielded esters that were very difficult to isolate because of their tendency to peptize in water and their poor solubility in organic solvents. Propionylation or butyrylation proceeded satisfactorily, although even these esters showed some tendency to become emulsified in water. Propionates and butyrates of the various pectic acids and of alginic acid swelled and dissolved slightly in methanol, ethanol, ether and chloroform. They were incompletely soluble in acetone, ethyl acetate, and pyridine, the insoluble fractions appearing as highly swollen gelatinous lumps. Analytical determinations of the soluble and insoluble fractions failed to reveal an explanation of the incomplete solubility. Gum tragacanth propionate was also found to be incompletely soluble in organic solvents, but gum arabic acetate and propionate were completely soluble in acetone, dioxane and chloroform to give clear solutions of low viscosity. White potato starch triacetate had a solubility behavior confirming the report of Whistler.[10]

The procedure described may be of general application for the acylation of polysaccharides which can be dissolved or gelatinized in formamide. The method possesses certain advantages over previously described techniques, in that no extensive pretreatment is required such as precipitation from aqueous solutions or pastes into alcohol or pyridine, and either an oven-dry

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[9] R. Speiser, C. R. Eddy and C. H. Hills, *J. Phys. Chem.*, **49**, 563 (1945).

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TABLE I

ACYLATION OF POLYSACCHARIDES

Polysaccharide	Viscosity[a]	Percentage		Ester	Polysaccharide, g.	Formamide, g.	Pyridine, g.	Acid anhydride, g.	Time, hr.	Temp., °C.	Percentage acyl		
		Methoxyl[b]	Uron. anhy.[c]								First Found[d]	First Calcd.[e]	Sec. ester
Citrus Pectin	3.0	10.7	81.6	Acetate	16	250	200	100	22	22	29.8	31.8	33.3
				Propionate	35	550	400	200	21	22	37.3	38.1	39.5
				Butyrate	16	175	200	150	6	30	42.8	43.5	45.1
Apple Pectic Acid	1.4	0.2	92.0	Propionate	25	375	300	200	24	23	39.0	39.6	40.1
Citrus Pectic Acid													
No. 1	3.8	.3	82.0	Propionate	35	400	300	200	5, 18	30, 22	40.2	39.6	41.2
				Butyrate	24	350	300	200	6	35	40.7	45.0	47.0
No. 2	2.7	.4	86.0	Propionate	18	360	200	100	22	23	39.4	39.6	40.4
				Butyrate	30	450	450	225	5, 16	30, 23	44.6	45.0	46.5
Alginic Acid	1.3		95.0	Propionate	30	350	200	200	6	30	35.3	39.6	39.0
				Butyrate	30	300	200	200	6, 18	30, 22	44.2	45.0	44.0
Gum Tragacanth				Propionate	18	350	250	200	20	22	44.6	[f]	44.9
Gum Arabic			29.0	Acetate	25	170	300	150	20	23	41.3	[f]	41.3
	0.23		16.7	Propionate	40	250	350	250	6	25	46.9		47.5
White Potato Starch				Acetate	25	250	400	150	5	40	41.9	44.8	44.7

[a] Viscosities were measured at pH 6, in 0.9 per cent. sodium chloride solution. [b] Methoxyl analyses were performed by E. P. Clark's modification of the Vieböck-Schwappach method [*J. Assoc. Off. Agr. Chem.*, **15**, 136 (1932); **22**, 100, 622 (1939)]. All samples were humidified over water to remove adsorbed ethanol and dried *in vacuo* before analysis. [c] Uronic anhydride analyses were performed by a modification of the Lefevre and Tollens method [McCready, Swenson and Maclay, to be published]. [d] 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 starch acetate was also analyzed by the Eberstadt saponification method: Genung and Mallatt, *ibid.*, **13**, 369 (1941). [e] Calculated acyl values for the pectic acid esters are for pure polygalacturonide diesters neglecting the non-uronide content. Theoretical acyl contents of the pectin esters were calculated for partially methylated polygalacturonide, assuming no loss of methyl ester in the esterification. The fact that the values obtained were higher than the calculated may be explained by the presence of non-uronide material, probably arabans or galactans which on esterification would yield the corresponding diester or triester. Theoretical acyl contents of the alginic acid esters were calculated for polymannuronide diesters. [f] Since the exact composition of gum arabic and tragacanth are not known, theoretical ester contents could not be calculated.

or an air-dry sample may be esterified directly. A low reaction temperature may be used, which should minimize degradation. The procedure has some disadvantages, in that esterification is usually not complete in one step and a second esterification is generally necessary to obtain complete acylation. The crude esters as prepared were usually slightly colored, probably as a result of by-product reaction of the formamide. The color could generally be removed by washing with water or whenever possible with ethanol and the purified esters had low nitrogen contents, usually less than 0.1%. The fact that formamide undergoes side reactions limits its usefulness to such relatively mild acylating agents as acid anhydrides. Benzoyl chloride and *p*-toluenesulfonyl chloride attack formamide in pyridine, yielding hydrogen cyanide and resinous material.

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Summary

A new technique has been described for the acylation of polyuronides, in which formamide is used as a solvent or gelatinizing agent prior to esterification with pyridine and an acid anhydride.

By this procedure propionates and butyrates of several pectic acids and of alginic acid have been prepared. Pectin acetate, propionate, and butyrate, gum arabic acetate and propionate, and the propionate of gum tragacanth have been prepared.

The method was found applicable to white potato starch and the starch triacetate was prepared.

The advantages and the limitations of the technique are pointed out and the possibility of wide application to the acylation of polysaccharides is discussed.

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