Other 1-aryl-2-chloropropanes, listed in Table I, were prepared similarly using bromobenzene, fluorobenzene, and anisole in place of chlorobenzene.

1-Chloro-2-(*p*-chlorophenyl)-propane.—The method was adapted from Truffault.[6] To a mixture of 380 g. (*ca.* 4.0 moles) of fuming sulfuric acid (10% oleum) and 337.5 g. (3.0 moles) of chlorobenzene there was added over a two-hour period with stirring and cooling, 153.0 g. (2.0 moles) of allyl chloride. The mixture was stirred overnight at room temperature. The reaction mixture was shaken with several times its volume of crushed ice, washed with water and dried over anhydrous calcium sulfate. The dried product was rectified to strip off lower boiling components. A higher fraction, boiling at 91–99° at 6 mm., was refractionated at 5 mm. pressure. Two distinct materials, boiling at 90.5–92.0° at 5 mm. and 94.0° at 5 mm., respectively, were obtained. The latter compound gave a negative test for active chlorine with alcoholic silver nitrate in the cold. Permanganate oxidation yielded *p*-chlorobenzoic acid, proving that it was 1-chloro-2-(*p*-chlorophenyl)-propane (reported by Truffault). The yield was 6%. The former compound, evidently an isomer (d^{20}_{20} 1.1801, n^{20}_D 1.5404, calculated for $C_9H_{10}Cl_2$, 37.5% Cl, found, 37.4% Cl), was not identified conclusively. It gave a positive test for active chlorine with alcoholic silver nitrate, but failed to yield a solid acid derivative on treatment with permanganate, dichromate, or nitric acid-silver nitrate. It was obtained in 7.5% yield.

Ammonolysis of Chloro Compounds to Primary Amines.—The preparation of benzedrine is typical of the method used for synthesis of the primary amines listed in Table II. Twenty-five grams (0.16 mole) of 2-chloro-1-phenylpropane was dissolved in 450 ml. of saturated alcoholic ammonia solution (125 g./l.) and sealed in an iron-pipe autoclave. The autoclave was mechanically rocked and was heated by an electric resistance winding. The charge was heated at about 160° for nine hours. It was then allowed to cool and filtered. The alcohol and excess ammonia were distilled, and the residue was made strongly basic with 6 *M* sodium hydroxide. The basic mixture was extracted four to five times with 20-ml. portions of benzene, and the aqueous residue was discarded. The benzene solution was next extracted three to four times with 15-ml. portions of 6 *M* hydrochloric acid to secure the amine as a solution of its hydrochloride. The aqueous acid solution was made basic with sodium hydroxide and extracted several times with benzene. The latter benzene solution was dried over anhydrous potassium carbonate. This benzene solution was distilled from a Claisen flask giving 11.1 g. of benzedrine boiling at 80–82° at 11 mm. The yield was 51%.

Ammonolysis of Chloro Compounds to Secondary Methylamines.—The technique for preparing the N-methylamines listed in Table II was identical to that used for synthesis of the primary amines, except that an alcoholic solution of methylamine (204 g. per liter) was used as the aminating agent.

Acknowledgment.—The authors wish to express their appreciation to the Abbott Laboratories for a grant which made this research possible.

Summary

The reaction of allyl chloride and benzene in the presence of ferric chloride to produce 2-chloro-1-phenylpropane, described by Nenitzescu and Isacescu, has been extended to four other aromatic compounds. Total yields of arylchloropropanes were 14 to 53%.

Truffault's method of adding aromatic compounds to allyl chloride in the presence of sulfuric acid was utilized for the preparation of 2-aryl-1-chloropropanes.

Nine arylchloropropanes have been treated with alcoholic ammonia to give 20–51% yields of the corresponding arylpropylamines. Five of the arylchloropropanes yielded 24–62% of the N-methylarylpropylamines on treatment with alcoholic methylamine.

LAFAYETTE, INDIANA RECEIVED DECEMBER 1, 1945

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

A Polymer Reaction Product of Tyrosine and Formaldehyde

BY ALFRED E. BROWN[2]

In a study of modified proteins[3] it was found that when the free amino groups of the protein were apparently completely acylated, the derivatives still had an appreciable formaldehyde-combining capacity as determined by a modification of the method of Nitschmann, *et al.*,[4] which involves acid hydrolysis of the formaldehyde-treated products, and distillation and titration of the formaldehyde. In addition to the acid-labile formaldehyde, it may well be that linkages stable to acid hydrolysis can be formed by proteins and formaldehyde.[5] The possibility that the l(−)-tyrosine[6] side chain is involved in binding formaldehyde has been mentioned.[7] We were interested, therefore, in investigating the manner in which the hydroxyphenyl group of tyrosine might react with formaldehyde as well as in the stability to acid hydrolysis of any linkages which might thus be formed. The reaction with tyrosine itself was first studied, with the expectation of subsequently using N-acylated tyrosine derivatives and peptides of tyrosine. It will be evident that the experimental conditions employed in the present work were different from those ordinarily used to harden proteins with formaldehyde.

Ullmann and Brittner[8] were able to prepare

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[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. Article not copyrighted.

[2] Present address: Milton Harris Associates, Washington, D. C.

[3] Gordon, Brown and McGrory, *Ind. Eng. Chem.*, **38**, 90 (1946).

[4] Nitschmann, Hadorn and Lauener, *Helv. Chim. Acta*, **26**, 1069 (1943); Wood, Swain and Kokes, to be published.

[5] Baudouy, *Compt. rend.*, **214**, 692 (1942).

[6] l(-)-tyrosine was used throughout the investigation so the prefix l(-) will be omitted henceforth.

[7] Carpenter and Lovelace, *Ind. Eng. Chem.*, **36**, 680 (1944); Nitschmann and Hadorn, *Helv. Chim. Acta*, **27**, 299 (1944).

[8] Ullmann and Brittner, *Ber.*, **42**, 2539 (1909).

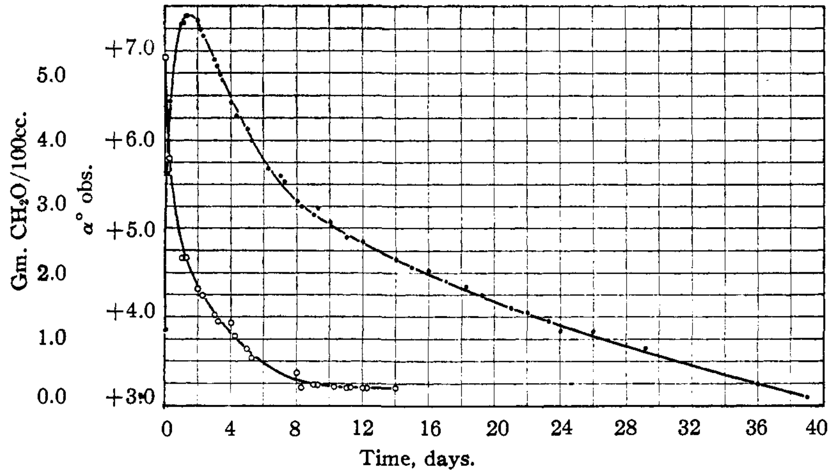

Fig. 1.—Formation of reaction product I as indicated by optical rotation (●) and decrease in free formaldehyde (o): original concentration of formaldehyde in mixture, 6.8 g./100 cc. (2 moles per mole tyrosine).

crystalline 2,6-dimethylol-*p*-cresol by the action of formaldehyde on *p*-cresol in concentrated aqueous alkali. By analogy one would expect that such a reaction could take place with tyrosine. Galeotti[9] heated a number of amino acids, including tyrosine, with formalin,[10] and obtained amorphous sticky masses, which were soluble in water. An amorphous product has also been prepared[11] by treating tyrosine with formaldehyde under alkaline conditions for one month at 37°. The product, like tyrosine, was reported to be only slightly soluble in water and soluble in alkali and acid, but it gave a negative ninhydrin test. It also gave a negative test for free formaldehyde and very low figures for free amino nitrogen. We have treated tyrosine with formaldehyde under conditions similar to those used for the preparation of dimethylol-*p*-cresol, and have isolated and characterized the amorphous reaction product.

The reaction in aqueous alkali was followed by measuring the optical rotation as well as the decrease in concentration of free formaldehyde in the reaction mixture (Fig. 1). The data indicate that one mole of formaldehyde is taken up rapidly; a second mole is taken up much more slowly. The rapid rise in positive rotation to a maximum is associated with the sharp decrease in concentration of free formaldehyde. The reaction product (I) was isolated as a yellow powder by acidifying the brown reaction mixture to *p*H 5.5. In no case were we able to obtain a crystalline product from the reaction mixture. This was later clarified by the observation that all the properties of the product isolated were in accord with the assignment to it of a polymeric structure.

The product, I, had $[\alpha]^{25}\text{D}$ +18.1° (*c* 0.9, 3 *N* sodium hydroxide) in contrast to $[\alpha]^{25}\text{D}$ −13.8° for tyrosine. Although I was amphoteric, it dissolved much more readily in alkali than in acid. Alkaline solutions were golden yellow, whereas acid solutions were colorless. I was practically insoluble in water (0.01%), but when reprecipitated from alkaline solutions, a good part of the material remained suspended in the supernatant liquid. Heating the suspension brought about the precipitation of I. The product had no definite melting or decomposition point. Air-dried I when heated at 105° in an oven continued to lose weight slowly. Loss of moisture to constant weight could be obtained by drying it over phosphorus pentoxide *in vacuo* at room temperature. The moisture so determined was slightly lower than that obtained by heating at 105°. The heated material had lost most of its solubility in 0.1 *N* alkali, whereas the product dried at room temperature was still completely soluble. The loss of a few molecules of water by condensation might explain this behavior.

X-Ray diffraction patterns of I indicated that the material was amorphous, consistent with a polymeric nature. Absorption curves shown in Fig. 2 demonstrate that the hydroxyphenyl nucleus of tyrosine was still present in I. The absorption maximum of I was at 284 mμ with the specific extinction coefficient[12] equal to 9.81; that of tyrosine was at 274 mμ, with the coefficient equal to 7.96.

Electrophoretic patterns of I in veronal buffer at *p*H 7.8 showed two definite peaks, indicating that I was not homogeneous. They were complicated by a difference between the ascending and descending patterns of a type which is usually interpreted as indicating complex formation between two interacting components. Heterogeneity of I was substantiated by fractionation of an alkaline solution with dilute acid. Although the larger part of the material precipitated at *p*H 5.5, small fractions were obtained at *p*H 4.5 and 3.5.

The polymeric nature of I was demonstrated further by its behavior on dialysis. Dialysis of a solution of I against distilled water for as long as six days yielded none of the material in the dialysate. Complete recovery of I by precipitation of the solution in the Cellophane casing indicated a high molecular weight.

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[9] Galeotti, *Biochem. Z.*, **53**, 474 (1913).

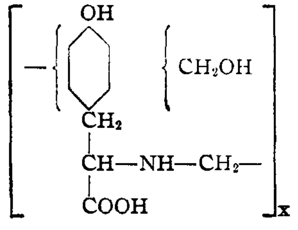
[10] Under strongly acidic conditions tyrosine reacts with formaldehyde to give an isoquinoline derivative [Pictet and Spengler, *Ber.*, **44**, 2030 (1911); Wellisch, *Biochem. Z.*, **49**, 173 (1913)].

[11] Holden and Freeman, *Australian J. Exptl. Biol. Med. Sci.*, **8**, 189 (1931).

[12] Density for 1-cm. layer of solution containing 1 g. per liter.

Although the experimental data show that 2 moles of formaldehyde reacted with 1 mole of tyrosine, none of the formaldehyde was acid labile. Hydrolysis of I with 1 *N* acid for as long as seven hours liberated no formaldehyde. Furthermore, the formaldehyde that had combined with tyrosine to form I was not removed by acid under the conditions used to determine formaldehyde in hardened proteins.[4] The formaldehyde combined with tyrosine in I was also more firmly bound than that in dimethylol-*p*-cresol, since either heat or acid liberates formaldehyde from this compound.[8]

I was devoid of amino nitrogen, as shown by a negative ninhydrin test, and confirmed by Van Slyke amino nitrogen analyses of 0.0–0.1%. Since the possible linkages $>N-CH_2-N<$ and $>N-CH_2-O-$ resulting from formaldehyde addition to the amino group are acid labile,[13] an acid-stable linkage of the type $>N-CH_2-CH<$ formed from the conventional $>N-CH_2OH$ by condensation with active hydrogen is indicated. That the nitrogen of I was secondary rather than tertiary was shown by the fact that addition of formaldehyde to an aqueous alkaline solution of I caused a drop in *p*H. A formol titration of I on a quantitative basis was not reliable because of the interference of the phenolic hydroxyl.[14] The total nitrogen content of I was 6.2–6.3% (depending on the preparation), giving an equivalent weight of 224 ± 2. The addition of 2 moles of formaldehyde to 1 mole of tyrosine with the elimination of 1 mole of water as indicated in unit I would yield an equivalent weight of 223. The bond adjacent to the ring in the skeleton formula denotes the loss of a hydrogen atom. Carbon and hydrogen values required for the empirical formula of I, $C_{11}H_{13}NO_4$, were in agreement with those obtained by analysis. Measurement of bromine substitution as used for the determination of tyrosine[15] gave an equivalent weight of 224 on the assumption that 2 moles of bromine reacted with 1 tyrosine unit. The mechanism of the reaction is open to speculation.



The chemical study of I was hampered by its insolubility in the common organic solvents tested. Attempts to synthesize derivatives yielded neither crystalline products nor amorphous products of constant composition. Amorphous acetyl derivatives prepared by various methods had nitrogen contents of 4.7–5.9% (I monoacetate, 5.28% N; I diacetate, 4.57% N), and the highly substituted derivatives gave negative Millon tests. Inorganic salts, picrates and methylated derivatives were also prepared, but none had a constant composition.

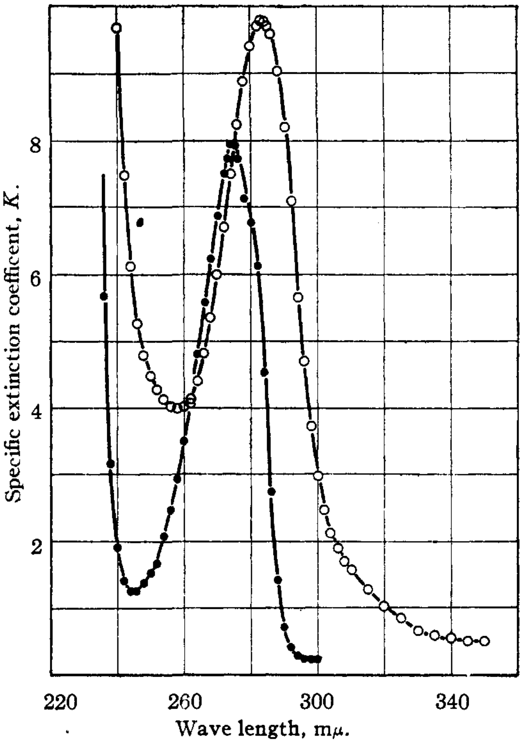


Fig. 2.—Ultraviolet absorption curves: ●, tyrosine, *p*H 5.8; o, reaction product I, *p*H 6.0.

Resinification of I could be accomplished by the same catalysts used for the formation of phenol-formaldehyde resins. Heating I at 105° decreased its solubility in 0.1 *N* sodium hydroxide considerably, but it was not resinified. No loss of carbon dioxide or formaldehyde could be detected. Heating I with addition of catalytic amounts of sodium hydroxide or ammonia gave light-brown, transparent, somewhat brittle resins; with hydrochloric acid a tough, opaque resin was obtained. Thus, an analogy may be drawn between I and phenol-formaldehyde resins in a low stage of condensation.

Experimental

Preparation and Isolation of the Tyrosine–Formaldehyde Reaction Product (I).—To 80 g. (0.44 mole) of tyrosine dissolved in 323 cc. (0.88 mole) of 2.74 *N* sodium hydroxide was added 26.6 g. (0.88 mole) of formaldehyde in the form of U.S.P. formalin. The stoppered flask containing the light-yellow solution was kept at 26°. After a few days the mixture became light brown. After ten days the free formaldehyde in the mixture had practically disappeared. The mixture (*p*H > 10) was filtered through sintered glass to re-

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[13] Feldman and Wagner, *J. Org. Chem.*, **7**, 31 (1942).

[14] Sörensen, *Biochem. Z.*, **7**, 45 (1908); Jodidi, THIS JOURNAL, **40**, 1031 (1918).

[15] Brown and Millar, *J. Chem. Soc.*, **89**, 145 (1906).

move some suspended particles (<0.2 g.), and the sirupy filtrate was diluted with 1 liter of water. With vigorous stirring the solution was adjusted to *p*H 5.5 by the dropwise addition of 6 *N* hydrochloric acid to *p*H 6.6 followed by the addition of 1.3 *N* hydrochloric acid. The large precipitate which formed was separated by centrifugation. On further acidification, the clear supernatant fluid gave a small additional precipitate (*ca.* 1 g.) at *p*H 4.5 and a final small precipitate (*ca.* 2 g.) at *p*H 3.5. These were discarded.

The large precipitate was washed twice with water and was then redissolved in approximately 360 cc. of 1 *N* sodium hydroxide. The solution was diluted to 1.5 liters, and again precipitated at *p*H 5.5. This was repeated once more. The centrifuged product was suspended in water and dialyzed against distilled water until the dialysate was free of chloride ion. The volume of the precipitate in the Cellophane casing had increased considerably and, on centrifugation, the supernatant liquid was turbid. This behavior of the product when washed with water in the presence of few inorganic ions is characteristic. The bulky material was washed by centrifugation three times with acetone (turbid wash liquid), and finally with ether. When dried in air, the product was a yellow powder; yield 55.5 g. (I).

Anal. Calcd. for $(C_{11}H_{13}NO_4)x$: C, 59.2; H, 5.87; N, 6.28. Found: C, 58.9; H, 5.75; N, 6.24.[16]

All the turbid supernatant wash liquids were combined and heated to 80°. The suspended material coagulated and separated rapidly, leaving a clear supernatant liquid. The material (10 g.) obtained in this manner was not mixed with I.

Optical Rotation of the Reaction Mixture.—The polarimeter sample was removed from the reaction mixture described above, maintained at 25 ± 1°, and its optical rotation was observed in a 2-dm. tube at various time intervals for forty days. After that time the sample had darkened somewhat, and the solution was viscous. The observed polarimetric readings are plotted in Fig. 1.

Decrease in Formaldehyde Concentration in the Reaction Mixture.—Immediately on mixing the alkaline solution of tyrosine and the aqueous formaldehyde, samples were removed for formaldehyde analysis. Subsequent samples were removed periodically until the amount of free formaldehyde in the reaction mixture had decreased almost to zero. Formaldehyde was determined both by the gravimetric method as modified by Wadsworth and Pangborn,[17] and by the volumetric method,[4] with concordant results. Data showing the decrease in free formaldehyde are plotted in Fig. 1.

Characterization of I.—I was insoluble in methanol, ethanol, ether, petroleum ether, chloroform and acetone. Although soluble in 1 *N* hydrochloric acid, it was resinified rather than dissolved by concentrated hydrochloric acid. The solubility of I in water at 26° was determined by shaking 65.2 mg. (14.8% moisture) with 50 cc. of twice-distilled water for twenty-four hours. Ten cc. of the filtrate (*p*H 6.48) when concentrated to dryness yielded 1.1 mg., corresponding to a solubility of 0.01%. I gave a positive Millon test, and had $[\alpha]^{25}\text{D}$ +18.1° (*c* 0.9, 3 N sodium hydroxide).

Hydrolysis of I with Acid.—0.22 g. of I was boiled with 200 cc. of 1 *N* sulfuric acid, and the volume of the solution was kept constant by periodic addition of water. Distillates collected at one-hour intervals for seven hours contained no formaldehyde. The same procedure with phosphoric acid also yielded negative results. On concentrating the 200 cc. of solution in the distilling flask to 10 cc. as carried out in the modified Nitschmann method,[4] no formaldehyde was found in the distillate.

Fractionation of I.—Five grams of I was dissolved in a mixture of 60 cc. of 0.1 *N* and 15 cc. of 1 *N* sodium hydroxide. The yellow solution was then diluted with 150 cc. of water, and 0.1 *N* hydrochloric acid was added dropwise to the stirred solution. The first turbidity which did not disappear on prolonged stirring developed at *p*H 6.4. On further acidification to *p*H 5.8, and standing overnight, a precipitate settled out. The clear supernatant liquid (*p*H 6.0) was acidified to *p*H 5.5, and again a precipitate formed. A small precipitate at *p*H 4.6 and a final one at *p*H 3.5 were obtained in the same manner.

Resinification of I.—To 0.5 g. of I in each of three test-tubes were added (1) 4 drops of concentrated ammonia, (2) 4 drops of 2 *N* sodium hydroxide, and (3) 4 drops of concentrated hydrochloric acid; a fourth tube (4) contained 0.5 g. I alone as a control. The four tubes were heated for thirty minutes at 115° and then for one hour at 100°. (1) and (2) yielded transparent, light-brown resins, (3) contained a tough, opaque resin, and in (4) was apparently unchanged powder. Powdered samples (0.2 g.) of the four products were shaken with 10 cc. of 0.1 *N* sodium hydroxide for twenty-four hours. Resins (1), (2), and (3) were completely insoluble. In contrast to unheated I, which dissolved completely in a few minutes, (4) had swelled considerably, but only 27% had passed into solution.

Acetylation of I.—Aqueous alkaline acetylations were carried out as follows: I (0.48 g., dry basis) was dissolved in 4 cc. of 1 *N* sodium hydroxide, and the solution was treated with 1.63 cc. of acetic anhydride in small portions, and with constant shaking. The mixture was heated

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[16] Values determined on an air-dried sample and corrected for a moisture content of 13.31% as found by drying over phosphorus pentoxide *in vacuo* at room temperature for five days. Using a moisture content of 14.08% as determined by heating at 105° for six hours, the corrected values are C, 59.4 and H, 5.69%.

[17] Wadsworth and Pangborn, *J. Biol. Chem.*, **116**, 423 (1936).

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.

Acknowledgment.—The author is indebted to Edith C. Gall for assistance in the laboratory, to F. R. Senti for X-ray patterns, to R. C. Warner for electrophoresis measurements, to M. L. Swain for the absorption curves, to C. L. Ogg for the microanalyses, and to W. G. Gordon and R. W. Jackson for their advice and interest in the investigation.

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.

PHILADELPHIA 18, PA. RECEIVED FEBRUARY 11, 1946

[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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(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

(2) J. F. Carson and W. D. Maclay, THIS JOURNAL, **67**, 787 (1945).

(3) V. C. Barry, T. Dillon and O'Muineachain, *Sci. Proc. Roy. Dublin Soc.*, **21**, 289 (1936).

(4) A. G. Norman, "The Biochemistry of Cellulose, the Polyuronides, Lignin, etc.," Oxford, 1937, p. 145.

(5) E. L. Hirst, J. K. N. Jones and W. O. Jones, *J. Chem. Soc.*, 1880 (1939).