removed at subsequent recorded times. The contents of the tubes were extracted with water and halide ion in the water extracts was determined by the Volhard method.

The percentage of reaction in the last tube removed varied from 75.3% (forty hours reaction time) in the case of *p*-nitrobromobenzene, to 7.0% (137.62 hours reaction time) in the case of *p*-bromoacetophenone.

Rate constants were determined from the equation[9]

$$kt = \frac{2.303}{b - 2a}\log_{10}\frac{(b - 2x)}{(a - x)} + C$$

where b is initial concentration of piperidine, a is initial concentration of the halogen compound, and x is concentration of the piperidinium halide product at time t (first tube removed from bath at t = 0). This mathematical expression is valid for the chemical equation

$$RX + 2C_5H_{10}NH \longrightarrow RNC_5H_{10} + C_5H_{10}NH_2X$$

When values of the term $2.303/(b - 2a) \log_{10} [(b - 2x)/(a - x)]$ were plotted against t, the points in every case fell virtually on a straight line.[10] Values of the slope were calculated by the Method of Zero Sum.[11]

B. Reactions with Sodium Methoxide.—The technique was generally the same as used for the reactions with piperidine. The same thermostat was used with temperature again 99 ± 0.5°. Each tube contained 0.00200 mole of the halogen compound (added as a solid) and 0.00200 mole of sodium methoxide in a total volume of 19.28 ml. When a tube was opened, its contents were added to 100 ml. of 50% methanol and unconsumed base was determined by titration with standard hydrochloric acid to the methyl red end-point. For one tube of each compound, this titration was followed by a conductimetric titration of halide ion, and it was observed that bromide liberated was equal to methoxide consumed in the case of the nitro and sulfone compounds.

Rate constants were calculated from the expression

$$1/(a - x) = kt + C$$

applicable to second order reactions in which the initial concentrations of both reactants are equal. Values of k were found by the Method of Zero Sum.[11]

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Summary

1. Rate constants for the reactions of some *p*-substituted bromobenzenes with piperidine at 99° have been determined; comparison of them shows that for this reaction four groups stand in the following order of activating influence: $NO_2 \gg CH_3SO_2 > CN > CH_3CO$.

2. Rate constants for the reactions of *p*-bromonitrobenzene and of *p*-bromophenyl methyl sulfone with methanolic sodium methoxide at 99° have been determined.

3. The rate constant for the reaction of *p*-chloronitrobenzene with piperidine at 99° has been determined; *p*-chlorobenzotrifluoride and *p*-chlorobenzophenone reacted too slowly with piperidine to allow kinetic study of the reactions.

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[9] *Cf.* Rheinlander, *J. Chem. Soc.*, **123**, 3099 (1923).

[10] The average deviation of points from the straight line was in every case less than 3% of the difference between the first and last values plotted.

[11] Campbell, *Phil. Mag.*, **39**, 177 (1920); **47**, 816 (1924).

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

Polysaccharide Aryl Carbamates[2]

BY IVAN A. WOLFF AND CARL E. RIST

Although aromatic isocyanates are regularly used for the characterization of a variety of alcohols, the reactions of these reagents with carbohydrates have not been extensively investigated. Carbanilates of several common sugars,[3] sugar alcohols,[4] glucosides,[5] and the 1-N-α-naphthyl carbamate of 2,3,4,5,6-0-pentamethyl-(*levo*)-sorbitol[6] have been prepared. Recently carbamate derivatives of cellulose and of partially esterified or etherified cellulose have been reported.[7]

This paper reports the preparation and some properties of the esters of corn starch, corn amylose and amylopectin with phenyl isocyanate and with α-naphthyl isocyanate. Tricarbanilates of waxy (glutinous) corn starch, white potato amy-

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

[2] Manuscript presented before the Division of Sugar Chemistry and Technology of the American Chemical Society at Chicago, Illinois, April 19–23, 1948.

[3] Maquenne and Goodwin, *Bull. soc. chim.*, [3] **31**, 430 (1904).

[4] Tessmer, *Ber.*, **18**, 968 (1885).

[5] Jolles and Botrini, *Gazz. chim. ital.*, **65**, 1217 (1935); Wolfrom and Pletcher, THIS JOURNAL, **62**, 1151 (1940); Hearon, Hiatt and Fordyce, *ibid.*, **66**, 995 (1944); Hearon, *ibid.*, **70**, 297 (1948); Reeves, *ibid.*, **70**, 259 (1948).

[6] Wolfrom and Gardner, *ibid.*, **65**, 750 (1943).

[7] Hearon, Hiatt and Fordyce, *ibid.*, **65**, 829, 833 (1943); Dyer and McCormick, *ibid.*, **68**, 986 (1946); Hearon and Lobsitz, *ibid.*, **70**, 296 (1948).

lose and amylopectin, glycogen, corn torrefaction dextrin, dextran from *Leuconostoc mesenteroides*,[8] corn β-amylase limit dextrin[9] and Schardinger β-dextrin[10] were also prepared. The tricarbanilate esters were different from the aliphatic esters previously studied in that they could be prepared from starch granules which had no pretreatment. Their optical rotations were negative in pyridine and were related to the degree of branching of the polysaccharide used. Starch tricarbanilate could be separated into its linear and non-linear components by the selective solvent action of ethyl acetate.

Corn starch granules reacted rapidly with phenyl isocyanate at 100° in the presence of pyridine to give a trisubstituted product. Esterification was substantially complete after two hours, but a reaction period of twenty-four hours gave products whose solutions in organic solvents showed greater clarity. The yield of trisubstituted derivatives was quantitative. Waxy corn starch, the corn and white potato starch components, Schardinger β-dextrin and the β-amylase limit dextrin reacted in a similar fashion. Dextran was converted to its triester in the pyridine-phenyl isocyanate medium, although the material was never completely in solution. Corn torrefaction dextrin was quite resistant to carbanilation. After twenty-four hours at 100° only 3.67% nitrogen had been introduced. The dextrin was rendered more reactive by solution in water and reprecipitation in ethanol.

Trisubstitution occurred readily when α-naphthyl isocyanate reacted with corn amylose, amylopectin or previously pasted and dried starch. Reaction with starch granules was, however, slower than in the case of phenyl isocyanate. In the presence of an excess of α-naphthyl isocyanate over that needed for trisubstitution, only two groups per anhydroglucose unit were introduced in twenty-four hours at 100°.

All of the esters were white amorphous solids which fused over a considerable temperature range of 220–300° (see Table I). The Schardinger β-dextrin tricarbanilate melted at 214–215°.

In contrast with the high positive optical rotations of corn starch and its aliphatic esters and ethers the tricarbanilates of the whole starch, corn amylose, and amylopectin had a levorotation in pyridine (see Table I). Even more striking was the 20° difference in the rotations of the amylose and the amylopectin derivatives. Since it is now believed that corn amylose is linear while amylopectin is branched, this rotational difference indicated that correlation might be expected between the structure of a 1,4-linked anhydroglucose polymer and the optical rotation of its tricarbanilate in pyridine. This expectation was borne out in the series of compounds listed in Table I. The potato amylose and amylopectin derivatives had rotations equal to those of the corn starch fractions. Waxy corn starch tricarbanilate had the same rotation as the amylopectin derivative to which it is closely related in structure.[11] Glycogen and corn β-amylase limit dextrin, which are thought to be approximately equally branched[12] and more so than amylopectin, gave derivatives with nearly the same rotations, but less negative than those of amylopectin. Using similar reasoning it would appear that the water-soluble corn torrefaction dextrin, prepared in the absence of catalyst, was more highly branched than amylopectin, but less so than glycogen. The rotation of the Schardinger β-dextrin carbanilate was not directly comparable to the rotations of the higher molecular weight polysaccharides.

TABLE I

OPTICAL ROTATIONS AND MELTING RANGES OF POLYSACCHARIDE TRICARBANILATES

Tricarbanilate of	$[\alpha]^{25}_D$, (C = 1), in Pyridine[a]	Morpholine	Melting range, °C.[b]
Corn amylose	−82.5°	− 7°	248; 259–265
Corn amylopectin	−62.0	− 4	250–260
Corn starch	−66.0	− 5	209; 222–262
Waxy corn starch	−61.0	− 3	250–260
White potato amylose	−82.5	− 7	245–265
White potato amylopectin	−61.0	− 6	245–260
Corn β-amylase limit dextrin	−35.0	+ 17	240–255
Glycogen	−31.5	+ 4	220; 240–260
Corn torrefaction dextrin	−46.0	+ 9	200; 226–245
Dextran	Insol.	+343	255; 288–298
Schardinger β-dextrin	+69.5	+ 22	214–215

[a] Values stated to the nearest 0.5°.

[b] Taken in capillary tubes. The first figure is the sintering point. Then the melting range is given.

Since the dextran tricarbanilate was sufficiently soluble only in morpholine for reading its optical rotation, the rotations of the other derivatives in morpholine were measured for comparison. The accuracy of these last-mentioned rotations was low because of their very small value. An outstanding difference could be noted, however (Table I), between the rotations of the predominantly 1,4-linked polysaccharide derivatives taken as a group and the large dextrorotation of the dextran carbanilate which contained an abundance of 1,6-linkages.[13] This phenomenon is being investigated as a possible test for the presence of different types of glycoside linkages in polysaccharides.

The optical rotations of the tri-α-naphthyl carbamates of the starch, amylose and amylopec-

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(8) Jeanes, Wilham and Miers, paper presented before the Division of Sugar Chemistry and Technology at the 112th meeting of the American Chemical Society, New York, N. Y., September 15–19, 1947.

(9) Hodge, Montgomery and Hilbert, *Cereal Chem.*, **25**, 19 (1948).

(10) McClenahan, Tilden and Hudson, THIS JOURNAL, **64**, 2139 (1942).

(11) Schopmeyer, Felton and Ford, *Ind. Eng. Chem.*, **35**, 1168 (1943).

(12) Meyer in "Advances in Colloid Science," ed. by E. O. Kraemer, Vol. 1, Interscience Publishers, Inc., New York, N. Y., 1942, pp. 159, 175.

(13) Levi, Hawkins and Hibbert, THIS JOURNAL, **64**, 1959 (1942).

tin of corn were +40, +50 and +36°, respectively. These positive values were unexpected in view of the negative rotations of the corresponding phenyl derivatives. The spread between the amylose and amylopectin α-naphthylcarbamates, while not as great as between their carbanilates, was substantially more than in the case of the aliphatic acid triesters.

The tri-α-naphthylcarbamates were not completely soluble in any organic solvent tested. They were partially soluble in pyridine, dioxane and morpholine. All of the higher molecular weight tricarbanilates were most soluble in pyridine and morpholine, less so in acetone, ethyl acetate, 1,4-dioxane and cold diethyl Cellosolve, and insoluble in other common organic solvents. The torrefaction dextrin and Schardinger β-dextrin carbanilates were readily soluble in most of the solvents tested. The tricarbanilates were often more soluble in cold than in warm solvents. This was shown most strikingly by the corn amylose derivative, which was completely soluble in diethyl Cellosolve at room temperature, but precipitated immediately on slight warming. Cooling of the mixture caused prompt re-solution.

Fractionation of corn starch tricarbanilate into the corresponding amylose and amylopectin derivatives has been accomplished by the selective solvent action of ethyl acetate. The soluble portion of the ester, constituting from one-sixth to one-quarter of the original material, had a rotation that indicated it to be the amylose ester, from 65 to 80% pure. After removal of the carbanilino groups, the reconstituted fractions were titrated potentiometrically with iodine.[14] The soluble fraction sorbed from 139 to 158 mg. of iodine per gram while the insoluble portion sorbed 19 mg. per gram, thus substantiating the analyses on the basis of optical rotation. This is the first known instance of successful solvent fractionation of a starch ester into its linear and non-linear components.

The close agreement between the optical rotation data and the fraction purities obtained in the potentiometric iodine titration suggested the possible use of the former as an analytical tool in determining the amylose–amylopectin ratio in a mixture. The successful operation of this procedure on a fairly pure amylose sample and on a material of rather low amylose content (whole starch) has been demonstrated above. The principle has also been applied to a synthetic sample composed of a mixture of equal parts by weight of corn amylose tricarbanilate and amylopectin tricarbanilate. The rotation of this mixture was −72.0° (pyridine, $C = 1$) indicating an amylose content of 49%. This procedure might advantageously be applied in cases where only very small amounts of material are at hand since the weight of sample is more than tripled by reaction with phenyl isocyanate.

Further studies on the reactions of carbohydrate materials with mono- and polyfunctional isocyanates are in progress.

Experimental

Materials.—The corn starch used was a high-grade commercial product. The white potato and waxy corn starches were extracted from their natural source in the pilot plant of this Laboratory. Amylose and amylopectin were separated by the butanol precipitation procedure,[15] and the amylose was recrystallized until the iodine sorption was 190 mg./g. or higher.[14] β-Amylase limit dextrin was prepared from an aqueous solution of corn amylopectin by allowing wheat β-amylase to react with it for eighteen hours.[9] The dextrin was isolated by precipitation with ethanol. Dextran from *Leuconostoc mesenteroides* was a water-soluble product of high viscosity prepared by allowing the organism to act for twenty-four hours on a 10% sucrose solution.[8] Corn torrefaction dextrin was prepared by heating corn starch in the absence of a catalyst for six hours at 185°. The product was completely water-soluble. It was pretreated by homogenization of an 8% aqueous solution in a Waring Blendor, filtration, and then isolation of the dextrin by precipitation in ethanol. Schardinger β-dextrin was kindly furnished to us by Dr. T. J. Schoch. Glycogen, phenyl isocyanate, and α-naphthyl isocyanate were Eastman Kodak Co. white label chemicals, used without further purification. Commercial pyridine having a 2° boiling range was dried over solid sodium hydroxide and distilled before use.

General Methods of Preparing the Tricarbanilate Esters.—The reactions of corn starch, waxy corn starch, corn and potato amylose and amylopectin, corn torrefaction dextrin, and corn β-amylase limit dextrin with phenyl isocyanate were carried out as in the following typical preparation. Nitrogen analyses on the products agreed with the calculated value for a tricarbanilate (calcd. for $C_{27}H_{25}N_3O_8$: N, 8.09) within ±0.1%.

Ten grams (0.055 mole) of waxy corn starch (moisture 10.76%) was suspended in 150 ml. of dry pyridine. Distillation through a 6-inch Vigreux column was carried out with stirring until 75 ml. had distilled. The amount distilled was then replaced with dry pyridine. Forty grams of phenyl isocyanate (0.34 mole) was added, and reaction was carried out for twenty-four hours at 100°. The clear, light yellow, viscous mass was precipitated in ethanol, washed three times with ethanol, and then dried, giving 28.9 g. of product (theoretical yield for the tricarbanilate 28.6 g.).

The course of the reaction of corn starch granules with phenyl isocyanate is shown in the following data:

Reaction time	% N in ester
14 min.	3.37
28 min.	5.54
57 min.	7.36
1 hr. 49 min.	7.82
4 hr. 32 min.	8.03

After fifteen minutes the reaction mixture was very viscous. No gross changes in viscosity occurred after this time.

Dextran tricarbanilate was prepared as above, but a reaction period of forty-eight hours was used. The reaction mixture in this case was light tan and somewhat viscous, but was not free of solid material at any time during the period of reaction.

Glycogen tricarbanilate was prepared by reaction of glycogen with phenyl isocyanate for twenty-four hours. After that time the mixture was filtered through a coarse Pyrex fritted glass funnel and the residue, which was estimated at approximately 10% of the starting material, was discarded. This was probably largely impurities in the glycogen. The filtrate was poured into ethanol, where-

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[14] Bates, French and Rundle, THIS JOURNAL, 65, 142 (1943); Wilson, Schoch and Hudson, *ibid.*, 65, 1380 (1943).

[15] Schoch, *ibid.*, 64, 2957 (1942).

upon the glycogen tricarbanilate formed a very fine precipitate. This ester was washed twice with ethanol, once with water, and then dried.

In the preparation of Schardinger β-dextrin tricarbanilate a reaction period of six hours at 100° was used. The esterification mixture was poured into four volumes of absolute ethanol, giving a clear solution, which was then poured into an equal volume of water. Eighty-three per cent. of the theoretical amount of crude tricarbanilate, m. p. 162–175°, precipitated. The crude product was soluble in hot isopropyl alcohol and reprecipitated (as round, non-crystalline particles) on cooling. Two reprecipitations gave pure Schardinger β-dextrin tricarbanilate, melting at 214–215°.

Anal. Calcd. for $C_{189}H_{175}O_{56}N_{21}$: N, 8.09. Found: N, 8.02.

Preparation of α-Naphthylcarbamates.—Corn starch (5.6 g., air-dried) was dried azeotropically with pyridine and reacted for twenty-four hours at 100°, in 100 ml. of pyridine, with 28 g. of α-naphthyl isocyanate. The tan, opaque reaction mixture was poured into ethanol, giving 16.1 g. of α-naphthyl carbamate, containing 5.61% N; calcd. for the triester 6.28% N.

A 7% cornstarch paste was stirred in a Waring Blendor for fifteen minutes, and the starch was then precipitated in ethanol, washed with ethanol, and dried. This pretreated starch, when treated with α-naphthyl isocyanate as above, gave starch tri-α-naphthylcarbamate.

Anal. Calcd. for $C_{39}H_{31}N_3O_8$: N, 6.28. Found: N, 6.29.

Corn amylose and amylopectin tri-α-naphthylcarbamates were prepared in a fashion similar to that for the starch derivative.

Optical Rotations.—All optical rotations were taken at 1% concentration. When an ester was incompletely dispersed in the solvent or when the solution was too turbid to see through in the saccharimeter, the mixture was homogenized in a Waring Blendor. These blended solutions always showed improved clarity. Their concentrations were determined by evaporating 10-ml. aliquots to dryness.

Fractionation of Corn Starch Tricarbanilate.—Five grams of the tricarbanilate prepared from pretreated corn starch was stirred at room temperature for twenty-four hours with 150 ml. of ethyl acetate. The mixture was then centrifuged and the soluble and insoluble portions were individually precipitated with 50% ethanol, washed with water, and dried. The soluble fraction weighed 1.0 g., $[\alpha]^{25}_D$ −78.5° (pyridine, $C = 1$). The insoluble fraction weighed 3.4 g., $[\alpha]^{25}_D$ −66.0° (pyridine, $C = 1$).

Two grams of the ethyl acetate-insoluble fraction and 0.54 g. of the soluble fraction were refluxed for twelve hours in 100 ml. and 50 ml., respectively, of 1.2 *N* sodium methoxide in absolute methanol. The regenerated fractions were washed twice with absolute ethanol, once in ethanol containing a small amount of glacial acetic acid, once with 90% ethanol, twice more with absolute ethanol, and then were dried. The iodine sorption of the regenerated fractions was 19 mg./g. and 158 mg./g., respectively.

In another similar fractionation, 1.3 g. of soluble tricarbanilate was obtained, $[\alpha]^{25}_D$ −75.0° (pyridine, $C = 1$). The recovered amylose from this material sorbed 139 mg. of iodine per gram.

A similar separation was effected with the tricarbanilate prepared from non-pretreated starch.

The use of trade names in this paper does not necessarily constitute endorsement of these products nor of the manufacturers thereof.

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Summary

1. Tricarbanilates have been prepared of corn starch, corn amylose and amylopectin, potato amylose and amylopectin, waxy corn starch, dextran, glycogen, β-amylase limit dextrin, corn torrefaction dextrin and Schardinger β-dextrin.

2. Tri-α-naphthylcarbamates of corn starch, amylose and amylopectin were also prepared.

3. The melting characteristics, solubility behavior and optical rotations of these derivatives were given.

4. In contrast with the usual aliphatic acid anhydrides, phenyl isocyanate reacted in the presence of pyridine with non-pretreated starch granules to give a fully esterified product.

5. The wide variation of up to 20° between the specific rotations of the tricarbanilates of branched and unbranched polysaccharides suggests a means of differentiation of polysaccharides of different structural types.

6. The fractionation of starch tricarbanilate by the selective solvent action of ethyl acetate into the component amylose and amylopectin esters was successfully accomplished.

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