B. With Ferric Oxide.—A similar experiment conducted with a mixture prepared from 7 g. of glycolide, 7 g. of chloroacetic acid, 28 g. of naphthalene and 50 mg. of ferric oxide yielded 5 g. of less pure acid, m.p. 108–110°.

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[CONTRIBUTION FROM THE STARCH AND DEXTROSE DIVISION, NORTHERN REGIONAL RESEARCH LABORATORY[1]]

Polysaccharide Aryl Carbamates. IV. Partly Substituted and Cross-linked Starch Carbamates[2]

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The preparation of partially substituted starch carbamates both in anhydrous pyridine and in aqueous medium is described. Gelatinization-resistant starches result when as little as one hexamethylenedicarbamyl cross-link for each 500 anhydroglucose units is introduced. Many of the starch carbamates still show the cross under polarized light and are microscopically indistinguishable from untreated corn starch.

In our recent publications the preparation and properties of completely substituted carbamate esters of several polysaccharides with phenyl isocyanate and with α-naphthyl isocyanate have been described.[3] The general lack of information concerning the reactions of isocyanates with amylaceous polysaccharides also was indicated. This paper reports the reaction of starch with hexamethylene diisocyanate, 2,4-tolylene diisocyanate, and with quantities of phenyl isocyanate such that less than complete substitution occurs.

Experimental

Materials.—The corn starch used was a high-grade commercial product. The potato starch was extracted at this Laboratory with distilled water from No. 1 commercial-grade Michigan potatoes. Phenyl isocyanate was Eastman Kodak Company white label grade used without further purification. The difunctional isocyanates were obtained from E. I. du Pont de Nemours and Company and were redistilled under reduced pressure before use. Their purities, determined by the method of Stagg,[4] were between 95 and 100%. Commercial pyridine (2° boiling range) was dried for several weeks over solid sodium hydroxide pellets and redistilled before use.

Preparation of Starch Carbamates in Pyridine.—Starch was usually dried by distilling pyridine from it until no more pyridine–water azeotrope could be removed. More anhydrous pyridine or other solvent was then added when desired, followed by the isocyanate, and reaction was carried out in an oil-bath, maintained at 100° for 6 hours. The product was isolated by pouring the reaction mixture into ethyl alcohol, washing the carbamated starch several times with ethyl alcohol, and drying. Analysis for nitrogen was by the Kjeldahl procedure.

Reaction of Starch with Isocyanates in Aqueous Medium.—To a stirred mixture of 100 g. of starch (as is basis) in 400 ml. of water, adjusted to the desired *p*H, was added the desired quantity of isocyanate. The suspension was allowed to stir for 2 hours, following which the product was separated by filtration, washed three times with water by alternate suspension and filtration, and finally allowed to air dry.

Measurement of Extent of Swelling of Products.—The amount of swelling undergone by starch carbamates in hot water was measured as follows: Two grams (as-is basis) of the substance, contained in a graduated 40-ml. centrifuge tube, was suspended in sufficient water to make a total volume of 40 ml. and the mixture was stirred for 20 minutes in a steam-bath. The volume of the swollen granules was read after a 24-hour settling period.

Results

Reaction of Starch with Phenyl Isocyanate.—When 0.03 to 0.5 mole of phenyl isocyanate per base mole of starch was used, substantially all of the added reagent combined with the starch (see Table I). As the degree of substitution increased, progressively greater granule damage was evident. While sample C still appeared microscopically like unchanged starch, some of the granules in D showed an enlargement of the hilum. In E and F increasing amounts of amorphous material were noted although many granules containing the cross in polarized light were still present.

TABLE I

REACTION OF 25 G. (AIR-DRY BASIS) CORN STARCH WITH INSUFFICIENT AMOUNTS OF PHENYL ISOCYANATE FOR COMPLETE SUBSTITUTION (100°, 6 HOURS)

Product	Mole C_6H_5NCO/anhydroglucose unit	N content of product[a]	Degree of substitution, acyl per C_6
A	0.03	0.25	0.03
B	.07	.45	.05
C	.13	.48	.06
D	.27	1.56	.21
E	.40	2.51	.37
F	.53	3.28	.53

[a] Corrected for 0.03% N in the original starch.

A progressive change was also noted in the X-ray diffraction patterns of these materials. Sample C had a typical "A" X-ray diffraction pattern. In D the "A" pattern was still present but the background was appreciably stronger and a ring close to the so-called 16 Å. ring of the "B" starch pattern appeared. This new ring became stronger in E, and in F it appeared to have the same intensity as the strongest line of the "A" part of the pattern.

All of the above materials had a hydrophobic surface, evidenced by their tendency to float on water and to become wetted only with difficulty. Products A through C pasted in hot water, although

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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.

[2] Presented before the Division of Carbohydrate Chemistry at the 124th national meeting of the American Chemical Society, Chicago, Illinois, September, 1953.

[3] I. A. Wolff and C. E. Rist, THIS JOURNAL, **70**, 2779 (1948); I. A. Wolff, P. R. Watson and C. E. Rist, *ibid.*, **74**, 3061, 3064 (1952); **75**, 4897 (1953).

[4] H. E. Stagg, *Analyst*, **71**, 557 (1946).

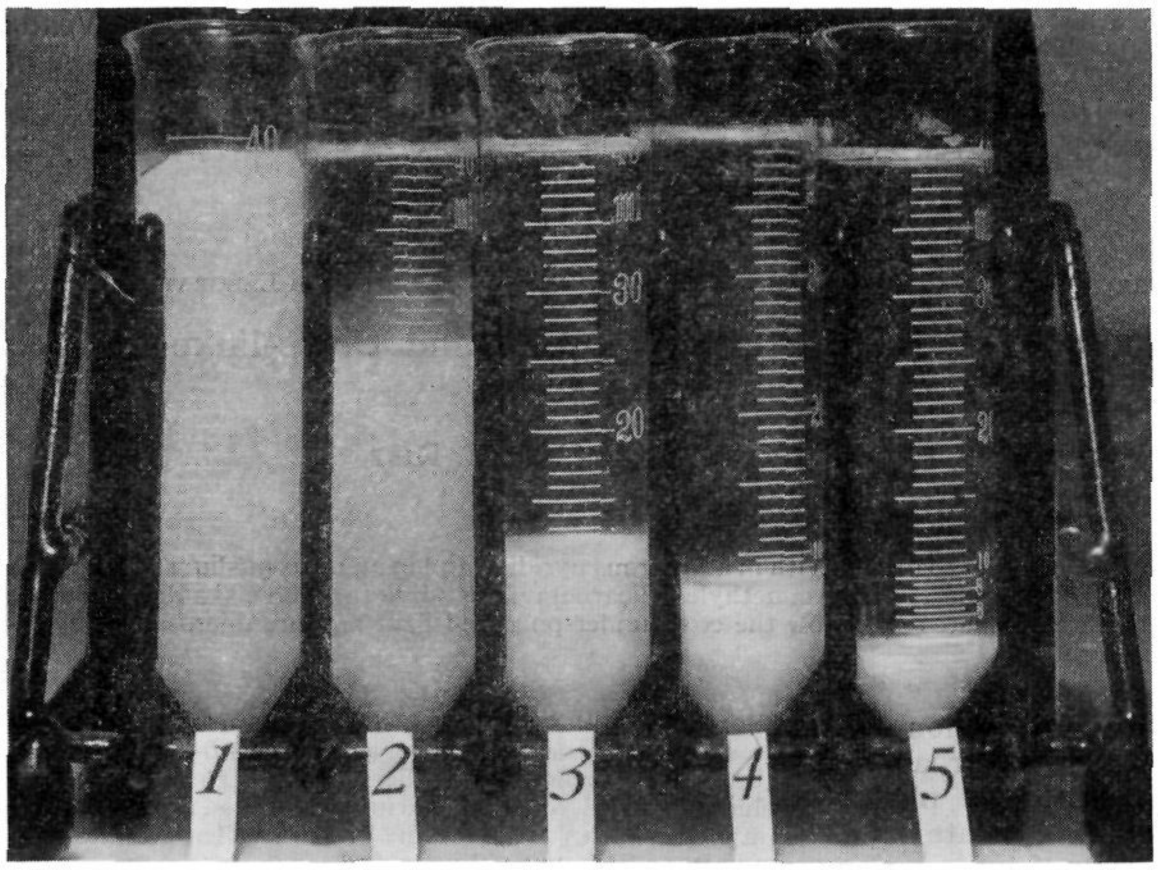

Fig. 1.—Appearance of gelatinization-resistant starches (2 g.) after heating in water for 20 minutes at 100° and allowing to settle for 24 hours: 1, control, unmodified corn starch; 2, product B, Table II; 3, product P, Table II; 4, product E, Table II; 5, product J, Table II.

less smoothly than did the original starch. The granules in products D, E and F swelled considerably but readily settled on standing. The swollen granules from 2 g. occupied 16, 13 and 11 ml., respectively.

The carbanilate grouping possessed no toxicity to cultures of *Aspergillus oryzae*, *Penicillium expansum* and *A. niger*, all of which grew well on a medium containing the modified starches C and D as carbon sources, in the presence of a suitable inorganic salt mixture.

A starch carbanilate having a degree of substitution of 1.43, and showing a minimum amount of granule damage was obtained by use of a reaction medium consisting of pyridine and diamyl ether (1:4 by volume) and containing 6 moles of phenyl isocyanate per mole of starch (24 hours at 100°). This product was incompletely soluble in any organic solvent tried, although it dissolved partially in pyridine, morpholine, dioxane, acetone, ethyl acetate and diethyl cellosolve. Approximately half of the granules still showed the cross under polarized light. In the swelling test described above the granules occupied a volume of 10 ml.

Substantially no reaction occurred between starch and phenyl isocyanate in toluene, triethylamine, or dimethylbenzylamine as replacement reaction media for pyridine. Since the reaction of aromatic isocyanates with aliphatic alcohols is base catalyzed,[5,6] the failure of the other tertiary amines to effect reaction indicates a specific activating effect of pyridine, which places the starch in a favorable condition for reaction. Lohmar and Rist[7] have observed a similar phenomenon in studies on the acetylation and phosphorylation of starch.

Reaction of Starch with Difunctional Isocyanates in Anhydrous Media.—Experiments on the reaction of starch with hexamethylene diisocyanate and toluene 2,4-diisocyanate are summarized in Table II. During the course of the reaction the starch granules either remained unchanged in appearance or became slightly swollen. The products, however, after treatment with ethanol were free-flowing, white powders which were microscopically indistinguishable from the original starch.

It is evident from the data of Table II that, in the lowest ranges of substitution, the nitrogen analyses are too high since the substitution indicated by that analysis exceeds the amount that it was possible to introduce (considering the amount of reagent added). This is undoubtedly due to traces of adsorbed pyridine, or to inaccuracies of the micro-Kjeldahl analyses in this low range. Of the products treated with hexamethylene diisocyanate, only A and K formed hydrogels in 5% concentration. Thus, for this derivative approximately 1 cross-link per 500 anhydroglucose residues is sufficient to inhibit complete granule gelatinization. With 2,4-tolylene diisocyanate a much higher degree of substitution is needed to confer water

TABLE II

REACTION OF STARCH WITH DIISOCYANATES IN DRY PYRIDINE

					Glucose residues per cross bridge	
					Calcd. from	
Product	Wt. starch g., air-dry basis	Reagent[a]	Ml.	N content, % dry basis	N content	reagent added[d]
A	125	HD	0.1	0.15	114	1150
B	25	HD	.05	.19	91	448
C	25	HD	.1	.24	71	224
D	25	HD	.25	.33	50	89
E	25	HD	.5	.43	38	46
F	25	HD	1.0	.67	25	22
G	25	HD	2.0	1.15	14	11
H	25[b]	HD	2.0	0.65	25	11
J	25	HD	10.0	2.31	6.5	2
K	25[c]	HD	5.0	0.08	213	4.5
L	125	TD	0.25	0.17	101	403
M	125	TD	2.0	0.32	53	50
N	25	TD	2.0	1.15	14	10
P	75	TD	10.0	2.38	6	6
R	25[c]	TD	5.0	0.08	213	4

[a] HD = Hexamethylene diisocyanate; TD = 2,4-tolylene diisocyanate. [b] Potato starch used in this experiment. [c] Toluene used as reaction medium. [d] Assuming complete reaction.

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[5] J. W. Baker and J. B. Holdsworth, *J. Chem. Soc.*, 713 (1947).

[6] D. S. Tarbell, R. C. Mallott and J. W. Wilson, THIS JOURNAL, **64**, 2229 (1942).

[7] R. L. Lohmar and C. E. Rist, *ibid.*, **72**, 4298 (1950).

resistance since even product M, having 50 chain units per cross-link, formed a gel in the standard swelling test. Perhaps the hexamethylenedicarbamyl cross-link fastens the starch chains into a more rigid position than the 2,4-tolylene bridge. Also, some of the 2,4-tolylene diisocyanate may react unifunctionally. Larger quantities of isocyanates confer a graded increase in the water resistance of corn starch as shown in Fig. 1. The granules in product J were swollen only a negligible amount in water at 100° and very little additional swelling occurred in an autoclave at 120°.

Under identical reaction conditions potato starch was substituted only one-half as much as corn starch by hexamethylene diisocyanate. The number of hydroxyl groups in the granule available for this reaction is thus variable for these two starch varieties.

Reaction of Corn Starch with Diisocyanates in Aqueous Medium.—Although isocyanates normally react with water to form substituted ureas, reactions have in some instances been carried out between isocyanates and amines in aqueous solution.[8] It was found that the gelatinization of starch could be profoundly modified by reaction with a diisocyanate in water. The effect of different reaction variables on the gelatinization behavior of corn starch is shown in Table III. From these data it can be seen that hexamethylene diisocyanate is more efficient than 2,4-tolylene diisocyanate in rendering starch swell-resistant. Equally good products can be obtained by use of reaction time periods from 5 minutes to 2 hours and temperatures from 5 to 50°. No advantage accrues from using 5% as much reagent as starch instead of 1%. The *p*H of the reaction mixture is important, the optimum effect being obtained at *p*H 10.5. These reaction products are not as uniformly swell-resistant as are the starches treated in anhydrous pyridine, since the supernatant liquids are always cloudy after settling of the swollen granules from a heated aqueous suspension. It is probable that on treatment of starch with hexamethylene diisocyanate in aqueous medium two types of reaction occur. There is some reaction of the reagent with the starch to form carbamate ester grouping and some coating of the starch granules by polyurea formed by the interaction of the diisocyanate with water. It was demonstrated that the swell-resistance of the treated starch was due at least partly to polyurea by extracting a treated sample with *o*-cresol, a known solvent for the urea. The swell-resistance of the treated starch was diminished, although not greatly so. The major reaction appeared then to be carbamate ester formation.

TABLE III

REACTION OF CORN STARCH WITH DIISOCYANATES IN WATER

Diisocyanates[a]	Amount, ml.	*p*H of suspension when reagent added	Reaction time, min.	Temp., °C.	Settling vol. in swelling test	% N in product, dry basis
TD	1	10.6	120	Ambient	33.5	0.26
HD	1	10.5	120	Ambient	12	.17
HD	1	10.5	5	Ambient	14	.16
HD	1	10.5	120	5	14	.15
HD	1	10.5	120	50	11.5	.18
HD	0.1	10.5	120	Ambient	34	.06
HD	5	10.5	120	Ambient	17	.71
HD	1	2.0	120	Ambient	35	.09
HD	1	4.0	120	Ambient	26.5	.12
HD	1	11.8	120	Ambient	31	.15

[a] HD = hexamethylene diisocyanate; TD = 2,4-tolylene diisocyanate.

Discussion

It is well known that cross-linking of a high polymeric material converts it to an insoluble, infusible state. It is particularly interesting in the case of starch that these cross-links are formed without disturbing the ordered arrangement of polysaccharide macromolecules in the starch granule (no change in X-ray diffraction pattern). Failure of the treated material to gelatinize may be a result of the insolubilization effected.

One theory of gelatinization[9] is that certain hydrogen bonds are broken by dissociated water molecules during the process. If the diisocyanates reacted at those sites, forming primary valence bonds where the weaker hydrogen bonds formerly existed, the resulting network could no longer be forced apart by the seepage of water between chains and a structure resistant to deformation would result. This may be an alternative explanation to the resistance to gelatinization of our products.

The diisocyanate-treated starches showed very limited swelling in 90% formic acid, 4 *M* calcium chloride, zinc chloride, or ammonium thiocyanate solutions, or in 0.5 *N* sodium hydroxide, all of which completely gelatinize untreated 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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