and a sealed Hershberg stirrer. A metal pail with mica insulation was placed around the flask. Liquid ammonia (700–750 ml.) was collected in a clear quart dewar and transferred to the flask. Sodium amide (1.0 mole, from 23 g. of sodium) was prepared in the liquid ammonia according to the procedure of Vaughn, Vogt and Nieuwland.[19] To the suspension of sodium amide in liquid ammonia was added the pyridine base to be alkylated. After this was added, a second Dry Ice condenser was fitted to the third neck of the flask. Through this condenser methyl chloride (51 g., 1.0 mole) was introduced into the reaction mixture from a small weighed cylinder. The rate of addition was usually rapid but slow enough to prevent boiling up into the condensers. The color usually changed to dark gray within 5–10 minutes after the addition. After the ammonia had evaporated (overnight), the mixture was treated dropwise with 50 ml. of water. The liquid was separated from the solid and the solid was dissolved in 150–200 ml. of water. The resulting solution or suspension was extracted twice with 50-ml. portions of ether. The ether was removed and the residue was combined with the liquid reaction product and dried over potassium hydroxide pellets. The product was rectified at atmospheric pressure in the column described in Method A.

Ethyl Nicotinate.—The procedure of Burrus and Powell[12] was used. The average yield of ethyl nicotinate, b.p. 92–93° at 7 mm., $n^{20}D$ 1.5034, for 16 runs was 73.6%. Burrus and Powell reported 61% ethyl nicotinate, b.p. 84° at 5 mm.

3-Acetylpyridine.—The procedure of Kolloff and Hunter[13] was used except for minor modifications. The yield of product, b.p. 65–66° (1 mm.), $n^{20}D$ 1.5341, was 72–73%.

Reduction of 3-Acetylpyridine to 3-Ethylpyridine.—The procedure of Fand and Lutomski[14] was used. The yield of product, b.p. 164.5–166°, uncor., $n^{20}D$ 1.5020, was 82.4%.

α,α-Dimethyl-3-pyridinemethanol.—A solution of 151 g. (1.0 mole) of ethyl nicotinate in 350 ml. of anhydrous ether was added with stirring during 2 hours to a solution of 3.25 moles of methylmagnesium iodide in 600 ml. of anhydrous ether. After refluxing for 3 hours the addition complex was decomposed by cautiously pouring the ethereal suspension into a mixture of 200 ml. (3.3 moles) of glacial acetic acid in 1 l. of cracked ice. The ether was evaporated by the heat of reaction. To the viscous mixture 600 ml. of water was added and the solution was thoroughly extracted with chloroform. The chloroform was removed and the product was distilled in a Vigreux column, b.p. 126° (8 mm.), $n^{20}D$ 1.5256 (supercooled); yield 62–78%; m.p. 53.5–55°; picrate, m.p. 149–150°. The following values are reported: m.p. 58°,[15] 53°[16]; b.p. 130° (11 mm.)[16]; picrate, m.p. 150°.[15]

3-Isopropenylpyridine.—3-Isopropylpyridine was prepared by the dehydration of α,α-dimethyl-3-pyridinemethanol by boiling for 0.5 hour with 27% sulfuric acid in glacial acetic acid.[15] The material was vacuum rectified in a 36-in. column, 12-mm. i.d., packed with $^3/_{16}$-in. glass helices. The average yield of product was 72%. The following constants were observed: b.p. 89° (25 mm.); $n^{20}D$ 1.5431, picrate, m.p. 155.5–156°, uncor. The following values are reported: b.p. 75° (10 mm.),[20] 187–188°[15]; $n^{25}D$ 1.5381[20]; picrate, m.p. 156°.[15]

Hydrogenation of 3-Isopropenylpyridine.—3-Isopropenylpyridine (83.6 g., 0.70 mole) was hydrogenated in the presence of 0.33 g. of platinum oxide catalyst during 25 hours at an initial pressure of 51 lb. A total of 57.8 lb. of hydrogen was taken up while the theoretical amount was 58.1 lb. The catalyst was filtered out and the product distilled: 65 g. boiled at 178–182°, uncor. Because of the wide boiling range this material was thought to contain some of the piperidine derivative. It was treated with a small amount of dilute hydrochloric acid (0.03 mole), dried over potassium hydroxide and calcium hydride, and distilled over phosphorus pentoxide, b.p. 179°.

Preparation and Properties of Monoalkylpyridines.—The results on the preparation and physical properties of the monoalkylpyridines are summarized in Tables I–III.

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(20) G. B. Bachman and D. D. Micucci, THIS JOURNAL, **70**, 2381 (1948).

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

Methylation and Ethylation of Corn Starch, Amylose and Amylopectin in Liquid Ammonia

BY J. E. HODGE, S. A. KARJALA[2] AND G. E. HILBERT

To obtain completely methylated corn starch fractions without severe degradation of the polyglucose chains, the authors adapted Freudenberg's liquid ammonia procedure. The cause of the degradation produced by Freudenberg's method was established and a way of avoiding it was found. Trimethyl ethers of corn starch, amylose and amylopectin of high intrinsic viscosity were prepared. Contrary to the findings of Freudenberg and Boppel, trimethyl amylose is distinctly different from trimethyl amylopectin in appearance, melting range, iodine sorption, viscosity characteristics, solubility, X-ray diffraction pattern, resistance to grinding and film-strength. Trimethyl corn starch was fractionated into trimethyl amylose (25%) and trimethyl amylopectin (75%) by means of their different solubilities in Diethyl Cellosolve. Triethyl ethers of corn starch, amylose and amylopectin were prepared for the first time. The disorganizing effect of liquid ammonia on starch granules was used to prepare dry granular starches dispersible in water. Use of this ammonia-treated starch allowed the omission of autoclaving in the starch fractionation procedure of Schoch.

Significant contributions to our knowledge of the structure of starch and other polysaccharides have been made by methylating the polysaccharide, hydrolyzing or methanolyzing the product, and determining the methylated sugars obtained. The importance of preparing a completely methylated polysaccharide without alteration of its structure is evident, but this goal has not been satisfactorily achieved. We have directed our experiments toward attaining this goal for starch and starch fractions.

The procedure of K. Freudenberg and co-workers[2a] for methylating starch in liquid ammonia is the most efficient.[3] However, this method has been avoided in favor of the laborious dimethyl sulfate–alkali procedures, because solutions of the ethers produced showed very low viscosities indicating

(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) Northwestern University, The Rheumatic Fever Research Institute, Chicago, Ill.

(2a) K. Freudenberg, H. Boppel and M. Meyer-Delius, *Naturwissenschaften*, **26**, 123 (1938); K. Freudenberg and H. Boppel, *Ber.*, **71**, 2505 (1938).

(3) Recently E. J. Bourne, K. H. Fantes and S. Peat, *J. Chem. Soc.*, 1109 (1949), have acknowledged the superiority of the Freudenberg procedure of methylation.

possible degradation of the polyglucose chains.[4] In this work the degradative action of sodium–ammonia solutions was minimized, so that starch ethers could be made with intrinsic viscosities as high as those of ethers produced in aqueous alkali with the exclusion of oxygen.

We found that the viscosity lowering occurring in Freudenberg's procedure was due to the action of sodium amide formed in sodium–ammonia solutions on standing. Sodium or potassium in liquid ammonia did not degrade methylated amylose, but the use of sodium amide or potassium amide did lead to a remethylated amylose of considerably lower viscosity. Hence, it is essential that the concentration of amide be limited by minimizing the excess of alkali metal and also by reducing the time allowed for its reaction with the starch. The beneficial effect of shortening the sodium treatments on the viscosities of the trimethyl ether derivatives is shown in Table I.

TABLE I

INTRINSIC VISCOSITIES OF TRIMETHYL CORN STARCH AND FRACTIONS

	Number of Na treatments	Total time of Na reactions, hr.	$[\eta]^a$	OCH_3,[b] %
Substance methylated: Starch A				
Freudenberg's	7	40	0.33	44.8
Improved	8	13	1.20	45.1
Pregelatinized[c] starch				
Freudenberg's	8	30	0.55	45.0
Improved	7	5	1.04	44.0
Amylose				
Freudenberg's	7	33	0.40	44.9
Improved	8	12	0.93	45.4
Amylopectin				
Freudenberg's	9	28	0.64	45.6
Improved	9	8	0.90	45.1

[a] $[\eta]$ is the limit $c \rightarrow 0\ \eta_{sp.}/c$ from viscosities of chloroform solutions, at 25.0°. [b] Calculated for $(C_6H_7O_2[OCH_3]_3)_x$: 45.6% OCH_3. [c] The starch was gelatinized in hot water and the hot paste was beaten in a Waring Blendor before precipitation in ethanol in the Blendor (an unpublished procedure of Jeanes, Deane, Whistler and Hilbert of this Laboratory).

to react with corn starch in liquid ammonia (as shown by the disappearance of the blue color of the solution) was related to the extent of disorganization of the micellar structures of the starch. This is shown in Table II together with the variations in time of reaction encountered with potato starch. When corn starch granules were sufficiently disorganized by the combined action of heat and mechanical shearing forces (IV, Table II), the rate and extent of methylation was nearly the same as that of the amylose and amylopectin fractions. The pretreatment of starch with moist liquid ammonia followed by ethanol (II, Table II)[5] yielded a partially disorganized starch that retained its granular form but did not show the cross characteristic of starch granules in polarized light. This starch gelatinized in cold water and could be completely acetylated in pyridine–acetic anhydride. Waxy corn starch granules were an exception in that they became highly swollen and lost their granular form in liquid ammonia.

Corn starch B (Table III), isolated from the grain with only a distilled water steep and not pretreated before methylation, was not methylated beyond 44.7% OCH_3 and 35% of the preparation was an insoluble gel in chloroform. After two remethylations of the recovered insoluble fraction, it did not become soluble in chloroform, nor was the methoxyl content increased. Apparently untreated starch will retain organized micellar structures throughout our methylation process. The action of sulfurous acid steep on Corn Starch A evidently weakened the associative forces so that a completely soluble product of higher methoxyl content was obtained (Table III). These results indicate that a disorganization of native starch granules by some method is necessary before complete methylation can be effected. The disorganization should not be accomplished during the methylation by imposing severe conditions; rather it should be done independently and, if possible, in a non-hydrolytic medium before methylation.

Amylopectin was not ethylated to the same extent as amylose under equivalent conditions. The

TABLE II

RATE OF REACTION OF STARCHES WITH SODIUM IN LIQUID AMMONIA[a]

		Viscosity of 3% aqueous paste,[b] Cps.	Time required for sodium substitution, min. Atoms of sodium added per C_6 unit 1.0	1.5	2.0
I	Untreated corn starch *A*	96	32	..	....
II	I pregelatinized in moist liquid ammonia and pptd. with ethanol	86	17	50	....
III	I pregelatinized in hot water and pptd. in ethanol using the Waring Blendor	45	4	6	32
IV	Same as III, except the hot paste was beaten in a Waring Blendor	17	3	5	12
V	Amylose of II		4	6	23
VI	Amylopectin of II		3–4	5–8	13–29[c]
VII	Untreated potato starch		180	..	....
VIII	VII pregelatinized as for III		7	23	....

[a] 10.0 g. of dry starch in 600 ml. of anhydrous ammonia at −35°. [b] In 80% water–20% dioxane at 90° MacMichael viscometer. [c] The rate of reaction of amylopectin was variable depending on the physical condition of the dry particles (method of precipitation).

The time required for a given amount of sodium same effect was noted in lesser degree with the methyl derivatives (Table III).[3] When ethylated

(4) (a) K. H. Meyer, M. Wertheim and P. Bernfeld, *Helv. Chim. Acta*, **23**, 865 (1940); (b) K. Hess, H. A. Schulze and B. Krajnc, *Ber.*, **73**, 1069 (1940); (c) R. W. Kerr, "Chemistry and Industry of Starch," 2nd edition, Academic Press, Inc., New York, N. Y., 1950, p. 202.

(5) This method of preparing a reactive granular starch in liquid ammonia, an original discovery of the authors in 1943, has also been used and described by other workers of this Laboratory.

TABLE III

TRIMETHYL AND TRIETHYL ETHERS OF CORN STARCH AND ITS FRACTIONS

	Total no. of treatments	Total Na reaction time, hr.	Alkoxyl, % OCH_3[a]	$[\alpha]^{25}D$ (c 1, $CHCl_3$)	$[\eta]$ ($CHCl_3$, 25°)
Methyl					
Amylose	8	11.5	45.4	+214°	0.93
Amylopectin	7	6.3	45.0	215°	0.93
remethyld.	9	8	45.1	214°	0.90
Starch A	8	13	45.1	215°	1.20
remethyld.	10	17	45.3	215°	1.15
Starch B	9	8	44.7	214°[b]	1.45[b]
remethyld.	11	9.3	44.7	.....	...
Ethyl			OC_2H_5[c]		
Amylose	9	37	54.5	+179°	0.80
Amylopectin	8	26	52.0	177°	0.57
reethyld.	11	32	52.8	.....	...
Starch A	8	40	53.9	180°	0.78

[a] Calculated for $(C_6H_7O_2[OCH_3]_3)_x$: 45.6% OCH_3. [b] For the soluble fraction (65%) only. [c] Calculated for $(C_6H_7O_2[OC_2H_5]_3)_x$: 54.9% OC_2H_5.

amylopectin containing 2.80 OC_2H_5/C_6 (nearly the end-point) was methylated, one treatment was sufficient to raise the degree of substitution to 2.90. This evidence of steric hindrance can be explained by the presence of relatively inaccessible regions either within the amylopectin molecule or between associated molecules. Other factors which tend to prevent complete methylation are the side-reactions of alkyl halides with liquid ammonia and the demethylation of highly methylated starch which occurs in sodium–liquid ammonia solutions.[4b] The existence of this reverse reaction indicates the need for using low concentrations of sodium in the last stages of etherification.[4b]

Experimental

Materials and Apparatus.—Starches A and B were isolated from Iowa 939 corn in this Laboratory, A with a 0.4% sulfur dioxide steep (46 hours at 52°, *p*H 1.7–3.7), and B with distilled water only (46 hours at 52°). The fatty material in both was reduced to 0.2% by extraction with methanol. The nitrogen contents of A and B were then 0.03 and 0.04%, respectively. Before methylation each starch was dried in a vacuum oven at 80°, 1 mm. pressure for 6 hours.

Commercial anhydrous liquid ammonia (min. purity 99.5%), C.P. grade sodium, potassium, methyl iodide and ethyl iodide were used.

The reaction vessel was a one-quart, rubber-stoppered, unsilvered dewar flask of tall cylindrical form, fitted with a stirrer and operated like the one illustrated by Scherer and Feild.[6] Filter sticks with plugs of coarse sintered glass sealed in at the ends of the long arms were introduced through the exhaust tube, and the supernatant liquor from the reaction mixture was collected in traps immersed in solid carbon dioxide–acetone. A glass aspirator connected to the series of traps was used to aid in drawing off the ammoniacal liquor.

Pretreatment of Starch with Liquid Ammonia.—The starch to be fractionated (10–12 pts. by wt.) was soaked in liquid ammonia (100 pts. by vol.) in an open dewar vessel for 15–30 minutes, then the mixture was carefully poured into 3 volumes of ethanol. When most of the ammonia had evaporated, the starch was filtered, restirred with ethanol, filtered and dried *in vacuo* over sulfuric acid and calcium chloride. It was sieved (150–200 mesh) to remove a small amount of horny particles. Analyses of defatted corn starch A before and after the liquid ammonia treatment gave: N, 0.03, 0.04; P, 0.012, 0.009; ash, 0.06, 0.05; fat (methanol extractables, soxhlet, 0.20, 0.12; alkali number,[7] 9.2, 8.5, respectively. Undefatted corn starch gave 0.6% methanol extractables before and 0.2% after the ammonia-alcohol treatment.

Fractionation of Ammonia-Pretreated Starch with Butanol.[8]—Water saturated with *n*-butanol (6 liters) was heated to 90° in a steam-jacketed bain-marie. A slurry of the ammonia-pretreated starch (180 g. dry weight) in 300 ml. of *n*-butanol was slowly added with vigorous mechanical stirring. The mixture was held at 90° with vigorous stirring for one hour, then the fractionation was continued by the method of Schoch.[9] The use of ammonia-pretreated starch allowed complete dispersal of the starch at 90° and, consequently, autoclaving was avoided.

The amylose fraction was recrystallized from *n*-butanol–water without autoclaving. The iodine sorption[10] of the recrystallized amylose was 174 mg./g., corresponding to about 87% pure linear fraction. The alkali number[7] was 19. The amylopectin, isolated by precipitating the centrifugate dropwise in a large volume of rapidly stirred methanol, gave an iodine sorption of 18 mg./g. and an alkali number of 5.5.

Procedure for the Methylation of Starch, Amylose and Amylopectin.—Ten grams of the dried starch or starch fraction was stirred with 600 ml. of anhydrous ammonia run directly from the tank into the dewar reaction vessel. The procedure of Freudenberg and Boppel[2] was followed with the exceptions noted: The temperature of the reaction mixture was −35°. In methylation steps no. (1) and (2) the sodium was added in small pieces at a rate controlled so that the amount of free sodium present in the reaction mixture did not exceed the amount that would react within the next 15 minutes. For amylose, amylopectin and pregelatinized starch the total amounts of sodium added in the first two steps were (1) 2.0 and (2) 1.8 atoms per C_6 unit. In the remaining sodium treatments the amounts of sodium added were (3) 1.4, (4) 0.8—then, after an inverted filtration and liquid ammonia wash—(5) 1.4, (6) 1.0, (7) 0.8 atom per C_6 unit. In no case were the sodium substitution reactions allowed to run for longer than one hour. The molar ratio of methyl iodide to sodium was 1.1 to 1 except for the last step before filtration when it was increased to 1.5 to 1. The time allowed for the methyl iodide to react was 1 to 1.5 hours. When further methylation was necessary, sodium treatments (6) and (7) were repeated after a second filtration and wash. The etherified starch was washed twice with liquid ammonia in the reaction vessel and allowed to dry in air and in a desiccator over sulfuric acid; yield 11–12 g. (88–95%). The product was purified in boiling water and finally by precipitation from chloroform solution dropped into petroleum ether; final yield 10–11 g. (80–88%).

Freudenberg and Boppel[2] found that gelatinization of potato starch to the state of homogeneity could not be allowed during the methylation. In this work with corn starch, gelatinization during the first methylation step (caused by sodium iodide present in the liquid ammonia after the reaction of methyl iodide) was found beneficial; it allowed subsequent sodium substitutions to proceed rapidly and no difficulty was encountered with the separation of the sodium-substituted methylated starch because of the gelatinization. Amylopectin was much more swollen than amylose in the reaction medium.

Ethylation of Starch, Amylose and Amylopectin.—The triethyl ethers were prepared by substituting ethyl iodide for methyl iodide and allowing two or more hours for the etherification reactions with the sodium derivatives. These ethers were not wet by water and were difficult to rid of the amine by-products. Purification was effected by dissolving in glacial acetic acid and precipitating in ammoniacal water.

Action of Potassium Amide on Methyl Amylose.—In two parallel experiments 5 g. of methyl amylose 44.9% OCH_3, $[\eta]$ 0.40, was suspended in 200 ml. of liquid ammonia at −35° and treated with 1.7 g. of potassium for 3 hours. In

(6) P. C. Scherer, Jr., and J. M. Feild, *Rayon Textile Monthly*, **22**, 607 (1941).

(7) T. J. Schoch and C. C. Jensen, *Ind. Eng. Chem., Anal. Ed.*, **12**, 531 (1940).

(8) Wolff, Olds, Karjala and Hilbert (unpublished procedure).

(9) T. J. Schoch, THIS JOURNAL, **64**, 2957 (1942).

(10) F. L. Bates, D. French and R. E. Rundle, *ibid.*, **65**, 142 (1943); E. J. Wilson, T. J. Schoch and C. S. Hudson, *ibid.*, **65**, 1380 (1943).

the first case, the free potassium metal was allowed to act on the methyl amylose; in the second case, the potassium was converted to potassium amide by adding 10 mg. of ferric chloride as a catalyst before adding the methyl amylose. Methyl iodide (2 moles) was the methylating agent. After remethylation with free potassium, the product contained 45.4% OCH_3, [η] 0.41; after remethylation with potassium amide, the product contained 45.5% OCH_3, [η] 0.16. Sodium amide showed the same effect.

Properties of the Ethers.—Whereas Freudenberg and Boppel[2] found no differences between trimethyl amylose and trimethyl amylopectin, our procedure with the improved fractionation technique of Schoch[9] produced these substances with several distinctly different properties. Trimethyl amylose of high viscosity was precipitated from solvents as tough, resilient threads that survived attrition; on the other hand, precipitated amylopectin ethers could be ground easily to a powder. The X-ray diffraction pattern of trimethyl amylose was sharp and distinct, characteristic of crystalline material; that of trimethyl amylopectin was diffuse, characteristic of amorphous material. The films cast from chloroform solutions of high-viscosity methyl amylose were strong and elastic; those from methyl amylopectin or methyl starch were weak and brittle. This difference in film strengths was reported by Meyer, Wertheim and Bernfeld[4] for corn starch fractions incompletely methylated in alkali with dimethyl sulfate.

An aqueous dispersion of trimethyl amylose in cold water gave a deep intense blue color with iodine; trimethyl amylopectin gave a weak greenish-blue color under the same conditions. In a series of methylated corn starches containing 0.8, 1.5, 2.3, 2.7 and 3.0 OCH_3/C_6, the iodine colors were blue, brown, greenish-blue, blue and deep blue, respectively.

The melting ranges of the two methylated starch fractions were also different. Trimethyl and triethyl amylose melted 40 to 50° higher than trimethyl and triethyl amylopectin. Interesting observations were made in connection with the melting ranges of the ethers. The methyl and ethyl derivatives were isolated after three stages of purification: (1) after washing with liquid ammonia, (2) after washing the product from (1) with boiling water, and (3) after precipitation of the product (2) from a solvent. Trimethyl amylopectin (m.p. 150–170°) and triethyl amylopectin (m.p. 160–180°) showed little or no change in the melting range on passing through the three stages of purification; on the other hand, trimethyl whole starch showed a lowering of the melting range from (1) 220–230°, to (2) 180–195°, to (3) 155–195°. Triethyl whole starch showed a similar drop from (2) 225–235° to (3) 185–195°. Trimethyl amylose gave for (1) above 240°, (2) 195–205° and (3) 185–195°; likewise, triethyl amylose gave for (1) above 240°, (2) 220–230° and (3) 205–220°. Since the melting range decreased on purifying whole starch and amylose ethers with solvents, it is assumed that a disaggregation of organized structures took place in these substances. Etherified amylopectin molecules, because of their highly branched, spherical and bush-like nature, would not be expected to associate extensively and, hence, would show no disaggregation by lowering of the melting range. Etherified whole starch, consisting of weakly associated alkylated amylose and amylopectin molecules, would be partially disaggregated by solvent action which would result in a lower melting range approaching that of the pure amylopectin ether. The amylose used in this study contained approximately 13% non-linear molecules by iodine titration; hence, it also could show a lowering of the melting range, either from a disaggregation of unlike molecules or from a disaggregation of associated linear chains.

Another difference in the two etherified starch fractions was shown in the shapes of the curves obtained when reduced viscosity, $\eta_{sp.}/c$, was plotted against concentration. Although the intrinsic viscosities of trimethyl amylose and trimethyl amylopectin were equal, 0.93 (Table III), the values of the reduced viscosities at 0.50% concentration, were 1.09 and 1.33, and at 1.00% concentration, 1.26 and 1.85, respectively. Thus, the amylose curves were nearly straight lines whereas the amylopectin curves showed sharp increases in reduced viscosity at the higher concentrations. In *m*-cresol solutions at 25°, 0.400% concentration, the reduced viscosity of trimethyl amylose prepared by our procedure was 2.07 and of trimethyl starch A, 2.52. These viscosity data may be compared directly with the data obtained by other procedures[4,11–14] which result in either incomplete methylation or much lower viscosities.

The trimethyl and triethyl derivatives of amylose, amylopectin and whole starch A were soluble in chloroform, benzene, glacial acetic acid, ethyl acetate, dioxane, pyridine and boiling ethanol; they were partially soluble in cold water, but the ethyl ethers were quite insoluble. Methyl and ethyl amylose were much less soluble than the corresponding amylopectin derivatives in cold ethanol, acetone, diethyl ether and Diethyl Cellosolve. These observations led to the fractionation of trimethyl and triethyl starch into linear and branched fractions by solvent action.

Fractionation of Trimethyl Corn Starch.—The fractionations by differential solubility in Diethyl Cellosolve were accomplished by (1) extraction and (2) crystallization methods: (1) Suspensions (3–5%) were stirred for 24 hours at 25°, the insolubles removed by centrifugation and restirred with fresh solvent for 6 hours at 25°. (2) Solutions (2–3%) at 80° were allowed to cool and stand at 25° for several days, the insolubles were removed by centrifugation and the solubles precipitated with petroleum ether. By the extraction method two corn starches, both pregelatinized before methylation, gave 25% insoluble fraction; however, methylated untreated starch granules gave 74% insoluble. By the crystallization method three pregelatinized corn starch ethers yielded 21, 21 and 24% precipitate and the methylated untreated granules likewise yielded 26% precipitate. Corn amylose containing 87% linear, 13% non-linear molecules by iodine titration yielded from the trimethyl amylose solution 85% "crystalline" precipitate, and a few per cent. more of insoluble matter precipitated on long standing.

Facts that led to the conclusion that the insoluble fraction from trimethyl starch was linear and the soluble fraction branched were: (1) the percentages of insoluble fraction found by the crystallization method corresponded to the percentage of amylose found in corn starch by Schoch[9] and others. (2) The appearance of the reprecipitated fractions (fibers *vs.* powder), their melting ranges, intensities of color with iodine, and strengths of the films they produced corresponded to those of authentic methyl amylose (for the insoluble fraction) and authentic methyl amylopectin. (3) The graph of $\eta_{sp.}/c$ *vs.* c for solutions of the insoluble fraction was a straight line of slight slope similar to that for methyl amylose solutions; the graph for the soluble fraction was a curved line of steeper slope similar to that for methyl amylopectin solutions. (4) The methoxyl contents of the insoluble fractions were higher (45.3–45.4%) than those of the methyl whole starch (44.7–45.1%), whereas those of the soluble fractions (44.5–44.8%) were lower; the same was true of the ethoxyl contents of fractions of an ethyl starch separated by diethyl ether. These results were as expected, since amylopectin is etherified to a slightly lower extent than amylose under equivalent conditions. (5) The X-ray pattern thrown by the powdered insoluble fraction was distinct and sharp, characteristic of crystalline material, whereas that of the soluble fraction was diffuse, characteristic of amorphous material.

Analytical Methods.—The alkoxyl contents of the ethers were determined by the micromethod of Vieböck and Schwappach[15] as modified by Clark.[16] For 63 methoxyl and 40 ethoxyl determinations, the average deviation from the average for duplicate and triplicate determinations was ±0.15% methoxyl and ±0.26% ethoxyl. The moisture contents (0.3–1.6%) of the ethers varied sensitively with changes in atmospheric humidity; hence, it was necessary to determine the moisture contents of the air-exposed samples immediately before the analyses. The alkoxyl contents were not corrected for ash, which for eight preparations ranged between 0.03 and 0.08%.

The viscosities of chloroform and *m*-cresol solutions of the ethers were determined at 25.0 ± 0.03° in Ostwald–Cannon–Fenske capillary viscometers at 1.0, 0.5, 0.25 and 0.125% concentrations. Kinetic energy corrections were

(11) W. Z. Hassid and R. M. McCready, THIS JOURNAL, **63**, 1632 (1941).

(12) W. N. Haworth, E. L. Hirst and M. D. Woolgar, *J. Chem. Soc.*, 177 (1935); W. N. Haworth, H. Kircher and S. Peat, *ibid.*, 619 (1943).

(13) E. L. Hirst and G. T. Young, *ibid.*, **951**, 1471 (1939).

(14) K. Hess and B. Krajnc, *Ber.*, **73**, 976 (1940); K. Hess and E. Steurer, *ibid.*, **73**, 1076 (1940).

(15) F. Vieböck and A. Schwappach, *Ber.*, **63**, 2818 (1931).

(16) E. P. Clark, *J. Assoc. Off. Agric. Chemists*, **15**, 136 (1932).

applied. The intrinsic viscosity, $[\eta]$, was obtained by graphic extrapolation of $\eta_{sp.}/c$ *vs.* c to zero concentration.

The use of trade names in this paper does not necessarily constitute endorsement of the products or of the manufacturers.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF SASKATCHEWAN]

Studies on Lignin by Means of Catalytic Hydrogenation of Aspen Wood and Wheat Straw[1,2]

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Thatcher wheat straw and aspen wood meals have been hydrogenated under conditions which effected complete solubilization of the lignin. The natures of the isolated lignin fractions of these two angiosperms were compared by techniques of separation based on varying degrees of acidity and chromatographic adsorption. Ultraviolet absorption spectra of the several lignin fractions indicated only minor differences, all curves being typical of other isolated lignins and basically aromatic. From straw, but not from wood, methanol is produced by hydrogenation in larger amounts than may be accounted for by the loss of methoxyl groups. From the aspen wood three lignin degradation products were isolated, 4-hydroxy-3-methoxyphenylethane, 4-hydroxy-3,5-dimethoxyphenylethane and 2-(4-hydroxy-3,5-dimethoxyphenyl)-ethanol in yields of 2.0, 2.6 and 0.37% (based on the Klason lignin content of the wood), respectively. It is suggested that the isolation of phenylethane derivatives indicates a β–γ linkage in the protolignin which is susceptible to cleavage under alkaline conditions but not so under neutral or mildly acidic conditions from which by a similar hydrogenation phenylpropane derivatives have been obtained previously.

Previous communications[3,4] have shown that the high pressure catalytic hydrogenation of maple wood meal converted the major part of the lignin fraction into chloroform-soluble oils, which retained the high methoxyl content of the protolignin and which gave all the indications of being aromatic in character. Pure compounds, representing lignin degradation products, were isolated and identified. They were all phenolic in nature, but their yield based on the original Klason lignin content was in no case very high. This method of isolation of lignin has advantages in the use of a starting material containing all of the lignin *in situ* and the stabilization, by reduction, of any reactive groups formed during the pressure cook, thereby minimizing possible secondary polymerization.

The application of this isolation technique to Thatcher wheat straw and aspen (*Populus tremuloides*) meals was undertaken for two reasons. Firstly, since these plant products are both representative of the angiosperm class, the chemical nature of the lignin should be basically similar[5] and similar degradation products should be obtained from each and it was thought of interest to determine if this were correct. Secondly, it was undertaken to study the similarity of the lignin fractions from the above two samples which had been maturing for approximately one-hundred-day and forty-year periods, respectively.

Solvent extracted samples of both wheat straw and aspen wood were hydrogenated in a dioxane–water medium (1:1), containing 3.0% sodium hydroxide in the presence of Raney nickel catalyst under an initial pressure of hydrogen of 3000 lb. per sq. in., for a period of two to three hours at 165–175°. The residual pulp and catalyst were separated in each case from the liquid components by filtration and negative Mäule and phloroglucinol tests on the pulps indicated complete lignin removal. The average yield of the pulp, determined after removal of the nickel by acid treatment, represented 40 and 51% of the original straw and wood, respectively. For each raw material triplicate hydrogenations were made. By analyzing separately the two-thirds of each of these runs and the combined one-thirds, the reproducibility of both the hydrogenation and the solvent extraction procedures (see below) was found to be no better than fifteen per cent.

The components of the alkaline solutions were separated by virtue of their varying degrees of acidity and thereby separate fractions were obtained which represented the chloroform-soluble neutral (A), chloroform-soluble (B) and insoluble (C) mildly acidic (phenolic or enolic), chloroform-soluble (D) and insoluble (E) strongly acidic, and chloroform-insoluble, ether-soluble neutral (F) reaction products. The proportions of each along with their methoxyl contents are given in Table I.

The mildly acidic fraction "B" of the hydrogenation products was chosen for more detailed study. An attempt was made to separate it by chromatographic fractionation on activated alumina from a chloroform solution. Successive elutions, each continued until no further material was removed, with chloroform, acetone, methanol and dilute alkali separated B into four distinct portions referred to as B-1, B-2, B-3 and B-4, respectively. The percentage of Fraction B and of the original Klason lignin as well as the OCH_3 content of each of these portions is given in Table II.

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(2) Presented, in part, before the Division of Cellulose Chemistry at the Meeting of the American Chemical Society, Chicago, Illinois, September 3–8, 1950.

(3) C. P. Brewer, L. M. Cooke and H. Hibbert, THIS JOURNAL, **70**, 57 (1948).

(4) J. M. Pepper and H. Hibbert, *ibid.*, **70**, 67 (1948).

(5) R. H. J. Creighton, R. D. Gibbs and H. Hibbert, *ibid.*, **66**, 32 (1944).