

form, and the combined filtrates evaporated to give a sirup which was methylated twice more with methyl iodide and silver oxide in the previous manner.

After distilling the methyl iodide, the silver oxide residue was extracted with ether (five 20-ml. portions). Concentration of the ethereal extract gave methyl 5-*O*-(2,3,4,6-tetra-*O*-methyl-*D*-galactopyranosyl)-2,3-di-*O*-methyl-*L*-arabinofuranoside as a yellow, non-reducing sirup (48.5 mg.) which showed $[\alpha]^{25}_D -45^\circ$ in methanol (*c* 1.6).³

Hydrolysis of Methyl 5-*O*-(2,3,4,6-Tetra-*O*-methyl-*D*-galactopyranosyl)-2,3-di-*O*-methyl-*L*-arabinofuranoside.—A solution of the methylated disaccharide (48.5 mg.) in 0.3 *N* sulfuric acid (3 ml.) was heated at 100° for 9 hr. The reaction mixture was neutralized (BaCO₃) and filtered, the salts being washed thoroughly first with water, then ethanol. Concentration of the filtrate gave a yellow sirup (38 mg.) which showed $[\alpha]^{25}_D +32^\circ$ in methanol (*c* 1.3).

Analysis of the sirup by paper chromatography, using solvent D, showed the presence of two components upon spraying either with ammoniacal silver nitrate or with *p*-anisidine, namely, 2,3-di-*O*-methyl-arabinose (*R_f* 0.43) and 2,3,4,6-tetra-*O*-methylgalactose (*R_f* 0.68).

Separation of the methylated components was achieved

by sheet paper chromatography (Whatman No. 1) using solvent D. In addition to the two components noted, a small amount of the reducing unhydrolyzed methylated disaccharide was obtained, *R_f* 0.79.

Identification of 2,3,4,6-Tetra-*O*-methyl-*D*-galactose.—The component corresponding to 2,3,4,6-tetra-*O*-methyl-*D*-galactose was eluted from the filter paper and concentrated to give a chromatographically pure sirup (13 mg.) which showed $[\alpha]^{25}_D +58^\circ$ in ethanol (*c* 0.4). It was converted in the usual manner to *N*-phenyl-*D*-galactopyranosylamine 2,3,4,6-tetramethyl ether, m.p. and mixed m.p. 196–197°, $[\alpha]^{25}_D -78^\circ$ in acetone (*c* 0.4), after recrystallization from ethanol; lit.⁸ m.p. 192°, $[\alpha]_D -77^\circ$ (acetone).

Identification of 2,3-Di-*O*-methyl-*L*-arabinose.—2,3-Di-*O*-methyl-*L*-arabinose was obtained as a colorless sirup (6.5 mg.) which showed $[\alpha]^{25}_D +92^\circ$ in water (*c* 0.3). It was characterized as its 1,4-di-*p*-nitrobenzoate, m.p. and mixed m.p. 149–152°.⁸

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ST. PAUL, MINNESOTA

[CONTRIBUTION FROM THE CEREAL CROPS SECTION, NORTHERN UTILIZATION RESEARCH BRANCH]

Starch Formate

BY I. A. WOLFF, D. W. OLDS AND G. E. HILBERT

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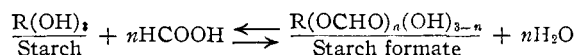
Formylation of starch under the conditions investigated is a reversible reaction; the extent of substitution is dependent upon the ratio of formic acid to starch and on the water content of the system. Appreciation of this fact is required by workers who use formylation of cellulose for accessibility studies and refer to amylaceous materials as standards for comparison, assuming the latter to be 100% accessible. Maximum degree of substitution of starch achieved was 2.3 acyl groups per anhydroglucose unit.

As part of a program at this Laboratory on the preparation of starch esters,² formyl derivatives were desired.

Gottlieb, Moe, Nickerson and their co-workers^{3–5} esterified starch, using 90% formic acid at room temperature and obtained esters having a degree of substitution approximating, but slightly greater than, one formyl group per anhydroglucose unit. Tarkow and Stamm,⁶ using 99% formic acid at 55° also report formation of essentially a monoformate, but in their studies a relatively low ratio of formic acid to carbohydrate was used. All of these authors indicate either exclusive reaction of the formic acid with primary hydroxyl groups or at least preferential, rapid formylation of this grouping. Browning and Sell⁷ state that their work "suggests formylation of secondary hydroxyl groups," though more slowly than primary hydroxyl groups. These authors conducted their experiments with 99.3% formic acid at 51° and demonstrated increased formylation of starch at higher formic acid to

starch ratios. Their conclusions are based on analytical data alone. Reaction products were not isolated. The use of data on the formylation of cellulose as a measure of its accessibility, with reference to amylaceous materials as standards,^{4–6} makes an understanding of the formylation of starch particularly pertinent.

In the studies reported here, starch was esterified at ambient temperature in excess formic acid containing different amounts of water. Formyl esters were isolated by alcohol precipitation at varying time intervals and analyzed for acyl content. Our results indicate that the formylation of starch under the conditions used is best depicted as a reversible reaction



in which equilibrium is reached in about 8 hr. Fortuitous choice of reaction conditions was apparently responsible for isolation of a monoformate by many earlier workers.

Anhydrous formic acid reacts with starch to give a monoformate in only one-fourth of the time needed with the 90% acid (Fig. 1). The final product obtained with this more concentrated acid approximates a diformate. Use of anhydrous starch, of higher proportions of formic acid relative to the starch or reformylation of an isolated "diformate" all raise the degree of formylation still further. The equilibrium nature of the reaction was demonstrated as follows: Sufficient water was added to the reaction mixture of dry starch with

(1) One of the Branches of the Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) (a) I. A. Wolff and C. E. Rist, *THIS JOURNAL*, **70**, 2779 (1948); (b) I. A. Wolff, D. W. Olds and G. E. Hilbert, *ibid.*, **73**, 346 (1951); (c) I. A. Wolff, D. W. Olds and G. E. Hilbert, *Ind. Eng. Chem.*, **43**, 911 (1951); (d) I. A. Wolff, P. R. Watson and C. E. Rist, *THIS JOURNAL*, **74**, 3061, 3064 (1952).

(3) D. Gottlieb, C. G. Caldwell and R. M. Hixon, *ibid.*, **62**, 3342 (1940).

(4) O. A. Moe, S. E. Miller and M. I. Buckley, *ibid.*, **73**, 4185 (1951).

(5) R. F. Nickerson, *Textile Res. J.*, **21**, 195 (1951).

(6) H. Tarkow and A. J. Stamm, *J. Phys. Chem.*, **56**, 266 (1952).

(7) B. L. Browning and L. O. Sell, *Textile Res. J.*, **23**, 939 (1953).

anhydrous formic acid after maximum reaction was obtained to lower the acid strength to 90%. The product then isolated corresponded to that expected if 90% formic acid had been used initially.

It was not possible to prepare starch triformate by removal of water with anhydrous calcium sulfate or azeotropically with benzene. The highest degree of substitution attained was 2.3 formyls per C_6 unit. However, failure to achieve preparation of the triester may still be simply a question of equilibrium, with the conditions employed being inadequate for removal of the last traces of water from the system. The large effects of small quantities of water in displacing the equilibrium toward the side of deacylation are demonstrated by the work of Hiller⁸ on a comparable cellulose-acetic acid system. In studying equilibrium combined acetic acid content of cellulose acetate in homogeneous, uncatalyzed reactions, Hiller found that (at 94.2°) presence of 0.5% water resulted in equilibrium degree of substitution of 2.75 acetyl groups per C_6 unit; 2.8% water in the system gave equilibrium substitution of 1.9.

Our results agree with those of other investigators in indicating preferential reactivity of the 6-hydroxyl group, as deduced from periodate analysis of "mono-" and "di-" formates. Starch monoformate consumed approximately one mole of oxidant per mole of anhydrohexose present. The starch diformate was oxidized by the periodate only to the extent of 0.15 mole of oxidant consumed per anhydroglucose unit. This fact indicates a fair degree of homogeneity for the diformate, although the possibility exists that some monoformate, with compensating amounts of triformate ester, may be present. In other studies we have shown⁹ that under conditions employed in the periodate oxidation analysis a minor, but definite and measurable, hydrolysis of formate esters can occur.

The free hydroxyl groups in starch formates can be esterified to give completely substituted mixed esters.

The solid starch esters developed a strong odor of formic acid within several days when left exposed to the laboratory atmosphere.

Experimental

Materials.—High grade commercial corn starch served as the carbohydrate raw material. Eastman Kodak Co. anhydrous formic acid (98–100%) and Merck and Co., Inc., reagent (approximately 90%) formic acid were used in the esterifications.

Analytical Procedures.—Formyl analysis on the starch esters was carried out by saponification at room temperature for 48 hr. with 0.25 *N* potassium hydroxide in aldehyde-free absolute ethanol. An excess of 0.25 *N* hydrochloric acid was added at the end of the saponification period and after several hours the samples were titrated to a phenolphthalein end-point with 0.1 *N* aqueous sodium hydroxide.

Esterification Methods.—Esterifications of the starch were carried out by adding the required amount of starch to formic acid which was being rapidly stirred mechanically at room temperature. Mechanical stirring was continued throughout the reaction period. Gelatinization always occurred in a short time. In about 2 hr. the mixture was practically homogeneous and free from gelled particles. From then on the mixture gradually became clearer and less viscous. At the end of the reaction period the esters were

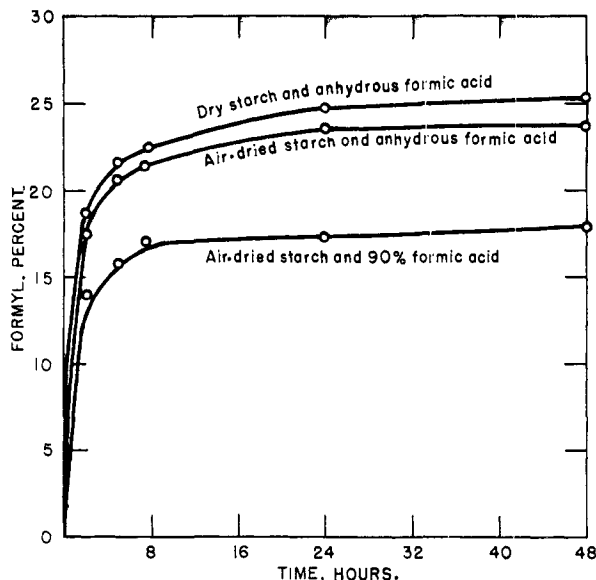


Fig. 1.—Esterification of starch with formic acid.

precipitated in absolute ethanol (Waring Blendor), washed three times in the Blendor with this solvent and finally dried *in vacuo* at 25 and 70°. The esters were all white powders.

Reaction of Air-dried Starch with 90% Formic Acid.—Thirty grams of air-dried starch was treated with 300 ml. of 90% formic acid. Samples were withdrawn at various time intervals, precipitated and analyzed:

Reaction time, hours	Formyl, % ^a
2	14.0
5	15.7
7.5	17.0
24	17.3
48	17.9

^a Calcd. for starch monoformate, $C_7H_{10}O_6$: 15.3% formyl.

Reaction of Air-dried Starch with Anhydrous Formic Acid.—Reaction of 30 g. of air-dried starch with 300 ml. of anhydrous formic acid and withdrawal of samples, as above, gave materials with the following analysis:

Reaction time, hours	Formyl, % ^a
2	17.75
5	20.6
7.5	21.4
24	23.65
72	24.2

^a Calcd. for starch diformate, $C_8H_{10}O_7$: formyl, 26.6.

If Drierite is added initially to a reaction mixture such as the above, the product after 5 hr. has 20.7% formyl and 1.05% ash.

Twenty-five grams of vacuum oven-dried starch formate (18.8% formyl) prepared by 3 hr. reaction of air-dried starch with anhydrous formic acid was reformylated for 4.5 hr. with 300 ml. of anhydrous formic acid. The formyl content of the product was 24.4%.

To test the effect of concentration 30 g. of air-dried starch was reformylated in 600 ml. of anhydrous formic acid. The course of the reaction was as shown:

Reaction time, hours	Formyl, %
2	20.1
5	23.2
7	24.3
24	27.3
48	28.0

The 24-hr. sample above (3.3 g. vac. oven dry wt.) was reformylated for 7.5 hr. with 80 ml. of anhydrous formic acid. This material was precipitated in diethyl Cellosolve,

(8) L. A. Hiller, Jr., *J. Polymer Sci.*, **10**, 385 (1953).

(9) I. A. Wolff, B. T. Hofreiter, P. R. Watson, W. L. Deatherage and M. M. MacMasters, *THIS JOURNAL*, **77**, 1654 (1955).

washed with that same solvent and dried *in vacuo*; formyl found: 29.5 (equivalent to a degree of substitution of 2.3 formyl groups per anhydroglucose unit). This small increase in formyl content indicates that in other formylations ethanolysis during precipitation and washing is not a factor in preventing complete substitution of hydroxyl groups with formyl.

Thirty grams of air-dried starch was formylated for 4 hr. in 300 ml. of formic acid. Benzene (200 ml.) was added in 70-ml. portions and removed by distillation *in vacuo*. Bumping occurred and the viscous paste containing the partially formylated starch did not mix well with the benzene. After the surface layer of benzene disappeared, 150 ml. of anhydrous formic acid was added, and the mixture was allowed to stand overnight. The formate then isolated contained 24.8% formyl, not significantly higher than if no benzene distillation had been used.

Reaction of Dry Starch with Anhydrous Formic Acid.—To 300 ml. of anhydrous formic acid was added gradually 30 g. of corn starch which had been dried overnight at 110° *in vacuo* in the presence of Drierite. Samples precipitated in ethanol after varying time intervals gave these analyses:

Time, hours	Formyl, %
2	18.7
5	21.7
7.75	22.6
24	24.8
48	25.4

Thirty grams of corn starch was formylated as above. One hundred milliliters of the mixture was precipitated after 24 hr. To the remainder 25 ml. of water was added and reaction was continued for 27 hr. longer. The remainder was then precipitated. The analysis of these products was: 24-hr. product, 26.1% formyl; 51-hr. product, 18.9% formyl.

The starch formates are soluble in pyridine and in formic acid but not in the other common organic solvents tested.

Optical Rotation of Starch Formates.—The rotation of a formyl ester with 1.3 formyl groups per C₆ (19.0% formyl) was $[\alpha]^{25}_D +180.6^\circ$ (pyridine, $c = 1$). A formate containing 2.1 formyl per C₆ (27.7% formyl) had $[\alpha]^{25}_D +166.5^\circ$ (pyridine, $c 1$).

Oxidation of Starch Formate with Periodate.—Samples of starch formate were treated with an excess of sodium periodate. After 24 hr., analysis for excess periodate was carried out.¹⁰ Data are summarized below.

Formyl/C ₆ unit	Wt. sample, g.	Starch formate	
		Periodate consumed (millimole)	Millimoles periodate per millimole repeat- ing unit
2.10	0.3050	0.21	0.15
1.16	.1449	.80	1.08

Carbanilation of Starch Formate.—To 19.0 g. of oven-dried starch formate (18.8% formyl, 1.28 formyl per C₆) in 125 ml. of dry pyridine was added 35 g. of phenyl isocyanate, and the mixture was heated for 6 hr. at 100°. The ester, isolated by precipitation with ethanol, had 5.99% N; calcd. 5.94% N. Similar preparation of other mixed esters from starch formates indicates that formyl esters are produced and that the formic acid is not held merely by sorption or solvation onto the carbohydrate.

Acknowledgment.—The authors are indebted to T. A. McGuire and C. H. Van Etten for assistance with the formyl analyses. The use of trade names in this paper does not necessarily constitute endorsement of these products or of the manufacturers thereof.

(10) Jackson (Adams, Editor-in-Chief), "Organic Reactions," Vol. II, John Wiley and Sons, New York, N. Y., 1944, pp. 341-375.

PEORIA, ILLINOIS

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PUBLIC HEALTH SERVICE}

1,2:3,5-Di-*O*-benzylidene- α -D-glucose

BY HARRY B. WOOD, JR., HARRY W. DIEHL AND HEWITT G. FLETCHER, JR.

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Condensation of D-glucose with benzaldehyde in the presence of zinc chloride-acetic acid gives a di-*O*-benzylidene-D-glucose identical with a substance which earlier authors obtained through successive benzylidenation and debenzoylation of 6-*O*-benzoyl-D-glucose diethyl dithioacetal. Evidence is presented showing the acetal to be 1,2:3,5-di-*O*-benzylidene- α -D-glucose.

In 1937, Papadakis¹ published a study of the behavior of 6-*O*-benzoyl-D-glucose diethyl dithioacetal with benzaldehyde in the presence of zinc chloride. Under the conditions which this author employed, the thioethyl groups were lost and a non-reducing *O*-benzoyl-di-*O*-benzylidene-D-glucose was obtained as the sole reaction product. Deacylation of the latter compound afforded a di-*O*-benzylidene-D-glucose, m.p. 163°, which was oxidized to a di-*O*-benzylidene-D-glucuronic acid, thus demonstrating that the di-*O*-benzylidene-D-glucose was unsubstituted at C₆ and that the benzoyl group in the starting material had not wandered in the process of cyclic acetal formation.²

(1) P. Papadakis, *THIS JOURNAL*, **59**, 841 (1937).

(2) Normally, O \rightarrow O acyl migrations progress toward, rather than away from primary positions although migrations in the opposite direction under acidic and somewhat violent conditions have been observed: R. C. Hockett, H. G. Fletcher, Jr., E. L. Sheffield and R. M. Goepf, Jr., *THIS JOURNAL*, **68**, 927 (1946); R. C. Hockett, H. G.

In the same year Wolfrom and Tanghe³ reported a more extensive study of the benzylidenation of 6-*O*-benzoyl-D-glucose diethyl dithioacetal. These authors were able to isolate a 6-*O*-benzoyl-di-*O*-benzylidene-D-glucose diethyl dithioacetal as an intermediate which, on further treatment with benzaldehyde and zinc chloride, afforded a non-reducing *O*-benzoyl-di-*O*-benzylidene-D-glucose melting at 160-160.5°. Debenzoylation gave a di-*O*-benzylidene-D-glucose melting at 163-165°. That Wolfrom and Tanghe's di-*O*-benzylidene-D-glucose and its benzoate were identical with the corresponding compounds prepared by Papadakis seems quite certain although the latter author unfortunately failed to report the rotations of his substances.

Recent investigations in this Laboratory have demonstrated, through experiments in the D-ribose

Fletcher, Jr., E. L. Sheffield, R. M. Goepf, Jr., and S. Soltzberg, *ibid.*, **68**, 930 (1946).

(3) M. L. Wolfrom and L. J. Tanghe, *ibid.*, **59**, 1597 (1937).