[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

Action of Formic Acid on Starch*

BY DAVID GOTTLIEB, C. G. CALDWELL AND R. M. HIXON

The use of formic acid as a solvent for starch in plant tissues has been suggested by Carr.[1] In a more recent publication, Alexander[2] suggested this solvent as a means of fractionating starch, the amylopectin being precipitated in the presence of a protective colloid. As far back as 1904 Kldiashvile[3] described the production of a starch formate, and a few years later Traquair[4] reported the formation of mono-, di- and triformates by changes in the reaction temperatures.

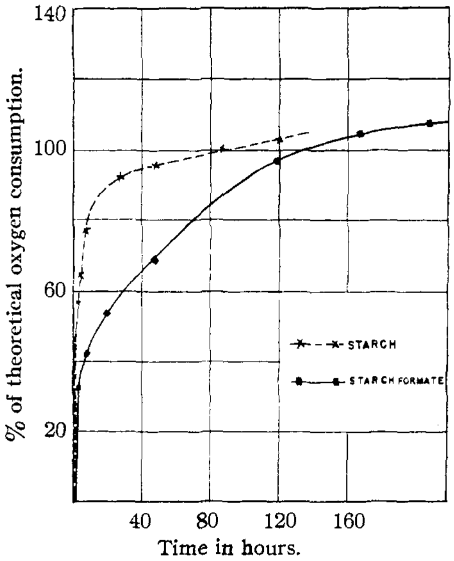

Fig. 1.—Oxidation of starch and starch formate by periodic acid.

In attempting to fractionate starch by Alexander's procedure, it was found that an ester of starch was formed which approximated the composition of a starch monoformate quite independent of the reaction conditions. The red color with iodine which Carr believed caused by the slight hydrolysis of starch by the acid and which Alexander considered to be the iodine color of the amylopectin was found to be due to the presence of the formyl group in the starch chain. This can be demonstrated by quantitative hydrolysis of the formate ester and recovery of starch giving the original blue iodine color. The di- and triformates reported by Traquair could not be obtained.

The existence of a definite monoformate of starch would be contrary to the general experience with other derivatives of starch and cellulose as summarized by Spurlin[5]:

> The substituents in a cellulose derivative are distributed among the hydroxyls according to the laws of chance, and the three sorts of hydroxyls are substituted to an extent relative to one another that is determined by the relative reactivities of the hydroxyls. . . and the nature of the reaction. . . .

Partial derivatives of starch have, however, been reported recently by Gomberg and Buchler[6] and by Tomecko and Adams.[7]

Oxidation of the starch formate with periodic acid (Fig. 1) and isolation of a monoformyl ester of the oxidized product in analogy with the work of Jackson and Hudson[8,9] on the structure of starch by this reagent would indicate that most of the formyl groups must be on carbon six of the glucose molecule. Conclusive proof of structure by methylation is precluded by the ease of hydrolysis of the formyl group. Tosylation followed by hydrolysis with hydrogen bromide in glacial acetic acid[10] gave a compound agreeing in composition with the expected bromo-diacetyl-ditosyl glucose. The latter, however, could not be crystallized. Amorphous derivatives of starch monoformate also were obtained which analyzed as monoformyl-diacetyl starch and monoformyl-ditosyl starch.

Formylation of limit dextrins gave a product approximating the composition of a monoformyl dextrin, thereby showing that mechanical packing of the glucose units was not the limiting factor in the production of a monoformate. Starch converted to the triacetate, saponified, and then treated with formic acid still gave a monoformate ester. This reaction and the product formed by periodic acid oxidation would indicate that the limitation of the reaction cannot be explained as due to secondary valences which muzzle positions two and three in the glucose chain.

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[1] Carr, *Science*, **69**, 408 (1929).

[2] Alexander, *J. Soc. Chem. Ind.*, **55**, 206 (1936).

[3] Kldiashvile, *J. Russ. Phys.-Chem. Soc.*, **37**, 421 (1905).

[4] Traquair, *J. Soc. Chem. Ind.*, **28**, 288 (1909).

[5] Spurlin, THIS JOURNAL, **61**, 2222 (1939).

[6] Gomberg and Buchler, *ibid.*, **43**, 1904 (1921).

[7] Tomecko and Adams, *ibid.*, **45**, 2698 (1923).

[8] Jackson and Hudson, *ibid.*, **59**, 2409 (1937).

[9] Jackson and Hudson, *ibid.*, **60**, 989 (1938).

[10] Hess and Pfleger, *Ann.*, **507**, 49 (1933).

Microscopic studies of extremely fine, precipitated threads indicated some molecular orientation, whereas the usual amorphous form shows no birefringence. With dilute solutions of the formate in formic acid, thin films of the monoformate can be produced on the surface of water. These films produce interference colors.

Experimental

Starch Monoformate.—Five grams of cornstarch was shaken with 50 ml. of 90% formic acid. The starch swelled slowly, jelled, and at the end of twenty-four hours reverted to a viscous solution. Upon pouring this solution into an excess of cold water a white plastic coagulum resulted. The precipitate was ground with 80% alcohol, washed till acid free and dried under reduced pressure at 70° giving a white amorphous powder. The formates of waxy maize starch and ball-milled starch were produced in a similar manner. The rotation of all preparations fell between the limits $[\alpha]^{25}\text{D}$ +2.09° to +2.16° in pyridine. The solid formate stained a characteristic red-brown color with iodine, but after saponification in dilute sodium hydroxide the recovered product gave the normal blue starch iodine color.

Anal. Calcd. for starch monoformate $C_7H_{10}O_6$: 15.26% formyl. Found for cornstarch: 16.19%; for waxy maize starch: 16.88%; for ball-milled starch: 16.88% formyl.

Attempts to obtain higher esters by increasing the reaction time to fifteen days raised the formic acid content of the ester to only 17.35%. Variation in the ratio of formic acid to starch likewise did not affect the composition of the reaction product. Increasing the temperature of the reaction to 85° for twenty-four hours produced so much hydrolysis that the reaction products could not be precipitated by water. Precipitation with alcohol gave a product of the following composition:

Anal. Calcd. for a dextrin monoformate $C_7H_{10}O_6$: 15.25% formyl. Found: 15.98% formyl.

Oxidation of Starch Formate.—One-tenth gram samples of starch formate were placed in Erlenmeyer flasks. Twenty-five ml. of periodic acid solution was added (prepared by dissolving 20.96 g. of sodium paraperiodate in 232.8 ml. of 0.9138 *N* sulfuric acid). The suspensions were allowed to stand at room temperature for lengths of time ranging from one-half to 240 hours, after which they were filtered and thoroughly washed. The filtrates were treated with an excess of potassium iodide and acidified with hydrochloric acid. The released iodine was titrated with sodium thiosulfate. Starch samples were run under similar conditions. A comparison of the amounts of periodic acid used in the oxidation of starch and its formate ester is shown graphically (Fig. 1). While the reaction was slower for the formate ester than for the original starch, it followed the same general course.

Isolation and Identification of Oxidation Products.—Eight grams of the formate was oxidized with periodic acid as described above. The suspended product was filtered, washed to remove all salts and dried under reduced pressure. A copper reduction number of 3581 was obtained for the oxidized formate as compared to 20.1 for the original formate ester and 7.9 for the original starch. Portions of the oxidized formate were hydrolyzed in 50% phosphoric acid for twenty-four hours at room temperature. Tests for the presence of formic acid in the oxidized formate were conducted according to the procedure of Klein and Wenzel[11] and a quantitative determination made using the apparatus described by Reid and Weihe.[12]

Anal. Calcd. for $C_7H_8O_6$: 15.26% formyl. Found: 13.98%.

One gram of the oxidized formate was suspended in 40 ml. of water and the mixture heated on a steam-bath for three hours. After filtering off any sediment, the mixture was placed in a pressure bottle with 5 ml. of *N* hydrochloric acid and kept at 99° for twenty hours. The solid decomposition products were filtered off and discarded. The filtrate was decolorized, neutralized with silver carbonate, and the excess silver precipitated with hydrogen sulfide. The filtrate was next treated with phenylhydrazine buffered with sodium acetate, and the amorphous yellow precipitate reprecipitated from alcohol; yield, 0.271 g. of crude glyoxalphenylosazone, or 21.5% of the calculated amount. Further purification by recrystallization from benzene proved exceedingly difficult and a yield of only 5% of pure glyoxal osazone was obtained, m. p. 170°.

Monoformyl-diacetyl Starch.—Five grams of starch monoformate was dissolved in 60 ml. of pyridine, 60 ml. of acetic anhydride added, the suspension shaken for five hours and poured into 95% alcohol. The purified white precipitate was saponified in an excess of sodium hydroxide and back-titrated with standard acid.

Anal. Calcd. for monoformyl-diacetyl starch $C_{11}H_{14}O_8$: 10.96 ml. of *N* NaOH. Found: 10.99 and 10.81 ml. of *N* NaOH.

Monoformyl-ditosyl Starch.—Five grams of starch formate was dissolved in 25 ml. of pyridine which had first been dehydrated by distilling over barium oxide. To the pyridine solution was added 25 g. of *p*-toluenesulfonyl chloride, 2 g. at a time over a period of one hour. The mixture was shaken for three days and then poured into cold water. The precipitate was ground under water, washed till free of pyridine, dried, and then extracted with ether for twenty-four hours. The sulfur content was determined by combustion in a pearl tube; tests showed the absence of chlorine.[10]

Anal. Calcd. for monoformyl-ditosyl starch $C_{21}H_{22}O_{10}S_2$: S, 12.85. Found: S, 12.71.

Bromo-diacetyl-ditosyl Glucose.—Ten grams of ditosyl-monoformyl starch was shaken with 200 g. of 32% hydrogen bromide in glacial acetic acid for twenty-one days, and then precipitated by pouring into a mixture of ice water and chloroform. The chloroform layer was washed with water until all traces of acid had disappeared, evaporated under reduced pressure, and the brown colored residue dissolved in 100 ml. of benzene. A precipitate was obtained by allowing the benzene solution to drop into a large excess of petroleum ether with constant stirring.

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(11) Klein and Wenzel, *Mikrochem.*, **5**, 70 (1932).

(12) Reid and Weihe, *Ind. Eng. Chem.*, *Anal. Ed.*, **10**, 271 (1938).

The precipitate changed from white to tan upon filtering and washing with petroleum ether. After drying it was analyzed for bromine and sulfur. The compound was soluble in toluene, xylene, benzene, chloroform, hot ethyl and hot methyl alcohol; insoluble in water, petroleum ether, and cold ethyl and methyl alcohol. Since the bromo-diacetyl-ditosyl glucose could not be obtained crystalline, a benzene solution was precipitated in 3 fractions to demonstrate homogeneity of the reaction product.

Anal. For bromo-diacetyl-ditosyl glucose $C_{24}H_{26}O_{11}S_2Br$:

	S, %	Br, %
Calcd.	10.21	12.75
Found: Fraction 1	10.20	12.59
Fraction 2	10.12	12.46
Fraction 3	10.17	12.65

Summary

1. The action of formic acid on starch produces an ester approximating a monoformate in composition.

2. Oxidation of the formate ester with periodic acid indicates that the formyl group is located primarily on carbon six of the glucose units.

3. Amorphous products analyzing as monoformyl-diacetyl starch and monoformyl-ditosyl starch were prepared.

4. Hydrolysis of the monoformyl-ditosyl starch yielded a compound which analyzed as bromo-diacetyl-ditosyl-glucose.

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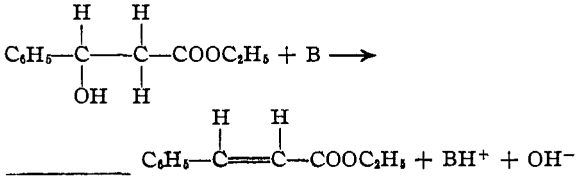
[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF DUKE UNIVERSITY]

The Mechanism of Elimination of Water from Organic Compounds in the Presence of Bases[1]

BY CHARLES R. HAUSER AND DAVID S. BRESLOW

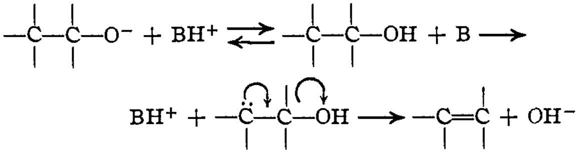
Apparently it is not generally recognized that bases can facilitate the elimination of water from organic compounds, although certain well-known reactions which presumably involve dehydration, for example, the aldol condensation of benzaldehyde with ketones or esters, are commonly effected by means of bases at ordinary temperatures.

Recently, we have stopped the reaction of benzaldehyde with ethyl acetate at the aldol stage.[2] It has now been shown that on standing with sodium triphenylmethyl in ether solution at room temperatures, the aldol, ethyl β-phenyl-β-hydroxypropionate, eliminates water to form ethyl cinnamate, whereas under comparable conditions but in the absence of the base the aldol appears to be quite stable. Also, it has been found that the elimination of water from ethyl β-phenyl-β-hydroxypropionate is facilitated by sodium ethoxide in ether. The elimination of water from the aldol by means of bases may be represented as follows, where B represents triphenylmethyl ion or ethoxide ion.



$$C_6H_5-\underset{OH}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-COOC_2H_5 + B \longrightarrow C_6H_5-\overset{H}{C}=\overset{H}{C}-COOC_2H_5 + BH^+ + OH^-$$

It is suggested that the mechanism of elimination of the elements of water from organic compounds in the presence of bases is analogous to the mechanism of the removal of hydrogen halide from alkyl halides by means of these reagents. In the latter case it is generally considered[3] that the hydrogen is attacked and removed as a proton, accompanied or followed by the release of halogen as halide ion. With hydroxyl compounds, however, it is necessary to take into account the fact that the hydroxyl hydrogen is much more active than the hydrogen attached to the carbon. The reactions may be represented by the equations



$$-\overset{H}{\underset{|}{C}}-\underset{|}{C}-O^- + BH^+ \rightleftarrows -\overset{H}{\underset{|}{C}}-\underset{|}{C}-OH + B \longrightarrow$$

$$BH^+ + -\underset{|}{\ddot{C}}-\underset{|}{C}-OH \longrightarrow -\underset{|}{C}=\underset{|}{C} + OH^-$$

Although the hydroxyl hydrogen is much more active than the hydrogen attached to the β-carbon atom, the acid-base reaction involving the hydroxyl hydrogen is reversible. The acid-base reaction involving the hydrogen attached to the β-carbon atom also may be reversible, but the carbanion readily loses hydroxyl ion to give the olefinic double bond, the latter reaction being essentially irreversible. The removal of the proton

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[1] This work was supported in part by a grant from the Duke University Research Council.

[2] Hauser and Breslow, THIS JOURNAL, 61, 793 (1939).

[3] See especially Hauser, *ibid.*, 62, 933 (1940).