adenosine triphosphate molecule, it was essential to have some estimate of the magnitude of the rate for the competing, non-terminal hydrolysis reaction at the ribose–phosphorus linkage. For this purpose, fructose-6-phosphoric acid (FPA) was selected as an available model compound to yield order of magnitude information on the hydrolysis rate of the primary sugar hydroxyl–phosphorus grouping.

Previous reports[2,3] on the hydrolysis of fructose mono- and diphosphates have indicated a greater lability toward aqueous hydrochloric acid of the 1-phosphate linkage as compared to the 6-grouping,[2] and a rate constant[3] for the relatively rapid hydrolysis of fructose-1-phosphate (in 0.1 N HCl at 100°) amounting to 1×10^{-2} sec.$^{-1}$. In the present work, the 6-phosphate ester hydrolysis was carried out under somewhat milder conditions, closely approximating those used in previous kinetic work[4] on the acid-catalyzed hydrolysis of triphosphoric and pyrophosphoric acids; reaction temperatures of 40 and 50° were employed, and at each temperature hydrolysis rates were determined at two levels of acidity. No additional inert salt was present in these rate runs beyond that introduced in the sample preparation procedure.

The stoichiometric hydrolysis reaction

$$C_3H_9O_5\text{–}CH_2OPO_3H_2 + H_2O \longrightarrow C_6H_{12}O_6 + H_3PO_4$$

was followed by colorimetric evaluation of inorganic phosphate evolved, using the method of Lowry and Lopez.[5] First-order rate constants were calculated for the exceedingly slow hydrolyses over roughly the first several per cent. of each reaction, corresponding to a total reaction time of about 10 days. Values for these constants and their observed deviations are summarized in Table I.

TABLE I

ACID-CATALYZED HYDROLYSIS OF FRUCTOSE-6-PHOSPHORIC ACID (FPA)

Run	Reaction temperature,[a] °C.	Initial concentrations Acid catalyst, N	FPA, M	k_1, sec.$^{-1} \times 10^5$
1	39.93	H_2SO_4, 0.0247	0.0210	1.09 ± 0.06
2	39.93	H_2SO_4, .0914	.0210	1.75 ± .07
3	50.23	H_2SO_4, .0247	.0210	4.11 ± .10
4	50.23	H_2SO_4, .0914	.0210	6.52 ± .47
5	39.93	HCl, .0853	.0210	1.26 ± .08

[a] Temperatures held constant to ±0.02°.

The data of Table I indicate first of all that the hydrolysis is markedly slower than that of the 1-phosphate ester, and subject to acid catalysis (compare runs 1 and 2, 3 and 4). Also, comparison of the rate constants for FPA with those for hydrolysis of pyrophosphoric and triphosphoric acids[4] under comparable conditions of acidity and temperature reveals that cleavage of the phosphate entity from FPA *is slower by a factor of 10^2 to 10^3* than hydrolytic cleavage of a single phosphate group from either polyphosphate species. This fact lends considerable support to the probability that acid-catalyzed hydrolysis of adenosine triphosphate under equivalent conditions will proceed largely by cleavage at the terminal phosphate grouping, without a significant contribution from slower hydrolysis at the internal sugar–phosphorus bond.

In this connection, it is also of considerable interest that both the acid-catalyzed[4,6] and enzymatically-catalyzed[7] hydrolysis of the related polyphosphate, triphosphoric acid, involve cleavage of a terminal phosphate residue.

Further, the data of Table I permit a rough comparison of the temperature coefficients for the hydrolysis of FPA at relatively low (0.025 N) and high (0.09 N) levels of added acid. For the temperature interval employed, both low acidity (runs 1 and 3) and high acidity (runs 2 and 4) determinations lead to virtually the *same* temperature coefficient for k_1, *i.e.*, a factor of about 3.7 for 10.3°. This would imply that both the catalyzed and uncatalyzed contributions to observed k_1 values have the same temperature dependence.

As a final observation, the results of run 5 employing HCl as catalyst (compared to 1 and 2 using H_2SO_4) point to the relative insensitivity of the hydrolysis to the nature of the strong acid used as catalyst.

Experimental

The barium salt of fructose-6-phosphoric acid was used directly as supplied by the Nutritional Biochemicals Corporation. Analyses for total hydrolyzable phosphate and for barium ion indicated a maximum purity of 95.5% with respect to barium fructose phosphate. Triply distilled water was used in all rate determinations.

In a given rate run, a weighed portion of barium salt was dissolved completely in about 25 ml. of water, giving a pale yellow solution. To this was added the equivalent quantity of sodium sulfate, in small portions with continual stirring. The required amount of a standard solution of acid catalyst was then added by pipet, with stirring. After standing in the cold for about five minutes, the mixture was filtered directly into the 50-ml. volumetric flask used as the reaction vessel, with about 15 ml. of water being used in the transfer and subsequent washing. The solution was then placed in the constant temperature bath, allowed to come to temperature, and made to volume with preheated water.

Aliquots (2 ml.) of reaction mixture were withdrawn at intervals of roughly 24 hours, delivered into about 40 ml. of water containing 0.4 millimole of sodium acetate, made to 50 ml., and finally analyzed colorimetrically[5] for inorganic phosphate. Concentrations of FPA were calculated from initial values and the subsequent analytical values for phosphate.

In general, one to two days of reaction time was allowed to elapse before the initial phosphate determination was made, to ensure the completion of an induction period noted occasionally.

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NAVAL MEDICAL RESEARCH INSTITUTE
BETHESDA 14, MARYLAND

The Electrolytic Preparation of Periodate Oxystarch

BY WILLIAM DVONCH AND C. L. MEHLTRETTER

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The usefulness of periodic acid in preparative work is limited by its high cost. Since periodic acid is prepared electrolytically,[1,2] it seemed probable

(1) E. Müller and O. Friedberger, *Ber.*, **35**, 2652 (1902).

(2) H. H. Willard and R. R. Ralston, *Trans. Electrochem. Soc.*, **62**, 239 (1932); A. Hickling and S. H. Richards, *J. Chem. Soc.*, 256 (1940).

that a practical electrolytic method for periodate oxidations might be developed wherein the periodate would be continuously regenerated *in situ*. Thus, only a fraction of the theoretical amount of periodic acid required for the oxidation would be needed. We report here the application of such a method to the oxidation of corn starch to oxystarch by known procedures for the electrolytic oxidation of iodic to periodic acid.[1,2]

The product is nearly identical with the oxystarch we prepared by the method of Purves.[3] Oxystarch was also prepared by oxidation with periodic acid as described by Jackson and Hudson.[4] Data on the preparation and characterization of the products by the three methods are given in Table I. Comparison of the dialdehyde content

TABLE I

ANALYSES OF PERIODATE OXYSTARCHES[a]

Oxystarch	Temperature of oxidation, °C.	Yield, %	$[\alpha]^{25}D$ (*c* 2, water)	Dialdehyde,[b] %	Dicarboxyl,[c] %	Yield brucine D-erythronate, %
Electrolytic	40–45	89	+17°	89		77
	40–45	91	+19°	89	0.2	
	20–25	85	+38°	81		45
Purves	12–25		+14°	89	.03	
	12–25	98	+15°	89		85
Jackson and Hudson	25	97	+ 9°	68	.1	

[a] All values on dry basis. [b] Estimated by oxime formation using Method A described by E. K. Gladding and C. B. Purves, *Paper Trade J.*, 116, 150 (1943). [c] Based on calcium acetate method described by E. C. Yackel and W. O. Kenyon, THIS JOURNAL, **64**, 121 (1942).

of the oxystarches made by the non-electrolytic methods substantiates Purves' contention that oxidation is selective only below 20° and at *p*H 2–5.[3c] The data for the electrolytic oxystarches prepared at 40–45° indicate that these restrictions do not apply to the electrolytic oxidation.

Jackson and Hudson[5] characterized their oxystarch and oxycellulose by hydrolyzing with 0.1*N* acid and identifying the D-erythrose present by oxidation with bromine to D-erythronic acid, isolated as the brucine salt. They obtained 22 and 15% yields from oxystarch and oxycellulose, respectively. These low yields were attributed in part to destruction of the material during hydrolysis and to incomplete degradation of the polymer. In substantiation of this, Pacsu[6] isolated the 2,4-dinitrophenylhydrazone of glyoxylic acid from periodate oxycellulose in 75% yield when bromine oxidation preceded hydrolysis in *N* acid. Accordingly, oxystarches prepared electrolytically and by Purves' method were oxidized by bromine and brucine D-erythronate isolated after hydrolysis (Table I).

Experimental

Electrolytic Oxidation.—The cell consisted of a battery jar (150 mm. in diameter by 150 mm. high) which contained a Coors' porous cup (90 mm. in diameter by 145 mm. high) as the anode compartment. The cathode was several turns of 1/4-in. o.d. lead tubing which served as a cooling coil. The anode of lead dioxide was prepared by oxidizing the lower 60 mm. of a 175-mm. by 25-mm. by 2-mm. lead strip, freshly roughened with a steel brush, in *N* sulfuric acid at 1.5 amperes for 30 minutes. The coated anode was then washed in water for 30 minutes.

The corn starch was prepared by extraction with methanol, drying over concentrated sulfuric acid, and equilibrating to room humidity after grinding to pass 60 mesh. Its moisture content and that of the oxystarches were determined by drying for eight hours in an Abderhalden dryer at 100°.

The anolyte was made up of 30.0 g. of corn starch, dry basis, and 10.0 g. of iodic acid (0.31 mole per anhydroglucose unit) in 300 ml. of water. The catholyte was 1200 ml. of 2% sulfuric acid which was sufficient to put the catholyte level several centimeters above that of the anolyte to diminish electroendosmosis and diffusion. The anolyte was well stirred as 3.0 amperes (0.12 ampere per sq. cm.) at 7–8 volts was passed for 8 hours 15 minutes (250% of theory).[7] The occasional addition of octyl alcohol to the anolyte was necessary to inhibit foaming.

At the end of the electrolysis, the anolyte was poured into a beaker and stirred one hour at which time all the periodic acid had reacted. The oxystarch was collected by filtration on a Buchner funnel, resuspended three times in 400 ml. of water in a Waring Blendor, twice in 400 ml. of acetone and then dried over concentrated sulfuric acid *in vacuo*. It was ground to pass 60 mesh and equilibrated to room humidity.

Bromine Oxidation and Isolation of Brucine D-Erythronate.[8]—A suspension of 9.8 g. of oxystarch, dry basis, was dissolved by heating on a steam-bath for two hours in 350 ml. of water. After the solution was filtered and diluted to 500 ml., 10 ml. of bromine was added, and the solution was then stored in the dark. Two days later, 4.5 ml. of bromine was added. The solution was allowed to stand two more days; the excess bromine was removed by aeration; and 50 ml. of 12 *N* hydrochloric acid was added. The solution was diluted to 600 ml. (1 *N* in hydrochloric acid) and heated for five hours on a steam-bath. The acids present were neutralized with barium hydroxide solution and an excess added to precipitate basic barium glyoxylate. The basic salt was removed by filtration after several days at 5°, and the excess barium hydroxide neutralized with carbon dioxide to precipitate barium carbonate. The procedure after this point followed that of Jackson and Hudson.[5]

The product resulting from this treatment was free-flowing and had the gross granular characteristics of the original corn starch. It showed no color with iodine and no birefringence.

NORTHERN REGIONAL RESEARCH LABORATORY
PEORIA, ILLINOIS[9]

(3) (a) D. H. Grangaard, J. H. Michell and C. B. Purves, THIS JOURNAL, **61**, 1290 (1939); (b) J. H. Michell and C. B. Purves, *ibid.*, **64**, 585 (1942); (c) D. H. Grangaard, E. K. Gladding and C. B. Purves, *Paper Trade J.*, **115**, 75 (1942).

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(5) E. L. Jackson and C. S. Hudson, *ibid.*, **60**, 989 (1938).

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(7) This is a current efficiency of 40%. Willard and Ralston, ref. (2), obtain similar values for the oxidation of iodic to periodic acid.

(8) We are indebted to Dr. Allene Jeanes for suggestions relating to this procedure (unpublished work of Allene Jeanes and C. S. Hudson).

(9) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

2-Phenyl-2-*p*-chlorophenyl-4-methylmorpholine

BY HENRY GILMAN AND CALVIN C. WANSER

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In a previous communication[1] the synthesis of several 2-substituted-4-methylmorpholines as possible antihistaminic agents was reported. Among the compounds prepared was 2,2-diphenyl-4-methylmorpholine, a cyclic analog of Benadryl. In view of the marked antihistaminic activity of certain 1-(*p*-halobenzhydryl)-4-methylpiperazines,[2,3] of

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