heated at 120–130°, the ethylenimine VIII was formed in 55% crude yield (from IV), m.p. 153–154°, or isolated as a second crystal form, m.p. 143–145° (Found: C, 63.4; H, 6.60; N, 5.20); NH absorption at 3.05 μ. By treatment with carbon disulfide and iodomethane under the conditions used to prepare II, compound VIII was converted back to IV, and with benzoyl chloride it gave the N-benzoyl derivative, m.p. 195–198° (Found: C, 68.1; H, 5.50; N, 3.95).

When the anhydromannoside V[10] was heated with excess ammonium thiocyanate in 2-methoxyethanol at 105–110°, the crystalline thiocyanohydrin VI was formed in 75–90% crude yield, m.p. 188–190° (Found: C, 55.8; H, 5.30; S, 9.61). Opening of the epoxide ring of V at C.3 to give the *trans*-diaxial product VI as the predominant product is assumed by analogy with other nucleophilic cleavages of I.[2] The conformational rigidity imposed by the 4,6-benzylidene ring obviously prevents the direct epoxide–episulfide transformation that commonly results from the action of thiocyanate ion on epoxides.[11] Mesylation of VI gave the sulfonate ester VII as a foam in 92% crude yield and treatment of VII, dissolved in 2-methoxyethanol, with aqueous sodium hydroxide[12] resulted in a 62% yield of the episulfide IX, m.p. 166° (Found: C, 60.2; H, 5.79; S, 11.5).

The potentially wide utility of VIII and IX for further synthetic transformations to compounds such as aminomercapto sugars and diamino sugars which may have interesting chemotherapeutic properties is under investigation.

The authors wish to thank Dr. B. R. Baker for valuable suggestions.

(10) H. R. Bolliger and D. A. Prins, *Helv. Chim. Acta*, **28**, 465 (1945).
(11) E. E. van Tamelen, THIS JOURNAL, **73**, 3444 (1951).
(12) L. Goodman and B. R. Baker, *ibid.*, **81**, 4924 (1959).

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ROLE OF HISTIDINE IN PHOSPHOGLUCOMUTASE. THE USE OF "RATE" AND "ALL-OR-NONE" ASSAYS

Sir:

The use of chemical modifications to elucidate the "active sites" of enzymes sometimes has resulted in the designation of a residue as "essential to enzyme activity." In most of these investigations, however, a decrease in activity by a factor of twenty would have been interpreted as "inactivation." It would seem desirable, therefore, to describe such residues as "essential to native enzyme activity" and to attempt to quantitate the decrease in activity caused by the modification in question. Accordingly different types of activity assays have been developed and tested in connection with methylene blue catalyzed photoöxidation of the enzyme phosphoglucomutase.

One of these was the conventional activity assay depending on the rate of the catalyzed glucose-1-phosphate (G-1-P) to glucose-6-phosphate (G-6-P) conversion. This assay measures an average *efficiency* of all enzyme species present. The other assay, an "all-or-none" assay, was different in that it measured that fraction of the enzyme capable of functioning at all. To do this, P^{32}-labeled enzyme was incubated with a large excess of G-1-P (or G-6-P) for time intervals sufficient to allow ample opportunity for the intact enzyme to undergo at least 750 complete turnovers. A modified enzyme retaining as little as 1/200 of its original activity would therefore lose essentially all its P^{32}-label during the assay.

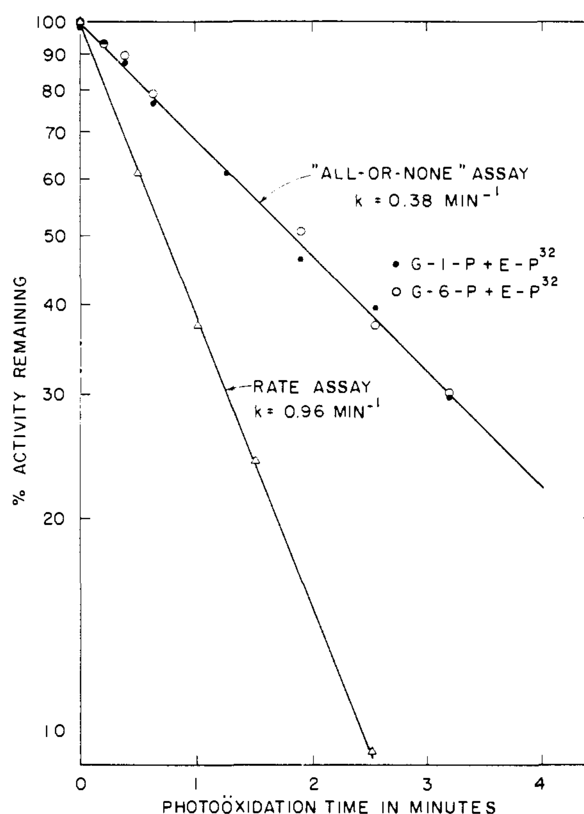

Fig. 1.—Loss of phosphoglucomutase activity on photoöxidation as measured by the "rate" assay (Δ) and the "all-or-none" assay (●, ○).

The activity remaining as a function of photoöxidation time measured by these two assays is shown in Fig. 1. The first order rate constants for activity loss from these plots are 0.96 min.$^{-1}$ for the "rate" assay and 0.38 min.$^{-1}$ for the "all-or-none" assay. The striking difference between these two plots can be explained in terms of an activity dependence on two amino acid residues, X and Y, independently oxidized at rates involving the constants 0.38 min.$^{-1}$ and about 0.6 min.$^{-1}$, respectively. If oxidation of X reduced enzyme activity by a factor of >200 while oxidation of Y affected activity to a considerably smaller extent, the "all-or-none" assay would be sensitive to oxidation of X while the rate assay would be sensitive to oxidation of both residues.

This explanation could be tested by an extension of the "rate" assay. The correlation between residues X and Y and activity by rate assay is expressed mathematically in equation 1. Here

$$A/A_0 = e^{-(k_X+k_Y)t} + F(e^{-k_Xt} - e^{-(k_X+k_Y)t}) \quad (1)$$

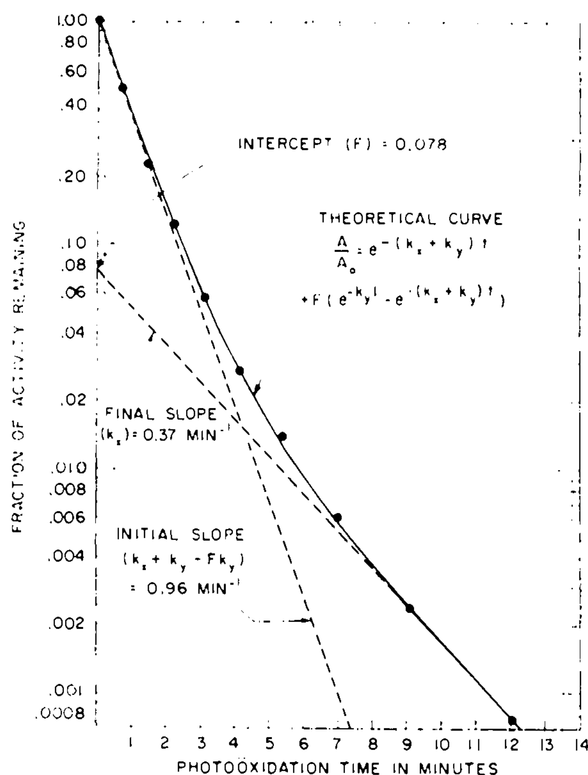

Fig. 2.—Loss of phosphoglucomutase activity on extensive photoöxidation as measured by the "rate" assay.

k_X and k_Y are the rate constants for oxidation of X and Y, and F is the fractional activity in the enzyme modification in which Y has been oxidized. The activity of the enzyme in which X has been oxidized is taken as zero, *i.e.*, undetectable by the present assay procedures. The equation gives a straight line on a semi-log plot only for small values of t in agreement with Fig. 1 but predicts a pronounced curvature at large values of t. When photoöxidation was followed to 99.9% loss of enzyme activity such curvature was found as shown in Fig. 2. Moreover the appropriate graphical analysis led to the evaluation of k_Y, k_X and F as 0.64 min.$^{-1}$, 0.37 min.$^{-1}$ and 0.078, respectively.

To identify the residue, Y, rate constants for oxidation of the various susceptible residues of the enzyme were determined. The first-order rate constant for oxidation of the accessible histidines was found to be 0.65 min.$^{-1}$. As noted previously[1] part of the histidine residues of this enzyme are relatively inaccessible to photoöxidation (k = 0.048 min.$^{-1}$).[2] Since the oxidation constants for other susceptible residues were considerably less than 0.64 (see Ray, *et al.*[3]), it seems quite certain that Y can be identified as an accessible histidine residue.

(1) D. E. Koshland, Jr., W. J. Ray, Jr., and M. J. Erwin, *Federation Proc.*, **17**, 1145 (1958).

(2) The rate constants are quite dependent on the detailed reaction conditions which will be described in the full publication. In general the photoöxidations were similar to those of Weil, James and Buehert, *Arch. Biochem. Biophys.*, **46**, 266 (1953), except for the use of much higher light intensities.

(3) W. J. Ray, Jr., H. G. Latham, Jr., M. Katsoulis and D. E. Koshland, Jr., THIS JOURNAL, **82**, 4743 (1960).

Both the comparison of the "rate" and "all-or-none" assays and the extension of the rate assay to large values of t lead to the same conclusion, that photoöxidation of a single accessible histidine residue reduces phosphoglucomutase activity by a factor of about 12. Moreover, this concordance suggests that these kinetic methods, which are not theoretically limited to phosphoglucomutase or photoöxidations may be useful in correlating structure with activity in other systems.

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CONFIGURATION AND OPTICAL ROTATORY DISPERSION OF ATROPISOMERIC BIARYLS[1]

Sir:

Rotatory dispersion curves have been useful in configurational correlations of compounds containing asymmetric atoms.[2] The availability of structurally symmetrical optically active biaryls of known absolute configuration[3] prompted us to secure the rotatory dispersion curves of over sixty compounds in this class. We now report the salient results of our investigation, which lead

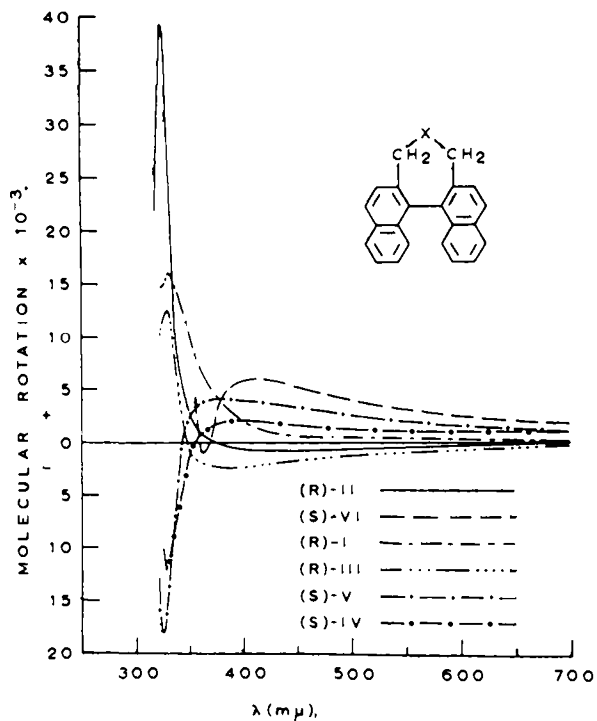

Fig. 1. Optical rotatory dispersion curves (dioxane) of: (R)-I (X = CO); (R)-II (X = $CHOCOCOC_6H_5$); (R)-III (X = CHOH); (S)-IV (X = $C(CO_2C_2H_5)_2$); (S)-V (X = O); (S)-VI (X = direct bond between CH_2 groups).

(1) Configurational Studies in the Biphenyl Series. IX (paper VIII, K. Mislow and P. A. Grasemann, *J. Org. Chem.*, **23**, 2027 (1958)) and "Optical Rotatory Dispersion Studies. XXXIX" (paper XXXVIII, C. Djerassi, W. D. Ollis and R. C. Russell, *J. Chem. Soc.*, in press).

(2) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, 1960; see especially Chapter 10.

(3) K. Mislow, *Angew. Chem.*, **70**, 683 (1958), and references cited therein.