Kinetic Procedure.—Solutions of the episulfide and triphenylphosphine of identical concentration were prepared and thermostated at 40 ± 0.1°. After thermal equilibrium had been reached the solutions were mixed. The progress of the reaction was followed by pipetting 1-ml. aliquots into a known excess of standard iodine solution (*ca.* 0.01 *N*) and rapidly back-titrating the excess iodine with standard triphenylphosphine solution (*ca* 0.01 *N*). Both standard solutions used a mixture of 75% benzene and 25% ethanol as the solvent. Two aliquots taken within the first 3 minutes were used to establish the initial concentration. The second-order rate constants were calculated from the equation $k = \left(\frac{1}{c} - \frac{1}{c_0}\right)\frac{1}{t}$. The data for a representative run on *cis*-2-butene episulfide are compiled below. The solvent used was N,N-dimethylformamide. The iodine concentration was 0.00935*N* and the iodine/triphenylphosphine titer was 0.91.

NEW BRUNSWICK, N. J.

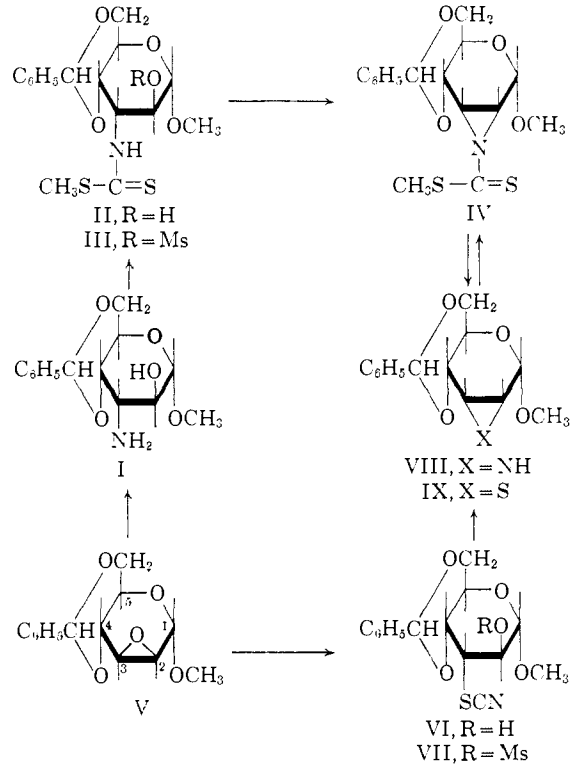
COMMUNICATIONS TO THE EDITOR

POTENTIAL ANTIRADIATION DRUGS. I. SUGAR ETHYLENIMINES AND EPISULFIDES.[1]

Sir:

Epoxides are among the most versatile intermediates in rare sugar synthesis.[2] In particular, those epoxides that are fused to sugar rings of either the pyranose or furanose form provide pathways for certain transformations that would otherwise be hindered or prevented by the very low S_N2 reactivity of the secondary sulfonate esters or secondary halides in sugar rings. The ethylenimines and episulfides that correspond to these fused epoxides ought to be equally interesting intermediates in sugar synthesis but have not been described previously.[3] In work directed toward the synthesis of sugars containing a β-mercaptoethylamine moiety, methods that should have general utility have been developed for the preparation of these unique sugar derivatives containing both types of three-membered rings, and are the subject of this Communication.

The 3-aminoaltroside I[4] with carbon disulfide and iodomethane in pyridine which contained triethylamine[5] gave a quantitative yield of the crude crystalline dithiocarbamate II, m.p.[6] 187–190° (Found: C, 52.2; H, 5.57; S, 17.2). Conventional sulfonylation in pyridine afforded 89% of the crystalline mesylate (III), m.p. 168–170° (Found: C, 46.0; H, 5.38; S, 20.7), which, when heated at 60° for 4–5 minutes with 1.10 equivalents of sodium methoxide in methanol–2-methoxyethanol, gave 65% of the thioacylated ethylenimine IV, m.p. 198–212° (Found: C, 54.4; H, 5.42; S, 18.4). Compound IV showed no infrared C=N absorption near 6.2 μ which would be expected for the isomeric thiazoline that would result from sulfur participation in the displacement.[7] The "complex neighboring group"[8] participation, then, went by predominant, if not exclusive, nitrogen attack in this instance to form a three-membered ring, although a pathway to the isomeric and less-strained five-membered thiazoline would seem to have been available. Reduction of IV with sodium borohydride–aluminum chloride in diglyme[9] gave an unstable solid which showed a positive nitroprusside reaction and SH infrared absorption at 3.9 μ and was evidently the result of reduction of the thiocarbonyl group. When this compound was



(1) This work was carried out under the joint auspices of the Office of the Surgeon General, Medical Research and Development Command, under Contract No. DA49-193-MD-268 and of the Cancer Chemotherapy National Service Center, National Cancer Institute, under Contract No. SA43-ph-1892. The opinions expressed in this manuscript are those of the authors and not necessarily those of either sponsoring agency.

(2) For a recent review, see F. H. Newth, *Quart Revs. (London)*, **13**, 30 (1959).

(3) A. M. Creighton and L. N. Owen, *J. Chem. Soc.*, 1024 (1960), recently have described the preparation of 5,6-dideoxy-5,6-epithio-1,2-O-isopropylidene-α-L-idose. The episulfide ring of this compound, however, is situated in the sugar ring side-chain, and would be expected to have a reactivity comparable to that of simple aliphatic episulfides.

(4) W. H. Myers and G. J. Robertson, THIS JOURNAL, **65**, 8 (1943).

(5) J. C. Crawhall and D. F. Elliott, *J. Chem. Soc.*, 2071 (1951).

(6) Melting points given are for the analytical samples.

(7) The thioacylethylenimide IV could be rearranged to the isomeric thiazoline, which showed strong infrared absorption at 6.25–6.4 μ (work to be published).

(8) S. Winstein and R. Boschan, THIS JOURNAL, **72**, 4669 (1950).

(9) H. C. Brown and B. C. Subba Rao, *ibid.*, **77**, 3164 (1955).

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.

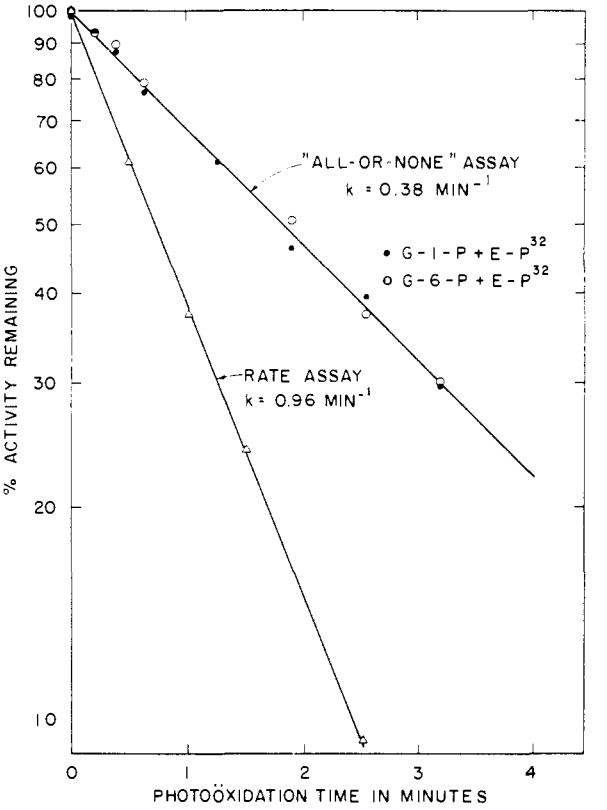

Fig. 1.—Loss of phosphoglucomutase activity on photooxidation as measured by the "rate" assay (△) and the "all-or-none" assay (●, ○).

The activity remaining as a function of photooxidation 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

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