white solid at room temperature over potassium hydroxide for 16 hours. For the animal experiments oxygen-free sterile water was added to each tube, and the solution was injected immediately. II was assayed with *Leuconostoc citrovorum* 8081[1] by aseptic addition to the culture medium and had an activity corresponding to 4 to 8 mγ per unit, which is about 2.5% of that of leucovorin and 5000 times that of pteroylglutamic acid. The effect of II in reversing the toxic effects of 4-aminopteroylglutamic acid (III) was quite marked. Injections into mice were made three times weekly[6] using 10 or 12 mice per group. With 10γ of III, average survival time was 4.9 days; with 10γ of III and 10γ or 20γ of I, all mice survived the 8-day assay period with respective weight gains of 0.3 g. and 3.5 g.; with 10γ of III and 30γ or 100γ of II all mice survived with respective weight gains of 1.3 g. and 3.3 g.; with 10γ of III and 30γ or 100γ of 10-formylpteroylglutamic acid the average survival times were respectively 4.5 days and 5.7 days. The results indicated that II had about one-third of the activity of I in reversing III and were confirmed by a second experiment. The inactivity of 10-formylpteroylglutamic acid is in contrast to the activity of II. The biological activity of II needs consideration in evaluating the effect of ascorbic acid in increasing the production of "citrovorum factor" from pteroylglutamic acid by liver slices of rats.[7]

The present observations enable some speculation to be made on the mechanism of the action of III. The formation of an imidazolinium ring at *p*H 2 by condensation of the 5-CHO group with the 10-position was postulated for I.[8] If, however, III formed an analog of I by reduction and formylation *in vivo*, an imidazole ring might form by condensation of the 5-CHO group with the 4-NH_2 group which distinguishes III from pteroylglutamic acid, giving rise to a compound which in contrast to I would be unable to reversibly transfer the "single-carbon fragment" represented by the 5-CHO group.

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THE TRANSGLUCOSIDASE OF *ASPERGILLUS ORYZAE*[1]

Sir:

In this communication we are reporting preliminary studies on a carbohydrate-synthesizing enzyme present in the filtrate of the mold *Aspergillus oryzae*.[2] Evidence is presented which shows that this enzyme is a transglucosidase,[3] *i.e.*, an enzyme capable of transferring glucose residues.

The enzymic digests were prepared by mixing appropriate amounts of the carbohydrate substrates with the enzyme, allowing the reaction to proceed at room temperature, and removing aliquots of the reaction mixture at varying time intervals. Next, the enzyme activity in these aliquots was destroyed by heat and finally the qualitative composition of the digest aliquots was ascertained by paper chromatography procedures.[4]

From pure maltose, the transglucosidase synthesizes the disaccharide isomaltose,[5] the trisaccharides 6-(α-D-glucosyl) maltose[6] and 6-(α-D-glucosyl) isomaltose[6] and a tetrasaccharide of unknown constitution. The mechanism postulated for the synthesis of these carbohydrates is termed transglucosidation and involves a transfer of the terminal glucose residue of maltose to the 6-position of a co-substrate saccharide. Phosphorylation is apparently not involved since the enzyme is without action on glucose and glucose 1-phosphate substrates.

Evidence for a transglucosidation mechanism was obtained from experiments[7] with C^{14} labelled glucose.[8] In the tracer study the enzyme was allowed to act on maltose in the presence of a small amount of labelled glucose. Examination of the digest for reducing sugars by paper chromatography showed that the distribution of synthesized compounds was essentially identical with that obtained for pure maltose. A radiogram[8] of the products showed the isomaltose and the 6-(α-D-glucosyl) isomaltose to be radioactive. Evidently the glucosyl units of maltose are transferred to radio-glucose to yield radio-isomaltose. The radio-isomaltose, in turn, functions as a glucosyl acceptor molecule in the synthesis of radio-6-(α-D-glucosyl) isomaltose. The non-radioactive reducing saccharides in the digest result from enzyme action on non-radioactive substrates.

(1) Journal Paper No. J-1949 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1116. Supported in part by a grant from the Corn Industries Foundation.
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SUBSTITUTED CYCLOÖCTATETRAENES FROM SUBSTITUTED ACETYLENES[1]

Sir:

We have found that copolymerization of mono- and disubstituted acetylenes with acetylene[2] leads to the formation of mono- and 1,2-disubstituted

(1) Supported in part by the Office of Naval Research under Contract N5ori-07822, Project Designation NR-055-96. Presented at the Twelfth National Organic Chemistry Symposium, Denver, Colorado, June 14, 1951.
(2) Under conditions used for the polymerization of acetylene to cycloöctatetraene: (a) W. Reppe, O. Schlichting, K. Klager and T. Toepel, *Ann.*, **560**, 1 (1948); (b) A. C. Cope and L. L. Estes, Jr., THIS JOURNAL, **72**, 1129 (1950).