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.

(6) A. L. Franklin, *et al.*, *Proc. Soc. Exp. Biol. Med.*, **67**, 398 (1948).

(7) C. A. Nichol and A. D. Welch, *Proc. Soc. Exp. Biol. & Med.*, **74**, 52 (1950).

(8) M. May, *et al.*, Abstracts of Papers, Am. Chem. Soc., 119th meeting, 5C, 1951.

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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.

(3) M. Doudoroff, H. A. Barker and W. Z. Hassid, *J. Biol. Chem.*, **168**, 725 (1947).

(4) D. French, D. W. Knapp and J. H. Pazur, THIS JOURNAL, **72**, 5150 (1950).

(5) E. M. Montgomery, F. B. Weakley and G. E. Hilbert, *ibid.*, **71**, 1862 (1949).

(6) D. French, *Science*, **113**, 352 (1951).

(7) Carried out in coöperation with Dr. S. Aronoff and his associates, Botany Div. of the Institute for Atomic Research, Ames, Iowa.

(8) S. Aronoff and L. Vernon, *Arch. Biochem.*, **28**, 424 (1950).

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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

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(2) Supplied by Dr. L. A. Underkofler, Chemistry Department Iowa State College, Ames, Iowa.

(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).

cycloöctatetraenes. Such copolymerizations apparently have not been described. It has been reported that phenylacetylene fails to polymerize, and that vinylacetylene yields a gel-like polymer,[3] although ref. 2a (p. 37) states that homologs of acetylene would lead to corresponding substituted cycloöctatetraenes.

In the copolymerizations, the substituted acetylene (20–50 g.) was included with the tetrahydrofuran solvent, nickel acetylacetonate and calcium carbide in a 1-l. stirred autoclave, which was pressured to 250–300 p.s.i. with acetylene and stirred and heated at 70–90° with periodic repressuring in the manner previously described[2b] for a reaction period of 7 to 12 hours. The product was steam distilled, and the substituted cycloöctatetraene was isolated from the steam distillate, or from the residue. The steam distillates contained benzene, cycloöctatetraene, an alkyl benzene (from copolymerization of the substituted acetylene with acetylene in a 1:2 ratio) and the substituted cyclooctatetraene (if volatile with steam). The less volatile substituted cycloöctatetraenes were isolated from the water-insoluble residue (largely cuprene) from the steam distillation by extraction with benzene in a Soxhlet apparatus. The substituted cycloöctatetraenes were isolated by fractional distillation, or through silver nitrate adducts, in yields of 16–25%. Phenylcycloöctatetraene and *n*-butylcycloöctatetraene were identified by direct comparison with authentic samples.[4] Methylcycloöctatetraene was isolated as a yellow liquid, b. p. 84.5° (67 mm.), $n^{25}D$ 1.5249, d^{25}_4 0.8978. (*Anal.* Calcd. for C_9H_{10}: C, 91.47; H, 8.53. Found: C, 91.17; H, 8.35). Quantitative reduction of methylcycloöctatetraene in the presence of platinum in acetic acid required 97% of four molar equivalents of hydrogen and yielded methylcycloöctane.

1,2-Dimethylcycloöctatetraene was isolated as a yellow liquid, b.p. 107° (96 mm.), $n^{25}D$ 1.5218 (*Anal.* Calcd. for $C_{10}H_{12}$: C, 90.85; H, 9.15. Found: C, 91.01; H, 9.21). Hydrogenation in the presence of 1% palladium on calcium carbonate in methanol resulted in the absorption of three molar equivalents of hydrogen and the formation of 1,2-dimethylcycloöctene, which was characterized by ozonization. Hydrogenation of the ozonide yielded 39% of decane-2,9-dione, which after recrystallization melted at 56–57°, and formed a dioxime (m.p. 131.5–132.5°). Both the diketone and dioxime were identical with authentic samples in melting point and mixed melting point.

Investigation of the preparation of substituted cycloöctatetraenes by the copolymerization of acetylene with substituted acetylenes, including derivatives containing various functional groups is being continued.

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(3) K. Kammermeyer, "Polymerization of Acetylene to Cycloöctatetraene," Hobart Publishing Co., Washington, D. C., 1947, p. 2 (based upon work of the group headed by Reppe).

(4) A. C. Cope and M. R. Kinter, THIS JOURNAL, **72**, 630 (1950); **73**, 3424 (1951); A. C. Cope and H. O. Van Orden, to be published.

ION-PAIR FORMATION IN ION EXCHANGE SYSTEMS

Sir:

When a movable, exchange ion forms an associated ion-pair with a fixed exchange group in an ion exchange resin, the formulation of the thermodynamic equilibrium constant must consider the unique conditions which exist in these systems. For example, consider the process, $A^- + R^+ = RA$, where A^- is a movable anion, R^+ the fixed exchange group, and RA the ion-pair. Since R^+ and RA are both fixed to the resin matrix, and are at finite distances of separation (7–10 Å.), they do not possess translational degrees of freedom and should be regarded as separate, solid phases. The exchanger system has four phases, the external solution (o), the internal solution phase (i), and the two solid phases R^+ and RA. Under these conditions, the dissociation constant $K_m = (m_{A^-})(\gamma_\pm)$, where m_{A^-} is the molality of the A^- ion in the solution phase,[1] and $\gamma_\pm$ the mean activity coefficient of R^+A^-; m_{R^+} and m_{RA} are set equal to unity.

Dissociation constants for this process have been written in the conventional manner as for solutions, $K'_m = (m_{A^-})(m_{R^+})/m_{RA}$.[2] A critical test of these equations is a comparison of calculated and experimental values for the variation in the selectivity coefficient, K_D, as a function of the fraction of the exchange capacity (X^i) taken up by an exchanging ion. Where ion-pair formation exists, the selectivity (which favors the ion-pair forming ion) should decrease as X^i_A increases according to K_m, but should increase according to K'_m.

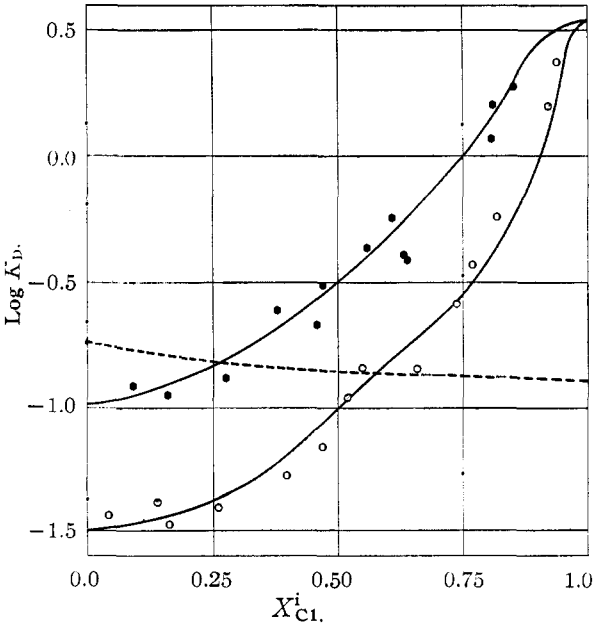

Fig. 1.—Variation in logarithm of K_D with fraction of exchange capacity in chloride state: experimental points for perchlorate–chloride exchange, O; for trichloroacetate–chloride exchange, ●; calculated curves by new theory (K_m) are thus ——; by old theory (K'_m) is – – –.

(1) H. P. Gregor, F. Gutoff and J. I. Bregman, *J. Colloid Sci.*, in press.

(2) D. K. Hale and D. Reichenberg, *Faraday Soc. Discussion*, **7**, 79 (1949).