4-Dimethylamino-2,2-diphenyl-3-methylbutanal Hydrochloride.—The reduction of 4-dimethylamino-2,2-diphenyl-3-methylbutanenitrile, 27.8 g. (0.1 mole), with lithium aluminum hydride in the above manner gave 7.5 g. of aminoaldehyde hydrochloride which was recrystallized from acetone–methanol, m.p. 187.8–192°.

Anal. Calcd. for: $C_{19}H_{23}NO \cdot HCl$: C, 71.81; H, 7.61; N, 4.40. Found: C, 71.75; H, 7.34; N, 4.47.

Catalytic reduction as indicated above gave 4-dimethylamino-2,2-diphenyl-3-methylbutanol hydrochloride, m.p. 200–201°.

Anal. Calcd. for $C_{19}H_{25}NO \cdot HCl$: C, 71.34; H, 8.19; Cl, 11.08. Found: C, 71.58; H, 8.39; Cl, 11.00.

Acknowledgment.—The authors are indebted to Messrs. M. E. Auerbach and K. D. Fleischer and staff for the analyses reported here.

STERLING-WINTHROP RESEARCH INSTITUTE
RENSSELAER, N. Y. RECEIVED JANUARY 4, 1951

COMMUNICATIONS TO THE EDITOR

PYRIDOXAL PHOSPHATE, THE COENZYME OF THIOETHER-CLEAVAGE

Sir:

In a previous report[1] the activation of certain preparations of the enzyme responsible for the cleavage of thioethers (*e.g.*, cystathionine with the formation of cysteine) by relatively large amounts of folic acid was described. Since that time, it has been found that derivatives of folic acid (conjugates and citrovorum factor) were without effect. The failure of these derivatives to activate the preparations and the limited results obtained in further studies with folic acid led us to consider other possibilities as to the identity of the dialyzable component. It has been found that minute amounts of pyridoxal phosphate[2] activated all preparations of the enzyme—fresh, aged or dialyzed. Maximal activation was obtained with 0.5 γ of pyridoxal phosphate per ml. of digest. Djenkolic acid,[3] 10 mg., and 1 ml. enzyme[4] in a total volume of 10 ml. 0.1 *M* sodium citrate were incubated for 30 minutes at 37° with amounts of pyridoxal phosphate varying from 0.1 to 10 γ per ml. With the fresh enzyme, maximal activity, 1.1 mg. of cysteine was obtained with 0.5 γ of pyridoxal phosphate; the control was 0.5 mg. of cysteine. After dialysis overnight against acetate buffer, 0.1 *M*, *p*H 5.5, the activity was reduced to 0.2 mg. of cysteine and was restored to 1.0 mg. of cysteine upon the addition of 0.5 γ of pyridoxal phosphate per ml. of digest. These amounts of pyridoxal phosphate are of the same order of magnitude as required for the transamination and decarboxylation enzymes and are compatible with the amounts predicted from the absorption spectrum of the enzyme.[1] It would appear, therefore, that pyridoxal phosphate is the coenzyme of the cleavage-enzyme.

When 10 mg. of pyridoxin and 50 mg. of adenosinetriphosphate were incubated in 10 ml. of saline with 1 ml. of homogenate of liver tissue (1 g. in 10 ml.) for 15 minutes, an apparent content of 5.5 γ of pyridoxal phosphate per ml. (activation of dialyzed enzyme) was found. The addition of folic acid was found to increase markedly the amount of coenzyme formed. It would appear probable, therefore, that the effects of folic acid and of adenosinetriphosphate[5] on the cleavage will be found to be concerned with the synthesis of pyridoxal phosphate or a closely related compound. It is of interest that the ultraviolet absorption of the purified enzyme[1] may be interpreted as that of protein and pyridoxal phosphate.[6]

These and related studies will be reported in detail in the near future.

(1) F. Binkley, THIS JOURNAL, **72**, 2809 (1950).
(2) Obtained from Dr. W. W. Umbreit.
(3) M. D. Armstrong and V. du Vigneaud, *J. Biol. Chem.*, **168**, 373 (1947). Djenkolic acid is an easily prepared substrate.
(4) F. Binkley and D. Okeson, *J. Biol. Chem.*, **182**, 273 (1950).
(5) F. Binkley, *J. Biol. Chem.*, **155**, 39 (1944).
(6) W. W. Umbreit, D. J. O'Kane and I. C. Gunsalus, *ibid.*, **176**, 629 (1948).

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RECEIVED APRIL 30, 1951

"CITROVORUM FACTOR" ACTIVITY OF TETRAHYDROPTEROYLGLUTAMIC ACID

Sir:

The preparation of leucovorin (I),[1] 5-formyl-5,6,7,8-tetrahydropteroylglutamic acid,[2,3] led to speculation as to its possible role in the transfer of "single-carbon fragments," following the suggestion which has been made for folic acid in such biological mechanisms.[4] It seemed feasible that I might be reversibly transformed to tetrahydropteroylglutamic acid (II) *in vivo* during such a process in which case II should have biological properties similar to those of I. II was synthesized by hydrogenation of 14.6 mg. of pteroylglutamic acid in 10 cc. of glacial acetic acid at room temperature, using 15 mg. of platinum oxide catalyst and a standard Ogg–Cooper micro-hydrogenation apparatus.[5] After 5.75 hours, reduction was complete; hydrogen uptake was 92.5% of the theoretical 2 moles. Subsequent operations were carried out under nitrogen to prevent oxidation. The catalyst was separated from the colorless solution of II by centrifugation, then aliquots were transferred to small test-tubes and vacuum-dried to a

(1) J. A. Brockman, Jr., *et al.*, THIS JOURNAL, **72**, 4325 (1950).
(2) E. H. Flynn, *et al.*, Abstracts of Papers, Am. Chem. Soc., 119th meeting, 18M (1951).
(3) B. Roth, *et al.*, in preparation.
(4) M. Gordon, *et al.*, THIS JOURNAL, **70**, 878 (1948).
(5) B. L. O'Dell, *et al.*, *ibid.*, **69**, 250 (1947).

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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RECEIVED MAY 31, 1951

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).

DEPARTMENT OF CHEMISTRY
IOWA STATE COLLEGE JOHN H. PAZUR
AMES, IOWA DEXTER FRENCH

RECEIVED MAY 17, 1951

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) 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.

(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).