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.

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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.

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(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

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(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).