The enzyme unit is defined as liberating 1 μg. of sialic acid[8] in 1 hr. at 37° from 3 mg. of ox brain mucolipid[2] in 1 ml. of 0.01 M Tris-acetate buffer of pH 6.6. At Stage 5, the protein sedimented with a single boundary having an s_{20} value near 1 S and could be crystallized as needles by being kept in concd. aqueous solution around 0° overnight; both criteria are not indicative of purity as shown by the table. Provisional determinations showed the final product (Stage 7) to have almost the same s_{20} value and a diffusion constant D_{20} well above 15×10^{-7}. It would seem that sialidase is an unusually small enzyme, conceivably with a molecular weight of below 10,000. The preparations are relatively stable at all stages except 7 following which they must be either lyophilized immediately or kept in frozen solution in order to preserve activity.

Treated with neutral Versene and subjected to exhaustive dialysis, the enzyme lost activity completely. The latter was restored by Ca^{++} ion[9] which was optimal near a 4 mM concentration. Mn^{++} or Co^{++} was equimolarly almost as effective, Cd^{++} or Mg^{++} restored 29% of the activity, Ba^{++} was ineffective. The activation pattern is, perhaps, in agreement with the assumption that the cation functions with the carboxylate groups of the enzyme and those of glycosidically bound sialic acid as the ligands.[10]

Loss of activity occasionally encountered during purification could be reversed entirely by treatment with Versene, dialysis and addition of Ca^{++}. The treatment of 70 μg. of enzyme with 5 micromoles of either iodoacetate or arsenite resulted in the almost complete loss of activity. Full inactivation was produced by Fe^{+++} or Hg^{++}, a 50% inhibition by Pb^{++}. Cysteine, thioglycolate, glutathione or cyanide did not restore enzymic activity.

In its action on mucolipid[2] the pH optimum of sialidase was found between pH 6.6 and 6.9; a lower secondary maximum was observed between pH 5.4 and 5.9. A Michaelis constant K_m of 4.1×10^{-2} M was calculated. Initial studies indicated that three out of four molecules of polymer-bound sialic acid are liberated by the enzyme. Two different experimental arrangements were employed for the study of the enzymic degradation of highly purified, homogeneous mucolipid preparations. When the release of free sialic acid[8] into a fixed volume of reaction mixture was followed, hydrolysis of the lipid polymer terminated with the liberation of 66% of the sialic acid within about 6 hr. When serial estimations of non-dialyzable sialic acid[11] were performed, the assay mixture being kept under conditions permitting the dialysis of liberated sialic acid, a 72% release was recorded, though more slowly.

We shall present later more detailed studies of this interesting enzyme which appears to be a protein of remarkably low molecular weight, possibly having sulfhydryl functions necessary for its action, and requiring Ca^{++}, Mn^{++} or Co^{++} ions for activity.

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(8) Sialic acid was determined according to L. Warren, *J. Biol. Chem.*, **234**, 1971 (1959).

(9) J. D. Stone, *Austral. J. Exp. Biol.*, **25**, 137 (1947).

(10) R. J. P. Williams, in Boyer, Lardy, Myrbäck (Editors), "The Enzymes," 2nd ed., **1**, 391 (1959).

(11) Polymer-bound sialic acid was determined according to I. Werner and L. Odin, *Acta Soc. Med. Upsal.*, **51**, 130 (1952).

ON THE MECHANISM OF THIAMINE ACTION

Sir:

A recent publication by Breslow and McNelis appearing under the above title,[1] which described the kinetic instability of 2-acetylthiazolium compounds, prompts us to report a similar observation we have made with 2-benzoylthiazolium salts.

The known 2-(α-hydroxybenzyl)-4-methylthiazole was prepared by a previous method.[2] Dichromic oxidation of this alcohol in acetic acid solution[3] gave 2-benzoyl-4-methylthiazole in 90% yield; m.p. 42–43°, calcd. for $C_{11}H_9NOS$: C, 65.00; H, 4.46; N, 6.89; S, 15.78. Found: C, 65.43; H, 5.05; N, 6.81; S, 15.75. The 2,4-dinitrophenylhydrazone, m.p. 218–220°, calcd. for $C_{17}H_{13}N_5O_4S$: C, 53.25; H, 3.41; N, 18.26; S, 8.36. Found: C, 53.21; H, 3.64; N, 18.53; S, 8.50. Quaternization of the 2-benzoyl-4-methylthiazole was found to be rather difficult in agreement with the findings of Breslow and McNelis for 2-acetyl-4-methylthiazole. However, some quaternization was accomplished when 0.85 g. of the ketone was refluxed with 15 ml. of methyl iodide and 10 ml. of dimethylformamide for 13 hours. Pouring the reaction mixture into 100 ml. of cold dry ether gave upon standing 0.21 g. (15% yield calculated as the methiodide) of crude crystals.

In order to test the methanolysis of this product, 50 mg. was dissolved in 1 ml. of methanol and immediately analyzed by gas chromatography. A peak was obtained which corresponded to a known sample of methyl benzoate. The volatile material was removed at 0.15 mm. from an 80° bath. Hydrolysis with base, and acidification with HCl gave 12 mg. of benzoic acid (theoretical 17.6 mg.), m.p. 122°.

These experiments show in agreement with those of Breslow and McNelis that 2-acylthiazolium salts can be solvolyzed very easily in methanol. The reaction of methanol with 2-acylthiazolium salts to give methyl benzoate is suggestive of a reaction of phosphate ion with 2-acetylthiamine to give acetyl phosphate. Thus 2-acetylthiamine is a possible in-

(1) R. Breslow and E. McNelis, THIS JOURNAL, **82**, 2394 (1960).

(2) H. Erlenmeyer, H. Baumann and E. Sorkin, *Helv. Chim. Acta*, **31**, 1978 (1948).

(3) M. Erne and H. Erlenmeyer, *ibid.*, **31**, 652 (1948).

termediate in the reaction of xylulose-5-phosphate with phosphate ion to give glyceraldehyde-3-phosphate and acetyl phosphate[4] catalyzed by the enzyme phosphoketolase and the coenzyme, thiamine pyrophosphate.[5]

(4) E. C. Heath, J. Hurwitz, B. L. Horecker and A. Ginsburg, *J. Biol. Chem.*, **231**, 1009 (1958).

(5) The authors gratefully acknowledge the support of the work by the American Cancer Society.

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TETRAFLUOROPYRIMIDINE[1]

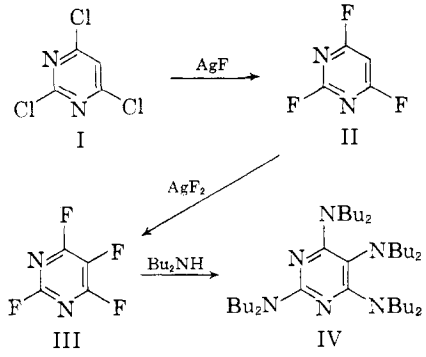
Sir:

To date there have been reported only two pseudo-aromatic perfluoro-N-heterocyclic parent compounds, namely, pentafluoropyridine and 2,4,6-trifluoro-s-triazine (cyanuric fluoride). The former compound was synthesized by defluorination of undecafluoropiperidine,[2] while cyanuric fluoride was obtained from cyanuric chloride (1) by means of potassium fluorosulfinate[3] or (2) with SbF_3Cl_2 (Swarts mixture).[4,5] Only the latter method led to exclusive formation of the desired perfluorinated triazine compound. Attempts to fluorinate other nuclear chlorinated heterocycles, such as chloropyrimidines, with SbF_3Cl_2 resulted in failure.[6]

In connection with an investigation of fluorinated heterocyclic compounds, we were interested in the synthesis of perfluorinated pyrimidine. Using 2,4,6-trichloro-pyrimidine[7] (I) as starting material, we employed silver fluoride (AgF) as the suitable inorganic fluorinating agent for the replacement of the chlorine atoms with fluorine. This selection was based upon the results of a comparative study of the effectiveness of SbF_3Cl_2, AgF, AgF_2 and HgF_2 upon certain chloro-s-triazines.[8] When compound I was refluxed and distilled from fresh AgF three times, 2,4,6-trifluoropyrimidine (II), b.p. 98°, was obtained in a 76% yield.

The conversion of II into tetrafluoropyrimidine (III) required the substitution of the hydrogen atom in 5-position by fluorine which was accomplished by means of silver difluoride (AgF_2). The reaction of II with AgF_2 either at reflux temperature or at 280° in an autoclave led to an incompletely fluorinated product; however, complete fluorination was achieved by carrying out the reaction in triperfluorobutylamine at 90°. Distillation of the reaction product gave III in 30% yield, b.p. 89°, $n^{25}D$ 1.3875 (calcd. for $C_4F_4N_2$: C, 31.60; F, 49.98; N, 18.42; found: C, 31.66; F, 49.70; N, 18.25). To our knowledge the direct replacement of hydrogen with fluorine in an aromatic system by means of silver difluoride in the liquid phase without subsequent addition of fluorine to the double bonds constitutes a novel procedure for such fluorination.

The identity of III was established by its reaction with di-*n*-butylamine to give tetra-di-*n*-butylaminopyrimidine (IV), b.p. 196° (0.3 mm.). Compound IV also was obtained from tetrachloropyrimidine. Experimental details and the description of the derivatives of II and III will be the subject of a subsequent publication.



(1) This article is based on work performed under Project 116-B of The Ohio State University Research Foundation sponsored by the Olin Mathieson Chemical Corporation, New York, N. Y.

(2) J. Burdon, D. J. Gilman, C. R. Patrick, M. Stacey and J. C. Tatlow, *Nature*, **186**, 231 (1960).

(3) D. W. Grisley, Jr., E. W. Gluesenkamp and S. A. Heininger, *J. Org. Chem.*, **23**, 1802 (1958).

(4) A. F. Maxwell, J. S. Fry and L. A. Bigelow, THIS JOURNAL, **80**, 548 (1958).

(5) E. Kober and C. Grundmann, *ibid.*, **81**, 3769 (1959); U. S. Patent 2,845,421.

(6) Unpublished results of E. Kober and H. Ulrich.

(7) M. Langermann and K. C. Banks, THIS JOURNAL, **73**, 3012 (1951).

(8) Unpublished results.

(9) Olin Mathieson Chemical Corporation, New Haven, Connecticut.

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THE STEREOCHEMISTRY OF THE SUCCINIC DEHYDROGENASE REACTION[1]

Sir:

The stereochemistry of the succinic dehydrogenase reaction was examined by Englard and Colowick in 1956.[2] From the data obtained on the exchange of the methylene hydrogen atoms with D_2O, it was concluded that the elimination of 2H is either random or *trans*. We now wish to report the results of some recent experiments which show that the elimination is not random, but is *trans* in nature.

Samples of fumaric acid and maleic acid were reduced catalytically with D_2 using Pd on charcoal as catalyst and ethyl acetate as solvent. The succinic acid obtained was oxidized by the Keilin–Hartree preparation of heart sarcosome.[3] An excess of ferricyanide was used to oxidize completely the succinic acid added. Although the sarcosome preparation contained fumarase, this would not affect the result since this enzymatic addition of water to fumarate has been shown to be stereo-

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(2) S. Englard and S. P. Colowick, *J. Biol. Chem.*, **221**, 1019 (1956).

(3) D. Keilin and E. F. Hartree, *Proc. Roy. Soc. (London)*, **B129**, 277 (1940). The grinding in a mortar was replaced by the use of a Waring Blendor.