solutions of acetic acid which were used, with contributions from hydrogen ion very minor and contributions from acetate ion still less important by a factor of 25.

Catalysis by acetic acid (or by hydrogen ion) is negligible in the 1:1 acetic acid–acetate ion buffers used for evaluating the catalytic coefficient for acetic ion, because doubling the acetic acid concentration on going to a 2:1 buffer ratio does not significantly change the rate. Table VII shows this. The data of Table VII were not used in evaluating the catalytic coefficients.

Only in the plots of first-order rate constant for 10:1 acetic acid–acetate ion buffers *vs.* buffer concentration could any curvature be observed, and it was too slight to permit the coefficient of the product term $k_p(HOAc)(AcO^-)$ to be calculated to even one significant figure. No curvature was observed in the plots for 1:1, 2:1 or 3:1 buffers nor in the plots *vs.* H_3O^+, HO^- or HOAc. However, these results are compatible with a coefficient for the product term of the order of $10^{-6}\ M^{-2}$ sec.$^{-1}$ or less.

Table VIII gives the first-order rate constants for racemization, deuterium exchange and tritium exchange. The catalytic coefficients are 10.59×10^{-6}, 2.111×10^{-6} and $1.037 \times 10^{-6}\ M^{-1}$ sec.$^{-1}$, respectively.

CAMBRIDGE 39, MASSACHUSETTS

COMMUNICATIONS TO THE EDITOR

AN ACTIVE ACETALDEHYDE-THIAMINE INTERMEDIATE

Sir:

Recently, Breslow has proposed a mechanism of action of thiamine and thiamine pyrophosphate (TPP) in which an α-hydroxyethyl group at the 2 position of the thiazole moiety is considered to be the active 2-carbon component ("active acetaldehyde") in various enzymatic reactions catalyzed by TPP as well as non-enzymatic reactions catalyzed by thiamine.[1]

In continuing earlier work[2] on the mechanism of thiamine action, we have synthesized DL-3-[(2-methyl-4-amino-5-pyrimidyl)methyl]-2-(1-hydroxyethyl)-4-methyl-5-(2-hydroxyethyl)-thiazolium chloride hydrochloride (I) and determined some of its growth-promoting and enzymatic activities.

2-(1-Benzoyloxyethyl)-4-methyl-5-(2-hydroxyethyl)-thiazole was prepared by the reaction of α-(benzoyloxy)-thiopropionamide with 3-acetyl-3-chloro-1-propanol in pyridine, b.p. 200–208° (0.7 mm.), $n^{23}D$ 1.5672. *Anal.* Calcd. for $C_{15}H_{17}NO_3S$: C, 61.83; H, 5.88; N, 4.81. Found: C, 61.66; H, 5.96; N, 5.04. Removal of the benzoyl group by potassium hydroxide in methanol at 50° yielded 2-(1-hydroxyethyl)-4-methyl-5-(2-hydroxyethyl)-thiazole, m.p. 80–81.5° cor. (from ethyl acetate). *Anal.* Calcd. for $C_8H_{13}NO_2S$: C, 51.31; H, 7.00; N, 7.48; S, 17.12. Found: C, 51.12; H, 7.02; N, 7.58; S, 17.30. Reaction of this thiazole with 2-methyl-4-amino-5-bromomethylpyrimidine hydrobromide in dimethyl formamide at 50–55° gave I as the bromide hydrobromide. Purification and conversion to the chloride hydrochloride (I) were accomplished by fractional crystallization from a methanol–acetone solution, by paper chromatography (R_f 0.54 in an isopropyl alcohol–hydrochloric acid–water system (170 ml. isopropyl alcohol, 41 ml. concentrated hydrochloric acid, water to 250 ml.) and by picrate formation, m.p. 225° dec. (uncor.) (from methanol–ether). *Anal.* Calcd. for $C_{14}H_{22}Cl_2N_4O_2S$: C, 44.09; H, 5.82; N, 14.69; Cl, 18.60; S, 8.41. Found: (dried at 78°, 0.2 mm.); C, 44.21; H, 6.06; N, 14.77; Cl, 18.46; S, 8.60. The ultraviolet spectra of I were similar to thiamine: in 0.005 *N* HCl, λ_{max} 245–257 mμ (*E* 13,901); in phosphate buffer, *p*H 7.0, λ_{max} 228–230 mμ (*E* 13,353) and 267.5–269.5 mμ (*E* 11,747); in phosphate buffer, *p*H 8.0 λ_{max} 234–236 mμ (*E* 17,347).

The activity of I in the reactivation of carboxylase was demonstrated. Alkaline-washed yeast deficient in TPP[3] contains thiaminokinase but apparently the cells are impermeable to adenosine triphosphate (ATP) inasmuch as the carboxylase activity is not restored by thiamine and ATP. However, if phosphoenolpyruvate (PEP), adenosine monophosphate (AMP), and thiamine are added to the preparation,[4] ATP is apparently generated from PEP and the resulting pyruvate is decarboxylated to acetaldehyde and carbon dioxide. The DL-hydroxyethylthiamine (I) can be substituted for thiamine in this system with equal activity.[5] With a soluble yeast carboxylase preparation possessing thiaminokinase, preliminary data

TABLE I

REACTIVATION OF CARBOXYLASE

Alkaline washed yeast 1.0 ml.; potassium phosphate buffer 0.05*M* *p*H 6.2; $MgCl_2$ 10 μmoles; total volume 2.0 ml.; time 45 minutes; temperature 30°

Additions	μmoles CO_2 evolved
PEP 10 μmoles	0
PEP 10 μmoles + AMP 3 mg.	0
PEP 10 μmoles + AMP 3 mg. + thiamine 30 μg.	8
PEP 10 μmoles + AMP 3 mg. + hydroxyethyl thiamine 30 μg.	8
Pyruvate 10 μmoles + TPP 4 μg.	9
Pyruvate 10 μmoles + thiamine 30 μg.	0
Pyruvate 10 μmoles + hydroxyethylthiamine 30 μg.	0

show that acetaldehyde is liberated from hydroxyethylthiamine. The growth-promoting activity[5] of I was found to be approximately 80% of the activ-

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(2) H. Koffler and L. O. Krampitz, *J. Bacteriol.—Proceedings*, 113 (1955).

(3) K. Lohman and P. Schuster, *Biochem. Z.*, **294**, 188 (1937).

(4) H. Weil-Malherbe, *Biochem. J.*, **33**, 1997 (1939).

(5) It must be emphasized that the hydroxyethylthiamine (I) employed in these tests is the DL form and very probably only one antipode is biologically active.

ity of thiamine when determined by microbiological assay with either *L. fermenti*[6] or *L. viridescens*.[7]

(6) H. Sarett and P. Cheldelin, *J. Biol. Chem.*, **155**, 153 (1944).

(7) R. H. Deibel, J. B. Evans and C. F. Niven, Jr., *J. Bacteriol.*, **74**, 818 (1957).

(8) This investigation at Western Reserve University was supported in part by a grant (Contract No. AT(30-1)-1050) from the Atomic Energy Commission, and in part by a research grant (E-253) from the United States Public Health Service.

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RECEIVED AUGUST 21, 1958

A STABLE DIPOSITIVE CARBONIUM ION[1]

Sir:

We wish to report the formation of a stable carbonium ion produced formally by loss of two anions from a single carbon atom. Trichloromethylpentamethylbenzene (I)[2] dissolves readily in 100% sulfuric acid (> 5 g./25 ml.) to give an intensely red solution (though neither benzotrichloride nor pentamethylbenzene are readily soluble in this solvent at 15°). The van't Hoff *i*-factor is 5 (4.97, 4.79, 4.96, 5.16) and is unaltered after 24 hours.[3] The spectrum in 100% sulfuric acid showed the following λ_{max} in mμ (ϵ): 545 (1031), 393 (16,160), 265 (3860) and 235 (5120), with an inflection at 382 mμ of slightly lower intensity than the 393 mμ band. Such solutions, when poured on ice or cold methanol, gave pentamethylbenzoic acid, m.p. 208–210°,[4] or its methyl ester, m.p. 67–67.5°, respectively, in essentially quantitative yield. When a stream of dry nitrogen was passed through a cold solution of I in 100% sulfuric acid, *two* moles of hydrogen chloride were collected in an alkaline trap within fifteen minutes; twelve more hours gave essentially no additional hydrogen chloride. The remaining solution had a spectrum identical with the original, an *i*-factor of 3 (2.91, 2.74) and when poured on ice gave pentamethylbenzoic acid essentially quantitatively; the third chlorine was accounted for in the water solution after hydrolysis.

Data to this point indicated that of the five particles produced when I dissolves in 100% sulfuric acid, two were hydrogen chloride, and that no drastic structural changes occurred. It seemed reasonable that the two hydrogens (for the hydrogen chloride) must come from the solvent, requiring that two bisulfate ions be produced, and that the fifth particle have two positive charges and be capable of producing a chloride ion and pentamethylbenzoic acid on hydrolysis.

It seemed necessary to demonstrate experimentally that indeed two bisulfate ions were formed. The elegant work of Gillespie and Wasif[5] clearly demonstrated that the principal conducting particle produced when bisulfates are dissolved in 100% sulfuric acid is the bisulfate ion, and that one can determine from molar conductivity the number of bisulfates produced per mole of solute. The molar conductivity[3] of solutions of I in 100% sulfuric acid (see Table) are consistent only with the production of two bisulfate ions.

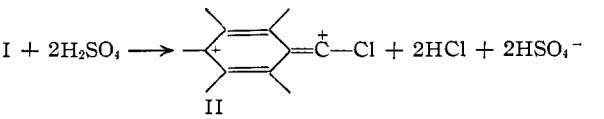
TABLE I

MOLAR CONDUCTIVITIES IN 100% H_2SO_4 AT 25°

M	$KHSO_4$	*o*-$C_6H_4(NH_3)_2$	I
0.05	...	304 (306)[a]	304
0.10	157 (156)[a]	212 (236)	213
0.20	122 (116)	164 (187)	155

[a] Values in parentheses are taken from Gillespie and Wasif, ref. 5.

The ionization of I in 100% sulfuric acid appears in accord with the equation



II

ion II having numerous contributing structures and being responsible for the color. Relief of steric strain and the need to produce a linear substituent may be factors in its formation. The same species (II) appears to be produced when I is dissolved in nitromethane containing a large molar excess of aluminum chloride (λ_{max} (ϵ) = 542 (1032), 395 (14,610), inflection at 385 mμ).

Extensions to related systems, and to other possible sources of dipositive carbonium ions are being investigated.

(1) First presented at the 7th Organic Reaction Mechanisms Conference, University of Chicago, Chicago, Illinois, September 3, 1958.

(2) We are indebted to Dr. R. J. Rolih, Standard Oil Company (Indiana), Whiting, Indiana, for samples of this compound, and for its method of synthesis.

(3) We are indebted to Professor James L. Dye for preliminary van't Hoff *i*-factor determinations, and for invaluable assistance with the cryoscopic and conductance measurements.

(4) O. Jacobsen, *Ber.*, **22**, 1215 (1889), reported 210 and 67° for the acid and methyl ester, respectively.

(5) R. J. Gillespie and S. Wasif, *J. Chem. Soc.*, 204, 209, 221 (1953).

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RECEIVED SEPTEMBER 17, 1958

TRIPHOSPHONITRILIC HEXA-ISOTHIOCYANATE[1]

Sir:

Many attempts have been made to replace the chlorine atoms of the phosphonitrilic chlorides by other structural units in order to prepare derivatives which might then be subjected to polymerization to yield elastomeric inorganic–organic polymeric products of greater hydrolytic and thermal stability. The chlorine atoms have been replaced partially and/or completely by the solvolytic action of water, alcohols, ammonia, amines, and most recently, of hydrazine.[2] Except for evidence that the chlorine atoms can be replaced by azide groups, no previous attempts have[3] been reported entailing introduction of other halogenoid groups

(1) This research was supported by Contract AF 33(616)-5486 with the Materials Laboratory of Wright Air Development Center, Wright-Patterson Air Force Base, Ohio. Reproduction of this Communication in whole or in part is permitted for any purpose of the United States Government.

(2) R. J. A. Otto and L. F. Audrieth, THIS JOURNAL, **80**, 3575 (1958).

(3) C. Grundmann and R. Rätz, *Z. Naturforschung*, **10b**, 116 (1955).