Anal. Calcd. for $C_{14}H_{14}O_5$: C, 64.12; H, 5.35. Found: C, 64.27; H, 5.57.

Treatment with 2,4-dinitrophenylhydrazine in methanol containing a trace of HCl gave the 2,4-dinitrophenylhydrazone which after three recrystallizations from ethyl acetate afforded orange plates, m.p. 230–231°, $\lambda_{max}^{CHCl_3}$ 375 mμ (ε 28,000).

Anal. Calcd. for $C_{20}H_{18}O_8N_4$: C, 54.29; H, 4.07. Found: C, 54.41; H, 4.32.

Pyrolysis of the adduct (1.0 g., 3.82 millimoles) diluted with 10 ml. of toluene at 400° afforded 740 mg. of a dark oil after removal of the toluene. After chromatography over Florisil and elution with 5% ether–benzene there was obtained 415 mg. of a yellow oil which had the desired infrared absorption at 3020(sh), 2980, 2850, 2750, 1720(broad) and 1605 cm.$^{-1}$. The oily aldehyde rapidly formed a 2,4-dinitrophenylhydrazone and was oxidized easily with potassium permanganate in acetone–water to yield nearly quantitatively colorless needles of dimethyl 4-carboxy-1,2-phthalate (**29**), m.p. 121–122° (lit.[22] m.p. 121°).

Anal. Calcd. for $C_{11}H_{10}O_6$: C, 55.46; H, 4.63. Found: C, 55.60; H, 4.24.

---

[22] I. Heilbron, "Dictionary of Organic Compounds," Oxford University Press, New York, N. Y., 1953, Vol. IV, p. 589.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND BIOPHYSICS, UNIVERSITY OF CALIFORNIA, DAVIS, CALIFORNIA]

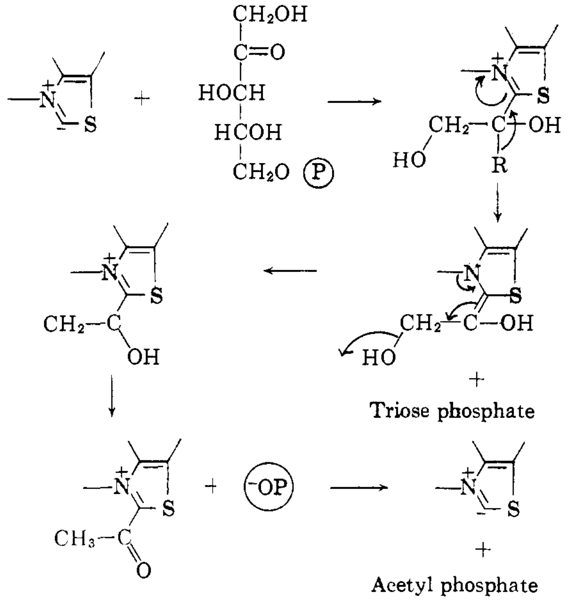
Mechanism of Thiamine Action: a Model of 2-Acylthiamine[1]

BY FRED G. WHITE AND LLOYD L. INGRAHAM

RECEIVED DECEMBER 4, 1961

2-Benzoyl-3,4-dimethylthiazolium iodide was prepared and its reactions were studied. This compound reacts very rapidly with methanol to produce methyl benzoate and 3,4-dimethylthiazolium iodide. The biological implications of this reaction are discussed.

The mechanism of all reactions catalyzed by thiamine can be explained in terms of a 2-thiazolium anion.[2] A logical extension of the mechanism for decarboxylation can be made to the phosphoketolase catalyzed reaction[3] in which xylulose-5-phosphate reacts with phosphate ion to give acetyl phosphate and glyceraldehyde-3-phosphate.



All reactions in this mechanism appear plausible except perhaps the last reaction in which 2-acylthiazolium ion reacts with phosphate ion to produce acetylphosphate. Such a reaction would not only require that 2-acylthiazolium ions be "energy rich" but also that they cleave rapidly upon attack by a nucleophile. In order to obtain information about the reactivities of 2-acylthiazolium compounds, 2-benzoyl-3,4-dimethylthiazolium iodide was prepared and its reactivity toward various nucleophiles was studied. 2-Benzoyl-3,4-dimethylthiazolium iodide was prepared by methylating 2-benzoyl-4-methylthiazole obtained by dichromate oxidation of 2-(α-hydroxybenzyl)-4-methylthiazole. The methylation of 2-benzoyl-4-methylthiazole was difficult. Difficulties in methylation prompted us to prepare the ethylene glycol ketal of 2-benzoyl-4-methylthiazole and to methylate this compound. The methylated ketal was quite stable in neutral ethanol (crystallized from ethanol) but was cleaved to benzoic acid in acidic aqueous solution. Later it was found that methylation of 2-benzoyl-4-methylthiazole in dimethylformamide gave fair yields of an unpure product while methylation in acetonitrile gave slightly poorer yields of a product of higher purity. A solution of methyl iodide and dimethylformamide was found to produce tetramethylammonium iodide under our reaction conditions, and presumably this was the impurity present in the product prepared in dimethylformamide. The impurity could be eliminated by recrystallization from acetonitrile.

Experimental

N,N-Dimethylformamide was placed over phosphorus pentoxide 3-6 days then distilled 2–4 times *in vacuo* (20–40 mm.) from phosphorus pentoxide. The distillate was stored in glass-stoppered flasks over phosphorus pentoxide. Immediately before use the dimethylformamide was distilled once *in vacuo* over potassium carbonate and once with no drying agent.

Practical acetonitrile was distilled (81–82°) over phosphorus pentoxide five or six times. Before use, the acetonitrile was distilled twice from potassium carbonate and once with no drying agent.

2-Benzoyl-4-methylthiazole.—2-(α-Hydroxybenzyl)-4-methylthiazole prepared according to Erlenmeyer, Baumann and Sorkin[4] was oxidized with sodium dichromate in glacial acetic acid as described by Erne and Erlenmeyer.[5] The white

---

(1) For previous papers in this series see F. White and L. L. Ingraham, *J. Am. Chem. Soc.*, **82**, 4114 (1960), and C. P. Nash, C. W. Olsen, F. White and L. L. Ingraham, *ibid.*, **83**, 4106 (1961).

(2) R. Breslow, *ibid.*, **79**, 1762 (1957); **80**, 3719 (1958).

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

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

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

crystalline product was recrystallized from ethanol–water yielding 6.0 g. (90%) of white crystals, m.p. 42–43°.

Anal. Calcd. for $C_{11}H_9NOS$: C, 65.00; H, 4.46; N, 6.89; S, 15.78. Found: C, 64.67; H, 4.89; N, 7.01; S, 15.86. 2,4-Dinitrophenylhydrazone had m.p. 218–220°.

Anal. Calcd. for $C_{17}H_{13}N_5OS$: C, 53.25; H, 3.42; N, 18.27: S, 8.36. Found: C, 53.21, H, 3.64; N, 18.53; S, 8.50.

Ketal of 2-Benzoyl-4-methylthiazole.—A solution of 2 g. of 2-benzoyl-4-methylthiazole, 2.5 g. of ethylene glycol and 0.3 g. of toluene sulfonic acid in 30 ml. benzene was azeotropically refluxed[6] for 10 hr., and 0.24 g. of potassium carbonate was added. The solid which separated was removed by filtration. The benzene was removed *in vacuo* yielding a pale yellow oil which was not purified further.

Methylation of the Ketal of 2-Benzoyl-4-methylthiazole.—A solution of 2.8 g. of ketal (oil) and 7 ml. of methyl iodide was placed in a heavy walled tube and sealed. The sealed tube was placed in an 85° water bath and after 10 hr. the methyl iodide was removed *in vacuo*. The residue was taken up in ethanol and undissolved material removed and washed with ethanol. The filtrate and wash were combined, an equal amount of ethyl ether added and the solution allowed to stand for 4 hr. The yellow crystals were removed and recrystallized from ethanol–ethyl ether yielding 2.77 g. (65%) of white platelets with m.p. 190–192°.

Anal. Calcd. for $C_{14}H_{16}INO_2S$: C, 43.20; H, 4.14; I, 32.60; N, 3.60; S, 8.24. Found: C, 43.43; H, 3.97; I, 32.49; N, 3.68; S, 8.44.

Preparation of 2-Benzoyl-3,4-dimethylthiazolium Iodide (I) in N,N-Dimethylformamide.—Approximately 60 ml. of dimethylformamide and 90 ml. of methyl iodide were distilled into a 30 ml. round bottom flask wrapped with aluminum foil and containing 2.3 g. of 2-benzoyl-4-methylthiazole. The reaction, protected with a calcium chloride–phosphorus pentoxide drying tube was refluxed at 50° for 18 hr. The excess methyl iodide was distilled off *in vacuo* (10–20 mm.) and the remaining dimethylformamide solution poured into 4 volumes of cold, dry ether. The orange crystals were washed with dry ether and dried *in vacuo* over phosphorus pentoxide and paraffin wax shavings. A yield of 0.61 g. (15%) of orange crystals which sinter at 150° and melt with decomposition at 157° was obtained. The product was recrystallized from 3:1 acetonitrile–ether, yielding orange needles, m.p.159–162° dec.

Anal. Calcd. for $C_{12}H_{12}INOS$: C, 41.75; H, 3.50; I, 36.76; N, 4.06; S, 9.29. Found: C, 41.80; H, 3.65; I, 36.82; N, 4.23; S, 9.19.

Formation of Tetramethylammonium Iodide.—When the above reaction was carried out without the ketone, 0.120 g. of white platelets was obtained which did not melt below 360°.

Anal. Calcd. for $C_4H_{12}IN$: C, 23.80; H, 5.95; I, 63.00; N, 6.95. Found: C, 24.04, H, 5.94; I, 63.42; N, 6.96.

Preparation of 2-Benzoyl-3,4-dimethylthiazolium Iodide in Acetonitrile.—A solution of 20 ml. of acetonitrile and 15 ml. of methyl iodide was distilled into a 100 ml. round bottom flask containing 1 g. of 2-benzoyl-4-methylthiazole and refluxed (protected by a calcium chloride–phosphorus pentoxide drying tube) for 21 hr. The excess methyl iodide and some acetonitrile was distilled off as an azeotrope (b.p. 28°). Purified, dry acetonitrile was added to give a volume of 70 ml., and freshly distilled dry ether (470 ml.) was added until persistent turbidity resulted and allowed to stand for 10 hr. The orange needles were separated, washed with dry ether and dried *in vacuo* over phosphorus pentoxide. The yield was 0.18 g. (15%). Recrystallization from acetonitrile–ether, as above, gave orange needles, m.p. 159–160° dec.

Anal. Calcd. for $C_{12}H_{12}INOS$: C, 41.75; H, 3.50; I, 36.76; N, 4.06; S, 9.29. Found: C, 41.67; H, 3.50; I, 36.78; N, 4.30; S, 9.20.

4-Methylthiazole.—4-Methylthiazole was prepared by the method of Kurkjy and Brown[7] using chloro-2-propanone. A yield of 17.6 g. (24%) of a pale yellow viscous liquid, b.p. 55–60°, 30 mm. was obtained.

3.4-Dimethylthiazolium Iodide.—A solution of 8.0 g. of 4-methylthiazole and 20 ml. of methyl iodide was allowed to stand overnight at room temperature. The 18.6 g. of pale yellow crystals (m.p. 112–114°) obtained was recrystallized to white crystals of constant melting point (117–118°) from methanol or ethanol. This melting point is lower than that reported by Breslow[8] 119–120° and Erlenmeyer, Baumann and Sorkin[4] 128–129°. The ultraviolet spectrum[9] was identical with that of 3,4-dimethylthiazolium iodide.

Ethanolysis of 2-Benzoyl-3,4-dimethylthiazolium Iodide.—To 50 mg. of I in a 4 ml. glass stoppered test tube was added 1 ml. of absolute ethanol yielding a pale yellow solution after standing 10 min., 0.5 ml. of water and one drop of 45% sodium hydroxide was added and the mixture heated, with air condenser for 15 min. During this time the ethanol distilled off. The reaction mixture was allowed to cool, 1 ml. of water was added and the pH was taken to approximately two with 6 *N* hydrochloric acid (2 drops). White crystals formed immediately. The crystals were removed by filtration after standing 3 hr. and dried, 14 mg. (80% m.p. 120–122°). Mixed melting point with benzoic acid was 120–122°.

Methanolysis of 2-Benzoyl-3,4-dimethylthiazolium Iodide.—To 110 mg. (I) in a 25 ml. glass-stoppered Erlenmeyer flask was added 2 ml. of anhydrous methanol. After standing 1 hr., 20 ml. of dry ethyl ether was added, white crystals separating immediately. These were removed by filtration yielding 71.6 mg. (93%) of white needles, m.p. 115–117°. The ultraviolet spectrum of the product was identical with known 3,4-dimethylthiazolium iodide. A yield of 33.9 mg. (87%) of benzoic acid, m.p. 121–122°, was recovered from the ether–methanol solution by removal of the ether and methanol and aqueous sodium hydroxide hydrolysis followed by acidification with hydrochloric acid.

Solvolysis of 2-Benzoyl-3,4-dimethylthiazolium Iodide in Water.—To 115.2 mg. of I was added 3 ml. of water. Crystals formed as I went into solution. After 3 hr., the crystals were removed by filtration and dried. A yield of 39.3 mg. (96%) of benzoic acid, m.p. 120–121°, was obtained. The ultraviolet spectrum of the water solution which had been extracted with ether to remove small amounts of benzoic acid was identical with that of known 3,4-dimethylthiazolium iodide.

Approximate Rate of Methanolysis of 2-Benzoyl-3,4-dimethylthiazolium Iodide.—Two tenths ml. of methanol at 25° were added to 4.6 mg. of I in a small separatory funnel. Twenty six seconds after the first drop of methanol, 0.4 ml. of water and 0.4 ml. ether were added, shaken and the ether layer separated. The ether layer was dried over calcium chloride and 50 μl. was analyzed by gas chromatography. A peak corresponding to 2.0 mg. (± 10%) of methylbenzoate was found (69% yield).

Gas Chromatography.—Gas chromatographic analyses were performed on a Willins aerograph using a diethyleneglycol adipate (LAC-446 20%) column operating at 154° and gas flow rate of 60 ml. per minute. The peak area was compared with the peak area of a standard solution to determine the amount of methyl benzoate.

Determination of Half-life of Spectrum of I in Methanol.—The solvolysis of I was followed spectrophotometrically by the decrease in optical density at the maximum attributable to the chromophore of I which is lost on solvolysis, Fig. 1. The apparatus used has been described by Marr and Marcus.[10] The recorder pen response time for the linear range was approximately 0.7 sec. After zeroing the instrument, the reaction cell was moved into the light path and the solvolysis mixture injected into the cell through the parafilm cover, by a syringe. The syringe and needle were calibrated to deliver a volume of solvolysis mixture so that the final volume in the cell would be 3 ml. The reference cell contained the solvolysis mixture. It was established that by careful injection, mixing was complete during the injection time which was of the order of 0.25 sec.

The half-life was estimated by taking one-half the difference between the starting and equilibrium optical density readings and determining the time required for this optical density change to occur. The half-lives of I in several

---

(6) R. D. Haworth and A. Lapworth, *ibid.*, **121**, 81 (1922).

(7) R. P. Kurkjy and E. V. Brown, *J. Am. Chem. Soc.*, **74**, 5778 (1952).

(8) R. Breslow, *ibid.*, **80**, 3719 (1958).

(9) A. E. Ruehle, *ibid.*, **57**, 1887 (1935).

(10) A. G. Marr and L. Marcus, *Anal. Biochem.*, **2**, 576 (1961).

TABLE I

HALF-LIFE OF SPECTRUM OF 2-BENZOYL-3,4-DIMETHYLTHIAZOLIUM IODIDE (1.68×10^{-4} MOLE/LITER) IN DIMETHYLFORMAMIDE–METHANOL SOLUTIONS AT 33°

Methanol, v./v. %	Approximate half-life, sec.
0.33	15
1.67	3
3.33	1
16.7	0.1

dimethylformamide–methanol solutions are shown in Table I.

Discussion

The results show that 2-benzoyl-3,4-dimethylthiazolium iodide cleaves to 3,4-dimethylthiazolium iodide and benzoic acid or alkylbenzoates in water or alcohol. These results are in agreement with those of Breslow and McNelis[11] who found that 3,4-dimethyl-2-(α-hydroxyethyl)-thiazolium iodide could be oxidized in nitromethane to a mixture of compounds giving infrared and ultraviolet spectra consistent with 2-acyl-3,4-dimethylthiazolium iodide. Upon addition of methanol or water a spectrum characteristic of 3,4-dimethylthiazolium iodide appeared.

The reaction of 2-benzoyl-3,4-dimethylthiazolium iodide with methanol is rapid. The gas chromatographic analysis of methyl benzoate after methanolysis of I indicates a half-life of less than 15 seconds. This 15 seconds includes the time of solution. Calorimetric measurements described elsewhere[16] give an approximate half-life of less than 5 seconds in methanol at 25°. The half-lives measured by the changes in ultraviolet spectrum in dimethylformamide–methanol solution show a half-life of approximately 1 second in 3% methanol at 33°. However, the spectral change may be the result of semi-ketal formation and not of carbon–carbon bond cleavage.[12] In water, crystals of benzoic acid were formed as fast as I dissolved.

The extreme reactivities of 2-benzoyl-3.4-dimethylthiazolium iodide toward methanol and water indicate that it is a very reactive compound, and therefore a 2-acylthiazolium ion may be a plausible intermediate in the phosphoketolase reaction. The extreme reactivity is discouraging to hopes of isolating the intermediate in the phosphoketolase reaction as was done for the 2-(α-hydroxyethyl)-thiamine in pyruvic decarboxylase[13] and pyruvic oxidase.[14]

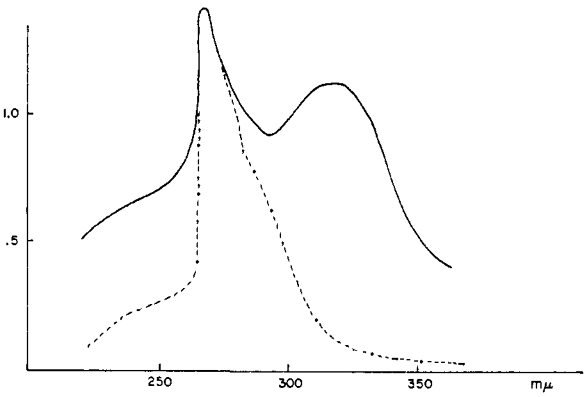
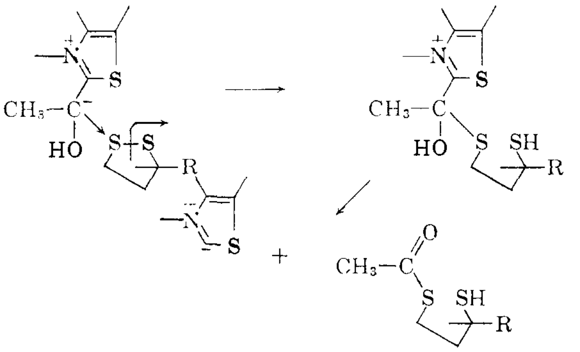


Fig. 1.—Spectrum of 2-benzoyl-3,4-dimethylthiazolium iodide in dimethylformamide. Concentration of 2-benzoyl-3,4-dimethylthiazolium iodide was 2.3×10^{-4} mole/liter. Solid line is spectrum in pure, dry dimethylformamide. The maxima are at 320 and 267 mμ. The dashed line is the spectrum after methanolysis of 2-benzoyl-3,4-dimethylthiazolium iodide yielding a single peak at 267 mμ.

2-Acetylthiamine has been proposed[15] as an intermediate in the pyruvic acid oxidation to acetyl lipoic acid. However, in this latter reaction it is easier to write a mechanism for the formation of a semi-thioketal of the ketone rather than the ketone itself. In addition the ketal would be a much less reactive intermediate than the 2-acetylthiamine and therefore probably easier to form by an oxidation. For example, the ethylene glycol ketal of 2-benzoyl-3,4-dimethylthiazolium iodide was much less reactive toward alcoholysis than the unsubstituted 2-benzoyl-3,4-dimethylthiazolium iodide.



Recent experiments have shown that the reaction of 2-benzoyl-3,4-dimethylthiazolium iodide with methanol is not only rapid but has a free energy of reaction of approximately -15 kcal.[16] Thus 2-acetylthiazolium ions are not only kinetically labile but are "high energy" compounds and both requirements for the reaction of 2-acetylthiamine with phosphate ion to produce acetylphosphate are fulfilled.

Acknowledgments.—We wish to thank Dr. Walter Hafferl for determining the approximate half-life of I in methanol, and the U. S. Public Health Service (Research Grant USPHS RG-8285) for generous support of this research.

---

[11] R. Breslow and E. McNelis, *J. Am. Chem. Soc.*, **82**, 2395 (1960).

[12] We wish to thank the reviewer of this paper for pointing out this possibility.

[13] H. Holzer and K. Beaucamp, *Angew. Chem.*, **71**, 776 (1959).

[14] P. Scriba and H. Holzer, *Biochem. Z.*, **334**, 473 (1961).

[15] M. L. Das, M. Koike and L. J. Reed, *Proc. Natl. Acad. Sci. (U. S.)*, **47**, 753 (1961).

[16] C. P. Nash, C. W. Olsen, F. G. White and L. L. Ingraham, *J. Am. Chem. Soc.*, **83**, 4106 (1961).