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Chemistry of the Tricyclic Form of Thiamine with Aldehydes in Basic Ethanol[1]

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Thiamine chloride hydrochloride **1** is converted to its neutral tricyclic form **2** when suspended in a solution of ethanol containing two equivalents of NaOEt (G. D. Maier and D. E. Metzler (1957) *J. Amer. Chem. Soc.* **79**, 4387). Upon the addition of benzaldehyde to this basic thiamine solution an intermediate accumulates which gives rise to 2-(1-hydroxyphenylmethyl)thiamine hydrochloride (HBT-HCl) **3** upon acidification of the reaction mixture with HCL after 5 min, and high yields of both 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-2-benzoyl-3a-methyl-perhydrofuro-[2,3-d]-thiazole (HBT ketone) **4** and benzoin when the reaction mixture is stirred for 5 h. Given that thiamine is unstable under these conditions such that it is converted completely to its tricyclic species, we argue that the 2-(1-hydroxylalkyl) thiamine salts, which have an increased base lability due to the addition of a C-2/N-3 torsional interaction to the base-labile thiazolium ring, would also not be expected to accumulate in appreciable concentrations under identical conditions. Based on product isolation and the synthesis of 2-benzoyl-3,4-dimethyl-5-ethoxycarbonyl-[2H]-thiazoline **12** in the reaction of 3,4-dimethyl-5-ethyoxycarbonylthiazolium iodide **11** with benzaldehyde in basic methanol, we conclude that the intermediate which accumulates during the reaction of the tricyclic form of thiamine with benzaldehyde is a 2-benzoylthiazoline **8** in equilibrium with a low concentration of its enol isomer **9**, i.e., the C-2α reactive "active aldehyde." Reasons for the increased catalytic power of thiamine under these conditions, as well as the relevance of this chemistry to the active site chemistry of thiamine pyrophosphate, are discussed.

INTRODUCTION

Subsequent to the work of Mizuhara and Breslow, early studies on the chemistry of thiamine as a model of the enzymic activity of thiamine pyrophosphate focused on the mechanism of its catalysis of benzoin-type condensations in basic aqueous solution (*1–3*). Recent work, however, has demonstrated that thiamine and compounds related structurally to thiamine are excellent catalysts of benzoin-type condensations in basic nonaqueous solution (*4, 5*). An interesting feature of the chemistry of thiamine under these conditions in basic nonaqueous solution is the enhanced instability of the thiazolium form. Maier and Metzler report that

[1] From the dissertation of Michael B. Doughty, Louisiana State University, 1982. For preliminary report on carbene chemistry of related thiazolium salts, see M. B. Doughty and G. E. Risinger (1982) *Abs. Amer. Chem. Soc.* **183**, 229.

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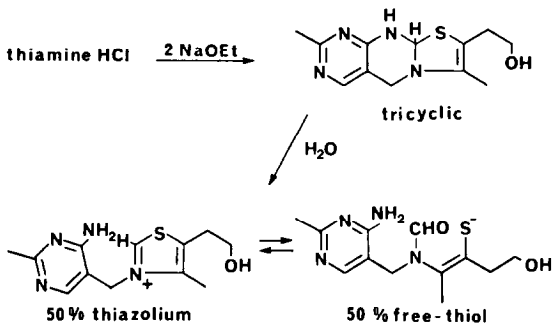
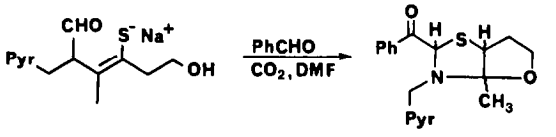


SCHEME 1

after neutralization of thiamine HCl with one equivalent of NaOEt in absolute ethanol, thiamine is converted quantitatively to its neutral tricyclic form **2** (Scheme 1) upon the addition of a second equivalent of NaOEt (*6*). As long as the neutral tricyclic form remains in a hydrophobic and nonaqueous environment it is stable relative to the positively charged thiazolium form and the negatively charged yellow form of thiamine. Upon addition to an aqueous solution the tricyclic form rapidly disproportionates, and within minutes forms an equimolar mixture of the thiazolium and free-thiol forms of thiamine (*6, 7*).

Given that as one moves from hydrophobic to aqueous solution the structure of thiamine can change such that the neutral tricyclic form predominates in media of low polarity, while the ionic thiazolium and free-thiol forms of thiamine predominate in more polar aqueous media, the question arises as to whether the structure of intermediates produced along the reaction path of the thiamine-catalyzed benzoin-type condensations might also change in going from aqueous to hydrophobic solvents. There is some circumstantial evidence which suggests that this is the case. Takamizawa *et al.* report that when the sodium salt of the free-thiol form of thiamine is heated in CO_2-saturated DMF in the presence of benzaldehyde, the neutral HBT ketone can be isolated (Eq. [1]), although in only 10% yield (*8*).

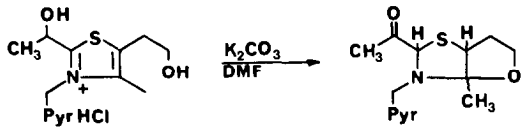


[1]

Additionally, Risinger and Karimian report that when 2-(1-hydroxyethyl)thiamine hydrochloride (HET-HCl)[3] is suspended in the more hydrophobic and aprotic

[3] Abbreviations used: HBT-HCl, 3-[(4-amino-2-methyl pyrimidin-5-yl)methyl]-5(2-hydroxyethyl)-2-hydroxyphenylmethyl-4-methylthiazolium chloride hydrochloride; HET-HCl, 3-[(4-amino-2-methyl pyrimidin-5-yl)methyl]-2-(1-hydroxyethyl)-5-(2-hydroxy-ethyl)-4-methylthiazolium chloride hydrochloride; HiBut-HCl 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-2-(1-hydroxy-2-methylpropyl)-4-methylthiazolium chloride hydrochloride; HBT ketone, 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-2-benzoyl-3a-methyl-perhydrofuro-[2,3-d]-thiazole; HiBut ketone, 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-2-isobutyryl-3a-methyl-perhydrofuro-[2,3-d]-thiazole; HET Ketone 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-2-acetoyl-2q-methyl-perhydrofuro-[2,3-d]-thiazole; TPP, thiamine pyrophosphate.

DMF with K_2CO_3 as base (Eq. [2]), HET ketone can be isolated in 70% yield (*9*).[4]



[2]

At least in these noncatalytic systems one can see that in going from basic aqueous to aprotic solution neutral forms of thiamine–aldehyde adducts can be stabilized relative to the ionic forms.

In an earlier series of papers we have shown that Metzler's tricyclic form of thiamine is a precursor to the C-2 deprotonated thiamine intermediate (*5*), and that in basic hydrophobic solution, conditions under which neutral thiamine–aldehyde products have been observed, this intermediate reacts as a nucleophilic carbene (*10*). In an extension of these studies, a critical question which we have attempted to answer experimentally is whether these neutral thiamine–aldehyde adducts might indeed form under catalytic and, if so, then to deduce their origin. Additionally, we suggest a rational interpretation of our data which helps to explain why thiamine is such an effective catalyst under these conditions in nonpolar media.

EXPERIMENTAL

General. Thiamine chloride hydrochloride (MCB) was purified by several washings with hot ethanol and dried at 100°C overnight in a ventilated drying oven prior to use. Triethylamine was purified by distillation over calcium hydride, and benzaldehyde was purified by extraction from K_2CO_3, followed by distillation over CaH. All other reagents and solvents were reagent grade. Matheson purity nitrogen was utilized in all experiments, and atmos-bags (I^2R) were routinely used to provide an inert atmosphere. For deoxygenating solutions, reaction vessels were connected to a Firestone valve equipped with nitrogen and vacuum lines; solutions were deoxygenated by alternating cycles of evacuating and flushing with zero-grade nitrogen.

Proton NMR spectra were recorded on a Varian A60A spectrometer and chemical shifts are reported using tetramethylsilane as a standard in organic solvents and HOD in aqueous solution. Ultraviolet spectra were recorded on a Beckman Model 25 spectrophotometer. The pH of aqueous solutions was measured on a Radiometer/Copenhagen PH82 standard pH meter. Melting points were determined with a Melt-Temp apparatus and are uncorrected. Mass spectral analyses were performed on a Hewlett–Packard HP5985 mass spectrometer. All elemental analyses were performed by Mr. Ralph Seab, scientific research specialist of the Louisiana State University Chemistry Department.

[4] In a reinvestigation of this chemistry we have found that in the conversion of HET to HET ketone in basic DMF, the reaction mixture must be kept cold (0°C) for high yields of HET ketone. Additionally, under these conditions HET ketone is isolated as a mixture of two diastereomers.

Synthesis of 3-[(4-amino-2-methylpyrimidin-5-yl)]methyl]-5-(2-hydroxyethyl)-2 hydroxyphenylmethyl-4-methylthiazolium chloride hydrochloride (HBT-HCl) **3.** A round-bottom flask was charged with thiamine chloride hydrochloride (5.0 g, 14.8 mmol) and absolute ethanol (75 ml), and the mixture was deoxygenated and cooled to 0°C. A sodium ethoxide solution was prepared in a dropping funnel by adding sodium (0.68 g, 30 mmol) to absolute ethanol (75 ml), and the base solution was deoxygenated and added dropwise, over 30 min, to the cooled reaction mixture. Benzaldehyde (3.0 ml, 30 mmol) was immediately added under a nitrogen atmosphere to this basic thiamine solution. The reaction mixture was stirred at 0°C for 5 min (during which time it went from light yellow to orange in color) and acidified to pH 3 with hydrogen chloride gas. The solution was warmed and suction filtered to remove salts and unreacted thiamine. The filtrate was roto-evaporated to a small volume, the liquid dissolved in 25 ml of water, and the water layer extracted with three 50-ml portions of chloroform to remove benzaldehyde and benzoin. The aqueous layer was roto-evaporated *in vacuo* to dryness, removing the last traces of water by roto-evaporating with absolute ethanol. The resultant yellow solid was suspended in cold ethanol (15 ml); the suspension was suction filtered, and the solid was washed with cold ethanol and dried *in vacuo*, yielding 4.2 g (63%) of HBT-HCl as a white solid. mp = 214°C dec, mp (lit) = 204–210°C dec (*11*). ^{1}H NMR (D_2O): δ 2.35 (s,3H,thiaz-**CH_3**), 2.40 (s,3H,pyr**CH_3**), 3.17 (t,2H,**CH_2**-CH_2OD), 3.93 (t,2H,CH_2-**CH_2**OD), 5.25 (br s,2H, bridge **CH_2**), 6.40 (s,1H,C2α-**H**), 6.62 (s,1H,pyr**H**), 7.30 (m,5H,phenyl-**H**).

Synthesis of 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)2-(1-hydroxy-2-methylpropyl)-4-methylthiazolium chloride hydrochloride (HiBuT-HCl) **6**. A reaction mixture of thiamine chloride hydrochloride (5.0 g) and NaOEt (0.68 g Na in EtOH) was prepared as described above for the synthesis of HBT-HCl. Isobutyraldehyde (2.7 ml) was added under a nitrogen atmosphere and the reaction mixture was stirred for 5 min (during which time the reaction mixture went from light yellow to yellow-green in color). The reaction was terminated by the addition of HCl gas to pH 3, and the acidic solution was filtered and roto-evaporated to dryness. The residue was dissolved in water, extracted with three 50-ml portions of chloroform, and the aqueous solution was roto-evaporated to dryness, removing the last traces of water by roto-evaporating with absolute ethanol. The resultant white solid was suspended in cold ethanol and the product was isolated by suction filtration yielding 4.4 g (72%) of HiBuT-HCl **6** as a white solid. mp = 229°C dec.

Anal. Calcd for $C_{16}H_{26}N_4O_2SCl_2$: C,46.94; H,6.40; N,13.69. Found: C,47.01; H,6.41; N, 13.72. ^{1}H NMR (D_2O): δ 1.11 (two d,6H,isobut-**CH_3**), 2.55 (s,3H,thiaz-**CH_3**), 2.76 (s,3H,pyr-**CH_3**), 3.35 (t,2H,thiaz-**CH_2**-), 4.06 (t,2H,-**CH_2**-OD), 5.10 (d, 1H,C2α-**H**), 5.75 (br s,2H,bridge **CH_2**), 7.45 (s,1H,pyr-**H**).

Synthesis of 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-2-benzoyl-3a-methyl-perhydrofuro-[2,3-d]-thiazole (HBT ketone) **4**. A round-bottom flask was charged with thiamine chloride hydrochloride (3.0 g, 9 mmol), freshly distilled benzaldehyde (1.8 ml, 18 mmol), and absolute ethanol (50 ml), and the mixture was deoxygenated and cooled to 0°C. A sodium ethoxide solution was prepared in a dropping funnel by adding sodium (0.41 g, 18 mmol) in ethanol (50 ml). This deoxygenated

sodium ethoxide solution was added dropwise to the round-bottom flask over 30 min, and the reaction mixture was stirred at 0°C for 5 h. The mixture was neutralized under a positive nitrogen pressure by the dropwise addition of glacial acetic acid (generally 0.5 ml), and this solution was stirred for 30 min at 0°C. The yellow solution was filtered—the solid contains salt, the *cis* isomer **4a** of the ketone, and some benzoin, and the filtrate contains the *trans* isomer **4b** of the ketone and benzoin.

The filtrate was flash evaporated to dryness, and the yellow solid was dissolved in chloroform and extracted with two 25-ml portions of water. The pH of the aqueous layer was adjusted to 8 with 5% sodium hydroxide and extracted with two 25-ml portions of chloroform. The combined chloroform layers were dried over sodium sulfate, filtered, roto-evaporated to a small volume, and HBT ketone was precipitated by dropwise addition of the chloroform solution into cold ethyl ether. The white solid was collected by suction filtration yielding 1.4 g (42%) of the *trans* isomer **4b** of the ketone (the chloroform/ether filtrate was saved for benzoin isolation). mp = 181°C dec, mp (lit) = 182–183°C dec (*8*). ir (KBr) υ_{max} cm^{-1}: 1695 (CO), 1660 (NH_2). ^1H NMR ($CDCl_3$): δ 1.67 (s,3H,C-**CH_3**), 2.06 (m,2H,C-**CH_2**-), 2.45 (s,3H,pyr-**CH_3**) 3.9 (br s,2H,pyr-**CH_2**-), 3.82–4.32 (3H,S-**CH**- and O-**CH_2**-), 5.47 (s,1H,C2-**H**), 6.48 (br s,2H,exchangeable-**NH_2**), 7.48 (m,3H,*m*- and *p*-aromatic-**H**), 7.78 (s,1H,pyr-**H**), 7.81 (m,2H,*o*-aromatic-**H**).

The initial precipitate containing the *cis* isomer was taken up in chloroform and filtered to remove salts. The chloroform solution was roto-evaporated to 50 ml and dropped into cold petroleum ether (50 ml). After formation of the yellow precipitate the solution was decanted and saved for benzoin isolation. The light yellow solid was dried *in vacuo*, and the ketone was recrystallized from chloroform/ether yielding 900 mg (25%) of the *cis* isomer of the ketone as a light yellow solid. mp = 148°C dec.

Anal. Calcd for $C_{19}H_{22}N_4O_2S$: C,61.60; H,5.98; N,15.12. Found: C,61.63; H,6.08; N,15.09. ir (KBr) υ_{max} cm^{-1}: 1680(CO), 1645(NH_2). mass spec: *m/e* (abundance,ion) 265 (28,M+-PhCO), 144 (11,265-pyrCH_2), 122 (100,pyrCH_2), 105 (8,PhCO). ^1H NMR ($CDCl_2$): δ 1.62 (s,3H,C-**CH_3**), 2.17 (m,2H,C-**CH_2**-), 2.47 (s,3H,pyr-**CH_3**), 3.48 (m,1H,S-**CH**-), 3.82 (br, s,2H,pyr-**CH_2**-), 4.54 (m,2H,O-**CH_2**-), 5.25 (s,1H,C2α-**H**), 6.4 (br s,2H,exchangeable-**NH_2**), 7.46 (m,3H, *o*- and *p*-aromatic-**H**), 7.89 (m,3H, pyr-**H** and *o*-aromatic-**H**).

The solutions containing benzoin were combined, roto-evaporated to dryness, and the yellow solid was dissolved in hot methanol (25 ml). Upon cooling, benzoin crystallized and was collected by filtration yielding 950 mg (50% based on initial benzaldehyde). mp = 136°C.

Synthesis of 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-2-isobutyryl-3a-methylperhydrofuro-[2,3-d]-thiazole (HiBuT ketone) **5.** A reaction solution of thiamine HCl, NaOEt, and aldehyde was prepared as above for the synthesis of HBT ketone except that isobutyraldehyde was substituted for benzaldehyde. The reaction mixture was stirred at 0°C under nitrogen for 5 h after which time it was neutralized with one equivalent of glacial acetic acid. This neutral solution was stirred at 0°C under nitrogen for an additional 30 min, and roto-evaporated to near dryness; the resultant oil was dissolved in chloroform and extracted with water.

The pH of the aqueous layer was adjusted to 8 with NaOH and extracted with two additional portions of chloroform (50 ml). The chloroform layers were combined, dried over sodium sulfate, roto-evaporated to a small volume, and the isomer of HiBuT ketone **5** in which the tetrahydrofuro-ring is apparently *trans* to the isobutyryl group (*8*) was precipitated by the addition of ether and collected by filtration yielding 1.5 g (35% yield) as a white solid. mp = 162°C dec, mp (lit) = 159–161°C dec (*8*). ir (KBr) ν_{max} cm^{-1}: 1710 (CO), 1660 (NH_2). ^1H NMR($CDCl_2$): 1.1 (two d,6H,isobut-$\mathbf{CH_3}$), 1.68 (s,3H,C-$\mathbf{CH_3}$), 2.0 (m,2H,C-$\mathbf{CH_2}$-), 2.48 (s,3H,pyr-$\mathbf{CH_3}$), 2.6 (m,1H,isobut-**CH**), 3.7 (m,1H,**CH**), 3.78 (br s,2H,pyr-$\mathbf{CH_2}$-), 4.1 (m,2H,-$\mathbf{CH_2}$-O), 4.63 (s,1H,C_2-**H**), 6.1 (br s,2H,exchangeable-$\mathbf{NH_2}$), 7.8 (s,1H,pyr-**H**).

Synthesis of 5-ethoxycarbonyl-4-methylthiazole. Thioformamide was prepared from formamide and phosphorus pentasulfide by the procedure of Erlenmeyer and Mensi (*12*). Ethyl-2-bromoacetoacetate was prepared by the following modification of a procedure of Smith (*13*). Ethyl acetoacetate (45 ml) was diluted in carbon tetrachloride (300 ml) and cooled to 0°C. Bromine (18 ml, 0.35 mol) in carbon tetrachloride (100 ml) was added dropwise over 2 h to the ethyl acetoacetate solution. During bromine addition, the reaction vessel was flushed with a steady stream of nitrogen to remove the developed hydrogen bromide gas. After bromine addition, the solution was washed with three 100-ml portions of saturated sodium bicarbonate solution, dried over sodium sulfate, filtered, and the solvent was roto-evaporated yielding 64 g (84%) of ethyl 2-bromoacetoacetate. bp = 55–60°C (0.5 mm); bp (lit) = 104–110°C (15 mm) (*13*).

A three-neck round-bottom flask was equipped with a dropping funnel and mechanical stirrer and charged with crude thioformamide in ethanol (15 ml). The solution was cooled to 0°C, and crude ethyl-2-bromoacetoacetate (64 g, 0.3 mol) was added dropwise over 2 h. The solution was stirred at 0°C overnight and then at room temperature for 24 h. The collected solid was reprecipitated from hot ethanol/ethyl ether yielding 30 g (38%) of 5-ethoxycarbonyl-4-methylthiazole hydrobromide (mp = 188°C). The free base was isolated by dissolving the hydrobromide in water, adjusting the pH of the solution to 10 with 10% sodium hydroxide, and extracting the free thiazole into chloroform. The crude product was purified by distillation. bp = 102°C (3 mm); bp (lit) = 215–220°C (*14*). ^1H NMR (CCl_4): δ 1.07 (t,3H,J = 7 Hz, ester-$\mathbf{CH_3}$), 2.45 (s,3H,thiaz-$\mathbf{CH_3}$), 4.05 (q,2H,J = 7 Hz, O-$\mathbf{CH_2}$-), 8.6 (s,1H,C-2 **H**).

Synthesis of 3,4-dimethyl-5-ethoxycarbonylthiazolium iodide **11**. Methyl iodide (8.9 ml, 0.029 mol) and 5-ethoxycarbonyl-4-methylthiazole (5.0 g, 0.029 mol) were refluxed in 2-butanone (60 ml) for 24 h. The reaction mixture was roto-evaporated to a small volume, and the solution was added dropwise into cold ethyl ether (100 ml). The light yellow solid was collected and reprecipitated from hot acetone yielding 7.5 g (85%) of **11** as a white solid. mp = 152–153°C dec; mp (lit) = 153°C dec (*15*); ir (KBr) ν_{max} cm^{-1}: 1575 (CO). ^1H NMR(D_2O):δ 1.42 (t,3H,J = 7 Hz, ester-$\mathbf{CH_3}$), 2.93 (s,3H,thiaz-$\mathbf{CH_3}$), 4.46 (s,3H,N-$\mathbf{CH_3}$), 4.48 (q,2H,J = 7 Hz,O-$\mathbf{CH_2}$-), 11.0 (s,1H,exchangeable-C-**H**).

Synthesis of 2-benzoyl-3,4-dimethyl-5-ethoxycarbonyl-[2-H]-thiazoline **12**. A three-neck, round-bottom flask was equipped with a reflux condenser, a magnetic stirring bar, and a Firestone valve. The apparatus was flamed while purging with

nitrogen and charged with freshly distilled triethylamine (0.68 ml, 9.6 mmol), freshly purified benzaldehyde (1.0 ml, 9.6 mmol), and anhydrous methanol (25 ml), and the solution was deoxygenated. The solution was brought to a gentle reflux, 3,4-dimethyl-5-ethoxycarbonylthiazolium iodide **11** (1.5 g, 4.8 mmol) was added over 30 min, and the red solution was refluxed under a positive nitrogen pressure for an additional hour. The product crystallized on cooling the reaction mixture and was collected, under nitrogen, by suction filtration. The crude product was recrystallized under nitrogen in ethyl ether/petroleum ether affording 0.96 g (69%) of **12** as orange-red crystals. mp = 152–153°C.

Anal. Calcd for $C_{15}H_{17}NO_3S$: C,61.84; H,5.81; N,4.81. Found: C,62.10; H,5.88; N,4.67. mass spec: *m/e* (abundance,ion) 291(3, M^+), 186 (97,M+-Phco), 158 (100,186-C_2H_4). ir (KBr): 1650, 1550. uv (methanol): ^{1}H NMR($CDCl_3$):δ 1.27 (t,3H,*J* = 6 Hz,ester-**CH_3**), 2.47 (s,3H,4-**CH_3**), 3.02 (s,3H,N-**CH_3**), 4.18 (q,2H,*J* = 6Hz, O-**CH_2**-), 6.12 (s,1H,C-2**H**), 7.60 (m,3H,*m*- and *p*-aromatic-**H**), 7.98 (m,2H,*o*-aromatic-**H**).

The mother liquor from this reaction was reduced by roto-evaporation and NMR and TLC analysis verified the production of benzoin in the reaction of **11** with benzaldehyde.

RESULTS

Although neutral thiamine–aldehyde adducts had not been isolated from reaction mixtures of thiamine and aldehydes in basic ethanol, we chose the conditions given in Table 1 for several important reasons. First and most importantly, Maier and Metzler demonstrate that ethanol is sufficiently nonpolar for thiamine to exist as its tricyclic form under the conditions of these studies (*6*). If one goes to more polar solvents, for example, methanol, then the tricyclic form dispropor-

TABLE 1

Rxn time	Workup[a]	Products (yields)[b]
5 min	HCl gas to pH < 3[c]	HBT-HCl **3** (63%), HBT ketone,[d] benzoin,[d] benzaldehyde
5 h	CH_3COOH pH 6.8[c]	HBT ketone **4** (67%), Benzoin (25%)
5 min	CH_3COOH, pH 6.8	Thiamine[e] (75%), HBT ketone,[d] benzoin[d]
5 h[f]	CH_3COOH, pH 6.8	Benzoin (256%),[g] HBT ketone (60%)

Note. Products isolated from reaction mixtures of dry thiamine hydrochloride (3 g, 0.089 M), sodium ethoxide (0.198 M), and freshly distilled benzaldehyde (0.20 M) in absolute EtOH.

[a] Solutions were acidified (or neutralized) and stirred an additional 30 min at 0°C prior to product isolation.

[b] Calculated based on initial thiamine HCl.

[c] Observed pH.

[d] Detected by NMR and TLC—low yields were not quantitated.

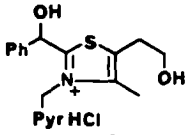
[e] Isolated as thiamine HCl.

[f] In the presence of 1.0 M benzaldehyde.

[g] The reaction mixture was diluted with chloroform and extracted with water (pH 3); benzoin in the chloroform layer was recrystallized from methanol.

tionates slightly between the yellow and thiazolium forms. Thus we predicted that ethanol should have the minimum hydrophobicity necessary for observation of neutral thiamine–aldehyde adducts.

Second, in an earlier paper we report that under these conditions (thiamine HCl, two equivalents NaOEt) the tricyclic form of thiamine is a precursor to the thiamine carbene via a proposed proton-transfer, elimination mechanism (*5*, *10*). Additionally, this thiamine carbene reacts with aldehydes, as evidenced by the isolation of the 2-(1-hydroxyalkyl)thiamine salts in excellent yields after acidification of the reaction mixture with HCl (*16*). These thiamine–aldehyde adducts are formed quite rapidly. When a reaction mixture of the tricyclic form of thiamine with benzaldehyde is acidified with HCl after only 5 min, 2-(1-hydroxybenzyl)thiamine hydrochloride **3** can be isolated in 65% yield (Table 1). HBT-HCl **3**



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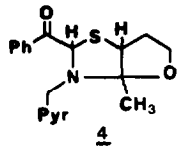
isolated from this reaction mixture was characterized by comparison with its known melting point and by its characteristic ^{1}H NMR spectrum. This rapid accumulation of thiamine–aldehyde derivatives in basic ethanol is in contrast to the relatively slow rates of formation and low yields of 2-(1-hydroxyalkyl)thiamine salts when thiamine is reacted with aldehydes in basic aqueous solution (*17*).

A characteristic phenomenon which can be observed when the tricyclic form of thiamine is reacted with benzaldehyde (under the conditions given in Table 1) is the dramatic change in color of the reaction solution. When benzaldehyde is added to a solution of the tricyclic form of thiamine (generated by the addition of two equivalents of NaOEt to thiamine HCl) the reaction mixture goes from the light yellow color characteristic of the tricyclic form to an orange-red color, with the color reaching maximum intensity after approximately 5 min. This color disappears rapidly upon acidification (or neutralization) of the reaction mixture, or fades slowly when the reaction mixture is stirred for 5 h. Of course under acidic conditions HBT-HCl **3** is isolated as a white solid. Thus the light-absorbing species must be a thiamine–benzaldehyde adduct which gives rise to the colorless HBT-HCl only under acidic conditions.

A second difference between the chemistry of **2** vs the chemistry of thiamine in basic aqueous solution is that when the tricyclic form of thiamine is reacted with benzaldehyde for 5 h (rather than 5 min) at 0°C, the enantiomers of the *cis* **4a** and *trans* **4b** isomers of HBT ketone can be isolated in 67% total yield (Table 1). Identification of **4a** and **4b** was based on their characteristic ^{1}H NMR spectra (e.g.,



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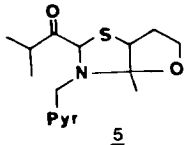
the "benzophenone split" of the aromatic protons; resonances assignable to the C-2 protons at δ 5.5 and 5.3 for **4b** and **4a**, respectively; broad resonances at δ 6.4 and 6.5 assignable to the exchangeable pyrimidine-NH_2 protons of **4a** and **4b**, respectively), ir spectra (carbonyl stretch at 1682 and 1695 cm^{-1} for **4a** and **4b**, respectively), and their identical mass spectral and elemental analyses. Our assignment of the *cis–trans* relationship between the benzoyl and THF ring is based on the assignment of Takamizawa *et al.* (*8*), and on their decomposition point, with the assumption that the isomer in which the benzoyl group is *cis* to the THF ring will be the most unstable. This tentative assignment awaits crystallographic confirmation. HBT ketone is also obtained as a disastereomeric mixture when the tricyclic form of thiamine is reacted with 10 equivalents of benzaldehyde (Table 1), but in this case benzoin can be isolated in 256% yield (based on initial thiamine), i.e., the reaction is catalytic.

Formation of acylperhydrofurothiazoles in basic ethanol is not limited to the reaction of the tricyclic form of thiamine with benzaldehyde. When thiamine HCl is reacted with isobutyraldehyde in basic ethanol and the reaction mixture is stirred for 5 h, the isobutyrylperhydrofurothiazole **5** is isolated in good yield, although only one of the diastereomers can be isolated by crystallization. Additionally, when this reaction mixture is acidified to pH 3 with HCl only 5 min after the addition of isobutyraldehyde to the tricyclic form of thiamine, 2-(1-hydroxyisobutyryl)thiamine HCl **6** is isolated as a white solid. Compounds **5** and **6** were identified based on their characteristic ^1H NMR and ir spectra, and in the case of **6** by comparison with a published literature melting point.



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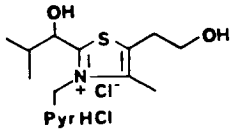

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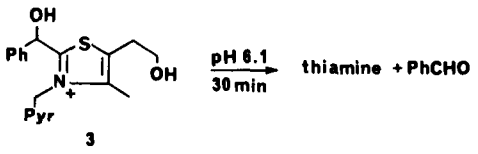
An interesting difference between the reaction of the tricyclic form of thiamine with isobutyraldehyde vs benzaldehyde is that in the former case, upon the addition of isobutyraldehyde to the tricyclic form of thiamine, the reaction mixture goes from the light yellow color of the tricyclic form to yellow-green in color; upon acidification the solution returns to light yellow in color. Thus although the nature of the light-absorbing species is dependent on the aldehyde R-group, this phenomenon has been observed in the reaction of thiamine with aldehydes in only basic nonaqueous solution.

Neutralization of the reaction mixture of thiamine HCl, two equivalents of NaOEt, and excess benzaldehyde after 5 min (Table 1) was run as a control and demonstrates two important principles concerning the intermediates formed. First, formation of HBT ketone must be time-dependent and is certainly not a result of the workup conditions. This conclusion is further supported by our observations that HBT ketone is stable to the basic reaction conditions, and to both the neutral workup with acetic acid and the acidic workup with HCl. Interestingly the product obtained after this neutralization is not HBT, but thiamine (Table 1)—HBT must be unstable even under neutral conditions (Eq. [3]).

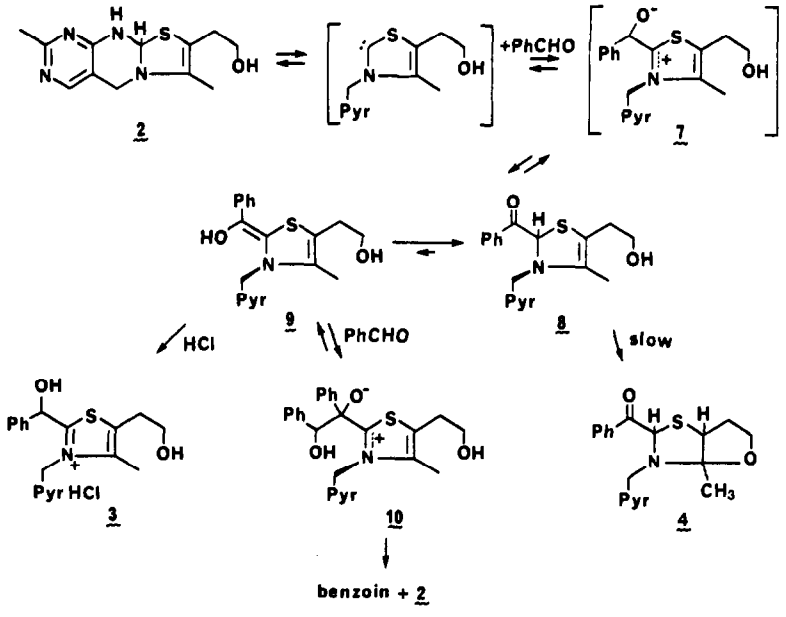


[3]

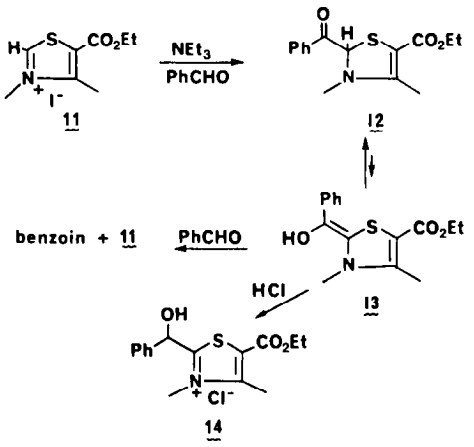
This result is not surprising when one considers that Breslow's mechanism demands that these intermediates be unstable even in aqueous solution (*1*), and that when one goes from aqueous to alcoholic solutions the rates of decomposition of 2-(1-hydroxyalkyl)thiamine salts increase by a factor of approximately 10^4–10^5 (*18*). Additionally, given the instability of HBT under even neutral conditions and the fact that the rate of decomposition of HBT increases with increasing pH, it seemed that HBT could not be a stable addition product under the more basic conditions of the reaction mixture (see Discussion).

At this point sufficient circumstantial evidence existed to suspect that the intermediate formed upon the addition of benzaldehyde to the tricyclic form of thiamine is the 2-benzoylthiazoline **8** in equilibrium with a low concentration of the enol isomer **9** (Scheme 2). The more logical mechanism for formation of HBT ketone **4** is the slow rate-limiting closure of the N-O ketal of **8**. The long wavelength absorbing species observed qualitatively would be explained by the existence of at least low concentrations of an extensively conjugated system such as enol **9**. The unstable HBT would then be formed (in high concentrations) upon acidification (or neutralization) by C-2α protonation of enol **9**, a reaction which would presumably drain the enol/ketone equilibrium.

Ideally, we felt it important to isolate the intermediates **8** and **9**. However, all attempts have failed apparently because of both our inability to crystallize these



SCHEME 2



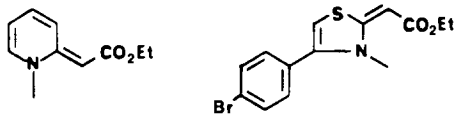
SCHEME 3

intermediates and their sensitivity to heat and oxygen. A second approach to this problem was to prepare an electron-deficient thiazolium salt. The rationale was that an electron-withdrawing group at the C-5 position of the thiazolium salt would be in conjugation with the free electron pair on nitrogen in both the ketone and enol isomers; such a substituent would be expected to stabilize the enol/ketone isomers, thus facilitating their isolation and characterization.

To test this prediction, 3,4-dimethyl-5-ethoxycarbonylthiazolium iodide **11** was synthesized by the *N*-methylation of 5-ethoxycarbonyl-4-methylthiazole, prepared by the condensation of thioformamide with ethyl α-bromoacetoacetate. When this salt was reacted with excess benzaldehyde containing two equivalents of NEt_3, and the reaction mixture cooled to 0°C, the 2-benzoylthiazoline **12** crystalized from the reaction mixture in 70% isolated yield as an orange-red solid (Scheme 3). Ketone **12** was identified by its characteristic ^1H NMR spectrum (e.g., "benzophenone split" of the aromatic protons, resonance at δ 6.12 assignable to the C-2 proton, and the upfield shift of the two methyl resonances relative to their positions in the starting thiazolium salt), ir spectrum (carbonyl stretch at 1650 cm^{-1}), and by its mass spectral and elemental analyses. Low yields of benzoin synthesis was also observed in this reaction by NMR and TLC analysis of the reaction mixture; benzoin is most likely produced by slow reaction of enol isomer **13** at the C-2α position with a second benzaldehyde. In preliminary NMR experiments directed at elucidation of the mechanism of formation of ketone **12**, we have observed that thiazolium salt **11** under identical conditions is converted completely to **12** after 30 min at 25°C with no detectable formation of the corresponding 2-(1-hydroxybenzyl)thiazolium salt **14**. An authentic sample of **14** was prepared by suspending ketone **12** in HCl-saturated DMF and was isolated as a white solid.[5]

[5] Salt **14** could not be purified to homogeneity due to its slow release of benzaldehyde to **11** even in acidic solution. However, it was identified, by its characteristic NMR spectrum: ^1H NMR(D_2O): δ (t,3H,J = 7.4 Hz,ester-$\mathbf{CH_3}$), 2.57 (s,3H,thiaz-$\mathbf{CH_3}$), 3.68 (s,3H,N-$\mathbf{CH_3}$), 4.27 (d,2H,J = 7.4 Hz,O-$\mathbf{CH_2}$-), 6.35 (s,1H,C-2α-**H**), 7.35 (m,5H,phenyl-**H**).

The homogeneous orange-red color of the 2-benzoylthiazoline **12** was surprising—the red color could not be removed with decolorizing charcoal, and on silica gel chromatography in several solvent systems the light-absorbing species eluted with the ketone product. Additionally this ketone was homogeneous as ascertained by elemental analysis. Appropriate model system compounds which absorb light at wavelengths greater than 300 nm are the anhydrobase forms of ethyl 2-pyridine acetate and ethyl 4-(*p*-bromophenyl)-3-methyl-2-acetate thiazolium ions.



The pyridyl derivative was isolated by Jones and Katritzky (*19*) as a yellow solid (λ max = 384 nm), and the thiazolyl anhydrobase was detected by a characteristic absorption band at 333 nm (*20*). Based on these and related model system compounds it is possible that the red color of thiazoline **12** is due to the presence of low concentrations of enol isomer **13** in equilibrium with the ketone.

DISCUSSION

Breslow's mechanism for the thiamine-catalyzed benzoin-type condensation as discerned from studies in basic aqueous solution stresses the stability of the aromatic thiazolium ring, but does not address the question of the possible importance of the reactivities of thiamine which are responsible for its instability in basic solution. This is in spite of the fact that under conditions maximum for the thiamine-catalyzed benzoin-type condensation in basic aqueous solution, thiamine exists as a 50% mixture of the thiazolium and ring-opened free-thiol forms (*21, 22*) (Scheme 1). Under these conditions in basic aqueous solution thiamine reacts with aldehydes to yield 2-(1-hydroxyalkyl)thiamine salts (*3, 17*) in apparent equilibria with their corresponding free-thiol forms. This instability of thiamine and 2-(1-hydroxyalkyl)thiamine salts in basic solution at the expense of the aromaticity of the thiazolium ring can be attributed to the formal positive charge at N-3, the partial positive charge at C-2, and the angle strain inherent in their planar structure (*10*).

The differences we have observed in the chemistry of thiamine with benzaldehyde in basic alcoholic vs basic aqueous solutions can be traced to the observations that aromatic thiazolium rings are unstable in basic solution. However, in contrast to the behavior of thiamine in basic aqueous solution, in basic ethanol hydrolysis of the thiamine thiazolium ring is precluded; in the absence of a mechanism for buffering the thiazolium ring thiamine is instead converted to its neutral tricyclic species (Scheme 1). This chemistry then represents a second mechanism, unique to thiamine, for releasing the positive charge and angle strain of the thiazolium ring.

If the thiazolium ring of thiamine is destabilized in basic ethanol resulting in its conversion to the neutral tricyclic species, then 2-(1-hydroxyalkyl)thiamine salts (HBT in particular), which have an increased base liability due to the addition of a C-2/N-3 torsional interaction (*23*), would also not be expected to exist in appreciable concentrations under identical conditions. Nevertheless, upon the acidification of a reaction mixture of the tricyclic form of thiamine with benzaldehyde, HBT-HCl **3** can be isolated in high yields (Table 1). Besides the tricyclic derivative of HBT, other neutral forms of HBT which could accumulate and give rise to HBT-HCl under acidic conditions is ketone **8** in equilibrium with at least a low concentration of enol **9** (Scheme 2). Although these intermediates have not been isolated, our observation that HBT ketone **4** is formed by the time-dependent closure of the *N,O* ketal of **8** is strong qualitative chemical evidence for its existence.

Additionally, the 2-benzoylthiazoline **12** has been isolated and characterized as the stable addition product in the reaction of the C-2 deprotonated intermediate of thiazolium salt **11** with benzaldehyde (Scheme 3). Synthesis of **12** is the first demonstration that thiazolium salts can react with aldehydes in basic alcoholic solution to yield stable nonaromatic derivatives. Of the three products, namely ketone **12,** enol **13,** and salt **14,** the 2-benzoylthiazoline **12** is the stable addition product—this result is not totally unexpected. The destabilizing reactivities of salt **14** (and salts **3** and **6**), i.e., the formal positive charge at N-2, the partial positive charge at C-2, the angle strain inherent in the planar thiazolium ring, and the C-2,N-3 torsional interaction, reactivities which activate thiazolium salts to hydrolysis in aqueous solution and to rapid formation of the tricyclic form (in the case of thiamine salts) in nonaqueous solution, are all absent in the 2-benzoylthiazolines. In ketone **12** (and **8**) C-2 and perhaps N-3 are sp^3-hybridized releasing the angle and torsional strain, and reduction of the thiazolium to the thiazoline ring removes the charge. Formation of the 2-benzoylthiazolines is indeed another mechanism by which the destablizing charge, angle strain, and torsional interactions of the aromatic 2-(1-hydroxyalkyl)thiazolium salts can be avoided in basic hydrophobic solutions.

Although ketone **12** is the major isomer observed, the enol isomer **13**, although not detectable at least by 60 MHz NMR, exists apparently in equilibrium with the ketone (Scheme 3). First, the long wavelength absorbance of **12** is best explained based on the absorptivity expected for low amounts of the enol isomer. Second, benzoin is produced in the reaction, apparently through the intermediacy of enol **13** (Scheme 3). Lastly, when ketone **12** is suspended in HCl-saturated DMF it is converted with loss of the red color to the 2-(1-hydroxybenzyl) salt **14**—rearrangement of ketone **12** to salt **14** must be through the intermediacy of enol **13** (Scheme 3). That the enol/ketone equilibrium lies to the ketone is not surprising when considering these equilibria in related ketones; as an example chloroacetone exists almost exclusively in the ketone form (*24*). Two additional structural features of the enol isomer which would argue against its stability relative to the ketone are (1) the sp^2-hybridized C-2 and N-3 must be strained due to inclusion in a five-membered ring, and (2) torsional interactions between the *cis*-vinylic and *N*-alkyl groups would increase the strain of the enol isomer. Note that each of these

destabilizing interactions could be released partially if N-3 is sp^3-hybridized, as opposed to sp^2-hybridized as argued by Schellenberger (*25*). A second point which bears mention is that in our preliminary NMR experiments mentioned under Results, we have observed that the rate of enolization of ketone **12** in basic deuterated methanol, as measured by the H-D exchange of the C-2 proton, is fast compared with the rate of synthesis of **12** from thiazolium salt **11** and benzaldehyde. This result is in agreement with the rapid rates of enolization of structurally related ketones (*24*); the 2-benzoylthiazolines, then, are precursors to the important "active aldehydes" via a rapid enol/ketone equilibrium.

Another important aspect of this work is that in spite of the fact that the active form of thiamine is slowly removed due to formation of the thermodynamically stable HBT ketone **4** (Scheme 2), the tricyclic form of thiamine is an excellent catalyst of the benzoin condensation, as evidenced by the 2.5 equivalents of benzoin isolated when thiamine is reacted with 10 equivalents of benzaldehyde in basic ethanol (Table 1). The rapid accumulation of benzoin under these conditions at 0°C in basic ethanol is in contrast to the relatively slow rate of formation and low yields of acyloin products observed when thiamine is reacted with aldehydes at even 40°C in basic aqueous solution (*21*). Currently, we are working on the kinetics of this system in order to discern the reasons for the catalytic power of thiamine under these conditions. Nevertheless, this effect is almost certainly due to stabilization of the neutral forms of thiamine in hydrophobic solution. Whether it be in stabilization of the neutral C-2 deprotonated intermediate **2** (see Scheme 2), in stabilization of ketone **8** in rapid equilibrium with the "active aldehyde" **9**, in stabilization of the neutral "transition state" structures such as **7** and **10**, and/or a possibility that we cannot rule out at this point, in destabilizing the thiazolium ring of HBT such that an equilibrium is set up between the tricyclic form of HBT and ketone **8** in equilibrium with enol **9**, we have been able to show that by placing thiamine in an environment where the nonaromatic chemistry is predominate thiamine is a superior catalyst in nonenzymic transformations.

Crosby and Lienhard conclude that important properties of thiamine pyrophosphate-dependent enzyme-active sites are that (1) they provide a hydrophobic environment for TPP and (2) they decrease (by unknown mechanisms) the pK_as of the C-2 proton of TPP and the C-2α hydroxyl proton of 2-(1-hydroxyethyl) thiamine pyrophosphate by three to four units (*18*). If their assessment is correct, then studies on the chemistry of thiamine in basic ethanol are more applicable to the study of the active site chemistry of TPP than studies on the chemistry of thiamine in basic aqueous solution. Because the 5-(2-hydroxyethyl) group of thiamine is pyrophosphorylated in nature, the only detail of Scheme 2 which is not available to enzyme systems is closure of the *N-O* ketal of **8** to HBT ketone **4.** In enzymatic systems formation of acylperhydrofurothiazoles such as **4** and **5,** compounds which are thermodynamically stable and inactive as catalysts, would result in dead-end complexes in which active thiamine would be lost. Perhaps a fortunate consequence of pyrophosphorylation of thiamine in nature is that it excludes formation of these catalytically inactive acylperhydrofurothiazoles.

CONCLUSION

By placing thiamine in aqueous solution where the ionic thiazolium and free-thiol forms of both thiamine and intermediate 2-(1-hydroxyalkyl)thiamine salts are stabilized relative to their neutral forms, Breslow and subsequent investigators were able to isolate and study the aromatic chemistry of thiamine in nonenzymic transformations. We have gone to the other extreme and placed thiamine in a solution in which the thiazolium ring cannot buffer thus forcing it into its neutral nonaromatic tricyclic form. We have concluded that under these conditions HBT cannot exist in appreciable concentrations even though intermediates do accumulate in high concentrations upon the addition of benzaldehyde to a solution of the tricyclic form of thiamine. Evidence presented indicates that the intermediate which accumulates is the nonaromatic benzoyl-thiazoline **8** in equilibrium with and a precursor to its enol isomer **9**, i.e., the C-2α reactive "active aldehyde." Formation of these 2-benzoylthiazolines appears to be another mechanism by which the destabilizing reactivities of 2-(1-hydroxyalkyl)thiamine salts can be avoided in basic nonaqueous solution. We have also demonstrated that this nonaromatic chemistry of thiamine endows it with a high catalytic potential in terms of the yields of benzoin which can be recovered. We assert that, at least in this model system chemistry, providing a pathway for effectively stabilizing the neutral forms of thiamine is sufficient for converting thiamine from a marginal to an efficient catalyst. In light of the proposal by Crosby and Lienhard on the factors responsible for the catalytic efficiency of TPP-dependent enzymes (*18*), we assert that enzymes might act by effectively destabilizing the thiazolium ring of TPP and 2-(1-hydroxyalkyl)TPP salts such that this nonaromatic nucleophilic carbene chemistry of thiamine could be exploited.

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