Synthesis and Properties of S-Aminomethylthiamine and N-Hydroxymethylthiamine

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S-Aminomethylthiamine: N-[(2-Methyl-4-amino-5-pyrimidinyl)methyl]-N-[1-methyl-2-(dialkylaminomethyl)thio-4-hydroxy-1-butenyl]formamide (IIIa--d) were successfully synthesized by the reaction of an alkaline solution of thiamine (I) with chloroform solution of chloromethylthiakylamine (VIA--d), which were freshly prepared from methylenebisdialkylamine (Va--d) and acetyl chloride.

The compounds (IIIa--d) were soluble in organic solvents and also soluble in water with the exception of IIIb.

When III was dissolved in water, it afforded a small amount of cyclobismethylenethiamine (II) and mostly decomposed to I. The behaviour of IIIa in water was confirmed by means of polarographic technique.

N-Hydroxymethylthiamine: 3-[(2-Methyl-4-hydroxymethylamino-5-pyrimidinyl)methyl]-4-methyl-5-(2-hydroxyethyl)thiazolium chloride (VII) was also prepared from thiamine monochloride [I-Cl] and formalin. Compound VII gave also II in the presence of diethylamine. The formation pathway of II from I was also discussed.

In the previous papers,20 we have reported the synthesis of a new class of thiamine derivative (cyclobismethylenethiamine): 2,7,13,18-tetramethyl-6,17-diformyl-8,19-di(2-hydroxyethyl)-dipyrimido[4,5-d; 4',5'-m]-1,10-dithia-3,7,12,16-tetraazacyclooctodeca-4,8,13,17-tetraene (II), which consists of eighteen membered heterocyclic ring with two thiamine and two formalin groups. II was prepared from thiamine (I) with formalin in the presence of secondary amines in an aqueous alkaline solution, and its properties as well as the structural elucidation were also investigated (Chart 1).

![Chart 1](attachment:chart1.png)

It is revealed that the addition of secondary amines such as diethylamine, piperidine and morpholine should be required for the formation of II, although any amine residue used is not involved in II. When a tertiary amine was used instead of the secondary amine, any reaction did not proceed at all. From the fact that an O-benzoyl derivative of II was also obtained from O-benzoylthiamine, the hydroxy group in the side chain of I would not directly play a role in the formation of II. Based on these results, a possible intermediate in the formation of II would be expected to be S-aminomethylthiamine (III) or N-aminomethylthiamine (IV), as shown in Chart 2.

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In order to clarify the formation pathway of II, we describe, in this paper, the synthesis and properties of S-aminomethylthiamine: N-[(2-methyl-4-amino-5-pyrimidinyl)methyl]-N-[1-methyl-2-(dialkylaminomethyl)thio-4-hydroxy-1-buteryl] formamide (III) and N-hydroxymethylthiamine: 3-[(2-methyl-4-hydroxymethylamino-5-pyrimidinyl)methyl]-4-methyl-5-(2-hydroxyethyl)thiazolium chloride (VII), which would be the precursor of IV.

It was reported in the previous paper\textsuperscript{3a} that the S-aminomethylation of I did not give III but II, according to the usual method which consists of the reaction of thiols with secondary amines and formalin.\textsuperscript{3} On the other hand, Böhme has reported that dialkylaminomethyl sulfide\textsuperscript{4a} is obtained by the reaction of thiol with chloromethylidialkylamine (VI)\textsuperscript{4b} prepared from methylenebisdiarylamine (V) with acyl halide.

In this study III was synthesized by the reaction of thiamine sodium salt (I-SNa) with VI as shown in Chart 3. An alkaline solution of I-Cl·HCl containing 3 equivalent of sodium hydroxide was added to a chloroform solution of chloromethylidialkylamine (VIa), freshly prepared from methylenebisdiarylamine (Va) and acetyl chloride according to the Böhme's method.\textsuperscript{4b} N-[2-Methyl-4-amino-5-pyrimidinyl]methyl]-N-[1-methyl-2-(diethylaminomethyl)thio-4-hydroxy-1-buteryl]formamide (IIa) was obtained as colourless prisms of mp 89–91\degree C from the chloroform layer in 65.0% yield. In the similar manner, IIIb–d were obtained the reactions of I-SNa and VIb–d, respectively.

The structures of IIIa–d thus obtained were confirmed by their elemental analyses, spectral data and qualitative tests as follows. The nuclear magnetic resonance (NMR) spectra


in deuterio-chloroform of IIIa–d showed signals in the range of 3.60 ppm to 3.98 ppm as each singlet which was attributable to the methylene protons between S and N (Table I). The spectrum of IIIa exhibited deuterium-exchangeable signals at 6.20 ppm (2H, broad singlet) and 3.50 ppm (1H, broad singlet), suggesting the presence of amino group at the 4 position in pyrimidine ring and hydroxy group in the side chain, respectively. Apparently, these NMR data reveal that IIIa–d are the products reacted selectively with the S atom of I. The above assignment was also supported by the ultraviolet (UV) spectra of IIIa–d, which showed maxima at 236 and 267–270 nm in ethanol due to the 4-amino pyrimidine moiety. Moreover, the qualitative tests of formalin were positive in both methods using chromotropic acid and 2,4-dinitrophenylhydrazine. Yields and physicochemical data were shown in Table I.

Table I. Yields and Physicochemical Data of S-Aminomethylthiamine

<table>
<thead>
<tr>
<th></th>
<th>N_R</th>
<th>Yield (%)</th>
<th>mp (°C)</th>
<th>Recryst. solvent</th>
<th>NMR (°)</th>
<th>UV ( \lambda_{max} ) nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>NEt_2</td>
<td>65.0</td>
<td>89–91</td>
<td>THF-ether</td>
<td>3.98</td>
<td>236, 270</td>
</tr>
<tr>
<td>b</td>
<td>NBut</td>
<td>68.4</td>
<td>83–85</td>
<td>AcOEt</td>
<td>3.96</td>
<td>236, 270</td>
</tr>
<tr>
<td>c</td>
<td>N</td>
<td>70.0</td>
<td>95–97</td>
<td>AcOEt</td>
<td>3.63</td>
<td>236, 267</td>
</tr>
<tr>
<td>d</td>
<td>N</td>
<td>49.0</td>
<td>113–115</td>
<td>THF-ether</td>
<td>3.60</td>
<td>236, 268</td>
</tr>
</tbody>
</table>

IIIa–d were easily soluble in organic solvents, such as tetrahydrofuran, dioxane, acetone, chloroform and ethanol and were also soluble in water with the exception of IIIb.

When IIIa was dissolved in water at room temperature, the pH, which indicated 9.2 immediately after dissolving, shifted gradually to 11.0, because of liberation of diethylamine from IIIa. After stirring for 15 hr, colourless precipitates were collected by filtration and identified with an authentic sample^{3a} of II by comparing IR spectra. Extraction of the filtrate with benzene and evaporation of the solvent gave a colorless oil, which was identified with an authentic sample of Va by IR and NMR spectra. Va would be formed by the reaction of formalin and diethylamine eliminated from IIIa. Further extraction with chloroform was carried out to obtain a trace of II dissolved in the aqueous solution. Yields of II and Va were 15.2% and 25.0%, respectively. Finally, the aqueous layer was acidified with 10% hydrochloric acid and was evaporated under reduced pressure to give I-Cl-HCl in 50.5% yield.

Since II was found to be decomposed to I in a strong alkaline region,^{3a} the pH value of an aqueous solution of IIIa was adjusted to the range 9.5–10.0 by bubbling carbon dioxide through the solution. After stirring for 15 hr, the reaction mixture was treated in the similar manner as above; yields of II, Va and I-Cl-HCl were 29.2%, 25.0% and 50.0%, respectively. IIIc was also treated in the same manner to give 14.4% of II, 15.0% of Vc and 36.0% of I-Cl-HCl, respectively (Chart 4).

From the result that a large amount of I-Cl-HCl was recovered from the aqueous solution of III, it would be interesting to know the behavior of III in water.

On the other hand, it has been well known^{5} that thiol type of I in polarogram indicates an oxidation wave in an alkaline solution. It has also been reported that an anodic wave

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is observed by the alkali decomposition of III. Based on the above knowledge, the use of a polarographic technique is advantageous to investigate the property of III. Then, the polarogram of an aqueous solution of IIIa was determined using a rotating Au-electrode and the oxidation wave was observed. As a result, the formation ratio of thiol type of I on the basis of the calibration curve of a standard thiamine were 70% after 2 min, and 90% after 20 min, respectively (Fig. 1).

From the above results it was found that S-C bond cleavage of III in water led to the decomposition to I. Since it was not agreeable to estimate III as an intermediate in the process to II, the isolation of IV was examined.

Although Mannich reaction of 4-aminopyrimidine compounds had been reported by Hirano et al., in this study the reaction of thiamine monochloride (I-Cl) with 1.0 molar ratio of formalin and 1.3 molar ratio of diethylamine was carried out at room temperature to obtain the corresponding N-Mannich base (IV). However, the expected compound IV was not obtained, but II was given in 28.2% yield. In this reaction, ring opening of I-Cl to thiol type would lead to the formation of II because the pH value in the reaction mixture shifted to 10. Further, the treatment of I-Cl with formalin and diethylamine hydrochloride in a weak acidic condition resulted in the recovery of the starting material.

Then, the preparation of N-hydroxymethylthiamine (VII) which would be the precursor of IV was attempted in this study, though Fumagalli, et al. had reported the synthesis of N,N'-dimethylothiamine by the reaction of I-iodide with excess formalin under boiling condition.

After the reaction of I-Cl with 5.0 molar ratio of 37% formalin in an aqueous solution was carried out at room temperature for 20 hr, acetone was added to the reaction mixture to separate out VII which was purified by reprecipitation from water-acetone to obtain colourless prisms, mp 116—118° (decomp.), in 65.0% yield. Compound VII was soluble in water and dimethyl sulfoxide and insoluble in other organic solvents. The qualitative tests of formalin and halogen ion were positive. The structure of VII was supported by elemental analysis and spectral data as follows. The UV spectrum of VII showed maxima at 240 nm and 265 nm in water. The NMR spectrum of VII in dimethyl- \(d_6\) sulfoxide showed a singlet at 9.70 ppm (1H) attributable to the C-6-proton of the thiazole ring, a broad triplet at 8.45 ppm (1H) due to \(-\text{NHCH}_2\text{OH}\) group, which disappeared by deuterium oxide treatment, and revealed a doublet at 4.85 ppm (2H) due to methylene protons of \(-\text{NHCH}_2\text{OH}\), which changed to singlet by deuterium oxide treatment. By irradiation of the methylene protons of \(-\text{NHCH}_2\text{OH}\) group, the signal of NH changed to singlet.

Then, the derivation to II from VII was examined. It is expected that the addition of secondary amines produces IV, followed by cyclization to II. According to expectation, II

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was obtained in 34.2% yield as a colourless powder when 1.0 molar ratio of sodium hydroxide and 0.5—1.0 molar ratio of diethyamine were added to an aqueous solution of VII. When 1.3 molar ratio of diethyamine was used instead of sodium hydroxide, II was also obtained in 85.5%. In the absence of diethyamine, II was not formed at all, even if the pH was adjusted to the optimum range (9.5—10.0) by use of triethyamine.

The results are briefly summarized as follows.

1. S-Aminomethylthiamine (III) was synthesized from I-SNa and chloromethyl-dialkylamine (VI) in a good yield.

2. S-C bond cleavage of III by hydrolysis in an aqueous solution was observed to give mainly thiamine (I) accompanied with methylenebisdialkylamine (V).

3. The partial formation of II from the above aqueous solution of III was attributed to the successive interaction between I and adducts of secondary amines with formalin, both of which were formed in the reaction mixture after S-C bond cleavage; i.e., this reaction condition was similar to that toward the formation of II, reported previously.2a

4. The above S-C bond cleavage of III was also supported by the polarographic method.

5. We failed in isolation of IV during the formation of II, by reacting I-Cl with formalin and diethyamine.

6. However, N-hydroxymethylthiamine (VII), which would be a precursor of IV, was obtained from I-Cl and formalin in a good yield.

7. VII was easily transformed to II by the addition of diethyamine.

8. In the absence of the secondary amines, VII did not give II at all even when the pH value was adjusted to the optimum range (9.5—10.0).

From these results, it seems reasonable to assume that II is formed not from III but from IV, thus a nucleophilic attack of the sulfur anion to the methylene group of −NCH₂N< leads to the cyclization, leaving the diethyamine out of the molecule. An outline of the proposed pathway is summarized in Chart 5. Bimolecular (intermolecular) ring closure for II would be more favourable than intramolecular cyclization because of a higher steric strain of the latter.

**Experimental**

**General Procedure for the Preparation of S-Aminomethylthiamine (IIIa—d)**—To a solution of I-Cl·HCl (0.06 mole) in H₂O (50 ml) was added an aqueous NaOH (0.18 mole) solution below 10° to prepare an aqueous solution of I sodium salt. On the other hand, a solution of CH₂COCl (0.06 mole) in ether was added dropwise to a solution of methylenebisdialkylamine (Vα—d) (0.066 mole) in ether to prepare a suspension of chloromethylidialkylamine (Vα—d₁), and then the suspension was dissolved by addition of CHCl₃. To the solution of VI in CHCl₃—ether, above I alkaline solution was added with vigorous stirring at −5—5°, and stirring was continued for 45 min at the same temperature. The organic solvent layer was separated, dried over Na₂SO₄ and evaporated in vacuo below 20°. The resulting oily residue was washed with n-hexane and ether to obtain a colourless powder of IIIa—d. Yields and physicochemical data were shown in Table I.

**S-Diethyaminomethylthiamine (IIIa)**—A solution of 20.20 g (0.06 mole) of I-Cl·HCl in H₂O (50 ml) was treated with 35.0 g (0.18 mole) of 20.6% NaOH to prepare a solution of I sodium salt. A suspension of chloromethylidialkylamine (Vα) in ether (100 ml) was prepared from 10.44 g (0.0066 mole) of methylenebisid-ethylamine (Vα) and 4.70 g (0.06 mole) of CH₂COCl. IIIa (14.3 g, 65.0%) was obtained as colourless prisms (from THF—ether) according to the general procedure cited above. Anal. Calcd. for C₁₇H₂₉N₂O₅S: C, 55.56; H, 7.06; N, 19.06; S, 8.73. Found: C, 55.66; H, 7.88; N, 18.69; S, 8.65. IR νₘα₈ max cm⁻¹: 3300, 3150, 1670, 3050, 1680, 1570, 1470, 960 cm⁻¹.

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8) All melting points were determined in capillaries and uncorrected. NMR spectra were taken on a Hitachi Perkin-Elmer R-20A spectrometer in CDCl₃ or DMSO-d₆ solution with tetramethyldisilane (TMS) as an internal standard. Chemical shifts are given in δ (ppm) values. Abbreviations used are s=singlet, d=doublet, t=triplet, m=multiplet, and br=broad. UV spectra were taken on a Hitachi EPS-3T spectrophotometer in EtOH or H₂O. IR spectra were taken in nujol mull on a Shimadzu IR-27G spectrometer. Polarograms were recorded on Yanagimoto P-8 type polarograph. A rotating Au-electrode (0.089 cm²) at 1000 rpm. was used. Potential values were referred to SCE.
S-Dibutylaminomethylthiamine (IIb)—IIb (14.5 g, 68.4%) was obtained from 16.8 g (0.05 mole) of I-Cl-HCl, 29.0 g (0.15 mole) of 20.6% NaOH, 14.8 g (0.055 mole) of methylenebisdibutylamine (Vb) and 3.90 g (0.05 mole) of CH₂COCl according to the general procedure. *Anal. Calcd. for C₁₃H₂₈O₂N₃S: C, 59.54; H, 8.81; N, 16.54; S, 7.57. Found: C, 56.51; H, 8.87; N, 16.93; S, 7.93.* IR νₘₐₓ cm⁻¹: 3300, 3150, 1670, 1640, 1595, 1085, 1000. NMR (CDCl₃) δ: 0.70—1.10 (6H, m, N(CH₂CH₂CH₂CH₂)₂), 1.15—1.50 (8H, m, N(CH₂CH₂CH₂CH₂)₂), 1.95 (3H, s, CH₃), 2.42 (3H, s, Pm-C₅H₅), 2.30—2.70 (6H, m, CH₂CH₂OH, N(CH₂CH₂CH₂)₂), 3.40 (1H, d, s, OH), 3.79 (2H, t, CH₂CH₂OH), 3.96 (2H, s, SCH₂N), 4.45 (2H, s, Pm-C₅H₅), 6.10 (2H, b, NH₂), 7.80 (1H, s, Pm-C₅H₅), 8.00 (1H, s, N-CHO).

S-Piperidinomethylthiamine (IIc)—IIc (16.0 g, 70.0%) was obtained from 20.2 g (0.06 mole) of I-Cl-HCl, 55.0 g (0.18 mole) of 20.6% NaOH, 12.0 g (0.06 mole) of methylenebispiperidine (Vc) and 4.70 g (0.06 mole) of CH₂COCl according to the general procedure. *Anal. Calcd. for C₁₃H₂₈O₂N₃S: C, 56.07; H, 7.70; N, 18.46; S, 8.45. Found: C, 56.43; H, 7.55; N, 18.00; S, 8.42.* IR νₘₐₓ cm⁻¹: 3300, 3150, 1680, 1645, 1600, 1110, 1050. NMR (CDCl₃) δ: 1.30—1.70 (6H, m, piperidine-CH₂CH₂CH₂), 1.95 (3H, s, CH₃), 2.45 (3H, s, Pm-C₅H₅), 2.40—2.72 (6H, m, CH₂CH₂OH, piperidine-CH₂CH₂OH), 3.63 (2H, s, SCH₂N), 3.78 (2H, t, CH₂CH₂OH), 3.90 (1H, d, s, OH), 4.50 (2H, b, s, Pm-C₅H₅), 6.15 (2H, b, NH₂), 7.80 (1H, s, Pm-C₅H₅), 8.00 (1H, s, N-CHO).

S-Morpholinomethylthiamine (IIId)—IIId (5.7 g, 49.0%) was obtained from 10.1 g (0.03 mole) of I-Cl-HCl, 17.5 g (0.09 mole) of 20.6% NaOH, 6.20 g (0.033 mole) of methylenebismorpholine (Vd) and 2.35 g (0.03 mole) of CH₂COCl according to the general procedure. *Anal. Calcd for C₁₃H₂₈O₂N₃S: C, 53.52; H, 7.13; N, 18.36; S, 8.41. Found: C, 53.54; H, 6.97; N, 18.11; S, 8.21. IR νₘₐₓ cm⁻¹: 3300, 3150, 1670, 1640, 1110, 1060. NMR (CDCl₃) δ: 2.0 (3H, s, CH₃), 2.47 (3H, s, Pm-C₅H₅), 2.40—2.75 (6H, m, CH₂CH₂OH, morpholine-CH₂CH₂OH), 3.60 (2H, s, SCH₂N), 3.45 (1H, b, OH), 3.58—3.90 (6H, m, morpholine-CH₂OCH₂, CH₂CH₂OH), 4.50 (2H, b, s, Pm-C₅H₅), 6.20 (2H, b, NH₂), 7.80 (1H, s, Pm-C₅H₅), 8.00 (1H, s, N-CHO).

**Behaviour of III in Water**—a) IIia (3.7 g, 0.01 mole) was dissolved in H₂O (30 ml) and the solution was stirred at 23—25° for 15 hr. The colourless precipitate was collected by filtration and washed with H₂O to give 0.40 g of II, mp 284—289° (decomp.). The filtrate was extracted with CH₂Cl₂ and then CHCl₃. The CH₂Cl₂ extract was dried over Na₂SO₄ and evaporated to give 0.2 g (25.0%) of Va. The CHCl₃ extract was washed with H₂O, dried over Na₂SO₄ and evaporated in vacuo. The residue was recrystallized from EtOH to give 0.05 g of II. Total yield of II was 15.2%. The aqueous layer was acidified with 10% HCl and evaporated under reduced pressure to obtain 1.70 g (50.5%) of I-Cl-HCl, which was identical with an authentic specimen.

b) To a solution of 1.84 g (0.005 mole) of IIIA in H₂O (15 ml) was often bubbled carbon dioxide to adjust to pH 8.9—10.2 with stirring. After stirring was continued at 23—25° for 15 hr, colourless precipitates were collected by filtration, washed with H₂O and dried to give 0.39 g of II, mp 284—289° (decomp.). The filtrate was treated with a similar manner as described above. Yields of II, Va and I-Cl-HCl were 29.2%, 25.0% and 50.0%, respectively.
c) To a solution of 2.85 g (0.0075 mole) of IIIc in H_2O (20 ml) was often bubbled carbon dioxide to adjust to pH 9.8—10.2 with stirring. After stirring was continued at 23—25°C for 15 hr, the reaction mixture was worked up in the same manner as described (a). Yields of II, Vc (bp, 75—81°C) and I—Cl·HCl were 14.4%, 15.0%, and 36.0%, respectively.

Polarograms of IIIa—To a solution of 185.5 mg (0.50 mmole) of IIIa in H_2O (100 ml) degassed previously was added 500 mg of KNO_3 as supporting electrolyte at 22°C under bubbling of N_2. The half wave potential (E_1/2) of IIIa showed +0.3 V. The results were shown in Fig. 1.

The Reaction of Thiamine Monochloride (I—Cl) with Formaldehyde in the Presence of Diethylamine—A mixture of 1.10 g (0.013 mole) of 37% formalin, H_2O (3 ml) and 0.95 g (0.013 mole) of Et_2NH was stirred for 30 min at 23—25°C, and the mixture was added to a solution of 3.0 g (0.01 mole) of I—Cl in H_2O (7 ml) at the same temperature. After stirring for 15 hr, the precipitates were collected by filtration, washed with water and dried to give 0.83 g (28.2%) of II.

N-Hydroxymethylthiamine (VII)—To a solution of 15.0 g (0.05 mole) of I—Cl in H_2O (30 ml) was added 20 g (0.25 mole) of 37% formalin and the reaction mixture was stirred at 23—25°C for 20 hr. Acetone (700 ml) was added dropwise to the reaction mixture under ice cooling to give colourless powder. Recrystallization from H_2O—acetone gave 10.70 g (65.0%) of VII—monohydrate, as colourless prisms, mp 116—118°C (decomp.). Anal. Calcd. for C_{8}H_{10}O_{5}N·SCl·H_2O: C, 44.75; H, 6.07; N, 16.06; S, 9.19; Cl, 10.16. Found: C, 44.53; H, 5.88; N, 15.90; S, 9.21; Cl, 10.28. IR (max cm^{-1}): 3500, 3350, 3200, 1610, 1580, 1560, 1300. NMR (DMSO-d_6) δ: 2.45 (3H, s, thiazole ring CH_3), 2.55 (3H, s, Pm—C_4—CH_3), 3.08 (2H, t, CH_2CH_2OH), 3.62 (2H, t, CH_2—CH_2OH), 4.85 (1H, b, OH), 4.85 (2H, b, d, NHCH_2), 5.40 (1H, b, OH), 5.60 (2H, s, Pm—C_4—CH_2), 8.18 (1H, s, Pm—C_4—H), 8.45 (1H, b, t, NH), 9.70 (1H, s, thiazole ring C_4—H).

General Procedure for the Derivation to II from VII—To a solution of VII (0.0026 mole) in H_2O (5 ml) was added 10.0% NaOH (0.0026 mole) and Et_2NH with stirring below 10°C. After stirring at 23—25°C for 15 hr, the precipitates (II) were collected by filtration, and the filtrate was extracted with CHCl_3 to obtain the last trace of II. Recrystallization from EtOH—H_2O afforded II as colourless prisms, mp 284—289°C (decomp.). The results were summarized in Table II.

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