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124. Suminori Umio, Takashi Kamiya, and Kazuma Nishitsuji : Synthesis of DL-Thiamic Acid.*1

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Van Eys, *et al.*[1] found that rabbit muscle α-glycerophosphate dehydrogenase contains a prosthetic compound, structure being as 4-methyl-5-(2-hydroxyethyl)-4-thiazoline-3-succinic acid, named later by "thiamic acid".[2] He also described the synthesis of racemic thiamic acid but no details were disclosed. In the present paper are reported synthesis of thiamic acid and some discussion about the ultraviolet spectra of thiamic acid and its synthetic intermediates.

The general method of thiazole synthesis was applied to this synthesis as shown in Chart 1.

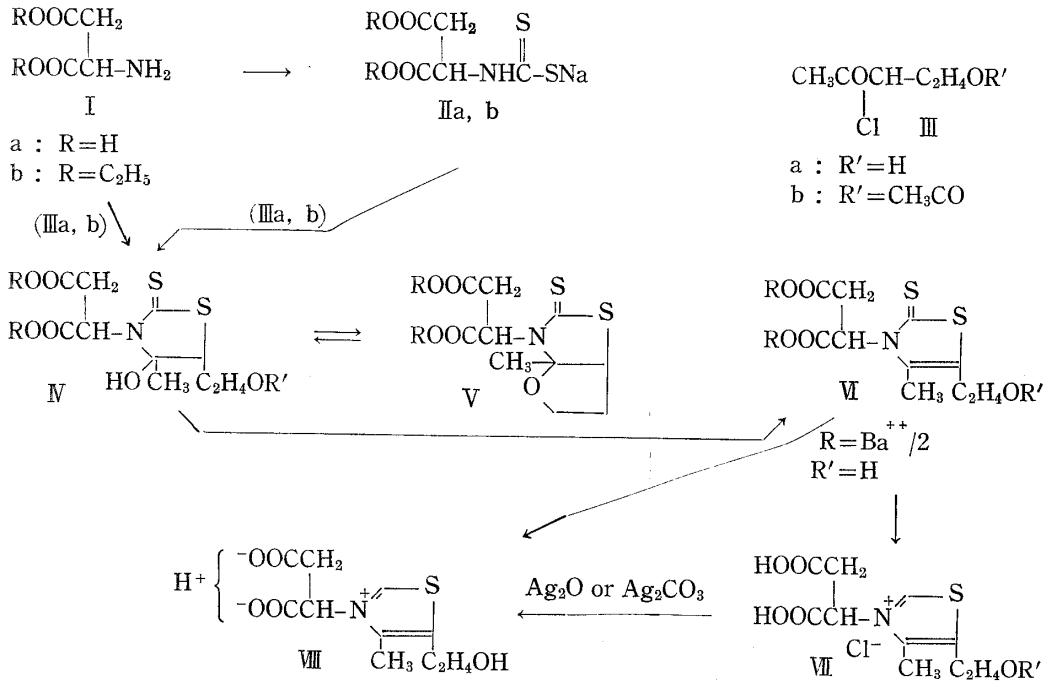

Chart 1.

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*1 This was presented at the 83 rd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, November, 1963.

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1) J. van Eys, *et al.* : J. Biol. Chem., **234**, 2308 (1959).

2) J. van Eys : Federation Proc., **19**, 26 (1960).

Racemic aspartic acid (Ia) or its ethyl ester (Ib) was condensed with carbon disulfide and ammonia in the usual manner to result in the dithiocarbamates (II : a,b), which were condensed with chloroketones (III : a,b) to yield 2-thiazolidinethiones (IV), which were also prepared directly by treating I with III and carbon disulfide in the presence of triethyl amine without isolating the intermediate. In the latter case the reaction proceeded in a shorter time and higher yield than in the former. In IV, R=H, R′=CH_3CO and R=C_2H_5, R′=CH_3CO products are oily and in R=R′=H being crystal of m.p. 110°. The 2-thiazolidinethiones (IV) were dehydrated without analysis*3 and ring-closed, when allowed to stand in a weak acidic solution, to yield tetrahydrofuro[2,3-*d*]-thiazole-2(3*H*)-thiones (V). V were immediately ring-opened in a stronger acidic solution and occasionally dehydrated to give 4-thiazoline-2-thiones (VI), which were also obtained by moderately heating IV or V in dilute acid, though in lower yields. IV were only dehydrated to VI when heated by itself in a water bath or treated with 10% hydrochloric acid at room temperature. The 4-thiazoline-2-thiones (VI) thus obtained were oxidized with hydrogen peroxide in hydrochloric acid to give hydrochloride of thiamic acid or its ester (VII). When VI contained such a hydrolysable group as CH_3COO, hydrolysis occurred partially during isolation of VII. Hydrochlorides (VII) were treated with freshly prepared silver oxide or silver carbonate to yield inner salt (VIII) and the hydrolysable group, if present in VII, was hydrolyzed simultaneously by use of the excess reagent.

The above described intermediates were amorphous and difficult to purify in most cases, especially in case a hydrolysable group is present and therefore the yield of thiamic acid was not satisfactory. For this reason an attempt was made to oxidize the neutral salt of VI, and successfully practiced: namely, the barium salt of VI, which is easily purified in crystalline state, is oxidized directly to betaine VIII with hydrogen peroxide in a good yield. In this case, a resulted sulfate ion was conveniently precipitated as barium sulfate. The barium salt of VI can be used for oxidation, without prior purification, by simply dissolving in water and removing the resinous substance. On the other hand, when the barium salt of V was oxidized with hydrogen peroxide, aspartic acid was obtained unexpectedly. It may be concluded from this evidence that the produced thiazolium compound (IX) was hydrolysed according to the scheme shown in Chart 2.

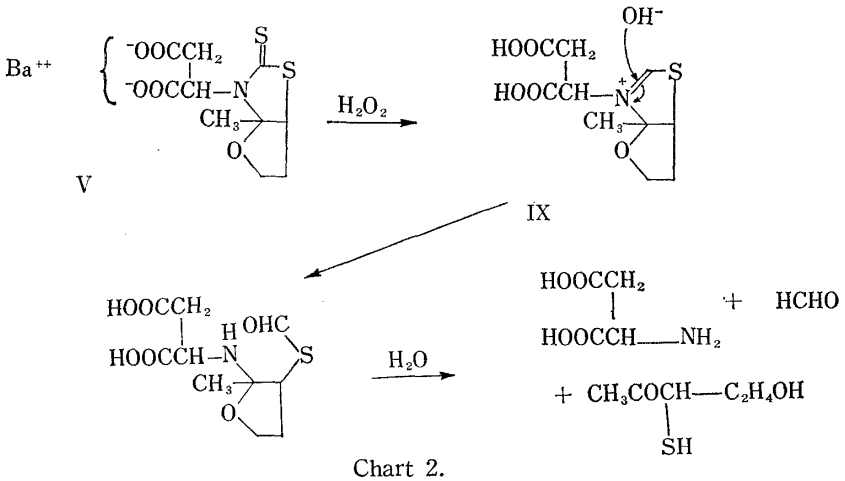


Chart 2.

*3 Similar thiazole derivatives were synthesized by H. Hirano: Yakugaku Zasshi, **75**, 244, 249, 251, 1182 (1954) and therefore, those 2-thiazolidinethiones (IV) were used directly without elementary analysis.

The inner salt of thiamic acid (Ⅷ) obtained by the above mentioned course is a crystalline compound having a melting point 167~168° (with decomposition) or 157~158° when slowly heated, and the ultraviolet absorption showed λ_{max} 261 mμ in an acidic solution, λ_{max} 236 mμ in an alkaline one, while van Eys[1,2] reported that thiamic acid had the ultraviolet absorption of λ_{max} 258 mμ in both acidic and alkaline media, and melting points or other physical constants being not reported. Furthermore, the ultraviolet absorption of the compounds presented in this papar do not agree with those of usual thiazolium salts, λ_{max} 226 mμ.[3] 2-Ethyl-4-methylthiazolium iodide, for instance, has λ_{max} =226 mμ in UV[3] and 5-(2-hydroxyethyl)-3,4-dimethylthiazolium chloride, synthesized material for reference has, λ_{max} 250~260 mμ as thiamic acid and its derivatives. In order to determine a relationship between structure and ultraviolet absorption of these compounds some model compounds were synthesized. Inspection of absorption spectra of these compounds led to conclusion that thiamic acid synthesized in this investigation must be represented by the structure mentioned above Ⅶ.

An analogous compound (XIV) to thiamic acid was synthesized in two ways (Chart 3), one of which was similar to the above mentioned thiamic acid synthesis, and both compounds obtained by each method are identical in melting points or infrared spectra, and further identified by mixed melting point determination. This indicates that the above synthetic route of thiamic acid is reasonable. Moreover, it was found that the analogous compound (XIV) showed a closely resembled absorption spectra to that of thiamic acid.

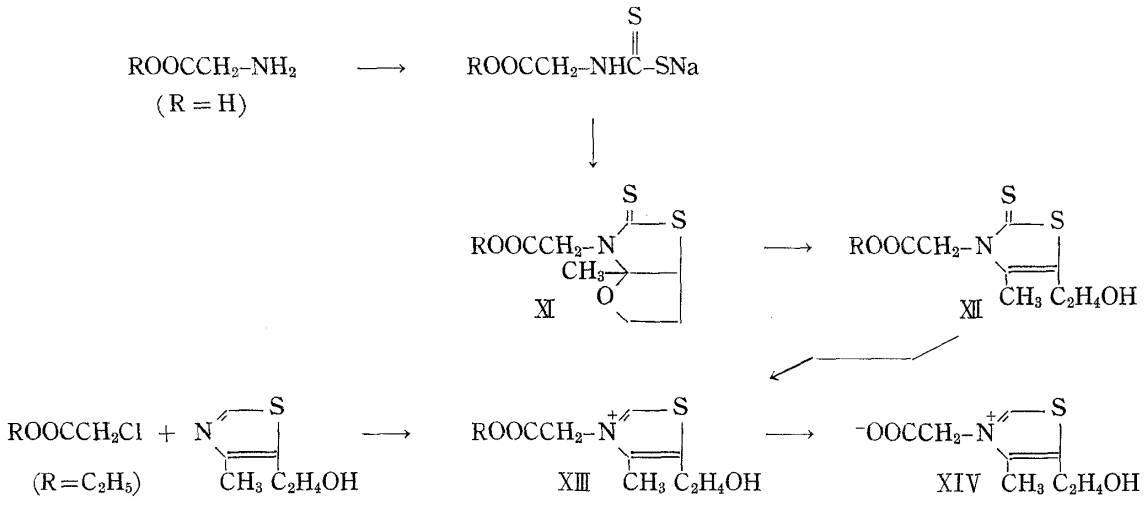


Chart 3.

Therefore, it can be said that the ultraviolet absorption of λ_{max} 261 mμ is a common property of the compounds of this type. Hereupon, the fact that the compounds of the type of thiamic acid have a different absorption spectrum with common thiazolium compounds, seems to suggest a contribution of following resonance structure.

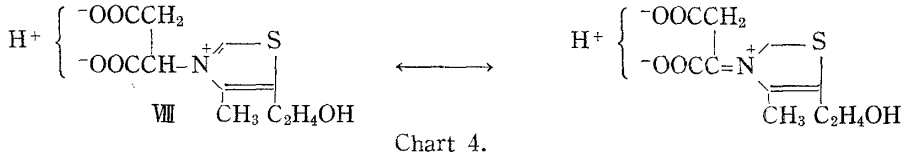
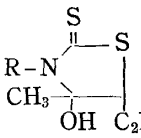


Chart 4.

3) A. E. Ruehle : J. Am. Chem. Soc., **57**, 1887 (1935).

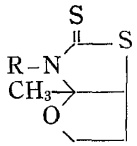
In Table I~IV, the ultraviolet spectra of several compounds are shown for comparison which might be quite helpful for confirmation of each structure.

TABLE I.



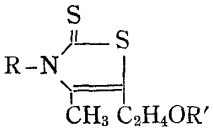
R	R′	λ_{max} mμ	Solvent
$HOOCCH_2$ $HOOCCH-$	CH_3CO	{251 {276	C_2H_5OH
$C_2H_5OOCCH_2$ $C_2H_5OOCCH-$	CH_3CO	{251 {276	C_2H_5OH
H	CH_3CO	275	C_2H_5OH

TABLE II.



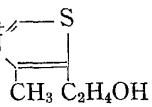
R	m.p. (decomp.) (℃)	λ_{max} mμ (ε)	Solvent
$HOOCCH_2$ $HOOCCH-$	184	{253 (8000) {278 (13320)	C_2H_5OH
$HOOCCH_2-$	125~126	{251 (8340) {275.5 (15100)	C_2H_5OH

TABLE III.



R	R′	m.p. (℃.)	λ_{max} mμ (ε)	Solvent
Ba^{++} {$^-OOCCH_2$ {$^-OOCCH-$	H	—	325 (11300)	H_2O
$HOOCCH_2$ $HOOCCH-$	CH_3CO	—	328	C_2H_5OH
$HOOCCH_2-$	H	58	324	C_2H_5OH
H	H	153	327	C_2H_5OH

TABLE IV.



R	m.p. (decomp.) (℃)	λ_{max} mμ (ε)	Solvent
H^+ {$^-OOCCH_2$ {$^-OOCCH-$	167	{230 (shoulder) {262 (43900)	H_2O
$^-OOCCH_2-$	178~179	{227.5 (33190) {261 (43200)	H_2O
CH_3 (chloride)	—	{226.5 (strong) {254 (weak)	H_2O

Experimental*4

2-Thioxo-4-hydroxy-4-methyl-5-(2-acetoxyethyl)-3-thiazolidinesuccinic Acid (IV, R=H, R′=CH_3CO)——To a mixture of 13.3 g. of DL-aspartic acid, 17.8 g. of 3-chloro-5-acetoxy-2-pentanone and 30 g. of triethylamine in 40 ml. of water and 100 ml. of methanol was added 10 ml. of carbon disulfide. An exothermic reaction occurred. The reaction mixture was stirred for 3 hr., occationally cooling to keep the reaction temperature less than 40°. After reaction was over, the reaction mixture was adjusted to pH 2 with 10% HCl, and extracted with ethyl acetate. The extract was washed with water, dried over anhydrous sodium sulfate, and the solvent was removed off to obtain 30 g. of an oily product. This was used without analysis to the following reactions.

Diethyl Ester of 2-Thioxo-4-hydroxy-4-methyl-5-(2-acetoxyethyl)-3-thiazolidinesuccinic Acid (IV, R=C_2H_5, R′=CH_3CO)——To a solution of 1.9 g. of diethyl DL-aspartate and 1.8 g. of 3-chloro-5-acetoxy-2-pentanone in 10 ml. of ethanol was added 1.1 g. of triethylamine and 1 ml. of carbon disulfide. The solution was stirred with occational cooling and allowed to stand overnight. Ethanol was removed from the solution *in vacuo*, and some water was added to the remainder. Precipitated oil was extracted with ether and extract was washed with water, dried, and ether was removed to obtain 4.2 g. of an oily product. This was used without analysis to the following reactions. IR ν_{max}^{KBr} cm^{-1} : 3400 (OH), 1435 (NHC=S).

2-Thioxo-4-hydroxy-4-methyl-5-(2-hydroxyethyl)-3-thiazolidinesuccinic Acid (IV, R=R′=H), and 2-Thioxo-3a-methylhexahydrofuro[2,3-*d*]thiazole-3-succinic Acid (V, R=H)——i) To a mixture of 6.5 g. of DL-aspartic acid, 6 g. of sodium hydroxide and 40 ml. of water, 4 ml. of carbon disulfide was added and the mixture was stirred for 18 hr. at room temperature. The excess carbon disulfide was removed *in vacuo* to obtain an aqueous solution of sodium N-(1,2-dicarboxyethyl)dithiocarbamate. To this solution was added a solution of 6.4 g. of 3-chloro-5-hydroxy-2-pentanone[4] in 32 ml. of water, and the solution was stirred for 15 minutes at 50°, and further 2 hr. at room temperature. The reaction mixture was adjusted to pH 5 with 10% HCl which resulted in precipitates (IV, R=R′=H), m.p. 115°. The reaction mixture was adjusted to pH 2 with 10% HCl to give crystals, m.p. 178~182°, which was recrystallized from aqueous ethanol to yield prisms of V (R=H), m.p. 185°. *Anal.* Calcd. for $C_{10}H_{13}O_5NS_2$: C, 41.24; H, 4.50; N, 4.81. Found : C, 41.31; H, 4.75; N, 4.59. IR ν_{max}^{Nujol} cm^{-1} : 1000, 1010, 1025 (tetrahydrofuran).

ii) To a solution of 1.3 g. of DL-aspartic acid, 13 g. of 3-chloro-5-hydroxy-2-pentanone and 3.0 g. of triethylamine in 4 ml. of water and 10 ml. of methanol, was added 1 ml. of carbon disulfide. An exothermic reaction occurred. The reaction mixture was stirred for 3 hr., occationally cooling to keep the reaction temperature less than 40°, adjusted to pH 2, and cooled to precipitate crystals, which were collected, recrystallized from aqueous ethanol to give 0.7 g. of (V, R=H), m.p. 185°. *Anal.* Calcd. for $C_{10}H_{13}O_5NS_2$: C, 41.24; H, 4.50; N, 4.81. Found : C, 41.35; H, 4.30; N, 4.63.

2-Thioxo-3a-methylhexahydrofuro[2,3-*d*]thiazole-3-acetic Acid (XI,R=H)——i) To a solution of sodium N-carboxymethyldithiocarbamate prepared from 1.5 g. of glycine, 1.6 g. of sodium hydroxide, 1.5 ml. of carbon disulfide and 11 ml. of water, 2.5 g. of 3-chloro-5-hydroxy-2-pentanone was added and the solution was treated in a manner similar to the experiment described above (i). Crystals obtained were pale yellow needles, m.p. 125~126°. *Anal.* Calcd. for $C_8H_{11}O_3NS_2$: C, 41.20; H, 4.76; N, 6.01; S, 27.42. Found : C, 41.36; H, 5.00; N, 5.89; S, 27.43.

ii) A mixture of 1.5 g. of glycine, 2.5 g. of 3-chloro-5-hydroxy-2-pentanone, 3 g. of triethylamine, 4 ml. of water, 10 ml. of methanol and 2 ml. of carbon disulfide was treated in a manner similar to the experiment discribed above (ii), to obtain 2.9 g. of pale yellow needles, m.p. 125~126°. *Anal.* Calcd. for $C_8H_{11}O_3NS_2$: C, 41.20; H, 4.76; N, 6.01; S, 27.42. Found : C, 41.38; H, 4.89; N, 5.81; S, 27.45.

2-Thioxo-4-methyl-5-(2-hydroxyethyl)-4-thiazoline-3-succinic Acid (VI, R=R′=H)——A solution of 30 g. of (V, R=H) in 30 ml. of 10% HCl and 60 ml. of methanol was refluxed for 30 minutes. After methanol was removed *in vacuo*, the solution was extracted with ethyl acetate, which was washed with water and dried. The solvent was removed from the solution to give a red oil, which was converted to barium salt with barium chloride. *Anal.* Calcd. for $C_{10}H_{11}O_5NS_2Ba$: N, 3.28; S, 15.03; Ba, 32.19. Found : N, 3.02; S, 14.80; Ba, 31.89.

2-Thioxo-4-methyl-5-(2-acetoxyethyl)-4-thiazoline-β-succinic Acid (VI, R=H, R′=CH_3CO)——A solution of 7.5 g. of 2-thioxo-4-hydroxy-4-methyl-5-(2-acetoxyethyl)-3-thiazolidinesuccinic acid in 30 ml. of *n*-buthanol was refluxed for 15 minutes, and *n*-buthanol was removed *in vacuo* to obtain 7.0 g. of an oily product.

2-Thioxo-4-methyl-5-(2-hydroxyethyl)-3-thiazolidineacetic Acid (XII, R=H)——A suspension of 1.0 g. of (XI, R=H) in 8 ml. of 10% HCl was heated at 70~80°, until the crystals disappeared. The solution was concentrated *in vacuo* and the remaining oil was triturated with ethanol. The crystals obtained was

*4 All melting points are uncorrected.

4) E. R. Buchmann : J. Am. Chem. Soc., **58**, 1803 (1936); J. R. Stevens, G. A. Stein : J. Am. Chem. Soc., **62**, 1045 (1940); T. Matsukawa, *et al.* : Yakugaku Zasshi, **71**, 720 (1951).

recrystallized from ethanol to give pale yellow prisms, m.p. 58°. *Anal.* Calcd. for $C_8H_{11}O_3NS_2$: C, 41.20; H, 4.76; N, 6.01. Found : C, 41.39; H, 4.87; N, 5.77.

Inner Salt of 3-(1,2-Dicarboxyethyl)-4-methyl-5-(2-hydroxyethyl)thiazolium Hydroxide (VIII)——i) To 5.0 g. of (Ⅵ, R=R′=H) were added 40 ml. of 3.5% HCl and 3.9 ml. of 30% H_2O_2 dropwise under ice cooling. The solution was stirred for 2 hr., until an oily material disappeared. The obtained solution was filtered and $BaCl_2$ was added to the filtrate until no further precipitate occurred. The solution was filtered and concentrated *in vacuo* at 40°. Absolute ethanol was added to the residue, the solution was filtered, concentrated *in vacuo* to obtain 4.0 g. of a thick oil of 3-(1,2-dicarboxyethyl)-4-methyl-5-(2-hydroxyethyl)thiazolium chloride (Ⅶ, R′=H). The oil (0.74 g.) was treated with a solution of 0.56 g. of Ag_2CO_3 in 6 ml. of water for 30 minutes. The precipitated AgCl and excess Ag_2CO_3 were filtered off, and the filtrate was passed by H_2S and filtered, concentrated *in vacuo* to obtain crystals of Ⅷ, which were identical with the hemihydrate prepared in the following experiment (ii).

ii) To a solution (pH 5~6) of 23 g. of (Ⅵ, R=R′=H) in 140 ml. of 10% $Ba(OH)_2$ was added 18 ml. of 30% H_2O_2 at 30~35°, and the solution was stirred for 3 hr. Precipitated $BaSO_4$ was filtered off and the filtrate was concentrated *in vacuo*. The residue was triturated with ethanol to obtain crystals, which was recrystallized from methanol to give prisms of hemihydrate, m.p. 167°. (decomp.). The hemihydrate lost water when dried on P_2O_5 at 50°. *Anal.* Calcd. for $C_{10}H_{13}O_5NS$: C, 46.33; H, 5.06; N, 5.40. Found : C, 46.61; H, 5.20; N, 5.46. Paper chromatography : solvent (BuOH-AcOH-H_2O=200:75:30) Rf 0.33 (ascending), 0.39 (descending).

Inner Salt of 3-Carboxymethyl-4-methyl-5-(2-hydroxyethyl)thiazolium Hydroxide (XIV)——i) A mixture of 3.0 g. of 4-methyl-5-(2-hydroxyethyl)thiazole[5] and 3.0 g. of ethyl chloroacetate was heated for 2 hr. on a water bath to obtain 6.5 g. of an oily 3-ethoxycarbonylmethyl-4-methyl-5-(2-hydroxyethyl)thiazolium chloride (XⅢ, R=C_2H_5). This was dissolved in water and to the solution was added freshly prepared Ag_2O. The mixture was stirred for 2 hr. at room temperature and precipitated AgCl was filtered off. The filtrate was passed by H_2S, filtered and concentrated *in vacuo* to obtain crystals, which was recrystallized from ethanol and acetone to give white crystalline (XⅣ), m.p. 178~179°. *Anal.* Calcd. for $C_8H_{11}O_3NS$: C, 47.76; H, 5.51; N, 6.96. Found : C, 47.60; H, 5.75; N, 6.78.

ii) To a suspension of pulverized (XⅡ, R=H) in 0.5 ml. of conc. HCl and 5 ml. of water, 1.4 ml. of 30% H_2O_2 was added dropwise under ice cooling. After the mixture was allowed to stand for an hour, the crystals disappeared. The obtained yellowish solution was filtered and $BaCl_2$ was added to the filtrate until no further precipitate occurred. The solution was filtered and concentrated *in vacuo* at 40°. Absolute ethanol was added to the residue, the solution was filtered, concentrated *in vacuo* to obtain a yellowish oil, which was reprecipitated twice from ethanol and ether to yield 600 mg. of a yellow oil of 3-carboxymethyl-4-methyl-5-(2-hydroxyethyl)thiazolium chloride (XⅢ, R=H). The oil (300 mg.) was treated with Ag_2O in a manner similar to the experiment described above (i) to obtain 100 mg. of crystals. m.p. 169~170°. (decomp.), which was identical with a specimen prepared in the above experiment (i).

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Summary

Thiamic acid, 3-(1,2-dicarboxyethyl)-4-methyl-5-(2-hydroxyethyl)thiazolium chloride, and an inner salt of its hydroxide were prepared from aspartic acid and 3-chloro-5-hydroxy-2-pentanone *via* thiazolidine-2-thione, tetrahydrofuro[2,3-*d*]thiazole-2(3*H*)-thione, and 4-thiazoline-2-thione. Some related compounds are also synthesized. The structure of thiamic acid was confirmed by their ultraviolet spectra.

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5) R. Maeda, Y. Sawa : Yakugaku Zasshi, **75**, 656 (1955); **76**, 301 (1956).