Chem. Pharm. Bull. 19(4) 759—763 (1971)

UDC 615.356 : 577.164.11.074

Studies on Pyrimidine Derivatives and Related Compounds. LXIX.[1] Sythesis of Thiamine Free Base[2]

Akira Takamizawa, Kentaro Hirai, Teruyuki Ishiba, and Itsuo Makino

Shionogi Research Laboratory, Shionogi & Co., Ltd.[3]

(Received October 8, 1970)

Reaction of thiamine sodium salt (III) with carbon dioxide afforded 2,6a-dimethyl-6a,8,9,9a,10a,11-hexahydro-5*H*-furo[2,3-*h*]thiachromine (VI). Further studies to investigate alternative synthetic routes to VI were carried out and the mechanism for the formation of VI was discussed.

In connection with the mechanism of cocarboxylase activity of thiamine, the authors have studied the chemical activity of the thiazole C_2 position in thiamine and have reported in several preceding papers, a new type of reaction of thiamine with a number of reagents (aldehydes, glyoxals, amines, phosphites, isocyanates, and isothiocyanates) occurring at the thiazole (Th) C_2 position. In these cases the reactions were carried out under the following conditions: thiamine sodium salt was treated with carbon dioxide, or thiamine halide was treated with triethylamine, in the presence of the appropriate reagent. On the other hand, Maier, *et al.*[4] reported that dihydrothiochrome (IV) was obtained as a solid, mp 128—129°, from the reaction of thiamine hydrochloride (I) with an equivalent amount of sodium ethoxide in ethanol. Since in their paper the compound IV obtained was not purified by recrystallization, and since we were unable to prepare IV following their procedure, we felt that IV might be very unstable. This prompted us to try the reaction of thiamine sodium salt (III) with carbon dioxide, and thus 2,6a-dimethyl-6a,8,9,9a,10a,11-hexahydro-5*H*-furo [2,3-*h*] thiachromine (VI, thiamine free base) was obtained as a stable new product. Further studies to investigate alternative synthetic routes to VI were then carried out. This paper describes the results of studies on the chemistry of VI and routes for its synthesis.

On passing carbon dioxide into a solution of III in dimethyl formamide (DMF) at room temperature, colorless crystals (VI) were obtained, which has a molecular formula, $C_{12}H_{16}ON_4S$, *i.e.*, two moles of HCl less than that of thiamine hydrochloride ($C_{12}H_{17}ON_4SCl \cdot HCl$). On treatment with hydrochloric acid, VI was reconverted into thiamine hydrochloride (I), therefore VI can be considered as a free base of thiamine hydrochloride.

The ultraviolet (UV) spectrum of VI showed maxima at 245 (log ε 3.96) and 285 mμ (log ε 3.84), closely resembling that of IV which showed maxima at 245 and 287 mμ,[4] suggesting that the pyrimidine nucleus of VI is a part of tricyclic system similar to that of IV. Since the melting point of VI 175° (decomp.) is different from that of IV, VI should be an isomer of IV. The infrared (IR) spectrum of VI showed absorption bands at 3246 (NH) and 1620 cm^{-1} (C=N). The nuclear magnetic resonance (NMR) spectrum (Fig. 1) in deuterochloroform showed peaks at τ value 2.05, 2.15 (s, 1H), 2.96 (b, 1H, NH), 4.00, 4.16 (s, 1H), 7.53, 7.56 (s, 3H, Pm-C_2-CH_3), indicating a mixture of two isomers. It was notable that the NMR spectrum of VI did not show proton signals for Pm-C_4-NH_2 and N-CHO which are specific signals of the

1) Part LXVIII: A Takamizawa, Y. Hamashima, H. Sato, and Y. Matsumoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 1576 (1970).
2) A part of this paper was presented at the 25th Annual Meeting of the Pharmaceutical Society of Japan at Sendai, October 1968.
3) Location: *Fukushima-ku, Osaka.*
4) G.D. Maier and D.E. Metzler, *J. Am. Chem. Soc.*, **79**, 4386 (1957).

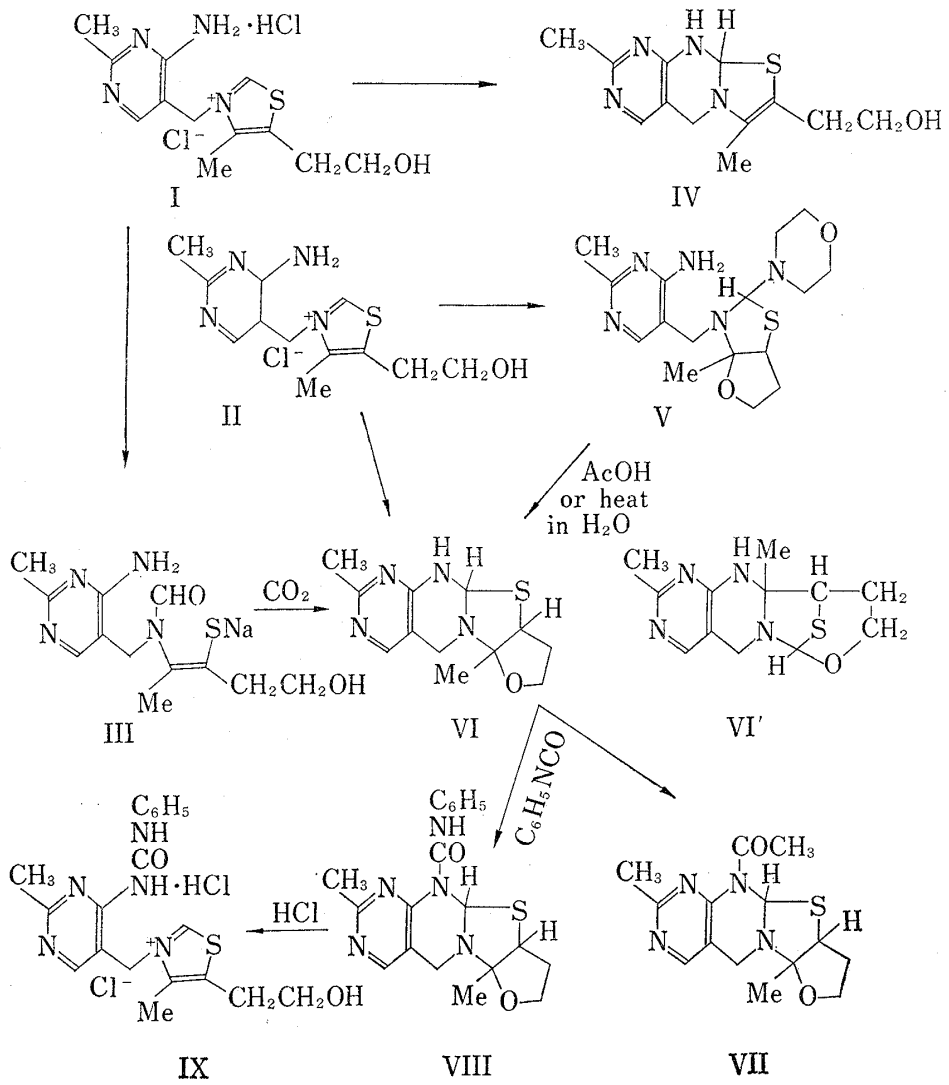


Chart 1

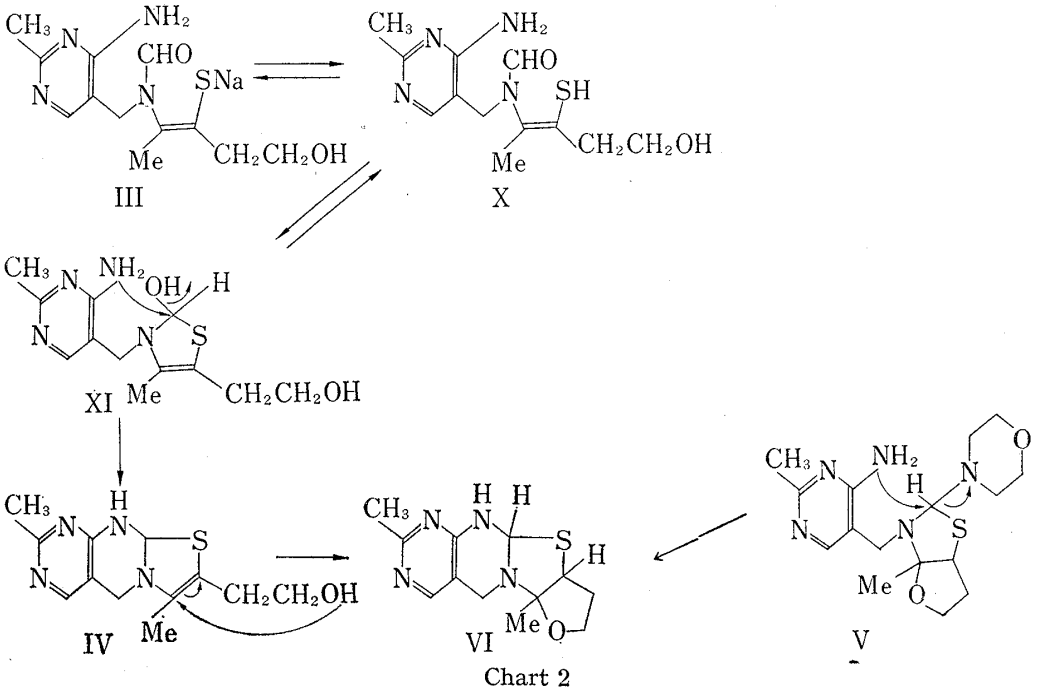


Chart 2

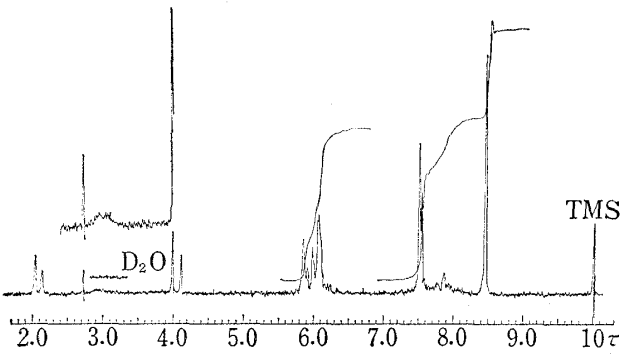


Fig. 1. NMR Spectrum of VI in $CDCl_3$

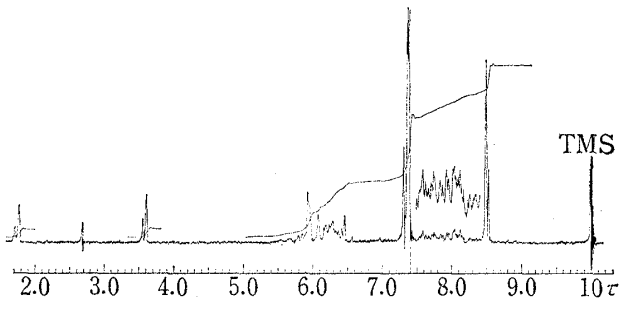
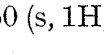


Fig. 2. NMR Spectrum of VII in $CDCl_3$

thiol type thiamine derivatives. It can be generally recognized[5,6] that the proton signals of the Th-C_5-CH_2CH_2OH system of thiamine and its derivatives are exhibited at τ value of about 6 and 7 as typical triplet patterns and that the proton signal of Th-C_4-CH_3 is shifted to a τ value of about 7.5—7.8. In the case of VI, however, the four protons of the Th-C_5-CH_2CH_2- system were recognized at τ values 5.87, 5.91, 6.00, 6.08 as complicated patterns and the proton signal of Th-C_4-CH_3 was shifted to a higher field (τ 8.45). These data gave strong support to the suggestion that, in accordance with our former experiments,[7,8] the structure of VI should contain a tricyclic ring system containing NH-CH system derived from Pm-C_4-NH_2 and the Th-C_2 position, and also a tetrahydrofuran ring system produced from the Th-C_5-CH_2CH_2OH group and the Th-C_4-C_5 double bond. Therefore, the structre of VI could be almost established as 2,6a-dimethyl-6a,8,9,9a,10a,11-hexahydro-5*H*-furo[2,3-*h*]thiachromine, though from the physicochemical properties it could not be completely ruled out that VI is the isomeric structure VI′. The mono acetate (VII) was obtained by the acetylation of VI with acetic anhydride. The UV spectrum of VII showed maxima at 234 and 279 mμ (log ε 3.94, 4.01) suggesting that the nucleus of VII is the same system as that of VI. The IR spectrum of VII showed a new absroption band at 1652 cm^{-1} being attributed to N-C=O group and no absorption band due to the NH or OH group. The NMR spectrum of VII in deuterochloroform showed that VII is a mixture of two geometrical isomers as shown in Fig. 2. In this spectrum it is notable that the peaks (s, 1H) are shifted to a lower field (τ values 3.56 and 3.62) than those of VI (τ 4.00 and 4.16), and that the proton signal of Th-C_4-CH_3 of VII is found at τ 8.50 and 8.53, the same chemical shift as that of VI. From the above data ,it is reasonable to assume the structure of VI having the NH-CH system and rule out the structure VI′ having the NH-C-CH_3 system. The 1:1 adduct (VIII) of VI and phenylisocyanate was obtained under the usual reaction condition. The UV spectrum of VIII showed maxima at 230, 252, and 282 mμ (log ε 4.08, 4.07, and 4.10). The IR spectrum of VIII showed a band at 1680 cm^{-1} (C=O) and the NMR spectrum, exhibiting signals at τ −2.50 (s, 1H, NH), 1.76, 1.86 (s, 1H, Pm-C_6-H), 2.5 (m, 5H, ), 3.40, 3.45 (s, 1H), 5.90 (s, 1H), 6.10—6.20 (m, 4H), 7.35 (s, 3H, Pm-C_2-CH_3), 8.48 (s, 3H, Th-C_4-CH_3), showed to be a mixture of two geometrical isomers. The structure of VIII had already been confirmed as the N-phenylcarbamoyl derivative of VI from the chemical evidence of the production of N-phenylcarbamoylthiamine chloride hydrochloride (IX) *via* VIII by aqueous hydrochloric acid solution, as we[9] have already reported. Thus it was confirmed that the st-

5) A. Takamizawa, S. Matsumoto, and S. Sakai, *Chem. Pharm. Bull.* (Tokyo), **17**, 128 (1969).
6) A. Takamizawa, S. Matsumoto, and S. Sakai, *Chem. Pharm. Bull.* (Tokyo), **17**, 343 (1969).
7) A. Takamizawa, K. Hirai, Y. Hamashima, S. Matsumoto, and T. Ishiba, *Chem. Pharm. Bull.* (Tokyo), **16**, 1210 (1968).
8) A. Takamizawa, K. Hirai, S. Matsumoto, S. Sakai, and Y. Nakagawa, *Chem. Pharm. Bull.* (Tokyo), **17**, 910 (1969).
9) A. Takamizawa, K. Hirai, S. Matsumoto, and T. Ishiba, *Chem. Pharm. Bull.* (Tokyo), **16**, 2130 (1968).

ructure of VI compries the NH-CH system formed from the Pm-C_4-NH_2 group and the Th-C_2-position, and a tetrahydrofuran ring produced from the Th-C_5-CH_2CH_2OH group and the Th-C_4-C_5 double bond. Since an inspection of a Dreiding model of VI indicates the 6a-methyl and 9a-hydrogen are in a *cis* relationship, the two isomeric structures of VI revealed by their NMR spectra were confirmed as the *cis* and *trans* isomers between 11- and 10a-hydrogens of the NH-CH$\langle^{S}_{N}$ system, dependent upon the ring forming orientation of the pyrimidopyrimidine nucleus. The ratio of isomers was estimated as 4:6 from the NMR spectrum. However, we could not isolate each isomer as pure crystals and so could not illustrate the geometrical structure of two isomers of VI in detail.

Next, we tried to develop an alternative synthetic pathway to VI in order to make clear the mechanism of its formation. It seems reasonable to consider that VI is formed *via* a pseudo-thiamine intermediate (XI) resulting from the thiol type of thiamine (X) produced on the reaction of thiamine sodium salt (III) with carbon dioxide. We had reported in the previous paper[10] that the 1:1 adduct at Th-C_2 position could be obtained by the reaction of thiamine chloride (II) with one molar excess of secondary amines (morpholine, piperidine, *etc.*) allowing the reaction mixture to stand at room temperature. We expected, therefore, that this reaction could also be used as a convenient route to VI, by reacting II with an amine whose basicity is a little greater than that of thiamine. This proved to be the case and VI was obtained from the reaction of II with an equimolar amount of morpholine. Furthermore, VI was also produced by the treatment of V with boild water or aqueous acetic acid solution. In this reaction, we support the mechanism by which VI was formed immediately from V on elimination of the morpholine molecule. Compound VI obtained under these reaction conditions was also a mixture of two geometrical isomers, estimated to be in a 5.5:4.5 ratio from NMR spectral data. The poor yield (below 50%) of VI obtained by any of these reaction routes, prevented any detailed proposals being made about the geometrical structural preference in the formation of VI.

In this paper we have confirmed the existence of the free base of thiamine, providing new information for the chemical and biochemical behavior of thiamine and for the consideration of the state of thiamine existing in nature.[11]

Experimental[12]

2,6a-Dimethyl-6a,8,9,9a,10a,11-hexahydro-5*H*-furo [2,3-*h*] thiachromine (VI)——a) To a suspension of 150 g of B_1Na in 1000 ml of dioxane was passed through an excess CO_2 for 8 hr at 10—15°. After removing the precipitate by filtration, dioxane was evaporated *in vacuo*. The residue was extracted with 250 ml of $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over Na_2SO_4 and evaporated. The oily residue was dissolved in $CHCl_3$ and purified by alumina column chromatography and recrystallized from acetone to give 19.5 g (21%) of colorless columnar crystals, mp 175° (decomp.). *Anal.* Calcd. for $C_{12}H_{16}ON_4S$: C, 54.55; H, 6.10; O, 6.06; N, 21.20; S, 12.13. Found: C, 54.89; H, 6.14; O, 6.42; N, 21.31; S, 11.86. UV λ_{max}^{EtOH} mμ (log ε): 245, 285 (3.96, 3.84). IR ν_{max}^{Nujol} cm^{-1}: 3246 (-NH), 1624 (C=N). NMR (τ): 8.45 (s, 3H, 6a-CH_3), 7.51, 7.53 (3H, 2-CH_3), 4.00, 4.16 (1H, 10a-H), 2.96 (s, 1H, 11-NH), 2.05, 2.15 (1H, 4-H).

b) To a suspension of 1.5 g ($\frac{1}{2}\times10^{-2}$ mole) of B_1Cl in 20 ml of MeOH was added 480 mg ($1\cdot\frac{1}{2}\times10^{-2}$ mole) of morpholine and the mixture was stirred at room temperature for 13 hr. After evaporating MeOH *in vacuo* the residue was extracted with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4

10) A. Takamizawa, K. Hirai, and Y. Hamashima, *Chem. Pharm. Bull.* (Tokyo), **16**, 1758 (1968).

11) Recently Risinger, *et al.* (G.E. Risinger, E.J. Breaux, and H.H. Hsieh, *Chem. Commun.*, **1968**, 841) suggested the formation of VI in the solution of thiamine in the presence of two equivalents of methoxide. However, no evidence has offered for the isolation of VI.

12) All melting points are uncorrected. All of the NMR spectra were taken with a Varian A-60 Spectrometer on the solution in deuterochloroform containing tetramethylsilane as an internal reference. Chemical shifts are expressed in τ values and coupling constants are in cycles persecond. Multiplicities of signals are represented as a s (singlet), d (doublet), t (triplet), b (broad), and m (multiplet).

and evaporated to leave crystals which were recrystallized from acetone giving colorless columnar crystals, mp 175° (decomp.), which were identical with the sample obtained above. Yield 144 mg (11%).

c) V (760 mg) was dissolved in 8 ml of H_2O and stirred for 1 hr at 50°. After cooling the reaction mixture was extracted with $CHCl_3$. The extract was treated in a similar way as above and 78 mg (14%) of VI was obtained.

d) To a solution of 1 g of V in 20 ml of H_2O was added 5 ml of 10% AcOH. After stirring for 1 hr at room temperature, the reaction mixture was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. The extract was treated in a similar way as above and 133 mg (18%) of VI was obtained.

11-Acetyl-2,6a-dimetyl-6a,8,9,9a,10a,11-hexanhydro-5*H*-furo[2,3-*h*] thiachromine (VII)——To a solution of 2.64 g of VI in 30 ml of pyridine (dried over KOH) was added 16.5 ml of acetic anhydride under ice cooling. The reaction mixture was stirred for 0.5 hr at 0—5°. After allowing to stand overnight at room temperature, the pyridine was removed *in vacuo* at 50° and the brown oily residue was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with 3% $NaHCO_3$ and H_2O successively, dried over Na_2SO_4 and evaporated. The residual crude product was purified by recrystallization from ether to give 795 mg (26%) of colorless crystals, mp 158—161°. *Anal.* Calcd. for $C_{14}H_{18}O_2N_4S$: C, 54.89; H, 5.92; O, 10.44; N, 18.29; S, 10.45. Found: C, 54.59: H, 5.93; O, 10.97; N, 18.12; S, 10.13. UV λ_{max}^{EtOH} mμ (log ε): 234, 279 (3.94, 4.01). IR ν_{max}^{Nujol} cm^{-1}: 1642 (-N-C=O). NMR (τ): 8.50, 8.53 (3H, 6a-CH_3), 7.36, 7.40 (3H, 2-CH_3), 5.93 (s, 2H, 5-CH_2), 3.56, 3.61 (1H, 10a-H), 1.71, 1.78 (1H, 4-H).

2,6a-Dimethyl-11-phenylcarbomoyl-6a,8,9,9a,10a,11-hexahydro-5*H*-furo[2,3-*h*] thiachromine (VIII)[9]——To a solution of 0.53 g of VI in 10 ml of dry DMF was added 0.72 g of C_6H_5NCO at room temperature. After stirring for 6 hr, the reaction mixture was allowed to stand overnight at room temperature. The reaction mixture was concentrated *in vacuo* at 70°. The residual crystals were washed with acetone and filtrated. Recrystallization from acetone gave 0.6 g (78%) of colorless prisms, mp 175—178°. *Anal.* Calcd. for $C_{19}H_{21}O_2N_5S$: C, 59.52; H, 5.52; O, 8.34; N, 18.27; S, 8.35. Found: C, 59.25; H, 5.61; O, 8.59; N, 17.95; S, 8.46: UV λ_{max}^{EtOH} mμ (log ε): 230, 251, 282 (4.08, 4.07, 4.10). IR ν_{max}^{Nujol} cm^{-1}: 1680 (-N-CO). NMR (τ): 8.48 (s, 3H, 6a-CH_3), 7.35 (s, 3H, 2-CH_3), 5.90 (s, 2H, 5-CH_2), 3.40, 3.45 (1H, 10a-H), 2.5 (m, 5H, C_6H_5), 1.76, 1.86 (1H, 4-H), −2.50 (b, 1H, NH).

The Conversion of VI into B_1Cl·HCl——After allowing to stand the solution of 528 mg of VI in 7.5 ml of 20% HCl for 2 hr at 50°, concentrated to one third of its original volume *in vacuo* at 30°. To the concentrate was added 10 ml of EtOH. After standing at 0° the precipitates were collected by filtration and washed with cold EtOH, to give 451 mg (60%) of crystals, mp 245° (decomp.), of which IR spectrum was identical with that of the authentic sample of B_1Cl·HCl.