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Studies on Pyrimidine Derivatives and Related Compounds. L.[1] Reactions of Thiamine with Aldehydes

Akira Takamizawa, Kentaro Hirai, Yoshio Hamashima, Saichi Matsumoto, and Teruyuki Ishiba

Shionogi Research Laboratory, Shionogi & Co., Ltd.[2]

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Reaction of thiamine–sodium salt (IV) with benzaldehyde in the presence of carbon dioxide afforded 2–benzoyl–3–(2–methyl–4–aminopyrimidin–5–yl–methyl)–3*a*–methylperhydrofuro[2,3–*d*]thiazole (Va) involving an unusual carbon–acylation at thiazole C–2 position of thiamine, and stereochemistry of the product was discussed. Va was also obtained from reaction of thiamine hydrochloride (I) with benzaldehyde in the presence of triethylamine under anhydrous condition. Some other aldehydes also reacted to furnish corresponding C–2 acylated thiamine derivatives.

Recently, special interests have been attracted upon the reactivity of thiazolium C–2 position of thiamine molecule, because it has been regarded as a reactive site of the thiamine action in some biochemical reactions.[3–5]

Few years ago, Miller and coworkers[6] reported that this position is enough reactive to form nucleophilic adducts with some aldehydes under a certain condition, and they described an example of the elegant synthesis of hydroxyethylthiamine (HET) (III) of which intermediacy in the course of pyruvate metabolism has now been accepted as a general concept.[7,8] The reaction condition described by these authors was close to the physiological one, for the condensation was carried out in an aqueous medium under the biologically acceptable pH values and at mild temperature. This reaction has been rationalized by nucleophilic addition of the thiazolium zwitterion (thiamine–ylid) (II) towards aldehydic carbonyl group[6] and extended to some other series of aldehydes.[9]

We have now studied the reactions of thiamine–sodium salt (IV) with aldehydes in the presence of carbon dioxide under nonaqueous condition, and wish to describe a novel type of reaction involving C–acylation at thiazole C–2 carbon atom of thiamine.

Reaction of benzaldehyde with ethanolic suspension of thiamine–sodium salt (IV) in the presence of dry carbon dioxide afforded several products which were separated by column chromatography over Silica Gel to give two compounds Va, $C_{19}H_{22}O_2N_4S$, mp 182—183°, and VI, $C_{12}H_{16}O_2N_2S_2$, mp 180—183°, accompanying the isolations of considerable amount of benzoin (VIII) and 3–(2–methyl–4–aminopyrimidin–5–yl–methyl)–4–methyl–5–(β–hydroxyethylthiazoline)–2–thione (SB_1) (VII). VI exhibited an absorption maximum at 347 mμ in its ultraviolet absorption (UV) spectrum, and acetic anhydride–pyridine treatment

1) Part XLIX: A. Takamizawa, K. Hirai, T. Ishiba, and Y. Matsumoto, *Chem. Pharm. Bull.* (Tokyo), **15**, 1294 (1967). A part of this work was presented in *Tetrahedron Letters*, **1967**, 5071.
2) Location: *Sagisu, Fukushima-ku, Osaka.*
3) R. Breslow, *Chem. & Ind.* (London), **1957**, 893.
4) R. Breslow, *J. Am. Chem. Soc.*, **79**, 1762 (1957).
5) R. Breslow, *Ann. N.Y. Acad. Sci.*, **98**, 445 (1962).
6) C.S. Miller, J.M. Sprague, and L.O. Kramptiz, *Ann. N.Y. Acad. Sci.*, **98**, 401 (1962).
7) R. Breslow, *J. Am. Chem. Soc.*, **80**, 3719 (1958).
8) G.L. Carlson and G.M. Brown, *J. Biol. Chem.*, **236**, 2099 (1961).
9) R. Oka and S. Yurugi, *Vitamins* (Japan), **32**, 570 (1965).

furnished a diacetate, mp 116—117°, indicating it should be identifiable with vitachrome which has been reported by Karrer and coworker.[10]

Va was easily convertible into thiamine hydrochloride (I) on treatment with hydrochloric acid, whereas it was quite stable in alkaline medium. Its infrared (IR) spectrum exhibited an absorption due to carbonyl group at 1687 cm^{-1}. In the nuclear magnetic resonance (NMR) spectrum (Fig. 1)[11] of this compound, the presence of singlet signal at 4.55 τ (1H) is attributable to the proton of the grouping $\overset{N}{S}\!>\!CH\text{–}CO\text{–}$, and an up-field chemical shift (8.32 τ) of methyl protons of the thiazole moiety indicated the saturated nature of the thiazole ring. Furthermore, presence of a broad signal at 3.6 τ (2H) which was easily disappeared by deuterium exchange and attributable to an amino group, combined with the fact that its UV spectrum exhibited absorption maxima at 243.5 mμ and 275.5 mμ, suggested that the original aminopyrimidine moiety was unchanged, thus an alternative structure IX for this compound should be eliminated. These results strongly suggested that the structure of Va should be established as 2-benzoyl-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]-thiazole which has been obtained by Hirano and coworkers[12] by reaction of 4-amino-5-aminomethyl-2-methylpyrimidine and 3-acetyl-3-mercapto-1-propanol and phenylglyoxal. The identity of Va with this compound was confirmed by direct comparison of their IR spectra.[13] Va was also obtainable in better yield when thiamine hydrochloride (I) was allowed to react with benzaldehyde in anhydrous N,N-dimethylformide (DMF) in the presence of triethylamine.

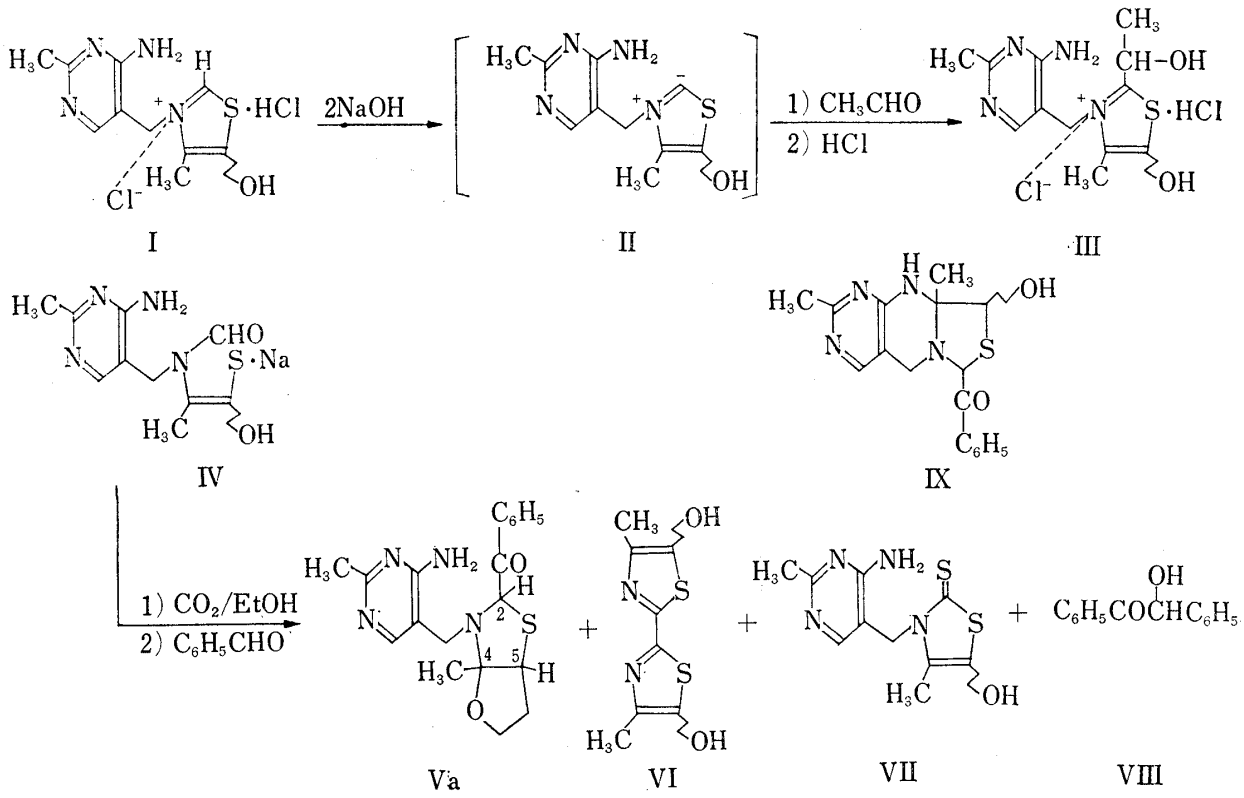


Chart 1

10) P. Karrer and M.C. Sanz, *Helv. Chim. Acta*, **26**, 1778 (1943).
11) All NMR spectra were taken on Varian A-60 instrument in deuteriochloroform solution (unless otherwise stated) containing tetramethylsilane as an internal standard.
12) Y. Oka, K. Yoshioka, and H. Hirano, Announced at the 15th Annual Meeting of Kinki-Branch of the Pharmaceutical Society of Japan, November (1965).
13) The IR spectrum of the authentic sample was offered by Dr. H. Hirano to whom the authors are indebted.

Reaction of acetaldehyde with IV in the presence of carbon dioxide afforded Vb, $C_{14}H_{20}O_2N_4S$, mp 143—146°, which was quite unstable on action of hydrochloric acid to be decomposed to thiamine hydrochloride (I) in nearly quantitative yield. Its IR spectrum exhibited a strong absorption due to ketonic carbonyl at 1720 cm^{-1}. In the NMR spectrum (Fig. 1) of this compound, it showed two singlets at 5.47 τ (1H) and 7.95 τ (3H) which are obviously attributable to the grouping $\overset{N}{S}\rangle CH-COCH_3$, and other signal patterns are quite analogous to those of Va indicating that the structure of this compound should be 2-acetyl-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]thiazole. Under the similar reaction conditions, isobutylaldehyde, *α*-furfural, *o*-hydroxybenzaldehyde and 2-hydroxy-1-naphthaldehyde afforded corresponding C-2 acylated thiamine derivatives, 2-isobutyryl-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]thiazole (Vc), mp 159—161°, 2-*α*-furoyl-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]thiazole (Vd), mp 151—153°, 2-(2-hydroxybenzoyl)-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]thiazole (Ve), mp 176—178° (174—175°),[14] and 2-(2-hydroxy-1-naphthoyl)-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]thiazole (Vf), mp 166—168°, respectively. These compounds all gave satisfactory elemental analyses and their NMR signal patterns were completely compatible with their structures (Fig. 1). Physical constants of these derivatives are listed in Table I.

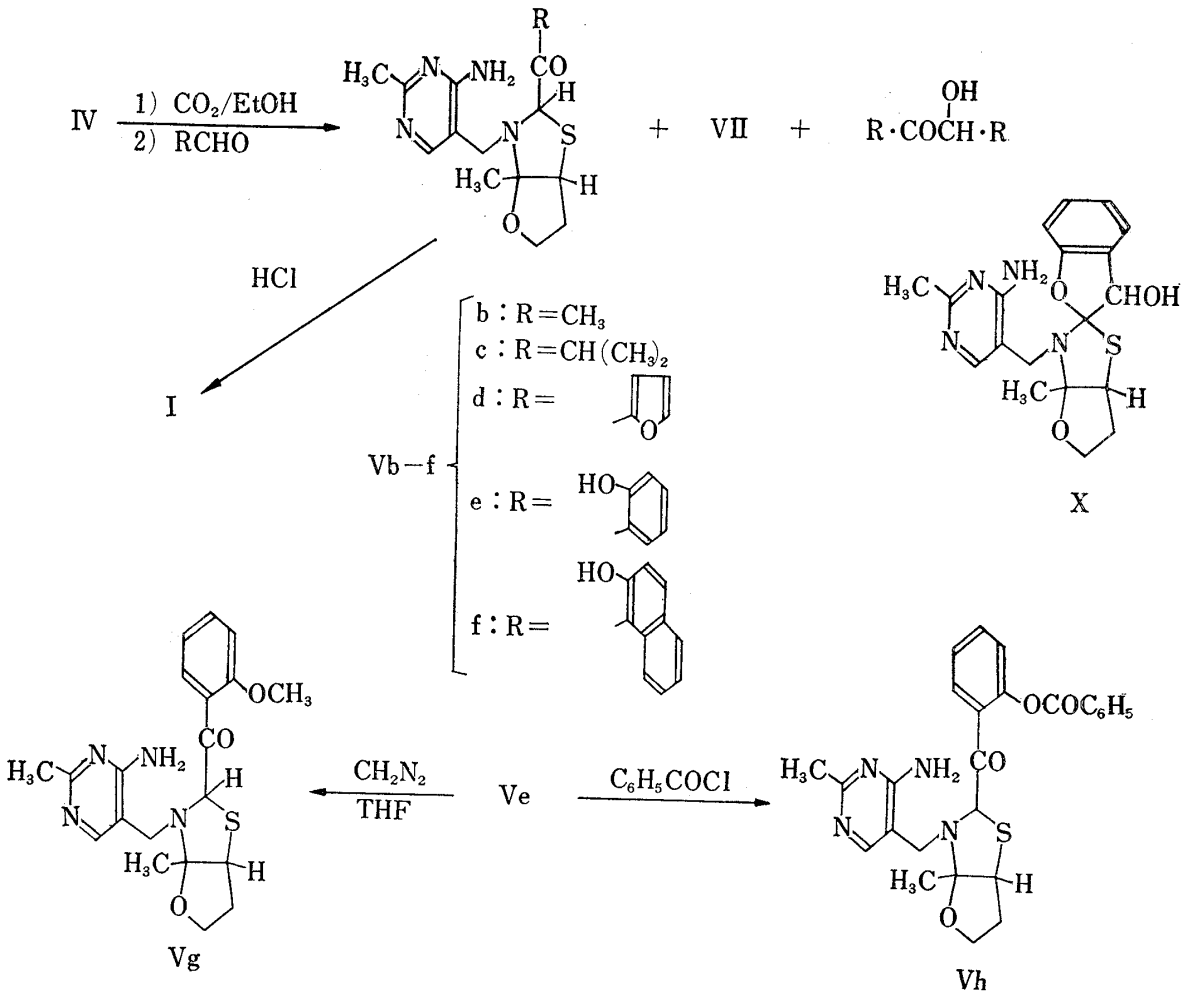
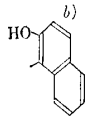
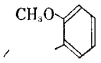
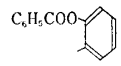


Chart 2

14) Ve has two different crystal forms. See experimental part.

Table I. Physical Constants of C-2 Acylated Thiamine Derivatives (Va—Vh)

	R	mp (C°)	IR ν_{max}^{Nujol} (CO) cm^{-1}	Chemical shift[a] C_4-CH_3	Chemical shift[a] C_2-H
Va	C_6H_5	182—183	1687	8.32	4.55
Vb	CH_3	143—146	1720	8.34	5.47
Vc	$CH(CH_3)_2$	159—161	1710	8.32	5.37
Vd		151—153	1675	8.30	4.75
Ve		176—178 [174—175]	1634 [1677]	8.32	4.52
Vf	 [b]	166—168	1695	—	—
Vg		137—140	1697	8.21	4.58
Vh		115—116	1680	8.44	4.72

a) τ values from tetramethylsilane (TMS) as an internal standard
b) NNR measurement was impossible because Vf was insoluble in $CHCl_3$ or DMSO.

Observations on the thin-layer chromatography of the reaction mixture always revealed that these C-2 acylated compounds were formed predominantly in each reaction, but the yields of purified products were not necessarily satisfactory probably owing to the loss during chromatographic purifications.[15]

The presence of carbonyl function in Ve (mp 176—178°) was not obvious in its IR spectrum owing to intramolecular hydrogen bonding with phenolic hydroxyl group and eventual overlapping of the carbonyl absorption with strong absorption band due to pyrimidine-amino group (1634 cm^{-1}). However, on treatment with diazomethane in tetrahydrofuran (THF), Ve afforded a corresponding methyl ether (Vg), $C_{20}H_{24}O_3N_4S$, mp 137—140°, which exhibited a sharp absorption band at 1697 cm^{-1} due to carbonyl group. Furthermore, its benzoate (Vh), $C_{26}H_{26}O_4N_4S$, mp 115—116°, which was obtained by treatment of Ve with benzoyl chloride in an aqueous chloroform solution containing excess potassium hydrogen carbonate, also exhibited a carbonyl absorption at 1680 cm^{-1}. Consequently, a possible alternative structure X for this compound must be ruled out unambigously.

There are three asymmetric carbon atoms in these C-2 acylated thiamine derivatives (Va—Ve), however these reactions always furnished stereochemically simple products without formation of any other stereoisomers. Supposing that these products (Va—Ve) have thermodynamically stable structure, ring junction between thiazolidine and perhydrofuran rings should be *cis*-fused and configuration of the acyl substituent at C-2 of the thiazolidine ring will probably be oriented so that the steric interactions around it might be minimum. So then, the C-2 acyl group is highly expected to have *trans* configuration to the perhydrofuran ring, and following experimental evidence provides a positive support for this assumption. Reduction of Va with calculated amount of sodium borohydride in methanol gave a dihydro-derivative (XI), $C_{19}H_{24}O_2N_4S$, mp 151—153°, whose IR spectrum showed no carbonyl absorption. In its NMR spectrum (Fig. 2), signals of singlet at 2.71 τ (5H) and a pair of doublets (J=3.3 cps) at 5.0 τ (1H) and 5.45 τ (1H) are obviously assignable to the grouping

15) See experimental part.

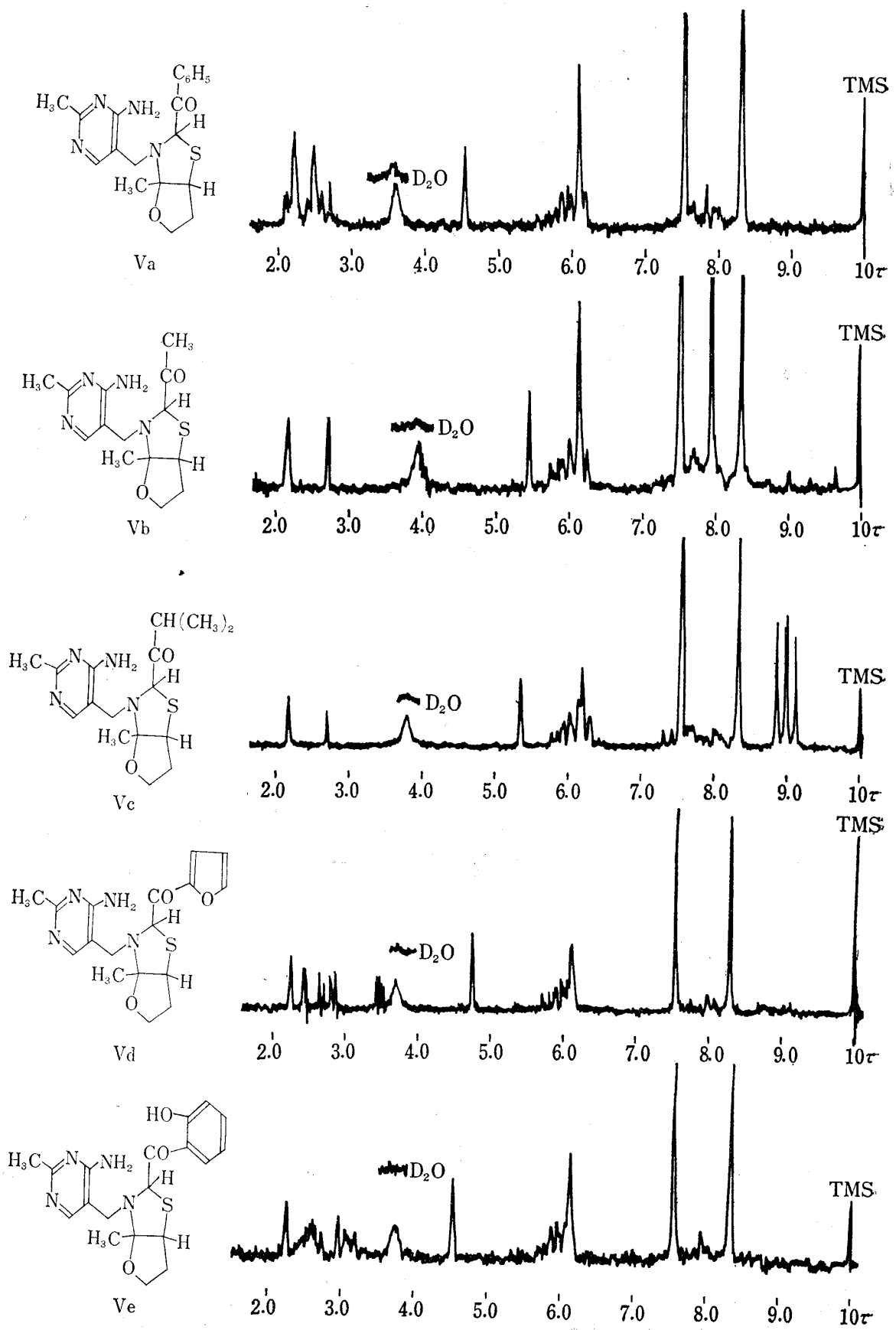


Fig. 1. NMR Spectra of C-2 Acylated Derivatives (Va—Ve) in $CDCl_3$ Solution

$\begin{matrix} N \\ S \end{matrix}\!\!>\!CH\text{–}\underset{\displaystyle OH}{\underset{|}{CH}}\text{–}C_6H_5$ indicating that the carbonyl group of Va was selectively reduced to alcohol. Moreover, the fact that the signal due to thiazolidine C–4 methyl protons (8.60 τ) was remarkably shifted to higher field (Δ=16 cps) compared with that of the original ketone (Va) showed its proximity to the carbonyl function of benzoyl group. Thus, the configuration of the benzoyl group of Va was established as *trans* orientation to the perhydrofuran ring as expected. All chemical shifts of the thiazolidine C–4 methyl groups in the acylated derivatives (Va—Vg) listed in Table I, are nearly constant values, which indicate that they have the same stereostructure as Va. On the other hand, sodium borohydride reduction of Ve and Vg resulted the formations of corresponding tetrahydroderivatives XII, as monohydrate, $C_{19}H_{26}O_3N_4S\cdot H_2O$, mp 172—176°, and XIII, as an oil, respectively. XIII was also obtainable from XII on methylation with diazomethane in tetrahydrofuran (THF). The NMR spectrum of XIII exhibited a doublet (J=6.0 cps) at 8.90 τ (3H) due to $>CH\text{–}CH_3$ grouping and a quartet (J=6.0 cps) at 7.24 τ (1H) was further split with small coupling constant ($J'\leqq 2$ cps) showing *trans* orientation of C–4 and C–5 protons resulted from the hydride attack to C–4 carbon from back side of the C–2 substituent. These stereospecificities of the reduction will supply a further evidence for the configuration of C–2 acyl group as described above.

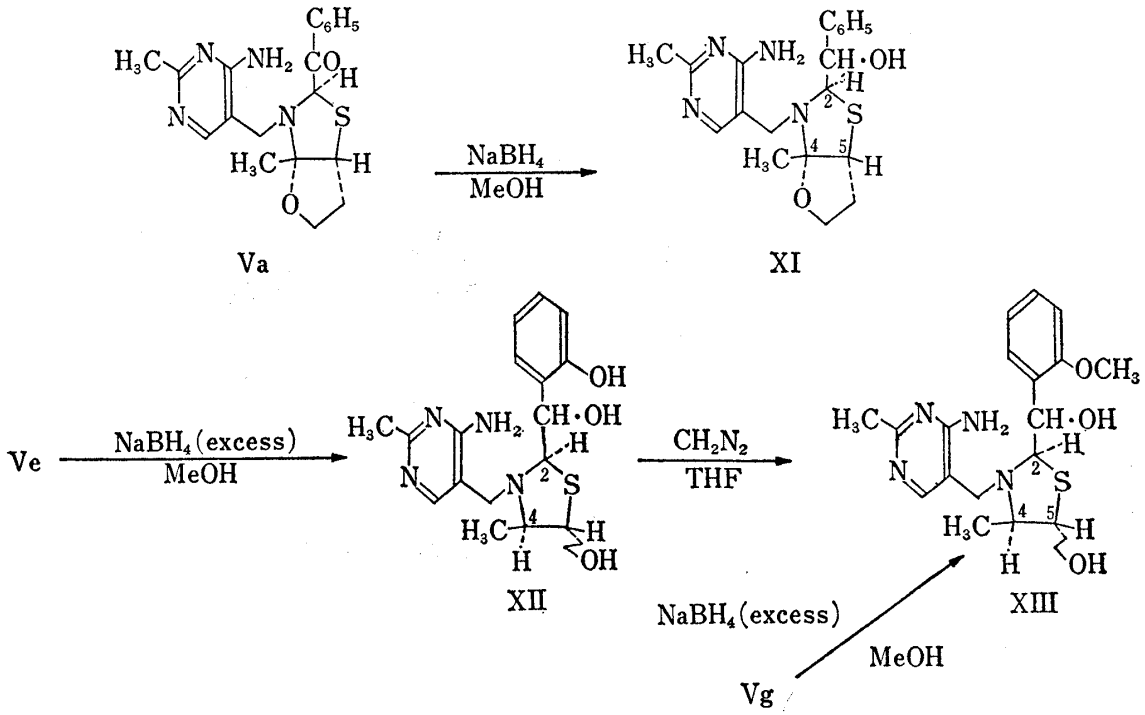


Chart 3

Thus, the unusual acylation reactions of thiamine with aldehydes have now been established, however, on the other hand, the reaction between thiamine hydrochloride (I) and benzaldehyde, when it was carried out in 50% aqueous ethanolic solution in the presence of two equimolar amounts of sodium hydroxide and subsequently treated with hydro-

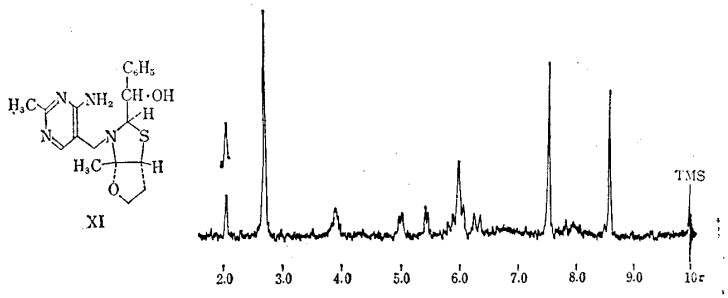
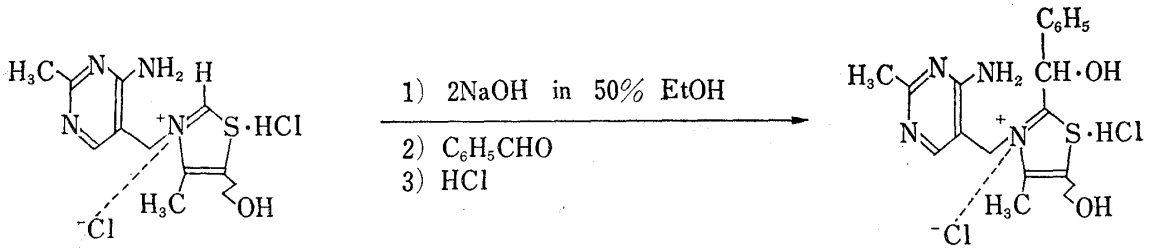


Fig. 2. NMR Spectrum of 2–Hydroxybenzyl–3–(2–methyl–4–aminopyrimidin–5–yl–methyl)–3*a*–methylperhydrofuro[2,3–*d*]thiazole (XI) in $CDCl_3$ Solution

chloric acid, afforded hydroxybenzylthiamine (HBzT) hydrochloride (XIV), as sesquihydrate, $C_{19}H_{24}O_2N_4SCl_2 \cdot {}^3/_2H_2O$, mp 204—209° (d), accompanying the formation of benzoin. XIV was quite stable to the action of hydrochloric acid as compared with Va which was completely decomposed to thiamine hydrochloride (I). The NMR spectrum[16] (Fig. 3) of XIV confirmed its structure clearly.



XIV

Chart 4

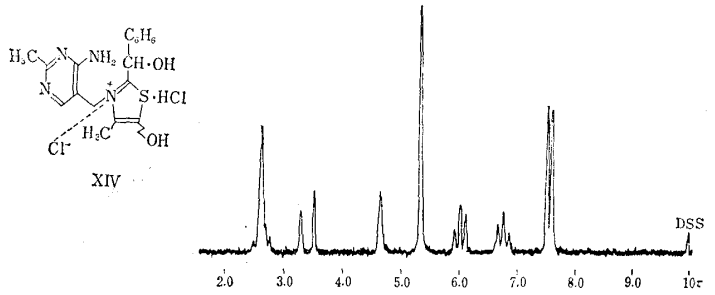


Fig. 3. NMR Spectrum of Hydroxybenzylthiamine-(HBzT) Hydrochloride (XIV) in D_2O Solution

Recently, transformation of the ketonic compound to the corresponding HET type one has been described by Hirano and coworkers.[17] In our reaction condition however the ketonic compounds Va—Vf were all stable not to undergo any isomerization reaction. On the other hand, the ketonic product Va was expected to be derivable from XIV, but every attempt to convert XIV into Va failed. Consequently, these two different products were considered to be the primary product in each reaction. Few examples of the anomalous C–acylation reaction of aldehyde with certain heterocyclic compound have been reported.[18,19] For example, reaction of N,N′–diphenylimidazolinium anion (XV) with benzaldehyde has been shown to give 2–benzoyl–N,N′–diphenylimidazolidine (XVI).[20]

These foregoing results indicate that thiamine exhibits two different reactivities towards aldehyde: the first is formation of HET type product under the Miller's condition[6] and the second is formation of C–2 acylated product under the condition described here. The authors now should like to piont out that these two different reactivities of thiazole C–2 position of thaimine molecule may be attributable to the inherent duplication of character as a carbenoid and as a ylid of the position. Namely, formation of the C–2 acylated thiamine derivative may be attributable to the reactivity of a nucleophilic carbene (XVIII),[20] and formation of HET type product on the other hand to that of a ylid (II) as indicated by Miller and coworkers.[6] Reaction of thiamine–sodium salt (IV) with aldehyde in the presence of carbon dioxide may probably be rationalized as illustrated in Chart 6. The reaction is possibly initiated with formation of pseudothiamine carbonate (XVII) and subsequent α–elimination of hydrogen carbonate will lead to XVIII which then react with aldehyde to give V *via* XIX as shown in path (A). On the reaction of thiamine hydrochloride (I) with aldehyde in the presence of base under anhydrous condition the reactivity of carbene (XVIII)

16) The spectrum was measured in deuterium oxide solution containing DSS as an internal standard.
17) Y. Oka, K. Yoshioka, and H. Hirano, *Chem. Pharm. Bull.* (Tokyo), **15**, 119 (1967).
18) H.W. Wanzlick and E. Schikora, *Chem. Ber.*, **94**, 2389 (1961).
19) H.W. Wanzlick and H.J. Kleiner, *Chem. Ber.*, **96**, 3024 (1963).
20) H.W. Wanzlick, *Angew. Chem.*, **74**, 129 (1962).

may also predominate rather than that of ylid (II), while under aqueous condition the ylid character may be preferable to form HET type product (XXI) after protonation of the intermediate (XX) as indicated in path (B).

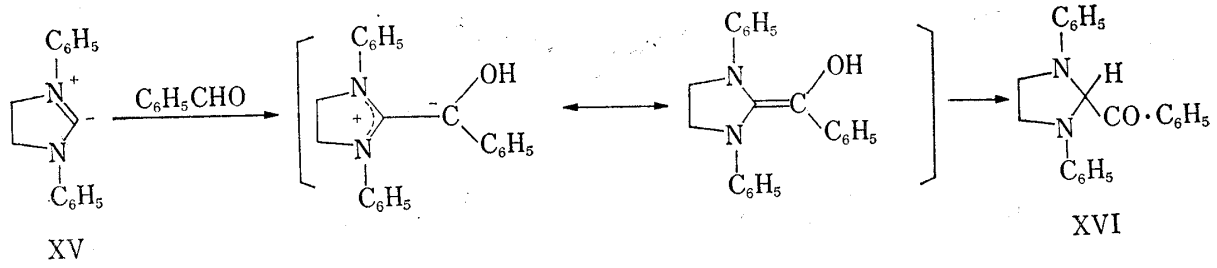


Chart 5

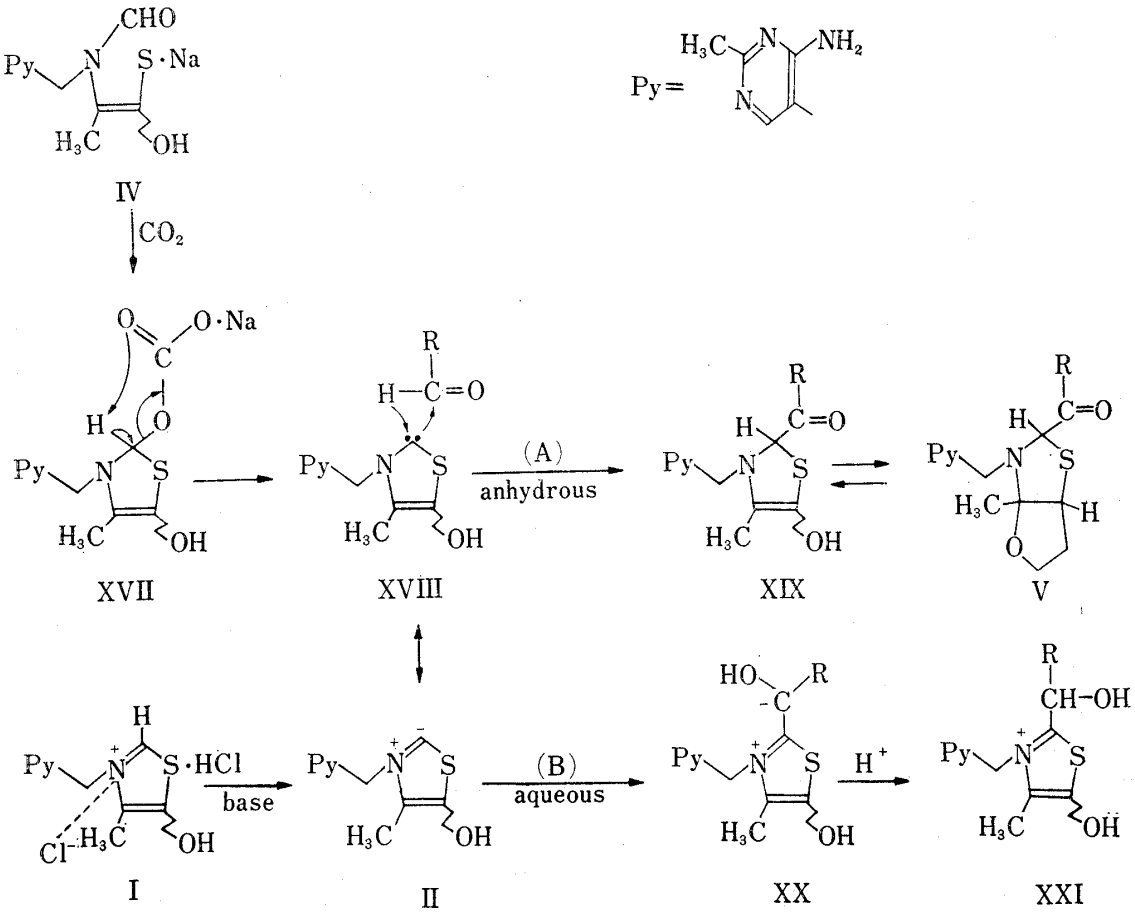


Chart 6

Experimental[21]

General Procedure for the Reaction of Thiamine-sodium Salt (IV) with Aldehyde in the Presence of Carbon Dioxide——To a suspension of thiamine-sodium salt (IV) in 99% EtOH, dry CO_2 gas was introduced for about 1 hr at room temperature with vigorous stirring. Aldehyde (2—3 equimol.) was added to the suspension and after having been stirred for about 4—5 hr, EtOH was removed by evaporation under reduced pressure and residual substance was extracted with $CHCl_3$. The $CHCl_3$ extracts, after concentration to dryness, were subjected to be chromatographed over SiO_2 (Davison, 60—200 mesh) column which was eluted with acetone, and corresponding C—2 acylated product was separated from other accompanying by-products.

21) All melting points are uncorrected.

2-Benzoyl-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]thiazole (Va)——4.25 g of IV was suspended in 40 ml of 99% EtOH and allowed to react with 2.12 g of benzaldehyde according to the general procedure cited above to yield an oily mixture which was subjected to be chromatographed over SiO_2. The first fraction gave benzoin (820 mg) and the second fraction gave vitachrome (VI) (150 mg), mp 180—182°, colorless prisms from acetone. *Anal.* Calcd. for $C_{12}H_{16}O_2N_2S_2$: C, 50.70; H, 5.67; N, 9.86; S, 22.53. Found: C, 50.32; H, 5.52; N, 9.79; S, 22.37. A portion of VI was acetylated with Ac_2O–pyridine according to the usual way to yield a diacetate, mp 116—117°, colorless prisms from ether (lit.[8] mp 116—116.5°). Va was obtained from the third fraction and recrystallization from acetone gave colorless prisms, mp 182—183°, 250 mg (6.7%). *Anal.* Calcd. for $C_{19}H_{22}O_2N_4S$: C, 61.60; H, 5.99; N, 15.12; S, 8.66; O, 8.64. Found: C, 61.28; H, 6.07; N, 16.00; S, 8.66; O, 9.03. IR ν_{max}^{Nujol} cm^{-1}: 1687 (CO), 1667 (NH_2), 1032, 975. UV λ_{max}^{EtOH} mμ: 243.5, 275. 5. NMR ($CDCl_3$)τ: 2.23 (1H, s) (Py–H), 2.1—2.6 (5H, m) (C_6H_5–), 3.6 (2H, broad) (NH_2), 4.55 (1H, s) $\left(\begin{matrix}N\\S\end{matrix}\!>\!CH\text{–}CO\text{–}\right)$, 6.11 (2H, s) (Py–$CH_2$–), 7.54 (3H, s) (Py–$CH_3$), 8.32 (3H, s) ($C_4$–$CH_3$). The final fraction gave SB_1 (VII) (500 mg). On standing in alcoholic–HCl solution for overnight at room temperature, Va was converted to thiamine hydrochloride (I) in almost quantitative yield.

Formation of Va from Thiamine Hydrochloride (I) and Benzaldehyde——6.74 g of I was added to dry DMF (40 ml) containing 12.14 g of NEt_3, and after having been stirred for 10 min at room temperature, 4.24 g of benzaldehyde was added to the mixture, then stirring was continued for 12 hr at 60°. After filtering the reaction mixture, the filtrate was concentrated to dryness under reduced pressure and residual oily substance was extracted with $CHCl_3$. The $CHCl_3$ extracts were combined, washed with water, dried over Na_2SO_4 and concentrated to leave a brown oil which was chromatographed over SiO_2 to give benzoin (2.1 g) and Va (0.8 g, 10.9%).

2-Acetyl-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]thiazole (Vb)——17 g of IV was suspended in 170 ml of 99% EtOH and allowed to react with 15 g of 80% CH_3CHO according to the general procedure to yield an oily mixture. Chromatographic separation of the mixture with SiO_2 gave vitachrome (VI) (60 mg) and Vb (374 mg, 3.0%) and the latter was recrystallized from acetone to give colorless prisms, mp 143—146°. *Anal.* Calcd. for $C_{14}H_{20}O_2N_4S$: C, 54.53; H, 6.54; N, 18.17; S, 10.39. Found: C, 54.37; H, 6.71; N, 18.17; S, 10.60. IR ν_{max}^{Nujol} cm^{-1}: 1720 (CO), 1669 (NH_2), 1028, 987. UV λ_{max}^{EtOH} mμ: 236.5, 275.5. NMR ($CDCl_3$) τ: 2.15 (1H, s) (Py–H), 3.95 (2H, broad) (NH_2), 5.47 (1H, s) $\left(\begin{matrix}N\\S\end{matrix}\!>\!CH\text{–}CO\text{–}\right)$, 6.13 (2H, s) (Py–$CH_2$–), 7.53 (3H, s) (Py–$CH_3$), 7.95 (3H, s) ($COCH_3$), 8.34 (3H, s) ($C_4$–$CH_3$). On standing in alcoholic–HCl solution for overnight at room temperature, Vb was completely converted to thiamine hydrochloride (I).

2-Isobutyryl-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]thiazole (Vc)——4.25 g of IV and 3.6 g of isobutylaldehyde in 40 ml of 99% EtOH were allowed to react according to the general procedure to yield SB_1 (VII) (55 mg) and Vc (120 mg, 3.6%) and the latter was recrystallized from acetone to give colorless needles, mp 159—161°. *Anal.* Calcd. for $C_{16}H_{24}O_2N_4S$: C, 57.11; H, 7.19; N, 16.66; S, 9.53. Found: C, 57.11; H, 6.93; N, 16.85; S, 10.00. IR ν_{max}^{Nujol} cm^{-1}: 1710 (CO), 1679 (NH_2), 1029, 981. UV λ_{max}^{EtOH} mμ: 235.5, 276. NMR ($CDCl_3$) τ: 2.18 (1H, s) (Py–H), 3.80 (2H, broad) (NH_2), 5.37 (1H, s) $\left(\begin{matrix}N\\S\end{matrix}\!>\!CH\text{–}CO\text{–}\right)$, 6.27 (2H, s) (Py–$CH_2$–), 7.55 (3H, s) (Py–$CH_3$), 8.32 (3H, s) ($C_4$–$CH_3$), 8.90, 9.05 (6H, d, d, J,J'= 7 cps) ($CH(CH_3)_2$).

2-*α*-Furryl-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]thiazole (Vd)——4.25 g of IV and 2.5 g of *α*-furfural in 45 ml of 99% EtOH were allowed to react according to the general procedure to give furoin (1.86 g) and Vd (200 mg, 5.6%) and the latter was recrystallized from acetone to give colorless pillars, mp 151—153°. *Anal.* Calcd. for $C_{17}H_{20}O_3N_4S$: C, 56.66; H, 5.99; N, 15.55; S, 8.80. Found: C, 56.03; H, 5.68; N, 15.13; S, 9.55. IR ν_{max}^{Nujol} cm^{-1}: 1675 (CO, NH_2), 1030, 980. UV λ_{max}^{EtOH} mμ: 235.5, 278. NMR ($CDCl_3$)τ: 2.25 (1H, s) (Py–H), 3.77 (2H, broad) (NH_2), 4.75 (1H, s) $\left(\begin{matrix}N\\S\end{matrix}\!>\!CH\text{–}CO\right)$, 6.15 (2H, s) (Py–$CH_2$–), 7.55 (3H, s) (Py–$CH_3$), 8.30 (3H, s) ($C_4$–$CH_3$).

2-(2-Hydroxybenzoyl)-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]thiazole (Ve)——8.5 g of IV and 5.0 g of *o*-hydroxybenzaldehyde in 100 ml of 99% EtOH were allowed to react according to the general procedure to yield SB_1 (VII) (230 mg) and Ve (780 mg, 11%). Ve was also obtainable in higher yield in a following way: 6.74 g of thiamine–hydrochloride (I) was allowed to react with 4.8 g of *o*-hydroxybenzaldehyde in 40 ml of CH_3CN in the presence of 12 ml of NEt_3 and stirred for about 10 hr at 40—50°. After evaporation of CH_3CN under reduced pressure, residual crystals were washed with H_2O for several times and then with acetone and finally with ether to give Ve as a white crystalline powder (3.2 g, 41%), mp 176—178°. *Anal.* Calcd. for $C_{19}H_{22}O_3N_4S$: C, 59.06; H, 5.74; N, 14.50; S, 8.28; O, 12.42. Found: C, 58.64; H, 6.10; N, 14.14; S, 8.21; O, 12.08. IR ν_{max}^{Nujol} cm^{-1}: 1634 (CO, NH_2), 1029, 970. UV λ_{max}^{EtOH} mμ: 213, 238 (shoulder), 259, 272 (shoulder), 333. NMR ($CDCl_3$)τ: 2.25 (1H, s) (Py–H), 3.74 (2H, broad) (NH_2), 4.52 (1H, s) $\left(\begin{matrix}N\\S\end{matrix}\!>\!CH\text{–}CO\text{–}\right)$, 6.12 (2H, s) (Py–$CH_2$–), 7.55 (3H, s) (Py–$CH_3$), 8.32 (3H, s) ($C_4$–$CH_3$). On recrystallization from MeOH Ve gave colorless needles, mp 174—175°. *Anal.* Calcd. for $C_{19}H_{22}O_3N_4S$: C, 59.06; H, 5.74;

N, 14.50; S, 8.28; O, 12.42. Found: C, 58.68; H, 5.87; N, 14.37; S, 8.34; O, 12.77. IR ν_{max}^{Nujol} cm^{-1}: 1677 (CO), 1636 (NH_2), 1030, 975. The mixed melting point with Ve (mp 176—178°) was remarkably depressed (160—165°) but both NMR ($CDCl_3$) and IR ($CHCl_3$) spectra were superimposable with those of Ve. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH), 3320, 3200 (NH_2), 1640 (NH_2), 1029, 937.

2-(2-Hydroxy-1-naphthoyl)-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]-thiazole (Vf)——12 g of IV was suspended in 100 ml of 99% EtOH and allowed to react with 9 g of 2-hydroxy-1-naphthaldehyde according to the general procedure. After evaporation of EtOH under reduced pressure, the residual substance was washed with water and collected. The collected crystals were washed again with water, subsequently with acetone and finally with ether to give Vf (3.1 g, 25%) as a white crystalline powder which was recrystallized from MeOH to give colorless prisms, mp 166—168°. *Anal.* Calcd. for $C_{23}H_{24}O_3N_4S$: C, 63.29; H, 5.54; N, 12.84; S, 7.33; O, 11.00. Found: C, 63.18; H, 5.56; N, 12.73; S, 7.44; O, 11.25. IR ν_{max}^{Nujol} cm^{-1}: 3380, 3165 (OH, NH_2), 1695 (CO), 1640 (NH_2), 1036, 995. UV λ_{max}^{EtOH} mμ: 225, 272, 340.

Methylation of Ve with Diazomethane: 2-(2-Methoxybenzoyl)-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]thiazole (Vg)——350 g of Ve (mp 176—178°) was dissolved in 15 ml of THF and to the solution excess amount of CH_2N_2 in ether solution was added and allowed to stand for 2 days in a sealed tube at room temperature. After removal of the solvents by evaporation under reduced pressure, residual crystals were recrystallized from acetone to give Vg as colorless prisms, mp 137—140° (270 mg, 75%). *Anal.* Calcd. for $C_{20}H_{24}O_3N_4S$: C, 59.99; H, 6.04; N, 13.99; S, 7.99; O, 12.00. Found: C, 59.97; H, 6.30; N, 13.96; S, 8.06; O, 12.70. IR ν_{max}^{Nujol} cm^{-1}: 1697 (CO), 1679 (NH_2), 1020, 975. UV λ_{max}^{EtOH} mμ: 212, 235 (shoulder), 274, 311. NMR ($CDCl_3$) τ: 2.26 (1H, s) (Py-H), 3.63 (2H, broad) (NH_2), 4.58 (1H, s) $\left(\begin{matrix}N\\S\end{matrix}\!>\!CH\text{–}CO\text{–}\right)$, 6.05 (2H, s) (Py–$CH_2$–), 6.24 (3H, s) ($OCH_3$), 7.55 (3H, s) (Py–$CH_3$), 8.21 (3H, s) ($C_4$–$CH_3$).

Benzoate of Ve: 2-(2-Benzoyloxybenzoyl)-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]thiazole (Vh)——240 mg of Ve (mp 176—178°) was dissolved in 5 ml of $CHCl_3$ containing *ca.* 5 ml of aqueous saturated solution of $KHCO_3$ and 1 g of benzoyl chloride was added drop-wise to the mixture under cooling in an ice bath (0—2°) with vigorous stirring. After two hr, the reaction mixture was extracted with $CHCl_3$, and combined $CHCl_3$ extract was washed with water, dried over Na_2SO_4 and evaporated to leave a crystalline residue which was recrystallized from AcOEt to give Vh as colorless prisms, mp 115—116° (195 mg, 64%). *Anal.* Calcd. for $C_{26}H_{26}O_4N_4S$: C, 62.53; H, 5.45; N, 11.22; S, 6.50; O, 14.59. Found: C, 62.37; H, 5.54; N, 11.25; S, 6.49; O, 13.56. IR ν_{max}^{Nujol} cm^{-1}: 1740 (CO), 1680 (CO), 1671 (NH_2), 1050, 1025. UV λ_{max}^{EtOH} mμ: 235, 277. NMR ($CDCl_3$) τ: 2.18 (1H, s) (Py-H), 3.94 (2H, broad) (NH_2), 4.72 (1H, s) $\left(\begin{matrix}N\\S\end{matrix}\!>\!CH\text{–}CO\text{–}\right)$, 6.17 (2H, s) (Py–$CH_2$–), 7.57 (3H, s) (Py–$CH_3$), 8.44 (3H, s) ($C_4$–$CH_3$).

$NaBH_4$ Reduction of Va: 2-Hydroxybenzyl-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-3*a*-methylperhydrofuro[2,3-*d*]thiazole (XI)——800 mg of Va was suspended in 10 ml of abs. MeOH and cooled to 0—2° in an ice bath. To the suspentsion, 54 mg of $NaBH_4$ was added with vigorous stirring, and after 30 min, MeOH was removed by evaporation under reduced pressure to give a colorless residue which was extracted with $CHCl_3$. The combined $CHCl_3$ extract was washed, dried over Na_2SO_4 and evaporated to leave crystals which were recrystallized from acetone to give XI as colorless prisms, mp 151—153° (540 mg, 62%). *Anal.* Calcd. for $C_{19}H_{24}O_2N_4S$: C, 61.72; H, 6.48; N, 15.05; S, 8.60; O, 8.59. Found: C, 61.02; H, 6.90; N, 15.03; S, 8.62; O, 9.35. IR ν_{max}^{Nujol} cm^{-1}: 3440 (OH), 1645 (NH_2), 1020, 983. UV λ_{max}^{EtOH} mμ: 233, 277. NMR ($CDCl_3$) τ: 2.07 (1H, s) (Py–H), 2.71 (5H, s) (C_6H_5–), 3.90 (2H, broad) (NH_2), 5.00 (1H, d, J= 3.3 cps) $\left(\begin{matrix}OH\\|\\C_6H_5\text{–}CH\text{–}\end{matrix}\right)$, 5.45 (1H, d, J=3.3 cps) $\left(\begin{matrix}N\\S\end{matrix}\!>\!CH\text{–}\right)$, 6.00 (2H, s) (Py–$CH_2$–), 7.55 (3H, s) (Py–$CH_3$), 8.60 (3H, s) ($C_4$–$CH_3$).

$NaBH_4$ Reduction of Ve: 2-(2-Hydroxyhydroxybenzyl)-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-4-methyl-5-*β*-hydroxyethylthiazolidine (XII)——250 mg of Ve (mp 176—178°) was suspended in 10 ml of MeOH and 100 mg of $NaBH_4$ was added to the suspension. After having been stirred for 4 hr at room temperature, MeOH was evaporated under reduced pressure to leave an oily residue to which 10 ml of aqueous saturated $(NH_4)_2SO_4$ solution was added and deposited crystals were collected and recrystallized from acetone to give XII as colorless prisms, mp 172—176° (185 mg, 71%). *Anal.* Calcd. for $C_{19}H_{26}O_3N_4S\cdot H_2O$: C, 55.87; H, 6.91; N, 13.72; S, 7.84. Found: C, 55.93; H, 6.94; N, 13.56; S, 7.85. IR ν_{max}^{Nujol} cm^{-1}: 3460 (OH), 1623 (NH_2), 1060, 1035. UV λ_{max}^{EtOH} mμ: 225, 277. NMR ($CDCl_3$) τ: 2.08 (1H, s) (Py–H), 3.70 (2H, s) (NH_2), 5.18 (1H, d, J=4.2 cps) $\left(\begin{matrix}OH\\|\\C_6H_5\text{–}CH\text{–}\end{matrix}\right)$, 5.62 (1H, d, J=4.2 cps) $\left(\begin{matrix}N\\S\end{matrix}\!>\!CH\text{–}\right)$, 6.36 (2H, s) (Py–$CH_2$–), 7.71 (3H, s) (Py–$CH_3$), 8.95 (3H, d, J=6.6 cps) (C_4–CH_3).

$NaBH_4$ Reduction of Vg: 2-(2-Methoxyhydroxybenzyl)-3-(2-methyl-4-aminopyrimidin-5-yl-methyl)-4-methyl-5-*β*-hydroxyethylthiazolidine (XIII)——200 mg of Vg was suspended in 10 ml of MeOH and 100 mg of $NaBH_4$ was added to the suspension. After having been stirred for 4 hr at room temperature, MeOH was

removed by evaporation under reduced pressure to leave an oily residue which was subjected to be chromatographed over SiO_2 and eluted with acetone to give XIII as colorless oil (190 mg, 93%). NMR ($CDCl_3$) τ: 2.12 (1H, s) (Py–H), 4.53 (2H, broad) (NH_2), 5.06 (1H, d, $J=5.7$ cps) $\left(C_6H_5\text{–}\overset{\overset{OH}{|}}{C}H\text{–}\right)$, 5.53 (1H, d, $J=5.7$ cps) $\left(\begin{matrix}N\\S\end{matrix}\!>\!CH\text{–}\right)$, 6.31 (5H, s) (Py–$CH_2$– and OCH_3), 7.24 (1H, q, d, $J_q=6.0$ cps, $J_d \leqq 2$ cps) (C_4–H), 7.58 (3H, s) (Py–CH_3), 8.90 (3H, d, $J=6.0$ cps) (C_4–CH_3).

Methylation of XIII with Diazomethane——120 mg of XII was dissolved in 5 ml of THF, and excess CH_2N_2 (ether solution) was added to the solution. After standing for two hours at room temperature, THF was evaporated under reduced pressure to leave an oil which was subsequently subjected to be chromatographed over SiO_2 and eluted with acetone to give a colorless oil (78 mg, 56%) which was identified with XIII by IR comparison.

Hydroxybenzylthiamine (HBzT) Hydrochloride (XIV)——3.37 g of thiamine hydrochloride (I) was added to 10 ml of EtOH and neutralized with *ca.* 8 ml of aqueous 10% NaOH solution to ajust the pH of the solution to 8.0—8.5. Then, 1.8 g of benzaldehyde was added and stirred for 6 hr at 40—50°. EtOH was evaporated under reduced pressure and remaining aqueous solution was washed with $CHCl_3$, and then acidified with concentrated hydrochloric acid (*ca.* 2 ml) under cooling in an ice bath, subsequently concentrated to dryness under reduced pressure to give a crystalline residue. The residue exhibited two spots positively responding upon Dragendorff reagent on its paper partition chromatogram (ppc) (*Rf* 0.72, 0.52).[22] Fractional recrystallizations gave recorvered thiamine hydrochloride (I) (2.1 g) and crude HBzT hydrochloride was obtained from MeOH soluble fraction. Repeated recrystallizations of the crude product yielded pure XIV as colorless prisms, mp 204—209° (d) (1.12 g, 25%). *Anal.* Calcd. for $C_{19}H_{24}O_2N_4SCl_2 \cdot \frac{3}{2}H_2O$: C, 48.52; H, 5.70; N, 11.91; S, 6.81; Cl, 15.07. Found: C, 48.52; H, 6.54; N, 12.20; S, 6.88; Cl, 15.04. ppc $Rf=0.72$.[22] UV λ_{max}^{EtOH} mμ: 239, 270. NMR (D_2O) τ: 2.65 (5H, broad singlet) (C_6H_5–), 3.29 (1H, s) (Py–H), 3.52 (1H, s) $\left(C_6H_5\text{–}\overset{\overset{HO}{|}}{C}H\text{–}\right)$, 4.64 (2H, s) (Py–$CH_2$–), 6.01 (2H, t) (–$CH_2$–OH), 6.77 (2H, t) (=C–$CH_2$–), 7.52 (3H, s) (Py–$CH_3$), 7.60 (3H, s) ($C_4$–$CH_3$). XIV was recovered unchanged on the treatment with EtOH–HCl solution for overnight at room temperature.

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22) Developed with the solvent system: iso–PrOH(17): HCl(4): H_2O(4).