Studies on Vitamin B₁ and Related Compounds. CX.¹⁾ Rearrangements of α -Hydroxybenzylthiamin and Its Homologues²⁾YOSHIKAZU OKA, SHOJI KISHIMOTO
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The structure of a product which is obtained by the reaction of thiamin with benzaldehyde was established as 2-[α -(4-amino-2-methyl-5-pyrimidinylmethyl)- α -hydroxybenzyl]-5-(2-hydroxyethyl)-4-methylthiazole (III). The mechanism of the reaction is discussed and the syntheses of several homologs of III as well as their chemical reactions are described.

In the preceding paper¹⁾ we described a novel cleavage of thiamin and its homologs by the reaction with aromatic aldehydes to afford 4-amino-2,5-dimethylpyrimidine (I) and 2-acylthiazoles. It was also pointed out that a small amount of unidentified compound was formed in the reaction. During the course of our investigations on the structure of this by-product, it has become clear that the compound can be formed by way of an interesting rearrangement of α -hydroxybenzylthiamin (HBzT).

The present paper deals with the structure of the by-product together with the mechanism of the rearrangement.

When thiamin chloride hydrochloride was treated with two mole equivalents of triethylamine in methanol and the solution was refluxed with an excess of benzaldehyde, a crystalline substance, C₁₉H₂₂O₂N₄S (III), mp 171–172° (decomp.), precipitated from the reaction mixture in a 4% yield along with I, 2-benzoyl-5-(2-hydroxyethyl)-4-methylthiazole (II) and benzoin. The elementary analysis and mass spectral data provided proof that III is an adduct of one mole each of thiamin and benzaldehyde, *i.e.*, an isomer of free base of HBzT. The nuclear magnetic resonance (NMR) spectrum of III showed no particular shift of the signals due to the protons on both the pyrimidine and thiazole moieties as compared with the signals of thiamin derivatives, except that the methylene protons of III had been shifted upfield and that an additional hydroxyl proton signal was observed at 3.65 τ . The upfield shift could be seen more obviously in the NMR spectra of III-mono-*o*-benzoate (V) and IV, a homolog of III, which was obtained by the reaction of 3-(4-amino-2-methyl-5-pyrimidinylmethyl)-4,5-dimethylthiazolium (VII) and benzaldehyde. In fact, the methylene signals of IV and V were found at *ca.* 6.7 τ as quartet ($J = 14$ Hz), moreover even V revealed the hydroxyl proton signal at 4.15 τ . These observations led us to speculate that III would be an isomer of HBzT, in which some structural change had occurred around the methylene group between the pyrimidine and the thiazole.

The most striking feature of III and its homologs was revealed in the mass spectra; *i.e.* they all showed an intense peak at m/e 123 which probably arises from the pyrimidine moiety. It should be mentioned that the m/e 122 peak, which we assigned to VIII, appears as one of the major peaks in most thiamin derivatives. Moreover, the peak at m/e 123 disappeared and a new peak at m/e 126 appeared when IV, a homolog of III, was deuterated with MeOD.

1) Part CIX: Y. Oka, S. Kishimoto and H. Hirano, *Chem. Pharm. Bull.* (Tokyo), **18**, 536 (1970).

2) This work was presented at The 89th Annual Meeting of The Pharmaceutical Society of Japan, Nagoya, April 1969.

3) Location: *Juso, Higashiyodogawa-ku, Osaka.*

Fig. 3. Ultra Violet Spectra of IV and XII in EtOH

— : XII   - - - : IV

Fig. 4. Nuclear Magnetic Resonance Spectrum of XII·H₂O in CDCl₃ at 100 Mc

—122), which in turn gives m/e 140 (XI) with loss of benzene and m/e 105 (benzoyl cation) with loss of 4,5-dimethylthiazole. The fragmentation to give m/e 140 also receives support by the existence of a metastable ion at m/e 90. The structure of III is therefore defined as 2-[α -(4-amino-2-methyl-5-pyrimidinylmethyl)- α -hydroxybenzyl]-5-(2-hydroxyethyl)-4-methylthiazole.

Further evidence for the structure comes from some chemical reactions. Although IV is considerably stable in alkaline or in dilute acid solutions, it is readily dehydrated in concentrated sulfuric acid to give a stable yellow substance. In view of a bathochromic shift in the UV spectrum (Fig. 3) and appearance of a vinyl proton signal at the expense of the methylene protons in the NMR spectrum (Fig. 4), the product was assigned 4-amino-5-[β -(4,5-dimethyl-2-thiazolyl)styryl]-2-methylpyrimidine (XII). Similarly, when IV was refluxed in 20% hydrochloric acid, it underwent both the dehydration and the hydrolysis of the amino group to give 5-[β -(4,5-dimethyl-2-thiazolyl)styryl]-4-hydroxy-2-methylpyrimidine (XIII). XIII was also given by treatment of XII with 20% hydrochloric acid.

Some homologs of III, prepared by the reaction of thiamin or its homologs with a variety of aromatic aldehydes, are summarized in Table I, and several dehydration products obtained by the reaction of them with 20% hydrochloric acid are listed in Table II.

The scope and limitations of the reactions to afford the rearranged compounds together with 2-alkyl-4-amino-5-methylpyrimidine and 2-acylthiazoles have been discussed in the preceding paper.¹⁾ From the arguments discussed, it is clear that III is a rearranged product arisen from HBzT and it is assumed that the 4-amino group in the pyrimidine ring may play an important role in this reaction. Moreover, neither the formation of III by the reaction of I and II nor the cleavage of III into I and II was observed under the conditions: This implies that the rearrangement presumably occurs competing with the cleavage reaction of HBzT into I and II.

In order to elucidate the mechanism of this novel reaction we first tried to clarify the role of the 4-amino group in the pyrimidine ring. We have found that oxythiamin (XV), in which the 4-amino group in thiamin is replaced by hydroxyl group, failed to undergo this reaction;

R ₁	R ₂	R ₃	mp (decomp.) (°C)	Yield (%)	Formula	Analysis (%)					
						Calcd.			Found		
						C	H	N	C	H	N
CH ₃	CH ₂ OH	H	184 ^{a)} 171—172 ^{b)}	4	C ₁₉ H ₂₂ O ₃ N ₄ S	61.59	5.99	15.12	61.38	5.95	15.40
CH ₃	CH ₂ OH	Cl	208—210 ^{a)} 158—161 ^{c)}	11	C ₁₉ H ₂₁ O ₂ N ₄ SCl	56.35	5.23	13.84	56.01	5.12	13.90
CH ₃	CH ₂ OH	Br	206	12	C ₁₉ H ₂₁ O ₂ N ₄ SBr	50.78	4.71	12.47	50.81	4.74	12.56
CH ₃	H	H	210—211	12	C ₁₈ H ₂₀ ON ₄ S	63.50	5.92	16.46	63.36	5.60	16.28
CH ₃	CH ₂ OH	CH ₃	210—212	4	C ₂₀ H ₂₄ O ₂ N ₄ S	62.47	6.30	14.57	62.19	6.37	14.43
CH ₃	H	Cl	216—218	11	C ₁₈ H ₁₉ ON ₄ SCl	57.66	5.11	14.97	57.68	4.98	14.72
CH ₃	H	Br	221	7	C ₁₈ H ₁₉ ON ₄ SBr	51.56	4.57	13.36	51.50	4.50	13.40
C ₂ H ₅	H	H	207—208	5	C ₁₉ H ₂₂ ON ₄ S	64.38	6.26	15.81	64.43	6.29	15.81

a) Recrystallized from dil. EtOH,

b) from acetone,

c) from CHCl₃.

R ₁	R ₂	mp (decomp.) (°C)	Yield (%)	Formula	Analysis (%)					
					Calcd.			Found		
					C	H	N	C	H	N
H	Cl	250	73	C ₁₈ H ₁₆ ON ₃ SCl	60.41	4.51	11.74	60.41	4.52	11.67
H	Br	274	84	C ₁₈ H ₁₆ ON ₃ SBr	53.75	4.01	10.44	53.99	4.18	10.41
H	H	279—281	56	C ₁₈ H ₁₇ ON ₃ S	66.85	5.30	12.99	66.65	5.21	12.98
CH ₂ OH	H	243	45	C ₁₉ H ₁₉ O ₂ N ₃ S	64.57	5.57	11.89	64.61	5.45	11.78

the fact suggests two possibilities for the role of the amino group. The one is that the amino group assists the addition of an aldehyde to the thiazolium ring to give HBzT; the second is that the amino group assists further rearrangements of HBzT to give III. According to the former possibility, the group should be indispensable for the formation of 2- α -hydroxyaralkylthiazolium compounds. Although our attempts to isolate α -hydroxybenzyl oxythiamin (XVI) from the reaction products of oxythiamin and benzaldehyde have thus far been unsuccessful, the possibility may be excluded in view of the fact that the reaction of 3,4-dimethyl-5-(2-hydroxyethyl)thiazolium iodide with benzaldehyde gives 3,4-dimethyl-2-(α -hydroxybenzyl)-5-(2-hydroxyethyl)thiazolium iodide.¹⁾

In order to obtain information on the second possibility, we attempted to synthesize XVI and its homologs by way of another route. Thus, according to the method for the synthesis of 2-acyldihydrothiamins which we reported earlier,⁵⁾ *p*-chlorophenylglyoxal, 5-aminomethyl-

5) Y. Oka, E. Imamiya and H. Hirano, *Chem. Pharm. Bull.* (Tokyo), **15**, 448 (1967).

4-hydroxyl-2-methylpyrimidine and 3-acetyl-3-mercapto-1-propanol were allowed to react and obtained 2-*p*-chlorobenzoyl-3-(4-hydroxy-2-methyl-5-pyrimidinylmethyl)-3a-methylperhydrofuro[2,3-*d*]thiazole (XVII). Treatment with phosphoric acid in ethanol⁵) successfully effected the conversion of XVII into *p*-chloro- α -hydroxybenzyl oxythiamin (XVIII). On the other hand, when HBzT was refluxed in 20% hydrochloric acid, the 4-amino group was hydrolyzed to afford XVI. We then examined the behaviors of XVI and XVIII in alkaline solutions.

The result, however, demonstrated that treatment of XVI and XVIII with triethylamine in methanol gave neither the rearrangement product similar to III nor 2-acylthiazoles. From these facts it is concluded that the 4-amino group plays a vital part in the reaction and participated in both the cleavage reaction of HBzT leading to I and II and the rearrangement to III as well. Hence we presumed the mechanism of the reaction to be as follows: The initial attack of the α -carbon attached to the 2-position of the thiazole ring by the amino group would effect a seven-membered ring intermediate with loss of water. Then cleavage of the bond between bridge-methylene and nitrogen of the thiazolium would take place to give a Schiff's base (XIX) by electron transfers as shown in Chart 4. Finally, the hydrolysis of XIX would lead to I and II, while further migration of XIX would give rise to III via a five-membered ring intermediate (XX).

Chart 4

Although it has been recognized that the 2-position of the thiazolium ring plays a biochemically important role and goes into reaction with pyruvic acid to give α -hydroxyethylthiamin (HET), relatively little attention has been directed to the importance of the amino group in the pyrimidine ring. Recently Schellenberger has pointed out that the amino group might act as an acceptor of the acetaldehyde from HET.⁶⁾ In view of Schellenberger's

6) A. Schellenberger, *Angew. Chem. Intern. Ed. Engl.*, **6**, 1024 (1967).

hypothesis it is interesting to note that in the present rearrangement the amino group went into reaction with the α -carbon attached to the 2-position of the thiazole ring.

Experimental

2-[α -(4-Amino-2-methyl-5-pyrimidinylmethyl)- α -hydroxybenzyl]-4,5-dimethylthiazole (IV)—To a solution of VII·HCl·Cl (45 g) in MeOH (375 ml) were added triethylamine (31.5 g) and benzaldehyde (31.5 g), and the mixture was refluxed for 5 hr on a steam bath. After evaporation of the solvent, to the residue was added 100 ml of water and extracted with AcOEt. The extract was shaken with 5% HCl (50 ml) and the aqueous layer⁷⁾ was neutralized with 10% NaOH to afford an oily material, which was taken up in EtOAc and evaporated to dryness. The residue was triturated with 50 ml of ether and the resulting solid was filtered. Recrystallization from CHCl₃-ether (1:1) gave 5.9 g of IV as needles. The yield and elemental analysis are listed in Table I.

General Procedure for the Preparation of Homologs of III—The preparation of III was described in the preceding paper.¹⁾ Several homologs of III (Table I) were prepared by similar procedures as in the case of III and IV; *i. e.* by the reactions of thiamin or its homologs with aromatic aldehydes. The general procedure is as follows: To a solution of thiamin or its homolog (0.1 mole) and triethylamine (20.2 g; 0.2 mole) in MeOH (250 ml) was added 0.2 mole of an aromatic aldehyde and the mixture was refluxed for 5 hr. After the solvent had been evaporated *in vacuo*, the residue was acidified with 10% HCl and extracted with AcOEt. The aqueous layer was neutralized with 10% NaOH and extracted with AcOEt. The extract was dried over Na₂SO₄ and evaporated. The residue was triturated with ether to give a homolog of III. In some cases when the product was not obtained as crystals, it was further purified by a column chromatography on silica gel. The results are summarized in Table I.

2-[α -(4-Amino-2-methyl-5-pyrimidinylmethyl)- α -hydroxybenzyl]-5-(2-benzoyloxyethyl)-4-methylthiazole (V)—To a solution of III (370 mg) in pyridine (4 ml) was added dropwise 0.3 g of benzoyl chloride with stirring and the mixture was allowed to stand overnight. After pyridine was removed under reduced pressure, the residue was neutralized with NaHCO₃ and extracted with AcOEt. Dried over Na₂SO₄, the extract was evaporated and the residue was dissolved in ether to afford crude crystals of V. Recrystallization from acetone-ether gave 150 mg of fine needles, mp 170–172°. *Anal.* Calcd. for C₂₈H₂₆O₃N₄S: C, 65.79; H, 5.52; N, 11.81. Found: C, 65.67; H, 5.44; N, 11.64.

Deuteration of IV—IV (200 mg) was dissolved in 99% MeOD (5 ml). The solution was allowed to stand for 2 hr and refluxed for 15 min. After removal of the solvent by evaporation, the residue was dissolved again in 5 ml of 99% MeOD, followed by the repetition of the above procedure, to give 180 mg of deuterated IV as colorless crystals. Mass spectrum of the compound showed peaks at *m/e* 219 (M⁺ – 122 + 1), *m/e* 140 (XI), *m/e* 126 (X + 3), *m/e* 105 (C₈H₅CO⁺) and *m/e* 77 (C₆H₅⁺).

4-Amino-5-[β -(4,5-dimethyl-2-thiazolyl)styryl]-2-methylpyrimidine (XII)—One gram of IV was dissolved in 20 ml of conc. H₂SO₄ and allowed to stand for 24 hr. The mixture was poured into ice water and neutralized with 10% NaOH. The precipitate was collected by filtration and recrystallized from acetone to give pale yellow needles, mp 183–184°. *Anal.* Calcd. for C₁₈H₁₈N₄S·H₂O: C, 63.50; H, 5.92; N, 16.46. Found: C, 63.59; H, 5.91; N, 16.49. UV $\lambda_{\text{max}}^{\text{EtOH}}$ $m\mu$ (ϵ) 351 (17100).

5-[β -(4,5-Dimethyl-2-thiazolyl)styryl]-4-hydroxy-2-methylpyrimidine (XIII)—i) XII (0.2 g) was dissolved in 10 ml of 20% HCl and refluxed for 3 hr. After removal of the solvent under reduced pressure, the residue was neutralized with 10% NaOH to give a yellow precipitate, filtration and recrystallization of which afforded 0.1 g of XIII as yellow needles.

ii) IV (4.9 g) was dissolved in 50 ml of 20% HCl and refluxed for 6 hr. The solution was cooled, neutralized with NaHCO₃ and extracted with CHCl₃. Evaporation of the extract and recrystallization of the residue from EtOH gave yellow needles. Melting point, yield and elemental analysis of this compound are listed in Table II together with those of other several homologs, which were also prepared by this method.

2-*p*-Chlorobenzoyl-3-(4-hydroxy-2-methyl-5-pyrimidinylmethyl)-3a-methylperhydrofuro[2,3-*d*]thiazole (XVII)—3-Acetyl-3-chloro-1-propanol (2.8 g) was heated with 10 ml of water on a steam bath for about 20 min to effect a clear solution. To the solution was added 8 ml of 10% NaOH saturated with H₂S. To the mixture were added a solution of 5-aminomethyl-4-hydroxy-2-methylpyrimidine prepared by the neutralization of its dihydrochloride (4.2 g) in water (6 ml) with 10% NaOH (16 ml) and a solution of *p*-chlorophenylglyoxal (4 g) in EtOH (25 ml). The mixture was stirred at room temperature for 3 hr to afford a brown precipitate, which was separated by decantation and extracted with CHCl₃. Dried over Na₂SO₄, CHCl₃ was removed by evaporation. The residue, dissolved in 5 ml of AcOEt, was allowed to stand overnight. Filtration of the resulting crystals and recrystallization from CHCl₃-acetone gave colorless needles,

7) The work up of the EtOAc layer gave 9.5 g of 2-benzoyl-4,5-dimethylthiazole.¹⁾

mp 156—157° (decomp.). Yield: 1.8 g (22%). *Anal.* Calcd. for $C_{19}H_{20}O_3N_3SCl$: C, 56.22; H, 4.97; N, 10.35. Found: C, 55.91; H, 4.83; N, 10.42.

2-(4-Chloro- α -hydroxybenzyl)-3-(4-hydroxy-2-methyl-5-pyrimidinylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium Diphosphate (XVIII)—To a 8.5% ethanol solution of H_3PO_4 (14 ml) was added 0.7 g of XVII. The mixture was refluxed for 1 hr to precipitate a gummy substance, which was separated by decantation and extracted with hot MeOH. The MeOH was evaporated to dryness and the residue, dissolved in a small portion of EtOH, was allowed to stand overnight. Filtration of the resulting crystals and recrystallization from MeOH-acetone gave 0.25 g of needles, mp 175—177° (decomp.). *Anal.* Calcd. for $C_{19}H_{26}O_{11}N_3SP_2$: C, 37.91; H, 4.35; N, 6.98. Found: C, 37.72; H, 4.59; N, 6.92.

2-(α -Hydroxybenzyl)-3-(4-hydroxy-2-methyl-5-pyrimidinylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium Chloride Hydrochloride (XVI)—To 8 ml of 20% HCl was added 1.8 g of HBzT chloride·HCl⁵ and the mixture was refluxed for 11 hr. After being decolorized with charcoal, the solution was evaporated *in vacuo*. The residue was dissolved in 3 ml of EtOH and undissolved NH_4Cl was removed by filtration. The filtrate was set aside overnight and resulting crystals were recrystallized from 90% EtOH to give 0.9 g (50%) of needles, mp 208—211° (decomp., sintered at *ca.* 120°). *Anal.* Calcd. for $C_{19}H_{23}O_3N_3SCl_2 \cdot H_2O$: C, 49.35; H, 5.45; N, 9.09. Found: C, 49.06; H, 5.57; N, 9.00.

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