Summary

Six among eight metabolites which were detected by paper chromatography, were isolated as crystalline compounds from the urine of rabbits administered thiamylal and all their structures were established as thiamylal ω-carboxylic acid, unchanged thiamylal, secobarbital, (ω-1)-hydroxythiamylal, secobarbital ω-carboxylic acid, and (ω-1)-hydroxysecobarbital, respectively. The fate of thiamylal ω-carboxylic acid, the main metabolite of thiamylal, was also studied and the possible *in vivo* metabolic map of thiamylal was speculated.

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77. Tanezo Taguchi and Kunitoshi Yoshihira : thionamides. I. Synthesis. Demethylation of their N,N-Dimethyl Derivatives by Sulfur.

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The direct oxidation of methylpyrazine (I) to pyrazinecarboxylic acid (V) has been found to be unfavorable for the preparation purpose, because of accompaniment of the ring openning reaction[1]. The oxidation has been improved by an indirect method[2] which is diagrammatically shown as A in Chart 1. An alternative procedure for the synthesis of the acid V, which is based on derivation of I to N,N-dimethylpyrazinecarbothionamide (II) followed by hydrolysis, was examined (see Chart 1. B).

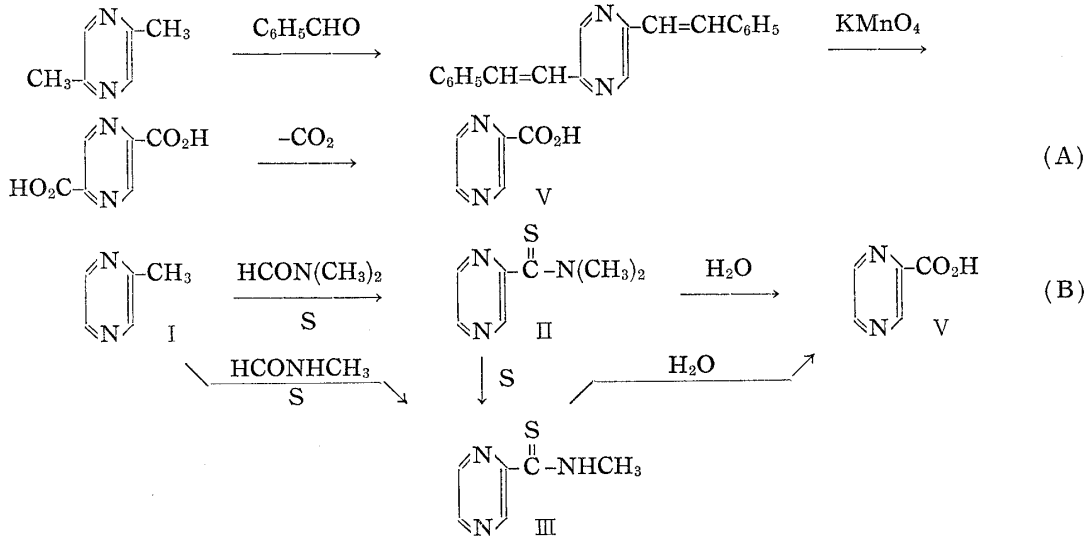


Chart 1.

*1 Katakasu, Fukuoka (田口胤三, 義平邦利).

1) S. Gabrier, A. Sonn : Ber., **40**, 4855 (1907).
2) K. Kaku : J.P. 271, 283 (1961).

Of several studies[3~8] which have been done for the derivation of active methyl or methylene to carbothionamide, a procedure by Wegler[3] was adapted here. Then I was submitted to the fusion with sulfur and dimethylformamide for yielding the N,N-dimethylthionamide (II). But the treatment produced also N-methyl pyrazinecarbothionamide (III), a product from demethylation reaction, besides the ordinal product II. Hence the main aim of the present study turned out to be focused on the demethylation reaction.

Results and reaction conditions in treatments of I with sulfur and dimethylformamide are summarized in Table I and consequently provides the following conclusion :

a) Temperature——The reaction required heating up to 150~170° or more before hydrogen sulfide gas evolved (see Exp. 1, 2). The temperature required is roughly identical with the altitude where sulfur exists in the S_x form.[9~12]

b) Solvent——*o*-Dichlorobenzene was used, because its boiling point is within the range of reaction temperature. But it was uneffective.

c) Catalyst——Iodine[13,14] and lead oxide[15] was used and only the former had an effect on lowering reaction temperature and increasing reaction rate. It will be because, as has been known[9], iodine serves to decrease the viscosity of sulfur.

d) Reaction time——The progress of reaction was roughly estimated by measurement of hydrogen sulfide gas evolving. The evolution of the gas barely reached 50% in theoretical amount after so long as 70 hour, while, when iodine was added as a catalyst, it reached 150% after 28 hour or less.

e) Products——The best yield of II was 13.6% with recovery of the starting material I in 41% (Exp. 10). Besides II, two products, $C_6H_7N_3S$ (III) and $C_{10}H_{10}N_4S$ (IV), were isolated from the same reaction mixture. The C_6 compound was identical with N-methylpyrazinecarbothionamide (III) which was prepared by action of sulfur and methyl-

Table I. Formation of N-Substituted Pyrazinecarbothionamides (II and III)

Materials—Methylpyrazine (I), 1 mol. : Dimethylformamide, 1.2 mol. : Sulfur, 3 mol.
Solvent—*o*-Dichlorobenzene.

Expt. No.	Temp. of bath (°C)	Time (hr.)	Catalyst (mol.)	Solvent (mol.)	Yield (%)			I recovered (%)
					II	III	IV	
1	120~140	52			—	—	—	67.0
2	150~170	70			0.48	—	—	32.1
3	180~200	70			3.3	0.65	0.03	30.5
4	180~195	70		5.2	3.5	—	0.05	31.0
5	180~195	70		1.7	4.5	—	0.06	37.0
6	180~195	70		2.5	5.6	1.9	0.1	39.2
7	180~195	70	I_2 : 0.01	4.0	11.6	2.7	0.15	33.0
8	180~195	70	I_2 : 0.01		11.5	2.8	0.11	31.0
9	170~180	25	I_2 : 0.04		12.5	6.2	0.11	49.0
10	150~160	28	I_2 : 0.06		13.6	5.4	0.1	41.0
11	185~200	60	PbO : 1.00		4.9	—	—	—

3) R. Wegler, E. Kühle, W. Schäfer : Angew. Chem., **70**, 351 (1958).
4) H. Saikachi, T. Hisano : Yakugaku Zasshi, **74**, 1318 (1954).
5) W. Schäfer, R. Wegler : D.B.P. 964, 142 (1953).
6) W. Schäfer, R. Wegler, G. Domagk : *Ibid.*, F 12, 625 (1953).
7) W. Schäfer, R. Wegler : *Ibid.*, P 191, 426 (1954).
8) *Idem* : U.S.P. 2,774,757 (1955).
9) R. Bacon, R. Fanell : J. Am. Chem. Soc., **65**, 639 (1943).
10) R.E. Powell, H. Erying : *Ibid.*, **65**, 648 (1943).
11) G. Gee : Trans. Faraday Soc., **48**, 515 (1952).
12) E.H. Farmer, F.W. Schipley : J. Polymer Sci., **1**, 293 (1947).
13) G. Doucherty, P.D. Hammond : J. Am. Chem. Soc., **57**, 117 (1935).
14) P. Shukla : J. Indian Ins. Sci., **10A**, Pt, **3**, 33 (1927).
15) M. Moore, T.B. Johnson : J. Am. Chem. Soc., **57**, 1287 (1935).

formamide on I or by action of phosphor pentasulfide and potassium sulfide on pyrazinecarboamide (see Chart 1 and Table III).

The C_{10} compound (IV) remains unidentified because the yield was too poor to be brought up as a product, however it is just supposed to be pyrazinylmethylsulfide on presuming from molecular formula and infrared spectrum (11.81 μ, 12.91 μ due to monosubstituted pyrazine ring).

The formation of III suggested that demethylation reaction, presumably due to the action of sulfur, was involved in the course. Therefore II was submitted to the fusion with sulfur at 200~230° for 50 hour and it was found that II existed in yield 4% in the reaction mixture which mostly had undergone decomposition further.

To examine the demethylation reaction again, the derivation of 2-picoline (VI) to the corresponding thionamide was attempted in the condition analogous to Table I, Experimental 2. The treatment gave N,N-dimethyl-picolinethionamide (VII) in 26.5%, N-methyl-picolinethionamide (VIII) in 13.2% and the starting material VI recovered in 16.7%. The use of iodine as a catalyst in the reaction was advantageous for the preparation of VII (yield 36.4%). The increase in addition of sulfur caused the increase in the formation ratio of the demethylation product (VIII) and also products decomposing further. An authentic sample of VIII was gained by fusion of VI with sulfur and methylformamide (see Chart 2).

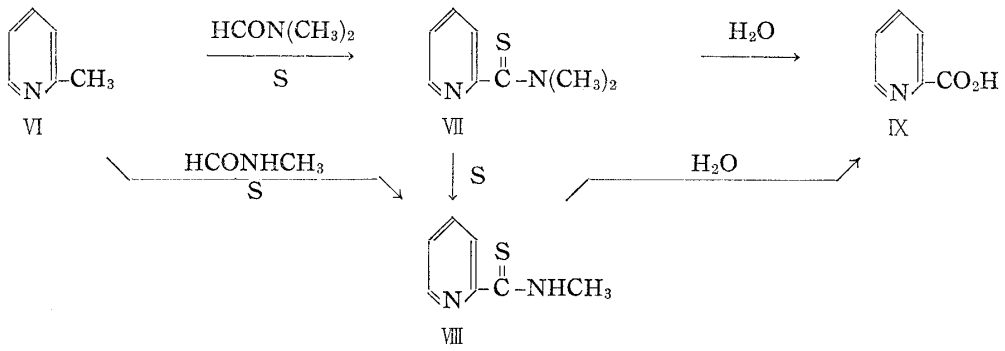


Chart 2.

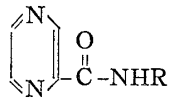
The fusion of VII with sulfur, also, caused demethylation to give VIII, the finding being analogous to the case of the pyrazine derivative (II).

Thus the demethylation reaction of N,N-dimethylthionamides by sulfur was generally established, but it was not clear that the reaction depended upon whether sulfur or hydrogen sulfide which arised from sulfur in the reaction course. To make it clear, hydrogen sulfide gas was introduced to fusing VII and the demethylation product (VIII) was obtained. Thus hydrogen sulfide, too, was effective for the demethylation reaction, however a possibility can not be excluded that the reaction arised from sulfur which was liberated from hydrogen sulfide in the reaction change. To convert I to V through II or III, alkaline hydrolysis of II and III was carried out and gave pyrazinecarboxylic acid (V) in yield 68.8 and 71%, respectively. The same treatments of the pyridine analogues (VII and VIII) gave picolinic acid (IX) in yield 66.9 and 73.3% respectively.

The activity of II against *M. tuberculosis* was examined in comparison with pyrazinecarboamide. The former was 4 times as effective as the latter and its toxity was LD_{50} 1180±15 mg./kg.[16] To test antitubercular activity, other N-alkyl derivatives of pyrazinecarbothionamide were synthesized on treatment of N-alkyl derivatives of pyrazinecarboamide with phosphor pentasulfide and potassium sulfide. Properties and yields of starting materials and products were shown in Tables II and III.

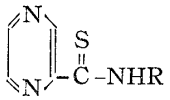
16) Tests by R. Inada, H. Takagi, Z. Hattori and I. Igarashi, Takamine Laboratory, Sankyo Co., Ltd.

Table II.



R	Appearance	m.p. (°C)	Yield (%)	Formula	Analysis (%)	Calcd.	Found
Methyl	colorless needles	105	71.3	known[17]			
Ethyl	〃	67	76.2	$C_7H_9ON_3$	C :	56.17	55.81
					H :	5.95	6.07
					N :	27.78	27.94
Propyl	〃	56	94.5	known[17]			
Isopropyl	〃	85.5	88.0	$C_8H_{11}ON_3$	C :	58.16	58.18
					H :	6.69	6.72
					N :	25.43	25.06
Butyl	〃	43	91.1	known[17]			
Isobutyl	colorless plates	61	97.5	$C_9H_{13}ON_3$	C :	60.32	60.25
					H :	7.24	7.30
					N :	23.43	23.22
Phenyl	〃	128	56.8	known[17]			

Table III.



R	Appearance	m.p. (°C)	Yield (%)	Formula	Analysis (%)	Calcd.	Found
Methyl	yellow plates	207	69.3	$C_6H_7N_3S$	C :	47.30	47.02
					H :	4.60	4.76
					N :	27.42	27.18
Ethyl	yellow needles	84	74.0	$C_7H_9N_3S$	C :	50.30	50.48
					H :	5.42	5.52
					N :	25.10	24.41
Propyl	〃	66	79.8	$C_8H_{11}N_3S$	C :	53.02	53.31
					H :	6.12	6.26
					N :	23.19	22.61
Isopropyl	yellow orange needles	79.5	72.2	$C_8H_{11}N_3S$	C :	53.02	52.67
					H :	6.12	6.05
					N :	23.19	22.81
Butyl	〃	61	94.9	$C_9H_{13}N_3S$	C :	55.29	54.99
					H :	6.69	6.59
					N :	21.60	21.14
Isobutyl	〃	62.5	79.5	$C_9H_{13}N_3S$	C :	55.29	55.74
					H :	6.69	6.74
					N :	21.60	21.28
Phenyl	reddish orange needles	139.5	62.0	$C_{11}H_9N_3S$	C :	61.37	61.47
					H :	4.17	4.32
					N :	19.51	19.27

Experimental[18]

The Simultaneous Formation of N,N-Dimethylpyrazinecarbothionamide (II) and N-Methylpyrazinecarbothionamide (III). General Procedure——The formation reaction was carried out in each of conditions described in Table I. The reaction mixture was filtered and the residue was extracted with boiling benzene for 2 hr. The filtrate and the benzene solution were combined and distilled to remove starting materials. The remainder was submitted to vac. distillation and the distillate at 140~150°/3 mm. was gathered. The distillate was dissolved in boiling benzene and fractionally concentrated to cause precipitations of crystalls. The first precipitate was recrystallized from EtOH to give yellow plates of m.p. 207°. A mixed m.p. with an authentic sample of III, which was prepared by a procedure described below, showed no depression. *Anal.* Calcd. for $C_6H_7N_3S$ (III) : C, 47.03; H, 4.60; N, 27.42.

17) O. Dalmer : U. S. P. 2, 149, 279 (1939).
18) All melting and boiling points were uncorrected.

Found : C, 47.02; H, 4.76; N, 27.18. The second precipitate was recrystallized from EtOH to give colorless needles of m.p. 207.5°. *Anal.* Calcd. for $C_{10}H_{10}N_4S$ (IV) : C, 55.02; H, 4.04; N, 25.67; mol. wt. 218.3. Found : C, 55.24; H, 3.40; N, 25.83; mol. wt. 221.5.

The remaining benzene solution was evaporated to dryness and the residue was recrystallized from H_2O to give yellow needles of m.p. 67°. *Anal.* Calcd. for $C_7H_9N_3S$ (II) : C, 50.28; H, 5.42; N, 25.13. Found : C, 50.17; H, 5.54; N, 25.17.

Yields of all products and recovery of I were shown in Table I.

N-Methylpyrazinecarbothionamide (III)——A mixture of I (9.6 g.), methylformamide (9.2 g.), sulfur (9.6 g.) and iodine (0.9 g.) was heated in an oil bath of 170~180° for 55 hr. Afterward the treatment was worked up just like that of the foregoing item to give III, yield 14.4%.

The Simultaneous Formation of N,N-Dimethylpicolinethionamide (VII) and N-Methylpicolinethionamide (VIII)——(a) A mixture of 2-picoline (VI) (93 g.), dimethylformamide (73 g.), and sulfur (128 g.) was heated in an oil bath of 160~170° for 50 hr. From the reaction mixture, 15.5 g. of VI and 8.4 g. of dimethylformamide were recovered by distillation. The remainder was extracted with boiling EtOH and evaporated to dryness. Vac. distillation of the extract gave orange yellow distillate of $b.p_{34\sim42}$ 120 ~210°. The distillate was dissolved in benzene and partitioned to two fractions by alumina chromatography (200~300 mesh, developer : benzene). The first fraction was evaporated to dryness and the residue was distilled under diminished pressure to give sticky and orange yellow liquor, $b.p_7$ 167°, Yield 44 g. (26.5%). Picrate, m.p. 128.5~129.5°. *Anal.* Calcd. for $C_{14}H_{13}O_7N_5S$ (VII·picrate) : C, 42.53; H, 3.31; N, 17.72. Found : C, 42.51; H, 3.15; N, 17.65. The second fraction was evaporated to dryness to leave yellow crystals which were recrystallized from EtOH, 20 g. (13.2%) of yellow needles, m.p. 79°. A mixed m.p. with an authentic sample of VIII, which preparation was described below, showed no depression. *Anal.* Calcd. for $C_7H_8N_2S$ (VIII) : C, 55.23; H, 5.29; N, 18.41. Found : C, 55.30; H, 5.37; N, 18.26. (b) When 0.06 mol. of iodine per mol. of VI was additionally used, it caused to save reaction time (14 hr.) and to increase yield of VII (36.4%).

N-Methylpicolinethionamide (VIII)——A mixture of 2-picoline (9.3 g.), methylformamide (5.8 g.), sulfur (9.6 g.) and iodine (0.1 g.) was treated exactly as heading (a) of the foregoing item to give yellow needles, m.p. 79°, yield 5.25 g. (34.4%). *Anal.* Calcd. for $C_7H_8N_2S$: C, 55.23; H, 5.29; N, 18.41. Found : C, 55.77; H, 5.37; N, 18.26.

Pyrazinecarboxylic acid (V)——(a) From II. To 3 mol. of 4*N* NaOH containing a small amount of EtOH was added 0.33 g. of II and heated on a water bath while stirring to go into a clear solution. Gaseous dimethylamine evolving during the reaction was introduced to an aqueous solution of HCl and identified. The alkaline solution was neutralized with 4*N* HNO_3 and then made strongly acidic with conc. HNO_3. The treatment caused the evolution of H_2S and the precipitation of colorless needles, which were recrystallized from H_2O with use of charcoal, m.p. and a mixed m.p. with an authentic sample of V 229°(decomp.), yield 0.17 g. (68.8%). *Anal.* Calcd. for $C_5H_4O_2N_2$ (V) : C, 48.39; H, 3.24; N, 22.58. Found : C, 47.97; H, 3.56; N, 22.61. (b) From III. III was treated just as heading (a) to give V, yield 0.20 g. (71%) from 0.32 g. of the starting material (III).

Picolinic acid (IX)——(a) From VII. Alkaline hydrolysis of VII was carried out just like the case of II. The solution was made strongly acidic with HCl, evaporated to dryness, extracted with warm MeOH and filtered. The filtrate was concentrated to a small volume. To the concentrated solution was added Me_2CO in drops to cause the precipitation of colorless needles, yield 0.95 g. (66.9%), m.p. and a mixed m.p. with an authentic sample of (IX·HCl) 216~217°. *Anal.* Calcd. for $C_6H_6O_2NCl$ (IX·HCl) : C, 45.15; H, 3.79; N, 8.77. Found : C, 45.03; H, 3.98; N, 8.87. (b) From VIII. VIII was treated exactly as (a) to give (IX·HCl), m.p. 216~217°, yield 73.3%.

Demethylation reaction. (a) N-Methylpyrazinecarbothionamide (III) from N,N-Dimethylpyrazinecarbothionamide (II)——To 1.68 g. of II was added 1.28 g. of sulfur and the mixture was heated in an oil bath. When temperature of the heating oil reached to about 240°, H_2S began to evolve violently. Then temperature was kept at 180~200° for 4 hr. After the evolution of the gas decayed at the end of the period, the reaction mass was extracted with boiling EtOH for 2 hr. The ethanolic solution was decolored with charcoal and concentrated to 10 ml. to deposit yellow crystals. Filtration followed by recrystallization gave yellow plates, yield 0.07 g. (4.3%), m.p. and a mixed melting point at 207° with an authentic sample of III.

(b) N-Methylpyridinecarbothionamide (VIII) from N,N-Dimethylpyridinecarbothionamide (VII)——Fusion of VII with sulfur was worked up just like (a). The reaction mixture was extracted with $CHCl_3$, filtered and purified by alumina chromatography (300 mesh, developer : $CHCl_3$). After removal of $CHCl_3$ repeated recrystallizations gave yellow needles, yield 20%, m.p. and a mixed melting point with an authentic sample of VIII 74~78°.

N-Alkylpyrazinecarbonamide——To 1 mol. of methyl pyrazinecarboxylate dissolved in 11 mol. of AcOEt was added 2 mol. of monoalkylamine and allowed to stand at room temperature for 24 hr. After removal of AcOEt and monoalkylamine, remaining crystalls were recrystallized from ligroin. Results are shown in Table II.

N-Alkylpyrazinecarbothionamide——To a solution of 1 mol. of N-alkylpyrazinecarboamide in 1.8 mol. of xylene was added 0.23 mol. of phosphor pentasulfide and 0.71 mol. of dry K_2S at 100° with violent agitation. Heating was continued for 5 hr. while stirring and the reaction mixture was immediately filtered. The residue was extracted with boiling benzene. The benzene solution was combined with the filtrate and evaporated to dryness. The remainder was several times recrystallized from EtOH. Results are shown in Table III.

This work was supported in part by the Grant-in-Aid for Scientific Research from the Ministry of Education, which the authors gratefully acknowledged. Thanks are also due to the Analytical Section of this Institute for the determinations of infrared spectra and for microanalyses and to Miss T. Hata for her technical assistance.

Summary

Methylpyrazine (I) and 2-picoline (VI) were converted to N,N-dimethylpyrazinecarbothionamide and N,N-dimethylpicolinethionamide (II and VII) respectively on fusion with sulfur in dimethylformamide. Iodine was found effective as a catalyst for the reaction. Besides the ordinal products (II and VII), the corresponding demethylated products were also isolated, thus indicating that the demethylation reaction, presumably due to the action of sulfur, was involved. The demethylation reaction was confirmed by the formation of N-methylpyrazinecarbothionamide and N-methylpicolinethionamide (III and VIII) from II and VII respectively on fusion with sulfur. II was 4 times more effective than pyrazinecarboamide against *M. tuberculosis* and its toxity was LD_{50} 1180± 15 mg./kg.

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78. **Morio Ikehara and Eiko Ohtsuka** : Studies on Coenzyme Analogs. XIV.*1 A New Phosphorylating Agent, Morpholinophosphorodichloridate.

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The recent progress in the field of nucleotide chemistry is due, at least partly, to the development of phosphorylating agent by many investigators.[1] In the authors' laboratory, various types of nucleotides were synthesized in order to elucidate the mechanism of action of several enzymes with their substrates. Analogs of nucleoside 5′-mono- and -triphosphate were synthesized from various nucleoside analogs as the substrate of snake venom 5′-nucleotidase[2,3] and myosin ATPase.[4,5] The protecting group of phosphorylating agent utilized for nucleotide synthesis in the past were removed either by hydrogenolysis or by alkaline hydrolysis. In order to phosphorylate

*1 Part XIII. M. Ikehara, A. Yamazaki, T. Fujieda : This Bulletin, **10**, 1075 (1962).

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