UDC 615.782-092.21

76. Hisao Tsukamoto, Hidetoshi Yoshimura, Hiroyuki Ide, and Shoin Mitsui: Metabolism of Drugs. XXXVI.*1 The Metabolic Fate of Thiamylal [5-Allyl-5-(1-methylbutyl)-2-thiobarbituric Acid]. (2).[1]

(*Institute of Pharmaceutical Sciences, Faculty of Medicine, Kyushu University**2)

None is known about the metabolic fate of thiamylal (5-allyl-5-(1-methylbutyl)-2-thiobarbituric acid) except the *in vitro* study using minced liver of a rat by Spector and Shideman,[2] although the fate of thiopental (5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid) was well established by several workers.[3]

It has been already shown in the previous paper[1] of this series that a carboxylic acid derivative was isolated as major metabolite from the urine of rabbits administered thiamylal and in addition, existence of many other metabolites were also observed by paper chromatographic analysis.

This paper presents isolation, characterization, and identification of these metabolites, and also discuss on its possible *in vivo* pathways.

Methods and Results

Isolation of Metabolites from the Urine of Rabbits Administered Thiamylal——Administration of the drug (totally given 30.6 g.) and extraction of the urine with AcOEt were performed as reported in the previous paper.[1] It was divided into two fractions, one of which was insoluble in sat. $NaHCO_3$ (T^a-fraction) and the other soluble in it (T^b-fraction).

1) **T^a-fraction**——This (6 g.) was dissolved in benzene and chromatographed through an alumina column (80 g.), in which effluents were fractionated by stepwise elution using the solvents of benzene, 0.5% Me_2CO in benzene, and 1% Me_2CO in benzene. Metabolite 1 which showed Rf value at 0.83~0.85 by the paper chromatography using the solvent system of BuOH, EtOH, conc. NH_4OH (4 : 2 : 1.2 v/v),*3 was first eluted with benzene, but it was failed to crystallize. After ending the elution of this metabolite, metabolite 2 (Rf 0.80~0.81)*3 came out still with benzene or with 0.5% Me_2CO in benzene. It was recrystallized from AcOEt to 1.15 g. of colorless crystals, m.p. 132°. The last fraction eluted with 1% Me_2CO in benzene (920 mg.) was seemed to be a mixture of metabolite 3 (Rf 0.72~0.73),*3 metabolite 4 (Rf 0.69~0.70),*3 and metabolite 5 (Rf 0.62~0.63),*3 considering from paper chromatographical results*3 and so, rechromatographed through a silica gel*4 column (20 g.), using the solvents, $CHCl_3$, $CHCl_3$-MeOH (49 : 1), and $CHCl_3$-MeOH (19 : 1) by stepwise elution. Metabolite 3 was first eluted with $CHCl_3$ and recrystallized from 30% aq. EtOH to colorless crystals, m.p. 99° (287 mg.). The second fraction, metabolite 4 which was eluted with $CHCl_3$-MeOH (49 : 1), was further purified by dissolving in *N* NaOH, reacidification, extraction with AcOEt, and treatment with charcoal to crystalline compound. It was recrystallized from hydrated AcOEt to 52 mg. of colorless crystals, m.p. 175°. $[\alpha]_D^{15}$ +119.3° (c=1.0, in EtOH). The last fraction, metabolite 5 was eluted with $CHCl_3$-MeOH (19 : 1) and recrystallized from AcOEt to 3 mg. of colorless crystals, m.p. 167°.

2) **T^b-fraction**——This fraction (5 g.) was dissolved in AcOEt and extracted again with sat. $NaHCO_3$ solution. The $NaHCO_3$ layer was acidified to pH 2 with conc. HCl. The solution was extracted with AcOEt, dried over Na_2SO_4, evaporated the solvent, and then chromatographed through a silica gel column (100 g.) using $CHCl_3$-MeOH (19 : 1), $CHCl_3$-MeOH (4 : 1), and $CHCl_3$-MeOH (1 : 1) as the effluent

*1 Part XXXV. H. Tsukamoto, H. Yoshimura, K. Tatsumi : This Bulletin, **11**, 421 (1963).
*2 Katakasu Fukuoka (塚元久雄, 吉村英敏, 井出博之, 三井勝允).
*3 Paper chromatographic study on these metabolites was described in reference 1.
*4 Silica gel (For chromatographic use, Kanto Chem. Co., Ltd.) was used.

1) H. Tsukamoto, H. Ide, E. Takabatake : This Bulletin, **8**, 236 (1960).
2) E. Spector, F.E. Shideman : J. Pharmacol. Exptl. Therap., **116**, 54 (1956), Biochem. Pharmacol., **2**, 182 (1959).
3) B.B. Brodie, *et al.* : J. Pharmacol. Exptl. Therap., **98**, 85 (1950); H.B. Wood, Jr., E.C. Horning : J. Am. Chem. Soc., **75**, 5511 (1953); J.R. Cooper, B.B. Brodie : J. Pharmacol. Exptl. Therap., **120**, 75 (1957).

solvents. Metabolite 7 (Rf 0.22～0.23)*3 was eluted first with $CHCl_3$–MeOH (19 : 1), which was recrystallized from hydrated AcOEt to 2.31 g. of colorless crystals, m.p. 158°. The next fraction, metabolite 8 (Rf 0.14～0.15),*3 eluted with $CHCl_3$–MeOH (4 : 1), was recrystallized from AcOEt to colorless crystals, m.p. 197° (26 mg.). The last fraction, metabolite 6 was eluted with $CHCl_3$–MeOH (1 : 1), which showed single spot at Rf 0.27～0.30 with (Mn)-reagent,*3 but failed to crystallize.

Characterization and Identification of Metabolites——1) Metabolite 1. It was failed to crystallize, but proved its existence with paper chromatography. It did not show any barbiturates color reaction, but indicated the possession of an double bond probably of allyl group with (Mn)-reagent. The Rf value was also quite reasonable for the acetylurea derivative but no further studies on this metabolite was undertaken.

2) Metabolite 2. It was identified with unchanged thiamylal by mixed m.p. test, paper chromatography, and IR spectrum.

3) Metabolite 3. It was identified with secobarbital (5-allyl-5-(1-methylbutyl)barbituric acid) by mixed m.p. test and IR spectrum.

4) Metabolite 4. The elemental analysis of this metabolite agreed with a hydroxythiamylal. *Anal.* Calcd. for $C_{12}H_{18}N_2O_3S$: C, 53.3; H, 6.67; N, 10.37. Found : C, 53.49; H, 6.70; N, 10.08. UV $\lambda_{max}^{Borate\ Buffer\ pH\ 10}$ 253, 305 mμ, and IR λ_{max}^{Nujol} μ : 2.81 (ν_{OH}); 3.12, 3.15 (ν_{NH}); 5.74, 5.87 ($\nu_{C=O}$); 6.07 ($\nu_{C=C}$); 10.22, 10.64 ($\delta_{CH=CH_2}$) indicated that this metabolite should be a hydroxythiamylal, in which one of the alkyl side chains would be oxidized to hydroxy alkyl group. The position of this hydroxy group was finally concluded from the fact that this metabolite produced a crystalline CH_3I, m.p. 117° by heating with 3*N* NaOH and I_2–KI solution. The structure of this metabolite should therefore be 5-allyl-5-(1-methyl-3-hydroxybutyl)-2-thiobarbituric acid.

5) Metabolite 5. The structure of this metabolite was established as 5-allyl-5-(1-methyl-3-hydroxybutyl)barbituric acid, which was also isolated from the urine extract of rabbits receiving secobarbital,[4] by the mixed melting point test and infrared spectrum.

6) Metabolite 6. This metabolite was also isolated from the urine of rabbit administered with metabolite 7, and the structure was presumed to be a ring-destroyed metabolite, 3-carboxypropyl allyl acetylthioureid as mentioned below.

7) Metabolite 7. This metabolite was identical with thiamylalcarboxylic acid which was described in the previous paper,[1] but the structure was not yet sufficiently clarified at that time. Attempt to convert this metabolite to secobarbital analogue with desulfuration reaction, was carried out as follows : 100 mg. of this metabolite was dissolved in cooled 1.1 equiv. NaOH solution (10 cc.), and 5 cc. of 6% H_2O_2 was added dropwise under stirring (5 min.). The alkaline solution was acidified to pH 4 with dil. HCl, and extracted with AcOEt. 48 mg. of purified crystals was obtained after crystallization from hydrated AcOEt. This compound, m.p. 197°, was identified with 5-allyl-5-(1-methyl-3-carboxypropyl)-barbituric acid[4] by mixed melting point and infrared spectrum. Metabolite 7 should therefore be 5-allyl-5-(1-methyl-3-carboxypropyl)-2-thiobarbituric acid.

8) Metabolite 8. It was established as 5-allyl-5-(1-methyl-3-carboxypropyl)barbituric acid[4] by its Rf value, melting point test, and infrared spectrum.

The Fate of Metabolite 7——It (540 mg.) was administered to the rabbits and treated with the same way as described above. About 400 mg. of urine extract was chromatographed through a silica gel column (2 g.) according to analogous method with the separation of T^b-fraction. As a result, most of the administered drug (346 mg.) was recovered unchanged from $CHCl_3$–MeOH (19 : 1) fraction of silica gel chromatography and only a trace of secobarbital carboxylic acid, which was detected by paper chromatography, was obtained. From $CHCl_3$–MeOH (1 : 1) effluent, 3 mg. of colorless needles, m.p. 110°, was obtained. Rf value of this compound on the paper chromatogram was 0.27～0.30 and identified with metabolite 6 by mixed paper chromatography. It did not show any barbiturates color reaction, but indicated the existence of a double bond with (Mn)-reagent and also of thioureid system with its ultraviolet spectrum UV λ_{max} mμ : 237, 292 (Borate buffer pH 10); 276 (EtOH).

From these results, this compound was presumed as ring-destroyed metabolite of thiamylal carboxylic acid, probably as 3-carboxypropyl allyl acetyl thioureid.

Discussion

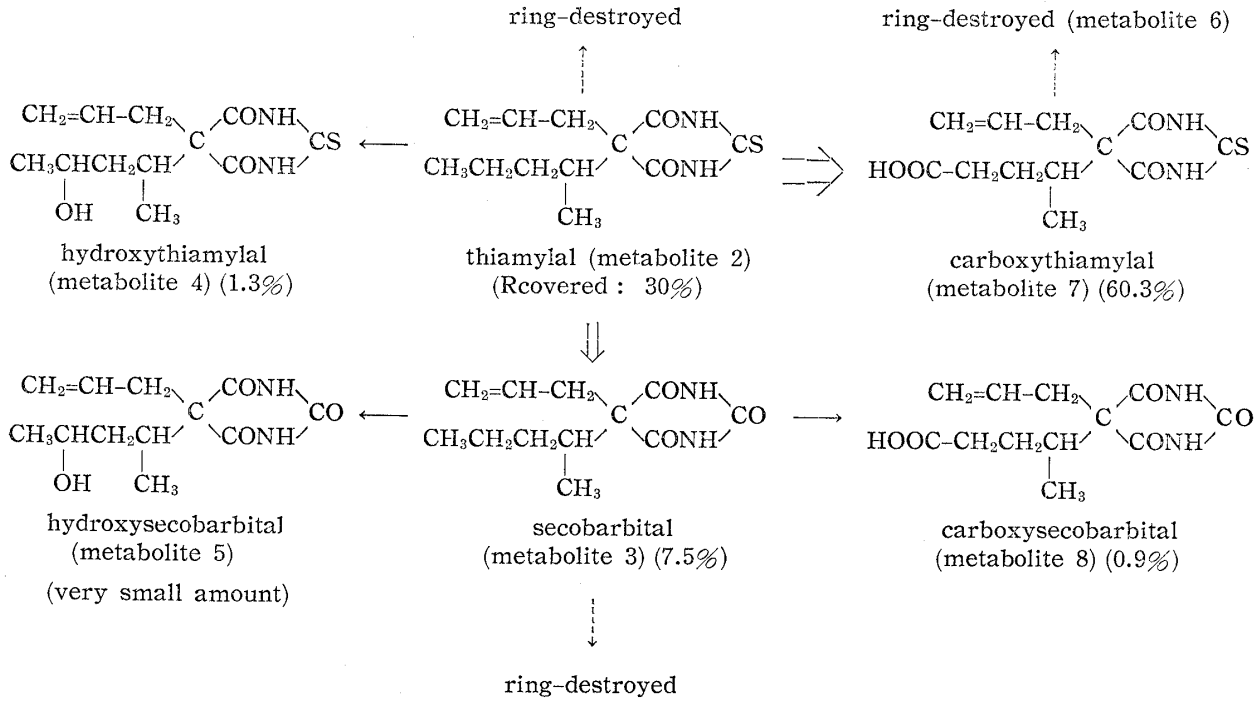
Spector and Shideman[2] reported the *in vitro* removal of sulfur atom from thiamylal by minced liver of a rat, producing secobarbital.

On the other hand, in this *in vivo* study using rabbits, thiamylal ω-carboxylic acid was isolated as a main metabolite, and as a minor product, (ω-1)-hydroxy compound

4) H. Tsukamoto, H. Yoshimura, H. Ide : This Bulletin, **11**, 9 (1963),

was isolated. There were also obtained another metabolites series as minor products, which were desulfurated to O-barbiturate. They were secobarbital, secobarbital ω-carboxylic acid and (ω-1)-hydroxy secobarbital. Moreover, some ring-destroyed metabolites could be observed by paper chromatography.

Considering from the amounts of these metabolites isolated here, and other results, the primary metabolites should be thiamylal ω-carboxylic acid, secobarbital, and (ω-1)-hydroxythiamylal. The possible pathways might be drawn as shown in Chart 1.



$$\% : \frac{\text{Amount of each metabolite}}{\text{Amount of all the isolated metabolites}} \times 100$$

* The thickness of arrows indicates a rough excretion rate of each metabolite and broken lines show a very little contribution to the metabolism.

Chart 1. The Possible Metabolic Pathways of Thiamylal in Rabbits

In order to confirm the secondary pathway, main metabolite of thiamylal, thiamylal ω-carboxylic acid was administered to rabbits and examined whether desulfuration would take place in this compound or not. As expected before, almost of thiamylal ω-carboxylic acid was recovered unchanged from the rabbits urine, and only a trace of secobarbital ω-carboxylic acid was isolated. There was another evidence, as reported in the previous paper,[4] that secobarbital was oxidized mainly to its ω-carboxylic acid. It could be therefore concluded that secobarbital ω-carboxylic acid was formed predominantly through primarily produced secobarbital in thiamylal metabolism.

From the present study, it is apparent that the metabolic fate of thiamylal was consist of four different pathways. The first one is ω-oxidation of side chain which was predominant in every case of thiobarbiturates and the next two are desulfuration reaction and (ω-1)-oxidation of side chain. To the last one, the authors may add ring rupture reaction.

The authers are deeply grateful to Mrs. S. Matsuba for elemental analyses, to Messers. H. Matsui and K. Hikita for the determination of IR spectra, and also to Yoshitomi Pharmaceutical Co., Ltd. for their supply of thiamylal and secobarbital.

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.

(Received June 15, 1962)

UDC 547.298.4'861

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

(*Institute of Pharmaceutical Sciences, Faculty of Medicine, Kyushu University*[*1])

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).

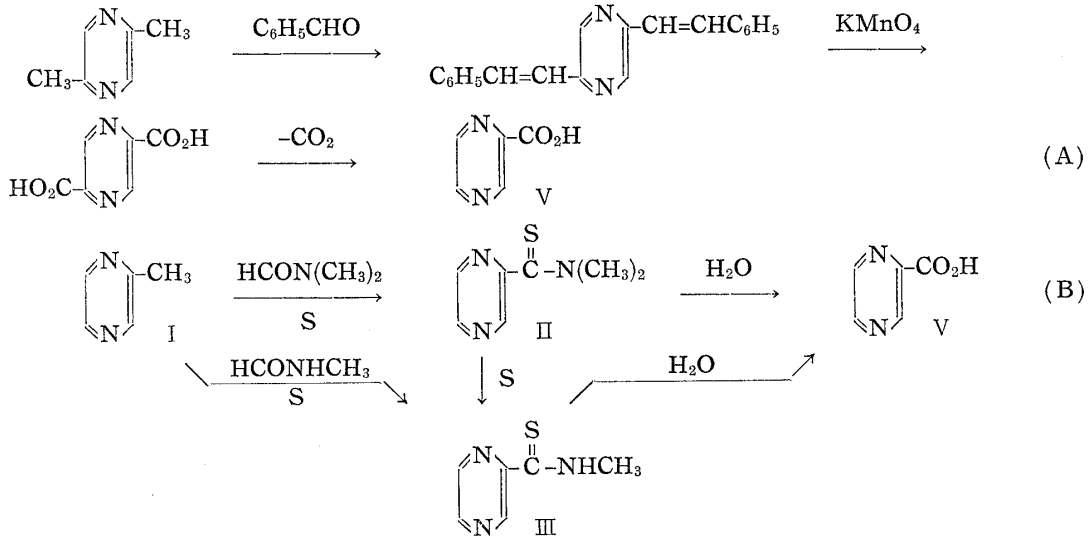


Chart 1.

*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).