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THE METABOLISM OF THE OCTAMETHYLENE-1,8-DITHIOL MOIETY OF *BIS*(*O*-ACETYLTHIAMINE)OCTAMETHYLENE-1,8-DISULFIDE IN RAT AND MAN*

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Abstract—Metabolic fate and urinary metabolites of the octamethylene-1,8-dithiol moiety of *bis*(*O*-acetylthiamine)-octamethylene-1,8-disulfide (BTOD) have been studied in rat and man. Following the i.v. administration of ^{35}S-labeled BTOD, the rat excreted about 75 per cent of the administered radioactivity in urine and 4 per cent in feces within 2 days. Upon the oral or i.p. administration, the rat excreted 44–50 per cent in urine and 35–45 per cent in feces. No significant radioactivity was found in the respiratory gas in either cases. Three metabolites were isolated by silicic acid chromatography from urine obtained after oral administration of the compound. The spectrometric data (infrared and nuclear magnetic resonance) demonstrated that their chemical structures were 1,8-*bis* (methylsulfinyl)octane, 1-methylsulfinyl-8-methylsulfonyloctane and 1,8-*bis*(methylsulfonyl)octane, respectively. The identity of these metabolites was conclusively established by comparison studies with authentic compounds which were chemically synthesized. These three metabolites were also recognized in the human urine after oral administration of nonlabeled BTOD. The excretion pattern of the radioactivity following the oral administration of ^{35}S-labeled octamethylene-1,8-dithiol was very similar to that obtained with BTOD. Identical metabolites were characterized in urine after i.p. administration of the dithiol. The present studies show that the sulfur in the compounds is metabolized solely by the methylsulfonyl pathway and conversion to inorganic sulfate does not occur in any appreciable amount.

BIS(*O*-ACETYLTHIAMINE)OCTAMETHYLENE-1,8-disulfide (BTOD)† is one of the new derivatives of thiol type thiamines synthesized by Hagiwara *et al.*[1] The compound exerts not only the vitamin activity equivalent to thiamine in rat growth assay but also it is better absorbed by the human subjects and laboratory animals upon oral ingestion when compared with thiamine-HCl.‡ Further, our studies have confirmed that the compound is readily converted to thiamine when administered to intact animals or incubated with tissue homogenates.‡ The conversion involves nonenzymatic reduction of disulfide bond as well as enzymatic hydrolysis of *O*-acetyl ester

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† *Bis*-(*O*-acetylthiamine)octamethylene-1,8-disulfide is 1,8-*bis*[2-(2-methyl-4-aminopyrimidin-5-*yl*) methylformamido-5-acetyloxy-2-penten-3-*yl* dithio]-octane (Fig. 1), abbreviated BTOD. BTOD labeled with ^{35}S at the sulfur of the octamethylene-1,8-dithiol moiety is designated as BTOD-^{35}S (outer).

‡ Z. Suzuoki, T. Matsuo and K. Furuno, unpublished.

presumably by nonspecific esterases. (Fig. 1). The present report concerns the metabolism of the octamethylene-1,8-dithiol moiety of the compound in rats and man. Namely, we have shown the urine to be the main excretory route and characterized the urinary metabolites as the methylsulfinyl and methylsulfonyl compounds. These results add further evidence for the occurrence of a new type of biotransformation by which foreign alkyl mercaptans are converted to methylsulfonyl compounds.[2-4]

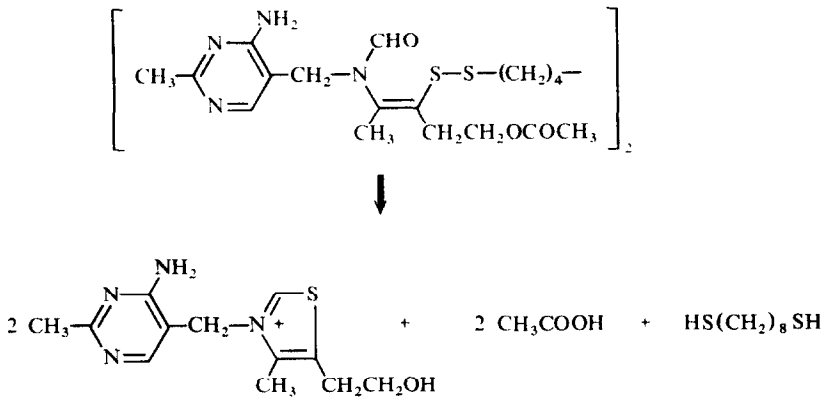

FIG. 1. Structural formula of BTOD and its conversion to thiamine in living matters.

MATERIALS AND METHODS

Preparation of bis(*O-acetylthiamine*)*octamethylene*-1,8-*disulfide*-^{35}S (*outer*) *and octamethylene*-1,8-*dithiol*-^{35}S

BTOD-^{35}S (outer) with the initial sp. act. of 527 μc/m-mole and octamethylene-1,8-dithiol-^{35}S with the initial sp. act. of 737 μc/m-mole were synthesized and supplied by Nishikawa and Toga, of the Radioisotope Laboratory, Chemical Research Laboratories of this Division. The chemical and radiochemical purity was checked by paper chromatography.

Synthesis of 1,8-bis(*methylthio*)*octane*, 1,8-bis(*methylsulfonyl*)*octane*, 1-*methylsulfinyl*-8-*methylsulfonyloctane and* 1,8-bis(*methylsulfinyl*)*octane*

1,8-*Bis*(methylsulfonyl)octane, 1-methylsulfinyl-8-methylsulfonyloctane and 1,8-*bis*(methylsulfinyl)octane are all new compounds and the authentic compounds have been synthesized starting with the known octane-1,8-dithiol[5] via the intermediate 1,8-*bis*(methylthio)octane as follows.

1,8-Bis(*methylthio*)*octane*. To a stirred and ice-cooled solution of octane-1,8-dithiol (9 g) dissolved in 10% aqueous sodium hydroxide (120 ml) was added dimethylsulfate (13 g) and the stirring was continued for 30 min. The solution was extracted with ethyl ether and the extract was washed with water, dried over anhydrous sodium sulfate, filtered and evaporated to yield yellowish oil (10·3 g) which was distilled *in vacuo* (20 mmHg) at 161–175° to result pure 1,8-*bis*(methylthio)octane (8·2 g, yield = 80%). (Anal. Calcd. for $C_{10}H_{22}S_2$: C, 58·19; H, 10·74. Found: C, 58·39; H, 10·55. NMR in $CDCl_3$ (τ): 7·52 (4H, triplet, two $-CH_2S$), 7·91 (6H, singlet, two CH_3S), 8·63 (12H, broad, $-(CH_2)_6-$).

1,8-Bis(*methylsulfonyl*)*octane*. To a stirred mixture of aqueous 30% hydrogen peroxide (11·3 ml) and formic acid (50 ml) was added 1,8-*bis*(methylthio)octane (2 g) at 60°. After 1 hr the solution was evaporated to yield white solids (2·6 g, yield = 81%)

of 1,8-*bis*(methylsulfonyl)octane which after recrystallization from ethanol exhibited m.p. 126°. (Anal. Calcd. for $C_{10}H_{22}O_4S_2$: C, 44·42; H, 8·20; S, 23·72. Found: C, 44·56; H, 7·98; S, 23·82. NMR in $CDCl_3$ (τ): 6·97 (4H, triplet, two -CH_2SO_2), 7·11 (6H, singlet, two CH_3SO_2), 8·59 (12H, broad, —$(CH_2)_6$—. IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 1135 and 1310 (sulfone).

1-*Methylsulfinyl*-8-*methylsulfonyloctane and* 1,8-bis(*methylsulfinyl*)*octane.* To a stirred and ice-cooled solution of 1,8-*bis*(methylthio)-octane (2 g) in acetic acid (20 ml) was added aqueous 30% hydrogen peroxide (2·5 g) and the stirring and cooling was continued for 1 hr and the mixture was allowed to stand overnight at room temperature. The solution was evaporated *in vacuo* and to the residue water (20 ml) was added and the mixture was made alkaline with the addition of solid sodium bicarbonate. The mixture was extracted with chloroform and the extract was washed, dried, filtered and evaporated to yield 1·8 g of white solids. Column chromatography on 75 g silica gel packed in the column (2·5 × 30 cm) using 50% ethyl acetate—acetone as a development solvent gave first 0·2 g of 1-methylsulfinyl-8-methyl sulfonyloctane, m.p. 79–81° (Anal. Calcd. for $C_{10}H_{22}O_3S_2$: C, 47·22; H, 8·72; O, 18·87; S, 25·21. Found: C, 47·28; H, 8·47; O, 19·14; S, 24·01. NMR in $CDCl_3$ (τ): 6·97 (2H, triplet, CH_2SO_2), 7·11 (3H, singlet, CH_3SO_2), 7·30 (2H, triplet, —CH_2SO), 7·43 (3H, singlet, CH_3SO), 8·59 (12H, broad, —$(CH_2)_6$—). IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 1030 (sulfoxide), 1135 and 1310 (sulfone) and second 1·1 g of 1,8-*bis*(methylsulfinyl)octane, m.p. 95–100° (Anal. Calcd. for $C_{10}H_{22}O_2S_2$: C, 50·73; H, 9·43; S, 26·89. Found: C, 50·58; H, 8·26; S, 26·78. NMR in $CDCl_3$ (τ): 7·30 (4H, triplet, two-CH_2SO), 7·43 (6H, singlet, two CH_3SO), 8·59 (12H, broad, —$(CH_2)_6$—). IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 1030 (sulfoxide).

Administration of the compounds and collection of the excreta

Male Sprague–Dawley rats, weighing about 200 g, were maintained on usual laboratory chow and after administration of the compounds the rats were kept individually in an all glass metabolism cage.

Experiments 1 and 2. To ascertain the excretory route, three rats were administered the HCl salt of BTOD-^{35}S (outer) (1·43 × 10^6 cpm equivalent to 2·5 mg/rat) in physiological saline (1 ml) i.v., i.p. or orally, respectively (Experiment 1). Larger amounts of the compound (4·04 × 10^6 cpm equivalent to 86 mg/rat) were orally administered to two rats in Experiment 2. The trapping system for possible volatile metabolites expired into the respiratory gas consisted of 4 washing bottles, of which two contained 50 ml of 1 N NaOH, followed by two bottles containing 50 ml of 1 N HCl. Urine and feces were collected at 12, 24, 48 and 72 hr after drug administration.

Experiment 3. For the isolation and identification of urinary metabolites, 24 rats were given the HCl salt of BTOD-^{35}S (outer) (30,100 cpm equivalent to 80 mg/rat) orally by stomach tube 4 times every other day. Urine was collected only for the first 24 hr after each administration and all the urine samples were combined for subsequent experiments.

Experiment 4. For time course studies on metabolites composition, a rat was orally given the HCl salt of BTOD-^{35}S (outer) (2·14 × 10^6 cpm equivalent to 80 mg/rat) and urine was collected at 3, 6, 24 and 48 hr after administration of the compound.

Experiment 5. Two rats were orally given octamethylene-1,8-dithiol-^{35}S (1·93 × 10^6 cpm equivalent to 0·5 mg/rat). Urine and feces were collected at 12, 24, 48 and 72 hr. Respiratory gas was trapped during the first 24 hr as described above.

Experiment 6. For the identification of urinary metabolites, a rat was i.p. injected octamethylene-1,8-dithiol-^{35}S ($1{\cdot}92 \times 10^6$ cpm equivalent to 0·5 mg) and the first 24 hr urine was collected.

Experiment 7. Three healthy adult men were orally given nonlabeled BTOD (500 mg/man) and the first 8 hr urine was collected and combined (2265 ml).

Chemical procedures for isolation and determination

Silicic acid column chromatography. Silicic acid column was prepared according to the method of Hanahan *et al.*[6] The solvent systems used for elution are demonstrated in Figs. 2 and 7 or Table 3.

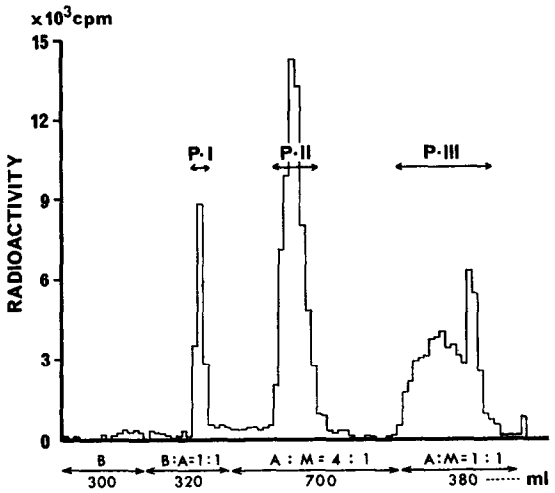

FIG. 2. Silicic acid column chromatography of the extract of urine obtained after administration of BTOD-^{35}S (outer) (Exp. 3).
Sample: $5{\cdot}9 \times 10^4$ cpm (3·69 g); Silicic acid-Hyflo Super Cel (2:1, w/w), 90 g; Column size 4 × 20 cm; Elution solvents: B = benzene, A = acetone, M = methanol.

Thin layer chromatography (TLC). Silica gel G thin layer plates ($7{\cdot}5 \times 7{\cdot}5$ or 20×20 cm) were prepared by the method of Stahl.[7] The solvent systems used are the mixtures of benzene-acetone-methanol (5:5:1, 3:3:1, 2:2:1 or 1:1:0, v/v). Compounds on chromatograms were detected by the iodine vapor method[8] and the radioactive compounds were detected as described below. The typical result is shown in Fig. 5.

Measurement of radioactivity. Radioactivity was counted in a liquid scintillation spectrometer, Packard TriCarb, Model 3000. Corrections were made, when necessary, for quenching, counting efficiency or natural decay. Urine aliquots (usually 0·1 or less) were placed in vials containing 10 ml of scintillation mix having the following formulation: 2,5-diphenyloxazole (PPO), 3 g; 1,4-*bis*[2-(5-phenyloxazolyl)]benzene (POPOP), 150 mg; naphthalene, 60 g; dioxane, 1000 ml and ethylcellusolve, 200 ml.

The radioactive sulfur in feces was determined by the modified method of Pirie.[9] Namely, an aliquot (about 500 mg) of finely ground feces with the small amounts of anhydrous sodium sulfate was placed in a beaker (100–200 ml) and were added

10 vol. of concentrated nitric acid—30% hydrogen peroxide mixture (3:1, v/v). The mixture was heated at 130° in order to decompose the organic substances completely. The procedure was repeated until the solution became clear and colorless. After evaporation of the solvent, the residue was dissolved in 10–20 ml of distilled water, and its aliquots were placed in vials and counted as described above. The recovery of the radioactivity added before the digestion was 86 ± 2 (S.D.)% for four fecal samples.

Radioactivity present on TLC plates was determined by scraping off the silica gel powder, successively, by 0·5 or 1·0 cm width, placing it in vial and counting by liquid scintillation technique. The recovery of the radioactivity from the chromatoplates usually exceeded 90 per cent and the error was less than 5 per cent. A typical result was illustrated in Fig. 5.

Determination of thiamine. Thiamine was determined by thiochrome method.[10] BTOD was determined as *O*-acetyl thiamine by the thiochrome method subsequent to the reduction. The complete reduction of BTOD to *O*-acetyl thiamine was achieved by adding 1 mg of cysteine.HCl to 1 ml of 0·2 M acetate buffer (pH 5·8) containing less than 10 μg test compound and allowing to stand at 60° for 30 min.

Instrumental analysis. The infrared spectra (IR) were measured in chloroform by Hitachi, Model EPI-S, spectrometer. The nuclear magnetic resonance (NMR) spectra were measured in $CDCl_3$ using $(CH_3)_4Si$ as an internal reference by Varian A 60 nuclear magnetic resonance spectrometer. The optical rotation measurement was performed in methanol by the polarimeter (Franz Schmidt & Haensch).

RESULTS

Fate of BTOD-^{35}S *(outer) in rats*

Following an i.v. dose of the compound, about 75 per cent of the administered radioactivity was excreted into the 48 hr urine, almost all within the first 12 hr, while only 4 per cent was found in the 48 hr feces (Table 1). When the compound was administered orally (2·5 or 86 mg/rat) or i.p., 44–50 per cent of the radioactivity was found in urine and 35–45 per cent in feces within the first 48 hr (Experiment 1 and 2). Any significant amount of the radioactivity was found in neither trapping solutions for respiratory gas.

Separation and identification of urinary metabolites of BTOD in rats

The combined sample of the urine obtained from Experiment 3 contained 39 per cent of the radioactivity administered. The sample was then saturated with sodium chloride and extracted 4 times with 3 vol. of chloroform. Ninety-seven per cent of the radioactivity was extracted into the solvent. The extract was then fractionated on the silicic acid column and the radioactivity was separated into three fractions, P-I, P-II and P-III (Fig. 2). The recovery of the radioactivity was 90 per cent in the chromatographic run. Thin layer chromatographic examination (benzene-acetone-methanol, 5:5:1, v/v) revealed that the fractions P-I, P-II and P-III gave distinct single radioactive spots, having $R_f = 0{\cdot}79$, 0·46 and 0·21, respectively. The white metabolites with m.p. 125–126° crystallized out with methanol from fraction P-I and with m.p. 113–116° with benzene from fraction P-III, respectively. The fraction P-II was further subjected to rechromatography on the silicic acid column and the radioactivity was eluted as a single radioactive peak with the solvent mixture of benzene-acetone-methanol (2:2:1, v/v). After evaporation of the solvent, the resulted solids were

TABLE 1. EXCRETION OF RADIOACTIVITY BY THE RAT AFTER ADMINISTRATION OF BTOD-^{35}S (OUTER) OR OCTAMETHYLENE-1,8-DITHIOL-^{35}S

Experiment	Exp. 1*						Exp. 2*		Exp. 5*	
Compound	BTOD-^{35}S (outer)						BTOD-^{35}S (outer)		Octamethylene-1,8-dithiol-^{35}S	
Dose (mg/rat)	2·5		2·5		2·5		86		0·5	
Route of admin.	i.v.		i.p.		p.o.		p.o.		p.o.	
Excretion (% of dose)	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
hr										
0–12	70·4	3·3	39·8	6·8	37·0	22·1	23·2 20·9	4·3 0·1	— 35·0	0 0
12–24	3·2	0·8	8·1	18·3	8·9	15·2	16·6 17·4	24·1 20·0	56·7† 8·3	39·3 35·6
24–48	1·7	0·1	2·0	16·9	2·3	7·9	4·2 6·1	6·3 18·7	0·7 1·0	6·9 1·4
48–72	0·7	0	0·3	0·4	0·3	0·2	— —	— —	0·2 0·1	0 0
Total	76·0	4·2	50·2	42·4	48·5	45·4	44·0 44·4	34·7 38·8	57·6 44·4	46·2 37·0

* Experiment 1 was made in a rat per dose and Exp. 2 and 5 were made in two rats, respectively.
† 0–24 hr urine.

analyzed as metabolite P-II. The IR spectra for three metabolites are shown in Fig. 3. The strong absorption peaks at 1136 and 1310 cm^{-1} characteristic to the sulfone group were shown in metabolites P-I and P-II. The strong absorption band at 1030 cm^{-1} corresponding to that of the sulfoxide group was recognized in metabolites P-II and P-III but not in P-I. Furthermore, the bands at 2780–2800 cm^{-1} due to the methylene

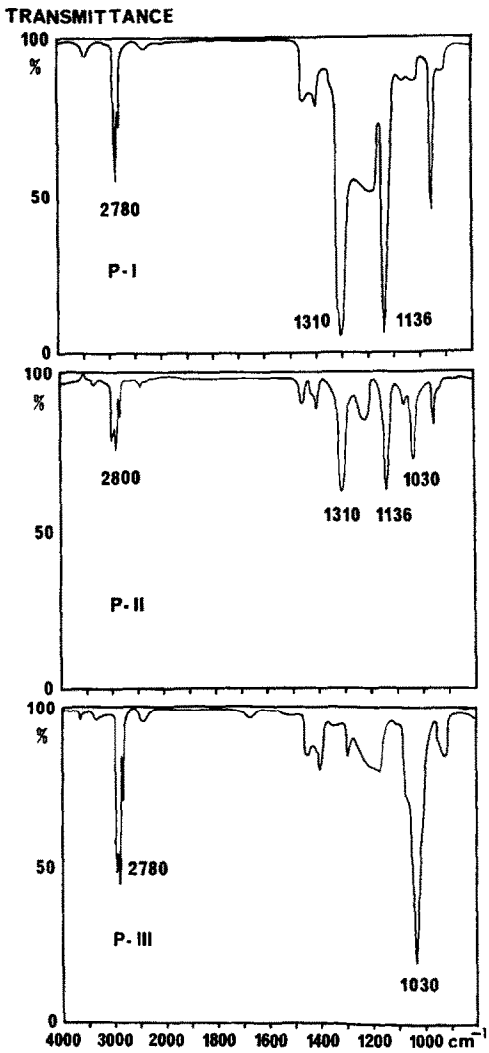

FIG. 3. Infrared spectra of metabolites P-I, P-II and P-III. Measured in $CHCl_3$.

group were shown in three metabolites. The NMR spectra of these metabolites are shown in Fig. 4. Metabolites P-I and P-II showed the peaks due to the protons in methylsulfonyl group, while metabolites P-II and P-III gave the peaks due to the protons in methylsulfinyl group. In addition to these characteristic peaks, all the metabolites exhibited the peaks of —$(CH_2)_6$—. On the basis of these spectrometric

data, the structures of P-I, P-II and P-III were proposed to be 1,8-*bis*(methylsulfonyl)octane, 1-methylsulfinyl-8-methylsulfonyloctane and 1,8-*bis*(methylsulfinyl)-octane, respectively. These presumed structures were confirmed by comparison with those of the authentic samples. Namely, the TLC behaviours, I.R. and NMR spectra of the metabolites were well coincident with those of the authentic samples. However,

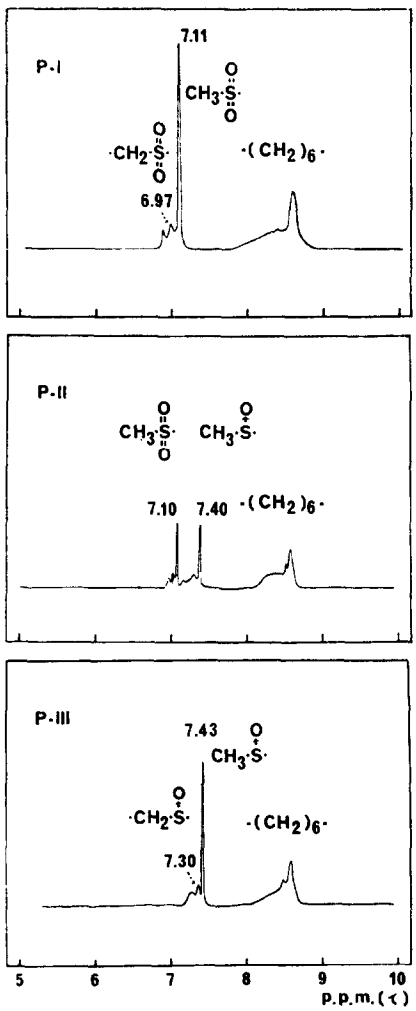

FIG. 4. Nuclear magnetic resonance spectra of metabolites P-I, P-II and P-III. Measured in $CDCl_3$.

1,8-*bis*(methylsulfinyl)octane isolated from urine gave m.p. 113–116° unlike the synthetic sample (m.p. 95–100°). Further, the metabolite was optically active with optical rotation of $\alpha_D^{22} = -17°$ (c = 0·8, in methanol), while the synthetic sample was inactive. The fact can be explained if we assume that the biological sulfoxidation is stereo-specific and the synthetic specimen is a mixture of diastereoisomers.

Time course changes of the urinary metabolites composition in rats

The urine samples obtained from Experiment 4 were directly analyzed for metabolites composition by TLC (Fig. 5). 1,8-*Bis*(methylsulfinyl)octane was dominant just after the administration, but thereafter 1,8-*bis*(methylsulfonyl)octane increased. (Fig. 6). Namely, in the first 3 hr urine, the ratio of three metabolites, 1,8-*bis*(methylsulfinyl)octane, 1-methylsulfinyl-8-methylsulfonyloctane and 1,8-*bis*(methylsulfonyl)-octane was about 9:4:1, while in the 6–24 hr urine it became about 1:1:5. The results

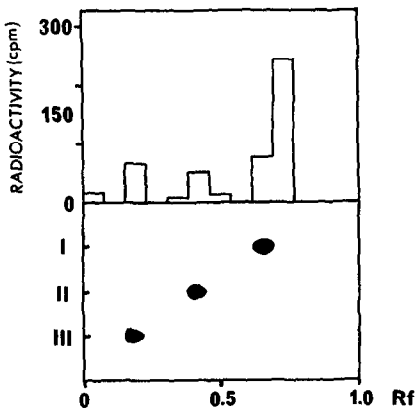

FIG. 5. Thin layer chromatogram of urinary metabolites.
Sample: 6–24 hr urine obtained from Exp. 4; Solvent system: benzene-acetone-metahnol (5:5:1, v/v); Authentic samples: I = 1,8-*bis*(methylsulfonyl)octane, II = 1-methylsulfinyl-8-methylsulfonyl-octane, III = 1,8-*bis*(methylsulfinyl)octane.

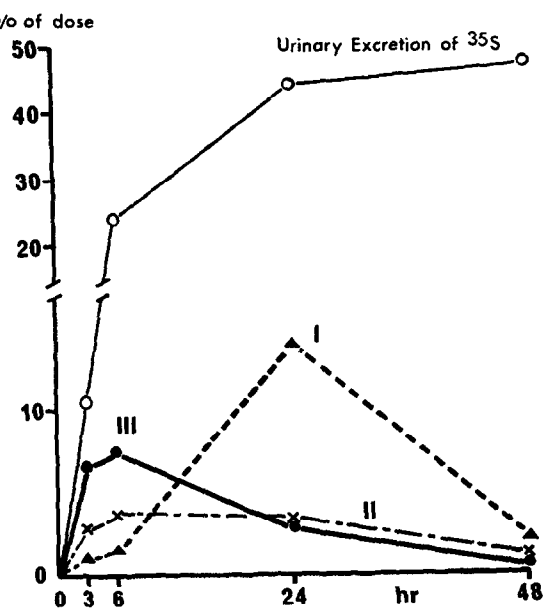

FIG. 6. Time course of metabolites composition in urine obtained after administration of BTOD-^{35}S (outer) (Exp. 4). I, II and III show 1,8-*bis*(methylsulfonyl)octane, 1-methylsulfinyl-8-methylsulfonyl-octane and 1,8-*bis*(methylsulfinyl)octane, respectively.

indicate that the formation of these three metabolites proceeds in a sequence of 1,8-*bis*(methylsulfinyl)octane → 1-methylsulfinyl-8-methylsulfonyloctane → 1,8-*bis*-(methylsulfonyl)octane.

Fate of octamethylene-1,8-dithiol-^{35}S in rats

The excretion route of octamethylene-1,8-dithiol-^{35}S was investigated in Experiment 5 and the results are very similar to those obtained with BTOD (Table 1). Namely, following the oral administration about half of the administered radioactivity was excreted into urine and about 40 per cent was found in feces and only 2·0 per cent was found in the expired gas.

Identification of urinary metabolites after administration of octamethylene-1,8-dithiol-^{35}S in rats

The first 24 hr urine sample obtained from Experiment 6 contained 54 per cent of the administered radioactivity. About 85 per cent of the radioactivity in the sample was extracted with chloroform. Thin layer chromatography of the extract revealed the three radioactive spots with the same R_f values as those of the authentic 1,8-*bis*(methylsulfinyl)octane, 1-methylsulfinyl-8-methylsulfonyloctane and 1,8-*bis*-(methylsulfonyl)octane, respectively. The identity was further confirmed by isotope

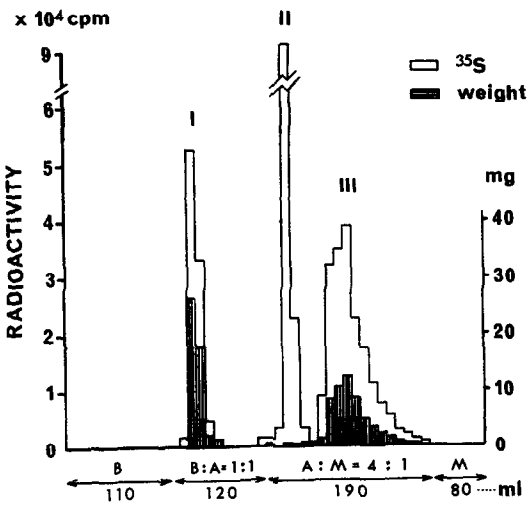

FIG. 7. Silicic acid column chromatography of the extract of urine obtained after administration of octamethylene-1,8-dithiol-^{35}S (Exp. 6).
Sample: $CHCl_3$ extract ($5·4 \times 10^5$ cpm) + authentic 1,8-*bis*(methylsulfonyl)octane (50·0 mg) + 1,8-*bis*(methylsulfinyl)octane (50·0 mg); Silicic acid-Hyflo Super Cel (2:1, w/w): 15 g; Column size: 2·4 × 8 cm; Elution solvents; B = benzene, A = acetone, M = methanol.

dilution. Namely, to an aliquot of the extract was added 50·0 mg 1,8-*bis*(methylsulfinyl)octane and 50·0 mg 1,8-*bis*(methylsulfonyl)octane, and the mixture was chromatographed on silicic acid column as described above. As shown in Fig. 7, the radioactivity was separated into three fractions, I, II and III. The radioactivity in fraction I was eluted with the authentic 1,8-*bis*(methylsulfonyl)octane and in fraction

III with the authentic 1,8-*bis*(methylsulfinyl)octane. Both compounds were recrystallized as white solids with constant specific radioactivity from fraction I and III, respectively (Table 2). Thin layer chromatographic examination (benzene-acetone-methanol, 5:5:1, v/v) showed that fraction II gave a single radioactive spot at $R_f = 0{\cdot}46$ identical to the authentic 1-methylsulfinyl-8-methylsulfonyloctane. From these data,

TABLE 2. IDENTIFICATION OF THE METABOLITE FROM FRACTION I AND III BY RECRYSTALLIZATION

Fraction	Number of recrys.	Solvent system	Total radio-activity (cpm)	Dry wt. (mg)	Sp. act. (cpm/mg)
	Origin	—	79,400	45·1	1,760
	1	Ethanol	55,000	40·4	1,360
I	2	Ethanol	28,120	20·5	1,370
	3	Ethanol	26,400	18·9	1,395
	4	Ethyl acetate	23,700	17·0	1,395
	Origin	—	128,640	49·1	3,320
	1	Ethyl acetate-petroleum ether*	58,460	19·2	3,020
III	2	Ethyl acetate-petroleum ether	35,560	12·0	2,970
	3	Benzene-petroleum ether*	23,720	8·3	2,860
	4	Benzene-petroleum ether	16,640	5·9	2,830

* The sample was recrystallized by dissolving in small volume of ethyl acetate or benzene, adding excess of petroleum ether and allowing to stand.

it was concluded that octamethylene-1,8-dithiol-^{35}S is metabolized in the same way as the ^{35}S-octamethylene-1,8-dithiol moiety of BTOD-^{35}S (outer) in rats.

Identification of the metabolites of BTOD in human urine

The urine obtained from Experiment 7 was concentrated to 150 ml by rotary evaporator *in vacuo* at 60° and extracted 4 times with 4 vol. of chloroform-methanol (3:1, v/v). The extract was then fractionated on silicic acid column under the essentially same conditions as described above. Effluent collected in each tube was checked for existence of the metabolites by TLC (Table 3). The spots corresponding to the

TABLE 3. SILICIC ACID COLUMN CHROMATOGRAPHY OF THE EXTRACTS OF HUMAN URINE AFTER ADMINISTRATION OF NONLABELED BTOD

Solvent for elution (v/v, ml)		Tube no. (fractions)	Dry wt. (mg)	TLC check* I	II	III
Benzene	260	1–17	50	—	—	—
Benzene-acetone (1:1)	240	18–23	18	—	—	—
		24–27 (A)	121	+	—	—
		28–33	31	—	—	—
Acetone-methanol (4:1)	450	34–40	112	—	—	—
		41–43 (B)	449	—	+	—
		44–49	3,675	—	—	—
		50–63 (C)	2,730	—	—	+
Acetone-methanol (1:1)	350	64–88	1,306	—	—	—

Column conditions: Silicic acid-Hyflo Super Cel (2:1, w/w), 75 g.
Column size, 3·3 × 22 cm. One tube, 15 g.
* Solvent system for TLC is benzene-acetone-methanol (5:5:1, v/v) and compounds on chromatoplates were detected by the iodine vapor method. I: 1,8-*bis*(methylsulfonyl)octane, II: 1-methylsulfinyl-8-methylsulfonyloctane, III: 1,8-*bis*(methylsulfinyl)octane.

authentic 1,8-*bis*(methylsulfonyl)octane, 1-methylsulfinyl-8-methylsulfonyloctane and 1,8-*bis*(methylsulfinyl)octane were detected in tube Nos. 24–27 (fraction A), Nos. 41–43 (fraction B) and Nos. 50–63 (fraction C), respectively. Fraction A was then applied on preparative TLC (benzene-acetone, 1:1, v/v) and the band corresponding to the authentic 1,8-*bis*(methylsulfonyl)octane ($R_f = 0{\cdot}66$) was eluted. White solids were yielded from the eluate which gave the identical I.R. spectrum with that of the authentic compound. Fraction C was rechromatographed on silicic acid column and white solids were obtained from the eluate with acetone-methanol (4:1, v/v). It gave a single spot at $R_f = 0{\cdot}47$, identical to 1,8-*bis*(methylsulfinyl)octane, in TLC (benzene-acetone-methanol, 2:2:1, v/v). The I.R. spectrum was also well coincident with that of the authentic 1,8-*bis*(methylsulfinyl)-octane. Although we failed to isolate the metabolite from fraction B because of the contamination with some usual urinary constituents, we confirmed the existence of 1-methylsulfinyl-8-methylsulfonyloctane by the TLC using several different solvent systems (benzene-acetone-methanol, 5:5:1, 3:3:1 or 1:1:0, v/v). From these results, it was concluded that the octamethylene-1,8-dithiol moiety of the compound is metabolized in man in the same way as in rats. The present results agree with our previous findings that there have been observed little species differences between rat, rabbit and man in the metabolism of the alkyl mercaptan moieties of thiamine propyl disulfide* and thiamine tetrahydrofurfuryl disulfide.†

DISCUSSION

First, we discuss on excretory route of the octamethylene-1,8-dithiol-^{35}S moiety of BTOD. When the compound was orally administered, the rats excreted about half of the dosed radioactivity in urine and nearly the same amount in feces within 48 hr, regardless of dosage size (Table 1). These results were different from those obtained with thiamine propyl disulfide or thiamine tetrahydrofurfuryl disulfide, since these alkyl mercaptan moieties were excreted solely into urine.[2–4] We have not yet studied on the nature of the fecal radioactivity and therefore we do not know how much of the compound is excreted unchanged into feces. Hence, the possibility is not excluded that a limited degree of intestinal absorption of the compound could be a factor causing such a fecal excretion. But, the possibility seems unlikely, because a substantial amount of the radioactivity was excreted into feces following the i.p. injection. However, the finding that such a fecal excretion was not observed upon the intravenous administration indicates that the problem is too complicated to be explained by a simple interpretation.

The present works also showed that most of the urinary radioactivity after the administration of BTOD-^{35}S or octamethylene-1,8-dithiol-^{35}S was identified as methylsulfinyl or methylsulfonyl compounds and the formation of radioactive inorganic sulfate was not observed at any rate. Neither unchanged BTOD nor *O*-acetyl thiamine was excreted in any appreciable amount into urine and this result is consistent with the data obtained from the previous experiments on the fate of the thiamine moiety of BTOD‡. As for the metabolites of foreign alkyl mercaptans we have

* H. Hirano, K. Nishikawa, S. Kikuchi, Suzuoki-Z. and M. Numata: Presented at the 168th meeting of Japan Vitamin B Research Committee in Nikko (10 August, 1966).

† H. Hirano, Suzuoki-Z., K. Murakami, S. Kikuchi, K. Nishikawa and M. Numata: Presented at the 169th meeting of Japan Vitamin B Research Committee in Kyoto (17 September, 1966).

‡ Z. Suzuoki, T. Matsuo and K. Furuno, unpublished.

established that about half of the propyl mercaptan moiety of thiamine propyl disulfide was metabolized as methylsulfonyl compounds[2, 3] and nearly 90 per cent of the tetrahydrofurfuryl mercaptan moiety of thiamine tetrahydrofurfuryl disulfide as methylsulfinyl or methylsulfonyl compounds.[4] We have further pointed out that there are two distinct metabolic pathways in the biotransformation of foreign alkyl mercaptans, of which one is responsible for formation of the methylsulfonyl compounds and the other leads to formation of inorganic sulfate, and that the former predominates in the metabolism of higher alkyl mercaptans while the latter contributes largely in the metabolism of lower mercaptans such as methyl mercaptan[11] and ethyl mercaptan.[12] The present results obtained with the octamethylene-1,8-dithiol-related compounds agree with these observations and offer an additional evidence for an implication of the methylsulfonyl pathway in the metabolism of sulfur-containing drugs.

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