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METABOLIC FATE OF BIS(3,5-DICHLORO-2-HYDROXYPHENYL)-SULFOXIDE (BITHIONOL SULFOXIDE)

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Abstract—Metabolic studies of bithionol sulfoxide using labeled compounds were carried out in rats. It was found that bithionol sulfoxide was not only oxidized to bithionol sulfone but also reduced to bithionol (sulfide). The metabolic fate of bithionol sulfone was greatly different from that of bithionol. Bithionol sulfone was excreted mainly in urine as 3,5-dichloro-2-hydroxyphenylsulfonic acid, whereas bithionol was excreted mainly in bile as a glucuronide conjugate.

The presence of 3,5-dichloro-2-hydroxyphenylsulfonic acid and inorganic sulfate was demonstrated in the urine of rats given bithionol sulfoxide. It was assumed that 3,5-dichloro-2-hydroxyphenylsulfonic acid was formed by the cleavage of the C–S bond of bithionol sulfone molecule and that inorganic sulfate was produced by the cleavage of the C–S bond of bithionol sulfoxide molecule. Metabolic study using ^{3}H-*bis*(2-hydroxyphenyl)-sulfone suggested that catechol was formed together with sulfonic acid by the cleavage of the C–S bond of bithionol sulfone.

It is considered from the above results that the metabolic fate of bithionol sulfoxide consists of 3 different pathways: the first is reduction of bithionol sulfoxide to bithionol (sulfide); the second is oxidation of bithionol sulfoxide to bithionol sulfone; and the third is cleavage of the C–S bond of bithionol sulfoxide.

BITHIONOL sulfoxide* (bis[3,5-dichloro-2-hydroxyphenyl]-sulfoxide) has been shown by Hamada[1] to have a stronger anthelmintic activity than bithionol (bis[3,5-dichloro-2-hydroxyphenyl]-sulfide). The present investigation was undertaken to elucidate the metabolic fate of the former. The results of this study showed that the sulfoxide was not only oxidized to bithionol sulfone (bis[3,5-dichloro-2-hydroxyphenyl]-sulfone) but also reduced to bithionol in the body of the rat. Therefore, the metabolic fates of ^{35}S-bithionol sulfone and ^{35}S-bithionol were also studied comparatively. Also, it was demonstrated that 3,5-dichloro-2-hydroxyphenylsulfonic acid and inorganic sulfate were formed from bithionol sulfoxide as metabolic end-products. This fact indicated that the cleavage of the C–S bond occurred in the body of the rat. To examine a metabolite produced together with 3,5-dichloro-2-hydroxyphenylsulfonic acid by the cleavage of the C–S bond of the bithionol sulfone molecule, a metabolic study using ^{3}H-bis(2-hydroxyphenyl)-sulfone as a structural analogue of bithionol sulfone was carried out.

EXPERIMENTAL

Preparation of labeled compounds. Reaction sequences used for the preparation of ^{35}S- or ^{3}H-labeled compounds are illustrated in Fig. 1. ^{35}S-bithionol sulfoxide [I] was

* Trade name is Bitin-S.

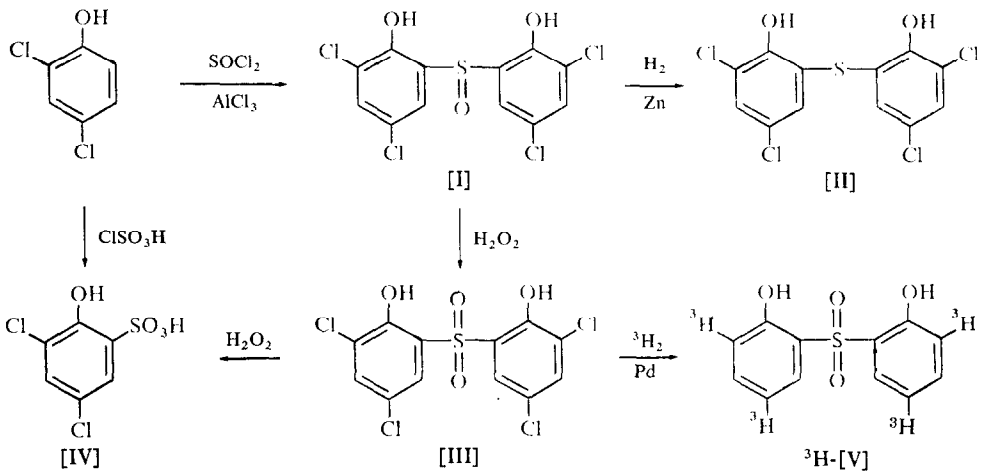

FIG. 1. Reaction sequences for preparation of labeled compounds.

prepared by the reaction of ^{35}S-thionyl chloride (20 mc) and 2,4-dichlorophenol in the presence of aluminum chloride.[2] The specific activity of ^{35}S-bithionol sulfoxide so prepared was 13·5 μc/mg, and its radiochemical purity was 99·0%.

^{35}S-bithionol [II] (sp. act., 7 μc/mg) was prepared by reduction of ^{35}S-bithionol sulfoxide with zinc dust according to the procedure of Gump and Vittucci.[2] ^{35}S-bithionol sulfone [III] (sp. act., 6 μc/mg) was prepared by the oxidation of ^{35}S-bithionol sulfoxide with hydrogen peroxide.[2]

^{35}S-3,5-dichloro-2-hydroxyphenylsulfonic acid [IV] (sp. act., 1·2 μc/mg) was prepared by the reaction of ^{35}S-chlorosulfonic acid and 2,4-dichlorophenol according to the procedure of Armstrong and Harrow.[3] When bithionol sulfone in acetic acid was heated under reflux with excess hydrogen peroxide, 3,5-dichloro-2-hydroxyphenylsulfonic acid was formed as one of the reaction products. 3,5-Dichloro-2-hydroxyphenylsulfonic acid was isolated as the potassium salt, and its i.r. spectrum was found to be identical with that of an authentic sample of potassium 3,5-dichloro-2-hydroxyphenylsulfonate.

^{3}H-bis(2-hydroxyphenyl)-sulfone [V] (sp. act., 100 μc/mg) was prepared by the catalytic hydrogenation of bithionol sulfone with tritium gas according to the procedure described in a previous paper.[4]

The radiochemical purity of all labeled compounds was demonstrated by paper chromatography and scanning of the chromatograms (most of the compounds were more than 98% pure). The solvent systems used for the chromatography are shown in Table 1.

Animal experiments. Male Wistar rats weighing about 200 g were used for all experiments. For administration the labeled compounds were suspended in 0·5% carboxylmethylcellulose (CMC) solution. Oral doses were 20 mg/kg for ^{35}S-bithionol sulfoxide and other labeled compounds. After administration of the labeled compounds, the rats were kept in cages constructed to permit the separate collection of urine and feces. Urine samples were collected in bottles containing 5 ml of toluene for 48 hr.

TABLE 1. ELECTROPHORESIS, PAPER AND THIN-LAYER CHROMATOGRAPHY OF ^{35}S-BITHIONOL SULFOXIDE AND ITS RELATED COMPOUNDS

Sample	Electrophoresis (cm)	Paper chromatography	Thin-layer chromatography	
		Solvent A*	Solvent B†	Solvent C†
Bithionol sulfoxide	0	0·81	0·24	0·46
Bithionol sulfone	0	0·81	0·56	0·69
Bithionol (sulfide)	0	0·90	0·86	0·88
Sulfonic acid‡	+7	0·60	0·09	0·25
Inorganic sulfate	+11	0·0	0·0	0·0
Urine§	0	0·0 (peak No. 1)		
	+4	0·28 (peak No. 2)		
	+7	0·60 (peak No. 3)		
	+11	0·81 (peak No. 4)		
		0·90 (peak No. 5)		

* Solvent system A is *n*-butanol:ethanol:water, 4:1:1 (v/v) for paper chromatography.
† Solvent system B is benzene:chloroform:ethyl acetate:*n*-propanol, 4:2:4:2 (v/v); and C is benzene:*n*-butanol, 1:1 (v/v) for thin-layer chromatography.
‡ 3,5-Dichloro-2-hydroxyphenylsulfonic acid.
§ Urine samples were collected for 48 hr after the oral administration of ^{35}S-bithionol sulfoxide to rats.

To investigate the biliary metabolites of ^{35}S-bithionol sulfoxide, ^{35}S-bithionol sulfone and ^{35}S-bithionol, bile was collected from rats anesthetized with pentobarbital after the oral administration of these compounds.

Separation and identification of metabolites. For the separation of biliary and urinary metabolites, solvent extraction, paper electrophoresis, paper and thin-layer chromatography were employed. Electrophoresis was carried out on filter paper, Toyo Roshi No. 51, in a 0·1 M sodium acetate solution, pH 7·1 (50 V/cm for 30 min). Ascending paper chromatography was carried out on Toyo Roshi No. 51 using solvent system A [*n*-butanol:ethanol:water, 4:1:1 v/v)]. Silica Gel G thin-layer plates (5·0 × 20 cm) were prepared by the method of Stahl,[5] using the solvent systems B [benzene:chloroform:ethyl acetate:*n*-propanol, 4:2:4:2 (v/v)], and C [benzene:*n*-butanol, 1:1 (v/v)].

DEAE-cellulose was used for the separation of glucuronides of bithionol sulfoxide and its metabolites from 3,5-dichloro-2-hydroxyphenylsulfonic acid. Glucuronides were eluted from the columns with 2 N-CH_3COOH in methanol and 3,5-dichloro-2-hydroxyphenylsulfonic acid was eluted with 2 N-HCl in methanol.[6] Enzymatic hydrolysis of the conjugates was effected with β-glucuronidase (Sigma Chemical Co.).

Inorganic sulfate was determined by precipitation as the barium salt according to the method of Folin[7] or as benzidine sulfate (Fiske's procedure).[8]

Biliary and urinary metabolites were identified both by their migration, as compared to chromatographed standards, and by co-crystallization with authentic compounds. The spots were visualized by spraying with 0·02% methyl red in ethanol or with Folin–Ciocalteau reagent. Quantification of the metabolites was accomplished by combining paper and thin-layer chromatography in solvent systems A and B.

Measurement of radioactivity. An Aloka liquid scintillation spectrometer LSC-502 equipped with an automatic quenching monitor system was used for assay of radioactivity. Detection of the metabolites on paper and thin-layer plates was carried out using an Aloka chromatogram scanner TRM-1B. Quantitative estimation of ^{35}S-

bithionol sulfoxide and its metabolites involved dividing the paper or thin-layer chromatograms into 5- or 10-mm sections, extracting each segment with 1 ml of 50% methanol in a counting vial and adding 15 ml of the scintillation fluid to the extract. The scintillation fluid used for aqueous samples was made by dissolving 7·0 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis-2-(5-phenyloxazolyl)-benzene in 1 l. of 50% ethanol-toluene.

RESULTS

When the 48-hr urine sample of a rat given ^{35}S-bithionol sulfoxide was subjected to paper chromatography in solvent system A, 5 radioactive components were observed, as shown in Table 1. About 30 per cent (peaks No. 1 and 2 and a part of peak No. 3) of the urinary radioactivity remained in the aqueous residue after the solvent extraction (ethyl acetate at pH 4·0).

Identification of inorganic sulfate. Electrophoretic studies of the urine sample showed that 2 strongly acidic compounds were present in the urine of the rat given ^{35}S-bithionol sulfoxide. The migration rate (+11 cm) of one of the two strong acids corresponded to that of the authentic sample of inorganic ^{35}S-sulfate. A paper chromatogram of the urine sample showed that about 20 per cent of the radioactivity applied to chromatogram occurred at the origin (peak No. 1) corresponding in R_f value to the authentic sample of ^{35}S-inorganic sulfate. When an excess of barium chloride or benzidine was added to aqueous extract of peak No. 1, about 85 per cent of the radioactivity was found to be precipitated as barium sulfate or benzidine sulfate. The identity was further ascertained by the following isotope dilution method. A measured quantity of Na_2SO_4 was added to the extract of peak No. 1 and then the sulfate was precipitated with ethanol. The precipitate was redissolved in water and then precipitated with ethanol. This procedure was repeated three times and a specific activity of each product was determined. The specific activity of each product was constant within the limit of experimental error. From these results, the metabolite present in peak No. 1 was largely identified as inorganic sulfate.

Identification of 3,5-dichloro-2-hydroxyphenylsulfonic acid. Electrophoretic examinations showed that the migration rate (+7 cm) of another strong acid corresponded to that of the authentic sample of ^{35}S-3,5-dichloro-2-hydroxyphenylsulfonic acid. A paper chromatogram showed that the R_f value of peak No. 3 (in Table 1) was similar to that (0·60) of the authentic sample of ^{35}S-3,5-dichloro-2-hydroxyphenylsulfonic acid. The identity was ascertained by the following isotope dilution technique: After removing inorganic sulfate from urine with the use of $BaCl_2$, the urine was passed through a column (1 × 20 cm) of Amberlite CG 50 (H^+ form). None of the applied activity was adsorbed on the column. The effluent and washings were concentrated to dryness *in vacuo* at 45–50°, and the residue was extracted with methanol. The methanol solution, which contained about 80 per cent of the urinary radioactivity, was put on a DEAE-cellulose column (1 × 20 cm), and it was found that the majority of the radioactivity was adsorbed on the ion exchanger. The column was washed with 2 N-acetic acid in methanol (this fraction contained bithionol, bithionol sulfoxide, bithionol sulfone and their glucuronides), and then eluted with 2 N-HCl in methanol. An electrophoretic check revealed that the eluate contained the strong acid metabolite (migration rate +7 cm), which had an R_f value of 0·60 in solvent system A. A measured quantity of 3,5-dichloro-2-hydroxyphenylsulfonic acid was added to the

elute, and the mixture was concentrated *in vacuo* at 45–50°. When a calculated amount of potassium chloride dissolved in a small amount of water was added to the concentrate, the potassium salt of 3,5-dichloro-2-hydroxyphenylsulfonic acid separated out in crystalline form. The product was recrystallized from water three times without a detectable change in specific activity. When the isotope dilution technique was also applied to the metabolites of peak No. 3, it was found that 55 per cent of the radioactivity (29 per cent of the urinary radioactivity) was present as ^{35}S-sulfonic acid. From these data, 1 of the metabolites in peak No. 3 was concluded to be 3,5-dichloro-2-hydroxyphenylsulfonic acid.

Identification of bithionol, bithionol sulfoxide and bithionol sulfone. The metabolites in peaks No. 4 and No. 5 were extractable into ethyl acetate at pH 4·0. The R_f value (0·80) of peak No. 4 in solvent system A was identical to that of the authentic sample of ^{35}S-bithionol sulfoxide. However, when the extract of peak No. 4 was subjected to thin-layer chromatography in solvent systems B and C, the radioactivity separated into two distinct spots, one of which corresponded in R_f value to bithionol sulfoxide and the other to the authentic sample of bithionol sulfone. Co-crystallization also confirmed that the metabolites of peak No. 4 were bithionol sulfoxide and bithionol sulfone.

The last metabolite (peak No. 5) had an R_f value (0·90) identical to that of the authentic sample of bithionol. Co-chromatography in solvent systems B and C revealed that the R_f value of this metabolite was identical with that of ^{35}S-bithionol. Co-crystallization also confirmed that it was bithionol.

Identification of glucuronides. Electrophoretic studies showed that another metabolite of peak No. 3 was a weak acid. The separation of this weak acid was accomplished by column chromatography on DEAE-cellulose. The effluent and washings (2 N-CH_3COOH in methanol) from the column were combined and concentrated to dryness *in vacuo*. The residue was dissolved in water and was then extracted with ether to remove bithionol, bithionol sulfoxide and bithionol sulfone. An electrophoretic check revealed that the remaining aqueous solution contained a weak acid which had an R_f value of 0·57 in solvent system A. When the weak acid metabolites were incubated with β-glucuronidase in the presence or absence of saccharo-(1:4)-lactone, which is known to be a potent inhibitor of β-glucuronidase,[9] it was found that they were hydrolyzed only in the absence of the inhibitor. This fact demonstrated that the weak acid metabolites were glucuronide conjugates.

Paper and thin-layer chromatography of the hydrolyzed products of the weak acid metabolites showed that about 70 per cent of the radioactivity was present as ^{35}S-bithionol and the remaining 30 per cent was a mixture of ^{35}S-bithionol sulfoxide and ^{35}S-bithionol sulfone.

The metabolite (about 10 per cent of the urinary radioactivity) of peak No. 2 could not be identified.

Quantification of these metabolites described above was established by combining paper and thin-layer chromatography, the results being shown in Table 2. The major metabolites in the urine of a rat given ^{35}S-bithionol sulfoxide consisted of the sulfonic acid, inorganic sulfate, bithionol and the glucuronide of bithionol.

Bile samples were also analyzed chromatographically for radioactive metabolites of ^{35}S-bithionol sulfoxide in the same manner as described for the urine sample. The same metabolites as in the urine were also found in the bile, but the relative percentages

TABLE 2. PERCENTAGES OF URINARY AND BILIARY METABOLITES OF ^{35}S-BITHIONOL SULFOXIDE*

Metabolites	Peak No.†	Urine‡ (3·8%) (%)	Bile§ (4·2%) (%)
Bithionol sulfoxide	4	4·5	1·0
Bithionol sulfone	4	2·5	0
Bithionol	5	14·5	2·0
Sulfonic acid‖	3	29·0	0·5
Inorganic sulfate	1	15·5	2·0
Glucuronide of bithionol sulfoxide	3	5·5	17·0
Glucuronide of bithionol sulfone	3	1·5	5·0
Glucuronide of bithionol	3	16·5	71·0
Unknown	2	10·5	1·5

* Data indicate percentages of the urinary and biliary radioactivity present as radioactive metabolites of ^{35}S-bithionol sulfoxide. Quantification of radioactive metabolites was carried out by combining paper and thin-layer chromatography in solvent systems A and B. Values in parentheses indicate the per cent recovery of the administered radioactivity.

† Peak No. represents radioactive peaks on the chromatogram when the urine or bile sample was developed in solvent system A. The R_f value of each peak No. is shown in Table 1.

‡ Urine samples were collected for 48 hr after the oral administration of ^{35}S-bithionol sulfoxide to rats.

§ Bile samples were collected for 8 hr after the oral administration.

‖ 3,5-Dichloro-2-hydroxyphenylsulfonic acid.

TABLE 3. PER CENT RECOVERY OF URINARY METABOLITES OF ^{35}S-BITHIONOL (SULFIDE), ^{35}S-BITHIONOL SULFOXIDE AND ^{35}S-BITHIONOL SULFONE*

Metabolites	Sulfide† (%)	Sulfoxide‡ (%)	Sulfone§ (%)
Total ^{35}S	3·1	3·8	4·3
Sulfonic acid‖	0·30	1·10	2·92
Inorganic sulfate	0·34	0·59	0·19
Glucuronide of sulfide†	1·52	0·63	0·37
Glucuronides of sulfoxide‡ and sulfone§	0·16	0·27	0·15
Sulfide†	0·33	0·55	0·13
Sulfoxide‡ and sulfone§	0·12	0·27	0·26
Unknown	0·33	0·40	0·28

* Urine samples were collected for 48 hr after the oral administration of corresponding ^{35}S-labeled compounds to rats. Quantification of radioactive metabolites was carried out by combining paper and thin-layer chromatography in solvent systems A and B. Data represent per cent recovery of the administered radioactivity.

† Bithionol.

‡ Bithionol sulfoxide.

§ Bithionol sulfone.

‖ 3,5-Dichloro-2-hydroxyphenylsulfonic acid.

of the metabolites in the bile were greatly different from those in the urine, as shown in Table 2. Approximately 70 per cent of the biliary radioactivity was found to be present as the glucuronide of ^{35}S-bithionol while only about 15 per cent of the urinary radioactivity was present as the glucuronide of bithionol. Only a small amount of bithionol sulfoxide was recovered in the unchanged form from urine and bile. This demonstrates that bithionol sulfoxide is extensively metabolized by the rat.

Excretion of radioactive metabolites of ^{35}S-bithionol, ^{35}S-bithionol sulfoxide and ^{35}S-bithionol sulfone. Table 3 shows the per cent recovery of urinary radioactive metabolites after the oral administration of ^{35}S-bithionol, ^{35}S-bithionol sulfoxide or ^{35}S-

bithionol sulfone to the rat. It is evident that the urinary excretion of radioactivity was very low for all of the compounds tested. Bithionol sulfoxide was found to be eliminated mainly into the feces (detailed data concerning the excretion of bithionol sulfoxide will be presented in the next paper). The per cent recovery of total ^{35}S in the 48-hr urine collection was the highest for bithionol sulfone, intermediate for bithionol sulfoxide and the lowest for bithionol. Considerable differences in the amounts of 3,5-dichloro-2-hydroxyphenylsulfonic acid, inorganic sulfate and bithionol glucuronide excreted were also observed. The percentages of 3,5-dichloro-2-hydroxyphenylsulfonic acid recovered were in the following order: bithionol sulfone > bithionol sulfoxide > bithionol, but the percentages of glucuronides formed were in the reverse. The urinary excretion of inorganic sulfate was the highest for bithionol sulfoxide and the lowest for bithionol sulfone. In contrast, the per cent recovery of total ^{35}S in the 8-hr bile samples was extremely high for bithionol as compared with those for bithionol sulfoxide and bithionol sulfone, as shown in Table 4. It was found

TABLE 4. PER CENT RECOVERY OF BILIARY METABOLITES OF ^{35}S-BITHIONOL (SULFIDE), ^{35}S-BITHIONOL SULFOXIDE AND ^{35}S-BITHIONOL SULFONE*

Metabolites	Sulfide† (%)	Sulfoxide‡ (%)	Sulfone§ (%)
Total ^{35}S	23·7	4·2	2·3
Sulfonic acid‖	0	0·02	0·04
Inorganic sulfate	0·08	0·09	0·01
Glucuronide of sulfide†	22·91	2·96	1·80
Glucuronides of sulfoxide‡ and sulfone§	0·41	0·93	0·31
Sulfide†	0·22	0·09	0·04
Sulfoxide† and sulfone§	0	0·04	0·04
Unknown	0·08	0·07	0·06

* Bile samples were collected for 8 hr after the oral administration of the corresponding ^{35}S-labeled compounds to rats. Quantification of radioactive metabolites was carried out by combining paper and thin-layer chromatography in solvent systems A and B. Data represent per cent recovery of the administered radioactivity.

† Bithionol.

‡ Bithionol sulfoxide.

§ Bithionol sulfone.

‖ 3,5-Dichloro-2-hydroxyphenylsulfonic acid.

that more than 90 per cent of the biliary radioactivity was excreted as glucuronides of bithionol, bithionol sulfoxide and bithionol sulfone, and that more than 70 per cent of the glucuronides was present as the glucuronide of bithionol for all of the compounds tested. This fact indicated that bithionol was more extensively conjugated with glucuronic acid than bithionol sulfoxide and bithionol sulfone, and more effectively excreted in the bile.

Identification of catechol. When the urine sample of the rat given ^{3}H-bis(2-hydroxyphenyl)-sulfone was subjected to paper chromatography in solvent system A, 5 radioactive components were observed, as shown in Table 5. The radioactive metabolites of peak No. 2 (R_f 0·28), which contained about 60 per cent of the urinary radioactivity, were assumed to be glucuronide conjugates, since they were converted to the original compound and its metabolites by treating with β-glucuronidase. A metabolite of peak No. 3 (R_f 0·58), which contained about 5 per cent of the urinary

TABLE 5. URINARY METABOLITES OF ^{3}H-bis(2-HYDROXYPHENYL)-SULFONE IN THE RAT*

Peak No.	R_f value	Recovery (%)	Corresponding compound
		(30·0)	
1	0·0	0·6	Unknown-1
2	0·26	18·6	Glucuronides† of sulfone, catechol‡ and guaiacol§
3	0·58	1·5	Sulfonic acid‖
4	0·80	1·3	Unknown-2
5	0·95	8·0	Unchanged sulfone

* The urine sample was collected for 24 hr after the oral administration of ^{3}H-sulfone (20 mg/kg) and analyzed by paper chromatography in solvent system A [*n*-butanol:ethanol:water, 4:1:1 (v/v)]. Data indicate per cent recovery of the administered radioactivity. Value in parentheses indicates the percentage of total radioactivity in the urine.

† Paper chromatography of hydrolyzed products showed that the glucuronides contained unchanged sulfone, catechol and *O*-methylated catechol (guaiacol) as aglycones.

‡ Catechol (R_f 0·56) was identified by co-chromatography in a solvent system of chloroform:acetic acid:water, 2:1:1 (v/v) and co-crystallization with an authentic sample of catechol.

§ Guaiacol (R_f 0·92) was identified in a manner similar to that described for catechol after it was converted to catechol by heating with 48 per cent HBr.

‖ 2-Hydroxyphenylsulfonic acid.

radioactivity, was identified as 2-hydroxyphenylsulfonic acid by co-chromatography and co-crystallization with an authentic sample of this acid.[10]

If ^{3}H-bis(2-hydroxyphenyl)-sulfone undergoes cleavage of the C–S bond in the rat, it may be possible that either ^{3}H-phenol or ^{3}H-catechol is produced along with the 2-hydroxyphenylsulfonic acid. To determine whether ^{3}H-phenol or ^{3}H-catechol is present in the urine of rat given ^{3}H-bis(2-hydroxyphenyl)-sulfone, an extract of peak No. 5 (R_f 0·95) was rechromatographed in solvent system D of [chloroform:acetic acid:water, 2:1:1 (v/v)]. Unexpectedly, the radioactive metabolite could scarcely be detected at a position on the chromatogram corresponding in R_f value to that of either authentic phenol (R_f 0·34) or catechol (R_f 0·56). However, when the metabolites of peak No. 2 were heated with 2 N-HCl under reflux and then rechromatographed in solvent system D, 0·6 per cent and 6·0 per cent of the radioactivity were found to be present as ^{3}H-catechol and ^{3}H-guaiacol respectively. Their identity as catechol and guaiacol was ascertained by the isotope dilution method. The fact that the amount of catechol was very small as compared with that of guaiacol might be accounted for by assuming that most of catechol formed from bis(2-hydroxyphenyl)-sulfone is converted *in vivo* to the *O*-methylated derivative (guaiacol), since it is well known that catechol and its derivatives are *O*-methylated *in vivo* by catechol *O*-methyl transferase (COMT).[11]

DISCUSSION

It was noted that urinary and biliary excretion of the radioactivity after the oral administration of ^{35}S-bithionol sulfoxide was very low, as shown in Tables 2 and 3. This fact may be considered to be because of binding of the drug to plasma proteins, since the highest concentration of the drug was found in the plasma and lasted for a long time when the distribution of the drug was studied in rats, and the drug added to the plasma could scarcely be recovered by ether extraction unless the plasma proteins were first precipitated by adding trichloroacetic acid. It is well known that high

and long-lasting concentration of sulfonamides in blood is because of binding of the drugs to serum protein and that sulfonamides bound to plasma protein do not permeate the glomerular membrane.[12]

The results of the metabolic study of ^{35}S-bithionol sulfoxide showed that very little unchanged bithionol sulfoxide was excreted by rats in urine or bile. This fact suggested that bithionol sulfoxide was extensively metabolized by the rat. The major metabolite in bile of a rat given bithionol sulfoxide was the glucuronide of bithionol, as shown in Table 2. Therefore, it was assumed that the main metabolic pathways for bithionol sulfoxide were reduction and glucuronide conjugation. Glucuronides of bithionol sulfoxide and bithionol sulfone were also detected in the bile, but the amounts of both were extremely small as compared to that of bithionol. This fact suggested that bithionol underwent glucuronide conjugation much more readily and hence was excreted into bile to a greater extent than bithionol sulfoxide and bithionol sulfone. This conclusion was supported by the fact that orally administered bithionol was much more efficiently excreted into the bile as a glucuronide conjugate than the orally administered bithionol sulfoxide and bithonol sulfone, as shown in Table 4.

In the present studies, bithionol sulfone has been definitely identified as a minor oxidation product of bithionol sulfoxide. The biological oxidation of sulfoxide has already been shown to occur in several drugs.[13–16] For example, Ellard[16] reported that considerable oxidation of bis(*p*-aminophenyl)-sulfoxide to bis(*p*-aminophenyl)-sulfone occurred in the human body.

The present studies on the urinary metabolites after the administration of ^{35}S-bithionol sulfoxide showed that about 30 per cent of the urinary radioactivity was eliminated as 3,5-dichloro-2-hydroxyphenylsulfonic acid and about 15 per cent as inorganic sulfate. This fact indicates that cleavage of the C–S bond occurs in the rat. At present, the reaction sequences by which 3,5-dichloro-2-hydroxyphenylsulfonic acid is formed are uncertain. However, it seems most reasonable to assume that 3,5-dichloro-2-hydroxyphenylsulfonic acid is produced by cleavage of the C–S bond of bithionol sulfone, since it was found that the per cent recovery of 3,5-dichloro-2-hydroxyphenylsulfonic acid was remarkably higher after bithionol sulfone administration than after bithionol sulfoxide, as shown in Table 3. The fact that a small amount of 3,5-dichloro-2-hydroxyphenylsulfonic acid was excreted in the urine after administration of bithionol might be explained by assuming that bithionol is converted to bithionol sulfone via bithionol sulfoxide. Therefore, it may be concluded that most of bithionol sulfone produced from bithionol sulfoxide is converted to 3,5-dichloro-2-hydroxyphenylsulfonic acid and then mainly excreted in the urine. From these results, it is evident that the metabolic fate of bithionol sulfone is quite different from that of bithionol; the former is converted mainly to 3,5-dichloro-2-hydroxyphenylsulfonic acid and then excreted in the urine, whereas the latter is converted mainly to glucuronide conjugate and is then excreted in the bile.

To obtain some information on the reaction mechanism involved in arylsulfonic acid formation, the metabolic study using ^{3}H-bis(2-hydroxyphenyl)-sulfone as a structural analogue of ^{35}S-bithionol sulfone was carried out. It was demonstrated that ^{3}H-catechol was formed together with ^{3}H-2-hydroxyphenylsulfonic acid by the cleavage of the C–S bond in ^{3}H-bis(2-hydroxyphenyl)-sulfone. Consequently, it may be considered that there are two kinds of reaction in the cleavage of the C–S bond of arylsulfone molecule; one is hydrolytic cleavage which produces arylsulfinic acid and

catechol as reaction products, and the other is oxidative cleavage which gives arylsulfonic acid and catechol. The results of the present study showed that arylsulfinic acid was not detected in the urine of rat given ^{35}S-bithionol sulfoxide. However, from this fact it may be premature to conclude that the cleavage of arylsulfone molecule is oxidative, because it may be possible that the arylsulfinic acid formed from arylsulfone is rapidly oxidized *in vivo* to arylsulfonic acid, just as hypotaurine is converted to taurine.[17]

Furthermore, the reaction sequences involved in inorganic sulfate formation are also uncertain. However, it seems most likely that inorganic sulfate is formed by the cleavage of bithionol sulfoxide, since the per cent recovery of urinary inorganic sulfate was higher after bithionol sulfoxide than after bithionol, as shown in Table 3. The fact that the smallest amount of inorganic sulfate was excreted in urine even after the administration of bithionol sulfone might be explained by assuming that a small amount of bithionol sulfone was reduced to bithionol sulfoxide. (It was reported that cysteine sulfinic acid was decomposed to inorganic sulfite by rabbit desulfinase.)[18] Therefore, if the cleavage of bithionol sulfoxide is oxidative, the arylsulfinic acid is produced as an intermediate product and is then converted to inorganic sulfite, which is then oxidized to inorganic sulfate.[19] If the cleavage of bithionol sulfoxide is hydrolytic, the arylsulfenic acid is produced as an intermediate, and is then oxidized to

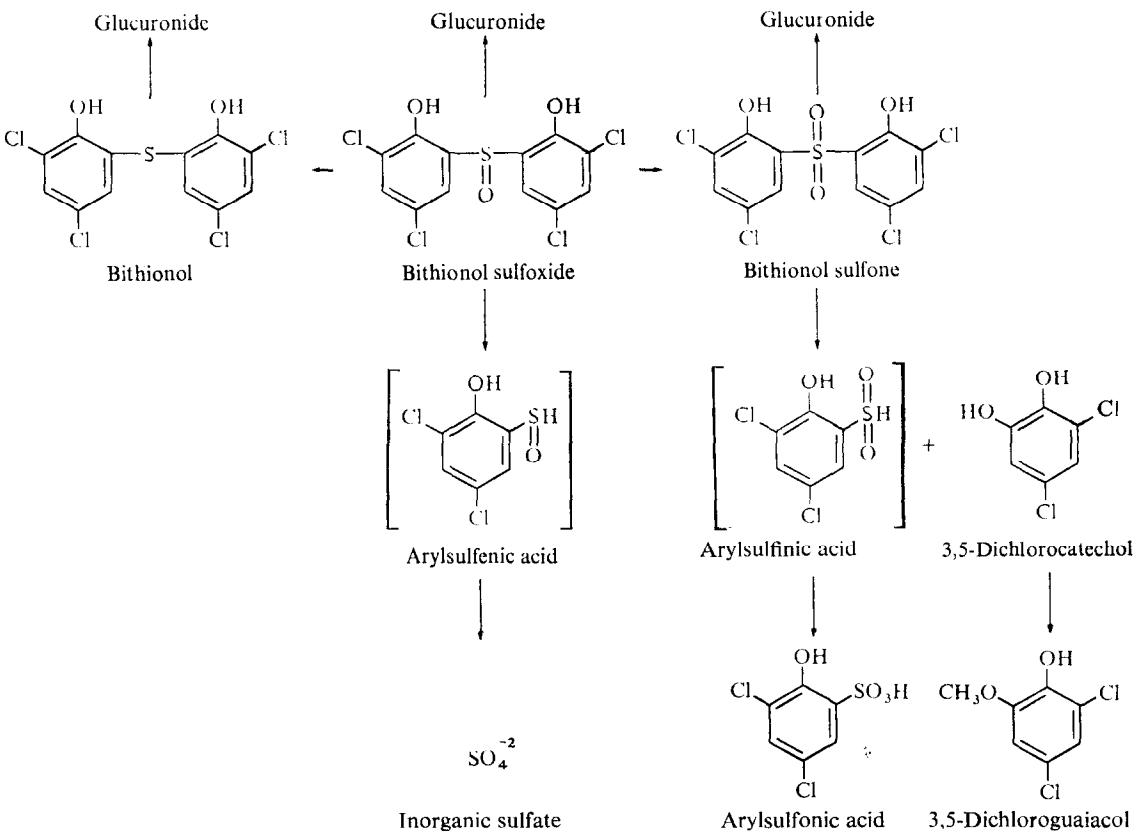

FIG. 2. Possible metabolic pathways of bithionol sulfoxide.

arylsulfinic acid. However, it has been demonstrated for the rat that inorganic sulfate does not arise from arylsulfonic acid. The proof consisted of showing that 98 per cent of the orally administered ^{35}S-3,5-dichloro-2-hydroxyphenylsulfonic acid was recovered in the unchanged form from the 24-hr urine of rats. Michael[20] studied the metabolism of alkyl benzene sulfonates in the rat and reported that the sulfonic acid moiety remained intact on the benzene ring.

From a consideration of the facts described above, it seems most reasonable to conclude that the metabolic fate of bithionol sulfoxide includes 3 different pathways as follows: (1) reduction of bithionol sulfoxide to bithionol, followed by glucuronide conjugation; (2) oxidation of bithionol sulfoxide to bithionol sulfone, followed by formation of 3,5-dichloro-2-hydroxyphenylsulfonic acid (arylsulfonic acid) and 3,5-dichlorocatechol, by the cleavage of C–S bond of bithionol sulfone; (It should be noted that the present finding of arylsulfonic acid formation from a sulfone is the first example of this type of drug metabolism). (3) cleavage of the C–S bond of bithionol sulfoxide, followed by formation of inorganic sulfate by cleavage of the C–S bond of the arylsulfinic acid or arylsulfenic acid. Therefore, the reactions shown in Fig. 2 may be proposed for the metabolism of bithionol sulfoxide. The metabolites in parentheses are tentatively postulated to be formed as intermediate products.

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