Some Metabolites of 1-Bromobutane in the Rabbit and the Rat

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1. Rabbits and rats dosed with 1-bromobutane excrete in urine, in addition to butylmercapturic acid, (2-hydroxybutyl)mercapturic acid, (3-hydroxybutyl)mercapturic acid and 3-(butylthio)lactic acid. 2. Although both species excrete both the hydroxybutylmercapturic acids, only traces of the 2-isomer are excreted by the rabbit. The 3-isomer has been isolated from rabbit urine as the dicyclohexylammonium salt. 3. 3-(Butylthio)lactic acid is formed more readily in the rabbit; only traces are excreted by the rat. 4. Traces of the sulphoxide of butylmercapturic acid have been found in rat urine but not in rabbit urine. 5. In the rabbit about 14% and in the rat about 22% of the dose of 1-bromobutane is excreted in the form of the hydroxymercapturic acids. 6. Slices of rat liver incubated with *S*-butylcysteine or butylmercapturic acid form both (2-hydroxybutyl)mercapturic acid and (3-hydroxybutyl)mercapturic acid, but only the 3-hydroxy acid is formed by slices of rabbit liver. 7. *S*-Butylglutathione, *S*-butylecysteinylglycine and *S*-butylcysteine are excreted in bile by rats dosed with 1-bromobutane. 8. Rabbits and rats dosed with 1,2-epoxybutane excrete (2-hydroxybutyl)mercapturic acid to the extent of about 4% and 11% of the dose respectively. 9. The following have been synthesized: *N*-acetyl-*S*-(2-hydroxybutyl)-L-cysteine [(2-hydroxybutyl)mercapturic acid] and *N*-acetyl-*S*-(3-hydroxybutyl)-L-cysteine [(3-hydroxybutyl)mercapturic acid] isolated as dicyclohexylammonium salts, *N*-toluene-*p*-sulphonyl-*S*-(2-hydroxybutyl)-L-cysteine, *S*-butylglutathione and *N*-acetyl-*S*-butylecysteinylglycine ethyl ester.

The high proportion of the bromine of administered 1-bromobutane that is excreted as bromide by rabbits and rats (Bray, Caygill, James & Wood, 1964) indicates that the compound is extensively metabolized by these animals. It is likely that the initial reaction is between 1-bromobutane and GSH, since it was shown that the injection of 1-bromobutane brought about a marked fall in the liver GSH content (Barnes, James & Wood, 1959). Butylmercapturic acid was isolated from the urine of rabbits and rats dosed with 1-bromobutane (Bray *et al.* 1964), and the excretion of a further sulphur-containing metabolite was detected. A preliminary report by James & Jeffery (1964a) suggested that this metabolite might be a hydroxybutylmercapturic acid, but its constitution was not established. Barnsley (1964b) isolated (2-hydroxypropyl)mercapturic acid from the urine of rats dosed with 1-bromopropane and later (Barnsley, 1966) showed that the compound was also a metabolite of 1-chloropropane, 1-iodopropane and 1,2-epoxypropane, and of 1-chloropropanol, in the

rat. The present paper describes a further investigation of the metabolism of 1-bromobutane. The nature of the hydroxybutylmercapturic acid has been established, and another type of metabolite, (3-butylthio)lactic acid, has been detected. The formation of 3-(methylthio)- and 3-(ethylthio)-lactic acid from methyl- and ethyl-cysteine respectively by yeast was described by Maw & Coyne (1966). The biliary excretion of metabolites of 1-bromobutane in the rat has also been investigated.

MATERIALS

All melting points are uncorrected. Carbon, hydrogen and nitrogen analyses were carried out by F. und E. Pascher, Bonn, Germany. Sulphur was determined by the flask method (see Macdonald, 1961). 1-Bromobutane and 1,2-epoxybutane were purchased. *S*-Butyl-L-cysteine and *N*-acetyl-*S*-butyl-L-cysteine (butylmercapturic acid) were prepared as described by Bray, Franklin & James (1959).

Butylmercapturic acid sulphoxide. Butylmercapturic acid (1.4g.) was dissolved in acetone (50ml.) and the solution cooled to 0°. Then 1.5ml. of a solution of H₂O₂ (100 vol.) was added and the mixture kept overnight at 0°. A small amount of insoluble material separated and was

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removed by filtration. The filtrate was evaporated under reduced pressure to give a viscous syrup. Chromatographic examination showed that the product contained no mercapturic acid. The syrup was dissolved in a mixture of acetone and ether, and then light petroleum (b.p. 40–60°) was added until a slight turbidity was produced. Crystals (0.29 g.) separated when the mixture was kept; these had m.p. 132–134° after recrystallization from acetone–light petroleum (b.p. 40–60°) (Found: S, 14.0; C₉H₁₇NO₄S requires S, 13.6%).

S-Butylglutathione. This was prepared by a method similar to that described by Roberts & Warwick (1958) for *S*-ethylglutathione. A mixture of GSH (2 g.) and water (9 ml.) was made just alkaline by the addition of 2*N*-NaOH. Then 2*N*-NaOH (3 ml.) and 1-iodobutane (3 ml.) were added with ethanol to give a homogeneous mixture. This was shaken for 5 hr. at room temperature and kept overnight. On the addition of ethanol, crystals (338 mg.) separated; these were washed with ethanol and ether. Recrystallization from aq. ethanol gave colourless *S*-butylglutathione, m.p. 216–218° (Found: N, 11.6; S, 8.5; C₁₄H₂₅N₃O₆S requires N, 11.6; S, 8.8%).

S-Butylcysteinylglycine. *N*-Acetyl-*S*-butylcysteine (2 g.) in ether was esterified by the addition of an ethereal solution of diazomethane. After evaporation of the solvent the syrupy ester obtained was converted directly into the hydrazide of *N*-acetyl-*S*-butylcysteine by treatment with hydrazine hydrate in ethanol. The crystals (0.9 g.) which separated were recrystallized from water and had m.p. 146° (Found: N, 17.8; S, 13.5; C₉H₁₉N₃O₂S requires N, 18.0; S, 13.7%). The hydrazide was converted into the azide and coupled with glycine ethyl ester as described by Le Quesne & Young (1950). The *N*-acetyl-*S*-butylcysteinylglycine ethyl ester formed (300 mg.) was recrystallized from ethanol–light petroleum (b.p. 40–60°) to give colourless crystals, m.p. 94–97° (Found: N, 9.1; S, 10.7; C₁₃H₂₄N₂O₄S requires N, 9.2; S, 10.5%). This ester (30 mg.) was heated under reflux at 100° with 2*N*-HCl (1 ml.) for 90 min. The product, when examined chromatographically, showed a major spot presumed to correspond to *S*-butylcysteinylglycine and smaller amounts of *S*-butylcysteine and glycine.

*N-Toluene-*p*-sulphonyl-*S*-(2-hydroxybutyl)-L-cysteine.* *L*-Cysteine hydrochloride (3.5 g.) in 2*N*-NaOH (40 ml.) was cooled in an ice–salt bath for 10 min. and then 1,2-epoxybutane (1.8 ml.) was added. The mixture was shaken mechanically in a stoppered flask for 1 hr. at 5°. The mixture was brought to room temperature and toluene-*p*-sulphonyl chloride (3.8 g. dissolved in ether) was added. This mixture was shaken for 2 hr. at room temperature and the ethereal layer was removed. The aqueous layer was acidified to pH 2 by the addition of conc. HCl, and an oil separated that solidified on being kept at 0°. The solid was purified twice by solution in 2*N*-NaOH and reprecipitation with acid. The product (900 mg.), which consisted of a major component (*R_F* 0.68 in solvent *B*) and a second sulphur-containing substance (*R_F* 0.50 in solvent *B*) (see Table 1 for the composition of solvents), was purified by streaking it on to paper sheets, which were developed for 16 hr. in solvent *B*. The zones containing the major component were located by spraying test strips cut from the sheets with the K₂Cr₂O₇–AgNO₃ reagent (see the Methods section). These areas were cut out and eluted with methanol acidified with 2*N*-HCl. The eluates were evaporated to dryness under reduced pressure and the residue was dissolved in the minimum

volume of 2*N*-NaOH. The solution was acidified and the crystals that separated from the cooled solution were collected and washed with water. After two recrystallizations from methanol the product (118 mg.) had m.p. 119–120°, [α]_D²⁰ + 47.2 ± 2° (c 0.24 in ethanol) (Found: C, 48.8; H, 6.2; N, 4.3; S, 18.0; C₁₄H₂₁NO₅S₂ requires C, 48.4; H, 6.1; N, 4.0; S, 18.5%).

The results of a nuclear-magnetic-resonance analysis (carried out and interpreted by Dr A. Melera of Varian A.-G., Zurich, Switzerland) were consistent with the hydroxyl group being attached to C-2 of the butyl chain and with the expected formula of the compound.

*N-Acetyl-*S*-(2-hydroxybutyl)-L-cysteine.* Method (a). *L*-Cysteine hydrochloride (1.8 g.) was dissolved in 2*N*-NaOH (20 ml.). 1,2-Epoxybutane (0.9 ml.) was added to the cooled solution, and the mixture was shaken for 2 hr. at 5°. Acetic anhydride (0.4 ml.) and 2*N*-NaOH (0.8 ml.) were then added to the cooled reaction mixture, and these additions were repeated four times at intervals of 10 min. The mixture was acidified to pH 2 with HCl 15 min. after the last addition, and repeatedly extracted with chloroform. The extract was washed with water, dried over anhydrous CaSO₄, and evaporated to give a syrup (0.8 g.). This was dissolved in acetone (5 ml.) and an ethereal solution of dicyclohexylamine was added until the pH of the reaction mixture reached 9. Crystals (0.4 g.) of the dicyclohexylammonium salt of *N*-acetyl-*S*-(2-hydroxybutyl)-*L*-cysteine separated. Three recrystallizations from acetone–ether gave fine needles (180 mg.), m.p. 146–148°, [α]_D²⁰ + 77 ± 7° (c 0.26 in water) (Found: C, 60.55; H, 9.3; N, 6.5; S, 7.8; C₂₁H₄₀N₂O₄S requires C, 60.6; H, 9.6; N, 6.7; S, 7.7%).

Method (b). The method described by James & White (1967) for the preparation of *N*-acetyl-*S*- β -hydroxyphenylethyl-*L*-cysteine was followed except that the reaction mixture was heated in a sealed tube at 100° for 6 hr. The product isolated as described in method (a) had properties identical with those of the compound obtained by that method.

*N-Acetyl-*S*-(3-hydroxybutyl)-L-cysteine.* But-1-en-3-ol was prepared by a Grignard reaction between methyl iodide acrolein, as described for the preparation of pent-1-en-3-ol in *Organic Syntheses*, (1955). The but-1-en-3-ol obtained had b.p. 97–99°/758 mm., n_D^{20} 1.4148. Morell & Auernheimer (1944) give b.p. 96.0–96.5°/745 mm., n_D^{20} 1.4137. But-1-en-3-ol (1.2 ml.), *N*-acetylcysteine (1.6 g.), dry chloroform (10 ml.) and a trace of benzoyl peroxide were stirred in a quartz flask for 2.5 hr. while being irradiated with ultraviolet light from a Hanovia lamp (Prescription Lamp, model 4; maximum emission at 313 m μ) placed 15 cm. from the flask (cf. Stacey & Harris, 1963). The reaction mixture was evaporated and the residue extracted with ether to remove some unchanged *N*-acetylcysteine. The remaining material was purified by chromatography on paper sheets in solvent *B*, as described above, and the eluted material was converted into the free acid and thence into its dicyclohexylammonium salt as described above. The product was recrystallized from ethanol–acetone to give crystals (104 mg.), m.p. 187–189° (Found: C, 60.7; H, 9.5; N, 6.6; S, 8.0; C₂₁H₄₀N₂O₄S requires C, 60.6; H, 9.6; N, 6.7; S, 7.7%). The infrared spectrum (KBr disk) was different from that of the 2-hydroxy isomer.

3-(Butylthio)lactic acid. *S*-Butylcysteine (0.34 g.) and acetic acid (5 ml.) were stirred mechanically during the addition of 0.14 g. of NaNO₂ in 2 ml. of water over 10 min.

The mixture was stirred for a further 5 min. and then continuously extracted with ether for 3 hr. The extract was evaporated *in vacuo* to remove solvent and acetic acid, and the product was streaked on to paper sheets, which were developed in solvent *D* for 16 hr. A sample strip sprayed with the $K_2Cr_2O_7$ - $AgNO_3$ reagent showed the presence of two major sulphur-containing compounds. The zones corresponding to the slower-running compound, which was present in the greater amount, were cut out and eluted. The product obtained on evaporation of the eluate was dissolved in water and passed down a Zeo-Karb 225 column (H^+ form), and the aqueous eluate from the column was evaporated *in vacuo* to an oil. Its chromatographic properties are given in Table 1. The compound was shown to be an acid by the positive reaction given with the 8-hydroxyquinoline sulphate- $ZnSO_4$ spray of Pesetz & Ferrero (1957) after chromatography in the ammoniacal solvents *B*, *C* and *D*. A sample of the acid was converted into the methyl ester by treatment with diazomethane. The retention times of the methyl ester were measured with gas-liquid chromatography columns I and II (see the Methods section). The infrared spectrum of the methyl ester in solution in CS_2 gave main peaks at 738, 1090, 1175, 1215, 1680, 1735 and 2851 cm^{-1} . The mass spectrum showed prominent peaks at *m/e* 192, 174 and 103. Mass measurement of these peaks fitted formulae $C_8H_{16}O_3S$, representing the molecular ion of methyl 3-(butylthio)lactate, $C_8H_{14}O_3S$, a substance formed by loss of water from the parent compound, and $C_5H_{11}S$, an expected fragment ($C_4H_9 \cdot S \cdot CH_3$) from methyl 3-(butylthio)lactate. A peak was also seen at *m/e* 88, which could arise by loss of CH_3 from $C_5H_{11}S$.

³⁵S-labelled yeast. Yeast was grown in a medium containing [³⁵S]sulphate as described by Williams & Dawson (1952).

METHODS

Animals, diet and dosage

Doe rabbits (2.5-3 kg.) and female rats (200-250 g.) were maintained as described by Bray *et al.* (1959). The rats used for the experiments with ³⁵S were maintained as described by James & White (1967).

Chromatography

Paper chromatography. Whatman 3MM paper was used and the chromatograms were developed by the descending method. The solvent mixtures used are described in Table 1. The chromatograms were developed for 16 hr. with solvents *A*, *B* and *C* and for up to 48 hr. with solvent *D*. The relative rates of movement of the reference compounds are given in Tables 1 and 3. The detecting reagents used were the $K_2Cr_2O_7$ - $AgNO_3$ reagent of Knight & Young (1958) for bivalent-sulphur compounds and the chloroplatinate reagent of Toennies & Kolb (1951) as modified by Barnsley, Thomson & Young (1964) for bivalent-sulphur-containing compounds and sulfoxides. The KI-HCl reagent of Thompson, Arnold & Morris (1963) was also used for sulfoxides. The ninhydrin reagent of Moffat & Lytle (1959) was used for amino acids and glutathione derivatives.

Thin-layer chromatography. Glass plates coated with silica gel (E. Merck A.-G., Darmstadt, Germany) 250 μ thick were used. The chromatograms were developed by the ascending method with solvent *G* (Table 3).

Gas-liquid chromatography. A Pye series 104 dual-flame-ionization chromatograph (W. G. Pye and Co. Ltd. & Cambridge) was used. Column packings were *E*, 5% QF₁ on acid-washed silanized Chromosorb W (James, Waring & White, 1967) and *F*, Fluoropak 80 coated with 10% Carbowax 207 terminated with terephthalic acid. The reference compounds and metabolites from urine were esterified with diazomethane before application to the column. The reference compounds that were isolated as the dicyclohexylammonium salts were desalted with Zeo-Karb 225 before esterification.

Experiments with rats fed with ³⁵S-labelled yeast

Samples of normal urine of rats fed with ³⁵S-labelled yeast and of urines from the same rats after dosing were acidified to pH 2 and extracted with ether. Samples of the two extracts and of the two urine residues from each rat were applied to paper sheets and spaced 2 in. apart. The developed chromatograms were dried, cut into strips and scanned by a Packard scanner with helium+butane (98.7:1.3) as the detector gas. The positions of the peaks obtained on the chart were compared with those of the reference compounds rendered visible with the chloroplatinate reagent. The strips were photographed by exposure to X-ray film for 2 weeks and were then dipped in the chloroplatinate reagent. Only traces of radioactive material were found in the ethereal extracts of the acidified urine of undosed rats; all the radioactive metabolites from dosed rats were extracted by ether.

Examination of urines

In general, untreated urines were examined by paper chromatography. Ethereal extracts were made from urines adjusted to pH 2 with HCl and examined by paper chromatography and, after esterification with diazomethane, by gas-liquid chromatography.

Determination of metabolites in urine

Urine was collected for the 24 hr. before dosing; after dosing urines were collected and assayed every 24 hr. until the metabolites were not detected. The doses were administered by stomach tube as suspensions in water; the amounts given are shown in Table 2.

Mercapturic acid. The chromatographic method of Bray *et al.* (1964) was used, but in some experiments the mercapturic acids were rendered visible by dipping the papers in the chloroplatinate reagent instead of spraying them with $K_2Cr_2O_7$ - $AgNO_3$. For 1-bromobutane measurements were made in solvent *D* as well as in solvent *C*. Calibration curves were constructed with either butylmercapturic acid or the dicyclohexylammonium salt of (2-hydroxybutyl)-mercapturic acid. Equimolar amounts of butyl- and (2-hydroxybutyl)-mercapturic acid gave the same areas on the chromatograms within the experimental error of the method ($\pm 7\%$). Equimolar amounts of butylmercapturic acid and (3-hydroxybutyl)mercapturic acid gave areas in the ratio 1.3:1 over the concentrations used. Allowance for this was made in calculating the results.

Keto acid. The method of Shen & Lewis (1946) was used with phenylpyruvic acid as standard.

Table 1. R_F values and retention times of reference compounds and of metabolites excreted in urine

The solvent mixtures used were: *A*, butan-1-ol-water-acetic acid-butyl acetate (24:10:5:2, by vol.); *B*, butan-1-ol saturated with aq. $2x-NH_3$; *C*, butan-1-ol-ethanol-aq. NH_3 (sp.gr. 0.88)-water (10:10:1:4, by vol.); *D*, butan-2-one saturated with aq. $2x-NH_3$. Details of the reagents used for detecting spots are given in the text. Methyl esters were prepared for gas-liquid chromatography on columns *E* and *F*, details of which are given in the text. Colours with the detecting reagents: C, cream; O, orange; W, white; Y, yellow. Other symbols: ne, not examined; ns, spot not seen.

	Paper chromatography										Gas-liquid chromatography			
	R_F values					Colour					Retention time (min.)			
	Solvent ...	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i> *	Reagent ...	$K_2Cr_2O_7$ - $AgNO_3$	Chloro- platinate	Column ...	Temp. ...	174°	196°	199°	233°
Reference compounds														
<i>N</i> -Acetyl- <i>S</i> -butyl-L-cysteine	(1)	0.89	0.32	0.63	1.0		O	W			8.4	3.6	18.8	6.2
3-(Butylthio)lactic acid	(2)	0.85	0.36	0.62	1.65		C	W	1.4, 1.65		—	—	1.85, 4.9	—
<i>N</i> -Acetyl- <i>S</i> -(2-hydroxybutyl)-L-cysteine	(3)†	0.79	0.15	0.48	0.30		O	W			—	7.8	—	21.2
<i>N</i> -Acetyl- <i>S</i> -(3-hydroxybutyl)-L-cysteine	(4)	0.76	0.10	0.45	0.21		O	W			—	9.3	—	28.8
<i>N</i> -Acetyl- <i>S</i> -butyl-L-cysteine sulphoxide†	(5)‡	0.74	0.10	0.47	0.23		†	Y			—	—	—	—
Metabolites of 1-bromobutane														
In rabbit urine	(1)	0.88	0.34	0.63	1.0		O	W	8.3	3.6	19.2	—	1.75, 4.8	6.2
	(2)	0.81	0.34	0.58	1.6		C	W	1.4, 1.65		—	—	—	—
	(3)†	0.74	0.15	0.46	0.21		{ O	W			—	7.8	—	21.2
	(4)	0.76	0.10	0.46	0.22		{ O	W			—	9.3	—	29.3
Isolated metabolite	(1)	0.88	0.29	0.62	1.0		O	W			—	9.3	—	28.8
In rat urine	(2)†	ns	0.30	ns	1.5		C	W	8.4	3.6	ne	—	ne	6.2
	(3)	0.76	0.15	0.45	{ 0.30		O	W	1.6		—	—	—	—
	(4)	ns	ns	0.47	0.22		O	W			—	7.8	—	21.6
	(5)‡	0.76	ne	0.46	ne		†	Y			—	9.3	—	28.3
Isolated metabolite	(1)	0.91	0.32	0.63	1.0		O	W			—	—	—	—
Metabolites detected on radioautograph	(2)†	\$	\$	\$	1.65		+	W			7.8, † 9.1	—	—	21.6, † 28.4
	(3)	\$	\$	\$	0.27		O	W			—	—	—	—
	(4)	0.77	0.10	0.45	0.22		O	W			—	—	—	—
Metabolite of 1,2-epoxybutane														
In rabbit urine	(1)	0.79	0.14	0.48	0.31		O	W			—	7.8	—	20.2
In rat urine	(2)	0.79	0.15	0.48	0.31		O	W			—	7.8	—	20.5

* The solvent was allowed to run off the paper. The rate of movement of the spots is related to that of butylmercapturic acid, which is taken as 1.0.

† Detected by KI-HCl reagent and by gas-liquid chromatography. Retention time on column *E* at 220° is 8.9 min.

‡ Only traces detected.

\$ Not distinguished, as it forms a composite spot with metabolite (1).

RESULTS

Detection and isolation of metabolites of 1-bromobutane in urine

The results obtained by paper chromatography and gas-liquid chromatography are given in Table 1. None of the metabolites was present in urine of undosed animals.

Metabolite 1. This was isolated and identified as butylmercapturic acid by Bray *et al.* (1964).

Metabolite 2. This compound, identified as 3-(butylthio)lactic acid, was easily extracted by ether from acidified urine and was clearly distinguished from butylmercapturic acid by the cream colour given with the potassium dichromate-silver nitrate reagent and the clear difference in R_F values in solvent *D*. Although this compound was readily detected in rabbit urine, only traces were found in rat urine.

The metabolite was separated by paper chromatography with solvent *D*, the appropriate zone being eluted with aqueous methanol. The eluate was evaporated under reduced pressure below 40° and the residue dissolved in water and passed down a Zeo-Karb 225 column (H^+ form) as described in the Materials section. Gas-liquid chromatography of the methyl ester gave two peaks with the same retention times on columns *E* and *F* as those found for synthetic methyl (butylthio)lactate. The main peaks in the infrared spectrum determined in CS_2 were 738, 1105, 1175, 1215, 1685, 1735 and 2851 cm^{-1} . The mass spectrum of the methyl ester of the metabolite showed a prominent peak at m/e 174, mass measurement of which fitted the formula $C_8H_{14}O_2S$, and a smaller peak at m/e 192 representing the molecular ion. Other peaks at m/e 103 and 88 corresponded to the fragments $C_4H_9 \cdot S \cdot CH_2$ and $C_3H_8 \cdot S \cdot CH_2$. This confirms that the ester was methyl (butylthio)lactate.

Metabolite 3. This compound, identified as *N*-acetyl-*S*-(2-hydroxybutyl)-L-cysteine, was detected on the radioautographs of chromatograms of rat urine in solvents *A*, *B*, *C* and *D*. It was only clearly recognized by the detecting reagents on paper chromatograms from rat urine in solvent *D* because it is accompanied by metabolite 4, *N*-acetyl-*S*-(3-hydroxybutyl)-L-cysteine, which has nearly the same R_F values in solvents *A*, *B* and *C* as metabolite 3 so that a composite spot is obtained. In the radioautographs with solvents *A*, *B* and *C*, two zones could be distinguished in the composite spot corresponding to the slightly different R_F values of the two isomers. Two peaks were also seen when the spot was examined in a Packard scanner. Metabolite 3 was detected by gas-liquid chromatography on columns *E* and *F*, which clearly distin-

guished between the 2- and 3-isomers. Only traces were detected in the urine of dosed rabbits. (2-Hydroxybutyl)mercapturic acid was also the major sulphur-containing metabolite of 1,2-epoxybutane in the rabbit and rat, and was identified by paper and gas-liquid chromatography.

Metabolite 4. This compound, identified as *N*-acetyl-*S*-(3-hydroxybutyl)-L-cysteine, was clearly distinguished by gas-liquid chromatography as a constituent of methylated extracts of acidified urines of dosed rabbits and rats.

(a) Isolation from rabbit urine. The urine of 11 rabbits that had each received 1-bromobutane (625mg.) was acidified with hydrochloric acid to pH 2 and extracted with ether to remove most of the hippuric acid and butylmercapturic acid, the hydroxybutylmercapturic acid being less readily soluble in ether. Ether was removed by aeration from the residual urine, which was then adjusted to pH 4.5. Charcoal (40g./500ml. of urine) was added and the mixture continuously stirred for about 30 min. The charcoal was filtered off, washed with water (2l.) and eluted with methanol (1.5l.) containing 5% of aq. ammonia, sp.gr. 0.88. The eluate was evaporated and the residue dissolved in water. On continuous extraction with chloroform a product containing the hydroxybutylmercapturic acid and some butylmercapturic acid was obtained. The mixture was separated on a cellulose powder column (50cm. $\times$ 3.5cm.) with solvent *C*. The fractions containing the hydroxybutylmercapturic acid were combined and purified by paper chromatography with solvent *C* and treatment of the eluate on a column of Zeo-Karb 225. The free acid obtained on evaporation was dissolved in acetone, and a solution of dicyclohexylamine in acetone was added until the mixture was alkaline (pH 9). The crystals that separated were recrystallized from acetone-ethanol to give the dicyclohexylammonium salt (50mg.), m.p. 186–187° unchanged by admixture with an authentic sample of the dicyclohexylammonium salt of *N*-acetyl-*S*-(3-hydroxybutyl)-L-cysteine (Found: C, 60.2; H, 9.3; N, 6.9; S, 8.3; $C_{21}H_{40}N_2O_4S$ requires C, 60.6; H, 9.6; N, 6.7; S, 7.7%). The infrared spectrum (potassium bromide disk) was identical with that of the synthetic compound. Gas-liquid chromatography showed that a trace of (2-hydroxybutyl)mercapturic acid was present.

(b) Isolation from rat urine. The urine of 12 rats that had each been dosed with 1-bromobutane (50mg.) was treated as described above except that the initial ether extraction was omitted. The hydroxy acid isolated as the dicyclohexylammonium salt (77mg.) had m.p. 155–157° after recrystallization from acetone-ether. Paper chromatography showed that it contained some butylmercapturic acid, and gas-liquid chromatography showed that,

Table 2. *Amounts of metabolites excreted in urine by rabbits and rats*

The results are means, expressed as percentages of the dose with the ranges in parentheses; the numbers of determinations are indicated by the superior numbers. N.D., not determined.

Compound administered ... Dose (m-moles /kg.) ...	Rabbit		Rat	
	1-Bromobutane 1.7	1,2-Epoxybutane 1.9	1-Bromobutane 2.3	1,2-Epoxybutane 2.5
Material determined				
Butylmercapturic acid	8 (8, 8) ²	0 ⁵	7 (5-10) ³	0 ⁵
Hydroxybutylmercapturic acid	14 (13, 16) ²	4 (2-5) ⁵	22 (17-26) ³	11 (7-16) ⁵
Keto acid	2 (1-3) ^{3*}	N.D.	N.D.	N.D.

*About half the material causing the increase observed was ether-soluble.

although both (2-hydroxybutyl)- and (3-hydroxybutyl)-mercapturic acid were present, the major component was the 3-hydroxy derivative.

(c) Isolation of hydrolysed metabolite 4 as the *N*-toluene-*p*-sulphonyl derivative. A fraction containing the hydroxybutylmercapturic acid was prepared from the urine of four rabbits as described above. It was dissolved in 2*N*-hydrochloric acid (15ml.) and heated at 100° under reflux for 3.5 hr. The solution was cooled, neutralized and concentrated under reduced pressure, and separated by paper chromatography with solvent *A*; the acid was eluted with methanol. Evaporation of the eluate gave a yellow solid (0.5g.) which was dissolved in 2*N*-sodium hydroxide (10ml.) and shaken with an ethereal solution of toluene-*p*-sulphonyl chloride (1g.) for 3 hr. Ether was separated from the reaction mixture, and the aqueous solution was acidified to pH 2 with dil. hydrochloric acid. The crystals that separated were recrystallized from aq. methanol to give needles (4.3mg.), m.p. 134°, $[\alpha]_D^{26} + 68.7 \pm 2^\circ$ (c 0.18 in ethanol). These properties were clearly different from those of synthetic *N*-toluene-*p*-sulphonyl-*S*-(2-hydroxybutyl)-L-cysteine. The infrared spectra of the isolated material (in Nujol mull) and that of the methyl ester (in CS₂) closely resembled, but were not identical with, those from the synthetic 2-hydroxy compound. The toluene-*p*-sulphonyl derivatives of the 2- and the 3-hydroxy compounds were indistinguishable by paper chromatography.

Metabolite 5. Traces of this compound, identified as butylmercapturic acid sulfoxide, were detected by paper chromatography in the urine of rats but not of rabbits. It was recognized by the yellow colour given with the chloroplatinate reagent and the positive reaction with the potassium iodide-hydrochloric acid reagent.

Determination of metabolites excreted in urine

The results are given in Table 2. The determination of butylmercapturic acid in solvents *C* and *D* gave the same result within experimental error,

so that the amount of (butylthio)lactic acid excreted by the rabbit did not affect the area of the butylmercapturic acid spot in solvent *C*.

The small increase in the excretion of keto acid observed was of doubtful significance, and no keto acid was detected on chromatograms of the urines or their ethereal extracts sprayed with DNP-hydrazine.

Excretion of metabolites of 1-bromobutane in rat bile

Rats were anaesthetized by the injection of Nembutal [0.3 ml. of a solution prepared by diluting veterinary Nembutal (Abbott Laboratories, Queensborough, Kent) with 4 vol. of 0.9% sodium chloride/100g. body wt.], and the bile duct was cannulated about 1 cm. above the entry into the duodenum. Normal bile was collected for 1½ hr. and then 60mg. of 1-bromobutane in 0.25 ml. of sunflower-seed oil was injected subcutaneously in the lumbar region. Urine and bile were then separately collected for about 6 hr. In some experiments saline was infused continuously into the tail vein (Barnes *et al.* 1959). Samples of the urine and the bile before and after dosing were examined by paper (solvents *A* and *C*) and thin-layer (solvent *G*) chromatography. Normal bile was spotted on to the reference compounds used. The results are summarized in Table 3. *S*-Butylglutathione, *S*-butylcysteinylglycine and *S*-butylcysteine were detected. Butylmercapturic acid and hydroxybutylmercapturic acid were not detected in the bile but were present in the urine of the dosed animals.

Experiments with tissues

Formation of *S*-butylglutathione. A rabbit was killed by a blow on the back of the neck, and the liver was quickly removed and ground with sand and three times its weight of sodium phosphate buffer, pH 8.0. The mixture was centrifuged at 2000*g* for 15 min., and 60 ml. of the supernatant was continuously shaken at room temperature with

Table 3. R_F values of reference compounds and of sulphur-containing metabolites excreted in the bile of rats dosed with 1-bromobutane

The solvent mixtures used were: *A* and *C*, as in Table 1; *G*, butan-1-ol-acetic acid-water (4:1:1, by vol.). Colours with ninhydrin: dB, dark brown; YB, yellow-brown; M, maroon. ns, no spot seen.

			R_F values			
Chromatography	...	...	Paper		Thin-layer	Colour with ninhydrin in solvent G
			A	C	G	
Reference compounds						
S -Butylcysteine			0.59	0.55	0.49	dB
S -Butylcysteinylglycine			0.53	0.47	0.47	YB
S -Butylglutathione			0.41	0.16	0.30	M
Butylmercapturic acid			0.89	0.63	0.67	—
(2-Hydroxybutyl)mercapturic acid			0.79	0.48	0.54	—
(3-Hydroxybutyl)mercapturic acid			0.76	0.45	0.54	—
Metabolites detected						
(a)			0.59	0.56	0.49	dB
(b)			ns	0.48	0.47	YB
(c)			0.41	0.16	0.30	M

12ml. of ethanolic 0.17M-1-bromobutane. The pH was adjusted to 4–4.5 by the addition of acetic acid, and the precipitate was removed by centrifugation. The supernatant was stirred with charcoal (2g.) for about 15 min. The charcoal was filtered off, washed with water, and eluted with methanol (100ml.) containing 5ml. of aq. ammonia, sp.gr. 0.88. The eluate was evaporated under reduced pressure at 40–50° to give a colourless residue, which was dissolved in aq. ethanol (0.3ml.). Paper chromatography revealed, in solvents *A* and *C* (Table 3) and *B*, a spot corresponding to *S*-butylglutathione (R_F 0.04 in solvent *B*), and, in solvents *B* and *C* only, a faint spot corresponding to *S*-butylcysteine (R_F 0.23 in solvent *B*). These spots were not present in liver extract without 1-bromobutane. *S*-Butylglutathione was detected in similar experiments with rabbit and rat liver at pH 7.4.

Formation of hydroxybutylmercapturic acid by liver slices. For the preparation of digests of rabbit or rat liver slices the method described by Bray *et al.* (1959) was used. For each experiment three digests were prepared, containing butylmercapturic acid (8mg.), *S*-butylcysteine (8mg.) and no substrate respectively in 20ml. of buffer, and to each was added 4g. of liver slices cut by machine. The digests were incubated for 4 hr. at 37°. The pH was then adjusted to 5.0 with acetic acid and the mixture homogenized in a Potter–Elvehjem homogenizer. The homogenate was heated at 70–75° for a few minutes, and the precipitated protein was removed by filtration. The filtrate was further acidified to pH 2 and continuously extracted with ether. Paper and gas-liquid chromatography of the extracts showed that (3-hydroxybutyl)mercap-

turic acid had been formed from butylmercapturic acid and from *S*-butylcysteine by slices of rabbit and rat liver, and that (2-hydroxybutyl)mercapturic acid was formed only by slices of rat liver.

DISCUSSION

The hydroxybutylmercapturic acid excreted by rabbits dosed with 1-bromobutane consisted chiefly of the (3-hydroxybutyl)mercapturic acid with a trace of the 2-hydroxy isomer. Rats, however, excreted both the 2- and the 3-hydroxy isomer, the latter predominating. The hydroxybutylmercapturic acid excreted by rabbits and rats dosed with 1,2-epoxybutane consisted of (2-hydroxybutyl)mercapturic acid only.

Butylmercapturic acid sulphoxide was detected in traces as a metabolite of 1-bromobutane in the rat but not in the rabbit. The presence of the sulphoxide was not detected by James (1961). Ethylmercapturic acid sulphoxide is a metabolite of ethylmercapturic acid. It is formed as a metabolite of bromoethane in the rat and is excreted in approximately the same amount as is ethylmercapturic acid (Barnsley *et al.* 1964). The excretion of *n*-propylmercapturic acid sulphoxide formed from 1-bromopropane by rats represents about 14% of the total sulphur-containing metabolites formed (Barnsley, Grenby & Young, 1966).

Maw & Coyne (1966) showed that *S*-methyl- and *S*-ethyl-cysteine were converted by yeast into 3-(methylthio)- and 3-(ethylthio)-lactic acid respectively and Harris, James & Needham (1968) found that *S*-butylcysteine was similarly converted into 3-(butylthio)lactic acid by yeast. The present work

that the rat converted *S*-methyleysteine into 2-hydroxy-3-(methylsulphinyl)propionic acid, the sulphoxide of 3-(methylthio)lactic acid. The excretion by a mental patient of a new sulphur-containing amino acid that consisted of the disulphide of cysteine and 3-mercaptolactic acid was described by Ampola *et al.* (1968).

The initial fate of 1-bromobutane in the rat resembles that of iodomethane (Johnson, 1966a), which combines with GSH in the liver, a considerable amount of the *S*-methylglutathione formed being excreted in the bile with a trace of *S*-methyleysteine. When 1-bromobutane is given, *S*-butylglutathione, *S*-butyleysteinyglycine and *S*-butyleysteine are excreted in bile. Boyland, Ramsay & Sims (1961) described the biliary excretion of naphthalene and 1,2-dihydronaphthalene in the form of derivatives of GSH, cysteinylglycine and cysteine.

Possible steps in the metabolism of 1-bromobutane are shown in Scheme 1. The initial step, probably catalysed by the glutathione *S*-alkyltransferase described by Johnson (1963, 1966b), results in the formation of *S*-butylglutathione (I) and the excretion of bromide. Compound I is converted into *S*-butyleysteine (III) via *S*-butyleysteinyglycine (II). *S*-Butylmercapturic acid (IV) is produced by the acetylation of compound III and is further metabolized to (2-hydroxybutyl)- and (3-hydroxybutyl)-mercapturic acid (V and VI). Compound VI is formed by both the rabbit and the rat, compound V appears to be formed in easily detectable amounts only by the rat. Although it has been shown that hydroxylation of butylmercapturic acid can occur, the possibility that the hydroxyl group is introduced into the alkyl chain at an earlier stage in the metabolism of 1-bromobutane has not been excluded. James & Jeffery (1964b) suggested that an epoxide might be an intermediate in the metabolism of 1-bromoalkanes and bromocyclohexane. Dehydrobromination of 1-bromobutane would give 1,2-epoxybutane (IX) which forms compound V but not compound VI in both rabbit and rat. The fact that the rabbit excretes only traces of compound V after receiving 1-bromobutane suggests that the 'epoxide pathway' is not used to a significant extent by this species, but this pathway might be used in the rat. Compound V is, however, formed by hydroxylation of butylmercapturic acid by slices of rat liver. It is perhaps relevant that the rat converts a greater portion of administered 1,2-epoxybutane into compound V than does the rabbit, and a similar difference was observed in the formation of β -hydroxyphenethylmercapturic acid from styrene oxide (James & White, 1967). Liver glutathione *S*-epoxidetransferase (Boyland & Williams, 1965) may be less active in the rabbit than in the rat.

The formation of (3-hydroxybutyl)mercapturic

acid is a further example of hydroxylation of an alkyl chain in the penultimate position (Brodie, Gillette & La Du, 1958). The conversion of butyl *p*-nitrophenyl ether into 3-hydroxybutyl *p*-nitrophenyl ether by rabbit and rat was reported by Yoshimura, Ida, Tsuji, Mori & Tsukamoto (1967).

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