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Biochemical Studies of Toxic Agents

THE METABOLISM OF 1- AND 2-BROMOPROPANE IN RATS

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1. (+)-*n*-Propylmercapturic acid sulphoxide, i.e. (+)-*N*-acetyl-*S*-*n*-propyl-L-cysteine *S*-oxide, was prepared as the dicyclohexylammonium salt, (−)-*n*-propylmercapturic acid sulphoxide was prepared as the free acid, and *S*-isopropyl-L-cysteine and isopropylmercapturic acid were also prepared. 2. The metabolism of 1- and 2-bromopropane was studied by radiochromatographic examination of the urine excreted by rats that had been fed with a diet containing ^{35}S-labelled yeast and then injected subcutaneously with these compounds. In addition to *n*-propylmercapturic acid and 2-hydroxypropylmercapturic acid, the excretion of which has already been reported, *n*-propylmercapturic acid sulphoxide was shown to be a metabolite of 1-bromopropane. Sulphur-containing metabolites of 2-bromopropane, if present in the urine at all, were there in very small amounts. 3. *n*-Propylmercapturic acid and isopropylmercapturic acid were isolated from the urine of rats that had been injected subcutaneously with *S*-*n*-propyl-L-cysteine and *S*-isopropyl-L-cysteine respectively.

1-Halogenopropanes are metabolized in animals to *n*-propylmercapturic acid, i.e. *N*-acetyl-*S*-*n*-propyl-L-cysteine (Grenby & Young, 1959, 1960), and other halogenoalkanes also give rise to alkylmercapturic acids (Bray & James, 1958, 1960; Thomson, Maw & Young, 1958; Thomson, Barnsley & Young, 1963; Bray, Caygill, James & Wood, 1964; Barnsley & Young, 1965). Other sulphur-containing metabolites derived from halogenoalkanes have been described. Bromo- and iodoethane are metabolized to ethylmercapturic acid sulphoxide (Thomson & Young, 1960; Barnsley, Thomson & Young, 1964), and 1-bromobutane and its higher homologues are converted into hydroxyalkylmercapturic acids (Bray *et al.* 1964; James & Jeffery, 1964) but not into the mercapturic acid sulphoxides (Bray & James, 1960). 2-Hydroxypropylmercapturic acid is formed from 1-bromopropane in the rat (Barnsley, 1964*a*), and it has now been shown that 1-bromopropane is also metabolized to *n*-propylmercapturic acid sulphoxide and that the latter metabolite and *n*-propylmercapturic acid are formed from *S*-*n*-propyl-L-cysteine. The metabolism of 2-bromopropane was also examined, and only occasionally was a trace of a sulphur-containing metabolite detected in the urine. It was shown, however, that *S*-isopropyl-L-cysteine, which would be formed by the *S*-alkylation of a cysteine residue by 2-bromopropane, is acetylated *in vivo* to give isopropylmercapturic acid.

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MATERIALS

Melting points are uncorrected. Elementary microanalyses were carried out by Weiler and Strauss, Oxford.

1-Bromo- and 2-bromo-propane (British Drug Houses Ltd., Poole, Dorset) were redistilled before use. The preparations of *S*-*n*-propyl-L-cysteine, propane-1-thiol mercury chloride and *n*-propylmercapturic acid were described by Grenby & Young (1960): *S*-*n*-propyl-L-cysteine, m.p. 245–247° (decomp.), $[\alpha]_D^{20}$ −22° (*c* 2 in water) (Found: C, 44·3; H, 7·9; N, 8·6; S, 19·8. Calc. for $C_6H_{13}NO_2S$: C, 44·1; H, 8·0; N, 8·6; S, 19·6%); *n*-propylmercapturic acid, m.p. 96°, $[\alpha]_D^{22}$ −19° (*c* 2 in water) (Found: C, 46·6; H, 7·2; N, 6·7; S, 15·9. Calc. for $C_8H_{15}NO_3S$: C, 46·8; H, 7·4; N, 6·8; S, 15·6%); propane-1-thiol mercury chloride, m.p. 181° (decomp.) (Found: C, 11·4; H, 2·4; S, 10·4. Calc. for C_3H_7ClHgS: C, 11·6; H, 2·3; S, 10·3%).

Preparation of S-*isopropyl*-L-*cysteine.* A procedure similar to method I described by Grenby & Young (1960) for the preparation of *S*-*n*-propyl-L-cysteine was used, but twice as much 2-iodopropane as 1-iodopropane was used to complete the alkylation of the cysteine. The product obtained from the reaction of 12·0g. of L-cysteine was recrystallized from ethanol–water (9:1, v/v) and then from acetone–water (9:1, v/v); the yield was 12·7g., m.p. 234–236° (decomp.), $[\alpha]_D^{26}$ −17° (*c* 2 in water) (Found: C, 44·3; H, 7·7; N, 8·8; S, 20·0. Calc. for $C_6H_{13}NO_2S$: C, 44·1; H, 8·0; N, 8·6; S, 19·6%).

Preparation of isopropylmercapturic acid. This compound

was prepared by three methods similar to those described by Grenby & Young (1960) for the preparation of *n*-propylmercapturic acid. Acetylation of *S*-isopropyl-L-cysteine in alkaline solution was carried out with acetic anhydride, and after crystallization from water and from benzene the product (yield 79%) had m.p. 134°, $[\alpha]_D^{26} - 6°$ (*c* 2 in water) (Found: C, 46·7; H, 7·3; N, 6·6; S, 15·9. $C_8H_{15}NO_3S$ requires C, 46·8; H, 7·4; N, 6·8; S, 15·6%). Products with virtually the same melting point, optical rotation and elementary analysis were obtained by acetylation of *S*-isopropyl-L-cysteine with keten in alkaline solution and by alkylation of *N*-acetyl-L-cysteine with 2-iodopropane in liquid ammonia.

Preparation of (−)-n-propylmercapturic acid sulphoxide. *n*-Propylmercapturic acid (3·49g.) was dissolved in 60ml. of water and oxidized with 1·94ml. of 30% (w/v) H_2O_2. After 24hr. the solution was evaporated at 45–50° under reduced pressure and the gum that remained was dissolved in ethanol and crystallized by the addition of ethyl acetate. A crude product (2·03g.) was obtained, $[\alpha]_D^{18} - 21 \pm 2°$ (*c* 1 in water). The mother liquor was set aside for the recovery of the dextrorotatory isomer. The crude product was recrystallized four times from a 0·5% (w/v) solution in ethanol–ethyl acetate (1:40, v/v) by the addition of light petroleum (b.p. 40–60°) and gave 0·40g. of product, m.p. 123·5–124° (decomp.), $[\alpha]_D^{19} - 59 \pm 2°$ (*c* 1 in water) (Found: C, 43·9; H, 6·9; N, 6·1; S, 14·6; equiv.wt. 222. $C_8H_{15}NO_4S$ requires C, 43·4; H, 6·8; N, 6·3; S, 14·5%; equiv.wt. 221). Further recrystallization did not increase the specific rotation of the product.

Preparation of the dicyclohexylammonium salt of (+)-n-propylmercapturic acid sulphoxide. The mother liquor set aside after the separation of crude (−)-*n*-propylmercapturic acid sulphoxide from the oxidation products of 15g. of *n*-propylmercapturic acid was evaporated and the residue was dissolved in acetone. Some crystalline material contaminated with gum was separated by the addition of light petroleum (b.p. 40–60°) and the solution was neutralized with dicyclohexylamine. The crude product that separated was crystallized several times from a 10% (w/v) solution in ethanol by the addition of acetone and the final product weighed 4·7g., m.p. 167° (decomp.), $[\alpha]_D^{18} + 1 \pm 1°$ (*c* 1·5 in water) (Found: C, 59·6; H, 9·3; N, 7·0; S, 8·4. $C_{20}H_{38}N_2O_4S$ requires C, 59·7; H, 9·5; N, 7·0; S, 8·0%).

Attempts to prepare the free dextrorotatory acid in crystalline form were not successful. The dicyclohexylammonium salt (21·3mg.) was dissolved in water and the solution was passed through a column (2cm. × 0·8cm. diam.) of Zeo-Karb 225 resin (H^+ form). The column was washed with 10ml. of water and the total eluate was evaporated under reduced pressure over P_2O_5. After drying over P_2O_5 *in vacuo* the product (11·7mg.) had a glassy appearance and was hygroscopic, $[\alpha]_D^{5} + 34 \pm 2°$ (*c* 1·2 in water).

Preparation of propane-2-thiol mercury chloride. The method used was similar to that described by Grenby & Young (1960) for the preparation of propane-1-thiol mercury chloride. The product (74% yield) had m.p. 213–216° (decomp.) (Found: C, 11·8; H, 2·7; Cl, 11·1; S, 10·3. C_3H_7ClHgS requires C, 11·6; H, 2·3; Cl, 11·3; S, 10·3%).

Preparation of ^{35}S-labelled yeast. A dried yeast preparation in which the sulphur-containing compounds were labelled with radioactive sulphur was obtained by the method of Knight & Young (1958). The specific activity was 0·37mc/g.

METHODS

Animals and dosing. Male rats were fed with a diet of rat cake [J. Murray and Sons (London) Ltd.] and water, and urine was collected separate from faeces during consecutive 24hr. periods after dosing. Compounds were administered by subcutaneous injection to animals that had been lightly anaesthetized with ether.

Chromatography. Chromatograms were prepared by the ascending method on Whatman no. 1 paper by using four solvent mixtures: *A*, butan-1-ol–1·33N-acetic acid (1:1, v/v; top layer); *B*, pyridine–water–aq. ammonia (sp.gr. 0·88) (20:2:1, by vol.); *C*, butan-1-ol–acetic acid–water (4:1:5, by vol.; top layer); *D*, butan-1-ol–pyridine–3N-ammonia (4:3:3, by vol.). Three detecting reagents were used: the $K_2Cr_2O_7$–$AgNO_3$ reagent of Knight & Young (1958), and the platinum reagent of Toennies & Kolb (1951) and ninhydrin, both as used by Barnsley *et al.* (1964).

Radiochromatography. Radiochromatograms were prepared with solvents *C* and *D*, and were scanned automatically as was described by Barnsley *et al.* (1964).

Measurement of radioactivity in eluate fractions from Amberlite CG-400 resin columns. Samples (40μl.) of each eluate fraction were applied to disks (2cm. diam.) of Whatman no. 1 paper, dried in a stream of warm air and counted for 2min. in the apparatus described by Hawkins & Young (1954).

RESULTS

Metabolism of 1-bromopropane

Four male rats (body wt. 200–210g.) were fed with the diet described by Maw (1953) to which ^{35}S-labelled yeast had been added (5%, w/w). Each rat received 18g. of the diet each day for 3 days. On the fourth day ^{35}S-labelled yeast was omitted from the diet and two rats were each injected with 1ml. of a solution of 1-bromopropane in arachis oil (40%, w/v). The other pair were used as controls and given arachis oil alone.

Chromatograms of the urine were developed in solvent system *C*, scanned for radioactivity and then treated with the platinum reagent (Table 1). Three metabolites were detected that were not present in the urine of control animals. Two of the metabolites have previously been isolated and identified as *n*-propylmercapturic acid (Grenby & Young, 1959, 1960) and 2-hydroxypropylmercapturic acid (Barnsley, 1964*a*), and the third metabolite, which gave a yellow spot with the platinum reagent, has now been identified as *n*-propylmercapturic acid sulphoxide.

Radiochromatography indicated that the sulphoxide and 2-hydroxypropylmercapturic acid were incompletely separated from each other, and so they were eluted with water from the appropriate areas of chromatograms (7·5cm. wide) on each of which 0·1ml. of urine had been applied as a streak. The eluates were chromatographed and shown to be free

Table 1. *Radiochromatographic examination of the urine excreted by rats in the first 24hr. period after they had been fed with a diet containing* ^{35}S*-labelled yeast and had then been injected subcutaneously with* 1*-bromopropane*

Details are given in the text. The R_F values of urine components are for peaks on radiochromatograms; the R_F values of synthetic compounds were obtained by reaction with the platinum reagent. The radioactivity found in the metabolites that were detected with the platinum reagent is given in parentheses as percentages of the total radioactivity in those metabolites.

	R_F in solvent *C*			
Urine of dosed rat 1	0·13	0·64†	0·73*	0·92*
		(33)		(67)
Urine of dosed rat 2	0·12	0·63†	0·74*	0·91*
		(35)		(65)
Control urine, rat 3	0·13	—	—	—
Control urine, rat 4	0·14	—	—	—
(+)- and (−)-*n*-Propylmercapturic acid sulphoxide		0·63†		
2-Hydroxypropylmercapturic acid			0·72*	
n-Propylmercapturic acid				0·90*

* White spot with the platinum reagent.
† Yellow spot with the platinum reagent.

Table 2. *Radiochromatographic examination of* n*-propyl*[^{35}S]*mercapturic acid sulphoxide separated from the urine of rats that had been fed with a diet containing* ^{35}S*-labelled yeast and then dosed with* 1*-bromopropane*

Details are given in the text. The R_F values of the metabolite and its derivatives were obtained from radiochromatograms; the R_F values of synthetic compounds were obtained by chemical tests.

	R_F					
Solvent	*C*	*D*	*C*	*D*	*C*	*D*
S-*n*-Propyl-L-cysteine			0·64*	0·55*		
n-Propylmercapturic acid					0·87†	0·64†
			After acid decomposition		After reduction	
Metabolite	0·63‡	0·45‡	0·67*	0·57*	0·89†	0·65†
(+)- and (−)-*n*-Propylmercapturic acid sulphoxide	0·63‡	0·43‡	0·66*	0·56*		0·64†

* White spot with the platinum reagent and purple with ninhydrin.
† White spot with the platinum reagent.
‡ Yellow spot with the platinum reagent.

from *n*-propylmercapturic acid, and were evaporated at 45° under reduced pressure. The metabolites obtained from 20 strip-chromatograms were dissolved in 1ml. of 0·1M-sodium formate buffer, pH4·0, and fractionated on a column (25cm. × 1cm. diam.) of Amberlite CG-400 resin (formate form; 200–400 mesh) with 0·1M-sodium formate buffer, pH4·0. Fractions (2ml.) were collected and metabolites were detected by measuring the radioactivity in 40μl. samples as described in the Methods section. Two peaks of radioactivity in fractions 28–44 inclusive (40% of the radioactivity eluted) and fractions 51–71 inclusive (60% of the radioactivity eluted) were detected. The metabolite in fractions 51–71 inclusive gave a red colour in the nitroprusside reaction (Thomson *et al.* 1963) and was identified as 2-hydroxypropylmercapturic acid. Fractions 28–44 inclusive were combined and passed through a column (10cm. × 1cm. diam.) of Zeo-Karb 225 resin (H^+ form). The column was washed with water until the pH of the eluate rose to 5 and the total eluate was evaporated at 45° under reduced pressure. The residue was dissolved in 1ml. of water and subjected to further tests (Table 2).

Samples of the solution were decomposed with hydrochloric acid or reduced with thioglycollic acid and the reaction products were compared with those of synthetic *n*-propylmercapturic acid sulphoxide. After 0·1 ml. of the solution had been heated with an equal volume of concentrated hydrochloric acid in a sealed tube at 100° for 3 hr. a compound was detected that reacted both with the platinum reagent and with ninhydrin and was chromatographically identical with *S*-*n*-propyl-L-cysteine. When 0·1 ml. of the solution had been heated with 5 μl. of thioglycollic acid in a sealed tube at 100° for 3 hr. material chromatographically identical with *n*-propylmercapturic acid was detected. In these tests the metabolite and the synthetic *n*-propylmercapturic acid sulphoxide behaved identically. Similar types of reaction have been described previously for methyl- and ethyl-mercapturic acid sulphoxides (Barnsley *et al.* 1964; Barnsley, 1964*b*).

Origin of n-*propylmercapturic acid sulphoxide.* It was shown that *n*-propylmercapturic acid sulphoxide was not formed from *n*-propylmercapturic acid after the excretion of the latter in the urine. The urine (3 ml.) from rats fed with radioactive yeast and injected with 1-bromopropane was acidified with concentrated hydrochloric acid and extracted with 3 ml. of ethyl acetate, and the extract was chromatographed on strips of paper as described above. Only one radioactive compound was detected (R_F 0·87 in solvent *C*) and this was chromatographically identical with *n*-propylmercapturic acid. It was eluted with water, the eluates were evaporated at 45° under reduced pressure and the residue was dissolved in 1 ml. of normal rat urine. The urine was divided into three portions, which were adjusted to pH 5·2, 6·4 or 8·5. Samples (20 μl.) were chromatographed immediately in solvents *C* and *D* and again after standing for 24 hr. at room temperature. In all cases only *n*-propylmercapturic acid was detected by scanning for radioactivity and with the platinum reagent.

Metabolism of 2-bromopropane

An experiment similar to that described for the metabolism of 1-bromopropane was carried out with two rats that had been fed with the radioactive diet and then dosed with 0·7 ml. of a 40% (w/v) solution of 2-bromopropane in arachis oil. Radiochromatography of the urine excreted in three consecutive 24 hr. periods after dosing and comparison with controls revealed no significant peaks of radioactivity that could be ascribed to metabolites of 2-bromopropane, and none was detected after application of the platinum reagent. When an ethyl acetate extract of acidified urine that had been excreted in the first 24 hr. period after dosing was chromatographed, traces of radioactive material with the same R_F as isopropylmercapturic acid were detected. This material also gave a white spot with the platinum reagent. When the experiment was repeated with rats that had been fed with a non-radioactive diet, material with the R_F of isopropylmercapturic acid was not always detected with the platinum reagent on chromatograms of extracts of acidified urine.

Metabolism of S-n-*propyl*-L-*cysteine*

Chromatographic studies. Two rats were each injected subcutaneously with 2 ml. of a solution (2·6% w/v) of *S*-*n*-propyl-L-cysteine in M-sodium hydrogen carbonate. The urine excreted in the first 24 hr. period (38 ml., pH 8) was chromatographed in solvent *C* and the chromatogram was dipped in the platinum reagent. Two metabolites were detected with R_F values 0·62 (metabolite 1) and 0·90 (metabolite 2). The metabolites were eluted from the appropriate areas of chromatograms (7·5 cm. wide) to each of which 0·1 ml. of urine had been applied. The eluates were evaporated under reduced pressure until the concentration of the solute was approximately equal to that in the original urine, and they were then subjected to reaction with hydrochloric acid and reduction with thioglycollic acid as described above. The eluates containing metabolite 2 were also oxidized with hydrogen peroxide [0·1 ml. of eluate and 0·1 ml. of 3% (w/v) hydrogen peroxide at room temperature for 3 hr.]. The results were consistent with the identification of metabolites 1 and 2 as *n*-propylmercapturic acid sulphoxide and *n*-propylmercapturic acid respectively, for the metabolites behaved identically with the synthetic compounds in these tests (Table 3). The excretion of *n*-propylmercapturic acid was confirmed by the isolation of this compound from the urine of rats dosed with *S*-*n*-propyl-L-cysteine.

Isolation of n-*propylmercapturic acid.* Sixty-six male rats (body wt. 205–240 g.) were each injected with 2 ml. of a saturated solution of *S*-*n*-propyl-L-cysteine in water, and urine was collected for 24 hr. after dosing. A second dose was then given and urine was again collected for 24 hr. The animals received a total of 5·0 g. of *S*-*n*-propyl-L-cysteine, and 1120 ml. of urine, pH 6·6, was collected. The urine was subjected to the extraction procedure described by Grenby & Young (1960), and the final product was obtained by crystallizing the crude gum from ethyl acetate at −20°. The product (0·87 g.) represented 14% of the compound administered and had m.p. 95° unchanged on admixture with synthetic *n*-propylmercapturic acid, $[\alpha]_D^{21}$ −18° (*c* 2 in water) (Found: C, 46·8; H, 7·0; N, 6·4; S, 15·5. Calc. for $C_8H_{15}NO_3S$: C, 46·8; H, 7·4; N, 6·8; S, 15·6%). The product was chromatographed and the chromatograms were sprayed with

Table 3. *Chromatographic examination of the metabolites obtained from the urine of rats that had been dosed with* S-n-*propyl*-L-*cysteine*

Details are given in the text. Chromatograms were treated with the platinum reagent and then with ninhydrin.

	R_F						
Solvent	*C*	*D*	*C*	*D*	*D*	*C*	*D*
S-*n*-Propyl-L-cysteine			0·65*	0·56*			
			After acid decomposition		After reduction	After oxidation	
Metabolite 1	0·62†	0·44†	0·64*	0·55*	0·64‡		
(+)- and (−)-*n*-Propylmercapturic acid sulphoxide	0·63†	0·44†	0·65*	0·55*	0·65‡		
Metabolite 2	0·90‡	0·63‡	0·65*	0·57*		0·61†	0·45†
n-Propylmercapturic acid	0·87‡	0·64‡	0·67*	0·56*		0·62†	0·42†

* White spot with the platinum reagent and purple with ninhydrin.
† Yellow spot with the platinum reagent.
‡ White spot with the platinum reagent.

the potassium dichromate–silver nitrate reagent. Single spots with the same R_F values as synthetic *n*-propylmercapturic acid were detected with R_F values 0·79 and 0·61 in solvents *A* and *B* respectively.

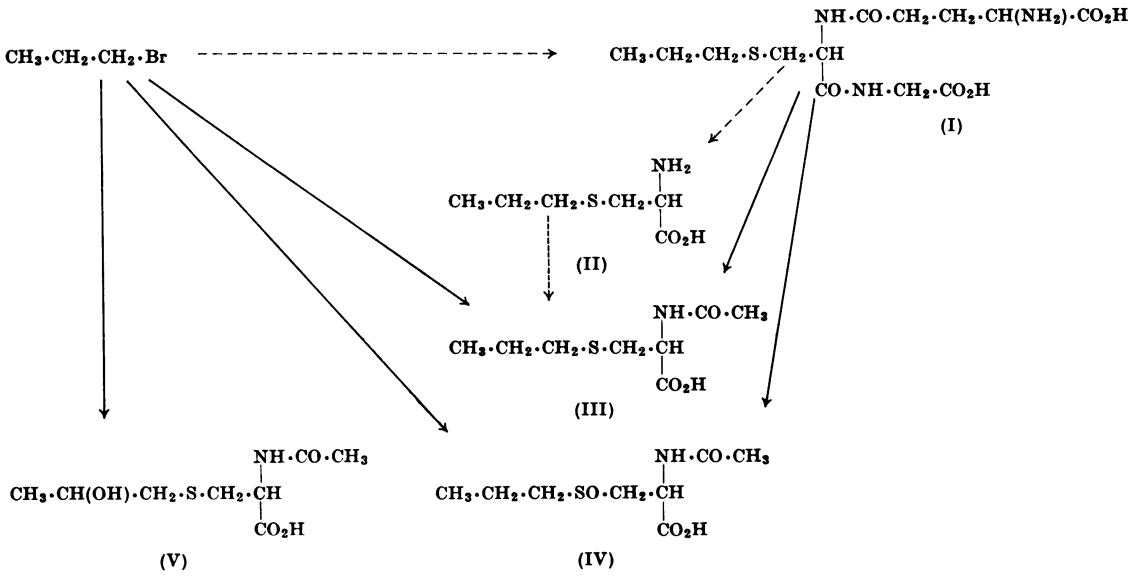
Hydrolysis of the biosynthetic compound with acid and alkali as was described by Grenby & Young (1960) gave reaction products identical with those derived from synthetic *n*-propylmercapturic acid. Alkaline hydrolysis of 0·291g. of biosynthetic material followed by treatment with mercuric chloride gave 0·154g. of propane-1-thiol mercury chloride, m.p. 182–183° (decomp.) unchanged on admixture with the synthetic compound (Found: C, 11·8; H, 2·1; Cl, 11·9; S, 10·4. Calc. for C_3H_7ClHgS: C, 11·6; H, 2·3; Cl, 11·4; S, 10·3%). After acid hydrolysis of 0·247g. of biosynthetic material, 0·086g. of *S*-*n*-propyl-L-cysteine was isolated, m.p. 240–241° (decomp.) unchanged on admixture with the synthetic compound, $[\alpha]_D^{21}$ −22° (*c* 2 in water) (Found: C, 43·8; H, 7·9; N, 8·5; S, 19·7. Calc. for $C_6H_{13}NO_2S$: C, 44·1; H, 8·0; N, 8·6; S, 19·6%). The product was chromatographically identical with *S*-*n*-propyl-L-cysteine, and had R_F values 0·37 and 0·30 in solvents *A* and *B* respectively.

Metabolism of S-*isopropyl*-L-*cysteine*

Isolation of isopropylmercapturic acid. Sixty rats (body wt. 210–250g.) were each injected subcutaneously with 2ml. of a solution of *S*-isopropyl-L-cysteine in water (4·6%, w/v). Urine was collected for 24hr., the dosing was repeated and urine was again collected. The combined urines (1010ml., pH 6·3) were extracted in a way similar to that used for the extraction of *n*-propylmercapturic acid, and the crude product was crystallized from a concentrated solution in ethyl acetate. The product (4·3g.) represented 31% of the compound administered and had m.p. 130° and m.p. when mixed with synthetic isopropylmercapturic acid 130–131°, $[\alpha]_D^{21}$ −7° (*c* 2 in water) (Found: C, 46·9; H, 7·2; N, 6·5; S, 15·5. $C_8H_{15}NO_3S$ requires C, 46·8; H, 7·4; N, 6·8; S, 15·6%). The isolated material was chromatographically identical with synthetic isopropylmercapturic acid. When chromatograms that had been prepared in solvents *A* and *B* were sprayed with the potassium dichromate–silver nitrate reagent single spots were detected with R_F values 0·79 and 0·61 respectively.

The biosynthetic compound (0·244g.) was hydrolysed with sodium hydroxide in a way similar to that described by Grenby & Young (1960) for the hydrolysis of *n*-propylmercapturic acid, and after treatment with mercuric chloride propane-2-thiol mercury chloride was separated from the reaction mixture. The yield after recrystallization from ethanol was 0·108g., m.p. 213–216° (decomp.) and m.p. when mixed with synthetic propane-2-thiol mercury chloride 212–215° (decomp.) (Found: C, 11·8; H, 2·1; S, 10·7. Calc. for C_3H_7ClHgS: C, 11·6; H, 2·3; S, 10·3%).

Acid hydrolysis of the biosynthetic compound was carried out with sulphuric acid as this acid was readily separable from the reaction mixture and this facilitated the isolation of pure *S*-isopropyl-L-cysteine. The metabolite (0·260g.) was refluxed for 6hr. with a mixture of 3ml. of water and 1ml. of concentrated sulphuric acid. The reaction mixture was diluted with water, solid barium hydroxide was added until the solution was alkaline and the excess was removed by adding 2N-sulphuric acid until the pH fell to 6. Barium sulphate was removed by centrifugation, the supernatant was evaporated



Scheme 1. Metabolism of 1-bromopropane. Conversions demonstrated in the animal body are shown by solid arrows, and a possible metabolic pathway is shown by broken arrows.

until crystallization occurred, and the product was recrystallized from water and then from a mixture of water and ethanol (1:3, v/v). The product weighed 0·043 g., m.p. 236–238° (decomp.) and m.p. when mixed with synthetic *S*-isopropyl-L-cysteine 235–237° (decomp.), $[\alpha]_D^{18}$ −16° (*c* 1 in water) (Found: S, 19·5. Calc. for $C_6H_{13}NO_2S$: S, 19·6%). The hydrolysis product was also chromatographically identical with *S*-isopropyl-L-cysteine and had R_F values 0·38 and 0·35 in solvents *A* and *B* respectively.

DISCUSSION

The metabolic reactions of 1-bromopropane are shown in Scheme 1. In the present work it has been established that 1-bromopropane is converted in the rat into *n*-propylmercapturic acid sulphoxide (IV). This metabolite is the third to be recognized, *n*-propylmercapturic acid (III) (Grenby & Young, 1959, 1960) and 2-hydroxypropylmercapturic acid (V) (Barnsley, 1964*a*) having been described previously. *n*-Propylmercapturic acid sulphoxide was not formed by oxidation of *n*-propylmercapturic acid after the excretion of the latter in the urine, for the sulphoxide was not detected by chromatographic examination of normal urine to which ^{35}S-labelled *n*-propylmercapturic acid had been added. *n*-Propylmercapturic acid sulphoxide represented about 14% of the total sulphur-containing metabolites of 1-bromopropane detected.

Glutathione has been recognized as a major source of cysteine residues in the biosynthesis of mercapturic acids (Barnes, James & Wood, 1959), and enzyme preparations that catalyse the *S*-alkylation of glutathione by alkyl halides have been described (Booth, Boyland & Sims, 1961; Johnson, 1963). Foxwell & Young (1964) have shown that *S*-*n*-propylglutathione (I) is converted *in vivo* into *n*-propylmercapturic acid and *n*-propylmercapturic acid sulphoxide, and it has now been shown that *S*-*n*-propyl-L-cysteine (II), which could arise by breakdown of the glutathione derivative, is also converted into the same two metabolites. One of these, *n*-propylmercapturic acid, was isolated in an amount that corresponded to 13·8% of the *S*-*n*-propyl-L-cysteine administered.

When the urine excreted by rats after they had been dosed with 2-bromopropane was examined by the same chromatographic and radiochromatographic methods that readily detected sulphur-containing metabolites of 1-bromopropane, only a trace of such a metabolite was detected and then only on some occasions. It therefore appears that either alkylation of thiol groups proceeds more slowly with 2-bromopropane than with 1-bromopropane or that hydrolysis of 2-bromopropane or the alkylation of groups other than thiol occurs, and that these factors preclude the formation of significant amounts of *S*-alkylated cysteine residues. Had the latter been formed the excretion of iso-

propylmercapturic acid might have been expected, for the acetylation of *S*-isopropyl-L-cysteine *in vivo* has been demonstrated. After the subcutaneous injection of *S*-isopropyl-L-cysteine a quantity of isopropylmercapturic acid was isolated that represented 31% of the material administered.

As a result of the present work it has become apparent that in addition to the formation of *n*-propylmercapturic acid the metabolism of 1-bromopropane resembles that of its lower and its higher homologues in other respects. Like bromoethane 1-bromopropane gives rise to a mercapturic acid sulphoxide, and like 1-bromobutane it is converted into a hydroxyalkylmercapturic acid.

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