The Formation of 2-Hydroxypropylmercapturic Acid from 1-Halogenopropanes in the Rat

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1. 2-Hydroxypropylmercapturic acid, i.e. *N*-acetyl-*S*-(2-hydroxypropyl)-*L*-cysteine, has been isolated, as the dicyclohexylammonium salt, from the urine of rats dosed with 1-bromopropane. 2. The formation of the same metabolite from 1-chloropropane, 1-iodopropane, 1,2-epoxypropane and 1-chloropropan-2-ol has been demonstrated by chromatographic examination of the urine excreted by rats after they had been dosed with these compounds. 3. (+)- and (-)-Dicyclohexylammonium 2-hydroxypropylmercapturate have been prepared by fractional crystallization of the mixture of isomers obtained by two methods: the reaction of 1,2-epoxypropane with *L*-cysteine followed by acetylation, and the reduction of 2-oxopropylmercapturic acid. 4. The following compounds have also been prepared: *S*-(3-hydroxypropyl)-*L*-cysteine, (+)- and (-)-*S*-(2-hydroxypropyl)-*L*-cysteine, dicyclohexylammonium 3-hydroxypropylmercapturate, (+)- and (-)-dicyclohexylammonium 2-hydroxy-1-methylethylmercapturate, and (+)- and (-)-dicyclohexylammonium 1-(ethoxycarbonyl)ethylmercapturate.

The halogenoalkanes give rise in animals to a general type of sulphur-containing metabolite, the alkylmercapturic acid. Methylmercapturic acid is formed from iodomethane (Barnsley & Young, 1965), ethylmercapturic acid from bromo- and iodo-ethane (Thomson, Barnsley & Young, 1963), propylmercapturic acid from 1-chloro, 1-bromo- and 1-iodo-propane (Grenby & Young, 1960), and 1-bromobutane and its higher homologues give rise to the corresponding alkylmercapturic acids (Bray, Caygill, James & Wood, 1964). Other sulphur-containing metabolites are excreted, the nature of which varies according to the alkyl group. Iodomethane gives rise to methylthioacetic acid and *N*-(methylthioacetyl)glycine (Barnsley & Young, 1965), and alkylmercapturic acid sulphoxides are formed from bromo- and iodo-ethane (Barnsley, Thomson & Young, 1964) and from the 1-halogenopropanes (Barnsley, Grenby & Young, 1966).

A further study of the metabolism of the 1-halogenopropanes has revealed the presence of another type of metabolite, which was isolated, as the dicyclohexylammonium salt, from the urine of rats dosed with 1-bromopropane and identified as 2-hydroxypropylmercapturic acid, i.e. *N*-acetyl-*S*-(2-hydroxypropyl)-*L*-cysteine. Part of this work was the subject of a preliminary publication (Barnsley, 1964).

MATERIALS

All melting and boiling points are uncorrected. Elementary analyses were carried out by Weiler and Strauss,

Oxford. The 1-halogenopropanes (British Drug Houses Ltd., Poole, Dorset) were redistilled, and 1-bromopropane was also fractionally distilled and had b.p. 71.0°/767 mm. Hg. Enzyme preparations were purchased from the following sources: acylase I from British Drug Houses Ltd.; crystalline catalase from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany; pig-kidney *D*-amino acid oxidase and *Crotalus adamanteus* venom (*L*-amino acid oxidase) from L. Light and Co. Ltd., Colnbrook, Bucks. Bromoacetone was prepared by the method of Levene (1943). After two fractional distillations the product (25% yield) had b.p. 39.5–40.1°/13 mm. Hg (Found: C, 26.6; H, 3.6; Br, 58.8. Calc. for C₃H₅BrO: C, 26.3; H, 3.7; Br, 58.3%). Propylmercapturic acid, its sulphoxides, and *S*-propyl-*L*-cysteine were described by Barnsley *et al.* (1966). 1-Chloropropan-2-ol was prepared from material containing 25% of 2-chloropropan-1-ol (British Drug Houses Ltd.) by repeated fractional distillation (Fickett, Garner & Lucas, 1951) and had b.p. 64.5°/75 mm. Hg. Some properties of the new synthetic compounds described below are given in Table 1.

Preparation of S-(3-hydroxypropyl)-*L*-cysteine. 3-Bromopropan-1-ol (23.7 g.) was added to a solution of *L*-cysteine hydrochloride (26.8 g.) in 100 ml. of 3.4 *N*-NaOH. Ethanol (35 ml.) was added to make the mixture homogeneous, and the solution was allowed to stand overnight at room temperature. The solution was adjusted to pH 5 with 5 *N*-HCl, the solution was evaporated under reduced pressure until the volume was reduced to 100 ml., and the crude product was precipitated by the addition of acetone. This material after drying over P₂O₅ *in vacuo* weighed 23.3 g., and contained cystine and NaCl. It was purified in the following way. Part of the crude product (5.0 g.) was dissolved in 100 ml. of water, and the solution was passed through a column (30 cm. long × 2 cm. diam.) of Zeo-Karb 225 resin

Table 1. *Properties of synthetic compounds*

Compound	m.p.	[α] _D [*]	Equiv. wt.		Elementary analysis (%)			
			Predicted		Predicted		Found	
			Predicted	Found	C	H	N	S
<i>S</i> -(3-Hydroxypropyl)-L-cysteine	198-203 [†]	-22 ± 2 [‡]	—	—	40.2	7.3	7.8	17.9
<i>S</i> -(2-Hydroxypropyl)-L-cysteine	188-193 [†]	-20 ± 2 [‡]	—	—	40.2	7.3	7.8	17.9
(+)- <i>S</i> -(2-Hydroxypropyl)-L-cysteine	190-192 [†]	+3 ± 2 [‡]	—	—	40.2	7.3	7.8	17.9
(-)- <i>S</i> -(2-Hydroxypropyl)-L-cysteine	189-191 [†]	-30 ± 2 [‡]	—	—	40.2	7.3	7.8	17.9
Dicyclohexylammonium 3-hydroxypropylmercapturate	175	-6 ± 1§	403	404	59.7	9.5	7.0	8.0
(+)-Dicyclohexylammonium 2-hydroxypropylmercapturate:								
Method 1	160	+9 ± 2§	403	403	59.7	9.5	7.0	8.0
Method 2	161	+8 ± 2§	403	405	59.7	9.5	7.0	8.0
(-)-Dicyclohexylammonium 2-hydroxypropylmercapturate:								
Method 1	169-170	-9 ± 2§	403	404	59.7	9.5	7.0	8.0
Method 2	170-170.5	-10 ± 2§	403	404	59.7	9.5	7.0	8.0
(+)-Dicyclohexylammonium 2-hydroxy-1-methylethylmercapturate	166.5-167	+7 ± 2§	403	406	59.7	9.5	7.0	8.0
(-)-Dicyclohexylammonium 2-hydroxy-1-methylethylmercapturate	165-166	-1.5 ± 1.5§	403	400	59.7	9.5	7.0	8.0
Dicyclohexylammonium 2-oxopropylmercapturate	166-167 [†]	-6 ± 1§	—	—	60.0	9.1	7.0	8.0
2-Oxopropylmercapturate 2,4-dinitrophenylhydrazone	160-160.5 [†]	—	—	—	42.1	4.3	17.5	8.0
(+)-Dicyclohexylammonium 1-(ethoxycarbonyl)ethylmercapturate	174.5-175.5	+48 ± 1	222¶	221	59.4	9.1	6.3	7.2
(-)-Dicyclohexylammonium 1-(ethoxycarbonyl)ethylmercapturate	148	-50 ± 1	221¶	221	59.4	9.1	6.3	7.2

* Measured at room temperature (18-22°).

† Decomp.

‡ c 1 in water.

§ c 1.5 in water.

|| c 2 in water.

¶ After saponification.

(H⁺ form). The column was washed with water until the pH of the eluate rose to 4–5, and then with a further 500 ml. of water. The amino acid was displaced from the column with 250 ml. of *N*-NH₃ solution, and the eluate was evaporated at 45–50° under reduced pressure. The product was crystallized from solution in 10 ml. of warm water by the addition of ethanol; yield 3.6 g.

Preparation of *S*-(2-hydroxypropyl)-L-cysteine. L-Cysteine hydrochloride (25 g.) was dissolved in 80 ml. of 2*N*-NaOH and 100 ml. of water, and the solution (pH 7.5) was cooled to 5°. 1,2-Epoxypropane (9.9 g.) and 50 ml. of ethanol were added, and after standing overnight at room temperature the solution was filtered to remove a little white solid that had separated. The filtrate was diluted to 500 ml. with water, and passed through a column (56 cm. long × 3 cm. diam.) of Zeo-Karb 225 resin (H⁺ form, 50–100 mesh). The column was washed with water until the pH of the eluate rose to 5, and then with a further 1 l. of water. The product was displaced from the column with 0.5*N*-NH₃ solution, and 1000 ml. of ammoniacal eluate was collected. The eluate was evaporated at 45–50° under reduced pressure and gave a crystalline product, which was recrystallized from solution in 20 ml. of hot water by the slow addition of 500 ml. of hot ethanol. The product weighed 20.1 g. The 1,2-epoxypropane used in the preparation was not optically active, and so the product was probably a mixture of diastereoisomers. Attempts to separate these by fractional crystallization from aqueous ethanol were not successful, but they were obtained by deacetylation of the resolved *N*-acetyl derivatives. The product of the reaction between 1,2-epoxypropane and L-cysteine was the required *S*-(2-hydroxypropyl)-L-cysteine and not *S*-(2-hydroxy-1-methylethyl)-L-cysteine, for after degradation of the product (0.268 g.) with Raney nickel and oxidation of the volatile reaction products (see the Methods section) acetone 2,4-dinitrophenylhydrazones was isolated (0.102 g.), which after recrystallization had m.p. 126°, unchanged on admixture with the authentic compound (Found: C, 45.6; H, 4.2; N, 22.9. Calc. for C₉H₁₀N₄O₄: C, 45.4; H, 4.2; N, 23.5%). Thin-layer chromatography of the crude 2,4-dinitrophenylhydrazones showed only the presence of the acetone derivative; propionaldehyde 2,4-dinitrophenylhydrazones was absent. When the degradation procedure was applied to *S*-(3-hydroxypropyl)-L-cysteine and to (+)- and (–)-dicyclohexylammonium 2-hydroxy-1-methylethylmercapturate, only propionaldehyde 2,4-dinitrophenylhydrazones was formed, in about 15% yield.

Preparation of (+)-*S*-(2-hydroxypropyl)-L-cysteine. From (+)-dicyclohexylammonium 2-hydroxypropylmercapturate (0.224 g.) a solution of the free acid was prepared (see the Methods section), and titrated with 0.1*N*-NaOH to pH 7.3. The final volume was 40 ml. Acylase I (20 mg.) was added and the solution was incubated at 37°. The pH of the solution fell slowly, and was brought back to 7.3 by the addition of 0.1*N*-NaOH at 6 hr. intervals. After 24 hr. the solution was passed through a column (5 cm. long × 1 cm. diam.) of Zeo-Karb 225 resin (H⁺ form, 50–100 mesh), the column was washed with 50 ml. of water, and the amino acid was then displaced from the column with 0.5*N*-NH₃ solution (30 ml.). The ammoniacal eluate was evaporated at 45–50° under reduced pressure and the residue was dissolved in 1 ml. of water. The solution was filtered, the filtrate was evaporated under reduced pressure at 45°, and the residue was crystallized from solution in 0.2 ml. of

water by the slow addition of acetone. The product was dried over P₂O₅ *in vacuo* and weighed 72 mg.

Preparation of (–)-*S*-(2-hydroxypropyl)-L-cysteine. (–)-Dicyclohexylammonium 2-hydroxypropylmercapturate (0.522 g.) was deacetylated enzymically as was described for the preparation of (+)-*S*-(2-hydroxypropyl)-L-cysteine, and 0.118 g. of product was obtained. A product with similar m.p., specific optical rotation and elementary analysis was obtained by the hydrolysis of a solution of 2-hydroxypropylmercapturic acid, obtained from (–)-dicyclohexylammonium 2-hydroxypropylmercapturate, with *N*-HCl at 100°. It appears therefore that the configuration of the 2-hydroxypropyl group is stable under these conditions.

Preparation of dicyclohexylammonium 3-hydroxypropylmercapturate. *S*-(3-Hydroxypropyl)-L-cysteine (9.5 g.) was dissolved in 100 ml. of water and 106 ml. of 2*N*-NaOH, and the solution was cooled to 5°. Acetic anhydride (10.8 g.) was added in 1 ml. portions, and the mixture was shaken vigorously and cooled to maintain the temperature at 5°. A further 106 ml. of 2*N*-NaOH and 10.8 g. of acetic anhydride were then added as before. The reaction mixture was passed in equal parts through two columns (45 cm. long × 3 cm. diam.) of Zeo-Karb 225 resin (H⁺ form, 50–100 mesh), the columns were washed with water until the pH of the eluates rose to 5, and the total eluates were combined and evaporated at 45–50° under reduced pressure. The residue, a colourless gum, was dried over NaOH pellets *in vacuo* and weighed 9.8 g. Attempts to crystallize the product were not successful, and a dicyclohexylammonium salt was therefore prepared. The gum (1.32 g.) was dissolved in 2 ml. of ethanol and 50 ml. of acetone, and a solution of 1.10 g. of dicyclohexylamine in 50 ml. of acetone was added. After standing at 5° for several hours the product was filtered, recrystallized from a mixture of ethanol and acetone, and dried over P₂O₅ *in vacuo*; yield 2.0 g.

Preparation of (+)- and (–)-dicyclohexylammonium 2-hydroxypropylmercapturate. Method 1. *S*-(2-Hydroxypropyl)-L-cysteine (8.4 g.) was acetylated as was described for the preparation of the 3-hydroxy isomer, and the product, isolated as the dicyclohexylammonium salt, weighed 13.0 g., $[\alpha]_D^{15} - 3 \pm 1^\circ$ (c 1.5 in water). The product was fractionally crystallized from solution in ethanol (about 7%, w/v) by the addition of acetone, and after repeated fractionation 4.2 g. of the laevorotatory isomer was obtained.

The mother liquors from which laevorotatory material had been crystallized were evaporated, and the crystalline residue that remained was repeatedly subjected to the crystallization procedure to separate more of the laevorotatory material. From the mother liquor of these crystallizations 2.5 g. of product, $[\alpha]_D^{15} + 3 \pm 1^\circ$, was obtained. Further purification of this material was difficult, and finally only 0.49 g. of a dextrorotatory isomer was obtained.

Identical products were obtained by causing 1,2-epoxypropane to react with *N*-acetyl-L-cysteine at pH 7, and converting the product into the dicyclohexylammonium salt. This was analytically pure and had the m.p. and specific rotation expected for a mixture of the isomers, but the infrared spectrum indicated the presence of an impurity that was only removed on fractional crystallization.

Method 2. Dicyclohexylammonium 2-oxopropylmer-

capturate (31.4g.) was dissolved in water (1l.), sodium borohydride (3.0g.) was added, and the solution was warmed to 50° on a water bath. After 2hr. the solution was cooled, filtered, and cations were removed from the filtrate. The acid solution obtained was evaporated at 60° under reduced pressure and gave a gum which was dissolved in acetone. The solution was evaporated, the residue was dissolved in 200 ml. of acetone, and the solution was filtered and then diluted to 1l. with acetone. Dicyclohexylamine was added until a sample of the solution, when allowed to evaporate on narrow-range indicator paper, showed an apparent pH 7. The solution was evaporated and the white residue was evaporated several times with methanol. If these evaporations were omitted crystallization did not occur at the next stage, probably owing to the presence of borate esters. The residue was dissolved in 140 ml. of hot ethanol, and crystallized by the addition of 1500 ml. of acetone. The product was filtered, dried *in vacuo*, and weighed 28.2g. It was fractionally crystallized from solution in ethanol by the addition of acetone as described in Method 1, and gave 10.2g. of (–)-dicyclohexylammonium 2-hydroxypropylmercapturate, m.p. 170–170.5°, unchanged on admixture with the laevorotatory product obtained by Method 1. (+)-Dicyclohexylammonium 2-hydroxypropylmercapturate (1.7g.) was obtained with m.p. 161°, unchanged on admixture with the dextrorotatory isomer obtained by Method 1. The infrared spectra of the products were identical with those of the products obtained by Method 1.

Preparation of (+)- and (–)-dicyclohexylammonium 1-(ethoxycarbonyl)ethylmercapturate. *N*-Acetyl-L-cysteine (6.96g.) was added slowly to a solution of 4.60g. of anhydrous Na₂CO₃ in 175 ml. of water. Ethyl 2-bromopropionate (7.72g.) dissolved in 125 ml. of ethanol was added, and after 16hr. at room temperature the solution was concentrated to 120 ml. by evaporation at 45° under reduced pressure. The solution was cooled to 5°, and conc. HCl (15 ml.) was added slowly with continuous stirring and cooling to maintain the temperature at 5°. The acid solution was extracted with seven portions (each 120 ml.) of CHCl₃ at 5°. The combined extracts were dried with anhydrous Na₂SO₄, evaporated at 45° under reduced pressure, and the residue, a pale-yellow gum, was dissolved in 75 ml. of acetone. Dicyclohexylamine (7.4g.) dissolved in 30 ml. of acetone was added and the solution was stood overnight at 5°. The material which separated was crystallized from solution in 45 ml. of ethanol by the addition of 940 ml. of acetone at 5°. The crude product [9.7g., $[\alpha]_D^{25} + 21 \pm 2^\circ$ (c 2 in water)] was recrystallized five times from solution in ethanol (33%, w/v) by the addition of 20 vol. of acetone and gave 4.9g. of product.

The mother liquor which remained after the separation of the crude dextrorotatory isomer was evaporated, the residue was dissolved in 10 ml. of ethanol, and acetone (125 ml.) and light petroleum (b.p. 40–60°) (150 ml.) were added. After standing at 5°, 3.4g. of laevorotatory material was separated, $[\alpha]_D^{25} - 39 \pm 1^\circ$ (c 2 in water). This was crystallized four times from solution in ethanol (40%, w/v) by the addition of acetone (20 vol.) and then, after the separation of some dextrorotatory material, light petroleum (b.p. 40–60°) (20 vol.). The product weighed 1.7g.

Preparation of (+)-dicyclohexylammonium 2-hydroxy-1-methylethylmercapturate. (+)-Dicyclohexylammonium 1-(ethoxycarbonyl)ethylmercapturate (2.47g.) was dissolved

in 60 ml. of water. Sodium borohydride (1.26g.) was added to the solution, which was heated at 70° for 1 hr. After the solution had been standing for 16hr. at room temperature, cations were removed and the acid solution obtained was evaporated at 50° under reduced pressure. The residue was extracted with four portions of acetone (5 ml. each), the extract was evaporated, and the residue was extracted with 2 vol. (5 ml. each) of acetone. The extract was titrated with a solution of dicyclohexylamine in acetone (10%, v/v) until one drop of the solution, when evaporated on narrow-range indicator paper, showed an apparent pH 7–8. The solution was evaporated, and methanol was repeatedly added to the residue and evaporated. The residue was dissolved in the minimum of hot ethanol and crystallized by the addition of acetone. The product (1.74g.) contained two minor impurities which were detected with the modified platinum reagent on chromatograms prepared in solvent B. These were not readily removed by recrystallization, and so the following procedure was adopted. From part of the crude product (0.91g.) a solution of the free acid was prepared, and brought to pH 7 with 0.1 N-NaOH. The solution was evaporated at 50° under reduced pressure. The residue was dissolved in ethanol, the solution was applied as a streak to six sheets of Whatman no. 3 paper (each 30 cm. wide), and after chromatography in solvent B the product was detected by treating test strips (1 cm. wide) with the modified platinum reagent. The appropriate areas of the chromatograms were eluted with water-ethanol (1:1, v/v), the eluate was evaporated under reduced pressure at 50°, and the residue was dissolved in 20 ml. of water. Cations were removed from the solutions and the acid solution obtained was evaporated under reduced pressure. The residue, a colourless gum, was evaporated several times with acetone, and was then dissolved in 25 ml. of acetone and titrated with a solution of dicyclohexylamine in acetone (10%, v/v) until one drop of the solution showed an apparent pH 7–8 when allowed to evaporate on narrow-range indicator paper. Light petroleum (b.p. 40–60°) (25 ml.) was added to the solution, which was stored at 5°. The product which crystallized was recrystallized from solution in 2.5 ml. of ethanol by the addition of acetone (80 ml.) and light petroleum (b.p. 40–60°) (40 ml.). The product weighed 0.49g.

Preparation of (–)-dicyclohexylammonium 2-hydroxy-1-methylethylmercapturate. This compound was prepared from (–)-dicyclohexylammonium-1-(ethoxycarbonyl)-ethylmercapturate (1.20g.) by the method described for the preparation of the dextrorotatory isomer. The product obtained after purification by chromatography and recrystallization weighed 0.61g.

Preparation of dicyclohexylammonium 2-oxopropylmercapturate. To 500 ml. of water cooled in an ice bath were added 49g. of *N*-acetyl-L-cysteine and 60 ml. of approx. 5 N-NaOH to bring the solution to pH 7. Acetone (250 ml.) was added, bromoacetone was run in with stirring in 1 ml. portions and, after each addition of bromoacetone, 5 N-NaOH was added to bring the pH to 7.5. The pH of the solution during the reaction varied between 5.5 and 7.5 and the temperature ranged from 13° to 15°. A total of 24 ml. of bromoacetone was used. The solution was evaporated under reduced pressure at 50–60° until the volume was reduced to 600 ml.; 40 ml. of conc. HCl was added and the solution was extracted with four 450 ml. portions of ethyl acetate. The combined extracts were dried over

anhydrous Na_2SO_4 and evaporated at 40–50° under reduced pressure. The residue, a yellow gum, was dissolved in acetone (400 ml.), and dicyclohexylamine was added until one drop of the solution, when allowed to evaporate on a narrow-range indicator paper, had an apparent pH 8. The solution was stood overnight at 5°, filtered and the crystals which were separated were washed with acetone and dried *in vacuo*. The product (43.2 g.) was recrystallized from solution in warm ethanol (70 ml.) by the addition of acetone (1200 ml.); yield 38.9 g.

A 2,4-dinitrophenylhydrazone of 2-oxopropylmercapturic acid was prepared. From an aqueous solution of dicyclohexylammonium 2-oxopropylmercapturate (0.404 g. in 10 ml. of water) a solution of the free acid was obtained, and to it 2,4-dinitrophenylhydrazine (0.223 g.), dissolved in a warm mixture of water (25 ml.), ethanol (15 ml.) and conc. HCl (20 ml.), was added. After 6 hr. at room temperature the product was filtered, washed with a little ice-cold water, and dried *in vacuo* over P_2O_5 . The product (0.312 g.) was recrystallized from a mixture of ethanol (10 ml.) and water (45 ml.); yield 0.246 g.

METHODS

Chromatography. Paper chromatograms were prepared by the ascending method with Whatman no. 1 paper and four solvent mixtures: A, butan-1-ol–acetic acid–water (4:1:5, by vol., top layer); B, butan-1-ol–pyridine–aq. 3N- NH_3 soln. (4:3:3, by vol.); C, phenol–water–aq. NH_3 (sp.gr. 0.88) [0.5 ml. of aq. NH_3 soln. (sp.gr. 0.88) was added to each 100 ml. of a solution of 500 g. of phenol in 125 ml. of water immediately before use]; D, propan-2-ol–water–aq. NH_3 soln. (sp.gr. 0.88) (20:2:1, by vol.). Thiocethers and sulfoxides were detected by dipping chromatograms in the platonic iodide reagent of Toennies & Kolb (1951) as modified by Barnsley *et al.* (1964), and amino acids were detected with ninhydrin (Barnsley *et al.* 1964).

Thin-layer chromatograms of the 2,4-dinitrophenylhydrazones derivatives of aldehydes and ketones were developed on silica gel G (E. Merck A.-G.) (0.25–0.30 mm. thick). The plates were dried, heated at 115° and allowed to cool in a drying cabinet for 30 min. Development was carried out with benzene–ethyl acetate (19:1, v/v) (15 cm. run). The disappearance of amino acids in the presence of amino acid oxidases in buffer solution was observed by chromatographing the reaction mixture on Amberlite ion-exchange paper WA-2 (H^+ form). Sodium formate buffer (pH 4.1, 0.1M) was allowed to rise to a height 10 cm. above the point of application, and the strip was then dried in warm air and dipped in the ninhydrin reagent. Neutral aliphatic amino acids had R_F values about 0.7–0.8.

Removal of cations from solutions of salts. A solution of the salt was passed over a column of Zeo-Karb 225 resin (H^+ form, 50–100 mesh; 1.5 ml. of resin/m-equiv. of cation), and the column was washed with water until the pH of the eluate rose to 5.

Infrared-absorption spectra. These were measured by the KBr-disk technique (0.5%, w/w, compound in KBr) in a Perkin–Elmer 237 infrared spectrophotometer.

Animals and dosing. Male rats (body wt. 180–240 g.) were fed with rat cakes [J. Murray and Sons (London) Ltd.], and had access to water at all times. They were lightly anaesthetized with ether while test compounds were injected subcutaneously in the lumbar region. Urine was

collected separately from faeces, and when not used immediately was stored at –25°.

Degradation of S-substituted cysteines with Raney nickel. Two tablets (0.5 g. each) of stabilized Raney nickel (British Drug Houses Ltd.) were freed from wax, and the nickel was distributed between three Pyrex tubes (7.5 cm. long $\times$ 1 cm. diam.). Excess of water was decanted off, and the sample (3–4 mg. dissolved in 1 ml. of water and 0.5 ml. of 0.5N-acetic acid) was added to each tube. The tubes were sealed, immersed in a boiling-water bath and continuously rotated for 6 hr. After cooling the tubes were opened (the contents were under pressure), and 10 μ l. samples were removed for chromatography of the amino acids. The remainder of the solution was filtered into a 25 ml. flask, diluted with 2 ml. of water and distilled. The vapour was led into a boiling mixture of water (5 ml.), conc. H_2SO_4 (0.5 ml.) and $\text{K}_2\text{Cr}_2\text{O}_7$ (0.01 g.), and the distillate from the oxidizing mixture was condensed and run into 1 ml. of a saturated solution of 2,4-dinitrophenylhydrazine in 2N-HCl. About 4 ml. of distillate was collected, and after standing for 30 min. the solution was extracted with 1 ml. of CHCl_3 . The extract was examined by thin-layer chromatography.

When the dicyclohexylammonium salts of mercapturic acids were examined, the sample (5–6 mg.) was dissolved in water (2 ml.), the solution was freed from cations, and the acid solution obtained was evaporated under reduced pressure. The residue was heated at 100° with 5 ml. of 5N-HCl in a sealed tube for 6 hr. The solution was evaporated to dryness at 50° under reduced pressure, and the residue was repeatedly evaporated with water to remove excess of HCl. The residue was dissolved in 1 ml. of water and 0.5 ml. of 0.5N-acetic acid; a 10 μ l. sample was removed for chromatography and the solution was treated with Raney nickel as described above.

Enzymic deacetylation. About 2 mg. of a dicyclohexylammonium salt was dissolved in 1 ml. of water, and the solution was passed through a column (1 cm. long $\times$ 0.5 cm. diam.) of Zeo-Karb 225 resin (H^+ form). The column was washed with 4 ml. of water, and 0.5 ml. of 0.1M-phosphate buffer, pH 7.2, was added to the total eluate. Acylase I (0.1 mg.) was added, and the solution was incubated at 35° for 16 hr. The solution was passed through a column (2 cm. long $\times$ 0.5 cm. diam.) of Zeo-Karb 225 resin (H^+ form), and the column was washed with 10 ml. of water. Amino acids were displaced from the column with 5 ml. of aq. 2N- NH_3 soln., and the eluate was evaporated at 50° under reduced pressure. The residue was dissolved in 0.5 ml. of water, and samples (20 μ l.) were removed for chromatography.

Oxidative deamination. To 0.1 ml. of a solution of the amino acid (5 mg./ml.) were added 0.3 ml. of L-amino acid oxidase (1.3 mg./ml. in 0.1M-phosphate buffer, pH 7.2) and 1 mg. of catalase. The solution was incubated at 35° for 4 hr. and a sample (10 μ l.) was chromatographed on Amberlite ion-exchange paper WA-2. A saturated solution (1 ml.) of 2,4-dinitrophenylhydrazine in 2N-HCl was added to the reaction mixture, and after 20 min. the solution was extracted with 2 ml. of CHCl_3 . The CHCl_3 extract was extracted with 1 ml. of aq. 2N- NH_3 soln. and the aqueous extract was chromatographed.

When the products of enzymic deacetylation were subjected to oxidative deamination, the whole of the solution (0.5 ml.) obtained from the deacetylation of 2 mg. of the

dicyclohexylammonium salt was used, and proportionately larger samples were removed for chromatography.

Incubation with hog-kidney D-amino acid oxidase (final concn. 6 mg./ml.) was carried out in pyrophosphate buffer, pH 8.5, final concn. 0.08 M.

Determination of the equivalent weight of dicyclohexylammonium salts. The sample of the salt (30 mg.) was dissolved in 2 ml. of water, and the solution was passed through a column (1 cm. long $\times$ 0.8 cm. diam.) of Zeo-Karb 225 resin (H⁺ form). The column was washed with four 2 ml. portions of water, and the total eluate was titrated under CO₂-free N₂ with 0.09 N-NaOH, a glass pH electrode being used. The equivalent weight of dicyclohexylammonium 1-(ethoxycarbonyl)ethylmercapturate was determined after saponification. Dicyclohexylammonium ions were removed from a solution of a sample (17 mg.) as described above, and to the eluate 1 ml. of 0.09 N-NaOH was added. The solution, held in a vessel with a lightly greased ground-glass stopper, was incubated at 35° for 16 hr. and excess of alkali was then back-titrated with 0.05 N-HCl.

RESULTS

Metabolism of 1-chloro-, 1-bromo- and 1-iodo-propane

Chromatographic studies. Male rats, body wt. 180–210 g., were each injected subcutaneously in the lumbar region with a 40% (w/v) solution of a 1-halogenopropane in arachis oil (1 ml./rat). The urine excreted by each rat in the first 24 hr. period after dosing was diluted to 20 ml. with water which was used to wash the urine-faeces separator, and 10 μ l. samples were chromatographed with solvent A. Chromatograms were dipped in the modified platinum reagent and all urine samples showed the presence of an unknown metabolite, which was separable from propylmercapturic acid and propylmercapturic acid sulphoxide (Table 2).

A sample (200 μ l.) of the urine of each dosed animal was applied as a streak to a strip (30 cm. wide) of Whatman no. 1 chromatography paper, and chromatographed with solvent A. The unknown metabolite was detected with the modified platinum reagent on test strips (1 cm. wide) cut

from each chromatogram, and was eluted with ethanol-water (1:1, v/v) from the appropriate areas. Some material at the leading and trailing edge of the streak had to be rejected because of its proximity to propylmercapturic acid and the sulphoxide. The eluate was evaporated under reduced pressure at 45°, and the residue was dissolved in 0.2 ml. of water. Chromatography of the eluates obtained from chromatograms of the metabolites of each 1-halogenopropane showed that the same unknown metabolite was formed in all cases, and after the eluates had been hydrolysed (0.1 ml. of eluate heated with 0.1 ml. of 5 N-hydrochloric acid at 100° for 4 hr. in a sealed tube) chromatography showed that the same amino acid was formed from each eluate. The unknown metabolite and its hydrolysate were chromatographically identical with 2-hydroxypropylmercapturic acid and *S*-(2-hydroxypropyl)-L-cysteine respectively, compounds with which they were identified after isolation of the metabolite (Table 3). Chromatograms of hydrolysates also showed the presence of traces of other amino acids but these were present on chromatograms obtained in a similar way from normal urine.

Isolation of the unknown metabolite from the urine of rats dosed with 1-bromopropane. Male rats (body wt. 180–240 g.) were each injected with 1 ml. of 40% (w/v) 1-bromopropane in arachis oil. The urine was collected for 24 hr. after dosing and stored at –25°. Over a period of 3 weeks 32 rats received by repeated dosing a total of 54 g. of 1-bromopropane, and a total volume of 1360 ml. of urine was collected. The pH of the urine was reduced to 4.5 with concentrated hydrochloric acid, and the urine was filtered and applied to a column (95 cm. long $\times$ 6.6 cm. diam.) of Amberlite CG-400 resin (formate form, 100–200 mesh) that had been equilibrated with 0.1 M-sodium formate buffer, pH 4.1. Sodium formate buffer, pH 4.1, was passed through the column, and fractions (500 ml.) were collected. The concentration of the buffer was

Table 2. *Chromatographic examination of the urine excreted by rats in the first 24-hr. period after they had been injected subcutaneously with a 1-halogenopropane*

Details are given in the text. Chromatograms were treated with the modified platinum reagent.

Compound injected	<i>R_F</i> of metabolites in solvent A			
1-Chloropropane	0.14*	0.61†	0.74*	0.90*
1-Bromopropane	0.13*	0.65†	0.72*	0.92*
1-Iodopropane	0.13*	0.62†	0.75*	0.89*
Control urine	0.12*			
Propylmercapturic acid sulphoxide		0.63†		
Propylmercapturic acid				0.90*

* White spot with the modified platinum reagent.

† Yellow spot with the modified platinum reagent.

Table 3. *Chromatographic examination of the unknown metabolite eluted from chromatograms of the urine of rats dosed with 1-halogenopropanes*

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

Solvent	R_F			
	A	B	A	B
2-Hydroxypropylmercapturic acid	0.74*	0.46*		
S-(2-Hydroxypropyl)-L-cysteine			0.33†	0.34†
Compound injected	Metabolite		Hydrolysed metabolite	
1-Chloropropane	0.72*	0.46*	0.35†	0.35†
1-Bromopropane	0.71*	0.45*	0.36†	0.34†
1-Iodopropane	0.75*	0.46*	0.33†	0.33†

* White spot with the platinum reagent.

† White spot with the platinum reagent and purple with ninhydrin.

increased linearly as described by Barnesley (1964) so that it had risen to 0.3M when 25 fractions had been collected, and elution was then continued with buffer at this concentration. The nitroprusside reaction (Thomson *et al.* 1963) was carried out on 2ml. samples of the fractions, and a positive reaction was obtained with fractions 27–34 inclusive. These fractions also gave a transient pink colour when nitroprusside was added to the alkaline solution. A sample (2ml.) of each fraction was shaken with 1ml. of Zeo-Karb 225 resin (H⁺ form, 50–100 mesh), filtered, and 1ml. of the filtrate was evaporated over phosphoric oxide *in vacuo*. The residue was dissolved in 0.05ml. of water, and 10 μ l. of the solution was chromatographed with solvent A. The chromatograms were treated with the modified platinum reagent, and spots between R_F 0.72 and 0.75 were obtained from fractions 26–34 inclusive. Propylmercapturic acid had R_F 0.90, and propylmercapturic acid sulphoxide had R_F 0.66.

Fractions 27–34 inclusive were combined and passed through a column (46cm. long $\times$ 6.6cm. diam.) of Zeo-Karb 225 resin (H⁺ form, 50–100 mesh), and the column was washed with water until the pH of the eluate rose to 5. The total eluate was evaporated at 50° under reduced pressure, and the residue was dissolved in 150ml. of water. The solution was shaken with 1g. of charcoal, filtered, and the filtrate was evaporated at 50° under reduced pressure. Chromatography of the residue with solvents A and B revealed the presence of a second component with R_F about 0.9 in both solvents. This material did not react with ninhydrin, and was always found when solutions of the metabolite (of synthetic or biosynthetic origin) were evaporated or allowed to stand for long periods. The residue was dissolved in water, neutralized and refractionated on a fresh column (45cm. long $\times$ 6.6cm. diam.) of Amberlite CG-400 resin in a

way similar to that already described. After desalting, the residue containing the metabolite weighed 1.3g., and from it a dicyclohexylammonium salt was crystallized (Thomson *et al.* 1963). A crude product, 0.24g., m.p. 150–155°, was obtained and after repeated recrystallization this gave 80mg. of product, m.p. 162–163° (C, 59.9; H, 9.4; N, 6.7; S, 7.9%; equiv. wt., 403). The product had no significant optical rotation.

When the isolation was repeated with the urine of 15 rats that had received a total of 25g. of 1-bromopropane, 22mg. of the metabolite was isolated, m.p. 163–164°. It had no significant optical rotation, and the infrared-absorption spectrum was identical with that of the material first isolated.

Isolation of the hydrolysis product of the unknown metabolite. Impure fractions of the dicyclohexylammonium salt of the metabolite were dissolved in water, cations were removed from the solution, and the acid solution obtained was evaporated under reduced pressure. The residue was dissolved in 12ml. of 6N-hydrochloric acid, and the solution was heated at 100° under reflux for 4hr. and then evaporated to dryness under reduced pressure. The residue was dissolved in water, the solution was passed through a column (10cm. long $\times$ 2cm. diam.) of Zeo-Karb 225 resin (H⁺ form, 50–100 mesh) and the column was washed with water until the pH of the eluate rose to 5. Amino acids were displaced from the column with 60ml. of aq. 2N-ammonia solution, and the ammoniacal eluate was evaporated at 45° under reduced pressure. The residue, a buff-coloured solid, was shown by chromatography in solvents A and B to contain at least two minor components, which reacted only with ninhydrin, in addition to the main component which reacted with both ninhydrin and the modified platinum reagent.

The residue was chromatographed on a column (60cm. long $\times$ 3cm. diam.) of cellulose with a mixture of butan-1-ol-acetic acid-water (12:3:5, by vol.), and 25ml. fractions were collected. The major component was detected by chromatography in fractions 18-21 inclusive. These were combined, evaporated at 50° under reduced pressure, and the residue was refractionated on a cellulose column (60cm. long $\times$ 2cm. diam.) with the same solvent. Fractions (10ml.) were collected. The required amino acid, which reacted with both ninhydrin and the modified platinum reagent, was recovered by evaporation from fractions 22-25 inclusive as a colourless solid which was recrystallized from aqueous ethanol. The product weighed 45mg., m.p. 190-191° (decomp.) (Found C, 40.6; H, 7.5; N, 7.5; S, 17.7%). It had no significant optical rotation. Titration of a sample (20mg.) in water (initial pH 5.3) with 0.24*N*-sodium hydroxide indicated an equivalent weight of 177 and the presence of a group ionizing with pK_a about 9.1.

Properties of the isolated metabolite and its hydrolysis product. The products obtained by degradation of the metabolite and its hydrolysis product with Raney nickel were chromatographed on Whatman no. 1 paper with solvents A, B and C, and chromatograms were treated with the modified platinum reagent and then with ninhydrin. No sulphur-containing compounds were detected. The metabolite, its hydrolysis product, and synthetic 2-hydroxypropylmercapturic acid and *S*-(2-hydroxypropyl)-L-cysteine all gave a product identical with alanine, and methionine gave a chromatographically distinct product, presumably 2-aminobutyric acid (Table 4).

2,4-Dinitrophenylhydrazones were prepared from the oxidation products of the steam-volatile compounds formed on Raney-nickel degradation

of the metabolite, (+)- and (-)-dicyclohexylammonium 2-hydroxypropylmercapturate, dicyclohexylammonium 3-hydroxypropylmercapturate and (+)- and (-)-dicyclohexylammonium 2-hydroxy-1-methylethylmercapturate. These were compared by thin-layer chromatography with the 2,4-dinitrophenylhydrazones of C₁-C₄ aldehydes and ketones. Formaldehyde, acetaldehyde, acetone, propionaldehyde, butan-2-one and butyraldehyde 2,4-dinitrophenylhydrazones had R_F 0.38, 0.40, 0.42, 0.48, 0.50 and 0.52 respectively. The metabolite and (+)- and (-)-dicyclohexylammonium 2-hydroxypropylmercapturate all gave a product with the same R_F as acetone 2,4-dinitrophenylhydrazone whereas the other compounds gave a product identical with propionaldehyde 2,4-dinitrophenylhydrazone. Traces of material with R_F values corresponding to formaldehyde and acetaldehyde 2,4-dinitrophenylhydrazones were occasionally formed from the degradation products of all compounds.

The metabolite was subjected to the action of acylase I and to acid hydrolysis, and the reaction products were chromatographed on Whatman no. 1 paper with solvents A and B. The chromatograms were treated with the modified platinum reagent and with ninhydrin, and in every case an amino acid was detected which was not separated from the product isolated from the hydrolysate of crude metabolite fractions or from *S*-(2-hydroxypropyl)-L-cysteine. It differed chromatographically from *S*-propyl-L-cysteine (Table 5).

The hydrolysed metabolite and the compound isolated after acid hydrolysis of crude metabolite fractions were subjected to the activity of L- and D-amino acid oxidase in the presence of catalase, and samples were chromatographed on Amberlite WA-2 ion-exchange paper. Treatment of the

Table 4. *Chromatographic examination of the amino acids formed on degradation of the hydrolysed metabolite with Raney nickel*

Details are given in the text. Chromatograms were dipped in the platinum reagent and then in ninhydrin. Those prepared with solvent C were washed with acetone before dipping.

Solvent.....	R_F		
	A	B	C
<i>S</i> -(2-Hydroxypropyl)-L-cysteine	0.35*	0.34*	0.78*
Alanine	0.29†	0.24†	0.59†
Degradation product of:			
Hydrolysed metabolite	0.30†	0.27†	0.59†
Isolated hydrolysis product	0.30†	0.26†	0.58†
<i>S</i> -(2-Hydroxypropyl)-L-cysteine	0.29†	0.26†	0.58†
Methionine	0.40†	0.31†	0.67†

* White spot with the platinum reagent and purple with ninhydrin.

† Purple with ninhydrin.

Table 5. *Chromatographic examination of the hydrolysis products of the metabolite*

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

Solvent	R_F					
	A	B	A	B	A	B
<i>S</i> -2-Hydroxypropyl-L-cysteine	0.34†	0.34†				
<i>S</i> -Propyl-L-cysteine	0.64†	0.56†				
Isolated hydrolysis product	0.36†	0.36†				
			Acid-hydrolysed		Hydrolysed with acylase I	
Metabolite	0.73*	0.45*	0.35†	0.38†	0.35†	0.34†
(+)-Dicyclohexylammonium 2-hydroxypropylmercapturate	0.75*	0.43*	0.36†	0.34†	0.36†	0.33†
(-)-Dicyclohexylammonium 2-hydroxypropylmercapturate	0.74*	0.43*	0.36†	0.36†	0.34†	0.33†

* White spot with the platinum reagent.

† White spot with the platinum reagent and purple with ninhydrin.

Table 6. *Action of L- and D-amino acid oxidase on the hydrolysed metabolite*

Details are given in the text. The disappearance of amino acids was observed by dipping chromatograms prepared on Amberlite WA-2 ion-exchange paper in ninhydrin. 2,4-Dinitrophenylhydrazones were chromatographed on Whatman no. 1 paper.

Solvent	Ninhydrin reaction after incubation				R_F of 2,4-dinitrophenylhydrazone			
	With L-amino acid oxidase		With D-amino acid oxidase		B		D	
	0	4	0	4	With L-amino acid oxidase	With D-amino acid oxidase	With L-amino acid oxidase	With D-amino acid oxidase
Time (hr.).....								
<i>S</i> -(2-Hydroxypropyl)- L-cysteine	+	—	+	+	0.88	0.82	—	—
DL-Alanine	++	+	++	+	0.75	0.51	0.75	0.51
Metabolite hydrolysed with acid	+	—	+	+	0.88	0.83	—	—
Metabolite hydrolysed with acylase I	+	—	+	+	0.89	0.84	—	—
Compound isolated from hydrolysed metabolite	+	—	+	+	0.90	0.81	—	—

chromatograms with ninhydrin showed that the compounds were destroyed by L-amino acid oxidase, but not by D-amino acid oxidase. Further, 2,4-dinitrophenylhydrazones were isolated from the reaction products formed with L-amino acid oxidase, and these were chromatographically identical with the product from *S*-(2-hydroxypropyl)-L-cysteine (Table 6).

Metabolism of 1,2-epoxypropane and 1-chloropropan-2-ol

Male rats (body wt. 190–220g.) were injected subcutaneously with 0.5ml. of 3.3% (w/v) 1-

chloropropan-2-ol in arachis oil or with 1ml. of 2% (w/v) 1,2-epoxypropane in arachis oil. The urine excreted by each rat in the first 24hr. after dosing was diluted to 20ml. with water-washings from the urine collector, and samples (20 μ l.) were chromatographed on Whatman no. 1 paper with solvents A and B. After dipping the chromatogram in the modified platinum reagent a metabolite was detected with the same R_F as 2-hydroxypropylmercapturic acid. The metabolite was eluted from strip chromatograms and hydrolysed with hydrochloric acid as previously described. Chromatograms of the hydrolysate were prepared with solvents A and B, and dipped in the platinum

Table 7. *Chromatographic examination of the metabolites excreted by rats in the first 24 hr. period after they had been injected subcutaneously with 1,2-epoxypropane or 1-chloropropan-2-ol*

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

Solvent	R_F			
	A	B	A	B
2-Hydroxypropylmercapturic acid	0.72*	0.45*		
<i>S</i> -(2-Hydroxypropyl)-L-cysteine			0.33†	0.35†
Compound injected	Metabolite		Hydrolysed metabolite	
1,2-Epoxypropane	0.74*	0.45*	0.36†	0.36†
1-Chloropropan-2-ol	0.75*	0.43*	0.34†	0.36†

* White spot with the platinum reagent.

† White spot with the platinum reagent and purple with ninhydrin.

reagent and then in ninhydrin. A compound was detected with both reagents which was not separable from *S*-(2-hydroxypropyl)-L-cysteine (Table 7).

DISCUSSION

The compound isolated after acid hydrolysis of crude fractions of the unknown metabolite of 1-bromopropane was an L-amino acid. When an aqueous solution (initial pH 5.3) was titrated with sodium hydroxide the presence of a group ionizing with pK_a about 9.1 was detected and, furthermore, the compound reacted with ninhydrin and this reaction disappeared after incubation with L-amino acid oxidase but not after incubation with D-amino acid oxidase (Table 6). Elementary microanalysis of the amino acid indicated an empirical formula $C_6H_{13}NO_3S$, in good agreement with the equivalent weight (177) determined by titration. Comparison of this with the empirical formula of the dicyclohexylammonium salt of the metabolite, $C_{20}H_{38}N_2O_4S$, suggested that during hydrolysis an acetyl group was lost, in keeping with the susceptibility of the metabolite to acylase I (Table 5).

The metabolite was an *S*-substituted derivative of cysteine, for on degradation of its hydrolysis product with Raney nickel alanine was formed (Table 4). On the assumption that the equivalent weight is the same as the molecular weight, the microanalyses indicated that the *S*-substituent was C_3H_7O , probably a hydroxypropyl group, and confirmation of this was obtained by oxidizing the volatile products of Raney nickel degradation and converting the oxidation products into 2,4-dinitrophenylhydrazones. The metabolite gave acetone 2,4-dinitrophenylhydrazone, showing that a 2-hydroxypropyl group was involved. The amino acid formed on hydrolysis of the metabolite was

stable towards acid, and it was assumed that the *S*-substituent had the structure (I) rather than (II),

(I)

(II)

for (II), a hemithioketal, would be acid-labile.

The results of these degradative studies suggested that the unknown metabolite was 2-hydroxypropylmercapturic acid (III). This was confirmed by synthesis.

(III)

Synthetic 2-hydroxypropylmercapturic acid was prepared by two methods, and resolved by fractional crystallization of the dicyclohexylammonium salt into dextro- and laevo-rotatory isomers. The formation of identical products by both methods (the reaction of 1,2-epoxypropane with cysteine followed by acetylation, and the reduction of 2-oxopropylmercapturic acid) showed that the reaction of 1,2-epoxypropane with cysteine gave rise to the *S*-(2-hydroxypropyl) derivative and not to the *S*-(2-hydroxy-1-methylethyl) derivative. This was confirmed by degradation of synthetic *S*-(2-hydroxypropyl)-L-cysteine with Raney nickel and oxidation of the reaction products. Acetone-2,4-dinitrophenylhydrazone was isolated from the oxidation products, whereas propionaldehyde 2,4-dinitrophenylhydrazone was isolated from the

other hydroxypropylmercapturic acids with acid-stable *S*-substituents (3-hydroxypropyl- and 2-hydroxy-1-methylethyl-mercapturic acid). (The identity of the metabolite with 2-hydroxypropylmercapturic acid and its distinction from the other hydroxypropylmercapturic acids was also demonstrated by Dr S. P. James by gas chromatography of the methyl esters.)

The infrared-absorption spectra of (+)- and (-)-dicyclohexylammonium 2-hydroxypropylmercapturate could not be distinguished from each other or from that of the dicyclohexylammonium salt of the metabolite. They did differ from the spectra of dicyclohexylammonium 3-hydroxypropylmercapturate and (+)- and (-)-dicyclohexylammonium 2-hydroxy-1-methylethylmercapturate, particularly in details in the range 700–1600 cm⁻¹.

The isolated metabolite and the isolated hydrolysis product of the metabolite had no significant optical rotation, showing that they were mixtures of the diastereoisomers derived from L-cysteine. The proportion of isomers in each isolated compound was not the same, however, for although the metabolite was isolated as approximately equal parts of the (+)- and (-)-dicyclohexylammonium salts, the hydrolysis product, *S*-(2-hydroxypropyl)-L-cysteine, consisted of about 90% of the (+)-isomer. This is consistent with the known solubility properties of (+)- and (-)-dicyclohexylammonium 2-hydroxypropylmercapturate. On fractional crystallization from ethanol–acetone mixtures, a procedure also used to purify the metabolite, a preferential enrichment of the laevorotatory isomer occurs. On the other hand, no resolution of (+)- and (-)-*S*-(2-hydroxypropyl)-L-cysteine was observed during the procedures used in its purification, and so it may well be that in the excreted metabolite the isomer which gives a dextrorotatory dicyclohexylammonium salt was predominant.

The recognition of 2-hydroxypropylmercapturic acid as a metabolite of the 1-halogenopropanes led to an examination of the metabolism of two other

compounds, 1,2-epoxypropane and 1-chloropropan-2-ol, which on chemical grounds were expected to give rise to the same metabolite, and this was supported by chromatographic evidence (Table 7). This compound was not found, however, among the metabolites of *S*-propyl-L-cysteine (Barnsley *et al.* 1966) or *S*-propylglutathione (C. J. Foxwell & L. Young, personal communication). It remains to be determined whether these observations indicate that in the metabolic formation of 2-hydroxypropylmercapturic acid an intermediate such as 1,2-epoxypropane or 1-halogenopropan-2-ol is formed from 1-halogenopropane before alkylation of a cysteine residue, or that after injection into the rat *S*-propyl-L-cysteine and *S*-propylglutathione do not have access to the site where hydroxylation occurs.

The metabolic formation of hydroxyalkylmercapturic acids from 1-halogenoalkanes may be a general reaction for James & Jeffery (1964) obtained evidence for the formation of 2-hydroxybutylmercapturic acid from 1-bromobutane.

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