The Metabolism of *S*-Methyl-L-Cysteine

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1. Methylsulphinylacetic acid, 2-hydroxy-3-methylsulphinypropionic acid and methylmercapturic acid sulfoxide (*N*-acetyl-*S*-methyl-L-cysteine *S*-oxide) were isolated as their dicyclohexylammonium salts from the urine of rats after they had been dosed with *S*-methyl-L-cysteine. 2. A fourth sulfoxide was isolated but not identified. 3. The excretion of sulphate in the urine of rats dosed with *S*-methyl-L-cysteine was measured. 4. The metabolism of *S*-methyl-L-cysteine by the hamster and guinea pig was examined chromatographically. 5. The preparation of the following compounds is reported: (–)-dicyclohexylammonium methylmercapturate sulfoxide; the dicyclohexylammonium salts of the optically inactive forms of 2-hydroxy-3-methylthiopropionic acid, 2-hydroxy-3-methylsulphinypropionic acid and methylsulphinylacetic acid.

A number of metabolites excreted in the urine of animals dosed with *S*-methyl-L-cysteine have been identified, and include sulphate in dogs (Pirie, 1932), rabbits (du Vigneaud, Loring & Craft, 1934) and man (Brand, Block & Cahill, 1937), and neutral sulphur compounds in the rabbit (du Vigneaud *et al.* 1934) and man (Brand *et al.* 1937). More recently some of the neutral sulphur metabolites excreted by rats have been identified as *N*-acetyl-*S*-methyl-L-cysteine, its *S*-oxide, *N*-(methylthioacetyl)glycine and methylthioacetic acid (Barnesley, 1964). The methyl group of *S*-methyl-L-cysteine is extensively oxidized to carbon dioxide and a very small proportion of the methyl carbon atom is incorporated into the methyl groups of choline in the rat (Horner & Kuchinskas, 1959).

A further study of the metabolism of *S*-methyl-L-cysteine in the rat using the ³⁵S-labelled compound has revealed the presence of a large amount of material chromatographically similar to *N*-acetyl-*S*-methyl-L-cysteine *S*-oxide, and experiments designed to isolate the material have shown that, in addition to this compound, three other metabolites are also present. These have been isolated as dicyclohexylammonium salts, and two of them have been identified as 2-hydroxy-3-methylsulphinypropionic acid and methylsulphinylacetic acid. The metabolism of *S*-methyl-L-cysteine in the hamster and guinea pig has also been investigated by chromatographic methods.

MATERIALS

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

Strauss, Oxford, and the Analytical Laboratory, School of Pharmacy, London, W.C. 1. The following compounds were described by Barnesley (1964): *S*-methyl-L-cysteine, methylmercapturic acid, *N*-(methylthioacetyl)glycine, (–)-methylmercapturic acid sulfoxide and the dicyclohexylammonium salt of (+)-methylmercapturic acid sulfoxide. Methylthioacetic acid was prepared by the method of Maw & du Vigneaud (1948) and had b.p. 126–128° at 22mm. Hg (Found: C, 33.9; H, 6.2; S, 29.6. Calc. for C₃H₆O₂S: C, 33.9; H, 5.7; S, 30.2%). 3-Methylthio-2-oxopropionic acid was prepared by a method similar to that of Parrod (1951), except that bromopyruvic acid was used as starting material. The product had m.p. 147° (decomp.); Parrod (1951) gave m.p. 149°. (Found: C, 36.2; H, 4.8; S, 23.9; equiv.wt. 133. Calc. for C₄H₆O₃S: C, 35.8; H, 4.5; S, 23.9%; equiv.wt. 134). Other general methods for the preparation of 2-oxo acids, such as those of Adickes & Andresen (1944) and Wegand, Steglich & Tanner (1962), failed to give the required product.

S-Methyl-L-[³⁵S]cysteine. This was prepared by a method similar to that described for the preparation of *S*-ethyl-L-[³⁵S]cysteine by Thomson, Barnesley & Young (1963). It had specific radioactivity 111 μC/m-mole, and appeared to be radiochemically pure on chromatography in solvents *A* and *B* (see below).

(±)-Dicyclohexylammonium 2-hydroxy-3-methylthiopropionate. This was prepared by the reduction of 3-methylthio-2-oxopropionic acid (0.60g.) with NaBH₄ (0.13g.) in solution in 20 ml. of aq. *N*-NH₃ at 35°. The reaction mixture was treated with excess of Zeo-Karb 225 resin (H⁺ form) and filtered, and the filtrate was evaporated. The residue, a colourless gum, was dissolved in methanol and the solution was evaporated. This evaporation with methanol was repeated six times, and the final residue was dissolved in methanol and neutralized with a solution of dicyclohexylamine in acetone. The solution was evaporated, and the residue was crystallized from ethyl acetate to give 0.76g. of product, m.p. 147.5–148° (Found: C, 60.9; H, 9.9; N, 4.1; S, 9.5; C₁₆H₃₁NO₃S requires C, 60.5; H, 9.8; N, 4.4; S, 10.1%). The product was not optically active.

($\pm$)-*Dicyclohexylammonium 2-hydroxy-3-methylsulphinylpropionate*. This was prepared by oxidizing dicyclohexylammonium 2-hydroxy-3-methylthiopropionate (0.407 g.) in solution in water (10 ml.) with 0.15 ml. of 30% (w/v) hydrogen peroxide. After 18 hr. the solution was evaporated, and the residue was crystallized from ethyl acetate and gave 0.302 g. of product, m.p. 141° (Found: C, 57.8; H, 9.3; N, 3.8; S, 9.1; $C_{16}H_{31}NO_4S$ requires C, 57.6; H, 9.4; N, 4.2; S, 9.6%). The product was not optically active and is presumed to be a mixture of two pairs of diastereoisomers.

($\pm$)-*Dicyclohexylammonium methylsulphinylacetate*. This was prepared by oxidizing methylthioacetic acid (2.12 g.) in aqueous solution with 2.4 ml. of 30% (w/v) hydrogen peroxide. After 24 hr. the solution was neutralized with dicyclohexylamine and evaporated to dryness, and the white residue that resulted was crystallized from solution in the minimum of hot ethanol by the addition of acetone. The product (4.54 g.) had m.p. 151° (decomp.) (Found: C, 58.9; H, 9.7; N, 4.6; S, 10.8; $C_{15}H_{29}NO_3S$ requires C, 59.4; H, 9.6; N, 4.6; S, 10.6%). The product was not optically active and is known to contain both sulphoxide isomers, for the enantiomeric forms prepared by another method have $[\alpha]_D^{29} \pm 2^\circ$ (Barnsley, 1968).

(-)-*Dicyclohexylammonium methylmercapturate sulphoxide*. This was prepared by neutralizing (-)-methylmercapturic acid sulphoxide with dicyclohexylamine, and crystallizing the product from solution in ethanol by the addition of acetone. The product had m.p. 168–169° (decomp.), $[\alpha]_D^{20} -67^\circ \pm 1.5^\circ$ (c 1.5 in water) (Found: C, 57.4; H, 9.2; N, 7.6; S, 8.6; $C_{13}H_{24}N_2O_4S$ requires C, 57.7; H, 9.2; N, 7.5; S, 8.6%).

METHODS

Animals and dosing. Male rats, of a black hooded or albino strain (body wt. 200–250 g.), and hamsters (body wt. 105–130 g.) were fed with Oxoid pasteurized mouse cubes. Guinea pigs (body wt. 330–340 g.) were fed with SG1 diet (pellet form) supplemented with cabbage. In experiments to determine sulphate excretion rats were fed with the diet described by Maw (1953). *S*-Methyl-L-cysteine was administered as an aqueous solution by subcutaneous injection, and each animal received 0.5 g./kg. body wt. Urine was collected separately from faeces and chromatographed immediately after collection. When required for isolation experiments urine was stored at -25° .

Paper chromatography. Paper chromatograms were developed on Whatman no. 3 paper by the descending method with butan-1-ol-acetic acid-water (12:3:5, by vol.) (solvent A), and by the ascending method with butan-1-ol-pyridine-aq. 3N-NH₃ (4:3:3, by vol.) (solvent B). Radioactive samples were chromatographed on strips of paper 2.5 cm. wide, and scanned for radioactivity with a Packard model 7200 radiochromatogram scanner. After the scanning for radioactivity, thio ethers and sulphoxides were detected by dipping the paper in the iodoplatinate reagent of Toennies & Kolb (1951) as modified by Barnsley, Thomson & Young (1964). Amino acids were detected after treatment with the iodoplatinate reagent by dipping the chromatogram in 0.2% (w/v) ninhydrin in acetone to which pyridine (2%, v/v) had been added.

Desalting of metabolite fractions. Cations were removed from solution by passing the solution through 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. When formic acid was present, the eluate was evaporated (in batches of 500 ml. or less) under reduced pressure at 45–50° and the residue was dried over pellets of NaOH *in vacuo*.

Infrared-absorption spectra. These were measured by the KBr disc technique in a Perkin-Elmer 237 infrared spectrophotometer.

Determination of the equivalent weight of salts. This was carried out by the method described by Barnsley (1966).

Reduction of sulphoxides with cysteine. The reduction of ethylmercapturic acid sulphoxide with thioglycolic acid was described by Barnsley *et al.* (1964). The need to use an excess of reductant, and the difficulty of removing this after the reaction, made the direct examination of the reaction products by chromatography difficult. Reduction of sulphoxides (which are not amino acids or bases) with cysteine was therefore adopted, and unwanted reaction products were removed with a cation-exchange resin. A freshly prepared solution of L-cysteine hydrochloride was neutralized to pH 7 with NaOH, and the solution was diluted to 0.3 M with water. A sample (0.1 ml.) of a neutral solution of the sulphoxide (0.02–0.03 M) was sealed with 0.1 ml. of the cysteine solution in a small Pyrex tube. The tube was heated for 5 hr. in a boiling-water bath, cooled and opened. The reaction mixture was passed through a column (0.5 cm. diam. $\times$ 1 cm.) of Zeo-Karb 225 (H⁺ form; 50–100 mesh) and the column was washed with four portions (0.2 ml. each) of water. The total eluate was mixed and 50 μ l. samples were chromatographed.

Acid decomposition of sulphoxides. The decomposition of sulphoxides with HCl is a complex reaction giving rise to numerous products (Synge & Wood, 1956; Ostermayer & Tarbell, 1960; Kenney, Walsh & Davenport, 1961). Among these products, thio ethers corresponding to the original sulphoxide are found (see also Barnsley *et al.* 1964) and the formation of these derivatives was used in the present work. A sample of the sulphoxide (about 0.03 M) was heated with an equal volume of conc. HCl in a boiling-water bath for 2 hr. A sample (10 μ l.) of the reaction mixture was chromatographed, and thio ethers were detected by dipping the chromatogram in the modified iodoplatinate reagent.

RESULTS

Metabolism of S-methyl-L-cysteine in the rat

Conversion of S-methyl-L-cysteine into sulphate. The excretion of total and inorganic sulphate in the urine of rats was measured by the procedure described by Maw (1953). Urine was collected for 24 hr. periods for 2 days, and each rat was then injected subcutaneously with 1.5 ml. of an aqueous solution of *S*-methyl-L-cysteine (0.5 M) at the beginning of the third 24 hr. period. The results (Table 1) show that there was a large increase in the excretion of inorganic sulphate equivalent to about 50% of the dose.

Chromatographic studies. Each of two black-hooded rats was dosed with about 0.6 m-mole of *S*-methyl-L-[³⁵S]cysteine in water, and urine excreted in each consecutive 24 hr. period after dosing

Table 1. *Excretion of sulphate by rats injected subcutaneously with S-methyl-L-cysteine*

For details see the text. Each rat received *S*-methyl-L-cysteine containing 24mg. of S at the beginning of the third day.

		Sulphate sulphur (mg.) excreted in 24 hr.				
	Day.....	1	2	3	4	5
Rat 1	Inorganic sulphate	5.9	5.4	16.4	5.6	6.7
	Ethereal sulphate	0.4	0.9	0.5	0.6	0.5
Rat 2	Inorganic sulphate	6.1	5.9	19.5	6.6	5.4
	Ethereal sulphate	0.5	0.4	0.6	0.4	0.6

was collected and diluted to 25ml. with water-washings from the urine-faeces separator. A sample of the urine (1ml.) was passed through a column (1ml.) of Zeo-Karb 225 resin (H^+ form) and the column was washed with water until the total eluate had a volume of 5ml. Chromatography of the total eluate (100 μ l.) with solvent *A* followed by scanning for radioactivity gave the results shown in Fig. 1.

The first double peak (I, Fig. 1) of radioactivity was probably principally sulphate, for the shape and R_F of the peaks was characteristic of those obtained with authentic radioactive sulphate and the amount of radioactivity in this fraction corresponded to that expected from independent measurements of sulphate excretion. Peak II occurred in the same region where a yellow reaction with the iodoplatinate reagent was obtained, and the presence here of four sulfoxides was later demonstrated by isolation experiments. Peak III, which was incompletely resolved into three components, corresponded to the region where the iodoplatinate reagent gave three white spots due to the presence of *N*-(methylthioacetyl)glycine, methylmercapturic acid and methylthioacetic acid. The identification of these metabolites was reported previously (Barnsley, 1964).

The radioactivity in each area corresponding to a peak on the radiochromatogram was computed from the area under the peak, corrected for background, and expressed as a percentage of the total radioactivity on the chromatogram. From the total area under peaks on the radiochromatogram the total radioactivity excreted in the first 24hr. after dosing was also calculated (Table 2). Radioactive metabolites were not detected in the urine in 24hr. periods after the first.

In these experiments urine was treated with Zeo-Karb 225 resin before chromatography to avoid trailing of the radioactive metabolites present, particularly those in peak II. This treatment would, however, have removed unchanged *S*-methyl-L-cysteine. When ammonia solution (3*N*) was passed over the resin and the eluate was chromatographed no radioactive metabolites were detected, indicating the absence of *S*-methyl-L-

Fig. 1. Radiochromatogram of the urine of rats dosed with *S*-methyl-L- $[^{35}S]$ cysteine. Reaction with the modified iodoplatinate reagent: Y, yellow spot; W, white spot. The radioactive material in each peak was identified as follows: I, sulphate; II, sulfoxides; III, thio ethers. For details see the text.

cysteine. In this respect the rats used in the present experiment gave different results from those reported previously (Barnsley, 1964). The albino strain did excrete unchanged *S*-methyl-L-cysteine.

Isolation of some metabolites. Twelve male albino rats were each injected with *S*-methyl-L-cysteine (1.5ml., 0.5*M*), and urine was collected for 24hr. after dosing. The dosing was repeated on two further occasions at 4 day intervals, and urine was stored at -25° until the final collection had been made. The total urine (volume 258ml.) was diluted with water to 400ml., the pH of the solution was then lowered to about 4.5 with formic acid, and the solution was applied to a column (47cm. $\times$ 5.8cm. diam.) of Amberlite CG-400 resin (formate form; 100–200 mesh) that had been equilibrated with sodium formate buffer (pH 4.0, 0.1*M* with respect to total formate). Elution was carried out with the same buffer and 500ml. fractions were collected. A sample of each fraction (2ml.) was shaken with 0.2ml. of Zeo-Karb 225 resin (H^+ form), 100 μ l. of the solution was chromatographed with solvent *A* and the chromatogram was treated with the modified iodoplatinate reagent. Fractions

6–11 inclusive gave yellow spots at R_F 0.53, fractions 10–14 inclusive gave yellow spots at R_F 0.47 and fractions 15–19 inclusive gave yellow spots at R_F 0.51.

Fractions 6–14 inclusive were combined, desalted and rechromatographed on a column of Amberlite CG-400 resin (formate form; < 200 mesh) with the same buffer as before. Fractions (25ml.) were collected, and chromatography of these showed that fractions 18–26 inclusive contained material that gave a yellow spot at R_F 0.53 and fractions 28–33 inclusive material that gave a yellow spot at R_F 0.47. Fractions 18–26 inclusive were combined (metabolite A), and fractions 28–33 inclusive were combined (metabolite B). Fractions 15–19 inclusive from the first fractionation were combined for the isolation of metabolites C and D.

Isolation and properties of metabolite A. Fraction A was desalted, and chromatographed on a column of cellulose (40cm. $\times$ 2cm. diam.) with butan-1-ol-ethanol-aq. 3N-ammonia (9:3:1, by vol.), and 25ml. fractions were collected. A sample (40 μ l.) of each fraction was applied to a strip of paper and dried, and the paper treated with the modified iodoplatinate reagent. Fractions 26–40 inclusive gave a yellow spot, and these fractions were combined and evaporated. The residue was dissolved in water, and cations were removed from the solution with Zeo-Karb 225 resin. The acid solution obtained was titrated with 2% (v/v) dicyclohexylamine in acetone until the apparent pH rose to 7, and the solution was evaporated to dryness. The residue (79mg.) was recrystallized from the minimum of hot chloroform by the addition of acetone, and gave a purest fraction (33mg.) of metabolite A, m.p. 160–161° (decomp.), $[\alpha]_D^{25} - 7^\circ$ (c 1 in water) (Found: C, 57.8; H, 8.7; N, 7.1; S, 8.1%). When the isolation was repeated, the product had m.p. and infrared spectrum identical with those of the material first isolated, but it had no significant optical rotation.

Metabolite A was a sulfoxide, for it gave a yellow spot with the modified iodoplatinate

reagent after chromatography, and was reduced on heating with cysteine (see the Methods section) to give a compound that was chromatographically identical with methylmercapturic acid. Acid degradation of the latter reaction product (or of the original metabolite) gave an amino acid that was chromatographically identical with *S*-methyl-L-cysteine (Table 3). These data are in agreement with the identification of the compound as dicyclohexylammonium methylmercapturate sulfoxide, and this was confirmed by comparison of infrared spectra, which were identical for the biosynthetic compound and the synthetic (+)- and (-)-compounds. The m.p. of the metabolite was lower than that of either (+)- or (-)-dicyclohexylammonium methylmercapturate sulfoxide, but accorded with that of mixtures of isomers.

Isolation and properties of metabolite B. Fraction B was desalted, chromatographed on cellulose, and the product was converted into the dicyclohexylammonium salt as described for metabolite A. The product (72mg.) was recrystallized from ethyl acetate and gave 61mg. of metabolite B, m.p. 157–158°, $[\alpha]_D^{25} + 57 \pm 2^\circ$ (c 0.5 in water) (Found: C, 57.8; H, 9.4; N, 4.2; S, 8.5%; equiv.wt. 335).

Metabolite B was a sulfoxide, for it gave a yellow spot with the modified iodoplatinate reagent on chromatograms, and after reaction with cysteine it gave a product that moved more rapidly on paper chromatograms than the original metabolite and that reacted as a thio ether with the modified iodoplatinate reagent. Acid decomposition of the metabolite gave a compound chromatographically identical with the thio ether produced on reduction with cysteine, but no material was produced that reacted with ninhydrin (Table 3). The analytical data suggested that the compound was the dicyclohexylammonium salt of an acid with the formula $C_4H_8O_4S$. The probable presence of the methylsulphinyl group indicated that the remainder of the molecule was possibly hydroxypropionic acid, and this was confirmed when it was found that the compound gave

Table 2. *Proportions of metabolites detected by radiochromatography of the urine of rats dosed with S-methyl-L-[^{35}S]cysteine*

The proportions of the metabolites were determined from the areas of each peak on the radiochromatogram, and the percentage of the dose excreted was calculated from the total radioactivity on the chromatogram. For details see the text.

	Percentage of the dose excreted*	Proportions of radioactivity in radiochromatogram peaks		
		I (sulphate)	II (sulphoxides)	III (thio ethers)
Rat 1	66	77	18	5
Rat 2	78	70	20	10

* This rapid method of estimating total radioactivity is subject to an error of $\pm 10\%$.

Table 3. *Chromatographic examination of metabolites A, B and C*

For details see the text. Only those reaction products that reacted with the iodoplatinate reagent are recorded.

Solvent mixture.....	R_F					
	A	B	After reduction		After acid decomposition	
			A	B	A	B
Metabolite A	0.55*	0.26*	0.83†	0.45†	0.46‡	0.30‡
Methylmercapturic acid sulphoxide	0.55*	0.23*	0.84†	0.45†	0.47‡	0.31‡
Methylmercapturic acid	0.83†	0.44†				
<i>S</i> -Methyl-L-cysteine	0.46‡	0.30‡				
Metabolite B	0.50*	0.22*	0.79†	0.40†	0.80†	0.41†
2-Hydroxy-3-methylsulphinyl-propionic acid	0.49*	0.22*	0.80†	0.40†	0.78†	0.41†
2-Hydroxy-3-methylthiopropionic acid	0.80†	0.39†				
Metabolite C	0.53*	0.21*	0.88†	0.38†	0.89†	0.35†, 0.58†
Methylsulphinylacetic acid	0.53*	0.19*	0.88†	0.36†	0.89†	0.38†, 0.60†
Methylthioacetic acid	0.87†	0.38†				

* Yellow spot with the modified iodoplatinate reagent.

† White spot with the modified iodoplatinate reagent.

‡ White spot with the modified iodoplatinate reagent and purple with ninhydrin.

a bright-yellow colour with Fe^{3+} ions in neutral solution. This reaction is typical of 2-hydroxy acids, and the metabolite was therefore identified as 2-hydroxy-3-methylsulphinylpropionic acid [$\text{CH}_3 \cdot \text{SO} \cdot \text{CH}_2 \cdot \text{CH}(\text{OH}) \cdot \text{CO}_2\text{H}$]. The infrared spectrum of the metabolite was identical with that of synthetic dicyclohexylammonium 2-hydroxy-3-methylsulphinylpropionate, but the latter had a lower m.p. and was a mixture of four stereoisomers. The metabolite and synthetic compound also gave the same products after reduction and acid decomposition (Table 3).

Isolation and properties of metabolites C and D. Fraction C was treated as was described for fraction A, and a total yield of dicyclohexylammonium salts of 0.81g. was obtained. This was extracted with 15ml. of acetone and a residue (0.12g.) was left that did not react with the iodoplatinate reagent on chromatograms. The acetone extract was evaporated to dryness and the residue obtained was fractionally crystallized from acetone. Two compounds were obtained that reacted like sulphoxides with the modified iodoplatinate reagent. That which crystallized first (metabolite C, 0.12g. of pure material) had m.p. 151° (decomp.) (Found: C, 59.5; H, 9.4; N, 4.8; S, 10.3%; equiv.wt. 302). It had no significant optical activity. The second metabolite (metabolite D, 0.056g.) had m.p. 140 – 140.5° (decomp.).

The elementary analysis of metabolite C (as the dicyclohexylammonium salt) indicated an empirical formula for the acid of $\text{C}_3\text{H}_6\text{O}_3\text{S}$, and on the assumption that the acid was monobasic and that it con-

tained a methylsulphinyl group it appeared to be methylsulphinylacetic acid ($\text{CH}_3 \cdot \text{SO} \cdot \text{CH}_2 \cdot \text{CO}_2\text{H}$). This was confirmed by reduction of the metabolite with cysteine and by degradation with hydrochloric acid, in which reactions the metabolite behaved identically with the synthetic compound. Acid decomposition gave two compounds that reacted like thio ethers one of them may have been methylthioacetic acid (Table 3). The synthetic and biosynthetic compounds also had identical infrared spectra.

Metabolite D was not characterized fully. It was very similar to methylsulphinylacetic acid chromatographically, and gave chromatographically similar sulphur-containing compounds after reduction and acid decomposition. The latter reaction, however, also gave rise to an amino acid.

Metabolism of S-methyl-L-cysteine in the hamster and the guinea pig

Hamsters and guinea pigs were injected subcutaneously with an aqueous solution of *S*-methyl-L-cysteine, and the urine excreted in the first 24 hr. after dosing was examined by paper and column chromatography as described in this and a previous paper (Barnsley, 1964) for the presence of metabolites that have been identified in the urine of rats. Methylsulphinylacetic acid and the unidentified metabolite D from rat urine have only been separated by fractional crystallization of their dicyclohexylammonium salts, and a chromatographic system for separation has not been found. They

are not distinguished therefore in the lists of metabolites given below and either or both may be present. The following metabolites were detected: Guinea pig: *N*-(methylthioacetyl)glycine, methylsulphinylacetic acid/metabolite D and 2-hydroxy-3-methylsulphinylpropionic acid; Hamster: methylmercapturic acid, methylmercapturic acid sulphoxide, 2-hydroxy-3-methylsulphinylpropionic acid and methylsulphinylacetic acid/metabolite D.

Stability of some sulphoxides in formic acid

The sulphoxides isolated in the present work were exposed during the process to formic acid, the strength of which was as high as 15*N* for periods during evaporation. As sulphoxides may racemize in acid solution (Iselin, 1961; Price & Oae, 1962) the possibility arose that this could have occurred during the isolation experiments. When a solution of (–)-methylsulphinylacetic acid in 0.1*N*-formic acid was evaporated in the same way and for the same period of time as was used in the isolation experiments, and the residue was re-dissolved in 0.1*N*-formic acid, there was no significant change in the optical rotation of the solution. On chromatography the compound appeared unchanged.

On the other hand, when (–)-dicyclohexylammonium methylsulphinylacetate was dissolved in 15*N*-formic acid and the solution was heated at 50°, the specific optical rotation of the solution fell from –33° to –17° over a period of 7 hr. and this fall in rotation was accompanied by the formation of material chromatographically similar to methylthioacetic acid. Under the same conditions the specific optical rotation of (–)-methylmercapturic acid sulphoxide fell from –128° to –114°, but no thio ethers were detected by chromatography.

DISCUSSION

It has been shown that in the rat *S*-methyl-L-cysteine is metabolized to inorganic sulphate and to four sulphoxide derivatives; methylmercapturic acid sulphoxide (I), 2-hydroxy-3-methylsulphinylpropionic acid (II), methylsulphinylacetic acid (III) and a compound (metabolite D) that has not been characterized. Sulphate is quantitatively the most important single sulphur-containing metabolite excreted in the urine (about 50% of the dose) and the sulphoxides, taken together as a group, constitute the next largest fraction (about 19% of the dose). The relative amount of each sulphoxide is not known with certainty.

Two thio ethers, methylmercapturic acid and methylthioacetic acid, corresponding to sulph-

oxides isolated in the present work, have previously been identified in the urine of rats dosed with *S*-methyl-L-cysteine, and so the possibility was investigated (in experiments not reported here) that sulphoxides were produced after excretion of the urine. Thio ethers corresponding to the sulphoxides isolated (except that corresponding to the unidentified metabolite D) were added to urine, and the solution was kept at room temperature for 24 hr., frozen at –25° for 6 days and then chromatographed and taken through the fractionation procedure. No sulphoxides were detected, and it was concluded that the sulphoxides were true metabolites and not artifacts.

Examination of the urine of hamsters and guinea pigs after they had been injected with *S*-methyl-L-cysteine has shown that they too excrete some of the metabolites produced by the rat. In guinea-pig urine methylthioacetic acid, *N*-(methylthioacetyl)glycine, 2-hydroxy-3-methylsulphinylpropionic acid and either or both of methylsulphinylacetic acid and the rat metabolite D were present. It is probable, though it has not been determined unequivocally, that extra inorganic sulphate was also excreted. The small amount or absence of *N*-acetylated cysteine derivatives in the urine of guinea pigs after dosing with other mercapturic acid precursors was observed previously by Bray, Franklin & James (1959). In hamster urine methylmercapturic acid, methylmercapturic acid sulphoxide, 2-hydroxy-3-methylsulphinylpropionic acid and methylsulphinylacetic acid/metabolite D were found, but methylthioacetic acid and its glycine conjugate were not detected. It is probable that in this animal too the excretion of extra inorganic sulphate occurred.

The results of the present work indicate that the sulphoxidation reactions observed *in vivo* may not be stereospecific, for the methylsulphinylacetic acid and methylmercapturic acid sulphoxide isolated were each a mixture of sulphoxide isomers. It seems unlikely that this was due to racemization during isolation, and under more drastic conditions (15*N*-formic acid at 50°) when the optical activity of a solution of (–)-methylsulphinylacetic acid did fall, the change was accompanied by the formation

of material chromatographically similar to methylthioacetic acid. This breakdown was not observed during the isolation of biosynthetic methylsulphinylacetic acid.

Unlike the other sulfoxides isolated from urine, 2-hydroxy-3-methylsulphinypropionic acid may have been predominantly one sulfoxide isomer, for the pure dicyclohexylammonium salt represented 80–90% of the crude product and had a high specific optical activity ($[\alpha]_D +57^\circ$). The synthetic compound has only been synthesized as a mixture of two pairs of diastereoisomers that have not been resolved, and so the optical purity of the biosynthetic material cannot be checked. A number of sulfoxides of biological origin have been isolated previously, and although chlorpromazine sulfoxide from dog urine was not optically active (Salzman & Brodie, 1956), ethylmercapturic acid sulfoxide was isolated as the pure dextrorotatory sulfoxide isomer from rat urine (Barnsley *et al.* 1964) and L-methionine sulfoxide as the dextrorotatory isomer from the blowfly (Lucas & Levenbook, 1966). From plant materials *S*-allyl-, *S*-methyl- and *S*-propyl-L-cysteine *S*-oxide have been isolated as dextrorotatory isomers (Fowden, 1964) and ω -methylsulphinyalkyl isothiocyanates as laevorotatory isomers (Klyne, Day & Kjaer, 1960).

The biosynthetic 2-hydroxy-3-methylsulphinypropionic acid may have the L-configuration about the hydroxy group if it is formed from an intermediate 2-oxo acid by reduction with an enzyme having the same stereospecificity as lactate dehydrogenase or the 2-hydroxy acid oxidase from animal tissue (Blanchard, Green, Nocito-Carrol & Ratner, 1946).

The chemical nature of the metabolites of *S*-methyl-L-cysteine described in this and a previous paper (Barnsley, 1964) suggests that they are derived by acetylation of the amino group, or transamination to give a 2-oxo acid that is either reduced to a 2-hydroxy compound or oxidatively decarboxylated to methylthioacetic acid. The latter may then conjugate with glycine. In addition, sulfoxidation may occur at one or several of these stages. Metabolic processes involving analogous reactions, except the sulfoxidation step, have been observed with sulphur-containing amino acids in several organisms. Hydroxy analogues of cysteine derivatives are produced by yeast (Maw & Coyne, 1966) and diatoms (Busby, 1966), and 2-hydroxy-4-methylthiobutyric acid promotes the growth of the rat on a diet low in sulphur-containing amino acids (Block & Jackson, 1932) and is converted into methionine by preparations from rat tissue (Langer, 1965). Methionine is converted by reactions that probably involve transamination and oxidative decarboxylation to

dimethyl- β -propiothetin in the marine alga *Ulva lactuca* (Greene, 1962) and to 3-methylthiopropionic acid in *Neurospora* (Galsworthy & Metzenberg, 1965). Ethionine is converted into 3-ethylthiopropionic acid in *Escherichia coli* (Faith & Mallette, 1966). It has not been determined if reactions of this type occur in the production of the major sulphur-containing metabolite of *S*-methyl-L-cysteine, i.e. inorganic sulphate.

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