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Biochemical Studies of Toxic Agents

THE METABOLIC FORMATION OF 1- AND 2-MENAPHTHYLMERCAPTURIC ACID

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1. Various derivatives of 1- and 2-methylnaphthalene, of the type $C_{10}H_7 \cdot CH_2X$, were administered to rats and the urines of the dosed animals were examined for the presence of 1- and 2-menaphthylmercapturic acid by chromatographic and isolation procedures. A similar, but more limited, series of experiments was carried out with rabbits. 2. All the compounds were administered by subcutaneous injection with the exception of *S*-(1- and 2-menaphthyl)-L-cysteine, which were added to the food. 3. 1-Menaphthylmercapturic acid was isolated from the urine of rats after the administration of 1-menaphthyl chloride, bromide, alcohol, acetate and benzoate, *S*-(1-menaphthyl)-L-cysteine and *S*-(1-menaphthyl)glutathione. 4. 2-Menaphthylmercapturic acid was isolated from rat urine after administration of 2-menaphthyl chloride, *S*-(2-menaphthyl)-L-cysteine and *S*-(2-menaphthyl)-glutathione, and was detected chromatographically after injecting 2-menaphthyl bromide. 5. The corresponding mercapturic acids were isolated after administering 1- and 2-menaphthyl chloride and 1-menaphthyl acetate to rabbits, but not after giving 1- and 2-menaphthyl bromide and 1-menaphthyl alcohol, although chromatographic evidence of mercapturic acid excretion was obtained after injecting these compounds.

The isolation of benzylmercapturic acid (i.e. *N*-acetyl-*S*-benzyl-L-cysteine) from the urine of dogs, rabbits and rats to which benzyl chloride had been administered (Stekol, 1938, 1939) provided the first evidence that aralkyl halides can give rise to formation of mercapturic acids in the animal body. Stekol (1941) also demonstrated that *p*-bromobenzyl bromide undergoes an analogous reaction in rats, and Bray, James & Thorpe (1958) showed that in the rabbit a number of other ω-halogenoalkylbenzenes form mercapturic acids. The excretion of 1- and 2-menaphthylmercapturic acids by animals that had been dosed with 1- and 2-menaphthyl halides, i.e. 1- and 2-(halogenomethyl)naphthalenes (cf. Heilbron & Bunbury, 1953), was reported in a preliminary communication by Hyde & Young (1965), and the present paper is a fuller description of this work together with an account of the formation of 1-menaphthylmercapturic acid in the rat after the administration of 1-menaphthyl alcohol, 1-menaphthyl acetate and 1-menaphthyl benzoate.

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MATERIALS

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

1-Menaphthyl chloride. This compound, m.p. 32°, was purchased from British Drug Houses Ltd., Poole, Dorset.

1-Menaphthyl bromide. The method of Kubiczek & Neugebauer (1950) was used to prepare this compound, m.p. 52–54° (Found: C, 59·7; H, 4·2; Br, 36·0. Calc. for $C_{11}H_9Br$: C, 59·7; H, 4·1; Br, 36·2%).

S-(*1-Menaphthyl*)-L-*cysteine.* A solution of 4·19g. of 1-menaphthyl chloride in 10ml. of 2-methoxyethanol was added to 4·1g. of L-cysteine hydrochloride in 25ml. of 2N-NaOH and the mixture was shaken for 3hr. The mixture was adjusted to pH6, and the precipitate that formed was filtered, washed with 60ml. of ether and then with 50ml. of water and crystallized from 200ml. of boiling aq. 50% (v/v) ethanol. The product (50% yield) had m.p. 197–198° (decomp.) (Found: S, 12·1. $C_{14}H_{15}NO_2S$ requires S, 12·3%).

1-Menaphthylmercapturic acid. This compound was prepared by two methods closely resembling methods I and II described by Grenby & Young (1960) for the preparation of *n*-propylmercapturic acid. Acetylation of *S*-(1-menaphthyl)-L-cysteine by keten in alkaline solution (method I) gave a product (23% yield) that had m.p. 176–177° (decomp.), $[\alpha]_D^{20}$ −48° (*c* 1·1 in ethanol) (Found: C, 63·7; H, 5·7; N, 4·9; S, 11·1. $C_{16}H_{17}NO_3S$ requires C,

63·4; H, 5·6; N, 4·6; S, 10·6%). A product with virtually the same melting point, optical rotation and elementary analysis was obtained in 73% yield when acetylation of *S*-(1-menaphthyl)-L-cysteine in alkaline solution was carried out with acetic anhydride (method II).

S-(1-*Menaphthyl*)*glutathione*. A solution of 2·0g. of 1-menaphthyl chloride in 20ml. of 2-methoxyethanol was added to 3g. of reduced glutathione dissolved in 30ml. of N-NaOH. The mixture was shaken for 3hr. and was then brought to pH 6 by the addition of 3N-HCl. The precipitate that formed at 4° was separated and crystallized from 750ml. of hot aq. 50% (v/v) ethanol. The product (yield 2·95g.) had m.p. 210° (decomp.), $[\alpha]_D^{20}$ +19° (*c* 0·11 in 3N-HCl) (Found: C, 55·5; H, 5·5; N, 9·5; S, 7·2. $C_{21}H_{25}N_3O_6S$ requires C, 56·3; H, 5·6; N, 9·4; S, 7·2%). *S*-(1-Menaphthyl)glutathione was also prepared by shaking 0·7ml. of 1-menaphthyl acetate in 10ml. of 2-methoxyethanol for 3·5hr. with 1·0g. of reduced glutathione in 10ml. of N-NaOH. The solution was acidified to pH 6 and left overnight at 4°. The precipitate was crystallized from aqueous ethanol (yield 0·490g.) and had m.p. 206–207° (decomp.), m.p. 209–210° (decomp.) in admixture with *S*-(1-menaphthyl)glutathione prepared from 1-menaphthyl chloride as described above (Found: S, 7·4%).

1-*Menaphthyl alcohol*. This compound was prepared by the method of Manske & Ledingham (1939), namely by the saponification of 1-menaphthyl acetate obtained by the interaction of 1-menaphthyl chloride, acetic acid and anhydrous sodium acetate. The product had m.p. 62° (Found: C, 83·2; H, 6·2. Calc. for $C_{11}H_{10}O$: C, 83·5; H, 6·3%). 1-Menaphthyl alcohol was also prepared by the method of Nystrom & Brown (1947) whereby 1-naphthoic acid was reduced with $LiAlH_4$ in ether. It had m.p. 62°, alone and when mixed with the product obtained by the method of Manske & Ledingham (1939).

1-*Menaphthyl acetate*. Acetylation of 1-menaphthyl alcohol with acetic anhydride in the presence of H_2SO_4 yielded 1-menaphthyl acetate as a colourless liquid, b.p. 182°/22mm. (Found: C, 77·6; H, 6·3. Calc. for $C_{13}H_{12}O_2$: C, 78·0; H, 6·0%).

1-*Menaphthyl benzoate*. Benzoylation of 1-menaphthyl alcohol with benzoyl chloride in pyridine yielded 1-menaphthyl benzoate, b.p. 230–234°/15mm., which crystallized on standing, m.p. 35–37° (Found: C, 82·2; H, 5·3. Calc. for $C_{18}H_{14}O_2$: C, 82·4; H, 5·3%).

2-*Menaphthyl chloride*. This compound was prepared by chlorination of 2-menaphthyl alcohol with thionyl chloride as described by Campbell, Anderson & Gilmore (1940). It had m.p. 48–49° (Found: C, 75·1; H, 5·3; Cl, 19·5. Calc. for $C_{11}H_9Cl$: C, 74·8; H, 5·1; Cl, 20·1%).

S-(2-*Menaphthyl*)-L-*cysteine*. The method of preparation was similar to that used for *S*-(1-menaphthyl)-L-cysteine. The product (30% yield) had m.p. 206–207° (decomp.) (Found: C, 64·3; H, 5·7; N, 5·2; S, 12·1. $C_{14}H_{15}NO_2S$ requires C, 64·3; H, 5·7; N, 5·4; S, 12·3%).

2-*Menaphthylmercapturic acid*. This compound was obtained in 33% yield by a procedure similar to method II used by Grenby & Young (1960) for the preparation of *n*-propylmercapturic acid. It had m.p. 148°, $[\alpha]_D^{20}$ −51° (*c* 0·86 in ethanol) (Found: C, 62·9; H, 5·3; N, 4·8. $C_{16}H_{17}NO_3S$ requires C, 63·4; H, 5·6; N, 4·6%).

S-(2-*Menaphthyl*)*glutathione*. This compound was prepared in 62% yield by the method used for the corresponding 1-menaphthyl derivative. It had m.p. 215–216° (decomp.) (Found: C, 55·7; H, 5·3; N, 9·6; S, 7·7. $C_{21}H_{25}N_3O_6S$ requires C, 56·3; H, 5·6; N, 9·4; S, 7·2%).

METHODS

Animals and dosing. Male black-hooded rats, body wt. 150–200g., were fed on a diet of rat cake [J. Murray and Sons (London) Ltd.] and water, and urine was collected separately from faeces during consecutive 24hr. periods from the time of dosing until at least 48hr. after dosing. The amounts of the test compounds administered to the rats are shown in Table 1. All the compounds were injected subcutaneously into the lumbar region as 25% (w/v) solutions in arachis oil, except *S*-(1- and 2-menaphthyl)-L-cysteine, which were added to the diet to the level of 2·5%, and *S*-(1- and 2-menaphthyl)glutathione, which were injected subcutaneously as 10% (w/v) solutions in aq. $NaHCO_3$. While being injected the rats were lightly anaesthetized with ether, and they received a single injection of a compound except for 1-menaphthyl alcohol and 1-menaphthyl benzoate, when each animal received two injections separated by 48hr.

Similar experiments to those with rats were conducted in which solutions of 1- and 2-menaphthyl chloride, 1- and 2-menaphthyl bromide and 1-menaphthyl acetate in arachis oil were injected subcutaneously into male rabbits, body wt. 2–3kg.

Chromatography. Chromatograms were developed on Whatman no. 1 paper by the ascending method at room temperature. The following solvent mixtures were used: *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.). The detecting agent used was the $K_2Cr_2O_7$–$AgNO_3$ reagent of Knight & Young (1958). The following R_F values were obtained with solvent mixtures *A* and *B* respectively: 1-menaphthylmercapturic acid, 0·88 and 0·69; 2-menaphthylmercapturic acid, 0·87 and 0·76; *S*-(1-menapthyl)-L-cysteine, 0·62 and 0·43; *S*-(2-menaphthyl)-L-cysteine, 0·56 and 0·50.

Isolation procedures. The urine was made just acid to Congo red by the addition of conc. HCl, and 10% (by vol.) of conc. HCl was then added. The acidified urine was shaken with four separate portions of chloroform, each equal to half the volume of the urine. The emulsions that formed during the shaking were separated by centrifuging. The combined chloroform extracts were concentrated to about one-tenth of their volume and were extracted by shaking with M-$NaHCO_3$. The aqueous layer was separated, acidified with conc. HCl and extracted with chloroform. The chloroform extract was evaporated to dryness. When a solid residue was obtained by this procedure it was extracted with boiling light petroleum (b.p. 60–80°) to remove any naphthoic acid and benzoic acid present, and the residue that remained was crystallized from aqueous ethanol to give the mercapturic acid. When the evaporation of the chloroform extract gave an oily residue this was purified on a cellulose column (3cm. × 60cm.) with, as the solvent mixture, either butan - 1 - ol – ethanol – benzene – aq. 3N-ammonia (6:3:3:1, by vol.) or butan-1-ol–ethanol–aq. 3N-ammonia (9:3:1, by vol.). From 0·75 to 1·5l. of solvent mixture was passed through the column and collected in 15–25ml. fractions. A drop of each fraction was tested on filter paper with the $K_2Cr_2O_7$–$AgNO_3$ reagent (Knight & Young, 1958) and those fractions that gave a positive reaction were evaporated to dryness. The residue

was extracted with boiling light petroleum (b.p. 60–80°) and the mercapturic acid was then crystallized from aqueous ethanol as described above.

In some experiments in which 2-naphthuric acid was present in the urine, acidification yielded a flocculent precipitate of this compound that was removed after the acidified urine had been left overnight at 4°.

1-Naphthoic acid (m.p. 161–162°) and 2-naphthoic acid (m.p. 182–183°) when present in the urine were separated from the light-petroleum (b.p. 60–80°) extract obtained during the isolation of the mercapturic acid. The light-petroleum extract was evaporated to dryness, and after the residue had been extracted with hot water it was crystallized from aqueous methanol to yield naphthoic acid.

RESULTS

Chromatographic studies. When the urine, and chloroform extracts of the acidified urine, of rats that had been dosed with the test compounds were examined by the chromatographic methods described above the following results were obtained. 1-Menaphthylmercapturic acid was detected after the administration of 1-menaphthyl chloride, 1-menaphthyl bromide, *S*-(1-menaphthyl)-L-cysteine, *S*-(1-menaphthyl)glutathione, 1-menaphthyl alcohol, 1-menaphthyl acetate and 1-menaphthyl benzoate. 2-Menaphthylmercapturic acid was detected after rats had been dosed with 2-menaphthyl chloride, *S*-(2-menaphthyl)-L-cysteine and *S*-(2-menaphthyl)glutathione. A trace of 2-menaphthylmercapturic acid was detected after the administration of 2-menaphthyl bromide, but none was found after the injection of 2-menaphthyl alcohol or 2-menaphthyl acetate. Faint positive tests for *S*-(1-menaphthyl)-L-cysteine were obtained after the administration of this compound and also after *S*-(1-menaphthyl)glutathione had been given.

In experiments with rabbits the corresponding mercapturic acid was detected in the urine after injection of each of the compounds tested, namely 1- and 2-menaphthyl chloride, 1- and 2-menaphthyl bromide, 1-menaphthyl alcohol and 1-menaphthyl acetate.

Isolation studies. The amounts of 1- and 2-menaphthylmercapturic acid isolated from the urine of rats after they had been dosed with the test compounds are shown in Table 1. In every case the identity of the isolated mercapturic acid was confirmed by its m.p. and by its mixed m.p. with the appropriate synthetic mercapturic acid, and by its chromatographic properties. The optical rotation and elementary analysis of almost every specimen of mercapturic acid isolated were also determined and provided confirmatory evidence of identity. The amounts of isolated 1- and 2-menaphthylmercapturic acid shown in Table 1 serve only as a rough guide to the amounts of these compounds present in the urine, for the chief purpose of the isolation studies was the separation of the mercapturic acids in analytically pure form, and this contributed to the losses that occurred. With one exception, for every 1- and 2-menaphthyl derivative for which chromatographic evidence of mercapturic acid formation in the rat had been obtained, the corresponding mercapturic acid was isolated from the urine of the dosed animals. The exception was 2-menaphthyl bromide, for after the administration of this compound all attempts to isolate 2-menaphthylmercapturic acid from the urine were unsuccessful.

In experiments with rabbits, 1-menaphthylmercapturic acid was isolated from the urine after the injection of 1-menaphthyl chloride and 1-menaphthyl acetate, but not after 1-menaphthyl bromide and 1-menaphthyl alcohol had been given. 2-Menaphthylmercapturic acid was isolated from rabbit urine after the administration of 2-menaphthyl chloride, but not after 2-menaphthyl bromide had been given.

DISCUSSION

Before the present investigation the synthesis and biosynthesis of 1- and 2-menaphthylmercapturic acid do not appear to have been studied. The finding

Table 1. *Amounts of* 1- *and* 2-*menaphthylmercapturic acid isolated from the urine of rats dosed with* 1- *and* 2-*menaphthyl compounds*

For experimental details see the text.

Compound administered	No. of rats	Total dose (g.)	Mercapturic acid isolated (g.)	Percentage conversion
1-Menaphthyl chloride	20	5·0	0·267	3·1
1-Menaphthyl bromide	28	7·0	0·060	0·6
S-(1-Menaphthyl)-L-cysteine	4	2·5	0·152	5·2
S-(1-Menaphthyl)glutathione	20	2·5	0·093	5·5
1-Menaphthyl alcohol	18	9·0	0·139	0·8
1-Menaphthyl acetate	8	1·9	0·052	1·8
1-Menaphthyl benzoate	15	7·5	0·060	0·7
2-Menaphthyl chloride	14	3·2	0·139	2·5
S-(2-Menaphthyl)-L-cysteine	8	5·0	0·009	0·2
S-(2-Menaphthyl)glutathione	6	0·9	0·029	4·8

that these mercapturic acids are excreted by rats and rabbits after the administration of 1- and 2-menaphthyl chloride was not unexpected in view of previous observations on mercapturic acid formation in animals that had been dosed with benzyl chloride (Stekol, 1938, 1939) and with *para*-substituted benzyl chlorides and unsubstituted ω-bromoalkylbenzenes (Bray *et al.* 1958). Chromatographic evidence was obtained in the present work that the injection of 1- and 2-menaphthyl bromide into rats and rabbits is followed by the excretion of the corresponding mercapturic acid, but this appeared to occur to a smaller extent than with 1- and 2-menaphthyl chloride. No observations were made on the metabolism of 1- and 2-menaphthyl iodide, as these compounds were found to decompose so readily as to render them unsuitable for use in animal experiments.

The possibility that administration of carboxylic acid esters of 1-menaphthyl alcohol might give rise to the excretion of 1-menaphthylmercapturic acid was tested by injecting 1-menaphthyl acetate into rats. 1-Menaphthylmercapturic acid was isolated from the urine of two groups of dosed rats in amounts representing 1·8% and 3·3% of the ester administered, and from the urine of rabbits in an amount corresponding to 1·0% of the injected ester. When 1-menaphthyl benzoate was injected into rats the mercapturic acid isolated from the urine corresponded to 0·7% of the ester administered.

Bray *et al.* (1958) showed that in rabbits benzyl chlorides and their alcohols are converted into benzoic acids, as would be expected from the occurrence of the reactions:

$$R\cdot CH_2Cl \rightarrow R\cdot CH_2\cdot OH \rightarrow R\cdot CO_2H$$

It appears probable that analogous reactions occur in the metabolism of menaphthyl halides and that menaphthyl esters also undergo hydrolytic cleavage in the organism. This is supported by various observations in the present work, e.g. 1-naphthoic acid was isolated from the urine of rats and rabbits after they had been injected with 1-menaphthyl bromide and acetate, and 2-naphthoic acid was isolated after 2-menaphthyl bromide had been given. When 1-menaphthyl alcohol was injected into rats both 1-menaphthylmercapturic acid and 1-naphthoic acid were isolated from the urine. As the amounts of mercapturic acid isolated corresponded to less than 1% of the compound administered, the possibility exists that they might have been formed from an impurity present in the 1-menaphthyl alcohol. This is most unlikely, however, for two specimens of 1-menaphthyl alcohol prepared by different routes (see the Materials section) were used in the animal experiments, and their properties indicated a high degree of purity and both gave rise to formation of 1-menaphthylmercapturic acid. It should be noted that Bray *et al.* (1958) found that no significant amounts of mercapturic acid were excreted when they studied the metabolism of benzyl alcohol and the alcohols corresponding to the various *para*-substituted benzyl chlorides that they had shown to give rise to mercapturic acid in rabbits.

The observation that *S*-(1- and 2-menaphthyl)-glutathione and *S*-(1- and 2-menaphthyl)-L-cysteine when administered to rats are converted into 1- and 2-menaphthylmercapturic acid is consistent with the hypothesis that these mercapturic acids can be formed *in vivo* by the general mechanism proposed by Barnes, James & Wood (1959) and Bray, Franklin & James (1959*a*,*b*), namely that the administered compound or an active derivative reacts with glutathione to give an *S*-substituted glutathione, which is broken down to an *S*-substituted cysteine that undergoes acetylation to give the mercapturic acid.

Some interest attaches to the metabolic formation of 1-menaphthylmercapturic acid from 1-menaphthyl acetate and benzoate, inasmuch as excretion of a mercapturic acid arising from the administration of a carboxylic acid ester does not appear to have been reported hitherto. The interpretation of this finding is complicated by the observation that 1-menaphthyl alcohol also gives rise to 1-menaphthylmercapturic acid in the organism. Though 1-menaphthyl acetate can aralkylate glutathione under strongly alkaline conditions, the question remains of whether such an ester can react directly with a thiol *in vivo*.

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