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Formation of Mercapturic Acids in Rats after the Administration of Aralkyl Esters

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1. Benzylmercapturic acid and hippuric acid were isolated from the urine of rats that had been injected subcutaneously with benzyl acetate. 2. 1-Menaphthylmercapturic acid and 1-naphthoic acid were isolated from the urine of rats after the subcutaneous injection of each of the following compounds: 1-menaphthyl alcohol and its acetate, propionate, butyrate and benzoate esters. 3. A quantitative method for determining 1-menaphthylmercapturic acid in urine was developed and used to measure the excretion of this compound in the urine of rats in the 4-day period after the subcutaneous injection of 1-menaphthyl alcohol and its acetate, propionate, butyrate and benzoate esters. 4. Chromatographic evidence was obtained for the presence of *S*-(1-menaphthyl)glutathione and *S*-(1-menaphthyl)-L-cysteine in bile collected from rats with cannulated bile ducts after the animals had been injected subcutaneously with each of the following compounds: *S*-(1-menaphthyl)glutathione, 1-menaphthyl acetate, propionate and butyrate. 5. Benzylmercapturic acid and 1-menaphthylmercapturic acid were isolated from the urine of rats that had been injected with sodium benzyl sulphate and sodium 1-menaphthyl sulphate respectively.

During an investigation of mercapturic acid synthesis in rats after the administration of compounds in the 1-menaphthyl series, i.e. derivatives of 1-methylnaphthalene of the type $C_{10}H_7 \cdot CH_2X$, it was found that 1-menaphthyl acetate and 1-menaphthyl benzoate both give rise to excretion of 1-menaphthylmercapturic acid (Hyde & Young, 1968). As no other examples of mercapturic acid formation from carboxylic acid esters *in vivo* have been described, the present work was undertaken to determine whether other carboxylic acid esters are metabolized in this way. The possibility that sulphuric acid esters give rise to mercapturic acids was also investigated, and it has been established that the injection of sodium benzyl sulphate and sodium 1-menaphthyl sulphate into rats is followed by the excretion of benzylmercapturic acid and 1-menaphthylmercapturic acid respectively in the urine of dosed animals.

MATERIALS

All melting points and boiling points are uncorrected. Elementary microanalyses were carried out by Weiler and Strauss, Oxford, U.K., and by the Microanalytical Laboratory, School of Pharmacy, London W.C.1, U.K.

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Sodium benzyl sulphate. A solution of 1.33g of benzyl alcohol in 10ml of pyridine was cooled in an ice-bath and 0.9ml of chlorosulphonic acid was added slowly with continuous stirring. The reaction was carried out in a closed flask. After the addition of the chlorosulphonic acid had been completed, stirring was continued for 2h during which time the temperature of the reaction mixture was allowed to rise to room temperature. On the next day the reaction mixture was cooled in an ice bath and 60% (w/v) NaOH was added with rapid stirring until the mixture was just alkaline to litmus. Ether (10 vol.) was added with stirring and the precipitate that formed was separated by centrifugation and was washed with ether. The precipitate was extracted with warm water–ethanol (1:4, v/v) and the crystalline material that separated when the extract was cooled was recrystallized from aqueous ethanol. The dried product weighed 1.78g (Found: C, 39.8; H, 3.6; ester sulphate S, 15.5. $C_7H_7NaO_4S$ requires C, 40.0; H, 3.4; ester sulphate S, 15.3%).

Benzylmercapturic acid. A solution of 7g of benzyl chloride in 15ml of 2-methoxyethanol was shaken for 3h at room temperature with 8g of *N*-acetyl-L-cysteine dissolved in 40ml of 10% (w/v) NaOH in water. The mixture was made acid to Congo Red by the addition of conc. HCl and after 30min the precipitate that had formed was separated by filtration, washed with water, and dissolved in chloroform. The chloroform solution was shaken with M-$NaHCO_3$ and the aqueous layer was separated and acidified to pH2 with 2M-HCl. The precipitate that formed was filtered, washed with water and crystallized twice from aqueous methanol. The product was dried over P_2O_5 *in vacuo* and weighed 12.1g and had m.p.

147°C. Stekol (1938) reported m.p. 147–148°C for benzylmercapturic acid (Found: C, 57.1; H, 6.0; N, 5.4; S, 12.4. Calc. for $C_{12}H_{15}NO_3S$: C, 56.9; H, 5.9; N, 5.5; S, 12.6%.)

1-*Menaphthyl alcohol.* This compound was prepared by two methods based on those of Manske & Ledingham (1939) and Nystrom & Brown (1947) as described by Hyde & Young (1968). The product obtained by the first method had m.p. 61–62°C (Found: C, 83.6; H, 6.3. Calc. for $C_{11}H_{10}O$: C, 83.5; H, 6.3%), and that obtained by the second method had m.p. 62°C (Found: C, 83.1; H, 6.3%). Manske & Ledingham (1939) reported m.p. 62°C for the compound.

1-*Menaphthyl acetate.* This compound was purchased from BDH Chemicals Ltd., Poole, Dorset, U.K., and was redistilled before use.

1-*Menaphthyl propionate, butyrate and benzoate.* Acylation of 1-menaphthyl alcohol with the appropriate acid chloride in the presence of pyridine was used to prepare these esters. 1-Menaphthyl propionate had b.p. 170–172°C/4–6mmHg (Found: C, 78.2; H, 6.6; sap. equiv. 216. Calc. for $C_{14}H_{14}O_2$: C, 78.5; H, 6.5%; sap. equiv. 214). 1-Menaphthyl butyrate had b.p. 175–176°C/4–6mmHg (Found: C, 79.4; H, 7.2; sap. equiv. 227. Calc. for $C_{15}H_{16}O_2$: C, 79.0; H, 7.0%; sap. equiv. 228). 1-Menaphthyl benzoate had m.p. 36–37°C (Found: C, 82.4; H, 5.3; sap. equiv. 261. Calc. for $C_{18}H_{14}O_2$: C, 82.4; H, 5.4%; sap. equiv. 262).

Sodium 1-*menaphthyl sulphate.* The method described for the preparation of sodium benzyl sulphate was used to prepare this compound. From 2.0g of 1-menaphthyl alcohol (prepared by the reduction of 1-naphthoic acid with lithium aluminium hydride) 1.71g of sodium 1-menaphthyl sulphate was obtained (Found: C, 47.4; H, 3.8; ester sulphate S, 11.8. $C_{11}H_9NaO_4S,H_2O$ requires: C, 47.5; H, 4.0; ester sulphate S, 11.5%).

1-*Menaphthylmercapturic acid.* This compound was prepared from 6.0g of 1-menaphthyl chloride and 6.0g of *N*-acetyl-L-cysteine by a method closely resembling that described above for the preparation of benzylmercapturic acid. The product weighed 7.2g and had m.p. 175.5°C (decomp.), $[\alpha]_D^{20} - 50°$ (*c* 0.5 in ethanol) (Found: C, 63.5; H, 5.8; N, 4.4; S, 10.6. Calc. for $C_{16}H_{17}NO_3S$: C, 63.4; H, 5.6; N, 4.6; S, 10.6%). Hyde & Young (1968) reported m.p. 176–177°C (decomp.), $[\alpha]_D^{20} - 48°$ (*c* 1.1 in ethanol) for 1-menaphthylmercapturic acid.

S-(1-*Menaphthyl*)-L-*cysteine and* S-(1-*menaphthyl*)*glutathione.* These compounds were prepared as described by Hyde & Young (1968).

METHODS

Animals and dosing. In the experiments with animals with cannulated bile ducts, August strain male rats, body wt. 250–300g, were used under the conditions described below. In all other experiments male black-hooded rats or male specific pathogen-free Wistar rats, approximate body wt. 200g, were used. They were fed on a diet of rat cakes [J. Murray and Sons (London) Ltd., London S.E.8, U.K.], had access to water at all times, and were housed in metabolism cages that permitted the collection of urine and faeces separately.

Except for sodium benzyl sulphate and sodium 1-menaphthyl sulphate, which were injected as described below, all the compounds administered were injected subcutaneously into the lumbar region as fine suspensions or as solutions in arachis oil. All rats were lightly anaesthetized with ether while being dosed.

Chromatography. Paper chromatograms were developed overnight at room temperature on Whatman no. 3 paper by the descending method. Separations by t.l.c. were carried out on glass plates coated with silica gel G (E. Merck A.-G., Darmstadt, Germany), 0.25mm thick in experiments conducted for identification purposes, and 0.5 and 1.0mm thick for preparative work.

The following solvent mixtures were used: A, butan-1-ol–acetic acid–water (12:5:3, by vol.); B, propan-1-ol–water–ammonia (sp.gr. 0.88) (80:19:1, by vol.); C, benzene–acetic acid (3:1, v/v).

Mercapturic acids and other bivalent sulphur compounds were detected on chromatograms by means of the $K_2Cr_2O_7$–$AgNO_3$ reagent of Knight & Young (1958) and by the platinum reagent of Toennies & Kolb (1951) as modified by Barnsley, Thomson & Young (1964). Amino acids were detected with the ninhydrin reagent prepared as described by Smith (1960). With this reagent it was possible to detect amino acids on paper chromatograms that had previously been dipped in the platinum reagent. Aromatic compounds were detected on thin-layer chromatograms with the formaldehyde–sulphuric acid reagent described by Le Rosen, Moravek & Carlton (1952). Chromatograms were viewed under u.v. light before being treated with any of the above reagents.

The following typical R_F values were obtained by means of paper chromatography with solvent mixtures A and B; *S*-(1-menaphthyl)-L-cysteine, A, 0.70; B, 0.63; *S*-(1-menaphthyl)glutathione, A, 0.54; B, 0.18. Typical R_F values obtained by t.l.c. with solvent mixtures A and C were as follows: 1-menaphthylmercapturic acid, A, 0.77; C, 0.34; 1-naphthoic acid, A, 0.69; C, 0.88.

Absorption spectra. U.v. absorption spectra of compounds in ethanol or dilute aqueous NaOH solution were measured with a Unicam SP.800 recording spectrophotometer or with a Hilger Uvispek spectrophotometer. I.r. absorption spectra of compounds as mulls in Nujol or in Kel-F oil no. 3 (BDH Chemicals Ltd.) or in KBr discs were recorded on a Perkin–Elmer model 237 spectrophotometer.

Determination of saponification equivalents of carboxylic acid esters. These were determined by refluxing the esters with excess of aqueous ethanolic 0.5M-NaOH and by titrating the excess of alkali with a standard HCl solution.

Determination of ester sulphate sulphur. A solution of the sulphate ester in 6ml of 2M-HCl was evaporated to dryness on a boiling-water bath and the residue was dissolved in water. The sulphate was precipitated as the benzidine salt, which was separated and titrated with 25mM-NaOH as described by Hawkins & Young (1954).

Determination of 1-*menaphthylmercapturic acid in urine.* The u.v.-absorption spectrum of 1-menaphthylmercapturic acid has a peak at 285nm and the extinction at this wavelength shown by solutions of the mercapturic acid in 20mM-NaOH was found to vary linearly with the concentration of the compound over the range 0–40μg/ml.

The following procedure was used to determine the amount of 1-menaphthylmercapturic acid in the urine excreted in a 24h period by a pair of male rats. The urine

was made 1M with respect to HCl and was left overnight at 4°C. It was then extracted three times by shaking with 50ml portions of chloroform and the chloroform extracts were combined and evaporated to dryness under reduced pressure. The residue was dissolved in 1.0ml of ethanol–diethylamine (9:1, v/v) and applied to the base line of a thin-layer plate (20cm×20cm) coated with silica gel 0.5mm thick. The chromatogram was developed in solvent mixture C and the silica gel in the area corresponding in position to 1-menaphthylmercapturic acid was removed and warmed gently for 10min with 50ml of 20mM-NaOH. A measured volume of this extract was diluted to 100ml with 20mM-NaOH and the extinction was measured at 285nm. When this procedure was applied to samples of rat urine to which 1-menaphthylmercapturic acid had been added in amounts ranging from 0.2 to 0.8mg/ml, the recoveries of mercapturic acid lay between 85 and 90%.

Cannulation of the bile ducts of rats. The bile ducts of male rats were cannulated by a method similar to that described by Boyland, Ramsay & Sims (1961). The operations were carried out by Dr D. J. Riches, Department of Anatomy, St Thomas's Hospital Medical School. The bile was collected by passing the cannula into a glass saddle-shaped vessel (Van Zyl, 1958) strapped with adhesive tape to the back of the animal. Each rat was housed in a metabolism cage that allowed the separate collection of urine and faeces and it had access at all times to a 2.5% (w/v) solution of glucose in 0.9% (w/v) NaCl. Urine and bile were collected for the 12h period before the injection of the compound under investigation, and thereafter for three successive 12h periods. When they were not examined immediately, the urine and bile samples were stored at −20°C until required.

RESULTS

Isolation of benzylmercapturic acid from the urine of rats injected with benzyl acetate and sodium benzyl sulphate

Benzyl acetate. Fifteen male rats were each injected subcutaneously with 1.0ml of a 20% (w/v) solution of benzyl acetate in arachis oil. The urine was collected for the following 48h and made 1M with respect to its content of hydrochloric acid by the addition of conc. hydrochloric acid. The acidified urine was left overnight at 4°C and the precipitate that formed was separated and extracted with 60ml of hot water. The extract was filtered and the filtrate, after addition of charcoal, was heated on a boiling-water bath. The charcoal was removed by filtration of the hot solution and the filtrate on cooling yielded 0.316g of needles, m.p. 188–188.5°C. The melting point was unchanged when the isolated compound was mixed with synthetic hippuric acid.

The acidified urine from which the hippuric acid had been removed was extracted three times by shaking with 200ml portions of chloroform. The combined chloroform extracts were evaporated to 100ml and extracted twice by shaking with 50ml portions of M-sodium hydrogen carbonate solution. The aqueous phase was separated, acidified with conc. hydrochloric acid, and the precipitate that formed was removed by filtration and dried *in vacuo* over phosphorus pentoxide. The dried solid was dissolved in 1.0ml of ethanol–diethylamine (9:1, v/v) and the solution was applied as a strip along the base line of two thin-layer plates with silica gel 1.0mm thick. The chromatograms were developed in solvent mixture C with synthetic benzylmercapturic acid as a marker. The area corresponding to the mercapturic acid was removed and heated with 50ml of ethanol for 10min. The hot solution was filtered and evaporated to dryness under reduced pressure. The solid thus obtained was crystallized twice from aqueous ethanol and gave 0.012g of product, m.p. 144–145°C, $[\alpha]_D^{22}$ −42° (*c* 1.0 in ethanol). The melting point was not depressed when the isolated compound was mixed with synthetic benzylmercapturic acid. The amount of compound isolated corresponded to 0.2% of the benzyl acetate administered.

Sodium benzyl sulphate. Twenty male rats were each given by subcutaneous injection 1.0ml of an aqueous solution containing 0.210g of sodium benzyl sulphate. The urine was collected for the periods 0–24 and 24–48h after dosing. The urine collected during the first 24h was made acid to Congo Red by the addition of conc. hydrochloric acid and left at room temperature overnight. It was then extracted four times by shaking with portions of ethyl acetate each equal to three times the volume of the acidified urine. The extracts were dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure. The residue was dissolved in 50ml of ethyl acetate and extracted twice with 10ml portions of M-sodium hydrogen carbonate solution. The extracts were combined, acidified with conc. hydrochloric acid and left overnight at 4°C. The crystalline precipitate that formed was separated by filtration and washed with a small volume of ether. It was dissolved in ethanol, and the solution was decolorized with charcoal, filtered, concentrated and hot water was added. The crystals that formed on cooling to 4°C were separated by filtration, washed with water and dried *in vacuo* over phosphorus pentoxide [0.418g, m.p. and mixed m.p. 146–147°C, $[\alpha]_D^{23}$ −51° (*c* 1.0 in ethanol) (Found: C, 56.7; H, 5.6; N, 5.5; S, 12.7%)]. Further evidence identifying the compound as benzylmercapturic acid was obtained by t.l.c. and by i.r. spectroscopy. The amount of mercapturic acid isolated corresponded to a conversion of 8.3% of the sodium benzyl sulphate injected. No significant amount of benzylmercapturic acid was found when the urine excreted in the period 24–48h after injection was examined as described above.

Isolation of 1-menaphthylmercapturic acid from the urine of rats injected with 1-menaphthyl alcohol and 1-menaphthyl esters of carboxylic acids

The following compounds were administered separately to rats by subcutaneous injection: 1-menaphthyl alcohol and its acetate, propionate, butyrate and benzoate esters. Each compound was administered to a group of 18 male rats, body wt. approx. 200g, in two doses separated by 48h, and the urine was collected for 96h after the first injection. Each dose of 1-menaphthyl alcohol consisted of 2.0ml of a 10% (w/v) suspension of the compound in arachis oil. Each dose of a 1-menaphthyl ester of a carboxylic acid consisted of 1.0ml of a 20% (w/v) solution of the ester in arachis oil.

1-Menaphthyl alcohol. The urine excreted by the rats in the 96h after the first injection was made 1M with respect to its content of hydrochloric acid and was left at 4°C overnight. It was then extracted three times by shaking with portions of chloroform each equal to the volume of the acidified urine. The combined chloroform extracts were evaporated to 100ml and were extracted twice with 50ml portions of M-sodium hydrogen carbonate. The extracts were then acidified with hydrochloric acid and the precipitate that formed was separated and dissolved in 2.0ml of ethanol–diethylamine (9:1, v/v). This solution was applied to the base line of four thin-layer plates coated with silica gel 1mm thick, and the chromatograms were developed in solvent mixture C. The areas of the chromatograms corresponding to 1-menaphthylmercapturic acid and 1-naphthoic acid were removed and each was heated on the water bath with 50ml of ethanol for 10min. The two ethanolic extracts were filtered, evaporated to dryness, and the solids obtained were crystallized from aqueous ethanol. The amount of 1-menaphthylmercapturic acid isolated was 0.144g [m.p. 176°C alone and in admixture with the synthetic compound; $[\alpha]_D^{20}$ −50° (*c* 0.5 in ethanol) (Found: C, 63.1; H, 5.8; N, 4.8; S, 10.7%)]. The 1-naphthoic acid isolated weighed 0.260g [m.p. 160–161°C alone and on admixture with the synthetic compound (Found: C, 77.1; H, 4.6. Calc. for $C_{11}H_8O_2$: C, 76.7; H, 4.7%)]. Both the isolated compounds were indistinguishable from their synthetic counterparts when they were examined by t.l.c. and by i.r. spectroscopy.

The procedures used for isolating 1-menaphthylmercapturic acid and 1-naphthoic acid from the urine of rats dosed with the carboxylic acid esters of 1-menaphthyl alcohol resembled closely those described above for the isolation of these compounds from the urine of rats dosed with 1-menaphthyl alcohol.

1-Menaphthyl acetate. The 1-menaphthylmercapturic acid isolated weighed 0.410g and had m.p. 176°C alone and on admixture with the synthetic compound and $[\alpha]_D^{21}$ −50° (*c* 0.5 in ethanol) (Found: C, 63.1; H, 5.8; N, 4.6; S, 10.8%). The 1-naphthoic acid isolated weighed 0.152g and had m.p. 161–162°C alone and on admixture with the synthetic compound (Found: C, 76.9; H, 4.8%).

1-Menaphthyl propionate. The amount of mercapturic acid isolated was 0.238g and had m.p. and mixed m.p. 176°C and $[\alpha]_D^{20}$ −51° (*c* 0.5 in ethanol) (Found: C, 63.3; H, 6.0; N, 4.2; S, 10.2%). The 1-naphthoic acid isolated weighed 0.138g and had m.p. and mixed m.p. 161°C and 161.5–162°C respectively (Found: C, 76.3; H, 5.1%).

1-Menaphthyl butyrate. The mercapturic acid isolated weighed 0.268g and had m.p. and mixed m.p. 175.5–176°C and $[\alpha]_D^{22}$ −51° (*c* 0.5 in ethanol) (Found: C, 63.5; H, 5.8; N, 4.2; S, 9.9%). The amount of 1-naphthoic acid isolated was 0.140g and had m.p. and mixed m.p. 161.5–162°C and 162°C respectively (Found: C, 76.2; H, 4.5%).

1-Menaphthyl benzoate. The weight of mercapturic acid isolated was 0.047g and had m.p. and mixed m.p. 175.5–176°C and $[\alpha]_D^{21}$ −50° (*c* 0.5 in ethanol) (Found: C, 63.4; H, 5.8; N, 4.7; S, 10.8%). The 1-naphthoic acid isolated weighed 0.052g and had m.p. and mixed m.p. 161.5–162°C (Found: C, 76.3; H, 5.1%).

Further confirmation of the identity of the compounds isolated in the above experiments was obtained by comparison of their i.r.-absorption spectra and their chromatographic behaviour with those of synthetic 1-menaphthylmercapturic acid and 1-naphthoic acid.

Quantitative studies of the excretion of 1-menaphthylmercapturic acid in the urine of rats injected subcutaneously with 1-menaphthyl alcohol and 1-menaphthyl esters of carboxylic acids

The compound (1mmol in 1.0ml of arachis oil) was injected subcutaneously into each rat of a group of four that were housed in pairs in metabolism cages. The urine was collected for a day before dosing and daily for 4 days after dosing, and each day's collection was analysed as described above. The total excretions of 1-menaphthylmercapturic acid by each group of four rats in the 4 days after injection (expressed as a percentage conversion of the compound administered) were as follows: 1-menaphthyl alcohol, 4.9; 1-menaphthyl acetate, 8.8; 1-menaphthyl propionate, 7.1; 1-menaphthyl butyrate, 7.1; 1-menaphthyl benzoate, 4.0. In general, the greatest excretion of the mercapturic acid occurred on the first day after dosing. In no case was excretion of mercapturic acid detected on the fourth day after dosing.

Isolation of 1-menaphthylmercapturic acid from the urine of rats injected with sodium 1-menaphthyl sulphate

Twenty male rats were each injected intraperitoneally with 2.0ml of an aqueous suspension containing an average of 0.185g of sodium 1-menaphthyl sulphate. The urine of the dosed animals was treated by a procedure similar to that described above for the isolation of benzylmercapturic acid from the urine of rats that had been injected with sodium benzyl sulphate. The urine excreted in the first 24h after dosing gave 0.194g of 1-menaphthylmercapturic acid which had m.p. and mixed m.p. 175–176°C and $[\alpha]_D^{23}$ −49° (*c* 1.0 in ethanol) (Found: C, 63.7; H, 5.6; N, 4.3; S, 10.5%). A further 8mg of mercapturic acid (m.p. and mixed m.p. 175.5–176.5°C) was isolated from the urine excreted in the second 24h period after dosing. The total 1-menaphthylmercapturic acid isolated corresponded to 5.0% of the sulphate ester administered.

Experiments with rats with cannulated bile ducts

After the intraperitoneal injection of naphthalene, 1,2-dihydronaphthalene and 1,2-epoxy-1,2,3,4-tetrahydronaphthalene into rats with cannulated bile ducts, Boyland *et al.* (1961) demonstrated the presence of glutathione derivatives of these compounds in the bile of the dosed animals together with various hydrolysis products of the glutathione conjugates. In the present work rats with cannulated bile ducts were dosed with 1-menaphthyl acetate, propionate and butyrate and *S*-(1-menaphthyl)glutathione, and their bile and urine were examined chromatographically for the presence of *S*-(1-menaphthyl)glutathione and products derived from it.

After the bile duct of a rat had been cannulated as described in the Methods section, control samples of bile and urine were collected for 12h. The rat was then dosed subcutaneously with 1.0ml of a 20% (w/v) solution of the 1-menaphthyl ester under test or with a suspension of 100mg of *S*-(1-menaphthyl)glutathione in 1.0ml of arachis oil. The urine was examined by t.l.c. in solvents A and C, and as in the experiments in which 1-menaphthyl compounds were administered to rats that had not had their bile ducts cannulated, the urine was found to contain 1-menaphthylmercapturic acid and 1-naphthoic acid. The bile was collected for the periods 0–12, 12–24, and 24–36h after dosing, and samples (25μl and 50μl) were examined by paper chromatography in solvents A and B. The results obtained for the four compounds were very similar, and those shown in Table 1 for 1-menaphthyl butyrate are typical. In the first and second 12h periods after dosing, evidence was obtained for the presence in the bile of *S*-(1-menaphthyl)glutathione and *S*-(1-menaphthyl)-L-cysteine, and two other compounds, both of which reacted with the platinum reagent of Toennies & Kolb (1951), and one of which also gave a positive reaction with ninhydrin. In the third 12h period after dosing only one of these compounds was detected, namely *S*-(1-menaphthyl)-L-cysteine. None of the compounds was detected in bile collected during the control period before dosing. Further evidence for the presence of *S*-(1-menaphthyl)-glutathione and *S*-(1-menaphthyl)-L-cysteine in bile collected from rats that had been dosed with 1-menaphthyl butyrate was obtained as described by Clapp (1967). The two compounds were first separated from the bile by means of paper chromatography. The *S*-(1-menaphthyl)-L-cysteine was converted into its phenylthiohydantoin and the chromatographic behaviour of this compound was shown to correspond to that of the authentic compound. The *S*-(1-menaphthyl)glutathione separated from bile was hydrolysed in hot 6M-hydrochloric acid and the presence of glycine, glutamate and *S*-(1-menaphthyl)-L-cysteine in the hydrolysate was shown by the chromatographic behaviour of the compounds and their phenylthiohydantoins.

Table 1. *Paper-chromatographic examination of bile collected from a rat after it had been injected with 1-menaphthyl butyrate*

For details see the text. Each R_F value shown corresponds to the site of positive reactions with both platinum and ninhydrin reagents, except those marked *, where only the platinum reagent gave a positive test. The R_F values of sites that reacted with the platinum reagent in the control bile have not been included.

	R_F	
Solvent system...	A	B
Bile, first 12h	0.70	0.62
	0.53	0.55
		0.42*
		0.17
Bile, second 12h	0.69	0.62
	0.54	0.54
		0.42*
		0.18
Bile, third 12h	0.69	0.63
S-(1-Menaphthyl)-L-cysteine	0.70	0.63
S-(1-Menaphthyl)glutathione	0.54	0.18

DISCUSSION

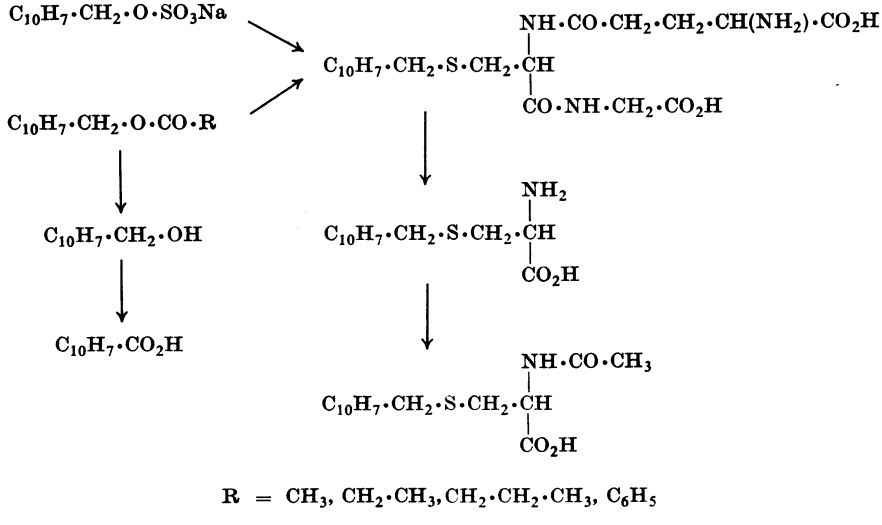
The observation that 1-menaphthyl alcohol and its acetate and benzoate esters give rise to the formation of 1-menaphthylmercapturic acid in the

animal body (Hyde & Young, 1968) has been confirmed in the present investigation, and has been extended to include 1-menaphthyl propionate, 1-menaphthyl butyrate and sodium 1-menaphthyl sulphate. It has also been shown that the administration of benzyl acetate and sodium benzyl sulphate is followed by the excretion of benzylmercapturic acid in the urine of the dosed animals.

The most likely pathway for the metabolism of carboxylic acid esters of benzyl alcohol and 1-menaphthyl alcohol would appear to be hydrolysis followed by oxidation of the alcohol to the corresponding acid. It is known that benzyl acetate is rapidly hydrolysed *in vivo* to benzyl alcohol which is then oxidized to benzoic acid (see Williams, 1959). Moreover, free and conjugated 1-naphthoic acid have been shown to be present in the urine of rats that have been dosed with 1-menaphthyl acetate and 1-menaphthyl benzoate (Hyde, 1964; Hyde & Young, 1968). In view of the ease with which these reactions occur it is not surprising that in the present work the results of quantitative studies of the urinary excretion of 1-menaphthylmercapturic acid by rats that had been dosed with carboxylic acid esters of 1-menaphthyl alcohol show that mercapturic acid formation is a minor metabolic pathway for these compounds. In no case did the amount of 1-menaphthylmercapturic acid found in the urine correspond to more than 9% of the ester injected.

There is now a considerable body of evidence in support of the hypothesis that in the animal body mercapturic acids can be formed by the interaction of glutathione and an administered foreign compound or a substance derived from it, to give an *S*-substituted glutathione, and that this is broken down to an *S*-substituted cysteine which undergoes acetylation to give the mercapturic acid (Barnes, James & Wood, 1959; Bray, Franklin & James, 1959*a*,*b*). In the present investigation evidence was obtained indicating that this pathway operates in the formation of 1-menaphthylmercapturic acid from carboxylic acid esters of 1-menaphthyl alcohol, for examination of bile collected from rats in the 24h period after they had been dosed with 1-menaphthyl acetate, propionate and butyrate showed the presence of *S*-(1-menaphthyl)glutathione and *S*-(1-menaphthyl)-L-cysteine. Two other compounds that were not present in control bile were found in the bile of rats that had been dosed with 1-menaphthyl esters; they appeared to be products of the breakdown of *S*-(1-menaphthyl)glutathione, for they were present in the bile of rats that had been dosed with this compound. They were also found to correspond to compounds detected by the chromatographic examination of the products of partial acid hydrolysis of *S*-(1-menaphthyl)glutathione (Clapp, 1967). Observations made on the metabolism of 1-menaphthyl esters in the present work are summarized in Scheme 1.

Enzyme studies by various workers (see Boyland & Chasseaud, 1969*a*) point to the existence of a group of *S*-glutathione transferases that bring about the formation of *S*-glutathione derivatives from various foreign compounds that have been shown to form mercapturic acids *in vivo*. Boyland & Chasseaud (1969*b*) have described a glutathione



Scheme 1. Metabolic changes undergone by 1-menaphthyl esters.

S-aralkyltransferase that catalyses the reaction of benzyl chloride with glutathione and they have obtained evidence indicating that this enzyme differs from glutathione *S*-alkyltransferase, *S*-aryltransferase, *S*-epoxidetransferase and an *S*-alkenetransferase. They suggest that the reaction of aralkyl esters with glutathione may be catalysed by glutathione *S*-aralkyltransferase and they found that a dialysed rat liver supernatant preparation catalysed at a low rate the reaction of glutathione with several aralkyl esters including 1-menaphthyl acetate (Boyland & Chasseaud, 1969*b*). They were unable to demonstrate an enzyme-catalysed reaction between glutathione and benzyl acetate, however, and it is noteworthy that although in the present work benzylmercapturic acid was isolated from the urine of rats injected with benzyl acetate, the amount of mercapturic acid obtained corresponded to only 0.2% of the benzyl acetate administered.

The observation that administration of 1-menaphthyl alcohol to rats is followed by the excretion of 1-menaphthylmercapturic acid (Hyde & Young, 1968) has been repeated in the present work, and an investigation of the metabolism of benzyl alcohol in rats (A. R. Morrison & L. Young, unpublished work) has yielded chromatographic evidence of the presence of benzylmercapturic acid in the urine of the dosed animals. These observations raise the possibility that liberation of the alcohol is a necessary step in the formation of mercapturic acids from aralkyl esters *in vivo*. Some light has been thrown on this question by the study of the catalysis of the reaction of sodium 1-menaphthyl sulphate and glutathione by an enzymic preparation obtained from a rat liver supernatant fraction (Gillham, Clapp, Morrison & Young, 1970). The amount of *S*-(1-menaphthyl)glutathione formed from glutathione and sodium 1-menaphthyl sulphate in the presence of the enzyme corresponded to the sulphate ions released and there was no significant liberation of sulphate ions when glutathione was omitted from the system. 1-Menaphthyl alcohol acted neither as a substrate for the aralkylation of glutathione in the presence of the enzyme nor as an inhibitor of the reaction between 1-menaphthyl sulphate and glutathione. Under these conditions it would appear that the ester rather than the alcohol served as the substrate for the enzyme.

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