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Metabolism of Polycyclic Compounds

16. THE METABOLISM OF 1:2-DIHYDRONAPHTHALENE AND 1:2-EPOXY-1:2:3:4-TETRAHYDRONAPHTHALENE*

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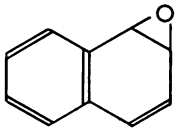
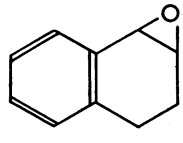
Previous work on the metabolism of naphthalene in animals has led to the suggestion that *trans*-1:2-dihydro-1:2-dihydroxynaphthalene and the mercapturic acid, *N*-acetyl-*S*-(1:2-dihydro-2-hydroxy-1-naphthyl)-L-cysteine (III) arise in the body through the same intermediate (Boyland & Sims, 1958; Sims, 1959; Booth, Boyland & Sims, 1960). The most probable structure for the intermediate would be the epoxide (I), which has not yet been prepared. The related epoxide, 1:2-epoxy-1:2:3:4-tetrahydronaphthalene (II), is available, however, and the present paper describes the metabolism of this compound and its related hydrocarbon, 1:2-dihydronaphthalene. Previous work (Pohl & Rawicz, 1919; Boyland & Solomon, 1955) indicates that 1:2-dihydronaphthalene is converted by animals into a substance which yields naphthalene on acidification, whereas the present work has shown that both 1:2-dihydronaphthalene and 1:2-epoxy-1:2:3:4-tetrahydronaphthalene are con-

* Part 15: Booth, Boyland & Sims (1960).



(I)



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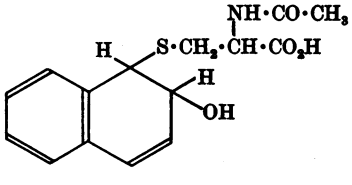
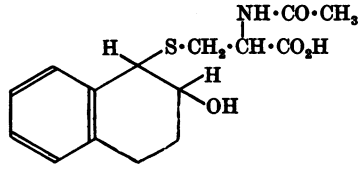


(III)



(IV)

verted into *trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene and a mercapturic acid of structure (IV). Dihydroxy compounds have been isolated from the urine of animals treated with indene (Brooks & Young, 1956) and with acenaphthylene (Brooks, Hopkins & Young, 1959).

EXPERIMENTAL

Melting points are uncorrected.

Paper chromatography

Except where stated, paper chromatography was carried out by downward development for 18 hr. on Whatman no. 1 paper with the following solvents: 1, butanol saturated with aq. 2N-NH_3 soln.; 2, butanol–propan-1-ol–aq. 2N-NH_3 soln. (2:1:1, by vol.); 3, butanol–acetic acid–water (12:3:5, by vol.); 4, butanol–acetic acid–water (2:1:1, by vol.). The dried chromatograms were examined under u.v. light and then treated with one of the following reagents: freshly diazotized *p*-nitroaniline (0·02% in 0·1N-HCl) followed by aq. 10% Na_2CO_3; 0·1M-$K_2Cr_2O_7$–acetic acid (1:1) followed by 0·1M-$AgNO_3$ (Knight & Young, 1958); aq. 2% (w/v) $NaIO_4$ followed, after 30 min., by Schiff's reagent (Brooks & Young, 1956); the platinic iodide reagent of Toennies & Kolb (1951); a solution of ninhydrin in acetone (0·2%) and heating the papers to 70° for 10 min. The first three reagents were applied to the papers by spraying and the last two by dipping the papers in the reagent. The $K_2Cr_2O_7$–$AgNO_3$ reagent was better than the platinic iodide reagent in the detection of small amounts of the sulphur-containing substances, and it could be used on papers previously treated with ninhydrin. The properties of compounds related to 1:2-dihydronaphthalene are listed in Table 1.

Amino acids were detected with ninhydrin on chromatograms developed with solvents 3 and 4 described above and also by conversion into their dinitrophenyl derivatives (Sanger, 1945) followed by chromatography on Whatman no. 7 paper with the solvents described by Phillips (1958).

Materials

1:2-*Dihydronaphthalene*. This was prepared by the method of Bamberger (1895) as an oil, b.p. 31° at 0·5 mm. Hg, and was purified as described by Rowe (1921). It was shown to be free of naphthalene by infrared analysis.

2-*Bromo*-1-*hydroxy*-1:2:3:4-*tetrahydronaphthalene*. This compound was prepared by the method of Straus & Rohrbacher (1921) and formed needles from ethanol, m.p. 112°.

1:2-*Epoxy*-1:2:3:4-*tetrahydronaphthalene* (II). The above-mentioned bromhydrin was converted into the epoxide (II) as described by Straus & Rohrbacher (1921), b.p. 85° at 1 mm. Hg, m.p. 21–22°.

(±)-trans-1:2-*Dihydroxy*-1:2:3:4-*tetrahydronaphthalene*. (±)-*trans*-1:2-Dihydro-1:2-dihydroxynaphthalene was hydrogenated in methanol at room temperature and atmospheric pressure in the presence of Adams catalyst. The product separated from benzene in flat needles, m.p. 111°. The diol (100 mg.) was heated under reflux with 2N-HCl (10 ml.) for 15 min. and the cooled solution extracted with ether. Removal of the ether afforded an oil, which on warming with semicarbazide hydrochloride and sodium

Table 1. *Paper chromatography of the metabolites of* 1:2-*dihydronaphthalene and* 1:2-*epoxy*-1:2:3:4-*tetrahydronaphthalene*

The solvents and the reagents are described in the Experimental section. The isomeric forms of the products could not be separated on paper chromatograms.

Metabolite	R_F				Colour with ninhydrin	Reaction with the $K_2Cr_2O_7$–$AgNO_3$ reagent	Colour with the $NaIO_4$–Schiff's reagent
	Solvent 1	Solvent 2	Solvent 3	Solvent 4			
S-(2-Hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine	0·23	0·57	0·53	0·72	Purple	+	Blue*
N-Acetyl-*S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine	0·25	0·56	0·81	0·88	.	+	Blue*
Methyl ester of *N*-acetyl-*S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine	0·85	0·91	0·88	0·91	.	+	Blue*
trans-1:2-Dihydroxy-1:2:3:4-tetrahydronaphthalene	0·79	0·87	0·85	0·86	.	–	Purple, turning blue
Glucosiduronate of *trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene	0·04	0·31	0·44	0·59	.	–	Pink†
Metabolite, possibly a cysteinylglycine derivative	0·15	0·39	0·70	0·79	.	+	Blue*

* After 4 hr. The reaction was not sensitive to small amounts of material.

† After 15 min.

acetate in water yielded the semicarbazone of 2-tetralone in prisms (from ethanol), m.p. 193° (Found: C, 64·8; H, 6·6. Calc. for $C_{11}H_{13}ON_3$: C, 65·0; H, 6·45%).

Reaction of 1:2-*epoxy*-1:2:3:4-*tetrahydronaphthalene with cysteine.* L-Cysteine hydrochloride (6 g.) and $NaHCO_3$ (3·6 g.), in water (25 ml.), were heated with 1:2-epoxy-1:2:3:4-tetrahydronaphthalene (II) (5·8 g.) to 60° for 15 min. with stirring. The mixture was kept at 0° for some hours and the solid, which had begun to separate during the reaction, was collected (9·8 g.). It was dissolved in a minimum volume of boiling water and the crystals which separated on cooling to room temperature were recrystallized several times from aq. ethanol to yield (−)-*S*-(2-*hydroxy*-1:2:3:4-*tetrahydro*-1-*naphthyl*)-L-*cysteine* (4·1 g.) in elongated plates, m.p. 217° (decomp.), $[\alpha]_D^{22} - 30 \pm 3°$ in 0·1 N-NaOH (*c*, 0·5) (Found: C, 58·5; H, 6·5; N, 5·1; S, 11·5. $C_{13}H_{17}O_3NS$ requires C, 58·4; H, 6·4; N, 5·2; S, 12·0%). The combined mother liquors were evaporated to 50 ml. under reduced pressure and the solid which separated was recrystallized several times from aq. ethanol to yield (+)-*S*-(2-*hydroxy*-1:2:3:4-*tetrahydro*-1-*naphthyl*)-L-*cysteine* (3·9 g.) in needles, m.p. 215° (decomp.), $[\alpha]_D^{22} + 44 \pm 3°$ in 0·1 N-NaOH (*c*, 0·5) (Found: C, 58·2; H, 6·3; N, 5·2; S, 11·7%). A mixture of the two isomers had m.p. 213° (decomp.).

The cysteine derivatives (0·5 g.) were separately heated under reflux with 5 N-HCl for 15 min. and the cooled solutions extracted with ether. The ethereal extracts were evaporated to yield oils, which in each case afforded the semicarbazone of 2-tetralone as prisms from ethanol, m.p. and mixed m.p. 191–193°. The mother liquors from the hydrolyses were shown to contain cysteine by paper chromatography.

When L-cysteine hydrochloride (2 g.) and $NaHCO_3$ (0·9 g.), in water (20 ml.) (which gave a solution of pH 4·5), were warmed with 1:2-epoxy-1:2:3:4-tetrahydronaphthalene (1·8 g.) for 30 min., an oil was formed which crystallized on cooling the mixture. The solid was recrystallized from benzene to yield (±)-*trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene (1·65 g.) in flat needles, m.p. and mixed m.p. 111°. The mother liquors were shown by paper chromatography to contain *S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine.

Reaction of 2-*bromo*-1-*hydroxy*-1:2:3:4-*tetrahydronaphthalene with cysteine.* 2-Bromo-1-hydroxy-1:2:3:4-tetrahydronaphthalene (15 g.), L-cysteine hydrochloride (10·5 g.) and NaOH (9 g.), in water (100 ml.) and acetone (150 ml.), were heated under reflux for 30 min. The solution was evaporated to 100 ml. under reduced pressure, acidified to pH 4 with conc. HCl and kept at 0° overnight. The solid was collected and separated into the two isomeric cysteine derivatives described above, the (−)-isomer (6·9 g.) forming elongated plates from aq. ethanol, m.p. 218° (decomp.), $[\alpha]_D^{22} - 30 \pm 3°$ in 0·1 N-NaOH (*c*, 0·5) and the (+)-isomer forming needles (5·4 g.) from aq. ethanol, m.p. 215° (decomp.), $[\alpha]_D^{22} + 44 \pm 3°$ in 0·1 N-NaOH (*c*, 0·5). The infrared spectra of the isomers were identical with those of the respective isomers obtained in the first preparation.

Acetylation of the isomeric cysteine derivatives. The derivatives (2 g.) (prepared by either of the above methods), each in 2 N-NaOH (10 ml.), were cooled to 0° and acetic anhydride (1 ml.) added dropwise over 15 min. with stirring. The solutions were acidified to pH 4 with 2 N-HCl and kept overnight at 0°. The (−)-isomer yielded a gum which could not be crystallized, whereas the (+)-isomer yielded a N-*acetyl* derivative which formed needles from water, m.p. 188°, $[\alpha]_D^{22} + 57°$ in 0·1 N-NaOH (*c*, 0·5) (Found: C, 57·9; H, 6·3; N, 4·5; S, 10·0. $C_{15}H_{19}O_4NS$ requires C, 58·2; H, 6·2; N, 4·5; S, 10·4%). Both isomeric *N*-acetyl derivatives were esterified with diazomethane in ether. That from the (−)-cysteine derivative yielded a gum, $[\alpha]_D^{22} + 77 \pm 5°$ in $CHCl_3$ (*c*, 0·5), and that from the (+)-derivative yielded a *methyl ester* which separated from benzene–light petroleum (b.p. 60–80°) in needles, m.p. 118°, $[\alpha]_D^{22} - 59 \pm 2°$ in $CHCl_3$ (*c*, 0·5) (Found: C, 59·4; H, 6·6; N, 4·45. $C_{16}H_{21}O_4NS$ requires C, 59·4; H, 6·5; N, 4·3%). Both esters could be eluted unchanged from an alumina column with benzene–ethanol (4:1, v/v). The *N*-acetyl derivatives, when treated with acetic anhydride in pyridine, which would acetylate the hydroxyl groups, yielded gums, both of which formed spots (at R_F 0·65 in solvent 2) on paper chromatograms sprayed with the $K_2Cr_2O_7$–$AgNO_3$ reagent.

Reaction of 1:2-*epoxy*-1:2:3:4-*tetrahydronaphthalene with* N-*acetylcysteine.* 1:2-Epoxy-1:2:3:4-tetrahydronaphthalene (II) (0·60 g.), *N*-acetylcysteine (0·68 g.) and $NaHCO_3$ (0·4 g.) in water (10 ml.) were heated to 60° with stirring. The solution was cooled to 0° and acidified to pH 4 with 5 N-HCl. The solid which separated was recrystallized from water to yield (+)-*N*-acetyl-*S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine (IV) (0·58 g.) in needles, m.p. and mixed m.p. 187°, $[\alpha]_D^{22} + 55 \pm 5°$ in 0·1 N-NaOH (*c*, 0·5) (Found: N, 4·45. Calc. for $C_{15}H_{19}O_4NS$: N, 4·5%). When the mother liquors were evaporated to 1 ml. under reduced pressure, an oil separated which could not be crystallized. On paper chromatograms it was indistinguishable from the mercapturic acid (IV).

Acid-hydrolyses of the cysteine and N-*acetylcysteine derivatives.* About 5 mg. of each derivative was heated to 100° with 2 N-HCl (1 ml.) for 5 min. The solution was cooled and an excess of 10 N-NaOH, ethanol (0·5 ml.) and ether (0·5 ml.) were added. On shaking a blue colour developed in the ether layer. This test is specific for 2-tetralone (Straus & Rohrbacher, 1921). All the cysteine and *N*-acetylcysteine derivatives mentioned above as well as *trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene gave positive reactions in this test.

The solutions obtained after heating the derivatives with conc. HCl for 4 hr. at 100° were examined for amino acids, either directly or after conversion into the 2:4-dinitrophenyl derivatives. All the cysteine and *N*-acetylcysteine derivatives described above gave rise mainly to a substance indistinguishable from cysteine, but on chromatograms treated with ninhydrin a number of small unidentified ninhydrin-positive spots were also found. All the derivatives gave rise to the same pattern of spots. If HBr (sp.gr. 1·7) was used in place of conc. HCl in the hydrolysis the numbers and sizes of these unidentified spots were decreased. Cystine was also detected as the 2:4-dinitrophenyl derivatives in these hydrolyses, but it might well be an artifact, since cysteine itself is partly converted into cystine under the conditions used.

Hydrogenolysis with Raney nickel catalyst. The *N*-acetylcysteine derivatives (IV) (50 mg.), in ethanol (10 ml.), were each heated under reflux with Raney nickel catalyst (W-2 grade) (500 mg.) for 2 hr. The Raney nickel was filtered off and the filtrates were diluted with water (50 ml.) and extracted with ether (2 × 20 ml.). The presence of a substance indistinguishable from 2-naphthol in the ether extracts was demonstrated by means of paper chromato-

graphy with the solvents and colour reactions described by Sims (1959). The aqueous phases were evaporated to dryness and the residues heated to 100° for 4 hr. with aq. HBr (sp.gr. 1·7). The solutions were evaporated to dryness and the presence of a substance indistinguishable from alanine in the residues was shown by paper chromatography, both directly and after conversion into a 2:4-dinitrophenyl derivative. No other amino acids were detected.

When *N*-acetyl-*S*-(1:2-dihydro-2-hydroxy-1-naphthyl)-L-cysteine (III) [isolated from the urine of rabbits treated with naphthalene (Boyland & Sims, 1958)] was similarly treated with Raney nickel catalyst followed by extraction with ether, 2-naphthol (but no 1-naphthol) was detected in the ether extract and the residue in the aqueous layer gave rise after hydrolysis with HBr to a substance indistinguishable from alanine.

Animal experiments

A rabbit (body wt. approx. 2 kg.) was given 1:2-dihydronaphthalene (0·5 g.) in arachis oil (0·5 ml.) daily for 12 consecutive days by intraperitoneal injection. Two rabbits were each given 1:2-epoxy-1:2:3:4-tetrahydronaphthalene (II) (200 mg.) in arachis oil (1 ml.) daily for 10 consecutive days. Six rats (body wt. approx. 200 g.) were each given 1:2-epoxy-1:2:3:4-tetrahydronaphthalene (II) (40 mg.) in arachis oil (100 ml.) daily for 10 days. In each experiment the urines were collected separately from the faeces, pooled and stored at 0°. The pooled urines were separately filtered, acidified to pH 4 with acetic acid, and activated charcoal (100 g.) was added with stirring. The charcoal was filtered off, and washed first with water (2 l.) and then with methanol (2 l.) containing 5% (v/v) of aq. NH_3 soln. (sp.gr. 0·88) followed by methanol–benzene (95:5, v/v) (1 l.). The methanol and methanol–benzene washings were combined and evaporated under reduced pressure. The gums thus obtained were chromatographed on cellulose-powder columns, each prepared from Whatman standard-grade cellulose powder (500 g.), with butanol–*cyclo*hexane–aq. 2N-NH_3 (9:2:1) as the developing solvent. Fractions of 250 ml. were collected, evaporated under reduced pressure and the residues (after examination on paper chromatograms) were treated as described below.

RESULTS

Examination on paper chromatograms of the urines and of the gums obtained after the treatment of the urines with charcoal showed that animals treated with either 1:2-dihydronaphthalene or with 1:2-epoxy-1:2:3:4-tetrahydronaphthalene (II) contained substances indistinguishable from *trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene, *N*-acetyl-*S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine (IV), *S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine and a third substance which gave positive reactions with the $K_2Cr_2O_7$–$AgNO_3$ reagent. The urine from the rabbit treated with 1:2-dihydronaphthalene also contained all the naphthalene metabolites reported in previous papers (Boyland & Sims, 1958; Sims, 1959). A portion of the urine of animals injected with 1:2-dihydronaphthalene yielded small amounts of naphthalene after acidification with hydrochloric acid, but none could be detected in the acidified urines of animals treated with 1:2-epoxy-1:2:3:4-tetrahydronaphthalene.

Metabolites of 1:2-*dihydronaphthalene*

Fractions 1 and 2 formed gums which contained both *trans*-1:2-dihydro-1:2-dihydroxynaphthalene (in small amount) and *trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene, together with a little 1- and 2-naphthol. The gums were combined and extracted three times with boiling *cyclo*hexane (25 ml.) and the combined extracts evaporated to 10 ml. volume. The crystals (25 mg.) which separated were recrystallized from benzene to yield (±)-*trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene (11 mg.) in needles, m.p. and mixed m.p. 111°. The mother liquors yielded a small amount of material of m.p. 113–114° which did not depress the m.p. of (−)-*trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene isolated from the urine of rabbits treated with 1:2-epoxy-1:2:3:4-tetrahydronaphthalene (see below). Fractions 2 and 3 contained a little 1-naphthyl sulphate together with the isomeric sulphuric esters of 1:2-dihydroxynaphthalene. Fraction 3 also contained a small amount of a substance indistinguishable from 1-naphthylmercapturic acid on paper chromatograms.

Fractions 4–7 contained the substance believed to be the mercapturic acid (IV). The fractions were combined and extracted with boiling water (3 × 10 ml.). The aqueous extracts were combined and evaporated to dryness and the residue was extracted with boiling chloroform (2 × 25 ml.). Removal of the chloroform afforded a gum (1·2 g.), which gave one spot on paper chromatograms indistinguishable from the mercapturic acid (IV). When a little of the gum was heated with 2N-HCl for 5 min. the test of Straus & Rohrbacher (1921) for 2-tetralone was positive and paper chromatography revealed the same pattern of ninhydrin-positive spots as those given by the synthetic materials. Hydrogenolysis with Raney nickel catalyst as described above yielded substances indistinguishable from 2-naphthol and alanine. When the gum was treated with acetic anhydride in pyridine, the product obtained was indistinguishable on paper chromatograms from the materials obtained by a similar treatment of the synthetic compounds. Esterification of the gum with diazomethane in ether, followed by chromatography of the product on an alumina column and the elution of the column with benzene–ethanol (4:1, v/v), yielded a gum, $[\alpha]_D^{22} + 83 \pm 5°$ in $CHCl_3$ (*c*, 0·5), which was indistinguishable on paper chromatograms from the synthetic methyl esters described above. The infrared spectrum (measured in carbon tetrachloride) of this gum was identical with those of the two synthetic methyl esters.

Fractions 8–10 formed gums which were shown by paper chromatography to contain substances indistinguishable from *N*-acetyl-*S*-(1:2-dihydro-2-hydroxy-1-naphthyl)-L-cysteine (III) and *S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine. Attempts to isolate the latter compound from the combined fractions were unsuccessful, but a little of the gum after heating to 100° with 2N-HCl for 5 min. gave a positive reaction in the Straus & Rohrbacher test for 2-tetralone.

Fractions 12–16 all contained a substance which gave positive reactions with the $K_2Cr_2O_7$–$AgNO_3$ reagent and negative ninhydrin reactions on paper chromatograms. The combined material from these fractions was applied along the base lines of Whatman no. 3MM chromatography paper and the chromatograms were developed for 18 hr. with solvent 3. The appropriate areas of the chromatograms were cut out and the new metabolite was eluted with hot water. Removal of the water under reduced pressure afforded a gum (80 mg.) which gave only one spot detectable with the $K_2Cr_2O_7$–$AgNO_3$ reagent. After being heated for 15 min. at 100° with 2N-HCl, the gum gave a positive test for 2-tetralone. The gum failed to react with 2:4-dinitrofluorobenzene, but after being heated with HBr (sp.gr. 1·7) at 100° for 4 hr. yielded substances on paper chromatograms indistinguishable from cysteine and glycine. When the hydrolysate was treated with 2:4-dinitrofluorobenzene, substances indistinguishable from the 2:4-dinitrophenyl derivatives of cysteine and glycine were detected on paper chromatograms. Similarly, hydrogenolysis with Raney nickel catalyst yielded a substance which did not react with 2:4-dinitrofluorobenzene, but which, after hydrolysis with HBr, yielded substances indistinguishable from alanine and glycine, and the products from the reaction of the hydrolysate with 2:4-dinitrofluorobenzene yielded compounds indistinguishable from the derivatives of these amino acids. A substance indistinguishable from 2-naphthol was also detected in the products from the hydrogenolysis. This evidence suggests that the substance is related to *S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteinylglycine.

Fractions 17–26 were combined and evaporated to about 20 ml. under reduced pressure, when crystals (170 mg.) separated. These were recrystallized from aq. ethanol to yield the ammonium salt of a *glucosiduronic acid* of (+)-*trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene in plates, m.p. 233° (decomp.), $[\alpha]_D^{22} - 74 \pm 2°$ in water (*c*, 0·5) (Found: C, 53·6; H, 6·7. $C_{16}H_{23}O_8$ requires C, 53·8; H, 6·5%). The salt gave a positive naphtharesorcinol test and a positive test for 2-tetralone after being heated with 2N-HCl. The salt was treated with β-glucuronidase at 37° in acetate buffer (pH 4·5) for 20 hr. and the reaction mixture extracted with ether to yield (+)-*trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene, separating from benzene in plates, m.p. 115°, $[\alpha]_D^{22} + 58 \pm 5°$ in ethanol (*c*, 0·5). The mother liquors, which contained small amounts of the glucosiduronic acids of *trans*-1:2-dihydro-1:2-dihydroxynaphthalene and of 1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene, were evaporated to dryness, the residue was dissolved in acetate buffer (pH 4·5) and treated with β-glucuronidase as before to yield a product which appeared to be mainly (+)-*trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene (75 mg.), separating from benzene in plates, m.p. 112–113°, $[\alpha]_D^{22} + 26 \pm 5°$ in ethanol (*c*, 0·5). The mother liquors were shown by paper chromatography to contain *trans*-1:2-dihydro-1:2-dihydroxynaphthalene.

Metabolites of 1:2-*epoxy*-1:2:3:4-*tetrahydronaphthalene*

The gums from fractions 1–3 of the urine of rabbits treated with 1:2-epoxy-1:2:3:4-tetrahydronaphthalene (II) were combined and extracted four times with boiling light petroleum (b.p. 80–100°) (20 ml.). The solid which separated from the fractions on cooling was recrystallized three times from benzene to yield (±)-*trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene (110 mg.) in flat needles, m.p. and mixed m.p. 111°, $[\alpha]_D^{22}$ 0° in ethanol (*c*, 0·5) (Found: C, 73·2; H, 7·5. Calc. for $C_{10}H_{12}O_2$: C, 73·1; H, 7·4%). The combined light petroleum and benzene mother liquors were evaporated to 5 ml. and the solid which separated was recrystallized three times from benzene to yield (−)-*trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene (85 mg.) in plates, m.p. 115°, $[\alpha]_D^{22} - 63°$ in ethanol (*c*, 0·5) (Found: C, 73·5; H, 7·6%). Booth & Boyland (1949) report m.p. 113°, $[\alpha]_D^{22} - 62°$ in ethanol (*c*, 0·5–1) for this isomer of the diol. The infrared spectra of the two isomers, measured in carbon tetrachloride, were identical.

Fractions 6–8 were combined and evaporated and the residues extracted three times with boiling water (25 ml.). The extracts deposited, on cooling, an unidentified acid which formed pale-yellow needles (95 mg.) from water, m.p. 231°. The mother liquor, which contained a substance indistinguishable from the mercapturic acid (IV), was evaporated and the residue twice extracted with boiling $CHCl_3$ (25 ml.). Removal of the $CHCl_3$ afforded a gum (1·1 g.) which was indistinguishable in all the tests described above from the corresponding product obtained from the 1:2-dihydronaphthalene urine. The gum yielded, with diazomethane in ether, a methyl ester $[\alpha]_D^{22} + 79 \pm 5°$ in $CHCl_3$ (*c*, 0·5), giving an infrared spectrum identical with those of the synthetic products.

Fractions 9–13 were combined and evaporated to about 50 ml. and the product (175 mg.) which separated was recrystallized from water to yield an unidentified acid in prisms, m.p. 257° (decomp.). The fractions were also shown by paper chromatography to contain a substance indistinguishable from *S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine.

Fractions 14–15 on evaporation yielded a gum which contained a substance giving a positive reaction with the $K_2Cr_2O_7$–$AgNO_3$ reagent, which was indistinguishable on paper chromatograms from the unidentified metabolite of 1:2-dihydronaphthalene which appears to be related to *S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteinylglycine. The substance was obtained as a clear gum (50 mg.) by paper chromatography on 3MM paper and gave tests for 2-tetralone, glycine and cysteine on acid hydrolysis, and for 2-naphthol, glycine and alanine after hydrogenolysis with Raney nickel catalyst.

Fractions 17–24 all contained a substance which was indistinguishable on paper chromatograms from the glucosiduronic acid of 1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene, but the compound was not isolated.

The urine of rats treated with 1:2-epoxy-1:2:3:4-tetrahydronaphthalene was not examined in detail, but compounds were detected in the various fractions from the cellulose column which were indistinguishable from *trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene, the mercapturic acid (IV) and the unidentified cysteinylglycine derivative described above. A small amount of (−)-*S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine, m.p. 217° (decomp.), was isolated from one of the fractions: the infrared spectrum was identical with that of the synthetic isomer.

DISCUSSION

The reaction of cysteine with 1:2-epoxy-1:2:3:4-tetrahydronaphthalene (II) appears to give mainly two stereoisomeric forms of a product which exist by virtue of the asymmetric carbon atom in the cysteine residue. Examination of the crude reaction mixture on paper chromatograms showed that a small amount of a ninhydrin-negative substance, which could be the product of the reaction of either the cysteine carboxyl or amino group with the epoxide, was present. Although the addition of the cysteine residue takes place in the 1-position, the possibility of some 2-addition is not ruled out since small amounts of the 2-isomer might be difficult to detect in the presence of large amounts of the 1-isomer. The structures of the isomeric forms of the cysteine derivative are confirmed by the formation of 2-tetralone and cysteine on acid hydrolysis and by the detection of 2-naphthol and alanine after the hydrogenolysis of the corresponding *N*-acetyl derivatives with Raney nickel catalyst. At pH 7 the reaction of an epoxide with a SH group proceeds more readily than reactions with carboxyl or amino groups. At pH 4·5, however, the formation of diol is the main reaction. Other epoxides, such as 1:2-epoxyindene, 1:2-epoxy*cyclo*hexane, 2:3-epoxy-1:2:3:4-tetrahydronaphthalene and epichlorhydrin all react with cysteine to give analogous cysteine derivatives (E. Boyland & P. Sims, unpublished work). The production of a 1-substituted 1:2:3:4-tetrahydronaphthalene derivative by the reaction of 2-bromo-1-hydroxy-1:2:3:4-tetrahydronaphthalene with cysteine probably proceeds through the intermediate formation of the epoxide (II). The hydroxyl group and the cysteine residue could be either *cis* or *trans* in respect to each other: since the epoxide (II) with water yields a *trans* product, the product with cysteine is probably also *trans*.

1:2-Dihydronaphthalene and 1:2-epoxy-1:2:3:4-tetrahydronaphthalene are converted in the body into both the stereoisomers of *trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene. Since the glucosiduronic acid of only the (−)-isomer has been isolated or detected, whereas the free diol is found both as the (+)-form and the racemate, it is probable that the first-formed diol is in fact the racemate, which would be expected from the non-enzymic opening of the oxiran ring. There is, however, little doubt that both 1:2-dihydronaphthalene and 1:2-epoxy-1:2:3:4-tetrahydronaphthalene form only one of the two possible isomeric forms of the mercapturic acid (IV) in the body. Experience with a mixture of equal amounts of both forms of the mercapturic acids has shown that that derived from (+)-*S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-cysteine can be separated easily from that derived from the (−)-form of the cysteine derivative. The fact that the metabolic production of the mercapturic acid derivatives leads to the formation of only one of the possible isomers indicates that the reaction in which some sulphur compound combines with the epoxide is probably enzymic.

The hydrogenolysis with Raney nickel catalyst of the mercapturic acids (III) (derived from naphthalene) and (IV) (derived from 1:2-dihydronaphthalene and 1:2-epoxy-1:2:3:4-tetrahydronaphthalene) to yield in each case 2-naphthol and, after hydrolysis, alanine, provides additional confirmation of their proposed structure and demonstrates the close relationship of the two metabolites. The metabolites differ in their reaction towards mineral acid, the mercapturic acid (III) being broken down rapidly in the cold to give mainly 1-naphthylmercapturic acid (by the loss of the elements of water) (Boyland & Sims, 1958), whereas the mercapturic acid (IV) is only decomposed by hot acid

to give 2-tetralone (by the loss of the *N*-acetylcysteine residue). In these reactions the mercapturic acids resemble the corresponding dihydroxy compounds, *trans*-1:2-dihydro-1:2-dihydroxynaphthalene, yielding mainly 1-naphthol, and *trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene yielding 2-tetralone.

The presence of *S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine in the urine of animals treated with 1:2-dihydronaphthalene and 1:2-epoxy-1:2:3:4-tetrahydronaphthalene is unexpected, since the urine of animals treated with naphthalene does not contain the related cysteine derivative, *S*-(1:2-dihydro-1:2-dihydroxy-1-naphthyl)-L-cysteine but only the mercapturic acid (III) (an acetylcysteine derivative). The third metabolite, detected in the urine of animals treated with 1:2-dihydronaphthalene, which yielded 2-tetralone, cysteine and glycine on acid hydrolysis and 2-naphthol, alanine and glycine after hydrogenolysis with Raney nickel catalyst, appears to be a derivative of *S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteinylglycine. *S*-Substituted cysteine and cysteinylglycine derivatives have been detected in the bile of rats dosed with either 1:2-dihydronaphthalene or naphthalene (E. Boyland & P. Sims, unpublished work) and probably arise from the breakdown of the corresponding *S*-substituted glutathione derivatives.

The unidentified acids of m.p. 231° and 257° (decomp.) isolated from the urine of rabbits dosed with 1:2-epoxy-1:2:3:4-tetrahydronaphthalene (II) appear to be related chemically. It is not certain that they are metabolites since they were not found in the urine of rats treated with the epoxide (II) or in that of rabbits treated with 1:2-dihydronaphthalene. Their structure is now under investigation.

The naphthalene metabolites detected in the urine of animals treated with 1:2-dihydroxynaphthalene probably arise through dehydrogenation of the dihydro compound in the body. Such dehydrogenations to aromatic compounds are well known. From a comparison of the sizes of the spots produced by the various metabolites on paper chromatograms it is estimated that 10–15% of the administered dihydronaphthalene is metabolized in this way. The substance which gave naphthalene with mineral acid could not be detected among the products from the cellulose-powder column.

It will be seen therefore that there are remarkable similarities between the metabolism of 1:2-epoxy-1:2:3:4-tetrahydronaphthalene and of 1:2-dihydronaphthalene on the one hand and between 1:2-dihydronaphthalene and naphthalene on the other, and it is probable that the epoxide (II) is an intermediate in the metabolism of 1:2-dihydronaphthalene. These similarities are in agreement with the hypothesis that the as yet unknown 1:2-dihydronaphthalene-1:2-epoxide is the actual intermediate in the metabolic conversion of naphthalene either into the corresponding diol or into mercapturic acid. Further evidence that this is so is presented in the following paper.

SUMMARY

1. The action of L-cysteine on 1:2-epoxy-1:2:3:4-tetrahydronaphthalene in neutral solution or on 2-bromo-1-hydroxy-1:2:3:4-tetrahydronaphthalene in alkaline solution yielded in each case the two isomeric forms of *S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine. The epoxide and *N*-acetylcysteine similarly gave two isomeric forms of *N*-acetyl-*S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine, which were also obtained by the acetylation of the cysteine derivatives.

2. Rabbits treated with either 1:2-dihydronaphthalene or 1:2-epoxy-1:2:3:4-tetrahydronaphthalene excrete both the optical isomers of *trans*-1:2-dihydroxy-1:2:3:4-tetrahydronaphthalene and one of the two isomers of *N*-acetyl-*S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine, together with small amounts of *S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-L-cysteine and a substance believed to be a *S*-(2-hydroxy-1:2:3:4-tetrahydro-1-naphthyl)-cysteinylglycine derivative. A glucosiduronic acid of *trans*-1:2-dihydro-1:2:3:4-tetrahydronaphthalene was also present.

3. Small amounts of all the known naphthalene metabolites were also present in the urine of rabbits after administration of 1:2-dihydronaphthalene.

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