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Metabolism of Polycyclic Compounds

THE METABOLISM OF 1,4-EPOXY-1,4-DIHYDRONAPHTHALENE IN RATS

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1. 1,4-Epoxy-1,4-dihydronaphthalene is converted by rats into 1,4:2,3-diepoxy-1,2,3,4-tetrahydronaphthalene, which was isolated from the urine. The synthesis of the diepoxide is described. 2. The monoepoxide also yielded a compound that is believed to be 1,4-dihydro-1,4-dihydroxynaphthalene, but no corresponding mercapturic acid was detected. A number of unidentified metabolites of the monoepoxide were detected that appear to arise by the hydroxylation of the diepoxide. 3. The monoepoxide is converted into the diepoxide by a rat-liver microsomal system. 4. 1,4-Epoxy-1,4-dihydronaphthalene does not appear to be an intermediate in naphthalene metabolism.

It has been shown that anthracene (Sims, 1964) and benz[*a*]anthracene (Boyland & Sims, 1964) are metabolized by animals at the meso-positions of the hydrocarbons to yield products that could have arisen via the intermediate formation of epoxydihydro compounds. Although these compounds have not been described, the related compound 1,4-epoxy-1,4-dihydronaphthalene (I) is known and it is now shown that it is converted by rats mainly into 1,4:2,3-diepoxy-1,2,3,4-tetrahydronaphthalene (II). A re-examination of the metabolism of naphthalene in rats failed to provide any evidence that the 1,4-monoepoxide is involved in naphthalene metabolism.

EXPERIMENTAL

All melting points are uncorrected.

Spectra. Absorption spectra were measured in ethanol on a Perkin–Elmer model 137 ultraviolet spectrophotometer and infrared spectra as mulls in Nujol on a Perkin–Elmer Infracord.

Chromatography. Paper chromatography was carried out on Whatman no. 1 chromatography paper by downward development for 18hr. in butan-1-ol–propan-1-ol–aq. 2N-NH_3 (2:1:1, by vol.). The dried chromatograms were examined in ultraviolet light and either sprayed with a solution of diazotized *p*-nitroaniline (0·2% in 4N-HCl), heated to 80° for 10min. and sprayed with 10% (w/v) Na_2CO_3, or dipped in the platinic iodide reagent of Toennies & Kolb (1951), or dipped into a 0·2% solution of ninhydrin in acetone and heated to 80° for 10min.

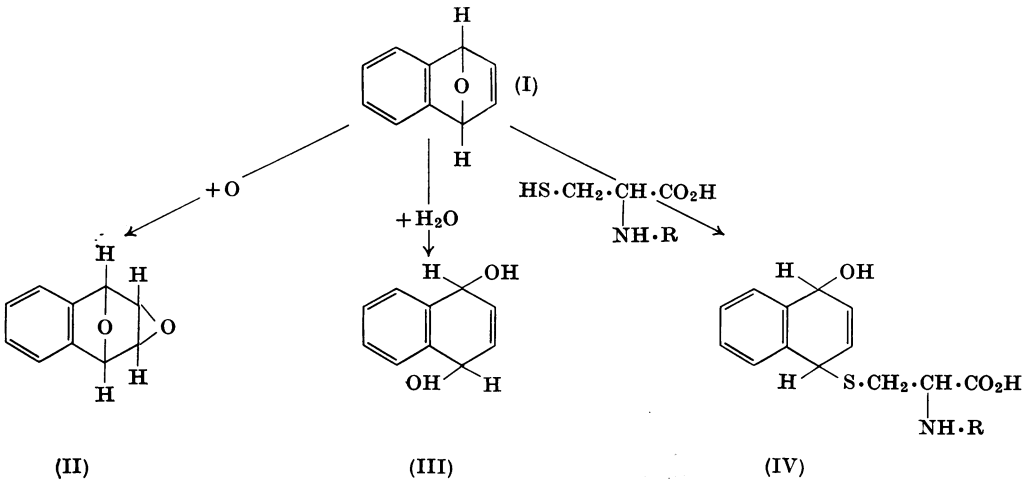
Thin-layer chromatograms were prepared by coating glass plates with a film of silica gel G (E. Merck A.-G., Darmstadt, W. Germany) of 0·25mm. thickness. The chromatograms were developed for 10cm. with either (a) benzene or (b) benzene containing 5% (v/v) of ethanol. They were sprayed with either (1) a 0·5% solution of 2,6-dichloroquinonechloroimide in ethanol followed by aq. 10% (w/v) Na_2CO_3 or (2) conc. HCl and heated in an oven at 80° for 10min., after which the chromatograms were examined and sprayed with reagent (1). Two-dimensional chromatograms were developed in the first direction for 10cm. with (a), sprayed with conc. HCl and heated in the oven to 80° for 10min. and developed in the second direction with (b). The dried chromatograms were sprayed with reagent (1).

Materials

1,4-*Epoxy*-1,4-*dihydronaphthalene* (see Scheme 1). This was prepared by the reaction of the benzyne intermediate with furan by the method outlined by Friedman & Logullo (1963), when it formed needles from light petroleum (b.p. 40–60°), m.p. 52–53°. Wittig & Pohmer (1956) give m.p. 55–56° for the epoxide.

The epoxide was decomposed on heating to 100° with 2N-HCl to yield a compound indistinguishable on thin-layer chromatograms from 1-naphthol. Attempts to convert the epoxide into 1,4-dihydro-1,4-dihydroxynaphthalene (III) by heating the compound under reflux in aqueous acetone or by treating the compound with dilute acid under a variety of conditions yielded either unchanged epoxide or 1-naphthol.

The epoxide (300mg.), in water (20ml.) and acetone (8ml.) containing $NaHCO_3$ (200mg.) and two drops of 2N-NaOH, was heated under reflux for 4hr. either with L-cysteine hydrochloride (300mg.) or with *N*-acetyl-L-cysteine (300mg.). In both experiments, 1,4-epoxy-1,4-dihydronaphthalene (I) was recovered in about 95% yield when the cooled solutions were extracted with ether. The aqueous layers were acidified to pH4 with acetic acid and activated charcoal (2g.) (British Drug Houses Ltd.) was added. The charcoal was filtered off and washed with water and the adsorbed materials were eluted with methanol (250ml.) containing 5% (v/v) of aq. NH_3 (sp.gr. 0·88). The small residues that were obtained on evaporation of the



Scheme 1. Possible reactions of 1,4-epoxy-1,4-dihydronaphthalene. R = H or CO·CH_3.

solvents formed gums that would not crystallize. These are presumed to be mixtures of the diastereoisomers of *S*-(1,4-dihydro-1-hydroxy-4-naphthyl)-L-cysteine (IV, R = H) and *N*-acetyl-*S*-(1,4-dihydro-1-hydroxy-4-naphthyl)-L-cysteine (IV, R = CO·CH_3) respectively. The cysteine derivative formed a spot of R_F 0·43 on paper chromatograms that gave a positive reaction with the platinic iodide reagent and a purple colour with ninhydrin and the *N*-acetylcysteine derivative formed a spot of R_F 0·46 that gave a positive reaction with the platinic iodide reagent but no colour with ninhydrin. Both products were unaffected by cold 2N-HCl, in contrast with the rapid decomposition that occurs when the related *S*-(1,2-dihydro-2-hydroxy-1-naphthyl)cysteine derivatives are similarly treated (Boyland, Ramsay & Sims, 1961). When the products were heated to 100° with 5N-HCl for 1hr., both yielded a product indistinguishable from 1-naphthol on thin-layer chromatograms and a product with the properties on paper chromatograms of *S*-(1-naphthyl)cysteine described by Boyland *et al.* (1961).

1,4:2,3-*Diepoxy*-1,2,3,4-*tetrahydronaphthalene* (II). 1,4-Epoxy-1,4-dihydronaphthalene (I) (5g.) and perbenzoic acid (7·5g.) in chloroform (200ml.) were kept for 2 days at room temperature. The solution was washed with an excess of aq. 2N-NaOH, dried over Na_2SO_4 and the chloroform removed under reduced pressure to yield 1,4:2,3-*diepoxy*-1,2,3,4-*tetrahydronaphthalene* (II) (4·8g.), separating from light petroleum (b.p. 60–80°) in plates, m.p. 91–92° (Found: C, 75·2; H, 5·0. $C_{10}H_8O_2$ requires C, 75·0; H, 5·0%), light-absorption max. at 218, 258, 264 and 271·5mμ, ε 3900, 420, 560 and 530 respectively.

The diepoxide was rapidly decomposed by cold aq. 2N-HCl to yield a brown amorphous product that was not identified. Dihydroxynaphthalenes, which might be expected as acid-decomposition products of the diepoxide, were not detected.

No substituted cysteine or *N*-acetylcysteine derivatives were obtained when the diepoxide was treated with cysteine or *N*-acetylcysteine as described above.

Enzymic and acid hydrolyses

Hydrolyses with the sulphatases of Taka-diastase (Parke, Davis and Co. Ltd.) were carried out in 0·1M-acetate buffer, pH 5·7, and hydrolyses with β-glucuronidase (Ketodase; Warner–Chilcott Laboratories) in 0·1M-acetate buffer, pH 5·0. The mixtures were incubated overnight at 37°. Acid hydrolyses were carried out by heating the material under examination with 5N-HCl on the steam bath for 15min. The products of the hydrolyses were extracted from the reaction mixtures with ether and examined on thin-layer chromatograms.

Animal experiments

Metabolism of 1,4-*epoxy*-1,4-*dihydronaphthalene* (*I*). Twenty male rats of the Chester Beatty strain (body wt. approx. 250g.) were each given 1,4-epoxy-1,4-dihydronaphthalene (I) (50mg.) in arachis oil (1ml.) by intraperitoneal injection every other day until 4g. of the epoxide had been administered. Higher doses than these caused the animals to become ill. The urines were collected until the day after the last injections and were pooled and stored at 4°.

The urine was filtered and acidified to pH 4 with acetic acid and activated charcoal (50g.) added. The charcoal was filtered off and washed with water (1l.) and the adsorbed material eluted with methanol (2·5l.) containing 5% (v/v) of aq. NH_3 (sp.gr. 0·88). The solvent was evaporated under reduced pressure and the residue chromatographed on a column prepared from cellulose powder (500g.) (Whatman standard grade), butan-1-ol–cyclohexane–aq. 2N-NH_3 (9:2:1, by vol.) being used as developing solvent. Fractions (100ml.) were collected and evaporated under reduced pressure and the residues examined on paper chromatograms. Those that contained similar products were combined to give four main fractions that were examined as described below.

Fraction 1, which formed a brown gum, was extracted three times with boiling light petroleum (b.p. 60–80°) (100 ml.). The combined extracts were evaporated to 5 ml. under reduced pressure and allowed to crystallize. The product (75 mg.) was recrystallized from light petroleum (b.p. 60–80°) in plates, m.p. 89° (Found: C, 74·7; H, 4·6. Calc. for $C_{10}H_8O_2$: C, 75·0; H, 5·0%). A mixture with 1,4:2,3-diepoxy-1,2,3,4-tetrahydronaphthalene (II) had m.p. 90–91°. The metabolite and the diepoxide were indistinguishable on thin-layer chromatograms and had identical infrared and ultraviolet spectra (light-absorption max. at 218, 258, 264 and 271 mμ, ε 3900, 420, 550 and 530 respectively).

The combined light-petroleum mother-liquors were evaporated to dryness and the residue was examined on thin-layer chromatograms. Three major products were present, the first of which was identical with the diepoxide described above. The second product yielded a compound indistinguishable from 1-naphthol after it had been chromatographed by the two-dimensional thin-layer chromatography technique described above and appears to be 1,4-dihydro-1,4-dihydroxynaphthalene (III) whilst the third, which yielded an unidentified brown substance on these chromatograms, appears, from its position on the chromatograms, to be a dihydrodihydroxy compound related to the diepoxide. The properties of these compounds are listed in Table 1.

Fraction 2 contained a compound, R_F 0·72, that had a dark-violet fluorescence in ultraviolet light and gave a brown colour with the *p*-nitroaniline reagent. Hydrolysis of the fraction with sulphatase yielded a compound indistinguishable on thin-layer chromatograms from the unidentified dihydrodihydroxy compound described above.

Fraction 3, in which mercapturic acids were expected, did not contain any metabolites that gave positive reactions with the platinic iodide reagent.

Fraction 4, which gave a positive reaction with naphtharesorcinol, was chromatographed on three sheets of Whatman no. 3MM chromatography paper, the chromatograms being developed downward for 18 hr. with butan-1-ol–propan-1-ol–aq. 2 N-NH_3. Three bands were recognized when test strips were sprayed with the *p*-nitroaniline reagent. These were cut from the chromatograms and the absorbed materials eluted from the paper with methanol containing 5% (v/v) of aq. NH_3 (sp.gr. 0·88). The first compound formed a spot, R_F 0·32, with a dark-violet fluorescence in ultraviolet light and which gave a brown colour with the *p*-nitroaniline reagent. No products were identified when the compound was incubated with β-glucuronidase. The second compound, R_F 0·28, gave a blue colour with the *p*-nitroaniline reagent. After hydrolysis with β-glucuronidase a compound was detected that was indistinguishable on thin-layer chromatograms from the compound detected in fraction 1 that is believed to be 1,4-dihydro-1,4-dihydroxynaphthalene (III). On two-dimensional thin-layer chromatograms the hydrolysate yielded a compound indistinguishable from 1-naphthol.

The third product, R_F 0·22, gave a brown colour with the *p*-nitroaniline reagent. After hydrolysis with β-glucuronidase, a compound was detected that was indistinguishable from the unidentified product found in fraction 1.

Metabolism of 1,4:2,3-*diepoxy*-1,2,3,4-*tetrahydronaphthalene* (II). Six rats were treated with the epoxide (100 mg.) in arachis oil, given by intraperitoneal injection every other day until a total of 2·4 g. had been administered. At this dose the epoxide was not toxic. The pooled urines were treated and examined as before. All the metabolites described above, except 1,4-dihydro-1,4-dihydroxynaphthalene (III) and its glucuronic acid conjugate, were detected. No mercapturic acids were found.

Metabolism of naphthalene. Two rats were each given naphthalene (100 mg.) in arachis oil (1 ml.) by intraperitoneal injection on 5 successive days. The pooled urines were examined for the metabolites described above. None was found although all the metabolites of naphthalene previously recognized were detected.

Microsomal oxidation of 1,4-*epoxy*-1,4-*dihydronaphthalene*. This was carried out with the rat-liver microsomal system described by Boyland, Kimura & Sims (1964).

Table 1. *Properties of compounds related to* 1,4-*epoxy*-1,4-*dihydronaphthalene on thin-layer chromatograms*

Chromatography was carried out as described in the text.

Compound	R_F		Colour		
				With conc. HCl and heating to 100°	
	In benzene	In benzene–ethanol (19:1, v/v)	With 2,6-dichloroquinonechloroimide–Na_2CO_3	Untreated	With 2,6-dichloroquinonechloroimide–Na_2CO_3
1-Naphthol	0·48	0·65	Violet	None*	Violet
1,4-Epoxy-1,4-dihydronaphthalene	0·45	0·88	None	None*	Violet
1,4:2,3-Diepoxy-1,2,3,4-tetrahydronaphthalene	0·15	0·48	None	Brown	Brown
Metabolite, probably 1,4-dihydro-1,4-dihydroxynaphthalene (III)	0·01	0·28	None	None*	Violet
Metabolite, probably a hydroxylated derivative of the diepoxide	0·00	0·30	None	Brown	Brown

* Purple colours were sometimes formed, which are presumably oxidation products of 1-naphthol.

An examination of the ether extract of the oxidation mixture on thin-layer chromatograms showed the presence of a compound indistinguishable from 1,4:2,3-diepoxy-1,2,3,4-tetrahydronaphthalene (II). No other metabolites were detected.

DISCUSSION

The results show that it is unlikely that naphthalene is metabolized by rats across the 1- and 4-positions to any appreciable extent. The isolation of 1,4:2,3-diepoxy-1,2,3,4-tetrahydronaphthalene (II) as a metabolite of the monoepoxide (I) is of interest, as apart from the chlorinated insecticides, aldrin and heptachlor, there appears to be no report of a relatively simple foreign compound being converted into an epoxy compound in the body. With aldrin (Bann, Decino, Earle & Sun, 1956) and heptachlor (Davidow & Radomski, 1953), the epoxides, which are almost insoluble in water, are deposited in the fatty tissue of the body and have not been detected in the urine. The diepoxide (II) isolated in the work described above is more soluble in water and is therefore excreted.

Neither 1,4-epoxy-1,4-dihydronaphthalene (I) nor 1,4:2,3-diepoxy-1,2,3,4-tetrahydronaphthalene (II) has any marked affinity for the enzyme that catalyses the addition of glutathione to many epoxides (Boyland & Williams, 1965) and neither is excreted as a mercapturic acid. Similarly, the epoxides show little or no chemical reactivity towards cysteine or *N*-acetylcysteine. The lack of reactivity of the diepoxide (II) is undoubtedly another reason why the compound is excreted in the urine. The related epoxides, 1,2-epoxy-1,2,3,4-tetrahydronaphthalene and 2,3-epoxy-1,2,3,4-tetrahydronaphthalene, react enzymically with glutathione (Boyland & Williams, 1965) and are excreted in the urine of treated animals as mercapturic acids (Boyland & Sims, 1960, and unpublished work).

1,4-Epoxy-1,4-dihydronaphthalene (I) is converted by rats into a compound that appears to be 1,4-dihydro-1,4-dihydroxynaphthalene (III) but this is only a minor metabolic pathway. The unidentified metabolites of the monoepoxide (I) all appear to have arisen by the further metabolism of the diepoxide (II) since they are excreted in the urine of animals dosed with this compound.

The detection of the diepoxide (II) in the microsomal oxidation of the monoepoxide (I) is the first direct evidence that microsomes are able to effect the epoxidation of double bonds of relatively simple compounds, although Wong & Terriere (1965) have shown that aldrin, isodrin and heptachlor undergo epoxidation in these systems.

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