Biochemical Studies of Toxic Agents

METABOLIC RING-FISSION OF *CIS-* AND *TRANS*-ACENAPHTHENE-1,2-DIOL

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1. The metabolism of *cis-* and *trans*-acenaphthene-1,2-diol has been studied after the administration of these compounds to rats by subcutaneous injection and by stomach tube. 2. 1,8-Naphthalic acid has been isolated as its anhydride from the urine of the dosed animals. 3. A spectrophotometric method for the determination of free and conjugated 1,8-naphthalic acid in urine has been developed and has been used in the study of the metabolism of the acenaphthene-1,2-diols. 4. The urine of rats dosed with *cis*-acenaphthene-1,2-diol by subcutaneous injection was shown by paper chromatography to contain both *cis-* and *trans*-acenaphthene-1,2-diol. Similar findings were obtained after the subcutaneous injection of *trans*-acenaphthene-1,2-diol.

Acenaphthylene is one of the few carbocyclic compounds that have been shown to undergo ring-fission in the animal body, for Hopkins, Brooks & Young (1962) were able to isolate 1,8-naphthalic acid, as its anhydride, from the urine of rats and rabbits after the administration of this hydrocarbon. In addition, they isolated *cis-* and *trans*-acenaphthene-1,2-diol from the urine of the dosed animals, and, in view of the suggestion (Young, 1950) that diols might undergo ring-fission *in vivo* to yield dicarboxylic acids, the possibility that the diols formed from acenaphthylene can give rise to 1,8-naphthalic acid in the animal body has now been investigated.

MATERIALS

All melting points are uncorrected. The melting point of naphthalic anhydride was determined by heating the compound either on a brass block or in a bath containing silicone fluid (MS 550; Hopkin and Williams Ltd., Chadwell Heath, Essex). Elementary microanalyses were carried out by Weiler and Strauss, Oxford. The Ring Index numbering of compounds (Patterson, Capell & Walker, 1960) has been used throughout this paper.

cis- *and* trans-*Acenaphthene-1,2-diol.* These compounds were prepared by the methods described by Hopkins *et al.* (1962) in which the Prévost (1933*a*,*b*) reaction was used. Acenaphthylene was treated with silver benzoate at room temperature to give the monobenzoate of *cis*-acenaphthene-1,2-diol. When the reaction was repeated at 80° the dibenzoate of *trans*-acenaphthene-1,2-diol was obtained. The diols were liberated by hydrolysis of the benzoates with methanolic KOH, and after recrystallization *cis*-acenaphthene-1,2-diol was obtained as needles, m.p. 207–208°, and (±)-*trans*-acenaphthene-1,2-diol as plates, m.p. 157–158°.

The synthetic diols used in this work were carefully purified, and each was tested for the presence of the other by paper-chromatographic methods, details of which are given below. With solvent mixtures *A* and *B* (see the Methods section) it was possible to detect *trans*-diol when 40μg. of a mixture containing 99·5% of *cis*-acenaphthene-1,2-diol and 0·5% of *trans*-acenaphthene-1,2-diol was chromatographed. When 40μg. of the synthetic *cis*-acenaphthene-1,2-diol was chromatographed under the same conditions, no *trans*-diol was detected. By the use of solvent mixtures *C* and *D* (see the Methods section) it was possible to detect the *cis*-diol in a mixture of 99·8% of *trans*-diol and 0·2% of *cis*-diol when 100μg. of the mixture was chromatographed. No *cis*-diol was detected when 100μg. of synthetic *trans*-acenaphthene-1,2-diol was chromatographed. It is therefore apparent that the *cis*-diol contained less than 0·5% of the *trans*-diol, and that the *trans*-diol contained less than 0·2% of the *cis*-diol.

1,8-*Naphthalic anhydride.* Acenaphthene was oxidized with $Na_2Cr_2O_7$ in acetic acid according to the method of Graebe & Gfeller (1892), and naphthalic anhydride was separated from the reaction mixture and purified as described by Hopkins *et al.* (1962). The product was obtained as colourless needles, m.p. 273–274°, which yielded *N*-anilinonaphthalimide, m.p. 215–216°, by interaction with phenylhydrazine (Hopkins *et al.* 1962).

Naphthalic anhydride was also prepared by the oxidation of *cis-* and *trans*-acenaphthene-1,2-diol with alkaline $KMnO_4$. Diol (0·05g.) was dissolved by heating in 200ml. of 2N-NaOH, and the solution was then cooled to room temperature. Aq. 1% (w/v) $KMnO_4$ was added slowly with stirring until a small excess was present. After the reaction mixture had stood at room temperature for 20min., the excess of $KMnO_4$ was removed by the addition of $Na_2S_2O_5$. The precipitate was removed by centrifuging, and was washed once with 200ml. of water. The washing and main supernatant solution were combined, brought to pH2 with conc. HCl, left at room temperature overnight and then extracted twice with equal volumes of ether. The

combined ether extracts were evaporated to dryness under reduced pressure, and the residue from each preparation was crystallized from aqueous acetone. Both preparations gave pale-yellow needles, m.p. 269–270°. The melting point was not depressed on admixture with naphthalic anhydride prepared by oxidation of acenaphthene with $Na_2Cr_2O_7$ in acetic acid, and the products prepared by the two methods gave the same infrared spectrum. By oxidation with alkaline $KMnO_4$, naphthalic anhydride was obtained in 76% yield from *cis*-acenaphthene-1,2-diol and in 84% yield from the *trans*-diol.

Disodium 1,8-naphthalate. This compound was prepared by treating naphthalic anhydride with 95% of the theoretical amount of NaOH necessary to form the disodium salt, and then removing the excess of naphthalic anhydride. To 5g. of naphthalic anhydride was added 1·92g. of NaOH in 250ml. of water. The mixture was heated under reflux in a boiling-water bath for 1hr., during which time nearly all the anhydride dissolved. The suspension was cooled and filtered to remove naphthalic anhydride, and the disodium salt of 1,8-naphthalic acid was precipitated from the filtrate by the addition of acetone. The disodium salt was separated and washed with acetone, and was obtained as a white solid (5·50g.), readily soluble in water. This product lost no weight when heated at 110°.

Acenaphthen-1-one. This compound was obtained as prisms, m.p. 119–120·5°, by the acid decomposition of *trans*-acenaphthene-1,2-diol as described by Hopkins *et al.* (1962).

METHODS

Animals and dosing. Male rats (body wt. 150–200g.) were used. They were of the Wistar strain or a black-hooded strain, and were housed in metabolism cages from which the urine was collected separately from the faeces. They were fed on rat cakes [J. Murray and Sons (London) Ltd.] and had access to water at all times.

In some experiments the acenaphthene-1,2-diol was administered by stomach tube and in others by subcutaneous injection. Each time a rat was given a diol by stomach tube it received 1·25ml. of a 20% (w/v) suspension of the diol in 1% (w/v) starch solution. When a diol was administered by subcutaneous injection each dose consisted of 1·0ml. of a 20% (w/v) suspension of the diol in arachis oil.

Urine was collected daily throughout the period of dosing and for a definite time after the last dose. The urine was filtered through glass wool and stored in the refrigerator.

Paper chromatography. cis- and *trans*-Acenaphthene-1,2-diol and 1,8-naphthalic anhydride were detected on paper chromatograms as fluorescent spots under ultraviolet light, after the chromatograms had been sprayed with ethanol–conc. H_2SO_4–water (18:1:1, by vol.) and heated (Hopkins *et al.* 1962). Four different solvent systems were employed, the compositions of which were: *A*, methanol–carbon tetrachloride (9:1, v/v); *B*, methanol–cyclohexane (9:1, v/v); *C*, benzene–carbon tetrachloride–water–aq. NH_3 (sp.gr. 0·88) (9:1:8:2, by vol.); *D*, benzene–carbon tetrachloride–water–acetic acid (9:1:8:2, by vol.). The components of systems *C* and *D* were shaken vigorously for 1–2min. and then allowed to separate for 30min. The upper layer of each was used as the mobile phase, and the lower layer as the stationary phase. Papers were equilibrated overnight in an atmosphere of the stationary phase. Whatman no. 1 paper was used, with the ascending method for systems *A* and *B*, and the descending method for systems *C* and *D*. The minimum quantity of either diol that could be detected was 0·2μg. Typical R_F values for *cis*- and *trans*-acenaphthene-1,2-diol in the four solvent systems are shown in Table 1.

Table 1. *Paper chromatography of* cis- *and* trans-*acenaphthene-1,2-diol*

Experimental details are given in the text.

Solvent system	R_F *cis*-Diol	R_F *trans*-Diol
A	0·69	0·77
B	0·70	0·77
C	0·53	0·23
D	0·62	0·25

Isolation of 1,8-*naphthalic acid.* This compound was isolated in the form of its anhydride from the acidified urine of dosed animals after the urine had been extracted under neutral conditions with ether to remove any free *cis*- or *trans*-acenaphthene-1,2-diol. The urine was adjusted to pH2 with conc. HCl and was then shaken with four separate portions of ether, each equal in volume to the urine. The combined ether extracts were evaporated to dryness and the residue so obtained was stirred with a small volume of ice-cold ethanol. The material that did not dissolve in the ethanol was separated and dissolved in warm 2N-NaOH. The alkaline solution was decolorized with charcoal and filtered. The precipitate that formed on acidifying the filtrate was crystallized from aqueous acetone. In some cases the product was recrystallized from hot conc. HNO_3 to which had been added one-tenth its volume of water, a procedure that had been shown to be advantageous in experiments with synthetic naphthalic anhydride. To ensure that the final product was in the anhydride form it was heated to constant weight at 110°.

Isolation of cis- *and* trans-*acenaphthene-1,2-diol.* These compounds were isolated from the urine of dosed animals, either before or after it had been treated with a rat-liver preparation containing β-glucuronidase, as described by Hopkins *et al.* (1962).

Determination of free and total naphthalic acid in urine. These determinations were based on the extinction shown by naphthalic anhydride at 339mμ. To determine free naphthalic acid the urine was clarified by centrifuging and it was then diluted with one-third its volume of water. To 1ml. of the diluted urine was added 5ml. of 0·45% $ZnSO_4$ solution followed by 3ml. of 0·1N-NaOH. The suspension that formed was mixed well and after 3min. it was centrifuged. The precipitate was washed once with 5ml. of M-$NaHCO_3$ and the washing was added to the supernatant solution, which was then brought to pH7 with 2N-HCl and washed twice by shaking with one-fifth its volume of ether to remove any acenaphthen-1-one that might have been present. The aqueous solution was then brought to pH2 with conc. HCl and after 1hr. at room temperature it was extracted by shaking six times with equal volumes of ether. The combined ether extracts were evaporated to dryness under reduced pressure. The residue obtained was heated at 110° for 1hr. and then dissolved in a known volume of butan-1-ol (analytical

grade) to give a solution containing about 15μg. of naphthalic anhydride/ml. After any undissolved matter had been removed by centrifuging, the concentration of naphthalic anhydride was determined by measuring the extinction of the butan-1-ol solution at 339mμ against a blank solution prepared by carrying out the procedures just described on urine from undosed rats. When the extinctions of solutions of naphthalic anhydride in butan-1-ol (5–25μg./ml.) were measured with a Hilger Uvispek spectrophotometer at 339mμ they varied linearly with the concentration of the anhydride. In experiments in which the method was tested by adding the disodium salt of 1,8-naphthalic acid to normal rat urine in amounts equivalent to 0·1, 0·2, 0·3, 0·4 and 0·5mg. of naphthalic anhydride/ml. the recoveries were 87, 101, 96, 98 and 99% respectively.

Total naphthalic acid was determined after acid hydrolysis. The urine was diluted with one-third its volume of water, and 1ml. of the diluted urine was heated under reflux in a boiling-water bath with 0·5ml. of 50% (v/v) H_2SO_4 for 4hr., conditions that had been found to give maximal liberation of naphthalic acid from its conjugates in urine. The hydrolysed urine was adjusted to pH8–9 with 40% (w/v) NaOH solution and allowed to stand overnight. Then 16ml. of 0·45% $ZnSO_4$ solution and 9·6ml. of 0·1N-NaOH were added and the precipitate was washed with 16ml. of M-$NaHCO_3$, and thereafter the procedure was as described above for the determination of free naphthalic acid.

When two rats were each injected subcutaneously with 1ml. of an aqueous solution containing 0·05g. of the disodium salt of 1,8-naphthalic acid, analysis of the urine collected during the next 4 days showed that the free naphthalic acid excreted during this period corresponded to 70% of that administered. The total naphthalic acid found after periods of hydrolysis of 1, 4, 6 and 8hr. corresponded to 77, 79, 79 and 78% respectively of the naphthalic acid administered. Naphthalic acid was stable under these conditions, for when normal rat urine to which 400μg. of disodium naphthalate had been added/ml. was hydrolysed for 8hr. recoveries of 98 and 99% were obtained.

RESULTS

Metabolism of cis-*acenaphthene*-1,2-*diol*

Isolation of 1,8-*naphthalic anhydride.* When a total of 7·2g. of *cis*-acenaphthene-1,2-diol was administered to 18 rats by subcutaneous injection, 0·147g. of naphthalic anhydride was separated from the urine of the dosed animals, m.p. 271–272°, mixed m.p. 271–272° (Found: C, 72·8; H, 3·2. Calc. for $C_{12}H_6O_3$: C, 72·7; H, 3·1%). By reaction with phenylhydrazine the compound from urine gave a product, m.p. 213–215°, and m.p. 214–215° when mixed with synthetic *N*-anilinonaphthalimide (m.p. 215–216°) (Found: C, 74·5; H, 4·2; N, 9·9. Calc. for $C_{18}H_{12}N_2O_2$: C, 75·0; H, 4·2; N, 9·7%). The ultraviolet and infrared spectra of the compound from urine and its behaviour on paper chromatograms corresponded to those of synthetic 1,8-naphthalic anhydride.

From the urine of 12 rats that had received a total of 6g. of *cis*-acenaphthene-1,2-diol by stomach tube, 0·106g. of naphthalic anhydride was obtained, m.p. 272–273°, mixed m.p. 272–273° (Found: C, 72·2; H, 3·2%).

Quantitative studies. Two black-hooded rats were injected subcutaneously with 0·093g. (0·5m-mole) of *cis*-acenaphthene-1,2-diol in 1ml. of arachis oil. By the use of the analytical procedure described above it was found that the excretion of naphthalic acid was virtually complete in the first 4 days after injecting the diol and that in this time the amounts of free and combined naphthalic acid in the urine corresponded to 9·2 and 0·4% respectively of the diol administered.

Paper-chromatographic studies. Two male black-hooded rats were each given by subcutaneous injection 0·2g. of *cis*-acenaphthene-1,2-diol in 1ml. of arachis oil, and two control rats were each injected with 1ml. of arachis oil. The urine from each pair of animals was collected for 24hr. and was then extracted continuously with ether for 6hr., after which the volume of the ether extract was adjusted to 100ml. The ether solution was shaken with 10ml. of 2N-sodium hydroxide to remove naphthalic acid and was then concentrated to 10ml. A third ether solution was prepared in the same way from a solution of a mixture of 5mg. of *cis*- and 5mg. of *trans*-acenaphthene-1,2-diol in 50ml. of normal rat urine. All three ether solutions were examined simultaneously by paper chromatography with solvent mixtures *A* and *B*. No diols were found in the extract of the urine from the control animals, but the extract of the urine from the animals dosed with *cis*-acenaphthene-1,2-diol showed the same results as were obtained with the extract of the reference urine containing *cis*- and *trans*-acenaphthene-1,2-diol.

After the free diols had been removed from the urine by ether extraction each urine was examined for the presence of conjugated diols. Dissolved ether was removed by warming the urine, after which the urine was incubated at pH 5·2 with a rat-liver preparation containing β-glucuronidase as described by Hopkins *et al.* (1962). The urine was then brought to pH 7–8 and examined, as described above, for the presence of free diols by using the reference solution obtained by ether extraction of normal rat urine to which *cis*- and *trans*-acenaphthene-1,2-diol had been added. The chromatograms were similar to those described above, and showed that when it was treated with rat-liver preparation the urine of rats dosed with *cis*-acenaphthene-1,2-diol yielded further amounts of the *cis*- and *trans*-forms of the diol.

Isolation of cis-*diol from urine.* When the urine of 16 rats that had received a total of 6·4g. of *cis*-acenaphthene-1,2-diol by subcutaneous injection was incubated with a rat-liver preparation containing β-glucuronidase it yielded 0·061g. of *cis*-diol,

m.p. and mixed m.p. 207–208° (Found: C, 77·2; H, 5·2. Calc. for $C_{12}H_{10}O_2$: C, 77·4; H, 5·4%). This material gave a diacetate, m.p. and mixed m.p. 130–131° (Found: C, 70·8; H, 5·3. Calc. for $C_{16}H_{14}O_4$: C, 71·1; H, 5·2%). In a similar experiment 0·159g. of *cis*-acenaphthene-1,2-diol, m.p. and mixed m.p. 206–207°, was isolated from the urine of 12 rats that had received a total of 6g. of the diol by stomach tube.

Attempts to isolate *trans*-acenaphthene-1,2-diol from the urine of rats that had been dosed with *cis*-acenaphthene-1,2-diol, either by subcutaneous injection or by stomach tube, were all unsuccessful, irrespective of whether the urine had been incubated with a rat-liver β-glucuronidase preparation.

Metabolism of trans-*acenaphthene*-1,2-*diol*

Isolation of 1,8-*naphthalic anhydride.* In an experiment in which 7·2g. of (±)-*trans*-acenaphthene-1,2-diol was administered to 18 rats by subcutaneous injection, 0·215g. of naphthalic anhydride, m.p. and mixed m.p. 270–271°, was separated from the urine of the dosed animals (Found: C, 72·6; H, 2·6%). This compound showed the same ultraviolet and infrared spectra and R_F values as synthetic naphthalic anhydride, and by reaction with phenylhydrazine it yielded *N*-anilinonaphthalimide, m.p. and mixed m.p. 214–215° (Found: C, 74·7; H, 4·3; N, 9·7%).

From the urine of rats that had received a total of 6·0g. of (±)-*trans*-acenaphthene-1,2-diol by stomach tube, 0·090g. of naphthalic anhydride, m.p. and mixed m.p. 271–272°, was obtained (Found: C, 73·1; H, 3·4%).

Quantitative studies. Two male black-hooded rats were injected subcutaneously with 0·093g. (0·5m-mole) of (±)-*trans*-acenaphthene-1,2-diol in 1ml. of arachis oil. Analysis of the urine collected in the next 4 days showed the presence of free and conjugated 1,8-naphthalic acid in amounts corresponding to 9·6 and 1·4% respectively of the diol administered. No naphthalic acid was detected in the urine excreted on the fifth and sixth days after injecting the diol.

Paper-chromatographic studies. The urine of rats that had each received a single dose of 0·2g. of (±)-*trans*-acenaphthene-1,2-diol by subcutaneous injection was examined by the procedures described above for experiments in which the *cis*-diol was administered. Both before and after hydrolysis with the rat-liver β-glucuronidase preparation, it was possible to detect *cis*- and *trans*-acenaphthene-1,2-diol in the urine of the dosed animals.

Isolation of trans-*diol from urine.* From the enzyme-hydrolysed urine of 16 rats that had received a total of 6·4g. of (±)-*trans*-acenaphthene-1,2-diol by subcutaneous injection was isolated 0·049g. of mixed (+)- and (±)-*trans*-diol, m.p. 142–147°, $[\alpha]_{5461}^{20}+23°$ (*c* 0·065 in benzene) (Found: C, 77·1; H, 5·6). Under the same conditions the urine of 12 rats that had received a total of 6·0g. of (±)-*trans*-acenaphthene-1,2-diol by stomach tube yielded 0·119g. of mixed (+)- and (±)-*trans*-diol, m.p. 144–147°, $[\alpha]_{5461}^{25}+49°$ (*c* 0·062 in benzene). It is noteworthy that the melting points and specific rotations of the isolated *trans*-diols resemble those obtained for the mixed (+)- and (±)-*trans*-acenaphthene-1,2-diol isolated after the administration of acenaphthylene to rats (Hopkins *et al.* 1962).

After enzymic hydrolysis the urine of the rats that had been dosed with a total of 6·4g. of (±)-*trans*-acenaphthene-1,2-diol by subcutaneous injection also yielded *cis*-acenaphthene-1,2-diol. The isolated diol weighed 0·036g., and its m.p., 206–207°, was not depressed on admixture with synthetic *cis*-acenaphthene-1,2-diol. It gave a diacetate, m.p. and mixed m.p. 130–131°.

Stability of cis- *and* trans-*acenaphthene*-1,2-*diol in rat urine*

The finding that 1,8-naphthalic acid can be separated as its anhydride from the urine of rats after the administration of *cis*- and *trans*-acenaphthene-1,2-diol made it necessary to determine whether any 1,8-naphthalic acid was formed in the urine, or during the isolation process, by breakdown of acenaphthenediols. This was tested by taking two 500ml. portions of normal rat urine and adding 0·425g. of *cis*-acenaphthene-1,2-diol to one and 0·425g. of the *trans*-isomer to the other. Each urine was saturated with diol by repeated gentle warming followed by mechanical shaking. The urines were then kept at 37° for 24hr. and each was then subjected to the treatment used to isolate 1,8-naphthalic acid. None was found.

The fact that *cis*-acenaphthene-1,2-diol was found in the urine of rats dosed with its *trans*-isomer and vice versa made it necessary to determine whether this was the outcome of an interconversion of the free diols occurring in the urine itself, and whether interconversion could be brought about by the liver preparation used to hydrolyse diol conjugates. Fresh normal rat urine was adjusted to pH 7–8 and in three 110ml. portions of the urine the following were dissolved: (1) 8mg. of *cis*-acenaphthene-1,2-diol; (2) 0·04mg. of *trans*-acenaphthene-1,2-diol; (3) 8mg. of *cis*-diol and 0·04mg. of *trans*-diol. The three urines were left at room temperature for 24hr. and each was then extracted by mechanical shaking for four 30min. periods with equal volumes of ether. The ether extracts from each urine were combined and evaporated under reduced pressure to 10ml. These solutions were examined by paper chroma-

tography with 100μl. of each solution and solvent mixtures *A* and *B*. The chromatogram from the extract of (1) showed no trace of *trans*-diol, whereas those from the extracts of (2) and (3) showed that *trans*-diol, alone and when mixed with 200 times as much *cis*-diol, can be readily detected by the procedure used.

Another experiment was carried out that was essentially the same as that just described except that the additions of diols were as follows: (1) 8mg. of *trans*-acenaphthene-1,2-diol; (2) 0·04mg. of *cis*-acenaphthene-1,2-diol; (3) 8mg. of *trans*-diol and 0·04mg. of *cis*-diol. When the ether extracts were chromatographed with solvent mixtures *C* and *D*, no *cis*-diol was found on the chromatogram of the extract of (1). The extracts of (2) and (3) showed that *cis*-diol, alone and when mixed with 200 times its weight of *trans*-diol, can easily be detected. It follows from these experiments that, if interconversion of the free acenaphthenediols occurs on standing in urine for 24hr., this takes place to the extent of less than 0·5%.

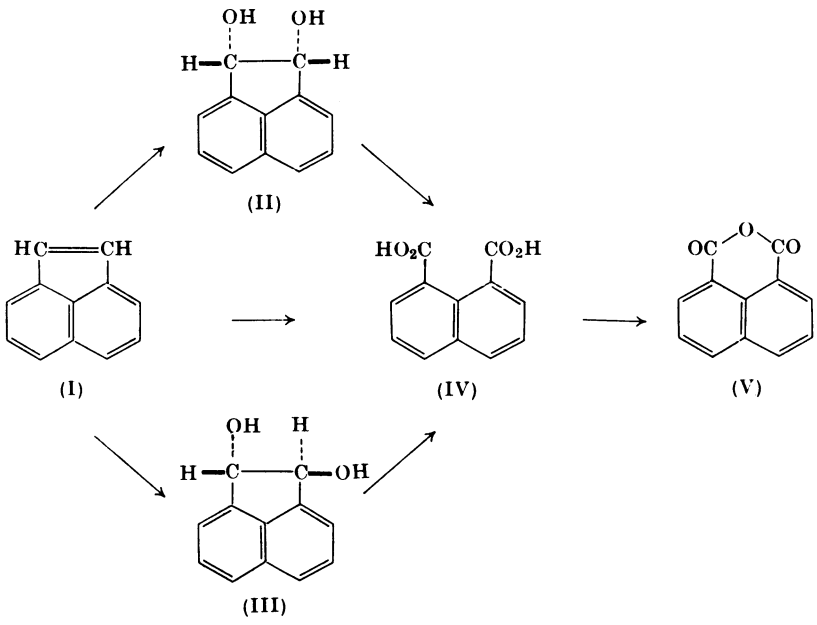
The possible effect of the rat-liver β-glucuronidase preparation on the interconversion of the free acenaphthenediols was tested in the following experiment. A solution of 16mg. of *cis*-acenaphthene-1,2-diol in 120ml. of water was prepared as well as another 120ml. of aqueous solution containing the same amount of the *trans*-isomer. The solutions were incubated at 37° for 24hr. with 0·2M-acetate buffer, pH 5·2, together with the liver preparation obtained as described by Hopkins *et al.* (1962). At the end of the incubation the suspensions were centrifuged, adjusted to pH 7–8 and extracted by mechanical shaking with four equal volumes of ether. The combined ether extracts from each solution were washed with 2N-sodium hydroxide to remove acids and, after being concentrated under reduced pressure, were examined by ascending paper chromatography with solvent mixture *B*. The results showed that, if interconversion of the free *cis*- and *trans*-diols occurred in the presence of the liver preparation, it took place to the extent of less than 0·5%. The experiments, however, do not exclude the possibility that interconversion of the diols resulted from hydrolysis of their conjugates (cf. Brooks & Young, 1956).

DISCUSSION

It has been suggested that dihydrodiols might serve as intermediates in the oxidative metabolism of cyclic hydrocarbons and that dicarboxylic acids might be among the products to which they give rise (Young, 1950; Hopkins & Young, 1961). Various hydrocarbons, e.g. naphthalene (Young, 1947; Booth & Boyland, 1949), anthracene (Boyland & Levi, 1935), phenanthrene (Young, 1947; Boyland & Wolf, 1950; Boyland & Sims, 1962) and indene (Brooks & Young, 1956), give rise to dihydrodiols in animals, but none of these hydrocarbons or the dihydrodiols has so far been reported to undergo ring-fission in the animal body. Benzene is known to be metabolized to muconic acid in animals (Jaffé, 1909; Bernhard & Gressly, 1941), and evidence has been obtained for its conversion *in vivo* into a dihydrodiol (Sato, Fukuyama, Suzuki & Yoshikawa, 1963). The dihydrodiol so formed, cyclohexa-1,3-diene-5,6-diol, has been synthesized (Nakajima, Tomida, Hashizume & Takei, 1956), but Tomida & Nakajima (1960) obtained no evidence for its conversion into muconic acid when they administered it to rats. Since Hopkins *et al.* (1962) showed that acenaphthylene (I) is converted in the rat into 1,8-naphthalic acid (IV) and into *cis*- and *trans*-acenaphthene-1,2-diol (II and III) and thereby undergoes ring-fission as well as forming dihydrodiols (see Scheme 1), it was decided to determine whether these dihydrodiols are metabolized in the rat to 1,8-naphthalic acid. Preliminary observations were made that indicated that this occurs (Hopkins & Young, 1961), and these have been confirmed and extended in the present work, in which *cis*- and *trans*-acenaphthene-1,2-diol were administered to rats by subcutaneous injection or by stomach tube and in every case 1,8-naphthalic acid was separated in the form of its anhydride (V) from the urine of the dosed animals.

The question arises whether the 1,8-naphthalic acid present in the urine was formed by a metabolic reaction within the tissues or whether it originated in some other way. It was shown that *cis*- and *trans*-acenaphthene-1,2-diol give 1,8-naphthalic acid when oxidized at room temperature by potassium permanganate in alkaline solution, but that in the absence of oxidant they are stable at the pH of rat urine. It was also shown that when *cis*- or *trans*-acenaphthene-1,2-diol was incubated at 37° for 24hr. in rat urine no 1,8-naphthalic anhydride was found when the urine was subjected to the treatment used to obtain this compound from the urine of rats that had been dosed with *cis*- or *trans*-acenaphthene-1,2-diol.

Although the formation of acids by the fission of diphenolic compounds by micro-organisms is well established (cf. Ornston & Stanier, 1964), the fission of dihydrodiols by micro-organisms does not appear to have been demonstrated. Nevertheless the possibility has to be considered that the conversion of the dihydrodiols into 1,8-naphthalic acid may occur as a result of microbial activity. The experiments just described, however, make it appear most unlikely that such a reaction occurred in the urine of the rats that had been dosed with *cis*- and *trans*-acenaphthene-1,2-diol. There remains



Scheme 1. Metabolic formation of 1,8-naphthalic acid.

the possibility that formation of 1,8-naphthalic acid was a result of the action of micro-organisms in the intestinal tract. Although the dihydrodiols were administered by subcutaneous injection this does not exclude the possibility that they were carried in the bile into the intestine and were there acted on by micro-organisms to give 1,8-naphthalic acid, which was absorbed and excreted in the urine.

Several groups of workers have demonstrated the occurrence in mammalian tissues of enzyme systems that bring about ring-fission. For example, Douglass, Shook, Weir & Hogan (1959) have shown the cleavage of gentisic acid by an enzyme system in rat liver, and Knox & Edwards (1955) have described an enzyme preparation from rat and guinea-pig liver that brings about ring-fission of homogentisic acid. The oxidation of *cis*- and *trans*-acenaphthene-1,2-diol to 1,8-naphthalic acid by rat tissues is at present under investigation in the authors' Laboratory, and microsomal preparations from liver have been shown to bring about this reaction. There is therefore little doubt that rat liver itself can bring about splitting of the five-membered ring of *cis*- and *trans*-acenaphthene-1,2-diol with the formation of 1,8-naphthalic acid.

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