Microsomal Ring-Fission of *cis*- and *trans*-Acenaphthene-1,2-diol

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1. The ring-fission of *cis*- and *trans*-acenaphthene-1,2-diol by rat liver microsomes was studied. 2. 1,8-Naphthalic acid was detected and isolated after microsomal incubations of the diols. 3. The accompanying reduction of NAD⁺ was followed spectrophotometrically. 4. The optimum pH for the microsomal reaction was 9.4 for the oxidation of the *cis*-diol and 9.8 for that of the *trans*-diol. 5. *p*-Chloromercuribenzoate and 2,4-dichlorophenol inhibited the reaction. 6. Possible mechanisms for the microsomal ring-fission, involving 1,8-naphthalic aldehyde, are discussed.

Though dihydrodiols are thought to participate as intermediates in ring-fission processes in bacteria, almost nothing is known of their role in processes of this type in animals. The ring-fission of benzene to muconic acid does not appear to involve the benzene dihydrodiol cyclohexa-1,3-diene-5,6-diol as intermediate, for Tomida & Nakajima (1960) showed that phenol and catechol, and not muconic acid, are excreted in the urine of rabbits injected with the diol. Further, Ayengar, Hayaishi, Nakajima & Tomida (1959) demonstrated with an enzyme preparation from rabbit liver that the diol is dehydrogenated, with the formation of catechol. Hopkins & Young (1966), however, showed that *cis*- and *trans*-acenaphthene-1,2-diol may well be intermediates in the metabolic ring-fission of acenaphthylene to 1,8-naphthalic acid, since they isolated the acid after injecting rats with the acenaphthenediols. Hopkins, Brooks & Young (1962) had isolated 1,8-naphthalic acid from the urine of rats dosed with acenaphthylene. The present paper is concerned with the conversion of *cis*- and *trans*-acenaphthene-1,2-diol into 1,8-naphthalic acid in the presence of rat liver microsomes, and lends support to the previous studies *in vivo* by Hopkins & Young (1966). A preliminary account of the work has already been published (Hopkins, 1966).

MATERIALS

All melting points are uncorrected. The melting point of naphthalic anhydride was determined by the use of an electrically heated brass block as a microscope stage; in this manner, the melting of only a few crystals of the material could be observed. Deionized water or double-glass-distilled water was used in enzyme experiments. The Ring Index

numbering of compounds (Patterson, Capell & Walker, 1960) is used throughout this paper. Elementary microanalyses were carried out by Weiler and Strauss, Oxford.

cis- and *trans*-Acenaphthene-1,2-diol, 1,8-naphthalic anhydride, disodium 1,8-naphthalate and acenaphthen-1-one were prepared by the methods described by Hopkins & Young (1966).

NAD⁺ and NADP⁺ were obtained as the tetrahydrate and sodium salt tetrahydrate respectively from the British Drug Houses Ltd. (Poole, Dorset). All other chemicals were also purchased from the British Drug Houses Ltd., except SKF 525A (β -diethylaminoethyl diphenyl-*n*-propyl-acetate hydrochloride), which was a gift from the Smith, Kline and French Research Institute (Welwyn Garden City, Herts.).

1,8-Naphthalic aldehyde hydrate was prepared by oxidation of *cis*- or *trans*-acenaphthene-1,2-diol with lead tetra-acetate, by the method of Criegee, Kraft & Rank (1933). *cis*-Diol (3.7 g.) and lead tetra-acetate (9.5 g.) were shaken in 100 ml. of dry benzene for 1 hr. at room temperature, after which the resulting precipitate of lead diacetate was removed by filtration. The filtrate was extracted twice with an equal volume of 2N-HCl. Needles were precipitated in the aqueous layers on standing. They were recrystallized twice from water to give the aldehyde hydrate in 39% yield as white needles, m.p. 133° (Found: C, 70.8; H, 4.8; Calc. for C₁₂H₈O₂.H₂O: C, 71.3; H, 5.0; H₂O, 8.9%). When heated to constant weight at 110°, 101.4 mg. of the compound lost 8.3 mg. (8.2%). The aldehyde gave the DNP-hydrazone as orange needles, m.p. 255° (decomp.). Criegee *et al.* (1933) reported m.p. 130° for 1,8-naphthalic aldehyde hydrate.

METHODS

Infrared spectroscopy. Samples were examined as Nujol mulls in a Perkin-Elmer model 237 infrared spectrophotometer.

Ultraviolet spectroscopy. The Hilger Uvispek spectrophotometer and the Unicam SP.800 spectrophotometer

were used. In experiments in which the reduction of NAD⁺ over a period of time was being measured, a cuvette carrier was used through which water at 37° from a constant-temperature bath was circulated. Cuvettes of 1 cm. light-path were used.

Preparation of subcellular fractions. Adult male rats of a black-hooded strain or a Wistar strain were used. In most experiments the procedures leading to the preparation of microsomes were as follows. Livers from two freshly killed animals were placed in 100 ml. of ice-cold 0.25 M-sucrose. All subsequent operations were performed at 4°. The suspension was homogenized, first in an Ato-Mix blender for 2–3 min. and then in a glass-Teflon homogenizer (A. H. Thomas Co., Philadelphia, Pa., U.S.A.). The homogenate was centrifuged in an MSE High-Speed 18 refrigerated centrifuge at 5000g for 10 min. (8 × 50 ml. rotor) to remove the nuclear fraction and cell debris. The supernatant fraction was then centrifuged at 15000g for 15 min. (8 × 50 ml. rotor). The resulting pellets were washed once by suspension in 0.25 M-sucrose and recentrifuged at 15000g for a further 15 min. The washings were combined with the 15000g supernatant fraction and centrifuged in an MSE Super-Speed 40 refrigerated ultracentrifuge at 100000g for 1 hr. (8 × 25 ml. rotor). The resulting microsomal pellets were washed by suspending them in 0.25 M-sucrose in the glass-Teflon homogenizer and resedimented by centrifuging them again at 100000g for 1 hr. Finally the microsomes were suspended for use in 0.25 M-sucrose to give a microsomal nitrogen concentration of 0.75–1.50 mg./ml.

In an experiment to compare the enzymic activity of various fractions of rat liver, the foregoing procedure was somewhat modified. The homogenate from one rat liver was made up to 60 ml. with 0.25 M-sucrose, and 50 ml. of this was subjected to differential centrifugation. The nuclear fraction was sedimented at 900g for 10 min. and the mitochondrial fraction at 7000g for 10 min. A lysosomal fraction was obtained from the mitochondrial supernatant suspension by centrifuging it at 20000g for 15 min. Microsomes were sedimented at 100000g. All fractions were washed by resuspension in 0.25 M-sucrose, and sedimented again at the appropriate *g* value, each washing being combined with the appropriate supernatant fraction. The fractions were finally suspended for use in 25 ml. of 0.25 M-sucrose.

Microsomes were examined by electron microscopy by Dr A. J. Palfrey, of the Department of Anatomy, St Thomas's Hospital Medical School, to whom the author is indebted, and were found not to be contaminated by lysosomes or mitochondria.

Incubations. Two types of incubation, *A* and *B*, were performed. In type *A*, *cis*- or *trans*-acenaphthene-1,2-diol (10 μmoles) dissolved in 30 ml. of 0.1 M-phosphate buffer (Na₂HPO₄-KH₂PO₄), pH 7.4, was gently shaken with 10 ml. of microsomal preparation at 37° in open flasks for 1.5 hr. When cofactors were used, 10 μmoles were incorporated as a solution in buffer in the microsomal preparation. Nicotinamide (300 μmoles) was also added with cofactors. In some experiments substances were investigated for any inhibitory action they might have on the microsomal ring-fission reaction. These substances were added to the incubation mixture in 10 μmole quantities as 1 ml. of a solution in water or 0.1 N-NaOH.

Incubations of the second type, *B*, were performed in a spectrophotometer cuvette at 37°. The cuvette contained

final concentrations of 0.2 mM-*cis*- or *trans*-acenaphthene-1,2-diol in 0.1 M-KH₂PO₄ adjusted to the required pH with N-NaOH, 0.2 mM-NAD⁺, 6 mM-nicotinamide and microsomes or other tissue suspension. The *E*₃₄₀ value of the mixture due to reduction of NAD⁺ was measured at suitable time-intervals against a complete reagent blank in which substrate (diol) was omitted. Disodium-1,8-naphthalate in 0.1 M-phosphate buffer did not absorb at 340 mμ.

Assay of 1,8-naphthalic acid. The method of Hopkins & Young (1966), in which 1,8-naphthalic acid in urine was detected and estimated as the anhydride, was modified in order to apply it to incubation mixtures. The microsomal reaction in an incubation of type *A* was stopped by bringing the pH to 11 with 2N-NaOH. The suspension was left at room temperature at this pH overnight, and was then adjusted to pH 6 with 2N-HCl to precipitate microsomal protein. The protein precipitate was removed by centrifugation and washed with water, the washing being added to the main supernatant solution. This was then brought to pH 2 with conc. HCl, and after 1 hr. was extracted four times with an equal volume of ether. The ether extracts were combined, dried over anhydrous Na₂SO₄ and then evaporated to dryness under reduced pressure. The residue obtained was heated at 110° for 1 hr. (to ensure complete conversion into the anhydride) before being dissolved in a known volume of butan-1-ol (analytical grade) to give a solution containing about 15 μg. of naphthalic anhydride/ml. Insoluble matter was removed by centrifuging, and the u.v. extinction of the solution was measured over the range 320–380 mμ against a complete reagent blank obtained from an incubation in which diol was omitted. The 1,8-naphthalic anhydride present could be determined by measuring the peak height at 339 mμ; diol had no extinction at 339 mμ. The efficiency of the assay was tested by incubating amounts of disodium 1,8-naphthalate equivalent to 100, 200, 300, 400 and 500 μg. of 1,8-naphthalic anhydride with NAD⁺, nicotinamide and rat liver microsomes. The subsequent extraction processes were carried out and recoveries of 108, 100, 93, 103 and 94% respectively were obtained. Acenaphthen-1-one was not detected in diol incubations. If it had been present, an ether extract of incubation mixtures after adjustment to pH 6 would have absorbed at 340 mμ: negligible extinction was found.

Isolation of 1,8-naphthalic anhydride. The isolation was performed with *cis*- or *trans*-acenaphthene-1,2-diol as substrate. The diol (0.15 m-mole) was incubated for 1.5 hr. at pH 7.4 with the microsomes from seven rat livers, in the presence of NAD⁺ (0.15 m-mole) and nicotinamide (4.5 m-moles), in a total volume of 600 ml. Thereafter the procedure followed that of the assay of 1,8-naphthalic acid, except that the ether extract was not evaporated to dryness. Instead, it was itself extracted twice with one-tenth of its volume of 2N-NaOH. The NaOH solutions were combined, brought to pH 2 with conc. HCl and left overnight at room temperature. White needles were precipitated. The suspension was extracted six times with an equal volume of ether, and the combined ether extracts were evaporated to dryness under reduced pressure. The residue was recrystallized from aqueous acetone to give pale-yellow needles of 1,8-naphthalic anhydride.

Paper chromatography. 1,8-Naphthalic anhydride was detected on paper chromatograms by the method of Hopkins *et al.* (1962). Two solvent systems were used,

namely methanol and methanol-carbon tetrachloride (9:1, v/v). Typical R_F values for naphthalic anhydride in these systems were 0.67 and 0.74 respectively.

Nitrogen determinations. The nitrogen content of subcellular fractions was determined by a micro-Kjeldahl method.

RESULTS

Microsomal formation of 1,8-naphthalic acid. The formation of 1,8-naphthalic acid from diol during incubations of type A occurred to the extent given in Table 1. The effect of cofactors on the microsomal oxidation of diol is also shown in Table 1. NAD^+ or NADP^+ increased considerably the conversion of *cis*-acenaphthene-1,2-diol into 1,8-naphthalic acid; the conversion of the *trans*-diol was increased

to a smaller extent. The activation caused by either cofactor was of the same order of magnitude. No naphthalic acid was detected after incubations in which microsomes inactivated by heating at 100° beforehand were used, either alone or in the presence of cofactors; similarly, no naphthalic acid was detected when microsomes were omitted from the incubations, whether cofactors were present or not.

The effect of substances tested as inhibitors of the microsomal diol oxidation is shown in Table 2. Only two of the substances investigated, namely *p*-chloromercuribenzoic acid and 2,4-dichlorophenol, inhibited the microsomal oxidation of the diols. Sodium azide and SKF 525A increased the oxidation.

Isolation of 1,8-naphthalic anhydride. The amount of 1,8-naphthalic anhydride isolated after incubation of 27.9 mg. (0.15 m-mole) of *cis*-acenaphthene-1,2-diol was 6 mg., representing a conversion of diol into naphthalic acid of 20.2%. The isolated material had m.p. 264° , not depressed on admixture with authentic 1,8-naphthalic anhydride, m.p. 271° . The u.v.-absorption spectrum of the isolated compound in butan-1-ol (analytical grade) showed maxima at 232, 328 and 339 $m\mu$ ($\log \epsilon$ 4.49, 4.09 and 4.09 respectively) and minima at 269 and 334 $m\mu$ ($\log \epsilon$ 3.07 and 4.05 respectively), and was not significantly different from that of synthetic 1,8-naphthalic anhydride. The i.r. spectrum of the compound and its behaviour on paper chromatograms corresponded to those of synthetic 1,8-naphthalic anhydride.

From the incubation of 27.9 mg. (0.15 m-mole) of *trans*-acenaphthene-1,2-diol, 2 mg. of 1,8-naphthalic anhydride was isolated, representing a conversion of diol into naphthalic acid of 6.7%.

Table 1. *Microsomal oxidation of cis- and trans-acenaphthene-1,2-diol to 1,8-naphthalic acid*

Experimental details are given in the text. The conversion shown is for the oxidation of diol into naphthalic acid. It is expressed both as a percentage and as the number of μ mole of diol converted/mg. of microsomal nitrogen/90 min. Results are given in some cases as the means $\pm$ s.d. for five experiments.

Substrate	Cofactor	Conversion	
		(%)	(μ mole/mg. of N/90 min.)
<i>cis</i> -Diol	None	6.8 $\pm$ 0.8	0.12 $\pm$ 0.01
	NAD^+	38.8 $\pm$ 5.5	0.54 $\pm$ 0.12
	NADP^+	32.2	0.56
<i>trans</i> -Diol	None	8.0 $\pm$ 1.3	0.13 $\pm$ 0.01
	NAD^+	17.6	0.20
	NADP^+	11.8	0.23

Table 2. *Effect of inhibitors on the microsomal oxidation of cis- and trans-acenaphthene-1,2-diol*

In these experiments, NAD^+ and nicotinamide were always included in the incubation mixture. The effect of the presence and absence of each inhibitor is compared. Conversion is expressed as μ mole of diol converted/mg. of microsomal nitrogen/90 min. The results are the mean values of two experiments. Other experimental details are given in the text.

Inhibitor (0.25 mm)	<i>cis</i> -Diol		<i>trans</i> -Diol	
	Conversion	Mean degree of inhibition (%)	Conversion	Mean degree of inhibition (%)
None	0.45		0.16	
<i>p</i> -Chloromercuribenzoic acid	0.09	80	0.03	81
None	0.62		0.15	
2,4-Dichlorophenol	0.14	77	0.05	67
None	0.43		0.16	
NaN_3	0.53	-23	0.19	-19
None	0.56		0.22	
SKF 525A	0.67	-20	0.30	-36

The m.p. of the isolated material was 263–265°, not depressed on admixture with authentic 1,8-naphthalic anhydride. The u.v.- and i.r.-absorption spectra and behaviour on paper chromatograms also corresponded to those of synthetic 1,8-naphthalic anhydride.

Reduction of NAD⁺ by microsomal diol ring-fission preparation. Incubations of type *B* enabled the reduction of NAD⁺ to be followed by observing the E_{340} value due to NADH. The reduction was most easily followed in the ring-fission of *cis*-acenaphthene-1,2-diol, since this was oxidized more readily than the *trans*-diol. A typical experiment, with 0.1M-phosphate buffer, pH 7.4, gave an initial rate corresponding to a reduction of 0.26 μ mole of NAD⁺/hr. The degree of reduction became approximately constant after 45 min., by which time 0.14 μ mole (24.1%) of the NAD⁺ present had been reduced. [The molar extinction coefficient of NADH is 6.22×10^3 mole⁻¹ cm.⁻¹ (Horecker & Kornberg, 1948).] No reduction of NAD⁺ was observed when microsomes were omitted from the incubation.

Observation of the reduction of NAD⁺ also permitted an investigation of the stability of the microsomal preparation to be made. After storage overnight at 4°, another portion of the same preparation as was used previously gave an initial rate of reduction not detectably different from that obtained before.

In addition, the inhibition caused by *p*-chloromercuribenzoic acid and 2,4-dichlorophenol, already shown by extraction and determination of 1,8-naphthalic acid, was also demonstrated in experiments involving the reduction of NAD⁺. The inhibitor was included in the incubation mixture at 0.2 mM final concentration. With *p*-chloromercuribenzoic acid, no extinction at 340 m μ could be detected, even after 30 min. 2,4-Dichlorophenol gave an initial rate of reduction of NAD⁺ of less

than one-third of that given by the same microsomal preparation when 2,4-dichlorophenol was absent. The degree of reduction became approximately constant after 25 min. in the presence of 2,4-dichlorophenol, after only 6.1% of the NAD⁺ present had been reduced, compared with a reduction of 30.4% in the absence of inhibitor.

Optimum pH of microsomal system. The optimum pH of the microsomal diol-oxidizing system was 9.4 for the oxidation of *cis*-acenaphthene-1,2-diol and 9.8 for the oxidation of the *trans*-diol. The variation in activity with pH was investigated by means of incubations of type *B*, with microsomes that had been stored overnight at 4° and with as buffer 0.1M-potassium dihydrogen phosphate adjusted to the required pH with N-sodium hydroxide. The initial rate of reduction of NAD⁺, expressed as change in E_{340} /min., was measured. A control experiment was performed by incubating the *cis*-diol, NAD⁺ and nicotinamide at pH 9.4 with microsomes inactivated by heating at 100° beforehand and employing subsequent procedures to extract and determine naphthalic acid as the anhydride. None was detected.

Subcellular distribution of diol-oxidizing activity. The reduction of NAD⁺ in the presence of *cis*-acenaphthene-1,2-diol was used to measure the diol-oxidizing activity of the following fractions of rat liver: nuclei, mitochondria, lysosomes, microsomes and cell sap (microsomal supernatant fraction). In addition, the activity of the whole homogenate was measured. These fractions were prepared as described in the Methods section. The cell sap was made up to 100 ml. with 0.25 M-sucrose. The initial rate of reduction of NAD⁺ was measured by incubations of type *B* at pH 9.4. Since the activity of some fractions was very low, the rates were determined by using the scale-expansion accessory of the Unicam SP.800 spectrophotometer in conjunction with an external recorder (Honeywell

Table 3. *Subcellular distribution of diol-oxidizing activity*

The initial rate of reduction of NAD⁺ by each fraction in the presence of *cis*-acenaphthene-1,2-diol was measured, and is expressed as change in E_{340} /min. The specific activity of each fraction is expressed as the change in E_{340} /min./mg. of nitrogen, and the total activity is the product of the specific activity and the total nitrogen content per fraction. For experimental details see the text.

Fraction	Initial rate of reduction of NAD ⁺	10 ³ × Specific activity	10 ³ × Total activity	% of combined total activity
Nuclei	0.010	7.27	262	9.3]
Mitochondria	0.008	7.43	200	7.1
Lysosomes	0.001	9.91	19	0.6
Microsomes	0.080	29.60	1999	70.6
Cell sap	0.004	3.56	350	12.4
Total			2830	100.0
Whole homogenate	0.047	8.07	2327	82.2

Brown Elektronik). As shown in Table 3, the highest diol-oxidizing activity, representing about 71% of the combined total activity of all the fractions, was found in the microsomal fraction of rat liver. This fraction also had the highest diol-oxidizing specific activity, with the lysosomal fraction next.

Lysosomes and mitochondria were also tested for diol-oxidizing activity after preincubation with Triton X-100, a detergent that enhances the activity of a number of enzymes in subcellular particles (Wattiaux & de Duve, 1956). No increased activity was found.

DISCUSSION

Hopkins & Young (1966) described the oxidative ring-fission of *cis*- and *trans*-acenaphthene-1,2-diol (I, R=H) by the rat with the formation of 1,8-naphthalic acid. Results similar to those of the work *in vivo* have now been obtained with an enzyme preparation from rat liver, and the diol-oxidizing activity has been shown to be localized in the microsomal fraction of the liver. These results support the suggestion of Young (1950) and Hopkins & Young (1961) that dihydrodiols may act as metabolic intermediates, for 1,8-naphthalic acid and *cis*- and *trans*-acenaphthene-1,2-diol are all products of the metabolism of acenaphthylene (Hopkins *et al.* 1962). Though ring-fission by bacterial preparations has frequently been demonstrated (see Hayaishi, 1962, and the review by Ribbons, 1965), few cases of such activity have been observed in mammalian systems.

Many investigations have shown that microsomal fractions are active in the oxidative metabolism of drugs. Molecular oxygen and reduced nicotinamide nucleotides generally seem to be required, and inhibition may be brought about by SKF 525A

(Brodie, Gillette & La Du, 1958; Conney & Burns, 1962; Hayaishi, 1962). In contrast, the diol ring-fission system described in the present paper was found to be enhanced by nicotinamide nucleotides in the oxidized form, and was not inhibited by SKF 525A. A requirement for molecular oxygen was not established, for a preliminary experiment conducted in an atmosphere of nitrogen gave no poorer conversion of diol into naphthalic acid than did the experiments in which flasks were open to the air. The amount of NAD⁺ added in all these experiments was the same, and was presumably in excess of that required for the extent of conversion that took place. Microsomal diol ring-fission activity therefore does not appear to fall into the category of typical drug-metabolizing reactions. Instead it may be a dehydrogenase type of activity. A diol dehydrogenase from rabbit liver having properties similar to those of the present microsomal system was described by Ayengar *et al.* (1959). Thus the diol dehydrogenase required NADP⁺, had a high pH optimum (9.0) and was inhibited by *p*-chloromercuribenzoate. The principal reaction that Ayengar *et al.* (1959) studied was the dehydrogenation of the dihydrodiol of benzene, namely cyclohexa-1,3-diene-5,6-diol, to catechol.

Though the stoichiometry of the reaction between the acenaphthene-1,2-diols and NAD⁺ has not been established, at least one of perhaps several steps in the oxidation of diol seems to involve the reduction of NAD⁺. Whether this is the diol ring-fission step is not known, and therefore no claim is made that results, such as the optimum pH of the microsomal diol-oxidizing system, that were obtained by measurement of NAD⁺ reduction, necessarily represent the optimum pH of a diol ring-fission enzyme. The matter is further complicated by a pronounced microsomal NADH-oxidizing activity found in the present work.

Though this activity could be inhibited to an extent of some 95% by 1mM-cyanide, no increase in the rate of NAD⁺ reduction was given on incubating diol with NAD⁺ and microsomes in the presence of cyanide. On the contrary, a slight diminution in the rate was observed. It may therefore be that the diol-induced microsomal reduction of NAD⁺ demonstrated in the present work outweighs the NADH-oxidizing activity, and that both are inhibited, albeit incompletely, by cyanide. Alternatively, the microsomal NADH-oxidizing activity may be inhibited in incubations with diol and NAD⁺. Preliminary experiments suggest, however, that only a small inhibition of microsomal NADH-oxidizing activity is given by diol or NAD⁺, and so the possibility remains that an intermediate in the conversion of diol into 1,8-naphthalic acid is responsible for blocking the oxidizing activity.

The route through which the acenaphthene-1,2-diols (I, R=H) pass to naphthalic acid is also a matter for some speculation. A simple dehydrogenation, involving the hydroxyl hydrogen atoms and accompanied by ring-fission, might give the compound 1,8-naphthalic aldehyde (II), which could act as an intermediate in the formation of 1,8-naphthalic acid. A compound having the properties of 1,8-naphthalic aldehyde hydrate was made in the present work, by the method of Criegee *et al.* (1933), and was incubated with microsomes and NAD⁺. 1,8-Naphthalic acid was subsequently isolated. Further, reduction of the NAD⁺ could be followed, though not in the absence of the microsomes. Though this work needs amplification, it does suggest that 1,8-naphthalic aldehyde may be an intermediate in the microsomal ring-fission of the acenaphthene-1,2-diols. Alternative modes of dehydrogenation could give instead structures (III) or (IV); these are structural isomers of 1,8-naphthalic aldehyde, and may indeed be less stable tautomeric forms of it. These compounds do not appear to have been described in the literature, and they may revert to the aldehyde. The hydroxyketone (III) might be formed from the acenaphthene diols by an alcohol dehydrogenase, whereas the 1,2-dihydroxy derivative (IV) might occur as the result of a diol dehydrogenase type of activity. The diol dehydrogenase described by Ayengar *et al.* (1959), which converts the benzenediol into catechol, would offer a precedent for the conversion of the acenaphthenediols into 1,2-dihydroxyacenaphthylene (IV). If the initial stage in the ring-fission of the acenaphthene-1,2-diols is a dehydrogenation, study of derivatives of the diols could be of help in discovering which hydrogen atoms are abstracted from the diol molecule. Thus the question whether

or not the non-hydroxy hydrogen atoms (I, R=H) are removed might be answered by incubation of a 1,2-disubstituted acenaphthene-1,2-diol, e.g. the 1,2-diphenyl compound (I, R=C₆H₅; Bachmann & Chu, 1936), with NAD⁺ and microsomes.

Inhibition of the acenaphthenediol ring-fission system is caused by *p*-chloromercuribenzoate, suggesting the presence of essential thiol groups in the enzyme system. 2,4-Dichlorophenol also inhibits the system. Some purification of the preparation is required before the properties of the enzyme system can be further investigated.

The significance of the present work appears to lie in the support offered for the proposed role of dihydrodiols as metabolic intermediates. Continued study of the enzyme patterns involved may lead to elucidation of the mechanisms of dihydrodiol metabolism.

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