Biochemical Studies of Toxic Agents

9. THE METABOLIC CONVERSION OF INDENE INTO *cis*- AND *trans*-INDANE-1:2-DIOL*

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The biological actions of indene have received little attention. Administration of the hydrocarbon to dogs with biliary fistulae was shown by Smith & Whipple (1930) to increase the flow of bile and also to produce marked toxic effects. The toxicity of indene was investigated by Cameron & Doniger (1939) in connexion with the presence of the compound in certain coal-tar-naphtha insecticides. They concluded that indene is not highly toxic. It was found, for example, that rats (180–200 g. body wt.) survived the subcutaneous injection of 0·5 g. of the compound. Rats developed fatty livers and died, however, after doses of 1·0 g. of indene. Indene was administered to rabbits by Böhm (1941), who found that a dose of 1·0 g. was tolerated when it was given by mouth to animals weighing 2–3 kg. Böhm reported that administration of the hydrocarbon was followed by an increased excretion of ethereal sulphate and glucuronide, an observation which suggests that indene undergoes hydroxylation in the animal body.

Studies of the metabolism of aromatic hydrocarbons (e.g. Boyland & Levi, 1935; Young, 1947; Boyland & Wolf, 1948; Booth & Boyland, 1949; Corner & Young, 1954, 1955) have shown that naphthalene, anthracene and phenanthrene give rise to dihydrodiols in addition to phenolic derivatives. These metabolites appear in the urine in the free state and in conjugated form. Indene is of interest because dihydrodiol formation might occur in the aromatic ring or at the double bond in the 5-membered ring. In the present work it has been shown that in the rabbit and the rat indene gives rise to indane-1:2-diol (1:2-dihydroxyindane) in which the aromatic ring is intact.

EXPERIMENTAL

Only male animals were used. Rabbits (2–3 kg. body wt.) were fed on cabbage together with rat cakes [J. Murray and Sons (London) Ltd.]. Rats were fed on rat cakes only. The animals had access to water at all times. They were housed in metabolism cages which permitted the collection of urine separate from faeces. Urine was collected daily and stored in the refrigerator.

Indene was purified by distillation and the fraction of b.p. 177–179° was used for dosing within 2 hr. of its collection. When rabbits received indene by stomach tube the dose was suspended in 10 ml. of water and washed down with a further 5–10 ml. of water. Undiluted indene was used for subcutaneous injection into rabbits and for administration to rats by stomach tube.

Reference compounds

Indene bromohydrin was prepared according to Suter & Milne (1940) and, when recrystallized from $CHCl_3$–light petroleum, formed needles, m.p. 129–130°. The direct conversion of indene bromohydrin into *trans*-indane-1:2-diol, as described by Porter & Suter (1935), gave, in our hands, only poor yields of diol. Satisfactory yields were obtained, however, from indene epoxide.

Indene epoxide was prepared by a modification of the procedure of Whitmore & Gebhart (1942) in which the ether-extraction and evaporation described by these authors were avoided in view of the ready volatility of the epoxide. Indene bromohydrin (25·9 g.), m.p. 125–127°, dissolved in a mixture of dioxan (60 ml.), ethanol (50 ml.) and water (15 ml.) was treated with 65 ml. of 1·91 N-KOH (1·02 mol. prop.) added during 25 min. with continuous shaking. The solution was allowed to stand for 5 min., and was then poured into ice water (500 ml.). The resultant oily precipitate crystallized on rubbing. The mixture was left overnight at 5°, and the solid was filtered off and washed with water. The product consisted of 14 g. of indene epoxide (87% yield), m.p. 31–32°. This substance readily sublimed under reduced pressure at a bath temp. of 50° to give prisms, m.p. 31–32°. It gave no precipitate with 2:4-dinitrophenylhydrazine in 96% ethanol containing 4% (v/v) H_2SO_4, under conditions suitable for the rapid formation of a precipitate from indan-2-one. The ultraviolet absorption curve of indene epoxide in 95% ethanol showed: $\lambda_{max.}$ 210, 259, 266, 272·5 and 274 mμ.; log ϵ, 3·78, 2·64, 2·69, 2·58 and 2·61 respectively.

Indene epoxide was converted into a mixture of *cis*- and *trans*-indane-1:2-diol by treatment with 1% (v/v) acetic acid on the steam bath (Loon, 1919). The *trans*-diol was separated from the mixture by virtue of its sparing solubility in $CHCl_3$, the $CHCl_3$ filtrate was evaporated to dryness *in vacuo*, and the *cis*-diol was obtained on recrystallization of the residue from *cyclo*hexane. In agreement with Loon, the principal product, obtained in 50% yield, was the *cis*-diol (m.p. 98–99°); the yield of *trans*-diol (m.p. 158–159°) was 13%. The yield of hydration products is probably lowered by the formation of indan-2-one. In an experiment in which indene epoxide was suspended in water and steam-distilled, indan-2-one was isolated from the distillate in 20% yield (as its 2:4-dinitrophenylhydrazone) (cf. Loon, 1919).

cis- and *trans*-Indane-1:2-diol were further characterized as their dibenzoates (Loon, 1919), prepared in the usual way with benzoyl chloride in pyridine at room temp. The

* Part 8: Corner & Young (1955).

cis-dibenzoate was separated by ether extraction, purified by recrystallization from ether and obtained as needles, m.p. 110–111°. The *trans*-dibenzoate could not be crystallized from the crude product, which was therefore chromatographed on alumina. The fraction eluted by light petroleum (b.p. 60–80°)–benzene (99:1) when recrystallized from methanol gave the dibenzoate, m.p. 77–78° (yield, 52%).

Indan-2-one was prepared from *trans*-indane-1:2-diol. The diol (580 mg.) dissolved in 2N-HCl (40 ml.) was heated on the steam bath under a reflux condenser for 1 hr. The reaction mixture, including the crystalline deposit in the condenser, was extracted with ether, giving a crude product from which indan-2-one (428 mg.), m.p. 54–56°, was isolated by chromatography on alumina. No other crystalline product was detected. Indan-2-one was also prepared from *cis*- and *trans*-indane-1:2-diol by refluxing in 7% (v/v) H_2SO_4 (Porter & Suter, 1935). The ketone, purified by steam distillation, had m.p. 58–60°: when kept at room temp. it decomposed with the formation of a yellow gum, but it was stable at 5° if stored in the dark. Indan-2-one was characterized as the oxime, which formed plates from $CHCl_3$–light petroleum (b.p. 40–60°), m.p. 151–152° (Porter & Suter, 1935), and as the 2:4-dinitrophenylhydrazone obtained from $CHCl_3$–ethanol as orange-yellow needles, m.p. 204–205° (decomp.) (Suter & Milne, 1940).

Paper-chromatographic procedure

The solvent mixtures and conditions used are given in Table 1.

Two methods of detecting 1:2-glycols on paper chromatograms were described by Buchanan, Dekker & Long (1950), and have been applied to the indane-1:2-diols. (1) The paper was dried and sprayed lightly with xylene, followed by lead tetraacetate in benzene (1%, w/v). It was advantageous to steam the paper at this stage to hydrolyse the reagent and reveal at once the white spots against a brown background. Although the reagent was not stable on storage, this disadvantage was overcome by using acetic acid as the solvent instead of benzene (Bush, 1955). However, the method was not very satisfactory for our purpose as 50 μg. of indane-1:2-diol was required to give a well-defined spot after development of the chromatogram.

Table 1. *Chromatographic separation of* cis- *and* trans-*indane*-1:2-*diol*

Whatman no. 1 paper and the descending method were used. Time of run: 16–18 hr. at room temp. Solvent mixtures (all proportions by vol.) were shaken vigorously for 1–2 min. and allowed to separate for 30 min. The upper layer was used as the mobile phase and the lower layer as the stationary phase. For methods of detection of spots see text.

Solvent system	R_F values	
	cis	*trans*
1. Benzene–water–ammonia (sp.gr. 0·88) (5:4:1)	0·28	0·05
2. Benzene–water–acetic acid (5:4:1)	0·36	0·06
3. Benzene–water–ethanol (5:4:1)	0·38	0·12
4. Benzene–water–ethyl acetate–acetic acid (4:4:1:1)	0·60	0·38
5. Water	0·70	0·69

(2) The dried chromatogram was sprayed with 2% (w/v) aqueous $NaIO_4$, left for 10–15 min. in the air, immersed in SO_2 vapour until the iodate and periodate were fully reduced to iodide, and finally sprayed with Schiff's reagent. Yellow spots slowly became visible against a background which changed from white to greyish-mauve. Even though colour development could be hastened by steaming the paper, it remained very slow, especially with the *trans*-diol. It was eventually found that excellent results were obtained by omitting the SO_2 treatment, when the yellow spots appeared as soon as the Schiff's reagent was applied. Under these conditions the final background colour was magenta.

In Table 1 are summarized R_F values for *cis*- and *trans*-indane-1:2-diol in five solvent systems.

Isolation of metabolites from the urine of rabbits dosed with indene by stomach tube

In one experiment with four rabbits the urine was collected daily for three successive 3-day periods. At the beginning of the second 3-day period the animals were dosed by stomach tube with indene (0·2 g./kg. body wt.) and a similar dose was given 1 day later. The total amount of indene administered was 3·7 g. The three 3-day batches of urine were examined separately by the following procedure.

Extraction of the urine at pH 7·5. The urine was filtered through glass wool and about 200 ml. of each 3-day batch of urine was reserved for treatment with acid (see below). The remainder of each batch (1–1·5 l.) was adjusted to pH 7·5 and extracted continuously with ether for 20 hr. The ether extract was washed with M-$NaHCO_3$ and with 2N-NaOH. Each washing was shaken twice with ether and the ethereal extracts were combined with the main extract and evaporated to dryness under reduced pressure. The urine extracts for days 1–3 and for days 7–9 yielded only about 20 mg. of oily residue, whereas that for days 4–6 yielded 280 mg. of partly crystalline material. This was chromatographed on 9 g. of alumina (Savory and Moore Ltd.) and yielded two well-separated crystalline fractions: (i) 22 mg. eluted by benzene–ether (9:1), and (ii) 87 mg. eluted by ether–methanol (95:5). Fraction (ii), when recrystallized from $CHCl_3$, gave prisms (46 mg.) of compound *A*, m.p. 157–159°, $[\alpha]_D$ +5° in ethanol (*c*, 1·02). Fraction (i) on recrystallization from ether–light petroleum (b.p. 40–60°) gave fine needles (10 mg.) of compound *B*, m.p. 109–111°, $[\alpha]_D$ +43° in $CHCl_3$ (*c*, 0·74).

Chromatographic separation, though convenient, was not essential, for compound *B* could be extracted from the crude mixture with boiling *cyclo*hexane or light petroleum, and compound *A* was obtainable by crystallization of the residue from $CHCl_3$. The characterization of compounds *A* and *B* is described below.

Extraction of the urine at pH 2. After the extraction at pH 7·5, each batch of urine was acidified with conc. HCl to pH 2 and a 500 ml. portion was extracted with ether for 20 hr. The ether extract was washed with M-$NaHCO_3$, N-NaOH and water. Each washing was shaken twice with an equal volume of ether, and these ether extracts were combined with the main ether extract and evaporated to dryness under reduced pressure. The extract from the urine of days 1–3 yielded only a trace of oil; that for days 4–6 gave 149 mg. of oily residue. Chromatography on alumina (6 g.) resulted in the separation of two sharply defined fractions of mainly crystalline character: (i) 13 mg.

eluted by benzene–ether (4:1) which on recrystallization from ether–*cyclo*hexane gave compound *B* (2 mg.), m.p. 105–107°, and (ii) 80 mg. eluted by ether–methanol (95:5) which on recrystallization from $CHCl_3$ gave compound *A* (28 mg.), m.p. 156–157°.

The ether-soluble acids in the $NaHCO_3$ extracts were examined by paper chromatography but no metabolites of indene were detected.

Isolation of metabolites from the urine of rabbits dosed subcutaneously with indene

Undiluted indene (1 g./kg. body wt.) was injected under the skin of the backs of three rabbits. After 2 days, the animals again received the same dose of the hydrocarbon. Urine was collected for 5 days after the first dosing. One rabbit died on the fifth day. The survivors eventually developed lesions at the sites of injection. The total amount of indene given was 15·3 g. The urine was extracted continuously with ether at pH 7·5 as in the experiments already described. The total crude residue from the combined extracts amounted to 440 mg., from which were separated 84 mg. of phenolic material and 265 mg. of neutral material. The latter was chromatographed on alumina (8 g.), affording compound *B* (139 mg. of crude fraction, yielding 96 mg. of product, m.p. 96–104°) and compound *A* (33 mg. of crude fraction, yielding 20 mg. of product, m.p. 155–158°).

Identification of metabolites isolated from the urine of rabbits dosed with indene

Various experiments were carried out in which the dose of indene administered by stomach tube ranged from 0·2 to 1·0 g./kg. body wt., and these led regularly to the isolation of compounds *A* and *B*. The identification of these metabolites was complicated by their occurrence as mixtures of optical isomers which tended to melt indefinitely. They were characterized as *trans*- and *cis*-indane-1:2-diol, however, by the following observations.

Compound A. Different samples of compound *A* had m.p.'s between 155° and 160°, and occasionally the melt remained cloudy until 175–180°: there was no depression in m.p. on admixture with (±)-*trans*-indane-1:2-diol of m.p. 158–159°. (Found: C, 71·6; H, 6·8. Calc. for $C_9H_{10}O_2$: C, 72·0; H, 6·7%.) $[\alpha]_D$ in ethanol varied between −7° and +5°. The compound reacted slowly with lead tetraacetate in acetic acid (1·15 mol. prop. of oxidant consumed in 20 hr.) and with periodic acid in aqueous ethanol (1:1) (0·95 mol. prop. of oxidant consumed in 25 hr.). Treatment of compound *A* (24 mg.) with 2N-HCl (5 ml.) on the steam bath for 40 min. yielded a crystalline product (17 mg., m.p. 58–60°) which was isolated by steam distillation and identified as indan-2-one by m.p. and mixed m.p. with an authentic sample (m.p. 58–60°). The derived 2:4-dinitrophenylhydrazone had m.p. 204–205° (decomp.) and gave no depression on admixture with an authentic specimen of the same m.p.

Compound B. The m.p. of compound *B* varied within the range 94–111°: the higher m.p.'s seemed to be associated with a higher degree of optical purity. Thus a sample of m.p. 94–96° had $[\alpha]_D$ +31° in $CHCl_3$ (*c*, 1·15), whereas one of m.p. 109–111° had $[\alpha]_D$ +43° in $CHCl_3$ (*c*, 0·74). (Found: C, 71·6; H, 6·5. Calc. for $C_9H_{10}O_2$: C, 72·0; H, 6·7%.) On admixture of various samples of compound *B* with (±)-*cis*-indane-1:2-diol of m.p. 98–99°, the m.p. was in no case depressed below that of the lower-melting component. Compound *B* reacted very rapidly with lead tetraacetate in acetic acid (1·03 mol. prop. consumed in 10 min.) and with periodic acid in aqueous ethanol (1:1) (0·95 mol. prop. consumed in 20 min.). Treatment of compound *B* (60 mg.) with benzoyl chloride (0·25 ml.) in pyridine (1 ml.) at room temp. overnight yielded an oily product which was purified by chromatography on alumina. A mixture of light petroleum (b.p. 60–80°) and benzene (90:10) eluted a crude crystalline fraction (95 mg., m.p. 89–92°) which was recrystallized from ether–light petroleum as prisms, m.p. 91–92°, $[\alpha]_D$ −32° in $CHCl_3$ (*c*, 1·26). (Found: C, 76·9; H, 5·2. Calc. for $C_{23}H_{18}O_4$: C, 77·1; H, 5·1%.) Treatment of compound *B* with 2N-HCl at 100° for 1 hr. resulted in the formation of indan-2-one, which was identified by comparison with an authentic sample.

In confirmation of these results, comparison of the ultraviolet absorption spectra of compounds *A* and *B*, and of the synthetic *trans*- and *cis*-indane-1:2-diol, measured in 95% (v/v) ethanol, showed them all to be identical over the range 210–300 mμ., with maxima at 211, 259, 265 and 271·5 mμ., log ε 3·94, 2·76, 2·92 and 2·94 respectively.

The rates of reaction of the diols with lead tetraacetate in acetic acid were also determined. The reactions were conducted at 21·5° with equimolar concentrations of diol and reagent, and the rate constants were derived by plotting the time of reaction against the inverse concentration of diol. The extremely rapid reaction of the *cis*-diol necessitated the use of low concentrations (0·00013M) of reactants: even so the time of half-reaction was only 15 sec. The course of the reaction was followed by titration with 0·002N-$Na_2S_2O_3$. Compound *B* and the synthetic (±)-*cis*-diol reacted at the same rate, $k_{21\cdot5}$ = 32 000 l./mole/min. Compound *A* and the synthetic (±)-*trans*-diol (0·013M) also reacted at the same rate, $k_{21\cdot5}$ = 0·52 l./mole/min. Criegee, Kraft & Rank (1933) recorded for k_{20} values of 27 800 and 0·467, respectively, for *cis*- and *trans*-indane-1:2-diol.

Products formed by acid treatment of the urine of rabbits dosed with indene

These experiments were carried out with the urine of rabbits dosed with indene by stomach tube (see above). The following treatment was applied to measured portions (approx. 200 ml. each) of (1) the urine of days 4–6, (2) the same urine after extraction with ether at pH 7·5 and at pH 2, as already described, and (3) the urine of days 7–9.

The urine was diluted with water (100 ml.) and steam-distilled, and 80–100 ml. of distillate (*a*) was collected. The urine was cooled and treated with sufficient 50% (v/v) H_2SO_4 to give a concentration of 7–8% (v/v) H_2SO_4, then refluxed gently for 1·5 hr. Any crystalline deposit forming in the condenser at this stage was dissolved in ether and combined with (*b*). The residual liquor was then steam-distilled and 150 ml. of distillate (*b*) collected. Each of the distillates was extracted four times with 20 ml. portions of ether, and the combined extracts were evaporated *in vacuo* at room temp., and finally at 0°, to 10–15 ml. The concentrate was decanted from ice, diluted with ethanol (10 ml.) and treated with a solution of 2:4-dinitrophenylhydrazine (12 ml., 20 mg./ml.) in 96% (v/v) ethanol containing 4% (v/v) H_2SO_4.

Precipitates were obtained only from the distillates (*b*) and only from the urine of days 4–6. These precipitates were identified as indan-2-one 2:4-dinitrophenylhydrazone by m.p. and mixed m.p. with an authentic specimen. The combined material was recrystallized from $CHCl_3$–ethanol and the product had m.p. 205–206° (decomp.) (Found: C, 57·7; H, 4·1. Calc. for $C_{15}H_{12}O_4N_4$: C, 57·7; H, 4·0.) Almost identical yields of phenylhydrazone were obtained from the ether-extracted and unextracted urine. The total amount isolated corresponded to approximately 25% of the dose of indene.

Isolation of metabolites from the urine of rats dosed with indene by stomach tube

Twenty-four rats (body weight 200–250 g.) were dosed with indene by stomach tube. Each rat received a single dose of 0·25 g. and urine was collected daily for 3 days after dosing.

The urine was extracted with ether (at pH 7·5) as described above. The combined extracts gave on evaporation a partly crystalline amber-coloured residue (630 mg.), which was extracted with 50 ml. of boiling light petroleum (b.p. 60–80°). The petrol-insoluble residue (380 mg.) was extracted three times with boiling *cyclo*hexane (50 ml. portions), and 210 mg. remained undissolved. The *cyclo*hexane extract, on cooling, deposited fluffy crystals, which when recrystallized from $CHCl_3$ gave 22 mg. of colourless prisms, m.p. 184–186°, $[\alpha]_D$ +31° in $CHCl_3$ (*c*, 0·75). The nature of this substance (compound *C*) is considered below. The petrol-soluble material, recrystallized from ether–light petroleum, gave compound *B* (32 mg.), m.p. 109–111° (alone and mixed with compound *B* from rabbit urine), $[\alpha]_D$ +44° in $CHCl_3$ (*c*, 1·01).

After removal of the crystalline products, the remaining material was recombined, and a neutral fraction (240 mg.) was separated and chromatographed on alumina (7 g.). This afforded a further 98 mg. of compound *B*, m.p. 108–111°. Although some solid was eluted at the point where compound *A* would have been expected to appear, no crystalline product could be isolated.

A portion of the extracted urine was treated with sulphuric acid as described above for the isolation of products formed by acid treatment of rabbit urine. Indan-2-one 2:4-dinitrophenylhydrazone was isolated in a yield representing 5% of the administered indene.

Nature of compound C *obtained from the urine of rats dosed with indene*

The substance of m.p. 184–186° showed no change in m.p. or appearance on recrystallization successively from $CHCl_3$ and acetone–light petroleum. Amounts of 20 μg. on paper could readily be detected by the periodate–Schiff's reagent method (see below). The resulting yellow spots closely resembled in character and in time of development those observed with (±)-*trans*-indane-1:2-diol. Paper chromatograms of compound *C* developed with solvent mixture 4 (Table 1) gave somewhat elongated spots with R_F value (0·34) slightly less than that (0·39) found for (±)-*trans*-indane-1:2-diol run simultaneously.

To test the homogeneity of compound *C* it was dissolved in benzene and chromatographed on alumina (1 g.). Elution with ether (350 ml.) gave 14 mg. of crystalline material, m.p. 184–186°; ether–methanol (9:1) eluted a further 5 mg. of product with the same m.p. These fractions, when separately recrystallized from $CHCl_3$, both yielded crystals of m.p. 185–186°. The two samples gave no depression in m.p. on admixture. The two fractions were combined, and a sample was taken for measurement of the rate of reaction with lead tetraacetate in acetic acid. From the time of half-reaction, the rate constant ($k_{21·5}$) was found to be 0·53 l./mole/min. The ultraviolet light absorption of compound *C* in 95% ethanol showed maxima at 211, 259, 265·5 and 272 mμ., log ε 3·92, 2·73, 2·92 and 2·95 respectively, and was not significantly different from that of (±)-*trans*-indane-1:2-diol over the range 210–300 mμ.

While the properties of compound *C* suggest that it may be (+)-*trans*-indane-1:2-diol, further evidence is required to confirm this.

Observations on the interconversion of cis- *and* trans-*indane*-1:2-*diol*

The interconversion of the diols was studied by isolating the products formed from each diol under various conditions, and by paper-chromatographic methods.

Effect of acid and base on the diols. *cis*- and *trans*-Indane-1:2-diol were each recovered in 90% yield after refluxing for 2 hr. in N-NaOH (in aqueous ethanol, 50%, v/v). Treatment of the *cis*-diol (200 mg.) with 0·02 N-H_2SO_4 on the steam bath for 45 min. gave a crude product (198 mg., m.p. 90–96°) from which *trans*-diol (15 mg., m.p. 153–156°) was isolated by recrystallization from $CHCl_3$. In a similar experiment, Hermans (1924) obtained a 7% yield of *trans*-diol.

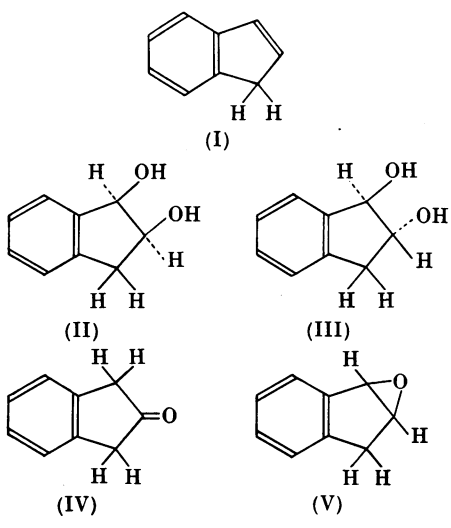
The interconversion of the diols was studied by heating *cis*- or *trans*-diol (200 mg.) on the steam bath in 20 ml. of 0·2 N-H_2SO_4 for 5 hr. The solution was then extracted continuously with ether for 2 hr. and the extract was evaporated to dryness *in vacuo* at room temp. The residue, which in each case exceeded 185 mg., was recrystallized from $CHCl_3$ to yield the *trans*-diol, and the mother liquor from this was evaporated to dryness to give crude *cis*-diol. This was purified by recrystallization from *cyclo*hexane. The identity of each of these products was established by m.p. and mixed m.p. Under these conditions the *cis*-diol gave rise to 57% *trans*- and 28% *cis*-diol, and the *trans*-diol afforded 59% *trans*- and 27% *cis*-diol. In the experiment starting with *cis*-diol, indan-2-one was also isolated in a yield of 2·5% as its 2:4-dinitrophenylhydrazone.

Stability of the diols in rabbit urine at different pH *values.* In these experiments a sample of normal urine obtained from a pair of rabbits, under the same conditions as in the metabolism experiments, was adjusted to the required pH with 2 N-HCl or 2 N-NaOH. Samples of the indane-1:2-diols (10 mg.) were dissolved in 20 ml. portions of the urine, and the solutions were left at room temperature in flasks open to the air, the pH being readjusted if

necessary. One day later the pH was brought to 7·5 and each solution was extracted continuously with ether for 3 hr. The extracts were evaporated to dryness *in vacuo*, the residues (8–10 mg.) were each dissolved in 1 ml. of acetone, and 0·10 ml. portions were applied to paper chromatograms. These were developed with solvent mixture 4 (Table 1). The spots obtained were compared with those found in parallel runs in which mixtures of the diols (95:5, w/w) were added to urine and treated in the same way. Experiments were performed with both *cis*- and *trans*-diol in urine at pH 5, 6·5 and 8–9. In no case was any isomerization detected.

DISCUSSION

Those aromatic hydrocarbons which are converted into dihydroaromatic diols in the animal body undergo hydroxylation at their more reactive bonds, and this suggested that in indene (I) the double bond in the 5-membered ring would be a site of biochemical oxidation. This has been shown to be so, for *cis*- and *trans*-indane-1:2-diol (II and III) were found in the urine of rabbits after administration of indene by stomach tube or by subcutaneous injection.



(I) (II) (III) (IV) (V)

The complete characterization of the isolated diols was rendered difficult by their occurrence as mixtures of optical isomers. The degree of optical homogeneity could not be estimated, since the two indane-1:2-diols do not appear to have been resolved into their enantiomorphic pairs. Contrary to the reports of Dox (1923) and Ingersoll (1944) no resolution of the (−)-menthylurethane of *trans*-indane-1:2-diol was recorded by Loon (1919). In the case of the *cis*-diol, the virtual identity of the specific rotations (43° and 44°) of the metabolites excreted by the rabbit and the rat suggests that these may approximate to the (+)-form. Apart from the question of their optical purity, the diols were identified as *cis*- and *trans*-indane-1:2-diol principally on the following evidence. (1) Both diols were converted by hot dilute acid into indan-2-one, characterized as its 2:4-dinitrophenylhydrazone. (2) The two diols, as well as authentic (±)-*trans*- and (±)-*cis*-indane-1:2-diol, all showed the same light-absorption, measured in 95% ethanol, over the range 210–300 mμ. (3) The isolated diols each consumed one molar equivalent of periodic acid, one reacting very slowly and the other very rapidly. Lead tetraacetate gave similar results, and the rate constants for the reactions in acetic acid were identical with those observed with (±)-*trans*- and (±)-*cis*-indane-1:2-diol under the same conditions.

From the small amounts of the diols isolated it appeared likely that other metabolites of indene were being excreted. The urine of the rabbits dosed by stomach tube contained material, not extracted by ether, which afforded indan-2-one (IV) when the urine was refluxed with H_2SO_4 (7%, v/v). The ketone was isolated in amounts representing about 25% of the indene administered. A simple explanation of this finding is that the ketone arises from conjugates of the diols with glucuronic acid or sulphuric acid. This view receives support from the increased excretion of 'glucuronide' which was observed with two pairs of rabbits after dosing them with indene (0·5 g./kg. body weight); the increments corresponded, on an equimolar basis, to 30 and 39% of the indene administered.

The formation of both *cis*- and *trans*-diols is noteworthy since the diols isolated as metabolites of aromatic hydrocarbons have been assigned the *trans*-configuration (Boyland, 1950; Cook, 1950). It appears unlikely that interconversion of *cis*- and *trans*-indane-1:2-diol occurs in the urine, for samples of each diol added to urine at pH 5, 6·5 and 8–9 underwent no detectable isomerization in 24 hr. The interconversion of these diols is normally achieved only at low pH (Hermans, 1924; Suter & Milne, 1940), and in the present work it was shown that both diols are converted by 0·2N-H_2SO_4 at 95–100° into an equilibrium mixture of *cis*- and *trans*-diol in the approximate ratio of 1:2. Although the direct isomerization of the diols in the urine appears unlikely, the possibility remains that the two diols isolated might have arisen from the breakdown in the urine of a derivative of one of them. Hydrolysis of a sulphate of one of the diols, for example, might be accompanied by some inversion of a hydroxyl group.

Boyland (1950) has drawn attention to the possible role of epoxides in the metabolic oxidation of aromatic hydrocarbons. Indene epoxide (V) undergoes cleavage by water to yield a mixture of

cis- and *trans*-diol, together with indan-2-one (Loon, 1919). So far, however, there is no experimental evidence to show that epoxide formation occurs in the animal body during the metabolism of indene.

SUMMARY

1. Indene has been administered to rabbits by stomach tube, and fractionation of ether extracts of the urine has yielded optically active *cis*- and *trans*-indane-1:2-diol in a total amount corresponding to about 5% of the dose. Acid treatment of the urine, either before or after ether extraction, yielded indan-2-one in amounts corresponding to about 25% of the dose of indene.

2. The same two diols have been isolated from the urine of rabbits dosed with indene by subcutaneous injection.

3. *cis*-Indane-1:2-diol has been isolated from ether extracts of the urine of rats dosed with indene by stomach tube. Indan-2-one has been obtained by acid treatment of the extracted urine.

4. The interconversion of *cis*- and *trans*-indane-1:2-diol has been studied, and the metabolic formation of the two diols is discussed.

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Studies of Sebum

6. THE DETERMINATION OF THE COMPONENT FATTY ACIDS OF HUMAN FOREARM SEBUM BY GAS–LIQUID CHROMATOGRAPHY*

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Relatively little information is available concerning the component fatty acids of human sebum. Engman & Kooyman (1934) made a brief study, using lead-salt separation and fractionation of the bromine derivatives of the liquid acids. They concluded that the free and combined fatty acid fractions of sebum contained both oleic and linoleic acids and that the combined acid fraction also contained arachidonic acid. They also demonstrated appreciable amounts of saturated products in the liquid acid fraction and concluded that branched-chain acids were possibly present. Ricketts, Squire & Topley (1951) examined the free fatty acids of human forearm sebum and were able to isolate pure barium oleate (identified by X-ray diffraction). They also reported a chromatographic examination of the saturated fatty acids performed for them by Dr G. A. Howard. This showed the

* Part 5: Wheatley (1954).