

The Metabolism of Tetralin

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1. [^{14}C]Tetralin was synthesized and fed to rabbits. 2. Of the radioactivity, 87–90% was excreted in the urine within two days and 0.5–3.7% on the third day. The faeces contained 0.6–1.8%. No radioactivity was found in the breath and negligible amounts were retained in the tissues. About 90–99% of an administered dose was accounted for. 3. The main metabolite in the urine was the glucuronide of α -tetralol (52.4%). Other conjugated metabolites were β -tetralol (25.3%), 4-hydroxy- α -tetralone (6.1%), *cis*-tetralin-1,2-diol (0.4%) and *trans*-tetralin-1,2-diol (0.6%). 4. β -Tetralone, α -naphthol, 1,2-dihydronaphthalene and naphthalene, previously reported as metabolites, are artifacts, and tetralin, α -tetralone, β -naphthol, 5-hydroxytetralin, and 6-hydroxytetralin are not metabolites. 5. The major metabolite of tetralin, α -tetralol and α -tetralone is the glucuronide of α -tetralol, which was isolated as *methyl (1,2,3,4-tetrahydro-1-naphthyl tri-O-acetyl- β -D-glucosid)uronate*; the major metabolite of β -tetralol and β -tetralone is the glucuronide of β -tetralol, which was characterized as *methyl (1,2,3,4-tetrahydro-2-naphthyl tri-O-acetyl- β -D-glucosid)uronate*. 5-Hydroxytetralin is conjugated with glucuronic acid, and was characterized as *methyl (5,6,7,8-tetrahydro-1-naphthyl tri-O-acetyl- β -D-glucosid)uronate*. 6-Hydroxytetralin is conjugated with glucuronic acid, and was characterized as *methyl (5,6,7,8-tetrahydro-2-naphthyl tri-O-acetyl- β -D-glucosid)uronate*. 6. A metabolic sequence accounting for the observed biological transformation products is proposed.

A considerable amount of reliable information is now available about the biological transformations of aromatic compounds, and, as a result of recent investigations (Elliott, Parke & Williams, 1959; Elliott, Tao & Williams, 1965*a,b*; Elliott, Robertson & Williams, 1966*a,b*) on the metabolism of cyclohexane, methylcyclohexane and decalin, their oxygenated derivatives (particularly the alcohols and ketones) and *tert*-butylcyclohexanone, the manner in which alicyclic compounds are metabolized by animals is now becoming clearer. No recent investigation, however, has been made of tetralin, which is particularly interesting because it contains within the same molecule an aromatic nucleus fused to an alicyclic one. Since the earlier studies by Schroeter & Thomas (1918), Pohl & Rawicz (1919) and Röckemann (1922) produced little real information about the metabolism of tetralin, some of the reported findings even being contradictory, the problem was reinvestigated. The present work gives a quantitative account of the metabolic transformation of tetralin, and shows that biological hydroxylation of the alicyclic ring occurs more readily than that of the aromatic ring and that the α -position in the alicyclic ring is much more readily oxidized than the β -position.

Further, it is shown that several compounds claimed as metabolites by previous workers are artifacts, but other compounds not previously shown to be metabolites of tetralin were isolated. On the basis of our findings a metabolic sequence is suggested.

MATERIALS

Tetralin. Tetralin (1,2,3,4-tetrahydronaphthalene) was from May and Baker Ltd., Dagenham, Essex, was twice redistilled and had b.p. 207.5° and n_D^{25} 1.5392, and the *o*-acrylbenzoic acid (Smith & Jones, 1948) had m.p. 154°. It gave a single peak with retention time 4.5 min. when chromatographed on a 10% diglycerol–Celite column. The i.r. spectrum corresponded to that of tetralin, hydroxyl and ketone absorption peaks being absent.

[^{14}C]Tetralin. 4[^{14}C]Phenylbutyric acid (m.p. and mixed m.p. 51°) was synthesized in 66% yield by Grignard carbonation of phenylpropylmagnesium bromide, by using $^{14}\text{CO}_2$ obtained from $\text{Ba}^{14}\text{CO}_3$ in an apparatus similar to that of Lemmon (see Calvin, Heidelberger, Reid, Tolbert & Yankwich, 1949). [1- ^{14}C] α -Tetralone was synthesized in 88% yield by ring closure of 4[1- ^{14}C]phenylbutyric acid (cf. Fieser & Hershberg, 1939). The acid (5g.) was kept for 16 hr. in 100 ml. of anhydrous HF. The product, [1- ^{14}C] α -tetralone, was checked by i.r. spectrum, and its DNP-hydrazone had m.p. and mixed m.p. 251°. [1- ^{14}C]Tetralin was prepared in 53% yield from [1- ^{14}C] α -tetralone (cf.

Martin, 1936). The ketone (3.36g.) in toluene (8ml.) and acetic acid (0.3ml.) was refluxed with 10g. of zinc amalgam and 25ml. of 10N-HCl for 24 hr., the product being separated from toluene by fractional distillation under reduced pressure. The overall yield, based on the $\text{Ba}^{14}\text{CO}_3$ used, was 31%, and the [^{14}C]tetralin obtained had b.p. 207° and n_D^{20} 1.5413, and was checked by i.r. spectrum and gas-liquid chromatography as described above.

Other compounds. 1,2-Dihydronaphthalene (Koch-Light Laboratories Ltd., Colnbrook, Bucks.), redistilled under reduced pressure, had n_D^{24} 1.5807; the dibromo derivative had m.p. 70°. Naphthalene (May and Baker Ltd.) had m.p. 80° and was recrystallized from ethanol. α -Tetralone (3,4-dihydro-2H-naphthalen-1-one) (Eastman Organic Chemicals, Rochester, N.Y., U.S.A.) was redistilled and had n_D^{26} 1.5668; its semicarbazone had m.p. 217°. β -Tetralone (3,4-dihydro-1H-naphthalen-2-one), synthesized by the method of Cornforth, Cornforth & Robinson (1942), had n_D^{26} 1.5550; its semicarbazone had m.p. 193°. (\pm)- α -Tetralol (1,2,3,4-tetrahydro-1-naphthol) was prepared by the catalytic hydrogenation of α -naphthol under 100 atm. for 9 hr. at 180° with copper chromite (Lazier & Arnold, 1943) as the catalyst. Phenols were removed with 10N-NaOH and ketones by chromatography on an alumina column, the ketonic material being eluted with light petroleum (b.p. 60–80°) and the α -tetralol finally recovered from the column with ethyl acetate. It had n_D^{22} 1.5634 and the i.r. spectrum showed hydroxyl but no ketone absorption peaks. Its phenylurethane had m.p. 121°. Its 3,5-dinitrobenzoate had m.p. 140° (Found: C, 59.8; H, 4.1; N, 8.6; $\text{C}_{17}\text{H}_{14}\text{O}_6\text{N}_2$ requires C, 59.6; H, 4.1; N, 8.2%). (\pm)- β -Tetralol (1,2,3,4-tetrahydro-2-naphthol), prepared by hydrogenation of β -naphthol (Dauben, McKusick & Mueller, 1948), had n_D^{25} 1.5630. The i.r. spectrum showed hydroxyl but no ketone absorption peaks. Its phenylurethane had m.p. 99°. Its 3,5-dinitrobenzoate had m.p. 155° (Found: C, 59.8; H, 4.3; N, 8.5%). 5-Hydroxytetralin (5,6,7,8-tetrahydro-1-naphthol) was a by-product of α -tetralol. Acidification of the alkali-soluble fraction yielded a precipitate that had m.p. 69° from light petroleum (b.p. 100–120°). Its 3,5-dinitrobenzoate had m.p. 156° (Found: C, 59.7; H, 4.3; N, 7.8%). 6-Hydroxytetralin (5,6,7,8-tetrahydro-2-naphthol) (Koch-Light Laboratories Ltd.) had m.p. 62° from light petroleum (b.p. 100–120°). Its 3,5-dinitrobenzoate had m.p. 162° (Found: C, 59.4; H, 4.3; N, 7.9%). (\pm)-*cis*-Tetralin-1,2-diol [(\pm)-*cis*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene], synthesized by the method of Strauss & Rohrbacher (1921), had m.p. 102°. (\pm)-*trans*-Tetralin-1,2-diol [(\pm)-*trans*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene] had m.p. 112°. *trans*-Tetralin-1,4-diol (*trans*-1,4-dihydroxy-1,2,3,4-tetrahydronaphthalene) (Argabright, Rider & Hanna, 1965) had m.p. 138°. 4-Hydroxy- α -tetralone (1,2,3,4-tetrahydro-4-oxo-1-naphthol) was prepared (cf. Boyland & Manson, 1951) by reducing 1,4-naphthoquinone with LiAlH_4 in dry ether in a Soxhlet apparatus. After decomposition of the complex and the excess of LiAlH_4 with 2N- H_2SO_4 , the ethereal layer was washed successively with water, 2N-NaOH and $\text{Na}_2\text{S}_2\text{O}_4$. The 4-hydroxy- α -tetralone in the uncrystallizable residue remaining after removal of the ether was isolated by twice subjecting the oil to a radial chromatographic separation (Elliott *et al.* 1966a) with silica gel H (E. Merck, Darmstadt, Germany). The resultant viscous liquid gave a single spot, R_F 0.45, on silica gel in

solvent system 3 (Table 2) when treated with Brady's reagent and phosphomolybdic acid. The i.r. spectrum showed hydroxyl and ketone absorption peaks. The *DNP*-hydrazone had m.p. 244° from ethanol (Found: C, 56.0; H, 4.3; N, 16.0; $\text{C}_{16}\text{H}_{14}\text{O}_5\text{N}_4$ requires C, 56.1; H, 4.1; N, 16.3%). α -Naphthol (British Drug Houses Ltd., Poole, Dorset), purified by distillation, had m.p. 94°; its 3,5-dinitrobenzoate had m.p. 217°. β -Naphthol (British Drug Houses Ltd.), similarly purified, had m.p. 123°; its 3,5-dinitrobenzoate had m.p. 210°. (+)-2-Hydroxytetralyl-mercapturic acid [(+)-*N*-acetyl-*S*-(2-hydroxy-1,2,3,4-tetrahydro-1-naphthyl)-L-cysteine], prepared by the method of Boyland & Sims (1960), had m.p. 187° and $[\alpha]_D^{25} +57^\circ$ in 0.1N-NaOH. Phthalic acid (Hopkin and Williams Ltd., Chadwell Heath, Essex) had m.p. 200° from hot water; its *p*-toluidide had m.p. 201°. The melting points, refractive indices and rotations of optically active compounds recorded agree with the values reported in the literature.

METHODS AND RESULTS

Animals and diet. Adult doe albino rabbits (1–2 kg.) were kept on a diet of 120g. of sweet potatoes (*Ipomoea reptans*) and 120g. of kangkong (*Ipomoea batatas*) daily. No additional water was given. The test compounds were administered by stomach tube followed by about 20 ml. of water.

Analytical methods. Glucuronic acid, ethereal sulphate and mercapturic acid were determined by the methods of Elliott *et al.* (1966a). The results are given in Table 1. Mercapturic acid was not found.

Infrared spectroscopy. Measurements were made in CHCl_3 with a JASCO Model I.R.S. spectrophotometer, (Japan Spectroscopic Manufacturing Co. Ltd., Hachioji City, Japan).

Thin-layer and paper chromatography. Plates were prepared as described by Elliott *et al.* (1966a,b) with silica gel H (E. Merck). Glucuronides were chromatographed in system 1 or system 2 (see Table 2) and located with naphtharesorcinol (Elliott *et al.* 1966a). Aglycones were chromatographed in solvent system 3, and were located with the phosphomolybdic acid reagent of Kritchevsky & Kirk

Table 1. *Glucuronic acid and ethereal sulphate excretion of tetralin and its derivatives*

Experimental details are given in the text. Three animals were used for each determination. The values given are averages for three experiments, with the ranges in parentheses.

	Dose (m-moles/ kg. body wt.)	Percentage of dose excreted as	
		Glucuronide	Ethereal sulphate
Tetralin	5.1	56 (54–61)	12 (10–14)
α -Tetralone	2.4	52 (39–65)	21 (11–36)
β -Tetralone	2.5	54 (50–60)	9 (7–12)
α -Tetralol	2.4	65 (63–67)	10 (8–13)
β -Tetralol	2.4	41 (39–44)	13 (8–19)
5-Hydroxytetralin	3.3	50 (46–55)	33 (31–36)
6-Hydroxytetralin	4.7	51 (48–55)	14 (13–16)

Table 2. *R_F* values of reference compounds and urinary glucuronides of tetralin and its derivatives

The solvent systems used were: system 1, butan-1-ol-acetic acid-water (2:1:1, by vol.); system 2, propan-1-ol-aq. NH₃ (sp.gr. 0.88) (7:3, v/v); system 3, hexane-ethyl acetate (1:1, v/v). The locating reagents used were: naphtharesorcinol (0.2% in acetone), for glucuronides; bromocresol green [0.04% in 60% (v/v) ethanol], for organic acids; ninhydrin (0.2% in acetone), for amino acids; Knight & Young (1958) spray, for mercapturic acids; diazotised *p*-nitraniline (0.2% in 0.1% HCl), for phenols; BaCl₂ (aq. 0.2% soln.) and sodium rhodizonate (aq. 0.6% soln.) for ethereal sulphates.

Compound	Reference compounds in system 3	<i>R_F</i> values			
		Glucuronides			
		Thin-layer chromatography		Paper chromatography	
		System 1	System 2	System 1	System 2
Tetralin	—	0.72, 0.82	0.50, 0.55	0.65, 0.79	0.64, 0.76
α-Tetralone	—	0.72, 0.82	0.50, 0.55	0.65, 0.79	0.64, 0.76
β-Tetralone	—	0.73, 0.81	0.52, 0.54	0.69, 0.78	0.65, 0.74
α-Tetralol	0.75	0.72, 0.82	0.50, 0.55	0.65, 0.79	0.64, 0.76
β-Tetralol	0.64	0.73, 0.81	0.52, 0.54	0.69, 0.78	0.65, 0.74
5-Hydroxytetralin	0.83	0.81	0.54	0.81	0.80
6-Hydroxytetralin	0.82	0.81	0.54	0.78	0.77
cis-Tetralin-1,2-diol	0.37	0.73	0.52	0.74	0.70
trans-Tetralin-1,2-diol	0.31	0.73	0.52	0.71	0.68
trans-Tetralin-1,4-diol	0.28	0.72	0.51	0.65	0.64
4-Hydroxy-α-tetralone	0.45	0.72	0.51	0.65	0.64
α-Naphthol	0.78	—	—	—	—

Table 3. *Distribution of radioactivity after a single dose of [1-¹⁴C]tetralin in rabbits*

The results are average values, with the ranges for four separate experiments in parentheses. Expired air was collected for 36–48 hr., and urine was collected until excretion of radioactivity had ceased (7–10 days).

	Radioactivity found (% of dose)
Dose (m-moles/kg. body wt.) ...	3.4 (1.6–4.8)
Dose (μC/animal) ...	76 (71–85)
Fraction	
Expired air	
[1- ¹⁴ C]Tetralin	< 0.2
[¹⁴ C]CO ₂	< 0.2
Tissues	0.07 (0.05–0.08)
Faeces	1.0 (0.6–1.8)
Urine	92 (88–98)
Total	94 (90–99)

(1952). The *R_F* values of the glucuronides of tetralin, tetralols, tetralones and the various reference materials are given in Table 2. Glucuronides were also chromatographed on Whatman no. 1 paper with solvent systems 1 and 2 and located with naphtharesorcinol. The *R_F* values are given in Table 2.

Radioautography. [1-¹⁴C]Tetralin (1.09 g.; 72.4 μC) was fed to a rabbit (1.66 kg.). The first fraction of urine collected, containing about 0.35 μC/ml., was placed on Whatman no. 1 paper and irrigated with solvent system 2. The dried chromatograms, after being placed in contact with Kodirex

No-Screen X-ray films for a fortnight, were sprayed for glucuronides, organic acids, amino acids, mercapturic acids, phenols and ethereal sulphates. Three spots were observed in solvent system 2, with *R_F* values 0.64, 0.70 and 0.76, giving a positive reaction with the glucuronide and organic acid sprays. No reactions were observed with the other sprays.

Measurement of radioactivity. Radioactivity was measured on solid samples of 'infinite' thickness, by using an end-window counter. Specific radioactivities were determined by comparison with a stable reference polymer (The Radiochemical Centre, Amersham, Bucks.), and sufficient counts were taken to give s.e.m. ± 2%.

Distribution of ¹⁴C in tissues and routes of excretion. [1-¹⁴C]Tetralin was administered by stomach tube in doses of 1.6–5 m-moles/kg., and the animals, after being dosed, were placed in a sealed Perspex metabolic chamber (Parke & Williams, 1950, 1953) for collection of urine, faeces and expired air separately in convenient fractions. The animals were kept in this chamber for 36–38 hr., and then removed and kept in open metabolic cages until urinary excretion of ¹⁴C had ceased. The rabbits were killed, and the tissues were homogenized in a Waring Blender and examined for radioactivity. As insignificant amounts were found (see Table 3), the radioactivities observed in the different tissues are not tabulated separately.

Excretion in the breath. The method of Elliott *et al.* (1959) was used. The ¹⁴CO₂ in each NaOH fraction was determined as Ba¹⁴CO₃, and the radioactivities of the ethanol fractions were estimated by counting liquid samples. The results are given in Table 3. As the recovery of radioactivity was practically quantitative it was not considered necessary to confirm the efficiency of the recovery of tetralin from the chamber.

Table 4. *Methods of determination of metabolites in urine*

Isotope diluents were added to 3–5 ml. of urine before enzyme or acid hydrolysis or simple equilibration. The diluent was recovered by (a) continuous extraction with ether, (b) steam-distillation, or (c) continuous extraction with chloroform, as described in the text. The specified derivatives were prepared as described by Smith & Jones (1948), by using proportionate quantities, and were recrystallized from the recommended solvents until constant in radioactivity.

Isotope diluent	Amount of diluent used (mg.)	Method of hydrolysis	Method of recovery	Derivative prepared
Tetralin	400	None	a	o-Aroylbenzoic acid
1,2-Dihydronaphthalene	390	None	b	Dibromo
Naphthalene	350	None	b	Picrate
α -Tetralone	300	None	b	Semicarbazone
β -Tetralone	300	None	b	Semicarbazone
α -Tetralol	500	Enzyme	b	3,5-Dinitrobenzoate
β -Tetralol	500	Acid	b	3,5-Dinitrobenzoate
5-Hydroxytetralin	300	Acid	b	3,5-Dinitrobenzoate
6-Hydroxytetralin	300	Acid	b	3,5-Dinitrobenzoate
cis-Tetralin-1,2-diol	100	Enzyme	a	None
trans-Tetralin-1,2-diol	100	Enzyme	a	None
trans-Tetralin-1,4-diol	100	Enzyme	a	None
1,2-Dihydroxy-1,2-dihydronaphthalene	200	Enzyme	a	None
4-Hydroxy- α -tetralone	120	Enzyme	a	DNP-hydrazone
α -Naphthol	300	Enzyme	a	3,5-Dinitrobenzoate
β -Naphthol	300	Acid	a	3,5-Dinitrobenzoate
2-Hydroxytetralylmercapturic acid	60	Acid	c	None
Phthalic acid	300	None	b	p-Toluidide

Table 5. *Metabolites of [1-¹⁴C]tetralin in the urine of rabbits after hydrolysis*

Experimental details are given in Table 4. The values given are averages for four experiments, with the ranges in parentheses. No unconjugated metabolites were found.

	Radioactivity found (% of dose)
Dose (m-mole/kg. body wt.) ...	3.4 (1.6–4.8)
Dose (μ C/animal) ...	76 (71–85)
Duration of experiment (days) ...	7–10
Metabolite sought	
α -Tetralol	53 (46–59)
β -Tetralol*	25 (21–31)
cis-Tetralin-1,2-diol	0.4 (0.4)
trans-Tetralin-1,2-diol	0.6 (0.5–0.7)
4-Hydroxy- α -tetralone	6 (6)
2-Hydroxytetralylmercapturic acid	0.05 (0.04–0.07)
Sum of radioactive metabolites sought	82 (80–83)
Total radioactivity in the urine	92 (88–98)

* After acid hydrolysis.

Excretion in the faeces. Faeces were combined and homogenized with water, and the radioactivity was determined by counting portions of the homogenate after they had been dried on planchets (Elliott *et al.* 1965b) (see Table 3).

Excretion in the urine. The total radioactivity in the

urine was determined by direct counting of liquid samples (Parke, 1956) (see Table 3).

Determination of metabolites in the urine. Fractions of urine containing significant amounts of radioactivity were combined, and portions were used for isotopic dilutions. Either simple equilibration or enzyme or acid hydrolysis was carried out (see below), and the equilibrated material was converted into a specified derivative, which was recrystallized from a suitable solvent until it was either of constant or of negligible radioactivity, and possessed the melting point given in the literature. The method adopted for the determination of each of the metabolites sought is detailed in Table 4, which also specifies the amount of diluent used, the method of hydrolysis and recovery adopted, and the derivative prepared. The metabolites found are listed in Table 5. Tetralin, 1,2-dihydronaphthalene, naphthalene, α -tetralone, β -tetralone, 5-hydroxytetralin, 6-hydroxytetralin, cis-tetralin-1,2-diol, trans-tetralin-1,2-diol, trans-tetralin-1,4-diol, (\pm)-1,2-dihydroxy-1,2-dihydronaphthalene, α -naphthol, β -naphthol and phthalic acid, which were also sought, were not found.

Simple equilibration. In these cases the urine (3–5 ml.), containing approx. 1 μ C, after addition of the specified amount of carrier, was adjusted to H7 and allowed to stand for 2 hr. before being extracted with ether or steam-distilled.

Enzyme hydrolysis. Preliminary experiments having shown that 98–99% of the radioactivity in urine hydrolysed with Ketodase (Warner-Chilcott Laboratories, Morris Plains, N.J., U.S.A.) could be removed by continuous extraction with ether, and chromatographic experiments having confirmed that the hydrolysis was quantitative,

Table 6. *Melting points and optical rotations of triacetyl methyl esters of glucuronides*

Compound fed	Parameters of triacetyl methyl esters of glucuronides	
	m.p.	$[\alpha]_D^{20-24}$ in CHCl_3
Tetralin	171–172°	–23°
α -Tetralone	171–172	–24
β -Tetralone	157.5–158	–13
α -Tetralol	171–172	–22
β -Tetralol	157.5–158	–13
5-Hydroxytetralin	140–141	–47
6-Hydroxytetralin	129	–38

the following standardized procedure was adopted for enzyme hydrolysis. Ketodase (10ml., containing 50000 Fishman units of β -glucuronidase) was added to portions of urine (3–5ml.) containing approx. $1\mu\text{C}$; the pH was readjusted to 5 if necessary, and the mixture was incubated for 24hr. at 37°. Carrier material (0.3–0.5g.) was then added and the mixture was allowed to stand for 2hr. to reach equilibrium. The hydrolysate was either extracted continuously with ether, or steam-distilled, the distillate then being continuously extracted with ether. After being washed with water the extract was dried with MgSO_4 , and after removal of the ether the residue was used for the preparation of the derivative specified in Table 4.

Acid hydrolysis. This was performed by adding carrier material (0.3–0.5g.) to 3–5ml. of urine mixed with an equal volume of 3N-HCl, and refluxing for 2hr. After being cooled and neutralized with NaHCO_3 , the mixture was either extracted continuously with ether or steam-distilled, the distillate being extracted with ether, and dried, as described above. After removal of the ether the appropriate derivative was prepared as described in Table 4.

Isolation of metabolites. Glucuronides were separated from the 24hr. urine by the basic lead acetate procedure of Kamil, Smith & Williams (1951), and, since the isolated gums could not be crystallized, triacetyl methyl esters were prepared. The R_F values of the glucuronides and the melting points and optical rotations of the triacetyl methyl esters are given in Table 6.

Characterization of aglycones. Each glucuronide was hydrolysed to establish the identity of its aglycone. Except in the cases of tetralin, α -tetralone and α -tetralol, the metabolites of which were decomposed by acid, and for which therefore the modified procedures described below were adopted, hydrolysis was effected with acid. Normally the glucuronide gums without further purification were refluxed with 30ml. of N-HCl on a glycerol bath at 130° for 1hr. The hydrolysate was then steam-distilled and the distillate was extracted with ether. Alcohols and phenols were esterified with 3,5-dinitrobenzoyl chloride in pyridine, and the resultant ester after treatment with Na_2CO_3 and HCl in water was recrystallized from ethanol or light petroleum (b.p. 100–110°). Ketones were characterized as DNP-hydrazones, by warming them with 3% DNP-hydrazine, washing the precipitates with acid and recrystallizing them from ethanol. The metabolite of β -tetralone was characterized as the known phenylurethane.

The separation and characterization of the metabolites

of each compound fed are described in the succeeding sections.

α -Tetralone

The ketone (6ml.) fed to six rabbits yielded 5g. of a glucuronide gum and 4.2g. of crude triacetyl methyl esters. Recrystallization from ethanol gave 0.01g. of pure methyl (1,2,3,4-tetrahydro-1-naphthyl tri-O-acetyl- β -D-glucosid)uronate, m.p. 171–172°, $[\alpha]_D^{23}$ –24° (c 1 in CHCl_3) (Found: C, 59.1; H, 5.8; $\text{C}_{23}\text{H}_{28}\text{O}_{10}$ requires C, 59.5; H, 6.1%).

Hydrolysis of the glucuronide gum. Heating with acid decomposed the aglycones to 1,2-dihydronaphthalene and naphthalene. Decomposition could be decreased by using lower temperatures and minimizing the time of contact with acid. Accordingly, the evaporated urine of six rabbits that had each been given 1ml. of tetralone was adjusted to pH 2 with 10N-HCl and continuously extracted with ether for 24hr. An oil (0.48g.) was obtained by steam-distillation of the residue remaining after evaporation of the ether, with the i.r. spectrum and R_F value (0.75 in solvent system 3) of α -tetralol. The 3,5-dinitrobenzoate prepared from the oil had m.p. and mixed m.p. 140° with α -tetralol 3,5-dinitrobenzoate. The liquid remaining after steam-distillation contained three substances; their R_F values were 0.31, 0.37 and 0.45 in solvent system 3, which are the R_F values of *trans*- and *cis*-tetralin-1,2-diol and 4-hydroxy- α -tetralone respectively. This mixture was then extracted with ether, and, after removal of the ether, the residue was separated by preparative chromatography. The band with R_F 0.45 was removed and extracted with ether. An oil (45mg.) was obtained that yielded a DNP-hydrazone with m.p. and mixed m.p. 244° with 4-hydroxy- α -tetralone DNP-hydrazone. In a blank experiment in which all conditions were identical except that α -tetralol replaced the glucuronide, only α -tetralol was recovered.

The same aglycones were shown to be present in the hydrolysate obtained by incubating the glucuronide gum (2g.) with Ketodase (10ml.), and the α -tetralol had $[\alpha]_D^{23}$ +7.0° (c 1 in CHCl_3) (lit. $[\alpha]_D$ +28.2°).

β -Tetralone

The ketone (4ml.) fed to four rabbits yielded 7g. of glucuronide gum and 6.5g. of crude triacetyl methyl esters. Recrystallization from ethanol gave 0.2g. of pure methyl (1,2,3,4-tetrahydro-2-naphthyl tri-O-acetyl- β -D-glucosid)uronate, m.p. 157.5–158°, $[\alpha]_D^{23}$ –13° (c 1 in CHCl_3) (Found: C, 59.5; H, 6.3%).

Hydrolysis of the glucuronide gum. β -Tetralone (0.5g.) fed to each of four rabbits yielded, after acid hydrolysis of the urine, an oil (0.9g.) whose phenylurethane had m.p. 117° and $[\alpha]_D^{25}$ +24° (c 2 in CHCl_3). Pickard & Kenyon (1912) reported for 1-ac-tetrahydro-2-naphthyl phenylcarbamate m.p. 119° and $[\alpha]_D$ –26.31° (c 5 in CHCl_3). A portion of the gum incubated with Ketodase yielded an oil, which contained three aglycones with R_F values 0.64, 0.37 and 0.31 (system 3) corresponding in position to β -tetralol, *cis*- and *trans*-tetralin-1,2-diol respectively. 4-Hydroxy- α -tetralone was not found.

α -Tetralol

The alcohol (6ml.) fed to six rabbits yielded 4g. of a glucuronide gum, and 4.2g. of crude triacetyl methyl esters.

Recrystallization from ethanol gave 0.25 g. of pure methyl (1,2,3,4-tetrahydro-1-naphthyl tri-*O*-acetyl- β -D-glucosid)uronate, $[\alpha]_D^{20} -22^\circ$ (c 2 in CHCl_3), m.p. and mixed m.p. 171–172° with the triacetyl methyl ester of α -tetralyl glucuronide obtained by feeding α -tetralone to rabbits.

Hydrolysis of the glucuronide gum. The glucuronide gum (3.5 g.) dissolved in 100 ml. of water adjusted to pH 2 and continuously extracted with ether for 24 hr. yielded an oil (0.5 g.), which was shown by thin-layer chromatography to contain four compounds with R_F values 0.75, 0.45, 0.37 and 0.31, corresponding to α -tetralol, 4-hydroxy- α -tetralone and *cis*- and *trans*-tetralin-1,2-diol respectively. Steam-distillation and continuous extraction of the oil with ether yielded 0.45 g. of α -tetralol, characterized by i.r. spectrum and R_F value 0.75 in solvent system 3. The 3,5-dinitrobenzoate had m.p. and mixed m.p. 140°. The liquid remaining in the flask after steam-distillation yielded a residue (0.07 g.) when further extracted with ether. By preparative thin-layer chromatography this yielded, from the band with R_F 0.45 in system 3, 4-hydroxy- α -tetralone; the DNP-hydrazone had m.p. and mixed m.p. 244°.

β -Tetralol

The alcohol (4 ml.) fed to four rabbits yielded a glucuronide gum (6.2 g.) and 5 g. of crude triacetyl methyl esters. Recrystallization from ethanol gave 0.20 g. of pure methyl (1,2,3,4-tetrahydro-2-naphthyl tri-*O*-acetyl- β -D-glucosid)uronate, $[\alpha]_D^{20} -13^\circ$ (c 1 in CHCl_3), m.p. and mixed m.p. with the triacetyl methyl ester of β -tetralyl glucuronide 157.5–158°.

Hydrolysis of the glucuronide. The gum obtained from feeding 6 ml. of β -tetralol to six rabbits was acid-hydrolysed and yielded 1.0 ml. of an oil, which had an i.r. spectrum identical with that of β -tetralol. Its 3,5-dinitrobenzoate had m.p. and mixed m.p. 155°. A portion of the gum that had been enzyme-hydrolysed was shown by thin-layer chromatography in system 3 to contain substances with R_F values 0.64, 0.37 and 0.31, corresponding to β -tetralol, *cis*- and *trans*-tetralin-1,2-diol respectively.

5-Hydroxytetralin

The phenol (3 g.) fed to three rabbits yielded 3.5 g. of the glucuronide gum and 3.2 g. of the crude triacetyl methyl esters. Recrystallization from ethanol gave 40 mg. of pure methyl (5,6,7,8-tetrahydro-1-naphthyl tri-*O*-acetyl- β -D-glucosid)uronate, m.p. 140–141°, $[\alpha]_D^{24} -47^\circ$ (c 1 in CHCl_3) (Found: C, 59.3; H, 6.2%). Acid hydrolysis of the glucuronide gum (2 g.) yielded 20 mg. of a phenol, m.p. 70°, R_F 0.83 in system 3, with an i.r. spectrum identical with that of 5-hydroxytetralin.

6-Hydroxytetralin

The phenol (3 g.) fed to three rabbits yielded 4.0 g. of a glucuronide gum and 4.2 g. of the crude triacetyl methyl ester. Recrystallization of the ester from ethanol gave 30 mg. of pure methyl (5,6,7,8-tetrahydro-2-naphthyl tri-*O*- β -D-glucosid)uronate, m.p. 129°, $[\alpha]_D^{22} -38^\circ$ (c 1 in CHCl_3) (Found: C, 59.2; H, 6.2%). Acid hydrolysis of the gum from feeding 3 g. of the phenol to two rabbits yielded 60 mg. of 6-hydroxytetralin, m.p. 62°, R_F 0.82 in system 3, and i.r. spectrum identical with that of 6-hydroxytetralin.

cis- and *trans*-Tetralin-1,2-diol, *trans*-tetralin-1,4-diol and 4-hydroxy- α -tetralone

Thin-layer chromatography of the urine of animals given each of these compounds showed that conjugation with glucuronic acid had occurred and that only one metabolite had been produced in each case (Table 2). Each compound (100 mg.) was administered to a rabbit. The isolated glucuronide gum was dissolved in acetate buffer (pH 5), incubated with Ketodase (2 ml.) for 24 hr. at 37° and continuously extracted with ether. Each aglycone was then separated by thin-layer chromatography. The R_F values of the hydrolysis products were the same as those of the parent compounds (Table 2).

Tetralin

Tetralin (6 g.) fed to six rabbits yielded a glucuronide gum (10 g.) and 4.6 g. of crude triacetyl methyl esters. Recrystallization from ethanol gave 68 mg. of a pure ester, $[\alpha]_D^{20} -23^\circ$ (c 1 in CHCl_3), m.p. and mixed m.p. with methyl (1,2,3,4-tetrahydro-1-naphthyl tri-*O*-acetyl- β -D-glucosid)uronate 171–172°.

Hydrolysis experiments. 1,2-Dihydronaphthalene was the only product obtained when the above triacetyl methyl ester was refluxed with acid. The urine from 12 rabbits each given tetralin (1 ml.) was concentrated in a rotary evaporator *in vacuo*. Inorganic matter was removed with methanol and, after removal of the methanol, the dried extract was separated by preparative thin-layer chromatography. Two spots reacting with naphtharesorcinol were observed, with R_F values 0.72 and 0.82 in system 1. The band having R_F 0.72 was removed and extracted with water. Incubation of the extract with Ketodase at 37° for 48 hr. yielded a material with R_F 0.45 in system 3 and i.r. spectrum identical with that of 4-hydroxy- α -tetralone. The band having R_F 0.82, when hydrolysed with Ketodase, gave four spots with R_F values 0.75, 0.64, 0.37 and 0.31 in system 3, reacting with phosphomolybdic acid but not with Brady's reagent, and corresponding in position to α -tetralol, β -tetralol and *cis*- and *trans*-tetralin-1,2-diol respectively.

One-third of the dried extract was dissolved in water (50 ml.) and adjusted with HCl to pH 4. After continuous extraction with ether for 24 hr. and removal of the ether, the oily residue (0.78 g.) was steam-distilled to give 0.7 g. of an oil having the i.r. spectrum of α -tetralol and yielding a 3,5-dinitrobenzoate with m.p. and mixed m.p. 140° with α -tetralyl 3,5-dinitrobenzoate. The urine remaining after the preliminary ether extraction yielded, after it had been refluxed with *N*-HCl for 3 hr. on an oil bath at 130° and the hydrolysate steam-distilled, an oil (0.5 g.) with the i.r. spectrum of β -tetralol, which yielded a 3,5-dinitrobenzoate with m.p. 155°, not depressed by mixture with β -tetralyl 3,5-dinitrobenzoate.

The remaining two-thirds of the extract was dissolved in water (200 ml.) adjusted to pH 2, which effected partial hydrolysis of all the glucuronides, and was then continuously extracted with ether for 48 hr. The ether was removed and α - and β -tetralol were recovered by steam-distillation. The liquid remaining in the distilling flask was extracted with ether. The residue after removal of the ether was separated by preparative thin-layer chromatography in solvent system 3. The band having R_F 0.45 when extracted

with ether yielded an oily substance (10mg.) with the i.r. spectrum of 4-hydroxy- α -tetralone. Its DNP-hydrazone had m.p. and mixed m.p. 244°.

DISCUSSION

The results show that in rabbits tetralin is oxidized preferentially in the alicyclic ring and that its metabolites in urine are conjugates of α -tetralol, β -tetralol, 4-hydroxy- α -tetralone, and *cis*- and *trans*-tetralin-1,2-diol. Since the site of hydroxylation always involves a saturated carbon atom the only possible mode of attack must be by substitution. A free-radical attack is excluded because β -tetralol was isolated, and Robertson & Waters (1946, 1948) have shown in their studies of the autoxidation of tetralin that only the α -methylene group is substituted. It is also unlikely that 1,2-epoxytetralin is an intermediate because Boyland & Sims (1960) have shown that 1,2-epoxytetralin produces mercapturic acids and *trans*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene. In the present experiments only small amounts of the *trans*-1,2-diols were isolated, the α - and β -tetralols predominating. Further, only traces of mercapturic acids were found, and these did not appear to arise from the tetralin fed.

In steroid metabolism there is evidence that specific hydroxylases are involved in hydroxylation of alicyclic rings through the direct and stereo-specific replacement of a ring hydrogen atom by an OH⁺ ion (Hayano, 1962), NADPH being the reducing agent. Elliott *et al.* (1966b) proposed a somewhat similar mechanism for the hydroxylation of the decalins, and it is now proposed that the same or closely related enzymes are concerned in the hydroxylation of tetralin.

Since tetralin is transformed in the rabbit into a number of metabolites, the problem is to decide their sequence. Consideration of the amounts of each metabolite produced offers little guidance. It is necessary to study the metabolism of each possible intermediate and all of the substances isolated in order to determine how they are related to each other.

It appears that α - and β -tetralone are first reduced *in vivo* to the corresponding alcohols. Tao & Elliott (1962) have shown that methyleyclohexanone is reduced, stereospecifically, by NADH and liver alcohol dehydrogenase. It would be reasonable to assume that NADH and a suitable enzyme are also involved in the metabolic transformation of α - and β -tetralone, and if this is so the model system developed by Elliott *et al.* (1965a) and Cheo, Elliott & Tao (1967) to account for the reductions of the alicyclic ketones they have studied might well serve to account for the reductions observed in the present work.

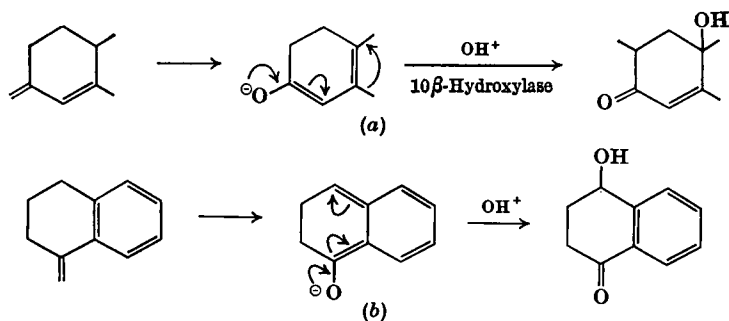
It also seems reasonable to assume that *cis*- and *trans*-tetralin-1,2-diol and 4-hydroxy- α -tetralone are terminal products because, when they are fed, the only substances found in the urine are their conjugates. Similarly, α - and β -tetralol appear to be intermediates despite the large amounts found, for, when either of these two substances is fed, one or other of the proposed terminal products is detected in the urine.

Since the diols are produced when α -tetralone, β -tetralone, α -tetralol or β -tetralol is fed, it is probable that a second hydroxylation occurs. Such a reaction has been demonstrated by Samuelsson (1959), who fed labelled chenodeoxycholic acid to rats and pigs and obtained both 6 α - and 6 β -hyocholic acid. Although a *trans*-diequatorial addition is the expected chemical reaction, *cis*-equatorial-axial additions have been observed *in vivo*. For example, Brooks & Young (1956) have shown that indene gives rise to both *cis*- and *trans*-indane-1,2-diol, and Hopkins, Brooks & Young (1962) have shown that acenaphthylene is converted into both *cis*- and *trans*-acenaphthene-1,2-diol.

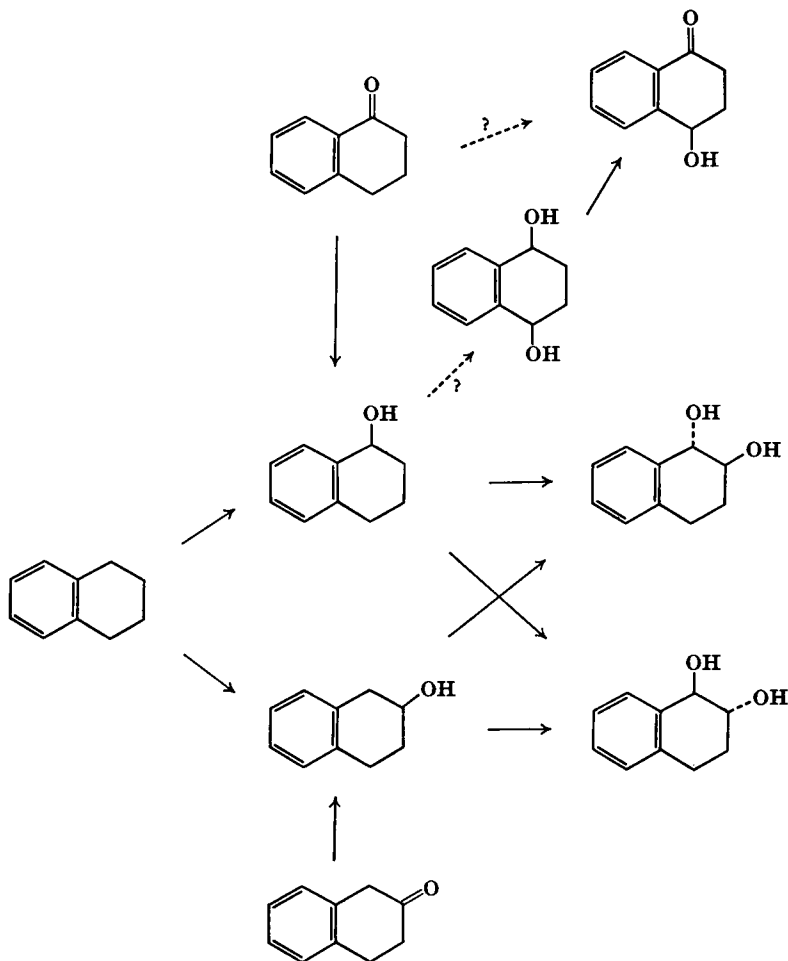
Although it would appear that 4-hydroxy- α -tetralone is a terminal product it is not certain whether α -tetralol or α -tetralone is its precursor. Experiments with β -tetralol and β -tetralone give little guidance, for neither of these substances produces 4-hydroxy- α -tetralone as a metabolite. In fact the metabolic pattern of the β -substituted compounds seems to be relatively uncomplicated. β -Tetralone is apparently reduced to β -tetralol by an oxidoreductase, and the β -tetralol is then presumably hydroxylated in the α -position to yield *cis*- and *trans*-tetralin-1,2-diol. Such a reaction sequence is in accord with chemical expectations, for the α -methylene group next to a benzene ring is highly reactive.

There are three possible ways in which 4-hydroxy- α -tetralone could be formed: (a) by oxidation of α -tetralol, (b) by hydroxylation of α -tetralone, or (c) by partial oxidation of a 1,4-diol intermediate. The evidence about the first possibility is somewhat conflicting for, although Hanson *et al.* (1953) have reported that Compound S can be converted by *Cunninghamella blakesleeana* into cortisone, Hanzke, Sebek & Murray (1960) have demonstrated that cortisol could be an intermediate. Similarly, the reported conversion of testosterone into 16-oxo-testosterone and 16 α - and 16 β -hydroxytestosterone (Herzog *et al.* 1960) does not clearly demonstrate the intermediary position of 16-oxotestosterone *in vivo*.

In support of the second possibility, the mechanism proposed by Hayano (1962) to account for the hydroxylation of certain steroids by 10 β -hydroxylase may be cited (see Scheme 1). This reaction



Scheme 1. (a) Mechanism for steroid 10 β -hydroxylation. (b) Proposed mechanism for hydroxylation of α -tetralone.



Scheme 2. Metabolism of tetralin in the rabbit. Unbroken arrows represent experimentally observed metabolic routes. Possible alternative metabolic routes are shown by broken arrows.

can occur with particular facility if the position being substituted is adjacent or vinylogous to the original keto group, the substrate being visualized

as undergoing reaction in the enol form. α -Tetralone might be hydroxylated in a similar way, the rearrangement ensuring a sufficiently high electron

density at the point under attack. Alternatively and perhaps even more simply, it may be considered that this hydroxylation and all others in positions 1 and 4 occur as a result of the activation of the α -methylene groups because they are next to an aromatic ring.

The main evidence favouring the third possibility is that in previous studies (Elliott *et al.* 1959, 1965a, 1966a) no evidence has ever been forthcoming that alicyclic alcohols can produce ketones, and in the present study it has similarly been found that, although α -tetralone produces α -tetralol with small amounts of 4-hydroxy- α -tetralone, the reverse does not occur. If this is the mechanism, the reaction sequence although complex is not enzymically impossible. It would involve a preliminary hydroxylation of tetralin or a preliminary reduction of the ketone to the alcohol, followed by a second hydroxylation in the 4-position, which by virtue of the highly reactive methylene group is particularly susceptible to such an attack. The finding that tetralin-1,4-diol yields 4-hydroxy- α -tetralone in the rabbit provides support for this proposed sequence.

Based on these considerations, a tentative scheme to account for the metabolism of tetralin is presented in Scheme 2.

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