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Metabolism of Methyldecalins

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1. Oral administration of *trans*- and *cis*-2-methyldecalin to rabbits increased the urinary glucuronide content. 2. The metabolite of the *trans* isomer was isolated and shown to be either *trans-cis*-6- or -7-methyl-2-decalol. The metabolite of the *cis* isomer was shown to be a 1:1 mixture of *cis*-6-methyl-2-decalol and *cis*-7-methyl-2-decalols, but the conformations of the hydroxyl groups were not determined. 3. It was concluded that a specific hydroxylase was responsible for the oxidations observed, and that this hydroxylase could be responsible for the metabolic oxidation of other simple alicyclic compounds.

It was found by Elliott, Robertson & Williams (1966) that hydroxylation *in vivo* of *cis*- and *trans*-decalins in rabbits occurred specifically at the β position (I), yielding mainly *cis-cis*-2-decalol or *trans-cis*-2-decalol respectively, which have the hydroxyl group in the equatorial conformation. The purpose of the present study was to examine the effect on metabolism of having a methyl group at the C-2 position in decalin. It was found that, as with the decalins, the methyl analogues were partly excreted in the urine as ether-type glucuronides (Table 1). Hydrolysis and extraction of the urine yielded secondary alcohols the characteristics of which had not previously been reported in the literature. Analysis of these aglycones by n.m.r. and mass spectrometry showed them to be either 6- or 7-hydroxy-2-methyldecalins. From these findings it was concluded that hydroxylation of the methyldecalins was effected by a specific enzyme.

MATERIALS

Melting points are uncorrected. Light petroleum (b.p. 60–80°C) was used for recrystallization and t.l.c.

cis-syn-2-Methyldecalin (II) was prepared by hydrogenation of 2-methylnaphthalene with W7 Raney nickel (Adkins & Billica, 1948) in ethanol at 150–200°C and 100 atm pressure. Fractional distillation (spinning band) of the product at 150°C and 15mmHg pressure gave the pure hydrocarbon. The i.r. spectrum was that of *cis-syn*-2-methyldecalin (Catalogue of Infra-red Spectral Data) as was the n.m.r. spectrum (Banas & Weitkamp, 1966).

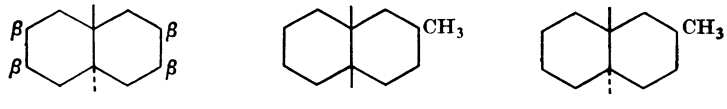
trans-syn-2-Methyldecalin (III) was prepared from the *cis* isomer by equilibration with anhydrous $AlCl_3$ (0.3M) for 16h (Zelinsky & Torowa-Polyak, 1932). Fractional distillation (spinning band) of the product gave the desired compound. The i.r. spectrum (Catalogue of Infra-red Spectral Data) and n.m.r. spectrum (Banas & Weitkamp, 1966) were those of *trans-syn*-2-methyldecalin.

2-Methylnaphthalene was purhcased from BDH Chemicals Ltd., Poole, Dorset, U.K.; 6- and 7-methylnaphthols were purchased from Aldrich Chemical Co. Inc., Milwaukee, Wis., U.S.A. Palladium (10%, w/w) on charcoal was purchased from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K.

METHODS

Animals and diet. White New Zealand × albino cross-bred rabbits weighing 1.8–3.5kg were used. Their daily diet consisted of 100g of Pelleted Rabbit Food (Drug Houses of Australia, Sydney, N.S.W., Australia) and unlimited water. Compounds were administered by stomach tube and followed by about 30ml of water.

Quantitative determination of glucuronide was carried out by the method of Hanson, Mills & Williams (1944), as modified by Paul (1951). No estimates were made of ethereal sulphate or mercapturic acid, since previous work



(I) (II) (III)

(I), *trans*-Decalin; (II), *cis*-2-methyldecalin; (III), *trans*-2-methyldecalin.

Table 1. *Excretion of the methyldecalins as glucuronides*

Experimental details are given in the text. The values given are averages with the ranges in parentheses; they are calculated from the increase in glucuronides in the urine after compared with before administration of the compounds.

Compound	No. of animals used	Dose (mmol/kg body wt.)	% of dose excreted as glucuronide
trans-2-Methyldecalin	6	2.2	43.5 (30.5–55.8)
cis-2-Methyldecalin	6	2.5	34.6 (15.8–46.5)

Table 2. R_F *Values on t.l.c. of urinary metabolites after hydrolysis*

The solvent system was *n*-hexane–ethyl acetate (3:1, v/v).

Compound administered	R_F values of metabolites	R_F values of isolated metabolites
trans-syn-2-Methyldecalin	0.12, 0.17, 0.22	0.12
cis-syn-2-Methyldecalin	0.12, 0.18, 0.22	0.12 (+10% of R_F 0.18)

(Elliott, Parke & Williams, 1959; Elliott, Tao & Williams, 1965; Elliott *et al.* 1966) had shown that they were unlikely metabolites of saturated alicyclic hydrocarbons.

Infrared spectroscopy. Measurements were made with a Perkin–Elmer model 21 spectrophotometer fitted with rock-salt optics. The hydrocarbons were prepared as liquid films and the metabolites as KBr discs or solutions in chloroform.

Thin-layer chromatography. Plates for t.l.c. were prepared as described by Elliott *et al*, (1966), with silica gel H or silica gel $HF_{254+366}$ (fluorescent) (E. Merck A.-G., Darmstadt, Germany). Light petroleum (b.p. 60–80°C)–ethyl acetate (3:1, v/v) was used as solvent for analytical and preparative t.l.c. Compounds were located with the phosphomolybdic acid reagent of Kritchevsky & Kirk (1952), or by examining for fluorescence under a u.v. lamp. With preparative t.l.c. the bands were located by their fluorescence or by exposing the dried chromatogram to iodine vapour.

Gas-liquid chromatography. A Hewlett–Packard F & M model 5750 gas chromatograph as described by Robertson & Hussain (1969) was used.

Nuclear-magnetic-resonance spectroscopy. Spectra were obtained by using Varian H-60 and Varian HA-100 n.m.r. spectrometers (Varian Associates, Palo Alto, Calif., U.S.A.). Compounds were dissolved in deuterated chloroform or benzene; tetramethylsilane was used as internal reference.

Mass spectrometry. Spectra were obtained on an A.E.I. MS-902 mass spectrometer [G.E.C.–A.E.I. (Electronics Ltd.), Barton Dock Road, Urmston, Manchester, U.K.].

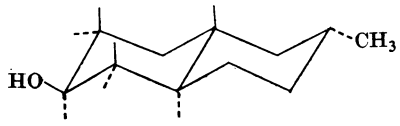
Dehydrogenation. The compound to be dehydrogenated was refluxed with 10% (w/w) palladium on charcoal. Neither solvent nor hydrogen-acceptor such as benzene or *p*-cymene (Linstead & Michaelis, 1934) were added as preliminary experiments showed that they produced mainly naphthalenes; this was also the case when platinum (5%, w/w) on alumina was used.

RESULTS

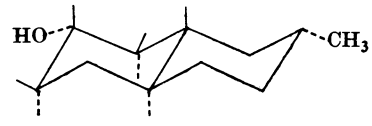
Characterization of aglycones. The urine was hydrolysed and then steam-distilled to partially purify the liberated aglycones, which were then extracted into ether. Further extraction of the urine with ether showed only traces of aglycones on t.l.c. R_F values of the metabolites are given in Table 2.

trans-syn-2-*Methyldecalin.* This hydrocarbon (1ml or 0.86g) was administered to each of six rabbits. After 24h 35% by weight of the dose was excreted as glucuronide, and a further 10.3% after 48h. Extraction of the steam-distillate gave a pale-yellow oil, t.l.c. of which showed the presence of three compounds of R_F values 0.12 (major), 0.17 and 0.22. Crystallization of the oil was achieved by adding an equal volume of *n*-pentane and placing the solution in a solid CO_2–acetone bath for several minutes. The resulting white solid had a minty odour, and after further recrystallization had m.p. 65–66°C; the 3,5-dinitrobenzoate had m.p. 86–88°C. The i.r. spectrum showed a strong absorption band at 3250nm. On mass spectral analysis the parent peak occurred at *m/e* 168, which together with the i.r. data suggested a formula of $C_{11}H_{19}OH$. The n.m.r. spectrum of the compound had a peak at 7.26τ that disappeared on exchange with deuterium oxide. From the integral trace it was calculated that there were 20 protons with the hydroxyl proton in the ratio of 1:20, confirming monohydroxylation. The other proton on the carbon carrying the hydroxyl group appeared as a seven-peak multiplet at 6.45τ in benzene solution. This value can be compared with that of 6.42τ for the axially located proton at C-3 in the spectrum of epiandrosterone (Bhacca & Williams, 1964); in the epimeric androstane (equatorial –H), the proton resonated at 5.85τ.

The conformation of a hydroxyl group can be determined from the observed splitting in the seven-peak multiplet. The latter can arise from a down-field axial proton in a cyclohexane ring interacting



(IV)



(V)

(IV), *trans-cis*-6-Methyl-2-decalol; (V), *trans-cis*-7-methyl-2-decalol.

with the four adjacent methylene protons so that the observed splitting arising from the interaction between the axial proton and its two equatorial neighbours is one-half of the observed splitting due to the diaxial interactions. Where the observed axial equatorial splitting is 5.5 ± 1 Hz, the hydroxyl is equatorial (Bhacca & Williams, 1964). In the present case, the observed splitting was 5 Hz. Thus the hydroxyl was equatorial, with two methylene groups on either side. There are two such positions in *trans*-2-methyldecalin, namely at C-6 or C-7. The compound then, using systematic nomenclature, was either *trans-cis*-6-methyl-2-decalol (IV) or *trans-cis*-7-methyl-2-decalol (V).

Mass spectroscopy failed to distinguish between the two possibilities. Oxidation of the alcohol yielded an oil whose i.r. spectrum showed a strong absorption band at 1720 nm, but no absorption at 3250 nm. The 2,4-dinitrophenyl derivative of this ketone had m.p. 146–148°C; the mass spectrum had the parent peak at the anticipated *m/e* 166. Dehydrogenation of 50 mg of the metabolite gave mainly 2-methylnaphthalene, identified as the picrate; naphthols were obtained in trace amounts only and of insufficient purity to allow their characterization. It was concluded that the metabolite was either *trans-cis*-6-methyl-2-decalol (IV) or *trans-cis*-7-methyl-2-decalol (V).

cis-syn-2-*Methyldecalin*. This compound (1 ml or 0.88 g) was administered to each of six rabbits. The glucuronide content of the urine collected 24 h later was equivalent to 29.4% of the hydrocarbon given, and to 34.8% after 48 h. Ether extraction of the steam-distillate yielded an oil which on t.l.c. showed the presence of three compounds of R_F 0.12, 0.18 and 0.22. The mixture, which failed to crystallize, was partially purified by preparative t.l.c. to yield a product consisting of 90% of compound of R_F 0.12 and 10% of R_F 0.18. The mixture could not be further purified by t.l.c. The i.r. spectrum showed a strong absorption band at 3325 nm, indicative of a hydroxyl group. Attempts to prepare crystalline derivatives were unsuccessful. Oxidation of the compound yielded an oil whose i.r. spectrum showed strong absorption at 1721 nm, but none at 3325 nm. Crystalline derivatives of this oil could not be prepared.

Dehydrogenation of 50 mg of the aglycone yielded a mixture of products of R_F values 0.13, 0.18 and 0.23. Those compounds of R_F values 0.18 and 0.23 fluoresced under u.v. light (The R_F values of 6-hydroxy- and 7-hydroxy-2-methylnaphthalenes were 0.23 and 0.24 respectively.) The mixture was applied to t.l.c. plates that were developed three times. The upper fluorescent band was isolated and extracted with ether to yield 1.5 mg of a light-brown solid. The solid could not be distinguished on t.l.c. from either 6- or 7-hydroxy-methylnaphthalene. The i.r. spectrum was similar to that of a 1:1 mixture of the two methylnaphthols. It was concluded that the metabolite of R_F 0.12 was a mixture of equal parts of *cis*-6-methyl-2-decalol and *cis*-7-methyl-2-decalol.

DISCUSSION

In previous studies on the oxidation *in vivo* of such simple alicyclic hydrocarbons as cyclohexane (Elliott *et al.* 1959), methylcyclohexane (Elliott *et al.* 1965) and decalin (Elliott *et al.* 1966), the main products were secondary alcohols having the hydroxyl groups in the equatorial, thermodynamically more favourable, conformation. The findings of the present investigation are consistent with this pattern of metabolism.

With cyclohexane, the main alcohol produced was cyclohexanol, and with methylcyclohexane *trans*-4-methylcyclohexanol, together with lesser amounts of *cis*-3- and *trans*-2-methylcyclohexanols. From these findings, it was uncertain whether hydroxylation had occurred by means of a random free-radical process or a specific hydroxylase. With decalin it had been shown (Jaffe, Steadman & McKinney, 1963) that chemical oxidation with peroxide (a free-radical process) yielded a mixture of 1-, 2- and 9-decalols whereas biological oxidation gave only 2-(β-)decalol. This suggested that decalin was oxidized *in vivo* by a specific hydroxylase. Decalin has four equivalent β-sites (I) so that the compound could have been hydroxylated while in any one of four similar orientations with respect to the enzyme. 2-Methyldecalin has only two equivalent β-sites, both of which were attacked as shown above. This would seem to reinforce the

possibility of oxidation being effected by a specific hydroxylase.

It may be that the same hydroxylase is responsible for the hydroxylation of all the alicyclic compounds mentioned above. The simpler, less space-filling molecules would have the possibility of presenting a number of equivalent or near-equivalent methylene groups at the hydroxylation site, giving an appearance of randomness to the hydroxylation process, whereas the larger, more demanding molecules have a more limited choice of orientation.

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