The Metabolism of the Isomeric Decalones

By T. H. ELLIOTT, J. S. ROBERTSON AND R. T. WILLIAMS

*School of Pharmacy, University of Singapore, Singapore, and
Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2*

(Received 3 January 1966)

1. The metabolism of ($\pm$)-*cis*-1-, ($\pm$)-*trans*-1-, ($\pm$)-*cis*-2- and ($\pm$)-*trans*-2-decalone in the rabbit has been investigated. 2. ($\pm$)-*cis*-2- and ($\pm$)-*trans*-2-Decalone were both reduced to racemic secondary alcohols, conformationally related to the ketones administered, and possessing an equatorial hydroxyl group. These alcohols were excreted in the urine as glucuronides in amounts equal to about half the dose administered. The glucuronides were isolated both as triacetyl methyl esters and as sodium salts. The ester obtained after feeding with ($\pm$)-*cis*-2-decalone proved to be *methyl (cis-cis-2-decalyl tri-O-acetyl- β -D-glucosid)uronate*, whereas ($\pm$)-*trans*-2-decalone yielded *methyl (trans-cis-2-decalyl tri-O-acetyl- β -D-glucosid)uronate*. The sodium salts were shown to be *sodium (cis-cis-2-decalyl glucosid)uronate* and *sodium (trans-cis-2-decalyl glucosid)uronate*. 3. Enzyme hydrolysis of the sodium salts and acid hydrolysis of the esters derived from ($\pm$)-*cis*-2-decalone yielded ($\pm$)-*cis-cis*-2-decalol, and of those from ($\pm$)-*trans*-2-decalone yielded ($\pm$)-*trans-cis*-2-decalol. 4. ($\pm$)-*cis*-1-Decalone was reduced mainly to ($-$)-*cis-cis*-1-decalol and excreted as [$(-)$ -*cis-cis*-1-decalyl glucosid]-uronic acid. A small amount of the corresponding (+)-isomer was produced, yielding [(+)-*cis-cis*-1-decalyl glucosid]uronic acid on isolation. Enzyme hydrolysis of these compounds gave the corresponding aglycones; both alcohols possessed an equatorial hydroxyl group. 5. ($\pm$)-*trans*-1-Decalone was reduced to (+)-*trans-trans*-1-decalol, with an equatorial hydroxyl group, and in smaller amount to (+)-*trans-cis*-1-decalol possessing an axial group. The former alcohol was excreted as [(+)-*trans-cis*-1-decalyl glucosid]uronic acid, and the latter as [(+)-*trans-cis*-1-decalyl glucosid]uronic acid. 6. From a knowledge of the conformations, and in some cases the absolute configurations, of the compounds administered and excreted, and by making the assumption that the coenzyme concerned in the reductions is NADH (or NADPH), an explanation of the above findings in terms of steric hindrance and thermodynamic stability is given.

There have been two previous systematic investigations of the mammalian metabolism of simple alicyclic compounds. Elliott, Parke & Williams (1959) studied the metabolism of cyclohexane, cyclohexanol, cyclohexanone and the cyclohexanediols. The fate of methylcyclohexane, the isomeric methylcyclohexanols and methylcyclohexanones has been investigated by Elliott, Tao & Williams (1965). These investigations revealed that these simple alicyclic compounds when administered to rabbits were mainly excreted in the urine as water-soluble ether-linked glucuronides. In the main, the aglycones obtained from the glucuronides were secondary alcohols, with the hydroxyl group in the equatorial conformation, i.e. in the thermodynamically more favoured conformation (Barton, 1953).

By assuming NADH to be the essential reducing

substance, the mechanism by which acetone is reduced enzymically to ethanol, as elucidated by Fisher, Conn, Vennesland & Westheimer (1953) and Vennesland (1958), was found to afford an explanation of the stereochemical reduction of the methylcyclohexanones.

This same mechanism has been assumed to be responsible for the stereospecificity of the mammalian reduction of the isomeric decalones. The results of feeding these ketones to rabbits are reported in this paper. A preliminary account of this work has been reported (Robertson & Elliott, 1965).

MATERIALS

Melting points are uncorrected. All compounds used were subjected to thin-layer chromatography and shown to be pure, except ($\pm$)-*trans-trans*-2-decalol, where about 3% of

impurity was present. The infrared spectra of the decalols were recorded, and found to be identical with those published by Hückel & Rashingkar (1960), Hückel & Riad (1960) and Prelog & Feldman (1958). Authentic samples of $(\pm)$ -*trans-trans*-1-, $(\pm)$ -*cis-cis*-1-, $(\pm)$ -*trans-cis*-1- and $(\pm)$ -*cis-cis*-2-decalol, purified by gas-liquid chromatography, were obtained through the courtesy of Dr H. Schumacher, Eidgenössische Technische Hochschule, Zurich, Switzerland.

$(\pm)$ -*trans-trans*-1-Decalol was prepared by hydrogenation of $(\pm)$ -*trans*-1-decalone with platinum oxide catalyst (Adams, Voorhees & Shriner, 1948) in acetic acid at 30° and 3–4 atm. pressure. From the oil remaining after isolation, crystals were obtained, which on recrystallization from light petroleum (b.p. 40–60°) had m.p. 59.5°. Hückel (1925) gives m.p. 63° and Prelog & Feldman (1958) give m.p. 57–58°.

$(\pm)$ -*trans-cis*-1-Decalol was separated by radial preparative chromatography (see the Methods section) from the mother liquor remaining after removing *trans-trans*-1-decalol. Extraction and recrystallization from light petroleum (b.p. 40–60°) gave crystals, m.p. 47.5–48.5°. Hückel (1925) gives m.p. 49°.

$(\pm)$ -*cis-cis*-1-Decalol was prepared from 1-naphthol by hydrogenation in the presence of Adams platinum oxide catalyst in acetic acid at 50–60° and 3–4 atm. pressure for 80 hr. Recrystallization from light petroleum (b.p. 40–60°) yielded needles, m.p. 93°.

$(\pm)$ -*trans-trans*-2-Decalol was prepared by hydrogenation of $(\pm)$ -*trans*-2-decalone by low-pressure hydrogenation of the *trans-trans* isomer. Purification by radial preparative chromatography gave a solid, m.p. 50–51°. Hückel, Mentzel, Brinkmann & Kamenz (1926) give m.p. 53°.

$(\pm)$ -*trans-cis*-2-Decalol was separated from a commercial preparation (L. Light and Co. Ltd., Colnbrook, Bucks.), containing mainly the *trans-cis* isomer, by recrystallization from light petroleum (b.p. 40–60°). Needles were obtained, m.p. 75°. Hückel (1925) gives m.p. 75°.

$(\pm)$ -*cis-cis*-2-Decalol was obtained from 2-naphthol by low-pressure hydrogenation (Adams platinum oxide catalyst in acetic acid) in the form of needles, m.p. 105°. Hückel (1925) gives m.p. 105°.

$(\pm)$ -*cis-trans*-2-Decalol was prepared by the isomerization of $(\pm)$ -*cis-cis*-2-decalol by refluxing in decalin in the presence of sodium for 8 hr. (Rodig & Ellis, 1961). The oil remaining after isolation was separated into two components by radial preparative chromatography, one of which proved to be *cis-trans*-2-decalol, m.p. 18°. Hückel *et al.* (1926) give m.p. 18°.

$(\pm)$ -*trans*-1-Decalone was prepared by heating a solution of $(\pm)$ -*cis*-1-decalone in a methanolic solution of sodium methoxide for 30 min., so converting it into the *trans* isomer. The product, after extraction and removal of solvent, was converted into the bisulphite compound, from which the ketone was liberated by alkali. Steam-distillation, extraction and distillation under reduced pressure yielded a viscous liquid, which set to large slab-like crystals, m.p. 32°. The infrared spectrum showed a strong ketonic absorption band at 1720 cm.⁻¹ and a diagnostic band at 900 cm.⁻¹ (Djerassi & Staunton, 1961), with no absorption at 950 cm.⁻¹, indicating the absence of the *cis* isomer; the 2,4-dinitrophenylhydrazone had m.p. 237.5–238°.

$(\pm)$ -*cis*-1-Decalone was prepared by the chromic acid oxidation in acetone (Bowers, Halsall, Jones & Lemm,

1953) of $(\pm)$ -*cis-cis*-1-decalol. The ketone, after extraction and removal of the solvent, was distilled under reduced pressure to yield an oil, n_D^{25} 1.4930. Hückel (1925) gives n_D^{20} 1.4936. The infrared spectrum showed a strong ketonic band at 1715 cm.⁻¹, and a diagnostic band at 950 cm.⁻¹ (distinction from *trans*-1-decalone, with a 900 cm.⁻¹ band and a minimum absorption at 950 cm.⁻¹; Djerassi & Staunton, 1961); the 2,4-dinitrophenylhydrazone had m.p. 238° (this is in fact the m.p. of the *trans* derivative, owing to *cis-trans* isomerization during recrystallization). Reduction of the ketone in methanol with methanolic 10% (w/v) NaBH₄ solution gave a quantitative yield of *cis-cis*-1-decalol, m.p. 93°.

$(\pm)$ -*trans*-2-Decalone was prepared by chromic acid oxidation of $(\pm)$ -*trans-cis*-2-decalol. The oil obtained after isolation and purification had n_D^{25} 1.429. Hückel (1925) gives n_D^{20} 1.4831. The infrared spectrum showed a strong absorption band at 1725 cm.⁻¹ and prominent bands at 1220, 1165 and 1120 cm.⁻¹. The semicarbazone had m.p. 191–192° (decomp.).

$(\pm)$ -*cis*-2-Decalone was prepared by chromic acid oxidation of $(\pm)$ -*cis-cis*-2-decalol. Isolation as the bisulphite compound, followed by alkaline decomposition, extraction and distillation under reduced pressure, yielded the ketone, n_D^{25} 1.4930. Hückel (1925) gives n_D^{20} 1.4935. The 2,4-dinitrophenylhydrazone had m.p. 155–156°. The infrared spectrum showed absorption bands at 1720, 1205 and 1175 cm.⁻¹. Reduction of a methanolic solution of the ketone with methanolic 10% (w/v) NaBH₄ solution gave a quantitative yield of *cis-cis*-2-decalol, m.p. 105°.

METHODS

Animals and diet. Doe chinchilla rabbits weighing 3–4 kg. were used. They were kept on a constant diet of 100g. of Diet S.G.1 (pellet form; Associated London Flour Millers Ltd., London, E.C. 3) and unlimited water.

Administration of dose. The compounds were administered by stomach tube followed by about 20 ml. of water.

Analytical methods. The following were used: glucuronic acid was determined by the method of Hanson, Mills & Williams (1944), as modified by Paul (1951), ethereal sulphate by the method of Sperber (1948) and mercapturic acid by the method of Stekol (1936).

General methods for the isolation of metabolites. Glucuronides were isolated by ion-exchange chromatography. Where possible, they were then purified by recrystallization from hot water. When the glucuronide was a gum, showing no tendency to crystallize, derivatives were prepared. These were either the triacetyl methyl ester prepared as described by Kamil, Smith & Williams (1952) or the sodium salt; the latter was further purified by radial preparative chromatography.

Sodium salts were prepared by adding excess of 20% (w/v) NaOH solution, with cooling, to the glucuronide gum. Excess of alkali was neutralized with a saturated solution of SO₂ in water. The solution obtained was evaporated to dryness and the salt extracted from the coloured residue with hot ethanol. Concentration of the ethanolic solution followed by chilling caused precipitation of the sodium salt. The process of dissolution in ethanol and precipitation were repeated until the product was shown by chromatography to be apparently a single substance. Where this technique was insufficient, the salt was

further purified by radial preparative chromatography, to constant optical rotation. In early experiments the sodium salts were hydrolysed with β -glucuronidase. When subsequently it was observed that hydrolysis by refluxing with N -HCl for 15 min. yielded the same aglycone, this method was then used exclusively. Triacetyl methyl esters were hydrolysed with N -HCl but required 3 hr. refluxing. The hydrolysate was steam-distilled, the distillate extracted with ether, dried and the ether removed, and then the residue was sublimed under reduced pressure. The crystalline material was recrystallized from light petroleum (b.p. 40–60°).

Isolation of glucuronide before ion-exchange chromatography. The urine from which the glucuronide was to be extracted was evaporated to dryness at a temperature not exceeding 50° in a rotary vacuum flash evaporator. The dried urine was extracted by shaking with hot methanol until the residue gave no reaction for glucuronide when tested with naphtharesorcinol. The methanol was then removed, and the residue dissolved in 150 ml. of water and passed into the resin column.

Ion-exchange chromatography. A 100g. portion of De-Acidite FF (Permutit) of chromatographic particle size in the Cl⁻ form was first converted into the OH⁻ form by treatment with 3% (w/v) NaOH. After being freed from alkali by washing with water, it was then converted into the acetate form with 10% (v/v) acetic acid and washed until free from excess of acid. A suspension of the acetate resin was poured into a glass column (60 cm. $\times$ 2.5 cm.) fitted at the exit with a tap and a plug of glass wool, to give a resin bed 20 cm. high. Increasing the bed to 30 cm. greatly reduced the flow rate.

A solution of the crude glucuronide was passed into the column. The column was well washed with water to get rid of urea, and then the glucuronide was desorbed by passing into the column a saturated solution of SO₂ in water. The solution of glucuronide was evaporated to dryness at 50°, and since the SO₂ came off first there was no danger of acid hydrolysis. The residue normally was light brown in colour, indicative of the purification achieved by this technique.

Thin-layer chromatography. Silica gel H, prepared according to Stahl for thin-layer chromatography (E. Merck A.-G., Darmstadt, Germany), was used throughout. Adsorption plates were either squares of glass (20 cm. $\times$ 20 cm.) for the determination of R_f values, or microscope slides (3 in. $\times$ 1 in.) for the rapid detection of metabolites. The thickness of the absorbent layer on the plates was maintained at 0.25 mm. Freshly spread plates were allowed to dry overnight in an oven maintained at 45–50°. Plates were developed in the standard tank (Desaga), the edges of which were coated with soft paraffin for polar solvents, and with a bentonite-glycerol paste for non-polar solvents. Slides were developed in Coplin jars of the type commonly used for holding slides during staining procedures; the jars were sealed with a rubber stopper.

Glucuronides were chromatographed with a system composed of butan-1-ol-aq. NH₃ (sp.gr. 0.88) (4:1, v/v) (system 1), aglycones and alcohols in a system consisting of hexane-ethyl acetate (9:1, v/v) (system 2). Glucuronides were detected by spraying the dried chromatogram with a solution composed of 0.2% (w/v) naphtharesorcinol in acetone or ethanol-85% phosphoric acid (5:1, v/v), and heating at 120–130° for 10 min., when they appeared as

blue-purple areas against a reddish-pink background. Alcohols were revealed as deep-blue spots against a green background when the dried chromatogram was sprayed with 10% (w/v) phosphomolybdic acid solution in ethanol (Kritchevsky & Kirk, 1952) and heated at 120–130° for 10 min.

Radial preparative chromatography. Glass plates (20 cm. $\times$ 20 cm.) were coated with a slurry of silica gel H in water, to give a layer 1 mm. thick. When dry, the latter was cut into columns, each 2 cm. wide. About 1.5 cm. from the bottom of the plate, each column was cut with a sharp chisel to produce a narrow waist 8 mm. broad and 15 mm. long. The solution to be chromatographed was applied to the bottom of the waist, a drop at a time, from a hypodermic syringe fitted with a fine-bore needle. A load of 5 mg. per column was found suitable.

After being dried, the chromatograms were developed either with system 1 for the sodium salts of glucuronides, or with system 2 for alcohols and aglycones.

One simple non-destructive detection technique was to view the developed chromatogram by transmitted light while it was still fully moist with solvent. The desired bands were rapidly delineated and, after evaporation of the developing solvent, were removed and extracted. Another was to expose the developed chromatogram, after removal of solvent, to iodine vapour long enough to allow the iodine to stain the organic compounds present. The areas desired were outlined and scraped off, and the iodine was allowed to desorb before extraction.

RESULTS

Experiments on urine

Of the four racemic ketones given, only ($\pm$)-*trans*-1-decalone appeared to possess any pharmacological action. At a high dose of approx. 5 m-moles/kg. this compound narcotized for 6 hr. two out of the three rabbits fed. There was no effect observed at the lower dose of approx. 2 m-moles/kg.

Qualitative experiments on urine. The urine collected at 24 and 48 hr. after feeding had pH 8–9, was of a reddish-brown colour and had a faint odour of mint. In all cases it gave an intense naphtharesorcinol reaction. The urine did not reduce Benedict's solution, nor did it give a precipitate with Brady's reagent. It gave a negative response when tested for phenols with ferric chloride solution and with 2,6-dichloroquinone-chloroimide.

Quantitative estimation on urine. Estimations for glucuronic acid, ethereal sulphate and mercapturic acid were carried out on urine samples before and after dosing; the results for glucuronic acid excretion are given in Table 1. Negligible amounts of ethereal sulphate and no mercapturic acid were detected.

Isolation and characterization of metabolites. The melting points and optical rotations of the pure isolated glucuronides, triacetyl methyl esters and sodium salts are given in Table 2. The R_f values of the glucuronides are given in Table 3. The R_f

values of the isomeric decalols are presented in Table 4, and the characteristics of the glucuronide aglycones are given in Table 5.

(±)-trans-1-Decalone

The ketone (2ml.) was administered to each of three chinchilla rabbits, and 4g. of semi-solid glucuronide was isolated, by ion-exchange chromatography, from the 24hr. urine. Chromatography of the urine revealed two spots giving a naphtha-resorcinol reaction, R_F 0.14 (major) and R_F 0.10

(minor) in system 1. Recrystallization of 3.5g. of the semi-solid glucuronide from hot water yielded 1.2g. of pure [(+)-trans-trans-1-decalyl β-D-glucosid]uronic acid monohydrate, m.p. 191–192° (decomp.), $[\alpha]_D^{18.5} -14.6 \pm 2^\circ$ (c 1 in methanol), R_F 0.14 (system 1) (Found: C, 55.5; H, 8.2. $C_{16}H_{26}O_7 \cdot H_2O$ requires C, 55.2; H, 8.1%).

Hydrolysis of the crude glucuronide. A 250mg. sample of the semi-solid glucuronide was dissolved in 1ml. of 0.4N-sodium hydroxide solution, then diluted to 2ml. with 0.4N-acetic acid to give pH 5. To this solution 1ml. of snail crop juice (*Helix pomatia*) was added, and a final dilution was made with water to 8ml. The well-mixed suspension was incubated at 37° for 48hr. Extraction with ether, drying, removal of solvent and sublimation under reduced pressure yielded 30mg. of an oil, the infrared spectrum of which revealed the presence of both trans-trans- and trans-cis-1-decalol, the former predominating. By comparison with the infrared spectra of standard mixtures of racemic trans-trans- and trans-cis-1-decalol, the relative concentrations were estimated at 4:1 of trans-trans to trans-cis isomer. Chromatography (system 2) revealed the presence of two alcohols, R_F 0.25 (major) and R_F 0.38 (minor), in about these same relative concentrations. The oil was separated into its components by radial preparative chroma-

Table 1. Glucuronic acid excretion of the isomeric decalones

Experimental details are given in the text. Three animals were used for each determination.

Compound administered	Dose (mmoles/kg. body wt.)	Percentage of dose excreted as glucuronide	
		Average	Range
(±)-trans-1-Decalone	1.62	58.4	53.6–63.4
(±)-cis-1-Decalone	1.67	56.8	42.0–65.7
(±)-cis-2-Decalone	4.23	51.0	41.0–62.0
(±)-trans-2-Decalone	3.28	49.4	41.0–58.5

Table 2. Melting points and optical rotations of isolated glucuronides, triacetyl methyl esters and sodium salts

Compound administered	Glucuronide		Triacetyl methyl ester		Sodium salt
	m.p.	$[\alpha]_D^{18-20}$ (in methanol)	m.p.	$[\alpha]_D^{18-20}$ (in chloroform)	$[\alpha]_D^{18-20}$ (in methanol)
(±)-trans-1-Decalone	191–192°	–14.6°	—	—	—
	(decomp.)	—	—	—	—
	174–175°	–3.0	—	—	—
(±)-cis-1-Decalone	(decomp.)	—	—	—	—
	182–183°	–87	—	—	—
	(decomp.)	—	—	—	—
(±)-cis-2-Decalone	173.5–174.5°	–47.3	—	—	—
	(decomp.)	—	—	—	—
(±)-cis-2-Decalone	—	—	162–164°	–37°	–42.5°
(±)-trans-2-Decalone	—	—	157–158	–23	–41.8

Table 3. R_F values of glucuronides

Compound administered	R_F (system 1)
(±)-trans-1-Decalone	0.14 (major) 0.10 (minor)
(±)-cis-1-Decalone	0.136
(±)-cis-2-Decalone	0.14 (major) 0.10 (minor)
(±)-trans-2-Decalone	0.132 (major) 0.09 (minor)

Table 4. R_F values of the isomeric decalols

Compound	R_F (system 2)
(±)-trans-trans-1-Decalol	0.25
(±)-trans-cis-1-Decalol	0.38
(±)-cis-cis-1-Decalol	0.25
(±)-cis-cis-2-Decalol	0.195
(±)-cis-trans-2-Decalol	0.35
(±)-trans-cis-2-Decalol	0.255
(±)-trans-trans-2-Decalol	0.185

Table 5. *Melting points, optical rotations and R_F values of glucuronide aglycones*

Compound administered	m.p.	$[\alpha]_D^{18-20}$ in methanol of aglycone obtained	R_F (system 2)
($\pm$)- <i>trans</i> -1-Decalone	80–81° 70–70.5	+35° +27	0.25 0.38
($\pm$)- <i>cis</i> -1-Decalone	68–69 91–92	–19.5 0	0.25 0.25
($\pm$)- <i>cis</i> -2-Decalone	105	0	0.195
($\pm$)- <i>trans</i> -2-Decalone	72–74	0	0.185

tography. Extraction of the combined bands of lower R_F yielded 10mg. of pure (+)-*trans-trans*-1-decalol, m.p. 80–81°, $[\alpha]_D^{20} + 35 \pm 2^\circ$ (c 1 in ethanol) (Found: C, 77.8; H, 11.9. $C_{10}H_{18}O$ requires C, 77.8; H, 11.8%). The infrared spectrum was the same as that of pure ($\pm$)-*trans-trans*-1-decalol, R_F 0.25 (system 2). No material was obtained after extraction of the combined bands of higher R_F .

Hydrolysis of the pure glucuronide. A 100mg. sample of pure glucosiduronic acid was subjected to enzyme hydrolysis under the same conditions as for the semi-solid glucuronide described above; 20mg. of pure (+)-*trans-trans*-1-decalol was obtained, having the characteristics described above.

The mother liquor remaining after separation of the [(+)-*trans-trans*-1-decalyl β -D-glucosid]uronic acid was acidified with 10% (v/v) sulphuric acid. After refrigeration for 14 days a white precipitate was obtained. Thorough washing with ice-cold water to remove sulphuric acid, followed by recrystallization from hot water, yielded 30mg. of [(+)-*trans-cis*-1-decalyl β -D-glucosid]uronic acid, m.p. 174–175° (decomp.), $[\alpha]_D^{19} - 3 \pm 2^\circ$ (c 1 in methanol), R_F 0.14 (system 1).

Hydrolysis of the glucuronide. Enzyme hydrolysis of this glucosiduronic acid (30mg.), followed by the usual purification procedures, yielded 5mg. of (+)-*trans-cis*-1-decalol, m.p. 70–70.5°, $[\alpha]_D^{19} + 27 \pm 2^\circ$ (c 0.5 in ethanol). Literature values are m.p. 71–72° and $[\alpha]_D + 34^\circ$ (in ethanol) (Prelog & Feldman, 1958). The infrared spectrum was identical with that of pure ($\pm$)-*trans-cis*-1-decalol. The R_F was 0.38 (system 2).

($\pm$)-*cis*-1-Decalone

A 1 ml. dose of this ketone was administered to each of three rabbits. Chromatography of the 24 hr. urine revealed one spot giving a naphtharesorcinol reaction, R_F 0.136 (system 1). From this urine 2g. of a semi-solid glucuronide was obtained by ion-exchange chromatography. Recrystallization from hot water produced 0.5g. of pure [(–)-*cis-cis*-1-decalyl β -D-glucosid]uronic acid monohydrate, m.p.

182–183° (decomp.), $[\alpha]_D^{19} - 87 \pm 2^\circ$ (c 1 in methanol) (Found: C, 55.1; H, 8.2. $C_{16}H_{26}O_7 \cdot H_2O$ requires C, 55.2; H, 8.1%). The R_F was 0.136 (system 1).

Hydrolysis of the pure glucosiduronic acid. A 100mg. sample of the glucosiduronic acid was hydrolysed with snail-crop juice as described above. Extraction, drying, removal of solvent and sublimation at 0.02mm. Hg pressure and 80° yielded 20mg. of pure (–)-*cis-cis*-1-decalol, m.p. 68–69°, $[\alpha]_D^{19} - 19.5 \pm 2^\circ$ (c 1 in chloroform). Literature values are m.p. 71–72° and $[\alpha]_D - 22^\circ$ (c 1 in chloroform) (Djerassi & Staunton, 1961). The infrared spectrum in carbon tetrachloride was identical with that of ($\pm$)-*cis-cis*-1-decalol. The R_F was 0.25 (system 2).

A 1g. sample of cruder glucuronide, m.p. 153–158° (decomp.), on recrystallization from hot water yielded 450mg. of pure [($\pm$)-*cis-cis*-1-decalyl β -D-glucosid]uronic acid monohydrate, m.p. 173.5–174.5° (decomp.), $[\alpha]_D^{19} - 47.3 \pm 2^\circ$ (c 1 in methanol) (Found: C, 55.4; H, 8.2. $C_{16}H_{26}O_7 \cdot H_2O$ requires C, 55.2; H, 8.1%). The R_F was 0.136 (system 1). Repeated recrystallization of this material did not alter the optical rotation or the melting point, showing that diastereomeric resolution of the compound was not possible under these conditions.

A 100mg. sample of this pure glucuronide was hydrolysed with snail-crop juice as described above. Extraction, drying, evaporation of the solvent and sublimation at 0.02mm. Hg pressure and 80° gave 20mg. of ($\pm$)-*cis-cis*-1-decalol, m.p. 91–92° unchanged by admixture with an authentic sample. The infrared spectrum was that of ($\pm$)-*cis-cis*-1-decalol. The R_F was 0.25 (system 2).

($\pm$)-*cis*-2-Decalone

A 2 ml. dose of this decalone was administered to each of three rabbits. Chromatography of the 24 hr. urine revealed two spots giving a naphtharesorcinol reaction; the R_F values were 0.14 (major) and 0.10 (minor) (solvent system 1).

Isolation of the glucuronide by ion-exchange chromatography yielded 5g. of gum that could

not be induced to crystallize. A 1.5g. sample of this gum was converted into the sodium salt. A 2.5g. sample of the gum was methylated and acetylated; after recrystallization from methanol, 200mg. of pure methyl [(±)-*cis-cis-2-decalyl* O-triacetyl-β-D-glucosid]uronate had m.p. 162–164°, $[\alpha]_D^{25} -37 \pm 2^\circ$ (c 1 in chloroform) (Found: C, 58.9; H, 7.2. $C_{23}H_{34}O_{10}$ requires C, 58.8; H, 7.3%).

Hydrolysis of the crude gum. A 1.0g. sample of the crude gum was hydrolysed by boiling with N-hydrochloric acid for 30min. After the normal isolation and purification procedures, 160mg. of pure (±)-*cis-cis-2-decalol* was obtained, m.p. 105°, $[\alpha]_D^{25} 0^\circ$ (c 1 in chloroform). The infrared spectrum was that of (±)-*cis-cis-2-decalol*. The R_f was 0.195 (system 2).

Hydrolysis of 100mg. of the sodium salt with β-glucuronidase yielded, after extraction and purification, 25mg. of (±)-*cis-cis-2-decalol*, m.p. 105°, indicating that acid hydrolysis had not altered the aglycone.

(±)-*trans-2-Decalone*

A 2ml. dose of this decalone was administered to each of three rabbits. Chromatography of the urine showed two spots giving a naphtharesorcinol reaction; the R_f values were 0.132 (major) and 0.09 (minor). Extraction of the glucuronide by ion-exchange chromatography yielded 4.8g. of a non-crystallizable gum. A 1.8g. sample of this gum was converted into the sodium salt. A 2g. sample of the remaining gum was converted into a triacetyl methyl ester, which after recrystallization from methanol yielded 150mg. of pure methyl [(±)-*trans-cis-2-decalyl* tri-O-acetyl-β-D-glucosid]uronate, m.p. 157–158°, $[\alpha]_D^{25} -23 \pm 2^\circ$ (c 1 in chloroform) (Found: C, 58.9; H, 7.4. $C_{23}H_{34}O_{10}$ requires C, 58.7; H, 7.3%).

Hydrolysis of the crude gum. The remaining 1g. of crude gum was hydrolysed by boiling with N-hydrochloric acid for 30min. Extraction and purification of the resulting aglycone gave 120mg. of (±)-*trans-cis-2-decalol*, m.p. 72–74° not depressed when admixed with pure material, $[\alpha]_D^{25}$

0° (c 1 in chloroform). The infrared spectrum was that of (±)-*trans-cis-2-decalol*.

Hydrolysis of the sodium salt with snail-crop juice yielded (±)-*trans-cis-2-decalol*, m.p. 74–75°, indicating that refluxing with acid had not affected the aglycone.

DISCUSSION

Administration of the racemic isomeric decalones to rabbits resulted in an increase in urinary glucuronide equivalent to 50–60% of the compound given (Table 1). The predominant metabolic products are equatorial alcohols that are optically active for the 1-decalols and racemic for the 2-decalols. Thus (±)-*trans-1-decalone* gave (+)-*trans-trans-1-decalol* and (+)-*trans-cis-1-decalol*; (±)-*cis-1-decalone* gave (–)-*cis-cis-1-decalol* and a small amount of (±)-*cis-cis-1-decalol*; (±)-*cis-2-decalone* gave (±)-*cis-cis-2-decalol*; (±)-*trans-2-decalone* gave (±)-*trans-cis-2-decalol* (Table 6).

This metabolic pattern is similar to that observed by Elliott *et al.* (1965), who investigated the methylcyclohexanones and methyleyclohexanols, and suggested that the stereospecific reduction of their series of ketones was explicable in terms of the hypothesis of Fisher *et al.* (1953) and Vennesland (1958) on the role of NADH in dehydrogenation reactions. Experiments *in vitro* (Robertson & Elliott, 1965) have shown that the decalones are reduced by NADH in the presence of horse-liver alcohol dehydrogenase (an 'A' class enzyme; Levy, Talalay & Vennesland, 1962), and Prelog (1959) has shown that a 'B' class enzyme isolated from *Curvularia falcata* utilizes NADPH in the reduction of decalones. By using the same hypothesis, models of the isomeric decalones, in their absolute configurations where established (Djerassi & Marshall, 1958; Djerassi & Staunton, 1961) (Figs. 1 and 2), can be placed in the transition state with an orientation that is either 'face-to-face' (Fig. 4) or 'head-to-tail' (Fig. 5) relative to the nicotinamide portion of the NADH molecule, also with its correct configuration (Cornforth, Ryback, Popják, Donninger & Schroepfer, 1962) (Fig. 3). It can be seen that, with

Table 6. *Major metabolic transformations of the isomeric decalones*

Compound administered	Configuration of aglycone	Conformation of –OH group
(±)- <i>trans-1-Decalone</i>	(+)- <i>trans-trans-1</i>	<i>eq</i>
	(+)- <i>trans-cis-1</i>	<i>ax</i>
(±)- <i>cis-1-Decalone</i>	(–)- <i>cis-cis-1</i>	{ <i>eq</i>
	(+)- <i>cis-cis-1</i>	
(±)- <i>cis-2-Decalone</i>	(+)- <i>cis-cis-2</i>	{ <i>eq</i>
	(–)- <i>cis-cis-2</i>	
(±)- <i>trans-2-Decalone</i>	(+)- <i>trans-cis-2</i>	{ <i>eq</i>
	(–)- <i>trans-trans-2</i>

Fig. 1. Isomeric *trans*-decalones and derived decalols. (I), (-)-*trans*-1-; (II), (+)-*trans*-1-; (III), (-)-*trans*-2-; (IV), (+)-*trans*-2-decalone. (V), (-)-*trans*-*cis*-1-; (VI), (+)-*trans*-*trans*-1-; (VII), (+)-*trans*-*cis*-1-; (VIII), (-)-*trans*-*trans*-1-decalol. (IX), (+)- or (-)-*trans*-*cis*-2-; (X), (+)- or (-)-*trans*-*trans*-2-; (XI), (+)- or (-)-*trans*-*trans*-2-; (XII), (+)- or (-)-*trans*-*cis*-2-decalol.

trans-1-decalone (Fig. 4), there is steric conflict between its cyclohexane ring and the carboxyamido moiety in one orientation (unfavourable alignment), but no conflict in the opposite orientation (favourable alignment). The formation of only (+)-*trans*-*trans*-1- and (+)-*trans*-*cis*-1-decalol is thus explicable. Considerably more (+)-*trans*-*trans*-1-decalol (equatorial hydroxyl group) is produced than (+)-*trans*-*cis*-1-decalol (axial hydroxyl group) (see above). If the only hindrance to reduction were the steric conflict mentioned above, one would expect the ketones in their favourable alignments to be equally reduced. Since this was not so, other factors must be controlling the reaction. (+)-*trans*-*trans*-1-Decalol has an equatorial hydroxyl group, and (+)-*trans*-*cis*-1-decalol an axial hydroxyl group; the latter is thermodynamically in a less stable conformation (Barton, 1953). On thermodynamic grounds, then, the ketones should be reduced to those alcohols

with an equatorial hydroxyl group. Since some axial alcohol was obtained, the reduction is not entirely dominated by favourable thermodynamic requirements.

Comparison of the formulae of the *trans*-2-decalones with those of the *trans*-1-decalones (Fig. 1) shows that, in the orientations depicted, the cyclohexane rings of the former are farther from the ketone group. It might therefore be expected that, on reduction of *trans*-2-decalone, both (±)-*trans*-*trans*-2- and (±)-*trans*-*cis*-2-decalol would be formed. However, only (±)-*trans*-*cis*-2-decalol was isolated. This compound has the hydroxyl group in the equatorial conformation, as compared with the *trans*-*trans*-2-isomer, which has an axial hydroxyl group. As steric hindrance was not a controlling factor in the reduction, thermodynamic factors must have played a dominant part, resulting in the production only of the more stable equatorial alcohols.

Fig. 2. Isomeric *cis*-decalones and derived decalols. (XIII), (-)-*cis*-1-; (XIV), (+)-*cis*-1-; (XV), (-)-*cis*-2-; (XVI), (+)-*cis*-2-decalone. (XVII), (-)-*cis-trans*-1-; (XVIII), (-)-*cis-cis*-1-; (XIX), (+)-*cis-trans*-1-; (XX), (+)-*cis-cis*-1-decalol. (XXI), (+)- or (-)-*cis-trans*-2-; (XXII), (+)- or (-)-*cis-cis*-2-; (XXIII), (+)- or (-)-*cis-trans*-2-; (XXIV), (+)- or (-)-*cis-cis*-2-decalol.

Fig. 3. Absolute configuration of the nicotinamide moiety of NADH, when attached (a) to an 'A' type enzyme and (b) to a 'B' type enzyme.

Fig. 4. Favourable (a) and unfavourable (b) 'face-to-face' alignments of *trans*-1-decalone with the nicotinamide moiety of NADH (partially overlaid).

Fig. 5. Favourable (a) and unfavourable (b) 'head-to-tail' approach (Prelog, 1959) of *trans*-1-decalone and the nicotinamide moiety of NADH. The configuration of the resulting alcohol is the same as that in the 'face-to-face' approach (see Fig. 4).

Inspection of the various possible orientations of the *cis*-1-decalones (Fig. 2) shows that, when the cyclohexanone ring is placed with its major plane in the plane of the paper, the cyclohexane ring lies either below or above that plane; the latter forms are reduced, the former are not. This suggests that

Scheme 1.

Fig. 6. Favourable (a) and unfavourable (b) 'face-to-face' alignments of *cis*-1-decalone with the nicotinamide moiety of NADH. Where the non-reacting ring of the decalone lies between its reacting ring and the nicotinamide ring (b), hydrogen transfer does not occur.

when the cyclohexane ring of the *cis*-decalones lies between the cyclohexanone ring and the nicotinamide ring the reactive centres are kept too far apart for reduction to occur. This results in the formation of the *cis-cis*-1- and *cis-cis*-2-decalols only.

A summary of the major metabolic pathways of the isomeric decalones is given in Scheme 1.

This work was supported (in part) by U.S. Public Health Service Grant no. GM-10042 from the General Medical Sciences Division.

REFERENCES

- Adams, R., Voorhees, V. & Shriner, R. L. (1948). *Org. Synth. (Coll. Vol.)*, 1, 463.
 Barton, D. H. R. (1953). *J. chem. Soc.* p. 1027.
 Bowers, A., Halsall, T. G., Jones, E. R. H. & Lemin, A. J. (1953). *J. chem. Soc.* p. 2548.
 Cornforth, J. W., Ryback, G., Popják, G., Donniger, C. & Schroepfer, G., jun. (1962). *Biochem. biophys. Res. Commun.* 9, 371.
 Djerassi, C. & Marshall, D. (1958). *J. Amer. chem. Soc.* 80, 3986.
 Djerassi, C. & Staunton, J. (1961). *J. Amer. chem. Soc.* 83, 736.
 Elliott, T. H., Parke, D. V. & Williams, R. T. (1959). *Biochem. J.* 72, 193.
 Elliott, T. H., Tao, R. C. C. & Williams, R. T. (1965). *Biochem. J.* 95, 70.
 Fisher, H. F., Conn, E. E., Vennesland, B. & Westheimer, F. H. (1953). *J. biol. Chem.* 202, 687.
 Hanson, S. W. F., Mills, G. T. & Williams, R. T. (1944). *Biochem. J.* 38, 274.
 Hüchel, W. (1925). *Liebigs Ann.* 441, 1.
 Hüchel, W., Mentzel, R., Brinkmann, E. & Kamenz, E. (1926). *Liebigs Ann.* 451, 109.
 Hüchel, W. & Rashingkar, R. B. (1960). *Liebigs Ann.* 637, 20.

- Hückel, W. & Riad, Y. (1960). *Liebigs Ann.* **637**, 33.
- Kamil, I. A., Smith, J. N. & Williams, R. T. (1952). *Biochem. J.* **50**, 235.
- Kritchevsky, D. & Kirk, M. R. (1952). *Arch. Biochem. Biophys.* **35**, 346.
- Levy, H. E., Talalay, P. & Vennesland, B. (1962). In *Progress in Stereochemistry*, vol. 3, p. 299. Ed. by de la Mare, P. P. D. & Klyne, W. London: Butterworths Scientific Publications.
- Paul, J. (1951). Ph.D. Thesis: University of Glasgow.
- Prelog, V. (1959). *Ciba Found. Study Group no. 2: Steric Course of Microbiological Reactions*, p. 79. Boston: Little, Brown and Co.
- Prelog, V. & Feldman, W. R. (1958). *Helv. chim. Acta*, **41**, 2396.
- Robertson, J. S. & Elliott, T. H. (1965). *Biochem. J.* **96**, 3P.
- Rodig, O. R. & Ellis, L. C. (1961). *J. org. Chem.* **26**, 2197.
- Sperber, I. (1948). *J. biol. Chem.* **172**, 441.
- Stekol, J. A. (1936). *J. biol. Chem.* **113**, 279.
- Vennesland, B. (1958). *Fed. Proc.* **17**, 1150.