Stereochemical Aspects of the Metabolism of the Isomeric Methylcyclohexanols and Methylcyclohexanones

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1. The seven isomeric optically inactive forms of methylcyclohexanol (i.e. 1-, and *cis*- and *trans*-2-, -3- and -4-) are excreted by rabbits mainly as glucuronides of the thermodynamically more stable forms of the alcohols. The eighth isomer, cyclohexylmethanol, however, undergoes aromatization *in vivo*, giving rise to benzoic acid and hippuric acid. The (±)-2-, (±)-3- and 4-methylcyclohexanones are reduced in the rabbit and excreted mainly as the glucuronides of the thermodynamically more stable forms of the corresponding methylcyclohexanols. 2. Racemic *cis*- and *trans*-2-methylcyclohexanol and 2-methylcyclohexanone are all excreted as conjugated *trans*-2-methylcyclohexanol. However, when the (±)-*cis*-alcohol or the (±)-ketone is fed, (+)-*trans*-2-methylcyclohexanol is excreted, whereas when the (±)-*trans*-alcohol is fed it is excreted as the (±)-*trans*-alcohol. 3. Racemic *cis*- and *trans*-3-methylcyclohexanol and 3-methylcyclohexanone are all excreted as conjugated racemic *cis*-3-methylcyclohexanol. *cis*- and *trans*-4-Methylcyclohexanol and 4-methylcyclohexanone are all excreted as conjugated *trans*-4-methylcyclohexanol. 4. The metabolic differences between the various methylcyclohexanols are explicable in terms of their conformations and of Vennesland's (1958) hypothesis of the role of NADH in dehydrogenation reactions.

During our investigation of the metabolism of methylcyclohexane (Elliott, Tao & Williams, 1965), it was considered probable that it would be metabolized in the body to one or other of the isomeric methylcyclohexanols in a manner similar to cyclohexane (Elliott, Parke & Williams, 1959). Although our primary aim in undertaking the present study was to obtain by biosynthesis a series of reference compounds for the study of methylcyclohexane, we were also particularly interested in the stereochemical potentialities of the methylcyclohexanols and methylcyclohexanones as substrates and the nature of their metabolic products.

In the present paper we report the results of feeding this series of alcohols and ketones to the rabbit. We also draw attention to the stereospecific nature of the metabolic processes and attempt to explain them in terms of the conformations of the substrates and Vennesland's (1958) hypothesis of the role of NADH in dehydrogenation reactions.

MATERIALS

Melting points are uncorrected. All materials were subjected to gas–liquid chromatography and shown to be pure, except (±)-*trans*-3- and *cis*-4-methylcyclohexanol where less than 3% of impurity was present. The infrared-absorption spectra of all the secondary methylcyclohexanols exhibited the diagnostic absorption bands described by Eliel & Lukach (1957), and the equatorial and axial absorptions were in agreement with those reported by Cole, Jefferies & Müller (1959), except that (±)-*trans*-2-methylcyclohexanol had an equatorial hydroxyl absorption at 3627 cm.$^{-1}$, which is the correct value (Dr A. R. H. Cole, personal communication).

The following alcohols were prepared according to the method of Eliel & Lukach (1957): (±)-*cis*-2-methylcyclohexanol, b.p. 165°, $n_D^{21\cdot7}$ 1·4639, $[\alpha]_D^{22\cdot3}$ 0°; 3,5-dinitrobenzoate, m.p. 99–100°; (±)-*trans*-3-methylcyclohexanol, b.p. 174°, $n_D^{20\cdot5}$ 1·4581 (literature value n_D^{20} 1·4583; see Macbeth & Mills, 1945; Goering & Serres, 1952), $[\alpha]_D^{21}$ 0°; 3,5-dinitrobenzoate, m.p. 108–109°; *cis*-4-methylcyclohexanol, b.p. 173°, n_D^{21} 1·4599; 3,5-dinitrobenzoate, m.p. 106–107°.

(±)-*trans*-2-Methylcyclohexanol was separated from a commercial mixture (British Drug Houses Ltd., Poole, Dorset) containing 67% of the *trans*-isomer by fractional recrystallization of the 3,5-dinitrobenzoate, once from heptane and twice from methanol. The pure ester, m.p. 117°, was hydrolysed for 16 hr. with 1·5 N-NaOH in aq. 80% (v/v) methanol, 2 moles of alkali being used/mole of ester. After the methanol has been removed, the alcohol was steam-distilled and recovered from the dried ethereal extract of the steam-distillate by fractionation under reduced pressure. The alcohol had b.p. 167°, $n_D^{24\cdot5}$ 1·4593, $[\alpha]_D^{24}$ 0°; 3,5-dinitrobenzoate, m.p. 116·5–117·5°.

(±)-*cis*-3-Methylcyclohexanol was obtained from a

cis–trans mixture (given by Howards of Ilford, Ltd., Essex) by preparing from it the pure hydrogen phthalate of the *cis*-isomer, m.p. 92–93°, which was recovered by steam-distillation in the presence of aq. 30% (w/v) NaOH. The recovered alcohol had b.p. 173°, n_D^{23} 1·4559 (literature value n_D^{20} 1·4573; see Macbeth & Mills, 1945; Goering & Serres, 1952), $[\alpha]_D^{24}$ 0°; 3,5-dinitrobenzoate, m.p. 98–99°.

trans-4-Methylcyclohexanol was obtained from a commercial sample (Eastman Organic Chemicals, Rochester, N.Y., U.S.A.) containing about 70% of the *trans*-isomer by fractional recrystallization from ethanol of its 3,5-dinitrobenzoate. Hydrolysis of the pure ester was effected by 1·5N-NaOH in aq. 80% methanol and the recovered alcohol had b.p. 174°, n_D^{23} 1·4542; 3,5-dinitrobenzoate, m.p. 141–142°.

1-Methylcyclohexanol was synthesized by the method of Zelinsky (1901). It had b.p. 154–154·5°, n_D^{22} 1·4580; 3,5-dinitrobenzoate, m.p. 132–133°.

Cyclohexylmethanol was prepared by the method of Gilman & Blatt (1941). It had b.p. 182–184°, $n_D^{23\cdot2}$ 1·4630; 3,5-dinitrobenzoate, m.p. 94–95°.

The following compounds were purchased and twice redistilled: (±)-2-Methylcyclohexanone (British Drug Houses Ltd.), b.p. 162–164°, $n_D^{19\cdot8}$ 1·4480, $[\alpha]_D^{22\cdot2}$ 0°; 2,4-dinitrophenylhydrazone, m.p. 137–138° (trace impurities were removed by gas–liquid chromatography on a Celite–diglycerol column). (±)-3-Methylcyclohexanone (Eastman Organic Chemicals), b.p. 169–171°, n_D^{25} 1·4430, $[\alpha]_D^{23}$ 0°; semicarbazone, m.p. 178–179°. 4-Methylcyclohexanone (Eastman Organic Chemicals), b.p. 168–171°, n_D^{24} 1·4429; 2,4-dinitrophenylhydrazone, m.p. 134–135°.

METHODS

Animals and diet. Rabbits weighing about 1–2kg. were used. They were kept on a constant diet consisting of 4oz. of sweet potatoes (*Ipomoea batatas*) and 4oz. of kangkong (*Ipomoea reptans*) daily.

Administration of dose. The compounds were administered in water by stomach tube.

Analytical methods. The following were used: determination of glucuronic acid by the method of Hanson, Mills & Williams (1944), as modified by Paul (1951); determination of ethereal sulphate by the method of Sperber (1948); determination of mercapturic acid by the method of Stekol (1936); determination of hippuric acid by formol titration (Quick, 1926); determination of total benzoic acid by the method of Kingsbury & Swanson (1921).

General method for the isolation of metabolites and their hydrolysis. All glucuronides were isolated from urine by the lead acetate precipitation method of Kamil, Smith & Williams (1951). The gum obtained on concentration of the solution after separation of the lead sulphide normally showed no tendency to crystallize. Consequently, it was methylated with an ethereal solution of diazomethane, and then acetylated with excess of acetic anhydride and pyridine and allowed to stand overnight. A semi-solid gum was obtained on pouring the crude ester into water, and, after successive treatments with saturated $NaHCO_3$, dilute HCl and washing with water until neutral, a solid ester was obtained that was recrystallized from ethanol to constant m.p. The glucuronides and triacetyl methyl esters were hydrolysed to establish the identity of the aglycone. In all cases, except for the glucuronide of 1-methylcyclohexanol, where enzymic hydrolysis proved more satisfactory, the hydrolysis procedure was to reflux with 25ml. of N-HCl in a glycerol bath maintained at 130° for 15–30 min. The hydrolysate was then steam-distilled and the distillate was extracted with ether, dried and the ether removed, and the residual alcohol was then esterified in pyridine with 3,5-dinitrobenzoyl chloride. The resultant ester was recrystallized from ethanol. The triacetyl methyl esters were treated similarly except that refluxing was prolonged to 2–3hr.

RESULTS

Experiments on urine

In all cases (except with cyclohexylmethanol) within 30min. after feeding the alcohols and ketones at a dose of approx. 5m-moles/kg. body wt., the animals showed signs of narcosis that lasted 2–5hr. The onset of narcosis was particularly rapid (within 5min. after feeding) with 1-methylcyclohexanol and lasted much longer.

Qualitative experiments on urine. The 24hr. urine had pH 8–9 and in all cases (except with cyclohexylmethanol) gave an intense naphtharesorcinol reaction. It did not reduce Benedict's solution and failed to give a precipitate with Brady's reagent. It gave a negative response when tested for phenols with ferric chloride solution and with 2,6-dichloroquinonechloroimide.

Quantitative estimations on urine. The results of the estimations of glucuronic acid, ethereal sulphate and mercapturic acid after the administration of the various methylcyclohexanols and methylcyclohexanones to rabbits are summarized in Tables 1 and 2. Table 3 illustrates the excretion of hippuric acid and total benzoic acid after the administration of cyclohexylmethanol.

Isolation and characterization of metabolites

The melting points and optical rotations of the pure glucuronide triacetyl methyl esters isolated are reported in Table 4, and those of the 3,5-dinitrobenzoates prepared from the secondary alcohol aglycones of the various glucuronides and their triacetyl methyl esters after hydrolysis are shown in Table 5.

(±)-trans-2-*Methylcyclohexanol.* The alcohol (1ml.) was fed to each of three rabbits. From the 18hr. urine was isolated a pale-yellow glucuronide gum (4g.), which, on methylation, gave 4g. of crude methyl ester. Acetylation yielded 4·8g. of solid triacetyl methyl ester, which, after three recrystallizations from ethanol, yielded 0·43g. of pure *methyl* [(+)-trans-2-*methylcyclohexyl tri*-O-*acetyl*-β-D-*glucosid*]*uronate*, m.p. 157–158°, $[\alpha]_D^{23}$ −9·8±1° (*c* 1·4 in chloroform) (Found: C, 55·8; H, 7·0; O, 37·4. $C_{20}H_{30}O_{10}$ requires C, 55·8; H, 7·0;

Table 1. *Glucuronic acid excretion of the isomeric methylcyclohexanols and methylcyclohexanones*

Experimental details are given in the text. The results given are average values, with the ranges for three separate determinations in parentheses.

Compound administered	Dose (m-moles/kg. body wt.)	Percentage of dose excreted as glucuronide
1-Methylcyclohexanol	4·7	74·1 (66·0–83·6)
(±)-*cis*-2-Methylcyclohexanol	5·1	71·4 (67·0–75·1)
(±)-*trans*-2-Methylcyclohexanol	6·0	84·8 (78·5–91·1)
(±)-*cis*-3-Methylcyclohexanol	5·5	55·7 (47·7–59·7)
(±)-*trans*-3-Methylcyclohexanol	5·8	73·7 (63·8–87·0)
cis-4-Methylcyclohexanol	5·8	73·8 (61·9–79·8)
trans-4-Methylcyclohexanol	6·0	81·1 (73·8–88·9)
Cyclohexylmethanol	7·5	0·4 (0·0– 1·0)
(±)-2-Methylcyclohexanone	4·6	72·3 (53·4–92·6)
(±)-3-Methylcyclohexanone	5·1	73·6 (49·8–89·7)
4-Methylcyclohexanone	5·4	80·3 (74·7–85·9)

Table 2. *Ethereal sulphate and mercapturic acid excretion of the isomeric methylcyclohexanols and methylcyclohexanones*

Experimental details are given in the text. The results given are average values, with the ranges for three separate determinations in parentheses

Compound administered	Dose (m-moles/kg. body wt.)	Percentage of dose excreted	
		As ethereal sulphate	As mercapturic acid
1-Methylcyclohexanol	4·7	0·6 (0·0–1·8)	0
(±)-2-Methylcyclohexanol*	4·6	3·3 (1·9–5·2)	0
(±)-3-Methylcyclohexanol†	4·6	0·9 (0·2–2·0)	0
4-Methylcyclohexanol‡	4·6	3·5 (0·9–6·7)	0
Cyclohexylmethanol	4·6	2·4 (1·6–3·3)	0
(±)-2-Methylcyclohexanone	4·6	0·9 (0·0–2·6)	0
(±)-3-Methylcyclohexanone	5·1	2·2 (0·5–4·0)	0
4-Methylcyclohexanone	5·4	1·1 (0·0–3·2)	0

* Containing approx. 67% of the *trans*-isomer and 33% of the *cis*-isomer (obtained from British Drug Houses Ltd.).

† Containing approx. 65% of the *cis*-isomer and 35% of the *trans*-isomer (obtained from Howards of Ilford, Ltd.).

‡ Containing approx. 70% of the *trans*-isomer and 30% of the *cis*-isomer (obtained from Eastman Organic Chemicals).

Table 3. *Excretion of hippuric acid and total benzoic acid after feeding with cyclohexylmethanol*

Experimental details are given in the text.

Rabbit no.	Dose (m-moles/kg. body wt.)	Percentage of dose excreted (calc. as benzoic acid)		
		Total benzoic acid	Hippuric acid	Free benzoic acid (by diff.)
1	6·5	60·6	1·6	59·0
2	7	63·9	3·5	60·4
3	9	68·4	6·6	61·8
	Average	64·3	3·9	60·4

O, 37·2%). The assignment of a positive rotation to the alcohol aglycone is based on experimental evidence described below.

Hydrolysis of the above triacetyl methyl ester. The pure ester (0·6g.), after hydrolysis and esterification with 3,5-dinitrobenzoyl chloride, yielded a product, which, after two recrystallizations from ethanol, gave (+)-trans-2-*methylcyclohexyl* 3,5-*dinitrobenzoate*, $[\alpha]_D^{24·8}$ +56·6° (*c* 0·8 in chloroform), m.p. 126–127° not depressed when mixed with the 3,5-dinitrobenzoyl derivative of the alcohol obtained from hydrolysis of either the glucuronide found in

Table 4. *Melting points and optical rotations of triacetyl methyl esters*

Compound administered	Properties of triacetyl methyl ester: m.p.	Properties of triacetyl methyl ester: $[\alpha]_D^{22-24}$ (in $CHCl_3$)
(±)-*cis*-2-Methylcyclohexanol	157–158°	−9·8°
(±)-*trans*-2-Methylcyclohexanol	157–158	−9·8
(±)-2-Methylcyclohexanone	157–158	−9·8
(±)-*cis*-3-Methylcyclohexanol	140·5–142	−26·9
(±)-*trans*-3-Methylcyclohexanol	140–141	−26·6
(±)-3-Methylcyclohexanone	140–141	−26·1
cis-4-Methylcyclohexanol	143	−33·0
trans-4-Methylcyclohexanol	142–143	−32·7
4-Methylcyclohexanone	142–143	−32·3
1-Methylcyclohexanol	109–110	−29·7

Table 5. *Characterization of metabolites*

Optical rotations were measured in chloroform; details of temperature and concentration are given in the text.

Compound administered	3,5-Dinitrobenzoate of alcohol: From the parent compound m.p.	From the glucuronide m.p.	From the glucuronide $[\alpha]_D$	From the crude triacetyl methyl ester m.p.	From the crude triacetyl methyl ester $[\alpha]_D$	From the pure triacetyl methyl ester m.p.	From the pure triacetyl methyl ester $[\alpha]_D$
(±)-*cis*-2-Methylcyclohexanol	100°	126°	+56·6°	—	—	—	—
(±)-*trans*-2-Methylcyclohexanol	117	117	0	117°	0°	126°	+56·6°
(±)-2-Methylcyclohexanone	—	126	+56·2	—	—	—	—
(±)-*cis*-3-Methylcyclohexanol	98	98	0	—	—	—	—
(±)-*trans*-3-Methylcyclohexanol	108	98	0	—	—	—	—
(±)-3-Methylcyclohexanone	—	98	0	98	0	96	+15·3
cis-4-Methylcyclohexanol	107	141	—	—	—	141	—
trans-4-Methylcyclohexanol	141	—	—	—	—	141	—
4-Methylcyclohexanone	—	—	—	—	—	141	—

the urine after feeding (±)-*cis*-2-methylcyclohexanol or that after feeding (±)-2-methylcyclohexanone (Found: C, 54·9; H, 5·3; N, 9·2. Calc. for $C_{14}H_{16}N_2O_6$: C, 54·5; H, 5·2; N, 9·1%).

Hydrolysis of the glucuronide. (±)-*trans*-2-Methylcyclohexanol (1·5ml.) was administered to two rabbits and the glucuronide was isolated from the 18hr. urine. After hydrolysis, the alcohol, worked up as described above, gave 1·4g. of crude 3,5-dinitrobenzoyl derivative.

Two recrystallizations yielded 0·7g. of pure (±)-*trans*-2-methylcyclohexyl 3,5-dinitrobenzoate, m.p. and mixed m.p. with an authentic specimen 116·5–117·5°, $[\alpha]_D^{20\cdot5}$ 0° (*c* 0·7 in chloroform).

Thus hydrolysis of the glucuronide yields racemic *trans*-2-methylcyclohexyl 3,5-dinitrobenzoate, whereas hydrolysis of the pure triacetyl methyl ester yields optically active (+)-*trans*-2-methylcyclohexyl 3,5-dinitrobenzoate. This is explicable if the triacetyl methyl ester undergoes diastereoisomeric resolution during recrystallization. To confirm this the following experiments were carried out. (±)-*trans*-2-Methylcyclohexanol (3ml.) was fed to each of six rabbits. This yielded 3·9g. of crude triacetyl methyl ester, which was repeatedly recrystallized. The optical activity progressively changed after each recrystallization, and finally became constant when the pure diastereoisomer separated. In addition, 1·8g. of the triacetyl methyl ester that had not been recrystallized was hydrolysed with dilute acid; the alcohol recovered from the steam-distillate gave, on esterification, a 3,5-dinitrobenzoyl derivative, which, after two recrystallizations, yielded pure (±)-*trans*-2-methylcyclohexyl 3,5-dinitrobenzoate, m.p. and mixed m.p. with an authentic specimen 116·5–117·5°, $[\alpha]_D^{23}$ 0° (*c* 0·8 in chloroform).

(±)-cis-2-*Methylcyclohexanol*. This alcohol (3ml.) was administered to three rabbits and, from the 24hr. urine, 3·5g. of gum was obtained; on methylation and acetylation it yielded 3·2g. of crude triacetyl methyl ester. Six recrystallizations from ethanol yielded 0·3g. of pure ester, m.p. 157–158° unchanged on admixture with methyl [(+)-*trans*-

2-methylcyclohexyl tri-*O*-acetyl-β-D-glucosid]uronate, $[\alpha]_D^{22}$ −9·8° (*c* 1·5 in chloroform) (Found: C, 56·0; H, 7·0; O, 37·1. Calc. for $C_{20}H_{30}O_{10}$: C, 55·8; H, 7·0; O, 37·2%).

Hydrolysis of the glucuronide. (±)-*cis*-2-Methylcyclohexanol (1·8ml.) was fed to two rabbits and the glucuronide gum (2g.) was isolated. On hydrolysis this gum yielded 0·5ml. of an alcohol, which, on esterification with 3,5-dinitrobenzoyl chloride followed by seven recrystallizations of the product from ethanol, yielded 0·1g. of pure *dextro*-rotatory 3,5-dinitrobenzoyl derivative, $[\alpha]_D^{23·5}$ + 56·5° (*c* 0·85 in chloroform), m.p. 126° not depressed when admixed with the 3,5-dinitrobenzoate of (+)-*trans*-2-methylcyclohexanol obtained from hydrolysis of pure methyl [(+)-*trans*-2-methylcyclohexyl tri-*O*-acetyl-β-D-glucosid]uronate (Found: C, 54·9; H, 5·4; N, 9·3. $C_{14}H_{16}N_2O_6$ requires C, 54·5; H, 5·2; N, 9·1%).

The *trans*-isomer obtained on feeding (±)-*cis*-2-methylcyclohexanol indicated that the less stable *cis*-form had inverted (at least in part) to the more stable *trans*-epimer. That this *cis–trans* inversion had occurred *in vivo* and not during the acid hydrolysis procedure was indicated by the following experiment.

(±)-*cis*-2-Methylcyclohexanol (1·4ml.) was refluxed with 25ml. of N-hydrochloric acid for 15min. in a glycerol bath maintained at 125–130°, and from the hydrolysate the alcohol was recovered (0·8ml.). This yielded, on esterification, 1·6g. of the crude ester. Two recrystallizations from ethanol gave 0·87g. of pure (±)-*cis*-2-methylcyclohexyl 3,5-dinitrobenzoate, m.p. and mixed m.p. with an authentic specimen 99–100°.

(±)-2-*Methylcyclohexanone*. The ketone (4ml.) was administered to four rabbits and the 18hr. urine was used for the isolation of the metabolite. The glucuronide gum yielded 5·5g. of crude triacetyl methyl ester, which, after recrystallization from ethanol, gave 0·7g. of pure ester, m.p. 157–158° not depressed when admixed with methyl [(+)-*trans*-2-methylcyclohexyl tri-*O*-acetyl-β-D-glucosid]uronate, $[\alpha]_D^{24}$ −9·8 ± 1° (*c* 1·1 in chloroform).

Hydrolysis of the glucuronide. (±)-2-Methylcyclohexanone (2ml.) was fed to each of two rabbits, and the glucuronide isolated from the urine was hydrolysed. The liberated alcohol gave, on esterification, 1·2g. of crude 3,5-dinitrobenzoyl derivative, which, after six recrystallizations from ethanol, yielded 0·1g. of pure material, $[\alpha]_D^{23}$ + 56·2° (*c* 0·8 in chloroform), m.p. 126–127° not depressed when mixed with the 3,5-dinitrobenzoyl derivative of the alcohol obtained from hydrolysis of the glucuronide found in the urine after feeding (±)-*cis*-2-methylcyclohexanol.

(±)-cis-3-*Methylcyclohexanol*. The alcohol (1ml.) was administered to each of three rabbits. The isolated glucuronide gum (4g.), on methylation and acetylation, yielded 5·5g. of the crude triacetyl methyl ester, which, after three recrystallizations, yielded 0·3g. of pure *methyl* [(+)-cis-3-*methylcyclohexyl tri*-O-*acetyl*-β-D-*glucosid*]*uronate*, m.p. 140·5–142°, $[\alpha]_D^{24}$ −26·9 ± 1° (*c* 2 in chloroform) (Found: C, 56·0; H, 6·9; O, 37·4. $C_{20}H_{30}O_{10}$ requires C, 55·8; H, 7·0; O, 37·2%).

The assignment of a positive rotation to the alcohol aglycone is based on the experimental evidence described below.

Hydrolysis of the glucuronide triacetyl methyl ester. The pure ester (0·9g.), when refluxed with N-hydrochloric acid for 2·5hr., yielded an alcohol, which, on esterification, gave 0·1g. of pure *dextro*-rotatory *cis*-3-methylcyclohexyl 3,5-dinitrobenzoate, $[\alpha]_D^{24}$ + 5·3° (*c* 0·6 in chloroform), m.p. 96–97° (see Macbeth & Mills, 1947; Goering & Serres, 1952). The melting point of the compound was depressed when mixed with (±)-*cis*-3-methylcyclohexyl 3,5-dinitrobenzoate (Found: C, 54·6; H, 5·3; N, 9·3. $C_{14}H_{16}N_2O_6$ requires C, 54·5; H, 5·2; N, 9·1%).

Hydrolysis of the glucuronide. The glucuronide gum obtained after administering 1·5ml. of (±)-*cis*-3-methylcyclohexanol to rabbits, on hydrolysis, yielded an alcohol, which, on esterification, gave 0·6g. of crude 3,5-dinitrobenzoyl derivative. After two recrystallizations from ethanol 0·24g. of pure (±)-*cis*-3-methylcyclohexyl 3,5-dinitrobenzoate was obtained, m.p. and mixed m.p. with an authentic specimen 98–98·5°, $[\alpha]_D^{21}$ 0° (*c* 0·8 in chloroform).

(±)-trans-3-*Methylcyclohexanol*. This alcohol (3ml.) was fed to two rabbits (1·5ml. each) and from the 24hr. urine was isolated 2g. of glucuronide, which gave 4·5g. of crude triacetyl methyl ester.

After the usual treatment, followed by five recrystallizations from ethanol, this yielded 0·25g. of pure ester, $[\alpha]_D^{22}$ −26·6 ± 1° (*c* 1 in chloroform), m.p. 140–141° not depressed on admixture with methyl [(+)-*cis*-3-methylcyclohexyl tri-*O*-acetyl-β-D-glucosid]uronate (Found: C, 56·1; H, 7·1; O, 37·1. $C_{20}H_{30}O_{10}$ requires C, 55·8; H, 7·0; O, 37·2%).

Hydrolysis of the glucuronide. (±)-*trans*-3-Methylcyclohexanol (1·4ml.) was fed to two rabbits and the 24hr. urine was worked up for the glucuronide. The gum, when hydrolysed with acid, yielded 0·6ml. of the alcohol, 0·4ml. of which was esterified, giving 0·03g. of pure (±)-*cis*-3-methylcyclohexyl 3,5-dinitrobenzoate after five recrystallizations, m.p. and mixed m.p. 98°, $[\alpha]_D^{20·5}$ 0° (*c* 1·6 in chloroform).

It would again appear that inversion from the less stable to the more stable epimer had occurred, as was the case with the corresponding 2-methylcyclohexanols. To exclude the possibility that this inversion had occurred during the hydrolytic procedure, (±)-*trans*-3-methylcyclohexanol (1ml.) was refluxed with 25ml. of N-hydrochloric acid

for 3hr. in a glycerol bath maintained at 120–130°. From the hydrolysate was recovered 0·6ml. of alcohol, which, on esterification, yielded 1·3g. of 3,5-dinitrobenzoyl derivative. After two recrystallizations from ethanol, a total of 0·7g. of pure (±)-*trans*-3-methylcyclohexyl 3,5-dinitrobenzoate was obtained, m.p. and mixed m.p. with an authentic specimen 108–109°.

(±)-3-*Methylcyclohexanone*. This ketone (5ml.) was fed to five rabbits and from the 22hr. urine there was obtained 7·5g. of glucuronide gum, yielding 7g. of crude triacetyl methyl ester. After two recrystallizations from ethanol, 0·9g. of pure ester was obtained, $[\alpha]_D^{24}$ −26·1° (*c* 3 in chloroform), m.p. 140–141° not depressed when mixed with pure methyl [(+)-*cis*-3-methylcyclohexyl tri-*O*-acetyl-β-D-glucosid]uronate.

Hydrolysis of the glucuronide gum obtained from feeding 2ml. of (±)-3-methylcyclohexanone to rabbits yielded, on hydrolysis, an alcohol, which, on esterification with 3,5-dinitrobenzoyl chloride, gave, after two recrystallizations, 0·8g. of pure (±)-*cis*-3-methylcyclohexyl 3,5-dinitrobenzoate, m.p. and mixed m.p. with an authentic specimen 98–98·5°, $[\alpha]_D^{20\cdot5}$ 0° (*c* 2·4 in chloroform).

To show that the optical activity of the aglycone of the pure glucuronide triacetyl methyl ester had arisen as a result of diastereoisomeric resolution, 3·8g. of the crude ester was prepared and the course of its purification in recrystallization was followed polarimetrically. Again progressive changes in optical activity of the compound occurred in successive recrystallizations until a constant value was obtained. Further, 2g. of the triacetyl methyl ester, without preliminary purification by recrystallization, when hydrolysed and esterified, yielded optically inactive *cis*-3-methylcyclohexyl 3,5-dinitrobenzoate, m.p. and mixed m.p. 98°, $[\alpha]_D^{23}$ 0° (*c* 1·1 in chloroform).

trans-4-*Methylcyclohexanol*. Three rabbits were each given 1ml. of *trans*-4-methylcyclohexanol. The 23hr. urine was collected and from it was obtained 6·5g. of crude glucuronide gum (with some fine needle crystals embedded). This yielded 6·3g. of crude triacetyl methyl ester, which, after two recrystallizations from ethanol, yielded 2·2g. of *methyl* (trans-4-*methylcyclohexyl tri*-O-*acetyl*-β-D-*glucosid*)*uronate*, m.p. 142–143°, $[\alpha]_D^{23\cdot2}$ −32·7±1° (*c* 3 in chloroform) (Found: C, 56·1; H, 7·0; O, 37·0. $C_{20}H_{30}O_{10}$ requires C, 55·8; H, 7·0; O, 37·2%).

Hydrolysis of the glucuronide triacetyl methyl ester. The pure ester (1·2g.) obtained from feeding *trans*-4-methylcyclohexanol was refluxed with N-hydrochloric acid for 5hr., and from the recovered alcohol was prepared the 3,5-dinitrobenzoyl derivative, which, after two recrystallizations, yielded 0·25g. of pure *trans*-4-methylcyclohexyl 3,5-dinitrobenzoate, m. p. and mixed m.p. with an authentic specimen 141–141·5°.

cis-4-*Methylcyclohexanol*. The alcohol (3ml.) was administered to three rabbits, and from the 23hr. urine was isolated 5g. of crude glucuronide gum, from which 5g. of the crude triacetyl methyl ester was obtained. This, after three recrystallizations, yielded 1·3g. of the pure ester, m.p. 143° not depressed on admixture with methyl [*trans*-4-methylcyclohexyl tri-*O*-acetyl-β-D-glucosid]uronate, $[\alpha]_D^{22}$ −33±1° (*c* 2·6 in chloroform) (Found: C, 56·0; H, 7·1. $C_{20}H_{30}H_{10}$ requires C, 55·8; H, 7·0%).

Hydrolysis of the triacetyl methyl ester. The ester (0·8g.), on being refluxed with 30ml. of N-hydrochloric acid for 5hr., yielded an alcohol, which, on esterification, gave, after three recrystallizations from ethanol, 0·13g. of pure *trans*-4-methylcyclohexyl 3,5-dinitrobenzoate, m.p. and mixed m.p. 141–141·5°.

Hydrolysis of the glucuronide gum. In another experiment, a total of 1·8ml. of *cis*-4-methylcyclohexanol was fed to two rabbits and the 22hr. urine was worked up for the glucuronide. This, when refluxed for 0·5hr. with N-hydrochloric acid, yielded 1ml. of alcohol, of which 0·5ml. was used for preparing the 3,5-dinitrobenzoyl derivative. After four recrystallizations from ethanol, 0·25g. of pure *trans*-4-methylcyclohexyl 3,5-dinitrobenzoate (m.p. and mixed m.p. 141–142°) was obtained, indicating that *cis–trans* inversion had again occurred.

cis-4-Methylcyclohexanol (1·2ml.), refluxed with 2N-hydrochloric acid for 0·5hr. followed by preparation of the 3,5-dinitrobenzoyl derivative of the resultant alcohol, yielded 1·1g. of pure *cis*-4-methylcyclohexyl 3,5-dinitrobenzoate, m.p. and mixed m.p. 106–107°.

4-*Methylcyclohexanone*. The ketone (4·5ml.) was fed to rabbits, and 9g. of glucuronide gum was isolated from the 24hr. urine. This was methylated and acetylated to yield 10g. of crude triacetyl methyl ester, which, after three recrystallizations, yielded 1·7g. of pure ester, m.p. 142–143° not depressed on admixture with methyl [*trans*-4-methylcyclohexyl tri-*O*-acetyl-β-D-glucosid]uronate, $[\alpha]_D^{24}$ −32·3±1° (*c* 3 in chloroform).

The above ester (0·5g.) was hydrolysed with 10ml. of N-hydrochloric acid for 4hr. The liberated alcohol, worked up in the usual manner, yielded *trans*-4-methylcyclohexyl 3,5-dinitrobenzoate on esterification (m.p. and mixed m.p. 141°).

With the isomeric 4-methylcyclohexanols and 4-methylcyclohexanone, the molecules are symmetrical, so that the question of optical segregation does not arise. However, *cis–trans* inversion once again occurred and the thermodynamically more stable *trans*-epimer was formed.

1-*Methylcyclohexanol*. This alcohol (5ml.) was

fed to rabbits and the basic lead acetate precipitation for isolation of the glucuronide was carried out on the 22hr. urine. The aqueous solution, after the separation of lead sulphide, was freeze-dried; the dried gum was methylated and acetylated, and, when the product was poured into water, a semi-solid gummy material was obtained; on prolonged treatment with sodium hydrogen carbonate this yielded 6g. of solid crude triacetyl methyl ester. Recrystallization from most of the common solvents was difficult. Ethanol appeared to be most suitable, but even in this solvent the material was rather soluble and crystal formation was slow. After five recrystallizations from a small amount of solvent, long needles of *methyl (1-methylcyclohexyl tri-O-acetyl-β-D-glucosid)uronate* were obtained (0·15g.), m.p. 109–110°, $[\alpha]_D^{23}$ −29·7° (*c* 1·1 in chloroform) (Found: C, 55·7; H, 7·1; O, 37·4. $C_{20}H_{30}O_{10}$ requires C, 55·8; H, 7·0; O, 37·2%).

Hydrolysis of the glucuronide. Acid, alkaline or neutral hydrolysis of the glucuronide was found to be unsatisfactory. Accordingly, hydrolysis with a β-glucuronidase preparation was attempted.

1-Methylcyclohexanol (2·5ml.) was fed to four rabbits. The combined urine was treated with lead acetate in the usual manner. The aqueous solution, after removal of lead sulphide, was adjusted to pH 8 with sodium hydrogen carbonate. The alkaline solution was then concentrated under reduced pressure at 40° to about 15ml. The concentrate was adjusted to pH 4·5 with acetic acid, and to this was added 10ml. of the centrifuged crop juice of the giant African snail (*Achatina fulica*). The mixture was left at room temperature (30°) for the 3 weeks found to be necessary in this case for effective hydrolysis. The alcohol was then extracted with ether, the ether removed and the residue esterified with 3,5-dinitrobenzoyl chloride. After four recrystallizations from ethanol, pure 1-methylcyclohexyl 3,5-dinitrobenzoate was obtained, m.p. and mixed m.p. 132°.

Cyclohexylmethanol. This alcohol (5ml.) was fed to five rabbits. The 24hr. urine had pH 9, gave a very weak positive naphtharesorcinol test, did not reduce Benedict's solution, gave no precipitate with Brady's reagent and showed no blue colour with 2,6-dichloroquinonechloroimide in sodium hydrogen carbonate solution. When attempts were made to isolate the glucuronide in the normal manner, after decomposition of the lead salt, a white granular crystalline material was deposited from the aqueous solution during concentration *in vacuo*. The mixture was cooled in ice and the crystalline material filtered off (0·5g.). The filtrate was concentrated to a small volume and cooled, and when no further crystals were deposited it was dried, giving a small amount of oil that was insufficient in quantity for characterization.

The white granular precipitate obtained during the concentration process had the properties of benzoic acid. After one recrystallization from hot water, the pure material, m.p. 121–122°, was obtained (mixed m.p. 121–122° with benzoic acid) (Found: C, 68·8; H, 4·9; O, 26·4. $C_7H_6O_2$ requires C, 68·8; H, 5; O, 26·2%).

On another occasion, the only metabolite similarly isolated was hippuric acid. Cyclohexylmethanol (4ml.) was fed to four rabbits and the aqueous solution (see above), on concentration, deposited white needles (0·5g.). These were filtered off from the ice-cooled solution and recrystallized once from water. They had m.p. 187·5° unchanged on admixture with hippuric acid.

Paper chromatography

The R_F values of the glucuronides as they occur in rabbit urine were determined by the descending technique at 21–23°, about 30μl. of urine being used. Papers (Whatman no. 1) were equilibrated for 2hr. with water where the system consisted of a single phase, and with the lower phase if there were two phases, and the upper layer was used for development. In a determination the solvent front was allowed to travel 25–30cm. from the origin. The chromatograms were sprayed with the naphtharesorcinol reagent (Elliott *et al.* 1965). Four solvent systems were used: *A*, butan-1-ol–pyridine–water (4:1:5, by vol.); *B*, butan-1-ol–acetic acid–water (4:1:5, by vol.); *C*, propan-1-ol–aq. ammonia (sp.gr. 0·91) (3:1, v/v); *D*, butan-2-one–acetic acid–water (4:1:5, by vol.). The R_F values of the glucuronides obtained after administering each of the isomeric methylcyclohexanones and secondary methylcyclohexanols were almost identical. Thus in solvent *A* the R_F value was 0·27, in solvent *B* it was 0·79, in solvent *C* it was 0·85 and in solvent *D* it was 0·88. The R_F values of the glucuronides of 1-methylcyclohexanol were 0·04 (faint) and 0·24 in solvent *A* and 0·36 (faint) and 0·75 in solvent *B*.

Gas–liquid chromatography

To confirm that the inversion of alcohols detected on feeding (±)-*cis*-2-, (±)-*trans*-3- and *cis*-4-methylcyclohexanol was effected *in vivo*, pure (±)-*cis*-2-, (±)-*trans*-3- and *cis*-4-methylcyclohexanol were each refluxed with N-hydrochloric acid in a glycerol bath maintained at 130° for 15min., and the alcohols recovered from the corresponding steam-distillates of the reaction mixtures were subjected to gas–liquid chromatography by using a column of 10% (w/w) diglycerol on Celite at 118°. No significant amount of inversion was detected.

A gas–liquid-chromatographic investigation was also carried out to investigate further the metabolites obtained after administration of the various methylcyclohexanones. (±)-2-, (±)-3- and 4-Methylcyclohexanone were fed to rabbits (approx. 5m-moles/kg. body wt.), and the isolated glucuronides were hydrolysed with N-hydrochloric acid at 130° for 15min. and with β-glucuronidase (Sigma type III) in acetate buffer, pH 5, at 37°.

Alcohols extracted at pH 7 from hydrolysis mixtures were chromatographed on diglycerol–Celite. By comparison with reference materials, the proportions of *cis*- to *trans*-alcohols obtained from the various feeds were found to be: (±)-2-methylcyclohexanone, *trans*/*cis* ratio 3·2:1 (acid hydrolysis) and 3:1 (enzyme hydrolysis); (±)-3-methylcyclohexanone, *cis*/*trans* ratio 14:1 (acid hydrolysis) and 15:1 (enzyme hydrolysis); 4-methylcyclohexanone, *trans*/*cis* ratio 5:1 (acid hydrolysis).

Thin-layer chromatograms (allowed to run 15cm. in benzene; Dhont & De Rooy, 1961) of 3,5-dinitrobenzoates prepared from the metabolically produced crude alcohol mixtures obtained from the various methylcyclohexanone feeds also indicated the presence of both *cis*- and *trans*-isomers in all cases. Thus 4-methylcyclohexanone gave two spots corresponding to *cis*-4-methylcyclohexanol (R_F 0·58) and *trans*-4-methylcyclohexanol (R_F 0·63), (±)-3-methylcyclohexanone gave *cis*-3-methylcyclohexanol (R_F 0·69) and *trans*-3-methylcyclohexanol (R_F 0·65); (±)-2-methylcyclohexanone gave *cis*-2-methylcyclohexanol (R_F 0·51) and *trans*-2-methylcyclohexanol (R_F 0·54). (In the last case, the chromatogram was allowed to run 36cm.) The spots corresponding to *trans*-2-, *cis*-3- and *trans*-4-methylcyclohexanol were much more intense than those of their epimers.

DISCUSSION

The isomeric methylcyclohexanones are all highly conjugated in the rabbit. Thus (±)-2-, (±)-3- and 4-methylcyclohexanone, administered at doses of 4·6, 5·1 and 5·4m-moles/kg. body wt. respectively, produce increases in glucuronic acid excretion corresponding to 72·3, 73·6 and 80·3% of the dose, and increases in ethereal sulphate excretion corresponding to 0·9, 2·2 and 1·1% of dose.

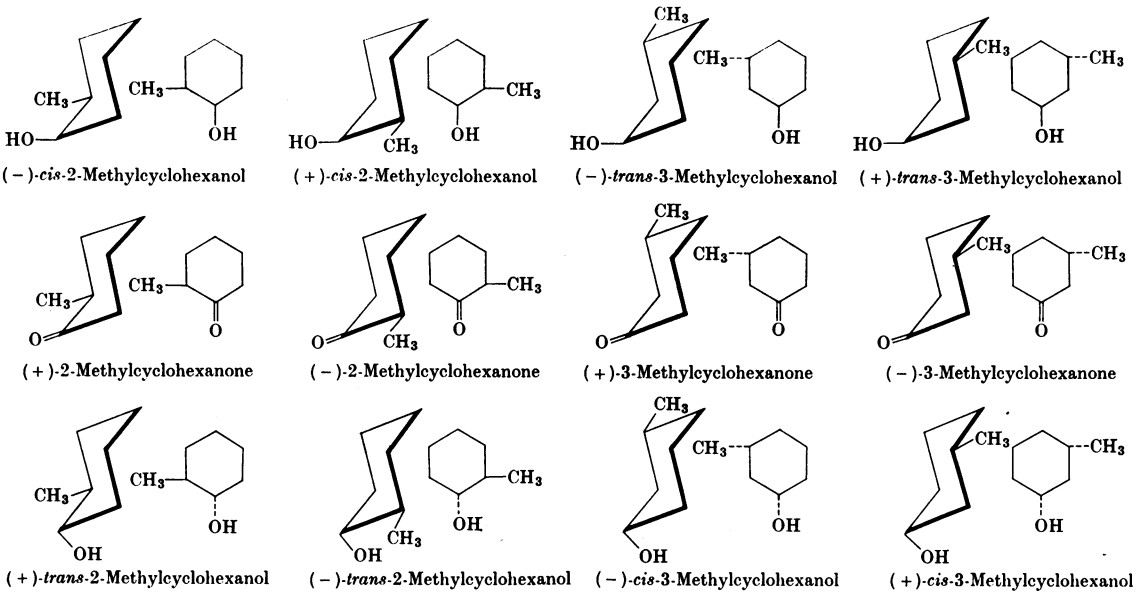
The principal metabolites appear, from paper-chromatographic evidence, to be glucuronides of the corresponding alcohols, and this was confirmed by acid hydrolysis of the glucuronides and characterization of the aglycones. In each case the thermodynamically more stable alcohol was obtained as the major metabolite (Table 6). Thus (±)-2-methylcyclohexanone was metabolized chiefly to *trans*-2-methylcyclohexanol, (±)-3-methylcyclohexanone to *cis*-3-methylcyclohexanol, and 4-methylcyclohexanone to *trans*-4-methylcyclohexanol. Whereas (±)-3-methylcyclohexanone is metabolized to give a racemic *cis*-3-methylcyclohexanol, (±)-2-methylcyclohexanone yields an optically active (+)-*trans*-2-methylcyclohexanol identical with that obtained in optically pure form from the hydrolysis of methyl [(+)-*trans*-2-methylcyclohexyl tri-*O*-acetyl-β-D-glucosid]uronate.

These stereochemical metabolic differences are consistently explicable in terms of the conformations of the substrates and of Vennesland's (1958) hypothesis of the role of NADH in dehydrogenation reactions. The preferred conformations of the 2- and 3-methylcyclohexanones and methylcyclohexanols are shown in Scheme 1. They are derived from the relative configuration determinations by Gough, Hunter & Kenyon (1926), together with the absolute configuration assignments for 2-methylcyclohexanone by Beard, Djerassi, Elliott & Tao (1962) and similar assignments for 3-methylcyclohexanone by Djerassi & Krakower (1959). A face-to-face contact, as suggested by Dixon & Webb (1958) with modification as proposed by Van Eys, San Pietro & Kaplan (1958), is visualized between the substrate and the coenzyme. In this way one only of the stereospecific hydrogens

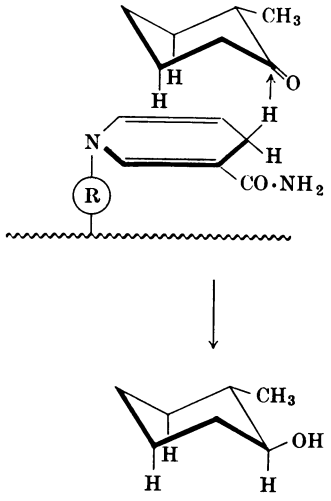
Table 6. *Major metabolic transformations of the methylcyclohexanols and methylcyclohexanones*

Compound administered	Configuration of aglycone: From crude glucuronide	Configuration of aglycone: From triacetyl methyl ester
(±)-*cis*-2-Methylcyclohexanol	(+)-*trans*-2-	(+)-*trans*-2-
(±)-*trans*-2-Methylcyclohexanol	(±)-*trans*-2-	(+)-*trans*-2-*
(±)-2-Methylcyclohexanone	(+)-*trans*-2-	(+)-*trans*-2-
(±)-*cis*-3-Methylcyclohexanol	(±)-*cis*-3-	(+)-*cis*-3-*
(±)-*trans*-3-Methylcyclohexanol	(±)-*cis*-3-	(+)-*cis*-3-*
(±)-3-Methylcyclohexanone	(±)-*cis*-3-	(+)-*cis*-3-*
cis-4-Methylcyclohexanol	*trans*-4-	*trans*-4-
trans-4-Methylcyclohexanol	*trans*-4-	*trans*-4-
4-Methylcyclohexanone	*trans*-4-	*trans*-4-

* The isolation of optically active aglycone was shown to be due to diastereoisomeric resolution.

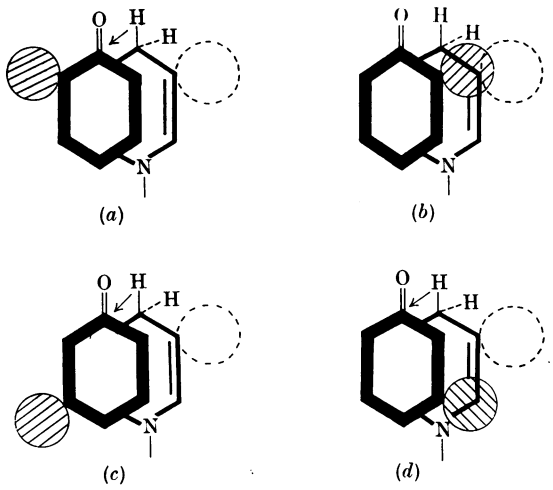


Scheme 1. Preferred conformations and absolute configurations of the 2- and 3-methylcyclohexanols and methylcyclohexanones.



Scheme 2. Conformational representation of proposed substrate–coenzyme interaction.

(Levy, Talalay & Vennesland, 1962) on C-4 of NADH can attack the carbonyl carbon of the substrate ketone. Further, since it has been shown (Tao & Elliott, 1962) that liver alcohol dehydrogenase mediates hydrogen transfer in this series of alcohols and ketones *in vitro* by using the same face of the coenzyme as does lactate dehydrogenase, (T. H. Elliott & R. C. C. Tao, unpublished work) it follows (Levy *et al.* 1962) that the A face of the coenzyme is used in the reaction. Then, assuming that the ketone is in its preferred conformation, it follows from the work of Cornforth, Ryback, Popják, Donninger & Schroepfer (1962) on the configuration of the nicotinamide ring that the face-to-face contact is as depicted in Scheme 2. Such a contact provides the substrate with good anchorage on the coenzyme surface and offers the most favourable conditions for direct hydrogen transfer (Fisher, Conn, Vennesland & Westheimer, 1953), possibly as H^- transfer or as a charge-transfer complex (Ingraham, 1962). If this is the general mechanism, then for the principal metabolite of (±)-2-methylcyclohexanone to be a *trans*-2-methylcyclohexanol with an equatorial hydroxyl group the attack must necessarily be directed towards C-1 of the substrate molecule on the side that has two axial hydrogens at C-3 and C-5. Further, since only the A face of the nicotinamide nucleotide ring is involved in the reaction, models show that the (+)-form of 2-methylcyclohexanone would be preferentially attacked (*a* in Scheme 3); for when (−)-2-methylcyclohexanone is similarly orientated to the specific reactive face of the nicotinamide nucleotide molecule there is a steric interaction between the methyl group of the substrate ketone and the carboxyamide group of the coenzyme of sufficient magnitude to prevent



Scheme 3. Suggested mode of interaction between nicotinamide moiety and (*a*) (+)-2-methylcyclohexanone, (*b*) (−)-2-methylcyclohexanone, (*c*) (+)-3-methylcyclohexanone and (*d*) (−)-3-methylcyclohexanone. Cross-hatched circle, $-CH_3$; broken circle, $-CO \cdot NH_2$.

effective hydrogen transfer (*b* in Scheme 3). Hence in a racemic mixture of the 2-ketone essentially only the (+)-isomer will be attacked, giving an optically active metabolite. While this steric interaction does not occur, e.g. with both forms of 3-methylcyclohexanone (*c* and *d* in Scheme 3), hydrogen transfer to either enantiomer occurs with equal facility, giving a racemic metabolite, and the attack must again have been on the side of the substrate molecule having two axial hydrogens at C-3 and C-5 since the *cis*-alcohol was produced.

Other reduction products. On the basis of this hypothesis, a maximum of 50% of the dose should be excreted as (+)-*trans*-2-methylcyclohexanol after administering (±)-2-methylcyclohexanone. Conjugation values show that 72% of the ketone was excreted conjugated with glucuronic acid, and therefore a metabolite or metabolites other than (+)-*trans*-2-methylcyclohexanol must have conjugated with glucuronic acid; on the basis of chromatographic evidence these can only be geometric or optically active isomers of (+)-*trans*-2-methylcyclohexanol. (−)-*trans*-2-Methylcyclohexanol could not have been present in more than traces, otherwise, being an enantiomer of (+)-*trans*-2-methylcyclohexanol, it would have produced, if present in substantial amounts, an unresolvable mixture of the two alcohols. However, (+)- and (−)-*cis*-2-methylcyclohexanol could have been present and lost in recrystallization during the isolation procedures. Indeed, the ratio of *trans*-2- to *cis*-2-methylcyclohexanol has been found by gas–liquid-chromatography to be 3:1. Similarly, after the administration of (±)-3-methylcyclohexanone to rabbits, the ratio of *cis*-3- to *trans*-3-methylcyclohexanol was 15:1. The ratio of *trans*-4- to *cis*-4-methylcyclohexanol after feeding 4-methylcyclohexanone was 5:1. The presence of *cis*-2-, *trans*-3- and *cis*-4-methylcyclohexanol as minor metabolites was also demonstrated by thin-layer chromatography. It is not known whether their presence implies a second enzyme system or a metabolic difference arising as a result of ring flexibility.

Metabolism of (±)-2-, (±)-3- *and 4-methylcyclohexanols.* This series of secondary alcohols, as Table 1 shows, is principally conjugated with glucuronic acid. There is an average increase of 73% of the dose administered (5·7m-moles/kg. body wt. average). This is comparable with the conjugation of the corresponding aliphatic secondary alcohols. For instance, heptan-3-ol and heptan-4-ol administered at a dose of 8m-moles/kg. body wt. to rabbits conjugated to the extent of 62 and 67% respectively (Williams, 1959). There are slight increases in ethereal sulphate excretion, but mercapturic acid output is not increased. Characterization of the alcohols resulting from hydrolysis of the isolated glucuronide gum (Table 5) as well as actual isolation of the pure triacetyl methyl ester of the glucuronide (Table 4) in each case had demonstrated that, though the thermodynamically more stable (±)-*trans*-2-, (±)-*cis*-3- and *trans*-4-methylcyclohexanol were excreted unchanged, the less stable (±)-*cis*-2-, (±)-*trans*-3- and *cis*-4-methylcyclohexanol were inverted to the corresponding more stable epimers. That these inversions did not occur during the refluxing process was indicated by the fact that the glucuronide triacetyl methyl ester isolated in each case was that of the more stable epimer. Confirmation was obtained from blank acid-hydrolysis experiments and by gas–liquid chromatography. Further, whereas (±)-*cis*-2-methylcyclohexanol gives rise to (+)-*trans*-2-methylcyclohexanol, (±)-*trans*-3-methylcyclohexanol gives rise to (±)-*cis*-3-methylcyclohexanol. These optical differences in behaviour are the same as those observed for the corresponding racemic ketones (Table 6), and this behaviour, taken in conjunction with the inversion patterns that have been demonstrated experimentally, suggests that the less stable epimeric alcohols most probably undergo inversion via a ketone, using a pathway similar to that described by Williams (1959) for aliphatic secondary alcohols.

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