The Metabolism of the Isomeric *tert.*-Butylcyclohexanones

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1. ($\pm$)-2-, ($\pm$)-3- and 4-*tert.*-Butylcyclohexanone are reduced in the rabbit to secondary alcohols, which are excreted extensively conjugated with glucuronic acid. 2. The major metabolite of ($\pm$)-2-*tert.*-butylcyclohexanone is (+)-*cis*-2-*tert.*-butylcyclohexanol, which has been isolated from the urine as [(+)-*cis*-2-*tert.*-butylcyclohexyl β -D-glucosid]uronic acid. The minor metabolite is (+)-*trans*-2-*tert.*-butylcyclohexanol. 3. ($\pm$)-3-*tert.*-Butylcyclohexanone is reduced mainly to ($\pm$)-*cis*-3-*tert.*-butylcyclohexanol, and to a smaller extent to ($\pm$)-*trans*-3-*tert.*-butylcyclohexanol. 4. 4-*tert.*-Butylcyclohexanone yields mainly the *trans*-alcohol, which is excreted in conjugated form and has been recovered from the urine as (trans-4-*tert.*-butylcyclohexyl β -D-glucosid)uronic acid. The *cis*-alcohol is formed to a minor extent and excreted in conjugated form. 5. The ratios of the amounts of *cis*- to *trans*-alcohols produced by the three ketones differed from the relative amounts of *cis*- and *trans*-alcohols produced by the corresponding methylcyclohexanones. 6. From these findings the suggestion is made that two orientations of ketone relative to coenzyme occur: alcohols with an equatorially orientated hydroxyl group are thought to be produced as a result of a 'face-to-face' interaction with NADH, and alcohols with axially orientated hydroxyl groups as a result of a 'perpendicular' interaction. Which will predominate is thought to depend on steric factors, particularly the size and position of alkyl substituents in the substrate.

In an investigation of the mammalian metabolism of the isomeric methylcyclohexanones, Tao & Elliott (1962) observed that the corresponding thermodynamically more stable alcohol with an equatorially orientated hydroxyl group was produced, and suggested that these results might be reasonably explained in terms of the conformations of the substrates and Vennesland's (1958) hypothesis on the role of NADH in dehydrogenation reactions, if it is assumed that a 'face-to-face' contact between the 'A' face of the coenzyme (Cornforth, Ryback, Popják, Donninger & Schroepfer, 1962) and the substrate occurs in the transition state. Since, however, the methylcyclohexanones are conformationally mobile, there is a possibility that the ketones reacted in the alternative (even though less thermodynamically stable) conformation, being converted into the more stable form after reduction, in which case, to account for the observed metabolic products it would have been necessary to postulate a 'perpendicular' orientation for the reactants in the transition state. To resolve this point it was decided to reinvestigate the problem by using the isomeric *tert.*-butylcyclohexanones, in which the alkyl substituent because of its bulk is invariably

equatorially orientated (Winstein & Holness, 1955) and the cyclohexane ring is effectively frozen in one conformation under all conditions of reaction. The results, reported below, support the view that substituted cyclohexanones are reduced in the forms that are conformationally most stable. However, it would seem that the orientation of coenzyme with substrate is not exclusively either 'face-to-face' or 'perpendicular' as was originally thought. Apparently both orientations are possible. Which predominates in any particular reaction appears to depend on steric factors.

MATERIALS

The isomeric ($\pm$)-2-, ($\pm$)-3- and 4-*tert.*-butylcyclohexanones used in the feeding experiments were prepared by the following method, which is an adaptation of that of Hueckel & Heyder (1963) for the preparation of ($\pm$)-4-*tert.*-butylcyclohexanone. In a typical preparation the appropriate *tert.*-butylphenol was hydrogenated at 130° under 100 atm. for 2 hr. with Raney nickel W.7 (Smith, Chadwell & Kirlis, 1955) as catalyst. After removal of phenols with NaOH, 30 g. portions of the dried product were oxidized with a mixture of 20 g. of $K_2Cr_2O_7$ and 26 g. of H_2SO_4 in 150 ml. of water, added dropwise over $\frac{1}{2}$ hr. at a temperature not exceeding 60°. The crude ketone was

separated from the reaction mixture by steam-distillation, and for 3- and 4-*tert*.-butylcyclohexanones it was purified via the bisulphite addition compound. 2-*tert*.-Butylcyclohexanone, which does not form a bisulphite addition compound, was purified by preliminary passage through an alumina column, prepared according to the recommendations of Reichstein & Shoppee (1949), with benzene as the eluting solvent, and making the final distillation under reduced pressure, collecting the fraction at b.p. 74°/10 mm.

(±)-2-*tert*.-Butylcyclohexanone was shown to be homogeneous by gas-liquid chromatography (retention time 2.2 min.) and thin-layer chromatography (R_F 0.83 in solvent system 2). The infrared spectrum showed a prominent characteristic absorption band at 1125 cm.⁻¹ (Goering, Reeves & Espy, 1956). It had b.p. 74°/10 mm. (literature b.p. 75–79°/12 mm.) and n_D^{25} 1.4540 (literature n_D^{25} 1.4570); the 2,4-dinitrophenylhydrazone had m.p. 163° (literature m.p. 162–162.5°).

(±)-3-*tert*.-Butylcyclohexanone was shown to be pure by gas-liquid chromatography (retention time 7.0 min.) and thin-layer chromatography (R_F 0.53 in solvent system 2). The infrared spectrum had a diagnostic absorption band at 1718 cm.⁻¹ (Djerassi, Warawa, Wolff & Eisenbraun, 1960), b.p. 90–92°/10 mm. (literature b.p. 96–98°/20 mm.) and n_D^{24} 1.4598 (literature n_D^{25} 1.4611); the 2,4-dinitrophenylhydrazone had m.p. 157.5° (literature m.p. 158–159°).

4-*tert*.-Butylcyclohexanone had m.p. 47–48° (literature m.p. 47.5–48.5°) and b.p. 94–96°/10 mm. (literature b.p. 90–92°/9 mm.). The infrared spectrum showed a typical ketone absorption band at 1720 cm.⁻¹ and the material was shown to be essentially pure by gas-liquid chromatography (retention time 7.2 min.) and thin-layer chromatography (R_F 0.53 in solvent system 2).

The isomeric alcohols used as reference materials were prepared by standard methods or as described below.

(±)-*cis*-2-*tert*.-Butylcyclohexanol was prepared by high-pressure hydrogenation of 2-*tert*.-butylphenol with Raney nickel W.7 as catalyst. It was purified by recrystallization of the acid phthalate, which on saponification yielded an alcohol with m.p. 54° (literature m.p. 56.8–57.7°) after three recrystallizations from light petroleum (b.p. 40–60°). Gas-liquid chromatography showed one peak (retention time 8.0 min.) and thin-layer chromatography showed, in addition to the *cis*-alcohol spot (R_F 0.77 in solvent system 2), a very faint spot corresponding to the *trans*-isomer (R_F 0.66). The infrared spectrum revealed a strong absorption band at 964 cm.⁻¹ (literature 966 cm.⁻¹) characteristic of the *cis*-isomer, and a minimum absorption at 1050 cm.⁻¹ corresponding to the *trans*-isomer. On admixture with the *trans*-isomer the m.p. was depressed from 54° to 43°.

(±)-*trans*-2-*tert*.-Butylcyclohexanol, m.p. 84.5° (literature 84–85°) was prepared according to the method of Goering *et al.* (1956). Examined by gas-liquid chromatography it gave one peak (retention time 9.0 min.). Thin-layer chromatography revealed one alcohol spot (R_F 0.66 in solvent system 2). The infrared spectrum revealed an absorption band at 1050 cm.⁻¹ characteristic of the *trans*-alcohol.

(±)-*cis*-3-*tert*.-Butylcyclohexanol was prepared by high-pressure hydrogenation of (±)-3-*tert*.-butylcyclohexanone with Raney nickel W.7 as catalyst, and was purified by recrystallization of the acid phthalate to constant m.p. (134°). Saponification of the ester with 30% (w/v) KOH yielded the alcohol, m.p. 40° (literature m.p. 40–41°) and

n_D^{25} 1.4671 (literature n_D^{25} 1.4660). It gave one peak with gas-liquid chromatography (retention time 12.4 min.). Thin-layer chromatography revealed one spot (R_F 0.28 in solvent system 3). The infrared spectrum exhibited a prominent absorption band at 1048 cm.⁻¹.

(±)-*trans*-3-*tert*.-Butylcyclohexanol was prepared as follows. Low-pressure hydrogenation of (±)-3-*tert*.-butylcyclohexanone with Adams' catalyst gave a *trans*-rich mixture of the corresponding alcohols. The *trans*-isomer was separated as the acid phthalate by recrystallization from a mixture of ethyl acetate and isohexane. Saponification of the pure ester (m.p. 154°) with 30% (w/v) KOH solution afforded pure (±)-*trans*-3-*tert*.-butylcyclohexanol, m.p. 63° (literature m.p. 60°). Examined by gas-liquid chromatography it gave one peak (retention time 8.75 min.). Thin-layer chromatography revealed one spot (R_F 0.35 in solvent system 3). The infrared spectrum showed a prominent band at 1125 cm.⁻¹.

cis-4-*tert*.-Butylcyclohexanol, m.p. 81–82° (literature m.p. 82–83°), was prepared as described by Eliel & Ro (1957). Gas-liquid chromatography revealed one peak (retention time 10.25 min.). It was shown to be pure by thin-layer chromatography (R_F 0.42 in solvent system 3). The infrared spectrum showed a strong characteristic absorption at 952 cm.⁻¹; the band at 1062 cm.⁻¹ diagnostic for the *trans*-alcohol was absent.

trans-4-*tert*.-Butylcyclohexanol, m.p. 81° (literature m.p. 81–82°), was separated from the *cis*-isomer by the method of Eliel & Ro (1957). Gas-liquid chromatography gave one peak (retention time 16.25 min.). Thin-layer chromatography gave one spot (R_F 0.31 in solvent system 3). The infrared spectrum showed a diagnostic band at 1062 cm.⁻¹ characteristic of the alcohol; the diagnostic absorption band of the *cis*-alcohol at 952 cm.⁻¹ was absent.

METHODS

Animals and diet. Adult doe albino rabbits weighing 1–2 kg. were used. They were kept on a constant daily diet consisting of 4 oz. of sweet potatoes (*Ipomoea reptans*) and 4 oz. of kangkong (*Ipomoea batatas*). No additional water was given.

Administration of dose. The liquid ketones were administered by stomach tube followed by about 50 ml. of water. The solid ketone was dissolved in arachis oil and administered similarly.

Analytical methods. The methods used for determination of glucuronic acid, ethereal sulphate and mercapturic acid were those employed by Elliott, Robertson & Williams (1966).

Thin-layer chromatography. Plates were prepared as described by Elliott *et al.* (1966) with silica gel G (E. Merck A.-G., Darmstadt, Germany) for glucuronides and silica gel H (E. Merck A.-G.) for the separation of alcohols. Glucuronides were chromatographed with system 1 [butan-1-ol–acetic acid–water (4:1:5, by vol.)] and were detected by spraying the dried chromatogram with a solution 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. The epimeric 2-*tert*.-butylcyclohexanols were separated in system 2 [light petroleum (b.p. 60–80°)–ethyl acetate (9:1, v/v)], and the epimeric 3- and 4-*tert*.-butylcyclohexanols were separated in system 3 [light petroleum (b.p. 60–80°)–ethyl acetate (7:3, v/v)]. The alcohols were

revealed by spraying with 10% (w/v) phosphomolybdic acid solution in ethanol and heating at 120–130° for 10 min. (Kritchevsky & Kirk, 1952).

Gas-liquid chromatography. Separations were performed on a Pye Argon Chromatograph with 10% diglycerol-Celite columns at 100°. The flow rate was 170–180 ml./min. (75 ml./min. for the 2-*tert.*-butylcyclohexanols), and 0.1–0.3 μ l. of the material in CHCl₃ was injected at a sensitivity setting of 3×10^{-9} .

Infrared spectroscopy. Measurements were made in CHCl₃ solution with a JASCO model IR-S spectrophotometer (Japan Spectroscopic Manufacturing Co. Ltd.) fitted with NaCl optics.

Optical-rotatory-dispersion measurements. Optical-rotatory-dispersion curves were made on a JASCO ORD/UV5 optical-rotatory-dispersion recorder. A full-scale rotation angle of 0.2° was chosen, requiring the corresponding analyser setting to be 0.224, thus giving an expansion factor (*P*) of 2000. The solution concentration was 1 mg./ml. and the sample cell had a path length of 0.1 dm.

Isolation and hydrolysis of metabolites. Glucuronides

were isolated from the 24 hr. urine by the basic lead acetate procedure of Kamil, Smith & Williams (1951). With ($\pm$)-2-*tert.*-butylcyclohexanone it was noted that the glucuronide separated as a solid from the acidified urine on concentration and standing. Accordingly for this ketone the preliminary lead acetate precipitation was not performed.

The isolated glucuronides were hydrolysed by heating with *N*-HCl for 10 min. on a glycerol bath at 130°.

RESULTS

Experiments on urine

After administration of the ketones the rabbits were narcotized within 10–15 min., the narcosis lasting for 4–5 hr. The effect was particularly marked with ($\pm$)-3-*tert.*-butylcyclohexanone, which caused convulsions lasting for 1 hr.

Qualitative experiments on urine. The 24 hr. urine in all cases had pH 8–9, was reddish brown

Table 1. *Glucuronic acid excretion of the isomeric tert.-butylcyclohexanones*

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

Compound administered	Dose (m-moles/ kg. body wt.)	% of dose excreted as glucuronide	
		Average	Range
($\pm$)-2- <i>tert.</i> -Butylcyclohexanone	4.3	76.5	71–82
($\pm$)-3- <i>tert.</i> -Butylcyclohexanone	4.3	93.5	93–94
4- <i>tert.</i> -Butylcyclohexanone	3.7	80.0	74–87

Table 2. *R_F values, retention times and optical rotations of glucuronide aglycones*

Experimental details are given in the text.

Compound administered	R_F	Retention time (min.)	$[\alpha]_D^{23-25}$ in CHCl_3 of aglycone obtained
$(\pm)$ -2- <i>tert.</i> -Butylcyclohexanone	0.77 (major)*	8.0	+20.6°
	0.66 (minor)*	9.0	+40.0
$(\pm)$ -3- <i>tert.</i> -Butylcyclohexanone	0.28 (major)†	13.5	0
	0.35 (minor)†	8.75	0
4- <i>tert.</i> -Butylcyclohexanone	0.31 (major)†	16.25	0
	0.42 (minor)†	10.25	0

* Solvent system 2.

† Solvent system 3.

Table 3. *R_F values and retention times of the isomeric tert.-butylcyclohexanols*

Experimental details are given in the text.

Compound	<i>R_F</i>	Retention time (min.)
($\pm$)- <i>cis</i> -2- <i>tert.</i> -Butylcyclohexanol	0.77*	8.0
($\pm$)- <i>trans</i> -2- <i>tert.</i> -Butylcyclohexanol	0.66*	9.0
($\pm$)- <i>cis</i> -3- <i>tert.</i> -Butylcyclohexanol	0.28†	13.5
($\pm$)- <i>trans</i> -3- <i>tert.</i> -Butylcyclohexanol	0.35†	8.8
<i>cis</i> -4- <i>tert.</i> -Butylcyclohexanol	0.42†	10.2
<i>trans</i> -4- <i>tert.</i> -Butylcyclohexanol	0.31†	16.3

* Solvent system 2.

† Solvent system 3.

and gave an intense naphtharesorcinol reaction. Chromatographed on thin-layer plates in solvent system 1 and sprayed with naphtharesorcinol, it gave one intense spot (R_f 0.80). The urine did not reduce Benedict's solution, nor did it give a precipitate with Brady's reagent. The test for phenols with ferric chloride solution was negative.

Quantitative estimations on urine. The results for glucuronic acid excretion are given in Table 1. Negligible amounts of ethereal sulphate and no mercapturic acids were detected.

Isolation and characterization of metabolites. The R_f values, retention times and optical rotations of the aglycones obtained by enzyme and acid hydrolysis of the glucuronides in the different urines are given in Table 2. For each ketone given two aglycones were observed on the chromatograms with R_f values and retention times corresponding to those of the respective *cis*- and *trans*-epimeric alcohols (Table 3) obtainable by reduction of the ketone.

The separation and characterizations of the individual metabolites of each ketone given are described in the succeeding sections.

($\pm$)-2-*tert*.-Butylcyclohexanone

Isolation and characterization of the major aglycone. The glucuronide, isolated from the concentrated urine of ten rabbits given a total of 11.2 ml. of the ketone, deposited as a white solid on standing; this, when repeatedly recrystallized from aq. 10% (v/v) ethanol with charcoal, yielded 1.9 g. of pure [(+)-*cis*-2-*tert*.-butylcyclohexyl β -D-glucosid]uronic acid, m.p. 167–168° and $[\alpha]_D^{25} + 3.4^\circ$ (c 2.67 in methanol) (Found: C, 54.7; H, 8.7. $C_{16}H_{28}O_7 \cdot H_2O$ requires C, 54.8; H, 8.6%). Acid hydrolysis of 1.68 g. of the pure glucuronide gave 0.32 g. of (+)-*cis*-2-*tert*.-butylcyclohexanol, $[\alpha]_D^{25} + 20.6^\circ$ (c 3.23 in chloroform, 0.5 dm.), which was shown by gas-liquid chromatography (retention time 8.0 min.) and thin-layer chromatography (R_f 0.77 in solvent system 2) to be homogeneous. The infrared spectrum was identical with that of racemic *cis*-2-*tert*.-butylcyclohexanol. A 78 mg. sample was esterified with phthalic anhydride in pyridine according to the method of Winstein & Holness (1955) to yield, after four recrystallizations from a mixture of isohexane (b.p. 56–70°) and ethyl acetate, 6.4 mg. of (+)-*cis*-2-*tert*.-butylcyclohexyl acid phthalate, $[\alpha]_D^{25} + 112^\circ$ (c 0.8 in chloroform, 0.25 dm.) (Found: C, 70.8; H, 8.2. $C_{18}H_{24}O_4$ requires C, 71.0; H, 8.0%). The m.p. of racemic *cis*-2-*tert*.-butylcyclohexyl acid phthalate was depressed from 160° to 138°. Final characterization of the aglycone was effected by oxidizing it to (–)-2-*tert*.-butylcyclohexanone as described by Djerassi, Hart & Warawa (1964). It had $[\alpha]_D^{25}$

–45.5° (c 2.75 in chloroform, 0.5 dm.). A negative Cotton effect was recorded and $[\alpha]_D^{25} - 1090^\circ$ (literature $[\alpha]_{317} - 1089^\circ$).

Isolation and characterization of the minor aglycone. Three rabbits were given a total of 4.2 ml. of ($\pm$)-2-*tert*.-butylcyclohexanone. Direct hydrolysis of the 24 hr. urine and recovery of the aglycones in the usual manner gave 0.95 g. of a mixture of the isomeric 2-*tert*.-butylcyclohexanols, shown to be *cis*-rich by thin-layer chromatography. The optical rotation of the mixture was +22.3°. A preparative thin-layer chromatographic separation (Elliott *et al.* 1966) of the crude hydrolysate on 1 mm. silica gel H plates (solvent system 2) gave 0.3 g. of a mixture of the alcohols, the optical rotation being 24.2°. This material (150 mg.), rechromatographed on 0.25 mm. silica gel H plates, yielded 56 mg. of material (R_f 0.78 in solvent system 2), $[\alpha]_D^{25} + 20.0^\circ$ (c 2.2 in chloroform, 0.25 dm.), having an infrared spectrum identical with that of *cis*-2-*tert*.-butylcyclohexanol. From this preparative separation a minor aglycone (11.4 mg.) was also separated (R_f 0.66) that was shown to be (+)-*trans*-2-*tert*.-butylcyclohexanol, $[\alpha]_D^{25} + 40^\circ$ (c 0.8 in chloroform, 1 dm.) (literature $[\alpha]_D + 44.2^\circ$). Its infrared spectrum was identical with that of ($\pm$)-*trans*-2-*tert*.-butylcyclohexanol.

($\pm$)-3-*tert*.-Butylcyclohexanone

Isolation and characterization of the major aglycone. Eight rabbits were given a total of 4.6 g. of ($\pm$)-3-*tert*.-butylcyclohexanone, and the glucuronide separated from the urine as an uncrystallizable gum, which, when hydrolysed, yielded an oily residue, $[\alpha]_D^{25} 0^\circ$, weighing 0.48 g. Examination by thin-layer chromatography and gas-liquid chromatography indicated that it was a mixture of *cis*- and *trans*-3-*tert*.-butylcyclohexanols, in which the *cis*-alcohol predominated. Ketones were shown to be absent by infrared spectroscopy. Esterification of the oil with phthalic anhydride in pyridine by the method of Winstein & Holness (1955) yielded, after four recrystallizations from a mixture of isohexane (b.p. 50–70°) and ethyl acetate, 0.11 g. of ($\pm$)-*cis*-3-*tert*.-butylcyclohexyl acid phthalate, m.p. and mixed m.p. with an authentic sample 133–133.5°. The m.p. of ($\pm$)-*trans*-3-*tert*.-butylcyclohexyl acid phthalate was depressed from 154° to 115°. It was not possible to isolate and characterize the minor aglycone (*trans*-3-*tert*.-butylcyclohexanol) that had been shown to be present by thin-layer chromatography.

($\pm$)-4-*tert*.-Butylcyclohexanone

Isolation and characterization of the major glucuronide. The mixture of glucuronides separated in the usual way from the urine of six rabbits that

had been given a total of 5.6g. of the ketone was recrystallized from boiling water with charcoal. After several recrystallizations, 0.16g. of pure (*trans*-4-*tert*.-butylcyclohexyl β -D-glucosid)uronic acid was obtained, m.p. 188–188.5° and $[\alpha]_D^{25}$ –48.4° (c 1.33 in methanol, 0.25dm.). A sample was dried for 24hr. *in vacuo* at 60° (Found: C, 54.7; H, 8.6. $C_{16}H_{28}O_7 \cdot H_2O$ requires C, 54.8; H, 8.6%).

Isolation and characterization of the aglycone. Five rabbits were given a total of 3.6g. of the ketone, and the glucuronides in the urine were isolated as a gum in the usual way. Acid hydrolysis yielded 0.8g. of a white solid mixture of aglycones. Ketonic materials shown to be present by infrared spectroscopy were removed with sodium hydrogen sulphite and the residue was shown by gas-liquid chromatography and thin-layer chromatography to consist of a *trans*-rich mixture of the 4-*tert*.-butylcyclohexanols, from which the *trans*-isomer was separated by repeated recrystallizations from light petroleum (b.p. 40–60°). The m.p. and mixed m.p. with an authentic sample of *trans*-4-*tert*.-butylcyclohexanol was 81°, depressed to 63° on admixture with a sample of the *cis*-alcohol. The infrared spectrum was identical with that of *trans*-4-*tert*.-butylcyclohexanol.

Determination of *cis/trans* ratios

The proportion of *cis*- to *trans*-alcohol in hydrolysates of the urine of animals given ($\pm$)-3- and 4-*tert*.-butylcyclohexanone was determined as follows. The total 24hr. sample of urine was mixed with 0.1 vol. of concentrated hydrochloric acid and heated under reflux for 10min. The aglycones were removed by steam-distillation and, after ether extraction and drying with anhydrous magnesium sulphate, were separated by gas-liquid chromatography on a Celite-diglycerol column. Estimations of the relative amounts of the isomeric alcohols were made by measurement of the areas under the curves. It was not possible to use the same method for ($\pm$)-2-*tert*.-butylcyclohexanone because the retention times of the two isomers were so similar. Infrared analysis was used instead. By measuring the relative percentage transmittances at 1050 cm^{-1} (diagnostic peak for the *trans*-isomer) and 966 cm^{-1} (diagnostic peak for the *cis*-isomer) of various mixtures of the *cis*- and *trans*-alcohols and comparing with these the transmittances at the same wavelengths of the acid hydrolysate of the urine of animals given ($\pm$)-2-*tert*.-butylcyclohexanone, it was possible to make an approximate estimate of the ratio (differing somewhat, however, from the ratio calculated from optical-rotation measurements). The results are given in Table 4. To confirm that the ratios observed reflected the proportions of *cis*- and *trans*-alcohols actually

Table 4. *Ratio of cis- to trans-alcohols in glucuronide hydrolysates*

Compound administered	<i>cis/trans</i> ratio
($\pm$)-2- <i>tert</i> .-Butylcyclohexanone	71:29*
($\pm$)-3- <i>tert</i> .-Butylcyclohexanone	74:26
4- <i>tert</i> .-Butylcyclohexanone	26:74

* The ratio calculated from the optical rotation of the crude aglycone is 88:12, and that from isolation experiments 84:16.

produced *in vivo*, ($\pm$)-*cis*-2-, ($\pm$)-*trans*-2-, ($\pm$)-*cis*-3-, ($\pm$)-*trans*-3-, *cis*-4- and *trans*-4-*tert*.-butylcyclohexanol were each refluxed with N-hydrochloric acid in a glycerol bath maintained at 130° for 30min., and the alcohols recovered from the corresponding steam-distillates of the reaction mixtures were subjected to gas-liquid chromatography. No perceptible inversion was detected.

DISCUSSION

Since the 2-*tert*.-butylcyclohexanones, in which the alkyl substituents are unambiguously equatorial (Winstein & Holness, 1955), possess a stereochemistry identical with that of the 2-methylcyclohexanones (Cheo, Elliott & Tao, 1966), and, since with these ketones there can be no question of ring inversion occurring, the general resemblance of their metabolism to that of the methylcyclohexanones confirms the validity of the assumption made by Elliott, Tao & Williams (1965) that alicyclic ketones react in their more stable conformations. The similarity of the metabolic patterns also confirms the conclusion arrived at in the earlier work on the methylcyclohexanones that the orientation of substrate with coenzyme must, at least for the major metabolites, have been 'face-to-face' if an 'A'-type enzyme (Cornforth *et al.* 1962) had participated.

However, the main interest lies more in the particular differences, especially those of a quantitative nature, than in the general similarities, as can be seen in a comparison of the metabolism of the 2-substituted ketones. Whereas the major metabolite of ($\pm$)-2-methylcyclohexanone is the (+)-*trans*-alcohol, the (+)-*cis*-alcohol being produced in minor amounts only (Elliott *et al.* 1965), the major metabolite of ($\pm$)-2-*tert*.-butylcyclohexanone is the (+)-*cis*-alcohol, the (+)-*trans*-alcohol being only a minor metabolite (Table 4). These quantitative differences must be explicable in steric terms, since the only difference in the two series of ketones is the size of the alkyl substituents.

Further, if a single enzyme system mediates the reductions occurring in the animal [and this now

appears a reasonable assumption to make since T. H. Elliott, E. Jacob & R. C. C. Tao (unpublished work) have reproduced these metabolic patterns occurring *in vivo* exactly with liver alcohol dehydrogenase, NADH and the various racemic or optically active ketones], then the formation of two metabolites [the (+)-*trans*- and (+)-*cis*-alcohols] can only mean that the substrate has been orientated in the transition state in two different positions relative to the coenzyme to produce the two different isomers.

Whether the *cis*- or *trans*-alcohol will predominate appears to depend on the relative ease with which the substrate can approach the coenzyme in one or other position. Examination of Catalin models demonstrates clearly that in the transition state only two orientations are possible for effective hydrogen transfer: a 'face-to-face' arrangement for the (+)-ketone producing the (+)-*trans*-isomer (equatorial hydroxyl group), as shown schematically by Elliott *et al.* (1965), and a 'perpendicular' arrangement for the (-)-ketone producing the (+)-*cis*-alcohol (axial hydroxyl group), the angular orientation of substrate relative to the coenzyme being as shown in Scheme 1.

Examination of the models also gives an indication why in the reduction of the ($\pm$)-*tert*-butyl ketone the (+)-*cis*-alcohol is the major metabolite.

It becomes apparent that the *tert*-butyl group, though not absolutely preventing approximation of the active centres in a 'face-to-face' orientation, renders it very much more difficult than would have been the case if the substituent had been a methyl group. However, if the models are placed with a 'perpendicular' arrangement no such relative steric hindrance is apparent, which would seem to indicate that, all things being equal, a 'face-to-face' orientation is the preferred mode, the 'perpendicular' orientation being utilized only if steric hindrances are of such magnitude as to prevent or limit effective hydrogen transfer. The ratios of *cis*- to *trans*-metabolites may thus be taken as providing an indication of the relative facility with which hydrogen transfer occurs in the alternative orientations.

The biological reduction of ($\pm$)-3-*tert*-butylcyclohexanone affords mainly racemic *cis*-3-*tert*-butylcyclohexanol and, to a smaller extent, racemic *trans*-3-*tert*-butylcyclohexanol (Table 4). For the racemic *cis*-alcohol to have been formed (+)- and (-)-*cis*-3-*tert*-butylcyclohexanol must have been produced in equal quantities. Similarly for the racemic *trans*-alcohol to have been formed (+)- and (-)-*trans*-3-*tert*-butylcyclohexanol must also have been produced in equal quantities. The configurational relationships of these four optical

Scheme 1. Conformational representation of proposed substrate-coenzyme interaction: (a) for a 'face-to-face' interaction; (b) for a 'perpendicular' interaction.

antipodes to each other and to (+)- and (-)-3-*tert*.-butylcyclohexanone can be deduced from the results of Djerassi *et al.* (1960), and it can be shown by the use of Catalin models that the enantiomeric ketones from which these alcohols have been formed possess conformations that offer no steric hindrance to effective hydrogen transfer when interacting either in 'perpendicular' or 'face-to-face' orientation. In such circumstances, when steric considerations do not favour the close approximation of one enantiomer rather than another, the (+)- and (-)-antipodes presumably approach the coenzyme with equal facility, preferentially assuming a 'face-to-face' orientation. Such an orientation, as was demonstrated for the 2-*tert*.-butylcyclohexanones, leads to alcohols with equatorial hydroxyl groups that in this case are (+)- and (-)-*cis*-3-*tert*.-butylcyclohexanol, both of which are apparently produced in equal amounts to afford racemic *cis*-3-*tert*.-butylcyclohexanol as the major metabolite. For similar reasons, (+)- and (-)-*trans*-3-*tert*.-butylcyclohexanone are also formed in equal amounts, and, since a 'perpendicular' orientation is apparently less favourable than a 'face-to-face' orientation, smaller amounts of the *trans*-3-alcohol are produced.

With 4-*tert*.-butylcyclohexanone, the question of optical segregation does not arise because the molecule is symmetrical, and, since the *tert*.-butyl substituent in position 4 does not in any way inhibit the close approximation of the reactive centres, the interacting molecules can assume either a 'face-to-face' or 'perpendicular' orientation. As the former appears to be preferred, the *trans*-

alcohol is found experimentally in relatively larger amounts than the *cis*-alcohol.

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REFERENCES

- Cheo, K. L., Elliott, T. H. & Tao, R. C. C. (1966). *J. chem. Soc. C*, p. 1988.
- Cornforth, J. W., Ryback, G., Popják, G., Donninger, C. & Schroepfer, G., jun. (1962). *Biochem. biophys. Res. Commun.* **9**, 371.
- Djerassi, C., Hart, P. A. & Warawa, E. J. (1964). *J. Amer. chem. Soc.* **86**, 82.
- Djerassi, C., Warawa, E. J., Wolff, R. E. & Eisenbraun, E. J. (1960). *J. org. Chem.* **25**, 917.
- Eliel, E. L. & Ro, R. S. (1957). *J. Amer. chem. Soc.* **79**, 5992.
- Elliott, T. H., Robertson, J. S. & Williams, R. T. (1966). *Biochem. J.* **100**, 393.
- Elliott, T. H., Tao, R. C. C. & Williams, R. T. (1965). *Biochem. J.* **95**, 59.
- Goering, H. L., Reeves, R. L. & Espy, H. H. (1956). *J. Amer. chem. Soc.* **78**, 4926.
- Hueckel, W. & Heyder, K. (1963). *Chem. Ber.* **96**, 220.
- Kamil, I. A., Smith, J. N. & Williams, R. T. (1951). *Biochem. J.* **50**, 235.
- Kritchevsky, D. & Kirk, M. R. (1952). *Arch. Biochem. Biophys.* **35**, 346.
- Reichstein, T. & Shoppee, C. W. (1949). *Disc. Faraday Soc.* **7**, 305.
- Smith, H. A., Chadwell, A. J., jun. & Kirsliis, S. S. (1955). *J. phys. Chem.* **59**, 820.
- Tao, R. C. C. & Elliott, T. H. (1962). *Biochem. J.* **84**, 38r.
- Vennesland, B. (1958). *Fed. Proc.* **17**, 1150.
- Winstein, S. & Holness, N. J. (1955). *J. Amer. chem. Soc.* **77**, 5562.