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# Metabolism of Camphors and Related Compounds

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1. The metabolism of (±)-norcamphor, (+)-camphor, (−)-camphor, (+)-epicamphor, (±)-camphorquinone, (±)-camphane-2,5-dione and camphane was investigated in rabbits. All the compounds except camphane-2,5-dione increased the content of glucuronide in the urine. 2. (±)-Norcamphor was reduced to *endo*-norborneol; (+)-camphor, contrary to expectation, was reduced to (+)-borneol, as well as being hydroxylated to (+)-5-*endo*-hydroxycamphor and (+)-3-*endo*-hydroxycamphor, 5-*endo*-hydroxycamphor being the predominant product. (+)-Epicamphor was reduced mainly to (+)-epiborneol; (±)-camphorquinone gave 3-*endo*-hydroxycamphor and 2-*endo*-hydroxyepicamphor, the former being the major metabolite. (±)-Camphane-2,5-dione was reduced to 5-*endo*-hydroxycamphor. Camphane was hydroxylated to borneol and epiborneol, the latter predominating. 3. An explanation of these findings is given in terms of steric hindrance and thermodynamic stability. 4. The possibility was investigated that NADH was involved in the reductions.

Kreiger (1962), after giving norcamphor to rabbits and hydrolysing the glucuronide excreted in urine, identified *endo*- and *exo*-norborneol as metabolites, and suggested that these two compounds were present in equal amounts.

From the urine of dogs that had been fed with (+)-camphor (Fig. 1), Schmeideberg & Mayer (1879) isolated three metabolites, which were termed '$\alpha$-camphoglycuronic acid', '$\beta$-camphoglycuronic acid' and 'uramidocamphoglycuronic acid', a nitrogenous compound; all three gave 'hydroxycamphors' on hydrolysis.

Asahina & Ishidate (1928) also administered (+)-camphor to dogs. Acid hydrolysis of the mixed camphoglycuronic acids excreted in the urine yielded '(+)-campherols', which on oxidation with chromium trioxide in acetic acid yielded a mixture of camphorquinone and camphane-2,5-dione. From this it was concluded that '(+)-campherols' was a mixture of 3-hydroxycamphor and 5-hydroxycamphor. In later experiments on the metabolism of (+)-camphor, Asahina & Ishidate (1933, 1934, 1935) isolated small amounts of *cis*- and *trans*-$\pi$-hydroxycamphor (8-hydroxy- and 9-hydroxycamphor) and camphor-$\pi$-carboxylic acid, and also showed that in the previously obtained '(+)-campherols' 5-hydroxycamphor predominated. The oxo group of (+)-camphor appeared not to undergo any change during metabolism. Shimamoto (1934) obtained 3-hydroxycamphor (15%), 5-hydroxycamphor (55%) and *trans*-$\pi$-hydroxycamphor (20%) from the urine of dogs. Reinartz, Zanke & Kuersch (1935) isolated a small amount of $\pi$-apocamphor-7-aldehyde in addition to 5-hydroxycamphor. There has been only one previous investigation of the metabolism of (+)-camphor in rabbits, by Shimamoto (1934), who isolated, after hydrolysis of urine, 5-hydroxycamphor and 3-hydroxycamphor, the former being the major metabolite. Mayer (1908) and Magnus-Levy (1906) investigated the metabolism of (±)-camphor and (−)-camphor respectively in dogs. They suggested that hydroxycamphors were the product of metabolism in both cases.

Reinartz, Zanke & Schaefers (1934) investigated the chemical nature of the metabolites of epicamphor in dogs. The main product was identified as 4-hydroxyepicamphor together with a small amount of $\pi$-hydroxyepicamphor (8-hydroxy- or 9-hydroxy-epicamphor).

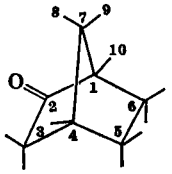



Fig. 1. (+)-Camphor. Those bonds directed towards the concave side of the cyclohexane 'boat' are termed *endo* and those towards the convex side *exo*. Substituents 8, 9 may be designated '$\pi$'; C-8 is *cis*-related and C-9 is *trans*-related to the oxo group.

Camphorquinone was found to be reduced to a mixture of 3-hydroxycamphor and 2-hydroxy-epicamphor in dogs (Reinartz & Zanke, 1934). It seemed that one oxo group could be reduced but not both.

Confusing reports about the metabolism of camphane-2,5-dione in dogs were made by Reinartz, Zanke & Faust (1934). This was reinvestigated by Ishidate, Kawahata & Nakazawa (1941), who isolated, after the hydrolysis of conjugated glucuronide, 5-hydroxycamphor as the only metabolite. The formation of this compound involved the reduction of one of the two oxo groups.

The metabolism of camphane was investigated by Hamalainen (1912). When given to dogs, it was thought to be hydroxylated at C-2 or C-6 to a mixture of (+)- and (−)-borneol, which were excreted as the corresponding glucuronides.

## MATERIALS

Melting points are uncorrected. Where a specific reference is not given, the literature values quoted for the physical constants of the compounds were taken from Heilbron, Cook, Bunbury & Hey (1965) and Rodd (1953). Light petroleum (b.p. 60–80°) was used for recrystallization and column chromatography.

(±)-*Norcamphor*. This compound was prepared by the method of Kleinfelter & Schleyer (1962) and had m.p. 92–94° (literature value 93–95°); the DNP-hydrazone had m.p. 130° and the semicarbazone 197° (literature values 130° and 198° respectively). The i.r. spectrum showed a strong absorption band at 1753 cm.$^{-1}$. T.l.c. indicated the homogeneity of the sample.

(±)-endo-*Norborneol*. Reduction of (±)-norcamphor in anhydrous ether with $LiAlH_4$ (Hirsjarvi, 1957) yielded, after extraction and recrystallization, (±)-*endo*-norborneol, m.p. 146–147° (literature value 149–150°).

(+)-*Camphor*. (+)-Camphor purchased from British Drug Houses, Sydney, N.S.W., Australia, contained some borneol, revealed by t.l.c. After oxidation with Jones reagent in acetone (Bowers, Halsall, Jones & Lemin, 1953), extraction and recrystallization, the product was homogeneous when examined by t.l.c., and had m.p. 178–179° (literature value 179·5°) and $[\alpha]_D^{20}$ +44·04° (*c* 5 in ethanol). Malkonen (1964) gave $[\alpha]_D^{20}$ +44·67° (*c* 10 in ethanol). The DNP-hydrazone had m.p. 175° (literature value 175°).

(−)-*Camphor*. The sample of this compound obtained from K & K Laboratories Inc., Plainview, N.Y., U.S.A., was found by t.l.c. and g.l.c. to contain about 40% of borneol. After oxidation as for (+)-camphor, extraction and recrystallization, it had m.p. 177–178° (literature value 178·6°) and $[\alpha]_D^{20}$ −41·65° (*c* 5 in ethanol).

(±)-*Camphor*. Camphor B.P. was found to contain a little borneol. Oxidation as for (+)-camphor, extraction and recrystallization yielded a compound with m.p. 175–176° (literature value 177–178·8°) and $[\alpha]_D^{20}$ 0·00° (*c* 10 in ethanol).

The i.r. spectra of (+)-, (−)- and (±)-camphor were identical and similar to that published by Malkonen (1964).

(±)-*Isoborneol*. This compound was prepared by reduction of (±)-camphor with $LiAlH_4$ in anhydrous ether (Noyce & Denny, 1950); on repeated recrystallization from light petroleum, the product had m.p. 208–210° (literature value 212°). T.l.c. and g.l.c. indicated the homogeneity of the product.

(±)-*Borneol*. This was obtained by reduction of (±)-camphor with sodium in ethanol, and purified by t.l.c. The borneol so obtained had m.p. 206–208° after sublimation (literature value 210·3°). The i.r. spectrum was identical with that published by Malkonen (1964).

*Epicamphor*. This was prepared by the hydroboration of bornylene (Berson, McRowe, Bergman & Houston, 1967). The mixture of borneol and epiborneol so obtained was oxidized with Jones reagent to yield a mixture of camphor and epicamphor, separation of which was achieved through the rate of semicarbazone formation (Huckel & Fechtig, 1962). The semicarbazone of epicamphor started separating after 30 min. whereas camphor took 6½ hr. to react. After 6½ hr. the semicarbazone of epicamphor was filtered off under suction and, after repeated recrystallization from ethanol, had m.p. 235–236° (literature value 237–238°). The semicarbazone was refluxed with aq. 10% (w/v) oxalic acid for 8 hr., and extraction of the steam-distillate yielded epicamphor, m.p. 180–181° and $[\alpha]_D^{20}$ +16·5° (*c* 2 in methanol). Huckel & Fechtig (1962) gave m.p. 186–187° and $[\alpha]_D^{20}$ −41·8° for the (−)-isomer and m.p. 182° for the (+)-isomer. The i.r. spectrum was similar to that published by Huckel & Fechtig (1962).

*Epiborneol*. This was prepared by reduction of epicamphor with sodium in ethanol; after purification by preparative t.l.c. it had m.p. 178–179° (literature value 181–182·5°). The i.r. spectrum resembled that published by Huckel & Fechtig (1962).

(±)-*Camphorquinone*. This was synthesized by the oxidation of (±)-camphor with $SeO_2$ (Evans, Ridgion & Simonsen, 1934). On recrystallization the product had m.p. 197–198° (literature value 199°).

(±)-3-endo-*Hydroxycamphor and* (±)-2-endo-*hydroxy-epicamphor*. These compounds were obtained as a mixture on reduction of (±)-camphorquinone by zinc in acetic acid (Manasse, 1897; Bredt & Ahrens, 1926; Huckel & Fechtig, 1962). The 2-*endo* compound was separated from the mixture by its ability to form a methyl ether with dry methanolic HCl, which on recrystallization from methanol had m.p. 142–144°. Bredt & Ahrens (1926) gave m.p. 133–134° for the (±)-isomer and m.p. 149–150° for the (+)-isomer. 3-*endo*-Hydroxycamphor was recovered unchanged and was purified by semicarbazone formation. Repeated recrystallization of the semicarbazone from ethanol yielded a product with m.p. 182–183° (literature value 182–183°). Hydrolysis of the semicarbazone by refluxing it with aq. 10% (w/v) oxalic acid gave a compound that on recrystallization had m.p. 196–197° (literature value 200°).

(±)-*Camphane-2,5-dione*. This was prepared by chromic acid oxidation of (±)-bornyl acetate in acetic acid–acetic anhydride (Asahina, Ishidate & Tukamoto, 1936; Malkonen, 1964). 5-Oxobornyl acetate on saponification with aq. 10% (w/v) $Na_2CO_3$ solution gave 5-oxoborneol, which on further oxidation with Jones reagent in acetone gave camphane-2,5-dione; this, after recrystallization, had m.p. 207–209° (literature value 210°). The i.r. spectrum showed an absorption band at 1757 cm.$^{-1}$ due to C=O stretching.

(±)-5-endo-*Hydroxycamphor*. This was prepared from camphane-2,5-dione by the method of Asahina & Ishidate (1934) and purified through formation of the acetate, which

was separated from unchanged diketone by passing the mixture through a silica-gel column and eluting the acetate with ether–light petroleum (1:49, v/v). Saponification with aq. 10% (w/v) $Na_2CO_3$ solution gave 5-*endo*-hydroxycamphor, which was further purified by preparative t.l.c. After recrystallization from light petroleum the product had m.p. 212–214°. Shimamoto (1934) gave m.p. 217°.

*Camphane.* This was prepared by Huang-Minlon (1946) reduction of (±)-camphor hydrazone in triethylene glycol. Camphor hydrazone was first prepared by refluxing a mixture of camphor (40g.), ethanol (150ml.), hydrazine hydrate [30ml. of a 100% (w/w) solution] and water (5ml.) for 32hr. In later experiments it was found that the addition of 5–10ml. of acetic acid to the reaction mixture facilitated the formation of the hydrazone, lowering the time of reaction to 16–20hr. T.l.c. of the product of the Huang-Minlon reaction revealed the presence of camphor and borneol. The mixture was purified by column chromatography on a column of silica gel (100–200 mesh) from which camphane was eluted with light petroleum. Recrystallization from light petroleum yielded white plates, m.p. 154–155° (literature value 158–159°). The i.r. spectrum showed no carbonyl absorption band.

## METHODS

*Animals and diet.* White New Zealand × albino cross-bred rabbits weighing 1·8–3·5kg. were used. Their daily diet consisted of 100g. of Pelleted Rabbit Food (Drug Houses of Australia, Sydney, N.S.W., Australia) and unlimited water. The test compounds, suspended in a 1% methylcellulose mucilage, were administered by stomach tube followed by about 25ml. of water.

Quantitative determination of glucuronide was carried out by the method of Hanson, Mills & Williams (1944), as modified by Paul (1951).

*Infrared spectroscopy.* Measurements were made with a Perkin–Elmer spectrophotometer, model 21, fitted with rock-salt optics. Camphor, epicamphor, norcamphor, camphorquinone, camphane-2,5-dione, borneol and epiborneol were prepared as 1–2% solutions in $CS_2$, and the other compounds as Nujol mulls.

*Thin-layer chromatography.* Plates were prepared as described by Elliott, Robertson & Williams (1966*a*,*b*) with silica gel H (E. Merck A.-G., Darmstadt, Germany). *n*-Hexane–ethyl acetate (3:1, v/v) was used as solvent for analytical and preparative t.l.c. Compounds were located with the phosphomolybdic acid reagent (Kritchevsky & Kirk, 1952). For preparative t.l.c. approx. 80–100mg. of the mixture was placed on the layer in a line rather than in spots. The bands were located by exposing the dried chromatogram to iodine vapour in a tank.

*Gas–liquid chromatography.* A Hewlett–Packard F & M model 5760 gas chromatograph equipped with a flame-ionization detector was used. The stationary phase was 10% UC-W 98 (Hewlett–Packard) on Chromosorb P (100–120 mesh). The column temperature was maintained at 150° during analysis, and $N_2$ was used as carrier gas. In quantitative analysis camphane was used as an internal standard.

*Hydrolysis of urine.* The 24hr. urines were pooled and acidified with 10M-HCl to pH1 and then refluxed for 6hr. After cooling the urine was extracted with ether. Any steam-volatile compound deposited in the condenser was collected by washing with ether.

*Experiments in vitro.* Equine liver alcohol dehydrogenase and NADH (Sigma Chemical Co., St Louis, Mo., U.S.A.) were used without further purification. A 160μg. portion of alcohol dehydrogenase was used for each experiment. NADH was dissolved in 0·03M-sodium phosphate buffer, pH7·0, and was used at a final concentration of 0·1mM. Solutions of cyclohexanone, (+)-camphor, (−)-camphor, (+)-epicamphor, (±)-norcamphor, (±)-camphorquinone and (±)-camphane-2,5-dione were made at a concentration of 0·3mM by dissolving suitable amounts of each separately in 0·03M-sodium phosphate buffer, pH7·0. A 1ml. sample of each of these solutions was used (final concn. 0·1mM). The reductions were followed by measuring the disappearance of NADH at 340nm. by using a Unicam SP.700 recording spectrophotometer. All reactions were carried out at 30° in 3ml. silica cuvettes (1cm. light-path) in a total volume of 3ml. The reaction was initiated by the addition of alcohol dehydrogenase. A blank containing all components except the substrate was run simultaneously and the initial rate of reduction of each substrate was compared with that of cyclohexanone. The results are given in Table 3.

## RESULTS

Of the compounds administered, (±)-norcamphor, (±)-camphorquinone and camphane did not appear to possess any pharmacological action. Epicamphor and camphor produced convulsions at a dose of 1g./rabbit. At 5min. after the administration of 500mg. of (±)-camphane-2,5-dione, the hind limbs became paralysed, and strong convulsions and death followed after 10min.

### *Determination of urinary glucuronides*

The urine collected at 24hr. and 48hr. after administration had pH8–9, and was usually of a reddish-brown colour. In all cases, except after the administration of (±)-camphane-2,5-dione, it gave an intense naphtharesorcinol reaction. Quantitative determination of glucuronic acid was carried out on urine samples before and after dosing; the results are given in Table 1.

### *Characterization of aglycones*

The glucuronides contained in the urine were hydrolysed to establish the identity of the aglycones. The hydrolysate was then extracted with ether. Aglycones were identified by melting-point determination, i.r. spectroscopy, t.l.c. and derivative formation. The separation and characterization of the metabolites of each compound given are described in the succeeding sections. $R_F$ values on t.l.c. of the compounds administered and of the metabolites isolated are given in Table 2.

(±)-*Norcamphor.* A 700mg. portion of the ketone was given to each of five rabbits. T.l.c. of the ether extract of hydrolysed urine revealed the presence of only one spot, $R_F$ 0·27. The compound was purified

Table 1. *Excretion of camphor and related compounds as glucuronides*

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

| Compound | No. of animals used | Dose (m-moles/kg. body wt.) | % of dose excreted as glucuronide |
|---|---|---|---|
| (±)-Norcamphor | 5 | 3·1–3·3 | 30·0 (24·3–43·6) |
| (+)-Camphor | 5 | 1·9–3·5 | 59·1 (55·3–65·8) |
| (±)-Camphor | 5 | 1·9–2·8 | 44·4 (41·1–47·7) |
| (−)-Camphor | 5 | 0·8–1·7 | 32·7 (24·0–40·0) |
| (+)-Epicamphor | 3 | 2·2–2·3 | 47·3 (45·9–49·7) |
| (±)-Camphorquinone | 7 | 1·3–2·5 | 38·3 (16·8–57·2) |
| (±)-Camphane-2,5-dione | 3 | 0·26–0·30 | 0 |
| Camphane | 5 | 1·8–4·0 | 14·2 (5·5–27·0) |

Table 2. *$R_F$ values on thin-layer chromatography of the compounds administered to rabbits and the metabolites isolated from their urine after hydrolysis*

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

| Compound | $R_F$ | Compound | $R_F$ |
|---|---|---|---|
| (±)-Norcamphor | 0·54 | Borneol | 0·57 |
| (+)-Camphor | 0·70 | Isoborneol | 0·61 |
| (+)-Epicamphor | 0·67 | Epiborneol | 0·49 |
| (±)-Camphorquinone | 0·36 | Epi-isoborneol | 0·55 |
| (±)-Camphane-2,5-dione | 0·38 | *endo*-Norborneol | 0·27 |
| | | 3-*endo*-Hydroxycamphor | 0·35 |
| | | 5-*endo*-Hydroxycamphor | 0·13 |
| | | 2-*endo*-Hydroxyepicamphor | 0·35 |

on a silica-gel column and eluted with ether–light petroleum (1:49, v/v). The i.r. spectrum of this material was identical with that of (±)-*endo*-norborneol. The product had m.p. 140–142° and $[\alpha]_D^{20}$ −1·01° (*c* 2·6 in chloroform). On oxidation with Jones reagent in acetone it gave norcamphor, $R_F$ 0·54; the DNP-hydrazone on recrystallization from ethanol had m.p. 129°, unchanged by mixture with the authentic compound.

(+)-*Camphor*. A 1·0g. portion of (+)-camphor was given to each of five rabbits. T.l.c. of the ether extract of the hydrolysed urine revealed the presence of three metabolites, $R_F$ 0·13, 0·35 and 0·57. These three compounds were separated by preparative t.l.c.

The compound of $R_F$ 0·57 was purified by sublimation under vacuum. T.l.c. revealed the presence of traces of a second compound of $R_F$ 0·61, which might have been isoborneol. The i.r. spectrum of the major metabolite was identical with that of authentic (±)-borneol. It had m.p. 203–205° and $[\alpha]_D^{20}$ +36·00° (*c* 2 in ethanol). On oxidation with Jones reagent in acetone, it gave camphor, $R_F$ 0·70, the DNP-hydrazone of which on recrystallization from ethanol had m.p. 175°, unchanged by mixture with the authentic compound.

The compound of $R_F$ 0·35 was recrystallized from light petroleum and shown to be 3-*endo*-hydroxycamphor by i.r.-spectrum correlation. The compound had m.p. 196–197° and $[\alpha]_D^{20}$ +18·3° (*c* 4·012 in chloroform). On oxidation with Jones reagent it gave (−)-camphorquinone, which on recrystallization from light petroleum had m.p. 198° and $[\alpha]_D^{20}$ +110·88° (*c* 1·55 in chloroform).

The compound of $R_F$ 0·13 was recrystallized from light petroleum. Its i.r. spectrum was identical with that of synthetic 5-*endo*-hydroxycamphor. It had m.p. 217–218° and $[\alpha]_D^{20}$ +41·05° (*c* 2 in ethanol). On oxidation with Jones reagent it gave (+)-camphane-2,5-dione, $R_F$ 0·38, which on recrystallization from light petroleum had m.p. 210–211° and $[\alpha]_D^{20}$ +115·8° (*c* 1·85 in ethanol).

(+)-Borneol, (+)-3-*endo*-hydroxycamphor and (+)-5-*endo*-hydroxycamphor were found to be present in the proportions 1·00:0·88:2·80, determined by g.l.c.

(−)-*Camphor*. A 300–500mg. portion of (−)-camphor was given to each of five rabbits. G.l.c. and t.l.c. of the ether extract of the hydrolysed 24hr. urine revealed the presence of four compounds, three of which were identified by melting-point determination, i.r. spectroscopy and derivative formation as borneol, 3-*endo*-hydroxycamphor and 5-*endo*-hydroxycamphor. The proportions of

these compounds as determined by g.l.c. were found to be 1·00:2·4:5·00.

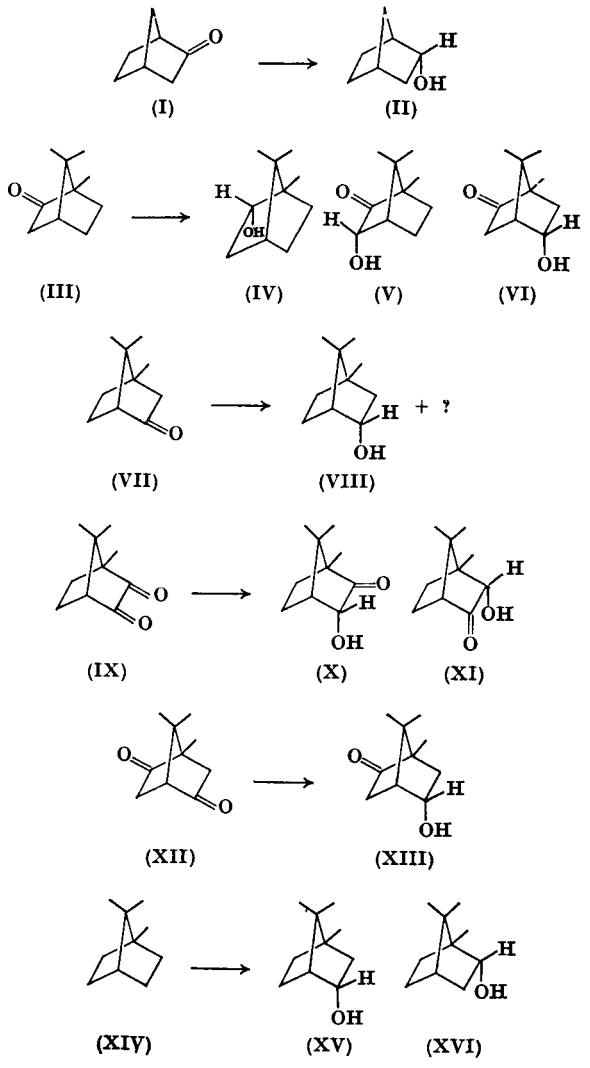
(+)-*Epicamphor*. A 700mg. portion of the ketone was given to each of three rabbits. T.l.c. of the ether extract of hydrolysed urine revealed the presence of two major metabolites, $R_F$ 0·49 and 0·14, but g.l.c. indicated the presence of three metabolites. The material of $R_F$ 0·49 corresponded to epiborneol (Table 2). It was separated by preparative t.l.c. and then sublimed. The i.r. spectrum of this material was identical with that of authentic (+)-epiborneol. It had m.p. 175–176° and $[\alpha]_D^{20}$ +1·81° (*c* 1·05 in toluene). The compound on oxidation gave epicamphor, $R_F$ 0·67. The DNP-hydrazone on recrystallization from methanol had m.p. 186–187°, unchanged by mixture with an authentic sample.

(±)-*Camphorquinone*. A 500–800mg. portion of the diketone was given to each of seven rabbits. The metabolites were isolated and chromatographed in the usual manner, and appeared to be a single compound. Huang-Minlon reduction of the hydrazone of this material gave a mixture of epiborneol, $R_F$ 0·49 (major), and borneol, $R_F$ 0·57 (minor), indicating that the metabolite was a mixture of 3-*endo*-hydroxycamphor and 2-*endo*-hydroxyepicamphor. On oxidation with Jones reagent the mixture gave camphorquinone, which on recrystallization from light petroleum had m.p. 197–198° and $[\alpha]_D^{20}$ −16·02° (*c* 2·25 in chloroform). The optical rotation suggested that (−)-camphorquinone had undergone less degradation than the (+)-isomer.

The 3-*endo*-hydroxycamphor was separated from 2-*endo*-hydroxyepicamphor through the formation of a methyl ether by using dry methanolic hydrogen chloride. The methyl ether of the latter compound, when recrystallized from methanol, had m.p. 149–150° and $[\alpha]_D^{20}$ +24·78° (*c* 3·1 in benzene). The i.r. spectrum of this material was identical with that of material prepared by synthesis.

The unchanged material was recovered by removing the methanol under vacuum, and then purifying the compound through semicarbazone formation. The semicarbazone on recrystallization from ethanol had m.p. 182–183°. On hydrolysis with aqueous oxalic acid it gave a compound that on recrystallization from light petroleum had m.p. 196–197° and $[\alpha]_D^{20}$ +2 73° (*c* 2·23 in ethanol). The i.r. spectrum of this material was identical with that of synthetic (±)-3-*endo*-hydroxycamphor.

(±)-*Camphane*-2,5-*dione*. A 100mg. portion of the compound was given to each of three rabbits. No pharmacological action was observed at this dosage. However, there was no increase in glucuronic acid excretion, which indicates that the metabolite of camphane-2,5-dione was not conjugated with glucuronic acid.




Scheme 1. Metabolism of camphors and related compounds in the rabbit. (I), Norcamphor; (II), *endo*-norborneol; (III), (+)-camphor; (IV), (+)-borneol; (V), (+)-3-*endo*-hydroxycamphor; (VI), (+)-5-*endo*-hydroxycamphor; (VII), (+)-epicamphor; (VIII), (+)-epiborneol; (IX), camphorquinone; (X), 3-*endo*-hydroxycamphor; (XI), 2-*endo*-hydroxyepicamphor; (XII), camphane-2,5-dione; (XIII), 5-*endo*-hydroxycamphor; (XIV), camphane; (XV), epiborneol; (XVI), borneol.

T.l.c. of the ether extract of the hydrolysed urine revealed the presence of one intense spot, $R_F$ 0·13, suggesting that the compound was 5-*endo*-hydroxycamphor (Table 2).

*Camphane*. A 0·5–1·0g. portion of camphane was given to each of five rabbits. T.l.c. of the ether extract of hydrolysed 24hr. urine revealed two spots, $R_F$ 0·49 (major) and $R_F$ 0·57 (minor). These two

compounds were separated by preparative t.l.c. The plates were developed twice to achieve good separation. The compound having $R_F$ 0·57 was purified by sublimation. The i.r. spectrum of this compound was identical with that of synthetic borneol. It had m.p. 202–204° and $[\alpha]_D^{20}$ 0·00° (*c* 2·1 in ethanol). On oxidation with Jones reagent in acetone it gave camphor, $R_F$ 0·70 (Table 2).

The compound having $R_F$ 0·49 had, after sublimation, m.p. 174–175° and $[\alpha]_D^{20}$ 0·00° (*c* 2·32 in toluene). There was a peak-to-peak correlation between the i.r. spectrum of this material and that of authentic epiborneol. On oxidation with Jones reagent it gave epicamphor, the DNP-hydrazone of which on recrystallization from methanol had m.p. 185–186°, unchanged on mixture with the authentic compound.

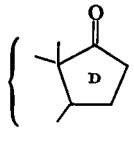
A summary of the metabolism of the camphors and related compounds is given in Scheme 1.

## DISCUSSION

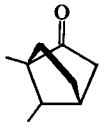
Except for camphane-2,5-dione, administration of the compounds investigated increased the urinary content of conjugated glucuronic acid (Table 1). Hydrolysis and extraction of the urine yielded secondary alcohols that had resulted from either reduction of an oxo group or hydroxylation of a methylene group. The two modes of metabolism are discussed separately.

*Hydroxylation.* As well as being reduced, (+)- and (−)-camphor were oxidized to the corresponding 5-*endo*-hydroxycamphor and 3-*endo*-hydroxycamphor, the former predominating in each case.

In general, alicyclic compounds are either specifically hydroxylated, e.g. the steroids (Hayano, 1962) and the decalins (Elliott *et al.* 1966*b*), or randomly hydroxylated, e.g. methylcyclohexane (Elliott, Tao & Williams, 1965*b*). Hydroxylation of steroids by hydroxylases is generally believed to occur through direct replacement of a specific ring hydrogen by a hydroxyl group, e.g. 11$\beta$-hydroxylases introduce a hydroxyl group into the 11$\beta$-position. In a discussion of enzyme induction by 17-oxo steroids (King, Mason & Morrison, 1964), reference was made to the existence of progesterone-induced ring-cleavage enzymes for (+)-camphor, and the similarity between the structure of camphor and the D-ring of steroids was noted.




17-Oxo steroid




(+)-Camphor

It is possible that steroid hydroxylases are concerned in the oxygenation of camphor and camphane. However, if this were so, then there should have been attack at a specific site leading to the formation of only one alcohol (or a pair of epimers). The present findings, that the compounds are hydroxylated at positions 2 and 3 (or 5 and 6), are not inconsistent with a free-radical mode of attack.

Whatever the mechanism of hydroxylation, the products were all *endo*-alcohols, suggesting that the hydroxyl group was attached from the *endo* side.

The preponderance of epiborneol over borneol in the metabolites of camphane is consistent with the less hindered nature of the 3-position in camphane. The 5-position in (+)- and (−)-camphor is more strained than is the 3-position, and 5-*endo*-hydroxycamphor is formed in greater amount.

The greater percentage excretion of metabolites as glucuronides when (+)-camphor was administered (Table 1) would indicate that it was more favourably sited on the enzyme than was (−)-camphor. Also, the greater production of hydroxylated metabolites over that produced by reduction would suggest that the body found hydroxylation the easier process.

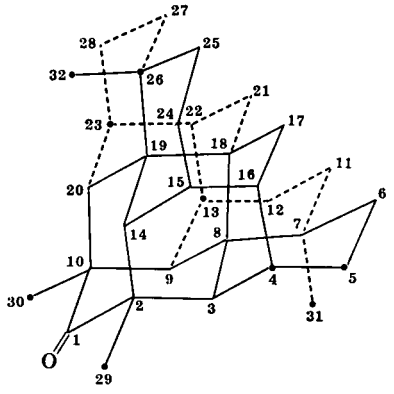
*Reduction.* If the pattern of reduction of the methylcyclohexanones (Elliott, Tao & Williams, 1965*a*) and of the decalones (Elliott *et al.* 1966*a*) were followed in the present series of compounds, the hydroxyl group would be expected to be in the thermodynamically stable form. In all cases, however, the hydroxyl group had the *endo* configuration (Table 2) irrespective of thermodynamic stability.

This parallels the results obtained when the same ketones were reduced with sodium in ethanol, a mode of reduction that was once thought to yield the thermodynamically more stable alcohol (Barton, 1950, 1953; Barton & Cookson, 1956; Klyne, 1954; Dauben & Pitzer, 1956). The finding that it does not (Ourissan & Rassat, 1960) may be explained in terms of the mechanism of reduction proposed by House (1965), where it is assumed that

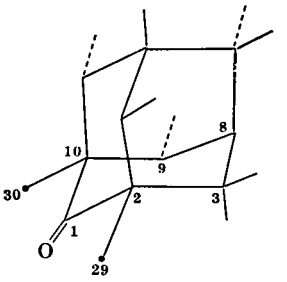
Table 3. *Relative rates of reduction of cyclohexanone, (±)-norcamphor and (±)-camphorquinone with NADH and liver alcohol dehydrogenase*

Experimental details are given in the text. (+)-Camphor, (−)-camphor, (+)-epicamphor and (±)-camphane-2,5-dione were not reduced under these conditions.

| Compound | Relative rate of reduction |
|---|---|
| Cyclohexanone | 100 |
| (±)-Norcamphor | 2·22 |
| (±)-Camphorquinone | 0·729 |




(*a*)



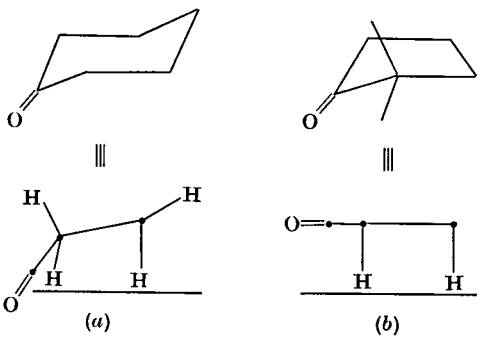

(*b*)

Fig. 2. (*a*) Diamond lattice section (Graves *et al.* 1965); (*b*) simplified diamond lattice section.

two molecules of ketone are required to produce one molecule of alcohol, and that the initial hydrogen attack is always towards the less hindered side. This is the *exo* side with norcamphor and *endo* side with camphor and similar compounds possessing a methyl group at the bridge in *cis* relationship to the oxo group. Usually the initial reduction product reacts with more starting ketone to produce the isomeric and more stable alcohol. If, however, the molecule is strained as is norcamphor, the initial reduction product does not react further, resulting in this instance in the formation of the less stable *endo*-norborneol. With camphor and similar molecules, initial reduction takes place from the less hindered *endo* side, and isoborneol should result. However, there now occurs such steric conflict between the oxygen of the newly reduced ketone and the *cis*-methyl group at the bridge that the developing hydroxyl group is forced into the *endo* configuration, resulting in the formation of borneol.

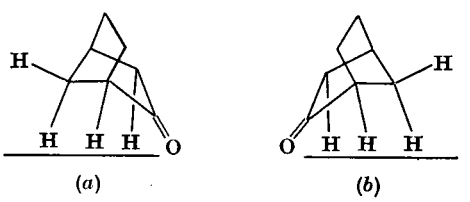
It is suggested that, in like manner, hydrogen attack *in vivo* is directed initially to the less hindered side, but strain energy and steric hindrance permit production only of *endo* alcohols. The finding that metabolically produced borneol contained possible traces of isoborneol tends to confirm that reduction was initially from the less hindered side.

Since many ketones that are reduced *in vivo* to secondary alcohols are similarly reduced *in vitro* by NADH or NADPH in the presence of liver alcohol dehydrogenase (Winer, 1958; Merritt & Tomkins, 1959; Prelog, 1963, 1964; Elliott *et al.* 1966*a*; Graves, Clark & Ringold, 1965), this combination was used in an attempt to reduce the ketones investigated. Of these compounds only norcamphor and camphorquinone were so reduced (Table 3), although all were reduced *in vivo*. For comparison, the reduction of cyclohexanone was also tested




(*a*) (*b*)

Fig. 3. Positioning of (*a*) cyclohexanone and (*b*) cyclopentanone on the enzyme surface; the former is reduced by liver alcohol dehydrogenase–NADH and the latter is not (see the text).




(*a*) (*b*)

Fig. 4. Positioning of (*a*) (+)-norcamphor and (*b*) (−)-norcamphor on the enzyme surface; the compounds are reduced by liver alcohol dehydrogenase–NADH (cf. Fig. 3*a*).

This difference in behaviour *in vitro* may be

explained in terms of the stereospecific requirements for hydrogen transfer in the liver alcohol dehydrogenase–NADPH system. These requirements have been summarized by Prelog (1964), in the form of his diamond-lattice-section theory, the validity of which has been substantiated by Graves *et al.* (1965). The theory requires, among other things, that when the proposed substrates are sited on the enzyme surface so that the carbonyl group assumes a specific orientation, the molecule fits into a diamond lattice (Figs. 2*a* and 2*b*), whether or not it has the form of a cyclohexanone chair. Reduction occurs unless there is a group larger than hydrogen at C-10 in the lattice.

For simplicity, the cyclohexanone moiety of the lattice is reproduced in Fig. 3(*a*), where it is shown attached to the enzyme surface by axial bonds at C-3 and C-5, and by equatorial bonds at C-2 and C-6. This shows that the carbonyl group lies well below the level of the enzyme surface, presumably at a distance that allows of reduction by NADH.

It is noteworthy that although cyclopentanone will fit the lattice and has only a hydrogen atom at C-2, it is not reduced by the enzyme (Graves *et al.* 1965). Fig. 3(*b*) shows that when the molecule is anchored by the bonds at C-3 and C-4, the carbonyl group is held away from the surface by the 'axial' bonds at C-2 and C-5, presumably preventing reduction (Graves *et al.* 1965).

Assuming that (±)-norcamphor is reduced from the *exo* side, the epimers will assume the orientation shown in Figs. 4(*a*) and 4(*b*). Although there are α-bonds at C-3 and C-7, neither prevents the carbonyl groups from extending below the level of the enzyme surface, since there is only one binding bond at C-3 or C-7. Since the equatorial bond at C-1 carries only a hydrogen atom, and the molecule appears to fit the diamond lattice, reduction would be expected. The lower rate of reduction compared with cyclohexanone must be due to as yet unknown steric factors.

(±)-Camphorquinone should be reduced from the *endo* side (see above). Since there is no hydrogen atom next to either of the carbonyl groups, the molecule can assume the same position as can cyclohexanone (Figs. 5*a* and 5*b*) whichever carbonyl group is being reduced. The preponderance of 3-hydroxycamphor over 2-hydroxyepicamphor is understandably due to the steric hindrance offered by the methyl group at C-1 of the substrate. The lower rate of reduction compared with norcamphor may well be due either to a carbonyl group or a methyl group at C-2 in the lattice, or to the 8- or 10-methyl groups of the substrate, depending on the particular epimer being reduced.

When camphor, epicamphor or camphane-2,5-dione are anchored on the enzyme surface (Fig. 6), all possess an axial bond α to the carbonyl group,

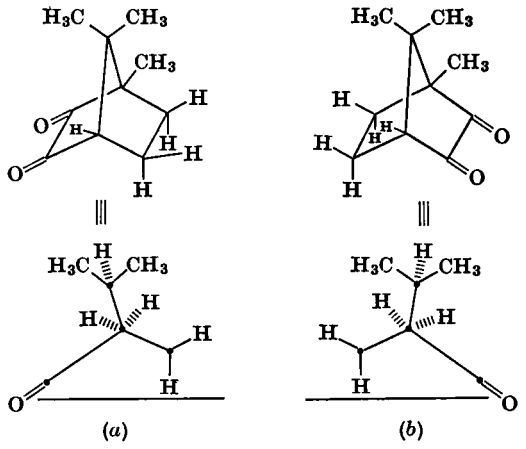



Fig. 5. Positioning of (*a*) (−)-camphorquinone and (*b*) (+)-camphorquinone on the enzyme surface; the compounds are reduced by liver alcohol dehydrogenase–NADH (cf. Fig. 3*a*).

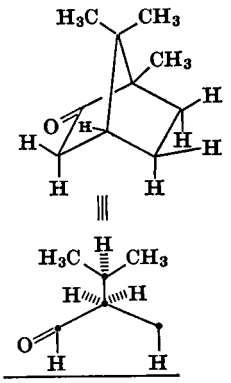


Fig. 6. Positioning of (+)-camphor on the enzyme surface; the compound is not reduced by liver alcohol dehydrogenase–NADH (cf. Fig. 3*b*).

which, as in cyclopentanone, is kept above and out of contact with NADH, so preventing reduction.

The conclusion is that, although the reduction of norcamphor and camphorquinone *in vivo* may be due to liver alcohol dehydrogenase and NADH, some other system must be responsible for the reduction of the other ketones.

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## REFERENCES

Asahina, Y. & Ishidate, M. (1928). *Ber. dtsch. chem. Ges.* **61B**, 533.

Asahina, Y. & Ishidate, M. (1933). *Ber. dtsch. chem. Ges.* **66**, 1673.

Asahina, Y. & Ishidate, M. (1934). *Ber. dtsch. chem. Ges.* **67**, 71.
Asahina, Y. & Ishidate, M. (1935). *Ber. dtsch. chem. Ges.* **68B**, 947.
Asahina, Y., Ishidate, M. & Tukamoto, T. (1936). *Ber. dtsch. chem. Ges.* **69**, 349.
Barton, D. H. R. (1950). *Experientia*, **6**, 316.
Barton, D. H. R. (1953). *J. chem. Soc.* p. 1027.
Barton, D. H. R. & Cookson, R. C. (1956). *Quart. Rev.* **10**, 44.
Berson, J. A., McRowe, A. W., Bergman, R. G. & Houston, D. (1967). *J. Amer. chem. Soc.* **89**, 2563.
Bowers, A., Halsall, T. G., Jones, E. R. H. & Lemin, A. J. (1953). *J. chem. Soc.* p. 2548.
Bredt, J. & Ahrens, H. (1926). *J. prakt. Chem.* **112**, 273.
Dauben, W. G. & Pitzer, K. S. (1956). In *Steric Effects in Organic Chemistry*, p. 47. Ed. by Newman, M. L. New York: John Wiley and Sons Inc.
Elliott, T. H., Robertson, J. S. & Williams, R. T. (1966*a*). *Biochem. J.* **100**, 393.
Elliott, T. H., Robertson, J. S. & Williams, R. T. (1966*b*). *Biochem. J.* **100**, 403.
Elliott, T. H., Tao, R. C. C. & Williams, R. T. (1965*a*). *Biochem. J.* **95**, 59.
Elliott, T. H., Tao, R. C. C. & Williams, R. T. (1965*b*). *Biochem. J.* **95**, 70.
Evans, W. C., Ridgion, J. M. & Simonsen, J. L. (1934). *J. chem. Soc.* p. 137.
Graves, M. H., Clark, A. & Ringold, H. J. (1965). *Biochemistry*, **4**, 2655.
Hamalainen, J. (1912). *Skand. Arch. Physiol.* **27**, 141.
Hanson, S. W. F., Mills, G. T. & Williams, R. T. (1944). *Biochem. J.* **38**, 274.
Hayano, M. (1962). In *Oxygenases*, p. 181. Ed. by Hayaishi, O. New York: Academic Press Inc.
Heilbron, I., Cook, A. H., Bunbury, H. M. & Hey, D. H. (1965). *Dictionary of Organic Compounds*. London: Eyre and Spottiswoode Publishers Ltd.
Hirsjarvi, P. (1957). *Ann. Acad. Sci. fenn.* A.II, no. 81, p. 5.
House, H. O. (1965). In *Modern Synthetic Reactions*, pp. 30–31, 54–55. New York: W. A. Benjamin Inc.
Huang-Minlon, (1946). *J. Amer. chem. Soc.* **68**, 2487.
Huckel, W. & Fechtig, O. (1962). *Liebigs Ann.* **645**, 101.
Ishidate, M., Kawahata, H. & Nakazawa, K. (1941). *Ber. dtsch. chem. Ges.* **74**, 1707.
King, T. E., Mason, H. S. & Morrison, M. (1964). *Proc. Symp. Oxidases and Redox Systems, Amherst, Mass.*, vol. 2, p. 835.
Kleinfelter, D. C. & Schleyer, P. von R. (1962). *Org. Synth.* **72**, 79.
Klyne, W. (1954). *Progr. Stereochem.* **1**, 36.
Krieger, H. (1962). *Acta chem. fenn.* **35B**, 382.
Kritchevsky, D. & Kirk, M. R. (1952). *Arch. Biochem. Biophys.* **35**, 346.
Magnus-Levy, A. (1906). *Biochem. Z.* **2**, 319.
Malkonen, P. (1964). *Ann. Acad. Sci. fenn.* A.II, no. 128, p. 1.
Manasse, O. (1897). *Ber. dtsch. chem. Ges.* **30**, 659.
Mayer, P. (1908). *Biochem. Z.* **9**, 439.
Merritt, A. D. & Tomkins, G. M. (1959). *J. biol. Chem.* **234**, 2778.
Noyce, D. S. & Denny, D. B. (1950). *J. Amer. chem. Soc.* **72**, 5743.
Ourissan, G. & Rassat, A. (1960). *Tetrahedron Lett.* no. 21, p. 16.
Paul, J. (1951). Ph.D. Thesis: University of Glasgow.
Prelog, V. (1963). *Colloq. Ges. physiol. Chem.* **14**, 288.
Prelog, V. (1964). *Pure appl. Chem.* **9**, 119.
Reinartz, F. & Zanke, W. (1934). *Ber. dtsch. chem. Ges.* **67B**, 548.
Reinartz, F., Zanke, W. & Faust, K. (1934). *Ber. dtsch. chem. Ges.* **67**, 1536.
Reinartz, F., Zanke, W. & Kuersch, M. (1935). *Ber. dtsch. chem. Ges.* **68B**, 310.
Reinartz, F., Zanke, W. & Schaefers, O. (1934). *Ber. dtsch. chem. Ges.* **67B**, 589.
Rodd, E. H. (1953). *Chemistry of Carbon Compounds*, vol. 2B. London: Elsevier Publishing Co.
Schmiedeberg, O. & Meyer, H. (1879). *Hoppe-Seyl. Z.* **3**, 422.
Shimamoto, T. (1934). *Sci. Pap. Inst. Phys. Chem. Res., Tokyo*, no. 529, pp. 52–58, 59–62.
Winer, A. D. (1958). *Acta chem. scand.* **12**, 1695.