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Metabolism of Camphanediols

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1. The metabolism of the camphane-2,3-diols and camphane was investigated in rabbits. All the compounds increased the content of glucuronide in the urine. 2. Both *trans*- and *cis*-diols gave rise to ketols; the *cis*-diols gave rise to *trans*-diols, but not vice versa; camphorquinone gave *cis*-diol, ketols and *trans*-diol; camphane did not give rise to diols. 3. The possibility is discussed that an enediol is the intermediate for ketol interconversions in the present series of compounds and in other series such as hydroxyoestrones and hydroxyindanones.

After administration to animals of cyclic hydrocarbons, mono- and di-hydroxylated metabolites, either free or conjugated, are frequently found in the urine. Of dihydroxylated metabolites, cyclohexane (Elliott, Parke & Williams, 1959), naphthalene (Corner & Young, 1954), anthracene (Booth & Boyland, 1949) and phenanthrene (Young, 1947; Boyland & Wolf, 1950) gave rise to *trans*-diols; indene (Brooks & Young, 1956), acenaphthylene (Hopkins, Brooks & Young, 1962) and tetralin (Elliott & Hanam, 1968) were hydroxylated to *cis*- and *trans*-diols. Foster (1962) has concluded that vicinal *trans*-diols are invariably intermediates in the metabolism of cyclic hydrocarbons.

In an investigation of the metabolism of camphane (Robertson & Hussain, 1969) the only metabolites detected were borneol and epiborneol. However, reference camphanediols were unavailable at the time, so the possibility of diol formation was not rigorously excluded. The four racemic camphanediols have now been prepared and used to show that diol formation is not a feature of camphane metabolism. The metabolic fate of the diols has also now been investigated.

MATERIALS

Melting points of the diols were obtained by the sealed-capillary-tube method, with a Kofler block. The capillary contained sufficient material to ensure that sublimation did not vitiate the melting-point determination. Melting points are uncorrected.

Camphane. This was prepared as described by Robertson & Hussain (1969) and had m.p. 156–157°C (literature value 158–159°C).

(±)-*Camphorquinone.* This was prepared by a slight modification of the method of Evans, Ridgion & Simonsen (1934). These authors' method gave camphorquinone containing about 20% unchanged camphor, which could be removed by careful column chromatography but with much diminished yield of product. However, increasing the SeO_2/camphor ratio from 6:5 to 8:5 and increasing reflux time from 3h to 30h decreased the unchanged camphor content to 5%. Recrystallization from benzene yielded yellow needles, m.p. 196°C (literature value 199°C).

(±)-2-endo-*Hydroxyepicamphor* (*I*) *and* (±)-3-endo-*hydroxycamphor* (*II*). These 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 dimolecular methyl ether with anhydrous methanolic HCl, which on recrystallization from cold methanol had m.p. 135–137°C; Bredt & Ahrens (1926) gave m.p. 133–134°C for the (±)-isomer and 149–150°C 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. 180–182°C (literature value 182–183°C). Hydrolysis of the semicarbazone, by refluxing with aq. 10% (w/v) oxalic acid, gave a compound that on recrystallization from a minimum volume of light petroleum had m.p. 195–197°C (literature value 200°C).

(±)-2-*endo*-Hydroxyepicamphor was recovered by stirring the dimer in conc. HCl at room temperature for 24h. The mixture was diluted with water, made alkaline and extracted with ether. Evaporation of the ether yielded a white solid, which on recrystallization from a minimum volume of light petroleum yielded needles with m.p. 209–211°C (literature value 211–212°C).

(±)-cis-2-endo,3-endo-*Camphanediol* (*III*). This was prepared by reducing (±)-2-*endo*-hydroxyepicamphor with $LiAlH_4$ in anhydrous ether at 0°C for 20h (Takeshita & Kitajima, 1959). T.l.c. of the product showed two spots of R_F values 0.4 and 0.3 (system 2), indicating the presence of '*cis*-diol' and '*trans*-diol' respectively (see below). The '*cis*-diol' was converted into the acetonide (Rupe & Thommen, 1947), which was separated from the unchanged *trans*-diol by chromatography on a silica-gel (100–200 mesh) column. Elution with ethyl acetate–light petroleum (9:91, v/v), evaporation of solvent and distillation at 70°C/2mmHg gave the acetonide; the i.r.

spectrum (liquid film) was identical with that of the acetonide of (±)-*cis*-2-*endo*,3-*endo*-camphanediol (Takeshita & Kitajima, 1959). Hydrolysis of the acetonide to the diol was attempted by addition of acetic acid (Angyal & Young, 1959), but this was unsuccessful, even under reflux conditions. Addition of a few drops of 10M-HCl to the mixture and further refluxing yielded a mixture of four products, R_F values 0.4, 0.45, 0.5 and 0.75 (system 2). The first and last spots had the same R_F values as '*cis*-diol' and acetonide respectively (Table 2). The other spots were most probably due to acetates of the '*cis*-diol', for they were not present after refluxing the mixture with 10% (w/v) NaOH in methanol. Hydrolysis was effected by refluxing for 4h in methanol containing 10% (v/v) of 10M-HCl. The reaction mixture was made alkaline and extracted with ether. Evaporation of the ether left an oily semi-solid. The oil was removed by short-path distillation at 100°C at atmospheric pressure. The solid remaining was sublimed at 110°C/2mmHg, yielding a white solid, m.p. 252–254°C [literature value 255–256°C for (±)-*cis*-2-*endo*,3-*endo*-camphanediol] and $[\alpha]_D^{20}$ 0.00° (*c* 2 in ethanol). The i.r. spectrum was identical with that for (±)-*cis*-*endo*-diol (Takeshita & Kitajima, 1959); g.l.c. showed one peak, retention time 9.0min.

The '*trans*-diol' was recovered from the column by elution with methanol; purification by repeated sublimation (nine times) at 110°C/2mmHg yielded a white solid, m.p. 255°C [literature value 254–256°C for (+)-*trans*-2-*endo*,3-*exo*-camphanediol]. The i.r. spectrum was identical with that of (+)-*trans*-2-*endo*,3-*exo*-diol (Takeshita & Kitajima, 1959).

(±)-cis-2-exo,3-exo-*Camphanediol* (*IV*). This was prepared by reducing (±)-camphorquinone with $LiAlH_4$ in anhydrous ether at −10°C for 20h (Takeshita & Kitajima, 1959). Recrystallization of the product from a minimum volume of light petroleum yielded fine platelets. G.l.c. indicated the presence of approx. 10% of '*trans*-diol'. The '*cis*-diol' was purified through acetonide formation as described for the *cis*-2-*endo*,3-*endo*-diol above. Repeated sublimation of the diol obtained at 110°C/2mmHg yielded a white solid, m.p. 259–261°C [literature value 256.6–258.5°C for (+)-*cis*-2-*exo*,3-*exo*-camphanediol] and $[\alpha]_D^{20}$ 0.00° (*c* 2 in ethanol). The i.r. spectra of the diol and acetonide matched those of (+)-*cis*-2-*exo*,3-*exo*-camphanediol and its acetonide (Takeshita & Kitajima, 1959). T.l.c. in systems 1 and 2 showed one spot of R_F values 0.25 and 0.4 respectively; g.l.c. showed one peak, retention time 9.0min.

(±)-trans-2-exo,3-endo-*Camphanediol* (*V*). This compound was prepared by hydrogenating (±)-3-*endo*-hydroxycamphor with Raney nickel W2 catalyst in ethanol at atmospheric pressure and room temperature (Angyal & Young, 1959). The reaction mixture showed two spots on t.l.c. of R_F values 0.05 and 0.25 (solvent system 1), corresponding to '*trans*-diol' and '*cis*-diol' respectively (Table 2). The '*cis*-diol' was removed by adding a solution of $NaIO_4$ (1g) in water (40ml) to a solution of the diol mixture (8g) in ethanol (20ml) at room temperature. After 0.5h, aq. 10% (w/v) KI solution was added and the mixture extracted with chloroform; the extract was washed with aq. 20% (w/v) $Na_2S_2O_3$ solution and evaporated to leave a white solid (7g). Recrystallization from light petroleum–acetone gave plates, m.p. 251–253°C [literature value 250.5–252°C for (+)-*trans*-2-*exo*,3-*endo*-camphanediol] and $[\alpha]_D^{20}$ 0.00° (*c* 2 in ethanol). The i.r. spectrum was identical with that of (+)-*trans*-2-*exo*,3-*endo*-camphanediol (Takeshita & Kitajima, 1959). G.l.c. of a sample showed one peak, retention time 12.0min, and t.l.c. showed one spot, R_F 0.05 (solvent system 1).

(±)-trans-2-endo,3-exo-*Camphanediol* (*VI*). This was prepared from (±)-2-*endo*-hydroxyepicamphor (Angyal & Young, 1959) and purified by using the procedures outlined for (±)-*trans*-2-*exo*,3-*endo*-camphanediol. The *trans*-diol obtained after recrystallization melted at 255°C [literature value 254–256°C for (+)-*trans*-2-*endo*,3-*exo*-camphanediol] and $[\alpha]_D^{20}$ 0.00° (*c* 2 in ethanol). The i.r. spectrum was identical with that of (+)-*trans*-2-*endo*,3-*exo*-camphanediol (Takeshita & Kitajima, 1959). T.l.c. and g.l.c. showed the presence of one compound having R_F 0.05 (system 1) and retention time 12.0min.

METHODS

Animals and diet. These were as described by Robertson & Hussain (1969). The diols were administered as suspensions in 1% methylcellulose mucilage; less water-soluble compounds were administered in solution in arachis oil.

Infrared spectroscopy and thin-layer chromatography. These were carried out as described by Robertson & Hussain (1969). For t.l.c. the solvents were light petroleum (b.p. 60–80°C)–ethyl acetate (3:1, v/v) (system 1) and light petroleum (b.p. 60–80°C)–ethyl acetate–ethanol (10:2:1, by vol.) (system 2).

Gas–liquid chromatography. The equipment described by Robertson & Hussain (1969) was used. The stationary phase was 12% Carbowax 20M on Chromosorb P (100–120 mesh). The column temperature was maintained at 160°C throughout. For quantitative analysis (±)-camphorquinone was used as internal standard.

Hydrolysis of urine. Before hydrolysis the 24h and 48h urine samples were extracted six times with equal volumes of ether to remove unconjugated metabolites. The urine, after removal of most of the ether in solution by using a rotary-film evaporator, was buffered to pH6.8–7.0 with 0.1M-Sörensen's phosphate and incubated at 37°C for 24h in the presence of β-glucuronidase (bacterial type II; Sigma Chemical Co., St Louis, Mo., U.S.A.) with the addition of a few drops of chloroform. After cooling, the urine was again extracted with ether.

RESULTS

The four diols sedated the animals, the *cis*-diols being more effective.

Determination and characterization of metabolites in the urine

In all cases the 24h urine 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.

Solutions of standard ketols and diols in 0.1M-

Table 1. *Excretion of* (±)-*camphanediols 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 (mmol/kg body wt.)	% of dose excreted as glucuronide
(±)-*cis*-2-*endo*,3-*endo*-Camphanediol	5	0.68–0.99	66±3 (60–70)
(±)-*cis*-2-*exo*,3-*exo*-Camphanediol	5	0.68–0.99	47±13 (14–87)
(±)-*trans*-2-*exo*,3-*endo*-Camphanediol	6	0.85–1.42	57±4 (39–67)
(±)-*trans*-2-*endo*,3-*exo*-Camphanediol	5	0.85–1.42	36±5 (20–57)

Table 2. R_F *values* (*t.l.c.*) *and retention times* (*g.l.c.*) *of alcohols, ketols and ketone derivatives of camphane*

Details of the systems used are given in the text.

Compound	R_F Solvent system 1	R_F Solvent system 2	Retention time (min)
(±)-*cis*-2-*exo*,3-*exo*-Camphanediol	0.25	0.4	9.0
(±)-*cis*-2-*endo*,3-*endo*-Camphanediol	0.25	0.4	9.0
(±)-*trans*-2-*exo*,3-*endo*-Camphanediol	0.05	0.3	12.0
(±)-*trans*-2-*endo*,3-*exo*-Camphanediol	0.05	0.3	12.0
(±)-2-*endo*-Hydroxyepicamphor	0.35	0.5	3.5
(±)-3-*endo*-Hydroxyepicamphor	0.35	0.5	3.5
(±)-Borneol	0.59		
(±)-Epiborneol	0.49		
(±)-Camphor	0.7		
(±)-*cis*-Diol acetonide		0.75	

Sörensen's phosphate buffer, pH 6.8, were incubated at 37°C for 24h in the presence of β-glucuronidase; ether extraction of the solution after incubation yielded the standards unchanged.

'*cis*-Diol' solutions in 0.1M-hydrochloric acid were incubated at 37°C for 24h. The diols were recovered unchanged, eliminating the likelihood of pinacol rearrangement occurring in the gastric juice of rabbits.

Metabolites were identified by melting-point determination, i.r. spectroscopy, t.l.c. and g.l.c. The fate of each compound administered is described below. R_F values and retention times of the compounds administered and of the metabolites isolated are given in Table 2.

The R_F values and retention times of the compounds within each of the following pairs were identical, and so could not be distinguished one from the other though the pairs were readily distinguishable (see Table 2): (*a*) 2-*endo*-hydroxyepicamphor and 3-*endo*-hydroxycamphor, i.e. 'ketol'; (*b*) *cis*-2-*endo*,3-*endo*- and *cis*-2-*exo*,3-*exo*-camphanediol, i.e. '*cis*-diol'; (*c*) *trans*-2-*endo*,3-*exo*- and *trans*-2-*exo*,3-*endo*-camphanediol, i.e. '*trans*-diol'.

Compounds within the pairs were designated 'ketol', '*cis*-diol' or '*trans*-diol' when their exact structure could not be determined.

The ratios of conjugated and unconjugated metabolites are given in Table 3.

Camphane. Portions (1g) of this hydrocarbon were administered to rabbits. T.l.c. (system 1) of the ether extracts of hydrolysed urine showed the presence of the previously reported borneol and epiborneol (Robertson & Hussain, 1969) with R_F values 0.59 and 0.49 (Table 2). The absence of 'ketol' and 'diols' was noted and confirmed by g.l.c.

(±)-cis-2-endo,3-endo-*Camphanediol.* A 400mg portion of diol was administered to each of five rabbits. T.l.c. of the ether extracts of both the unhydrolysed and the hydrolysed urine revealed three spots not present in the ether extracts of unhydrolysed and hydrolysed control urine. The metabolites had R_F values 0.25 (major), 0.35 and 0.05 (system 1) and 0.4, 0.5 and 0.3 (solvent system 2), corresponding exactly to the R_F values of '*cis*-diol', 'ketol' and '*trans*-diol' respectively (Table 2). G.l.c. of the ether extracts confirmed the presence of these three metabolites with retention times of 9.0, 3.5 and 12.0min (Table 2).

Table 3. *Proportions of conjugated and unconjugated metabolites in urine of rabbits 24h after administration of camphanediols*

The proportions were determined by quantitative g.l.c., with camphorquinone as standard.

	Relative proportions of metabolites					
	Conjugated			Unconjugated		
Compound administered	'Ketol'	*'cis*-diol'	*'trans*-diol'	'Ketol'	*'cis*-diol'	*'trans*-diol'
cis-2-*endo*,3-*endo*-Diol	0.8	2.6	0.1	0.6	3.0	0.1
cis-2-*exo*,3-*exo*-Diol	1.0	4.0	0.5	1	4.0	0.7
trans-2-*exo*,3-*endo*-Diol	0.2	—	1.3	0.3	—	4.2
trans-2-*endo*,3-*exo*-Diol	0.1	—	1.0	0.2	—	1.5

Since the major metabolite seemed to be '*cis*-diol', isolation was attempted by acetonide formation. The mixture of metabolites was refluxed in anhydrous acetone in the presence of a few drops of sulphuric acid. Column chromatography yielded a yellow oil, which after purification had an i.r. spectrum that matched that of the acetonide of the *cis*-2-*endo*,3-*endo*-diol and which on hydrolysis yielded a white solid, m.p. 257°C and $[\alpha]_D^{20}$ 0.00° (*c* 2 in ethanol). The i.r. spectrum of this solid was identical with that of synthetic *cis*-2-*endo*,3-*endo*-camphanediol.

Further elution of the column with ethyl acetate–light petroleum (b.p. 60–80°C) (1:5, v/v) yielded a small quantity of 'ketol', characterized by a strong absorption band at 1760nm and a medium band at 3450nm, indicating the presence of both carbonyl and hydroxyl groups, and by its R_F values 0.35 (system 1) and 0.5 (system 2).

Final elution of the column with methanol yielded a small quantity of '*trans*-diol', with R_F values 0.05 (system 1) and 0.3 (system 2); the i.r. spectrum showed absorption at 3460nm but none at 1760nm.

(±)-cis-2-exo,3-exo-*Camphanediol*. A 400mg portion of this diol was administered to each of five rabbits. T.l.c. and g.l.c. of the ether extracts of unhydrolysed and hydrolysed 24h urine showed the presence of three metabolites of R_F values 0.35, 0.25 and 0.05 (system 1) and retention times of 3.5, 9.0 and 12.0min, corresponding to the values of 'ketol', '*cis*-diol' and '*trans*-diol' respectively.

The major metabolite, the '*cis*-diol', was isolated as the acetonide, which on purification had an i.r. spectrum identical with that of the acetonide of synthetic (±)-*cis*-2-*exo*,3-*exo*-camphanediol and quite distinct from that of the acetonide of the *cis*-2-*endo*,3-*endo*-diol.

The 'ketol' and '*trans*-diol' metabolites were isolated from the chromatographic column, as described for the *cis*-*endo*-diol, and characterized by R_F values. Too little (10mg, impure) of the 'ketol' was obtained for effective purification and subsequent i.r. analysis. However, Huang-Minlon (1946) reduction of the 'ketol' gave a product that on t.l.c. showed two spots corresponding to the R_F values of borneol (0.59) and epiborneol (0.49) (Table 2). Thus the 'ketol' was a mixture of 2-hydroxyepicamphor and 3-hydroxycamphor. The configurations of the hydroxyl groups in the original ketol are uncertain, since under the alkaline conditions of the reaction isoborneol can be converted into the more stable borneol (Flemming & Woodward, 1968).

(±)-trans-2-exo,3-endo-*Camphanediol*. A 500mg portion of this diol was administered to each of five rabbits. T.l.c. (system 1) and g.l.c. of the ether extracts of unhydrolysed and hydrolysed 24h urine showed the presence of two metabolites, R_F 0.05 and retention time 12.0min (major) and R_F 0.35 and retention time 3.5min, corresponding to '*trans*-diol' and 'ketol' respectively (Table 2).

On column chromatography of the mixture the 'ketol' fraction (8mg) was eluted first. After subjection to Huang-Minlon (1946) reduction, the product showed one spot on t.l.c. of R_F 0.59 (system 1) corresponding to that of borneol (Table 2). Thus the 'ketol' was a 2-hydroxyepicamphor; again the configuration of the hydroxyl radical was rendered uncertain by the reaction conditions.

The compound of R_F 0.05, on recovery from the column by eluting with methanol and purification, had m.p. 251–254°C and $[\alpha]_D^{20}$ 0.00° (*c* 2 in ethanol). The i.r. spectrum was identical with that of (±)-*trans*-2-*exo*,3-*endo*-camphanediol.

(±)-trans-2-endo,3-exo-*Camphanediol*. A 500mg portion of this diol was administered to each of five rabbits. T.l.c. (system 1) and g.l.c. of the unhydrolysed and hydrolysed 24h urine showed the presence of two compounds, R_F 0.05 and retention time 12.0min (major) and R_F 0.35 and retention time 3.5min, corresponding to '*trans*-diol' and 'ketol' respectively (Table 2).

Separation and purification of the '*trans*-diol'

gave a white solid, m.p. 253°C and $[\alpha]_D^{20}$ 0.00° (*c* 2 in ethanol). The i.r. spectrum was that of *trans*-2-*endo*,3-*exo*-camphanediol. On Huang-Minlon (1946) reduction the 'ketol' (6mg) gave a product with the same R_F value (0.59) as borneol (Table 2).

(±)-2-endo-*Hydroxyepicamphor*. A 700mg portion of this ketol was administered to one rabbit. Ether extracts of unhydrolysed and hydrolysed 24h urine showed on t.l.c. (system 1) and g.l.c. the presence of 'ketol', R_F 0.35 and retention time 3.5min, and '*trans*-diol', R_F 0.05 and retention time 12.0min; '*cis*-diol' was not present.

(±)-2-endo-*Hydroxyepicamphor and* (±)-3-endo-*hydroxyepicamphor mixture*. A 700mg portion of this ketol mixture was administered to a rabbit. G.l.c. and t.l.c. of the ether extracts of unhydrolysed and hydrolysed 24h urine also showed the presence of 'ketol' and '*trans*-diol' only; '*cis*-diol' was absent.

(+)-*Camphor*. An ether extract obtained from acid-hydrolysed 24h urine collected after administration of (+)-camphor to rabbits was available (Robertson & Hussain, 1969). Examination of the extract by g.l.c. and t.l.c. showed the presence of 'ketol' only, as reported by Robertson & Hussain (1969); diols were not present.

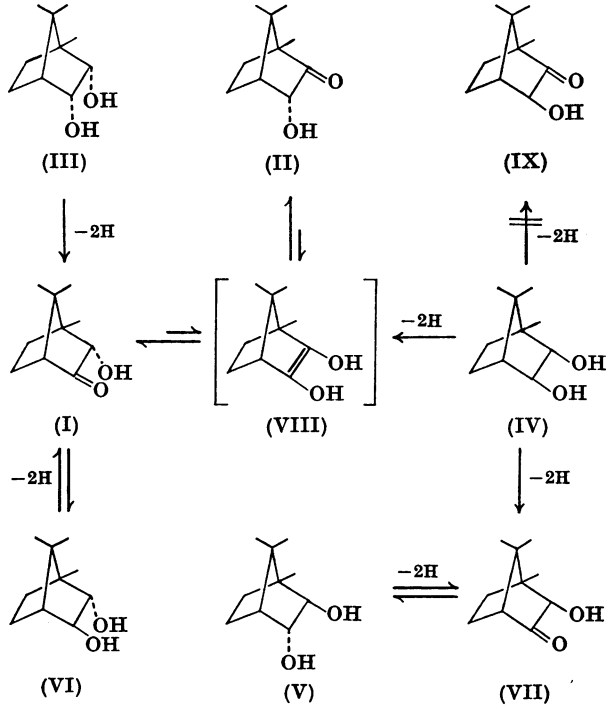
(±)-*Camphorquinone*. An ether extract of acid-hydrolysed 24h urine collected after administration of (±)-camphorquinone to rabbits was available (Robertson & Hussain, 1969). G.l.c. showed the presence of the previously reported 'ketol' (Robertson & Hussain, 1969), and in addition a small amount of '*cis*-diol', retention time 9.0min, and a smaller amount of '*trans*-diol', retention time 12.0min.

DISCUSSION

All compounds administered increased the urinary content of conjugated glucuronic acid. With regard to the metabolism of camphane, the previously reported borneol and epiborneol were detected (Robertson & Hussain, 1969), but there was no evidence of the presence of 2,3-diols. It can be concluded that vicinal diol formation is not a feature of camphane metabolism.

For a period of 24h after the oral administration of (±)-camphane-2,3-diols to rabbits the original compounds and their metabolites were excreted in the urine both free and combined with glucuronic acid.

The two *trans*-diols were partly metabolized to



Scheme 1. Metabolism of camphanediols in rabbits; (I), 2-*endo*-hydroxyepicamphor; (II), 3-*endo*-hydroxycamphor; (III), *cis*-2-*endo*,3-*endo*-camphanediol; (IV), *cis*-2-*exo*,3-*exo*-camphanediol; (V), *trans*-2-*exo*,3-*endo*-camphanediol; (VI), *trans*-2-*endo*,3-*exo*-camphanediol; (VII), 2-*exo*-hydroxyepicamphor; (VIII), enediol; (IX), 3-*exo*-hydroxycamphor.

'ketols', whereas the two *cis*-diols were partly metabolized to 'ketols' and to '*trans*-diols'. The quantities of these 'ketols' and '*trans*-diols' isolated were so small that they could not be fully characterized. However, it is known that groups attached at C-2 are less reactive than if attached to C-3, owing to shielding by the C-10 methyl group (Chittenden & Cooper, 1970). Also, the 2-*exo* conformation seems more inert than the 2-*endo*: bornyl acetate (*endo*) is more readily hydrolysed than isobornyl acetate (*exo*) (Lipp & Bund, 1935). Angyal & Young (1959) compared the rates of alkaline hydrolysis of the diacetates of the diols; those having a 2-*exo*-acetate reacted more slowly than those having a 2-*endo*-acetate. It seems reasonable to assume that groups attached to C-2, especially those with an *exo* configuration, are at least as unreactive under physiological conditions as they are *in vitro*. The lower reactivity *in vivo* of an oxo group at C-2 has been previously noted (Robertson & Hussain, 1969).

trans-*Diols*. (±)-Camphane-2-*endo*,3-*exo*-diol (VI) was dehydrogenated *in vivo* to give 2-*endo*-hydroxyepicamphor (I). This dehydrogenation was thought to be reversible, since after the administration of 2-*endo*-hydroxyepicamphor to rabbits '*trans*-diol' was detected in the urine. Huang-Minlon (1946) reduction of the 'ketol' metabolite yielded borneol, thus confirming the site of dehydrogenation (see Scheme 1).

The (±)-*trans*-2-*exo*,3-*endo*-diol (V) also gave some 'ketol', presumably 2-*exo*-hydroxyepicamphor (VII) (Scheme 1). By analogy with the 2-*endo*,3-*exo*-diol, this reaction was thought to be reversible. Huang-Minlon (1946) reduction of the 'ketol' again yielded borneol. As explained above, under the basic conditions of the Huang-Minlon reaction 2-*exo*-hydroxyepicamphor would be reduced to borneol instead of the otherwise expected isoborneol.

cis-*Diols*. (±)-Camphane-2-*endo*,3-*endo*-diol (III) yielded 'ketol' and '*trans*-diol' metabolites, presumably 2-*endo*-hydroxyepicamphor (I) and *trans*-2-*endo*,3-*exo*-diol (VI) respectively. Since '*cis*-diols' were not detected in the urine of rabbits dosed with (±)-2-*endo*-hydroxyepicamphor, it was thought that, unlike the *trans*-diols, the dehydrogenation of *cis*-diols was irreversible. The metabolic fate of *cis*-2-*endo*,3-*endo*-diol is outlined in Scheme 1.

The most interesting diol is (±)-camphane-2-*exo*,3-*exo*-diol (IV). It is the most unstable of the four diols, owing to steric conflict between the 2-*exo*-hydroxyl and the 8- and 10-methyl groups, and between the 3-*exo*-hydroxyl and the C-8 methyl groups, as well as the mutual conflict between the *cis*-related hydroxyl groups.

The *cis*-*exo*-diol gave rise to 'ketol' and '*trans*-diol' metabolites, as had the *cis*-*endo* isomer. However, Huang-Minlon (1946) reduction of the 'ketol' yielded both borneol and epiborneol. This could imply that the diol had been dehydrogenated at both C-2 and C-3 to yield 3-*exo*-hydroxycamphor (IX) and 2-*exo*-hydroxyepicamphor (VII). The formation of the latter ketol is understandable in the light of the discussion above on the 2-*exo*,-3-*endo*-diol (V), i.e. dehydrogenation at C-3; Huang-Minlon (1946) reduction would cause isomerization of isoborneol to the found borneol. However, formation of 3-*exo*-hydroxycamphor by dehydrogenation at C-2 contradicts the assumption made above with regard to the inertness of a hydroxyl group there. One explanation is to assume that, after dehydrogenation at C-3, the resulting 'ketol' (VII) isomerizes to an enediol (VIII); alternatively, the enediol may have been formed directly by the dehydrogenation of the diol from its unhindered *endo* face along the C-2–C-3 bond. This high-energy species then equilibrates to give a mixture of the more stable 2- and 3-*endo*-hydroxy ketols.

Such an enediol intermediate has been postulated in the formation of 3-*endo*-hydroxycamphor and 2-*endo*-hydroxyepicamphor by zinc–acetic acid reduction of camphorquinone (Rodd, 1969).

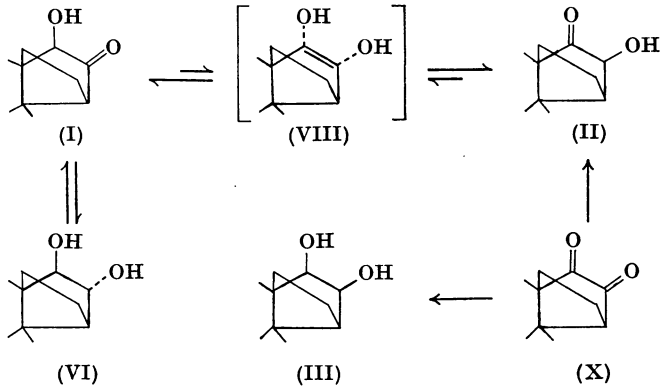
The expected '*trans*-diol' metabolites of the *cis*-*exo*-diol would be both *trans*-2-*exo*,3-*endo*-diol (V) and *trans*-2-*endo*,3-*exo*-diol (VI), resulting from reduction *in vivo* of 2-*exo*- and 2-*endo*-hydroxyepicamphor (VII and I) respectively.

The 3-*endo*-hydroxycamphor (II) formed, which is also a metabolite of camphor (Robertson & Hussain, 1969), is not expected to form a diol, since diol was not detected as a metabolite of camphor. This behaviour reinforces the suggestion above that reaction at C-2 is hindered. This hindrance would also make unlikely the formation of 3-*exo*-hydroxycamphor (IX).

The proposed formation of an enediol intermediate in ketol interconversions is applicable to a number of situations. It provides an alternative explanation for the formation of both 2-*endo*-hydroxyepicamphor and 3-*endo*-hydroxycamphor by the reduction of camphorquinone (X) *in vivo* (Robertson & Hussain, 1969). The 2-*endo*-hydroxy ketol was thought to be formed by reduction of the 2-oxo group, which is not consistent with the observation that this group was not reduced during the metabolism of bornane-2,5-dione. It is now thought that the reduction at C-2 was only apparent, and that after reduction at C-3 isomerization to the enediol occurred followed by equilibration to a mixture of 2- and 3-*endo*-ketols (Scheme 2).

The *cis*-diol obtained from camphorquinone was almost certainly the more stable *cis*-*endo*-diol (III).

Interconversion between ketols and vicinal diols has been demonstrated both *in vivo* and *in vitro* with hydroxyoestrones and oestriols (Marrian,



Scheme 2. Metabolism of camphorquinone (X) in rabbits. The formulae are drawn to represent the D ring of steroids.

Loke, Watson & Panattoni, 1957; Layne & Marrian, 1958; Brown & Marrian, 1957; Breuer & Nocke, 1958, 1959; Nocke, Breuer & Knuppen, 1961).

Nocke *et al.* (1961) proposed that 16-oxo-oestradiol-17β was converted into 16β-hydroxyoestrone via 16-oxo-oestrone. Having regard to the suggested scheme for the metabolism of camphorquinone, an alternative explanation is that the conversion goes through the enediol oestra-1,3,5(10),16-tetraene-3,16,17-triol.

2-Hydroxyindan-1-one was the ketol necessary for the interconversion of *cis-* and *trans*-indane-1,2-diol (Lewis, 1966*b*). It was also observed (Lewis, 1966*a*) that *cis-* and *trans*-indane-1,2-diol were present in the urine of rabbits and rats dosed with either indan-1-one or indan-2-one, indicating that the ketol formed from the latter ketone, i.e. 1-hydroxyindan-2-one, is converted into 2-hydroxyindan-1-one. It is suggested that once again this interconversion could occur via an enediol Δ^1-indane-1,2-diol.

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