Studies in Detoxication

69. THE METABOLISM OF ALKYLBENZENES: *n*-PROPYLBENZENE AND *n*-BUTYLBENZENE WITH FURTHER OBSERVATIONS ON ETHYLBENZENE*

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An account of our studies on the metabolic fate of toluene, ethyl-, *iso*propyl- and *tert.*-butyl-benzenes has been given in earlier papers (Smith, Smithies & Williams, 1954*a*, *b*, *c*; Robinson, Smith & Williams, 1955; Robinson & Williams, 1955). The present paper deals with *n*-propyl- and *n*-butyl-benzenes. These two compounds were previously studied by Thierfelder & Klenk (1924), who showed that *n*-propylbenzene was oxidized in rabbits to yield benzoic acid (excreted as hippuric acid) and *n*-butylbenzene to yield phenylacetic acid (excreted as phenaceturic acid). It was suggested that about 60–65% of these compounds were oxidized to acids and it was concluded that these alkylbenzenes underwent ω-oxidation initially and then, by β-oxidation of the resulting ω-carboxylic acid, they were converted into benzoic or phenylacetic acid according to the number of carbon atoms in the alkyl side chain. However, Smith *et al.* (1954*a*) found that *n*-propyl- and *n*-butyl-benzenes gave rise to appreciable amounts of non-reducing conjugated glucuronic acids, and suggested that the alkyl side chains of these compounds were oxidized in other positions as well as the ω- to yield secondary alcohols which were excreted conjugated with glucuronic acid. Both propyl- and butylbenzene could be expected to be oxidized at the α-position, since this is activated by its proximity to the benzene ring (cf. ethylbenzene, Smith *et al.* 1954*b*). Furthermore, it has been observed that alkyl side chains in barbiturates (Maynert, 1952) and the *n*-butyl chain in the antirheumatic drug, phenylbutazone (butazolidine) (Burns *et al.* 1955), are oxidized in the $(\omega-1)$-position. This type of oxidation could also occur with *n*-propyl- and *n*-butyl-benzene. It will, in fact, be shown here that both these compounds are oxidized in rabbits at the α- and $(\omega-1)$-positions of the alkyl side chain. Further observations on the metabolism of ethylbenzene have now shown that this compound undergoes ω-oxidation, although the major process is α-oxidation.

* Part 68: Parke (1956).

EXPERIMENTAL

Reference compounds. Secondary alcohols were identified by oxidizing them to ketones. The 2:4-dinitrophenylhydrazones of propiophenone, benzyl methyl ketone, butyrophenone, benzyl ethyl ketone and methyl 2-phenylethyl ketone were prepared (for references see Heilbron & Bunbury, 1953). The characteristics of the spectra of these compounds are given in Table 1. Methyl [(−)-ethylphenylcarbinyl tri-*O*-acetyl-β-D-glucosid]uronate, m.p. 127° and $[\alpha]_D^{20}-80°$ in $CHCl_3$, was a sample previously prepared (Smith *et al.* 1954*c*).

The azlactone of hippuric acid with *p*-dimethylaminobenzaldehyde has been described (Gaffney, Schreier, DiFerrante & Altman, 1954). Attempts to prepare the corresponding azlactone of phenaceturic acid were unsuccessful. The azlactone of cinnamoylglycine was prepared from *p*-dimethylaminobenzaldehyde and cinnamoylglycine in the presence of acetic anhydride in the usual manner. The 4-p-*dimethylaminobenzal*-2-(2-*phenylvinyl*)-5-*oxazolone* formed deep-red needles, m.p. 194°, from acetone. (Found: C, 75·1; H, 5·8; N, 8·7. $C_{20}H_{18}O_2N_2$ requires C, 75·4; H, 5·7; N, 8·8%; light absorption, $\lambda_{max.}$ 255, 320 and 490 mμ. with $\epsilon_{max.}$ 12000, 20000 and 61000 respectively in ethanol.)

Animals and diet. Chinchilla rabbits weighing 2·5–3·5 kg. were used throughout this work. They were kept on a constant diet consisting of Bruce & Parke's diet no. 18 (Associated London Flour Millers). Compounds were administered by stomach tube.

Determination of hippuric acid

Gaffney *et al.* (1954) described a micro-method which involves the formation of a coloured azlactone from hippuric acid and *p*-dimethylaminobenzaldehyde. Hippuric acid is separated from urine by paper chromatography and the azlactone is formed on the paper, then eluted for estimation. We have modified this procedure so that the estimation can be carried out on the urine without paper chromatography.

Reagents. Ethyl acetate (May and Baker, Ltd., 98% esters) and 7% (w/v) *p*-dimethylaminobenzaldehyde (A.R. British Drug Houses Ltd.) in acetic anhydride (May and Baker Ltd.; not less than 96% at assay) were used. The *p*-dimethylaminobenzaldehyde should not give any red colour on dissolving it in acetic anhydride and if necessary should be purified by crystallization from benzene. The concentration of *p*-dimethylaminobenzaldehyde necessary for optimum colour production had been determined in a separate experiment.

Determination in pure solution. Aqueous solutions of hippuric acid containing 50–250 μg./ml. were prepared and 1 ml. of each solution was shaken vigorously with 5 ml. of ethyl acetate in a 15 ml. centrifuge tube. The mixture was centrifuged for 2·5 min. and 1 ml. samples (in triplicate) of the clear extract were evaporated to dryness in 6 in $\times \frac{5}{8}$ in. test tubes in an oven at 110°. The dry residue (a trace of water here gives high results) in the tube was treated with 3 ml. of the *p*-dimethylaminobenzaldehyde solution and the tube was then heated in an oil bath at 120° for 0·5 hr. This temperature (which should be carefully controlled) and time had been found to give the best results in a separate experiment. The tube was cooled to room temp., 2 ml. of ethyl acetate was added to dilute the coloured product and the yellowish-brown colour of the solution was measured in a SP. 600 Unicam spectrophotometer at 460 mμ. against a blank test prepared similarly, except that water was substituted for the hippuric acid solution. The calibration curve was a straight line, and the error in the recovery of concentrations of hippuric acid from 150 to 250 μg./ml. ranged from $\pm 10\%$ at the lower level to $\pm 5\%$ at the higher concentrations.

Determination in rabbit urine. Urine collected during 24 hr. was brought to pH 2–3 with conc. HCl (about 0·5–2 ml.) and then diluted to 200 ml. The diluted urine (1 ml.) was then extracted with 5 ml. of ethyl acetate and treated as described above. The recovery of hippuric acid added to urine in quantities corresponding to 15–50 mg./day was within $\pm 10\%$. In one set of determinations on normal rabbit urine (113 samples on diet 18), the output of apparent hippuric acid was 20–81 mg./day (average, 43 mg.). Beer, Dickens & Pearson (1951) found that rabbits excreted 5–90 mg./day as benzoic acid on a cabbage diet.

Large excesses (100:1, w/w) of amino acids (glycine, alanine, serine, lysine, cystine, methionine, ornithine, histidine, tyrosine and tryptophan) added to the urine did not interfere. The effect of phenaceturic acid was irregular but it did not interfere if it were present in an amount not greater than half that of the hippuric acid. The interference from phenaceturic acid was less if the colour from hippuric acid were measured at 470 mμ. instead of 460 mμ. At both these wavelengths, the extinction of the hippuric acid colour is the same. When the estimation procedure is carried through with phenaceturic acid instead of hippuric acid, the resulting extract shows a relatively low maximum absorption at 435 mμ.

Paper chromatography of glycine conjugates

The separation of hippuric and phenaceturic acids (20 μg. or less in water) on paper was achieved with benzene–acetic acid–water (1:1:2, by vol.). Whatman no. 4 paper (descending chromatography) was used. The time of run was 5 hr. at room temp. The paper was then dried and sprayed with a 10% solution (w/v) of *p*-dimethylaminobenzaldehyde in acetic anhydride. When the paper was heated in an oven at 120° for 2 min. or before an electric fire, hippuric acid showed up as an orange-red spot (R_F 0·18) and the phenaceturic acid as a yellow spot (R_F 0·28). Cinnamoylglycine could also be separated from these compounds since under the same conditions it showed up as a deep-red spot (R_F 0·34). Phenylpropionylglycine also separated from hippuric and phenaceturic acids but it overlapped with cinnamoylglycine, and gave a bright-yellow spot (R_F 0·37). Mixtures of the first three conjugates were readily separated and the

Table 1. *Absorption spectra of the 2:4-dinitrophenylhydrazones of certain ketones*

2:4-Dinitrophenylhydrazone of	M.p.	Cryst. form	Spectra in ethanol: Authentic sample $\lambda_{max.}$ (mμ.)	Authentic sample $\epsilon_{max.} \times 10^{-3}$	Urinary sample $\lambda_{max.}$ (mμ.)	Urinary sample $\epsilon_{max.} \times 10^{-3}$
Ethyl phenyl ketone	191°	Red plates	375, ~260	26·4, 11·6	375, ~260	26·8, 13·0
Benzyl methyl ketone	153–154°	Deep-yellow needles	360, ~260	22·4, 11·0	360, ~260	22·7, 12·0
Phenyl propyl ketone	194–195°	Red plates	375, ~260	27·5, 13·0	375, ~260	27·5, 13·0
Benzyl ethyl ketone	145°	Deep-yellow needles	360, ~260	23·5, 12·0	—	—
Methyl 2-phenylethyl ketone	128°	Crimson prisms	360, ~260, 230	22·0, 10·7, 16·2	360, ~260, 230	22·0, 11·3, 16·5

components detected by this procedure in pure solution and in urine. The urine was first acidified to about pH 2, about 0·01 ml. was placed at the starting line, dried and then irrigated with the solvent. Normal rabbit urine gave a faint spot corresponding to hippuric acid, but none corresponding to phenaceturic acid. Rough estimates of the amounts of phenaceturic acid in a given urine were obtained by comparison of the urinary spot with those obtained from standard amounts of phenaceturic acid.

ISOLATION OF METABOLITES

From ethylbenzene

Isolation of phenaceturic acid (see also Table 2). A total of 17·8 g. of ethylbenzene was fed to ten rabbits and the urine was collected for 2 days. The urine (1500 ml.) was acidified to pH 2 with 2N-H_2SO_4 and continuously extracted with ether for 16 hr. The extract was evaporated and the residue, which contained glucuronides, was dissolved in water (10 ml.) and kept overnight. The crystals (2·8 g.) which were deposited were washed with a few drops of water and dissolved in 25 ml. of hot water. From the solution at 30° crystals of hippuric acid (1·5 g.) were separated, m.p. and mixed m.p. 189–190°. (Found: N, 7·6. Calc. for $C_9H_9O_3N$: N, 7·8%.) Further cooling to 20° gave impure phenaceturic acid (0·8 g., m.p. 138–140°). After two recrystallizations from ethyl acetate, pure phenaceturic acid, m.p. and mixed m.p. 144°, was obtained. (Found: N, 7·3. Calc. for $C_{10}H_{11}O_3N$: N, 7·3%.) Further ether extraction of the urine for 2 days yielded a further 0·8 g. of mixed crystals, and the mother liquors from the first extraction 0·4 g. These fractions were mixed and the impure phenaceturic acid (m.p. 135–140°) was separated from them. This was then hydrolysed by boiling for 4 hr. with N-NaOH. On acidification phenylacetic acid separated, which on recrystallization from light petroleum (b.p. 60–80°) had m.p. and mixed m.p. 75–76°.

From n-*propylbenzene*

Isolation of ethylphenylcarbinyl glucosiduronic acid. A total of 16 ml. (13·8 g.) of *n*-propylbenzene was fed to eight rabbits and the urine was collected for 24 hr. It was acidified to about pH 2 with dilute H_2SO_4 and continuously extracted with ether for 60 hr. The extract was evaporated to a syrup which was triturated with a little water and kept overnight to allow hippuric acid to separate. The acid was filtered off and the filtrate evaporated to a gum. This was dissolved in 100 ml. of ether containing a little methanol and then poured into 500 ml. of light petroleum (b.p. 40–60°). The glucuronide gum (10 g.) which was precipitated was methylated with diazomethane and then acetylated with pyridine and acetic anhydride. The product on pouring into water gave 8 g. of crude triacetyl methyl esters. Fractional crystallization from absolute ethanol yielded 2·5 g. of pure methyl [(−)-ethylphenylcarbinyl tri-*O*-acetyl-*β*-D-glucosid]uronate, m.p. and mixed m.p. 125° and $[\alpha]_D^{20}$ −82° in $CHCl_3$ (*c*, 1). (Found C, 58·3; H, 6·2. Calc. for $C_{22}H_{28}O_{10}$: C, 58·7; H, 6·2%.) Other small crystalline fractions were obtained with $[\alpha]_D$ −60 to −70° in $CHCl_3$, but the main residue was a gum with $[\alpha]_D$ −27°, which was very soluble in ethanol and did not crystallize. This was suspected to contain methyl [(+)-ethylphenylcarbinyl tri-*O*-acetyl-*β*-D-glucosid]uronate, whose rotation would be expected to be about −15°, and methyl [(+)- and (−)-benzylmethylcarbinyl tri-*O*-acetyl-*β*-D-glucosid]uronate, whose rotations are known to be −24° and −46° respectively (Smith *et al.* 1954*c*). We failed, however, to isolate the glucosiduronic acid of either form of benzylmethylcarbinol in a crystalline state, but proof of the presence of this carbinol was obtained by converting it into the ketone (see below).

Isolation of propiophenone and benzyl methyl ketone. The experiment described above was repeated to the stage of precipitating the glucuronide gum with light petroleum (the yield of hippuric acid, m.p. and mixed m.p. 186°, was 2·8 g. or 14·6% of the dose on the assumption that it was mainly from propylbenzene). The glucuronide gum (10 g.) was dissolved in a little water, treated with charcoal and filtered. The filtrate was diluted to 500 ml. with 0·1M acetate buffer of pH 4·6, and incubated for 48 hr. at 37° with 0·5 ml. of brown locust-crop liquor (Robinson, Smith & Williams, 1953) containing *β*-glucuronidase. The mixture was then made alkaline with solid K_2CO_3 and extracted with 3 × 100 ml. of ether. The extract was dried over anhydrous Na_2SO_4 and the ether was evaporated, leaving about 0·5 ml. of oily residue of carbinols. This was oxidized by keeping overnight in 10 ml. of pyridine containing 1 g. of chromium trioxide. The product was diluted with an excess of ice-cold 2N-HCl and the solution extracted with 3 × 50 ml. of ether. Evaporation of the ether left a small quantity of oil smelling of ketones. Benzyl methyl ketone reacts with sodium bisulphite, but propiophenone is inert. The oil was therefore treated with 2 ml. of saturated aqueous sodium bisulphite solution. After 5 hr. the small amount of crystals which had formed was filtered and converted into the 2:4-dinitrophenylhydrazone. After recrystallization from ethyl acetate, 10 mg. of the deep yellow 2:4-dinitrophenylhydrazone of benzyl methyl ketone was isolated, m.p. and mixed m.p. 153°. Its absorption spectrum ($\lambda_{max.}$ 360 mμ.) was identical with that of the authentic compound (see Table 1). (Found: N, 17·7. Calc. for $C_{15}H_{14}O_4N_4$; N, 17·8%.) The filtrate from the bisulphite compound contained the bulk of the ketonic material obtained in the oxidation. From it there was isolated the red plates of propiophenone 2:4-dinitrophenylhydrazone (0·5 g.), m.p. and mixed m.p. 191°, with an absorption spectrum ($\lambda_{max.}$ 375 mμ.) identical with the authentic compound. (Found: N, 17·9%.) The isolation of these compounds proved the presence of ethylphenylcarbinol and of benzylmethylcarbinol in the glucuronide gum, but gave no indication of their stereochemical form.

From n-*butylbenzene*

Isolation of phenaceturic acid, butyrophenone and methyl 2-phenylethyl ketone. Attempts at crystallizing the glucuronide fraction from the urine of rabbits dosed with butylbenzene failed. *n*-Butylbenzene (16 ml.) was fed as described above for *n*-propylbenzene, and the same procedure was followed. Instead of hippuric acid, phenaceturic acid was separated in a yield of 1·9 g. (10% of the dose) with m.p. and mixed m.p. 145° after recrystallization from water. The glucuronide gum (8 g.) was again obtained by the light-petroleum treatment and incubated with locust-crop liquor as above. About 1 ml. of carbinols was thus obtained, and this was oxidized with chromic oxide in pyridine. The methyl ketones were again separated as the bisulphite compounds and eventually 20 mg. of the crimson prisms (from ethyl acetate) of methyl 2-phenylethyl ketone 2:4-dinitrophenylhydrazone was obtained, m.p. and mixed m.p. 127° ($\lambda_{max.}$ 360 mμ.). (Found: N, 17·3. Calc. for $C_{16}H_{16}O_4N_4$:

N, 17·1%.) The ketonic material which did not react with bisulphite was converted into the 2:4-dinitrophenylhydrazone, and after recrystallization the red plates of butyrophenone 2:4-dinitrophenylhydrazone were obtained, m.p. and mixed m.p. 194° ($\lambda_{max.}$ 375 mμ.). (Found: N, 17·1%.) The isolation of the two dinitrophenylhydrazones proved the presence of methyl-phenylethylcarbinol and phenyl-*n*-propylcarbinol in the glucuronide gum. Benzylethylcarbinol, the other possible metabolite of *n*-butylbenzene, was not detected.

Detection of p-*hydroxyphenylacetic acid.* Smith *et al.* (1954*a*) reported that rabbits receiving butylbenzene excreted ethereal sulphate corresponding to 5% of the dose. This did not occur with ethylbenzene and the figure of 2% ethereal sulphate reported for propylbenzene was probably not significant. It was therefore possible that butylbenzene underwent aromatic hydroxylation and a probable metabolite is, therefore, *p*-hydroxyphenylacetic acid. This acid has been reported to be a constituent of normal rabbit urine (Bray, Thorpe & White, 1950), in which it was identified chromatographically on paper by its R_F value and colour reactions. In the present work *n*-propanol–NH_3 soln. (sp.gr. 0·88) (7:3), amongst other solvent mixtures, was used with Whatman no. 1 or 4 paper (descending chromatography). In this solvent it had R_F 0·36 and separated well from other constituents reacting with the reagents. The spraying reagents used were diazotized *p*-nitraniline and diazotized sulphanilic acid (Bray *et al.* 1950) and a dilute aqueous solution of Brentamine Fast Blue B Salt (Imperial Chemical Industries Ltd.); the coloured spots obtained were bluish red, red, and yellowish red respectively.

The urine output for 24 hr. (100 ml.) of a rabbit which had received 2 ml. of butylbenzene orally was heated under reflux for 2 hr. with 30 ml. of conc. HCl. The cooled urine was extracted with 3 × 150 ml. of ether. The acids in the extract were transferred to 10% NaOH (2 × 15 ml.). The alkaline solution was acidified and extracted with ether. A concentrate of the last ether extract was used for paper chromatography. The same extraction method was used on normal rabbit urine. Normal rabbit urine gave weak but definite spots corresponding to *p*-hydroxyphenylacetic acid. After butylbenzene the spots appeared to be about ten times stronger than those found with normal urine. This strongly suggests that *p*-hydroxyphenylacetic acid is a minor metabolite of butylbenzene.

DISCUSSION

Metabolism of ethylbenzene. Ethylbenzene is an important intermediate in the production of the styrene used in the plastics industry. In the U.S.A. in 1954, the output of ethylbenzene was 800 million lb. weight, which is more than any other intermediate listed in the U.S. Tariff Commission's statistics (Anon. 1955). According to Browning (1953) no systematic injury from ethylbenzene in man has been reported, but its acute toxicity is slightly more than that of benzene. The metabolites of ethylbenzene are in general relatively non-toxic, and there is no evidence that it is metabolized to phenolic substances which could produce chronic toxic effects as in the case of benzene. Its main metabolites in rabbits are hippuric acid and methylphenylcarbinyl glucosiduronic acid, which are produced in roughly equal amounts and account for at least 60–70% of the dose. Its minor metabolites are mandelic acid (2%) and, as shown in this paper, phenaceturic acid (10–20%). The results of our investigations suggest the scheme shown in Fig. 1 for the metabolism of ethylbenzene; the major routes are indicated by thick arrows. The figures in Table 2 suggest that methylphenylcarbinol is the precursor of hippuric acid since approximately the same amount of this acid is excreted whether ethylbenzene or the carbinol, a known metabolite of ethylbenzene, is fed. Acetophenone is also a precursor of hippuric acid, but our earlier work (Smith *et al.* 1954*b*) has shown that this ketone is reduced to methylphenylcarbinol *in vivo*, and need not be included in the scheme. It was not detected in the breath or urine of rabbits receiving ethylbenzene. Mandelic acid is apparently a minor end product of ethylbenzene metabolism and is not converted into hippuric acid. It is also a known minor metabolite of methylphenylcarbinol (Thierfelder & Klenk, 1924) and is presumably produced from ethylbenzene via the carbinol. The main process of ethylbenzene metabolism is thus initially oxidation of the activated α-carbon atom. The actual mechanism of this initial oxidation is as yet unknown. However, oxidation of the ω-methyl group of ethylbenzene does take place to a small extent, since phenaceturic acid can be isolated from the urine. 2-Phenylethanol (see Table 2) and phenylacetaldehyde are presumably intermediates in its formation.

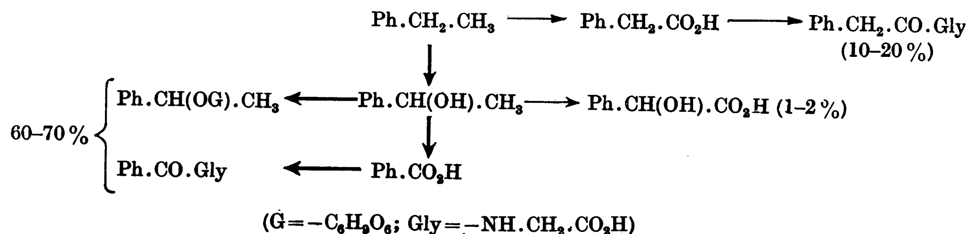


Fig. 1

Metabolism of n-propylbenzene. The metabolites of *n*-propylbenzene are hippuric acid, and the glucuronides of ethylphenylcarbinol and benzyl methylcarbinol. The results shown in Table 2 suggest the scheme shown in Fig. 2 for its metabolism.

What we believe to be major routes are indicated by thick arrows, minor routes by thin arrows, and hypothetical routes by broken arrows. Glucuronide and hippuric acid excretion account for about 60–70 % of the *n*-propylbenzene fed. Ethylphenylcarbinyl glucosiduronic acid can be readily isolated from the urine, but ethylphenylcarbinol itself is not readily converted into hippuric acid (see Table 2). Conjugated benzylmethylcarbinol was also detected as a metabolite of propylbenzene, but appeared to be present in the urine in lesser amounts than ethylphenylcarbinol. However, benzylmethylcarbinol is more readily converted into hippuric acid than the α-carbinol. It is to be noted that ethyl phenyl ketone and benzyl methyl ketone are equivalent to the carbinols as far as hippuric acid production is concerned. We conclude therefore that the degradation of propylbenzene to hippuric acid is mainly through benzylmethylcarbinol rather than ethylphenylcarbinol. Another possible route of degradation is by ω-oxidation to β-phenylpropionic acid, which can be subsequently β-oxidized to benzoic acid. According to Raper & Wayne (1928), in the dog 77 % of the administered β-phenylpropionic acid (122 mg./kg.) is excreted as hippuric acid. With doses of about 500 mg./kg. in the same animal Quick (1928)

Table 2. *Excretion of hippuric acid and phenaceturic acid by rabbits receiving alkylbenzenes and related compounds orally*

Results are quoted as the average for three animals, with range in parentheses; the superior figure indicates the number of experiments if other than three. The excretion of metabolites was complete in 24 hr. after dosing. Phenaceturic acid was detected by paper chromatography and roughly estimated; percentage range in parentheses indicates rough estimate as percentage of dose, + + a large amount, + a minor amount, – absent.

Compound	Formula	Dose (m-mole/kg.)	Hippuric acid excretion (% of dose)	Phenaceturic acid excretion	Glucuronic acid conjugation (% of dose)
Benzoic acid	$Ph.CO_2H$	0·75	79 (76, 81)[2]		
Toluene	$Ph.CH_3$	3·0	74 (63–94)		
Ethylbenzene	$Ph.CH_2.CH_3$	3·0	31 (24–36)	(10–20 %)	32*
2-Phenylethanol	$Ph.CH_2.CH_2.OH$	2·0	3† (3–4)	+ +	7*
(±)-Methylphenylcarbinol	$Ph.CH(OH).CH_3$	2·0	28 (26, 30)[2]	–	50*
Acetophenone	$Ph.CO.CH_3$	2·0	19 (17–21)	–	47*
(±)-Mandelic acid	$Ph.CH(OH).CO_2H$	2·0	0 (0–1)	–	
n-Propylbenzene	$Ph.CH_2.CH_2.CH_3$	3·0	15 (13–16)	–	47 (39–56)
β-Phenylpropionic acid	$Ph.CH_2.CH_2.CO_2H$	2·0	47 (41–57)[5]	–	
Cinnamic acid	$Ph.CH{:}CH.CO_2H$	2·0	74 (71–77)	–	
(±)-Ethylphenylcarbinol	$Ph.CH(OH).CH_2.CH_3$	2·0	6 (6–7)	–	54*
Ethyl phenyl ketone	$Ph.CO.CH_2.CH_3$	2·0	8 (7–11)	–	
(±)-Benzylmethylcarbinol	$Ph.CH_2.CH(OH).CH_3$	2·0	19 (17–21)	–	43*
Benzyl methyl ketone	$Ph.CH_2.CO.CH_3$	2·0	20 (14–24)	–	
n-Butylbenzene	$Ph.CH_2.CH_2.CH_2.CH_3$	3·0	2† (0–4)	(15–25 %)	51 (42–60)[2]
γ-Phenylbutyric acid	$Ph.CH_2.CH_2.CH_2.CO_2H$	2·0	3† (2–4)	(80–90 %)	
(±)-Phenylpropylcarbinol	$Ph.CH(OH).CH_2.CH_2.CH_3$	2·0	0 (0–1)	–	72 (66–77)
Phenyl propyl ketone	$Ph.CO.CH_2.CH_2.CH_3$	2·0	4 (2–5)	–	
(±)-Benzylethylcarbinol	$Ph.CH_2.CH(OH).CH_2.CH_3$	2·0	1 (0–2)	–	64 (58–68)
Benzyl ethyl ketone	$Ph.CH_2.CO.CH_2.CH_3$	2·0	4 (3–5)	–	
(±)-Methyl 2-phenylethylcarbinol	$Ph.CH_2.CH_2.CH(OH).CH_3$	2·0	0 (0–0)	(15–20 %)	68 (64–71)
Methyl 2-phenylethyl ketone	$Ph.CH_2.CH_2.CO.CH_3$	2·0	6 (5–7)	+	

* These figures are for doses of 4 m-moles/kg. quoted from Smith *et al.* (1954*a*, *b*, *c*).
† The significance of these values is discussed in the text.

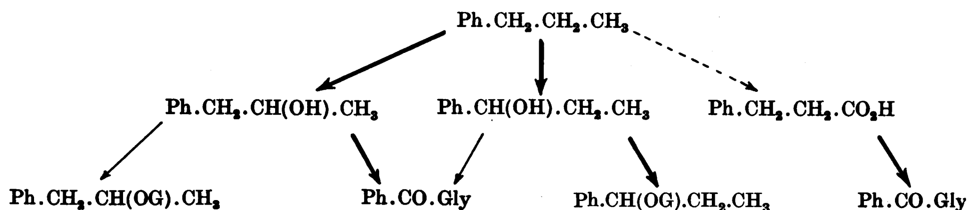


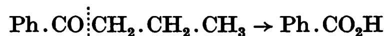
Fig. 2

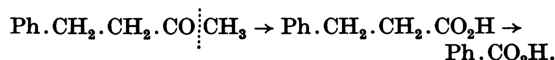
reported the hippuric acid excretion to be only 28% of the dose. In rabbits at 300 mg./kg., Table 2 shows the hippuric acid formation to be about 50% of the dose. With cinnamic acid, the hippuric acid output in the rabbit is 74% of the dose (Table 2) and is the same as that reported by Raper & Wayne (1928) for the dog.

Cinnamoylglycine has been reported as a trace metabolite in dogs of β-phenylpropionic acid, cinnamic acid and β-hydroxy-β-phenylpropionic acid (cf. Dakin, 1911 and earlier papers). We have shown that this conjugate can be readily detected by paper chromatography. If cinnamoylglycine is a trace metabolite of β-phenylpropionic acid and the latter is an intermediate in propylbenzene metabolism, then cinnamoylglycine could be a trace metabolite of propylbenzene. This glycine conjugate was not detected as a metabolite of propylbenzene, β-phenylpropionic acid or cinnamic acid in the rabbit.

Metabolism of n-*butylbenzene*. The main metabolites of this compound appear to be phenylpropyl- and methylphenethyl-carbinylglucuronides, and phenaceturic acid, and a consideration of all our observations suggests the scheme shown in Fig. 3 for its metabolism. The formation of γ-phenylbutyric acid has not been proved, but we consider it to be a possible reaction, since ω-oxidation of alkyl chains is a known reaction and γ-phenylbutyric acid is known to be oxidized to phenaceturic acid (Raper & Wayne, 1928). Both phenylpropyl- and methyl-2-phenylethyl-carbinols are formed, but only the latter is converted into phenaceturic acid. The third possible carbinol, benzylethylcarbinol, was not detected in the urine and when fed it was not a precursor of phenaceturic acid but was largely excreted conjugated with glucuronic acid (see Table 2). It is probably not a metabolite of butylbenzene. The routes of degradation of the side chain of butylbenzene are therefore by $(\omega-1)$-oxidation and probably by ω-oxidation. α-Oxidation does take place but does not lead to phenylacetic acid.

It is to be noted in Table 2 that phenyl propyl, benzyl ethyl, and methyl 2-phenylethyl ketones give rise to a small but probably significant (as shown by paper chromatography) hippuric acid output (about 4%) which is not observed with the corresponding carbinols; otherwise, these ketones appear to be mainly converted into the glucuronides of the corresponding carbinols (see Smith *et al.* 1954*c*). It is thus possible that these ketones undergo a minor metabolic reaction leading to benzoic acid. Loss of the alkyl residue in phenyl propyl and methyl 2-phenylethyl ketones could lead to benzoic acid thus:





With benzyl ethyl ketone, loss of the alkyl residue would yield phenylacetic acid:

However, it is to be noted from Table 2 that 2-phenylethanol, which is mainly metabolized to phenylacetic acid, also gives rise to a small apparent hippuric acid output (3–4%).

Penultimate versus terminal oxidation. Since it has been shown that hippuric acid could arise from propylbenzene by ω- or $(\omega-1)$-oxidation and phenaceturic acid could arise similarly from butylbenzene, it is pertinent to inquire whether one or both modes of oxidation result in the degradation of these two alkyl chains. Although α-oxidation is important in the degradation of ethylbenzene to hippuric acid, it is not important in the propyl and butyl derivatives, since ethylphenylcarbinol yields little hippuric acid and phenylpropylcarbinol no phenaceturic acid *in vivo*. In any case, α-oxidation is probably a special reaction of alkylbenzenes due to the presence of the benzene ring. ω-Oxidation is a known reaction of alkyl chains of six or more carbon atoms and numerous examples are available, especially in the fatty acid series (see Weitzel, 1951). $(\omega-1)$-Oxidation of the butyl side chain to form a 3-hydroxybutyl chain has been shown to occur in 5-butyl-5-ethylbarbituric acid (butobarbitone) in the dog (Maynert, 1952) and in 4-butyl-3:5-dioxo-1:2-diphenylpyrazolidine (butazolidine) in man (Burns *et al.* 1953, 1955). The experiments of Titus & Weiss (1955) on the fate of [^{14}C]-5-ethyl-5-(1-methylbutyl)barbituric acid (pentobarbi-

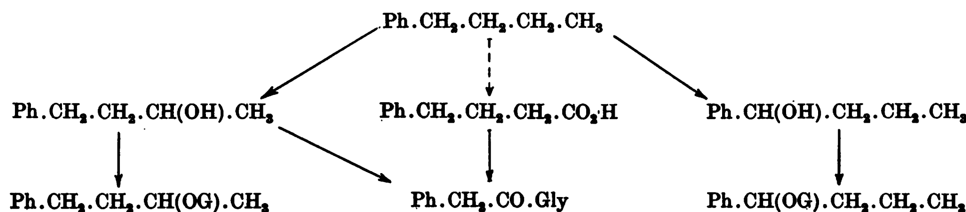


Fig. 3

tone) in the dog suggest that the butyl chain undergoes very little ω-oxidation, since 75·6 % of the dose was excreted as the $(\omega-1)$-oxidation product, 5-ethyl-5-(3-hydroxy-1-methylbutyl)barbituric acid, and only 4·5 % as the ω-oxidation product, 5-ethyl-5-(3-carboxy-1-methylpropyl)barbituric acid.

Our present results, however, do not permit a decision as to whether ω- or $(\omega-1)$-oxidation is the more important in the degradation of propyl- and butyl-benzenes. It is significant, however, that the degradation of the $(\omega-1)$-carbinols is of the same order as that of the corresponding alkylbenzenes.

SUMMARY

1. A study has been made of the fate of ethylbenzene, *n*-propylbenzene and *n*-butylbenzene in the rabbit.

2. About 60–70 % of ethylbenzene undergoes α-oxidation to yield methylphenylcarbinol glucuronide and hippuric acid, and 10–20 % undergoes ω-oxidation to yield phenaceturic acid.

3. About 50 % of propylbenzene is excreted as the glucuronides of ethylphenylcarbinol and benzylmethylcarbinol, and about 15 % as hippuric acid. The main precursor of hippuric acid appears to be benzylmethylcarbinol.

4. About 50 % of butylbenzene is excreted as the glucuronides of methyl-2-phenylethylcarbinol and phenylpropylcarbinol, and about 20 % as phenaceturic acid. The precursor of phenaceturic acid appears to be methyl-2-phenylethylcarbinol; phenylpropylcarbinol is not a precursor. Derivatives of benzylethylcarbinol were not detected as metabolites of butylbenzene, and this carbinol is not a precursor of phenaceturic acid.

5. The three alkylbenzenes have been proved to undergo α-oxidation; propyl- and butyl-benzenes also undergo $(\omega-1)$-oxidation of the alkyl side chain. No direct evidence for the ω-oxidation of propyl- and butyl-benzenes has been obtained, but its occurrence cannot be ruled out.

6. A method modified from Gaffney *et al.* (1954) has been described for the determination of hippuric acid in urine.

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REFERENCES

Anon. (1955). *Chem. Engng News*, **33**, 3124.
Beer, C. T., Dickens, F. & Pearson, J. (1951). *Biochem. J.* **48**, 222.
Bray, H. G., Thorpe, W. V. & White, K. (1950). *Biochem. J.* **46**, 271.
Browning, E. (1953). *Toxicity of Industrial Organic Solvents*, p. 63. London: H.M. Stationery Office.
Burns, J. J., Rose, R. K., Chenkin, T., Goldman, A., Schulert, A. & Brodie, B. B. (1953). *J. Pharmacol.* **109**, 346.
Burns, J. J., Rose, R. K., Goodwin, S., Reichenthal, J., Horning, E. & Brodie, B. B. (1955). *J. Pharmacol.* **113**, 481.
Dakin, H. D. (1911). *J. biol. Chem.* **9**, 127.
Gaffney, G. W., Schreier, K., DiFerrante, N. & Altman, K. I. (1954). *J. biol. Chem.* **206**, 695.
Heilbron, I. & Bunbury, H. M. (1953). *Dictionary of Organic Compounds*. London: Eyre and Spottiswoode.
Maynert, E. W. (1952). *Fed. Proc.* **11**, 625.
Parke, D. V. (1956). *Biochem. J.* **62**, 339.
Quick, A. J. (1928). *J. biol. Chem.* **77**, 581.
Raper, H. S. & Wayne, E. J. (1928). *Biochem. J.* **22**, 190.
Robinson, D., Smith, J. N. & Williams, R. T. (1953). *Biochem. J.* **53**, 125.
Robinson, D., Smith, J. N. & Williams, R. T. (1955). *Biochem. J.* **59**, 153.
Robinson, D. & Williams, R. T. (1955). *Biochem. J.* **59**, 159.
Smith, J. N., Smithies, R. H. & Williams, R. T. (1954*a*). *Biochem. J.* **56**, 317.
Smith, J. N., Smithies, R. H. & Williams, R. T. (1954*b*). *Biochem. J.* **56**, 320.
Smith, J. N., Smithies, R. H. & Williams, R. T. (1954*c*). *Biochem. J.* **57**, 74.
Thierfelder, H. & Klenk, E. (1924). *Hoppe-Seyl. Z.* **141**, 13.
Titus, E. & Weiss, H. (1955). *J. biol. Chem.* **214**, 807.
Weitzel, G. (1951). *Hoppe-Seyl. Z.* **287**, 254.

Maleic Anhydride in the Study of Naturally Occurring Isomers of Vitamin A

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During the past ten years, several *cis* isomers of vitamin A have been described (Robeson & Baxter, 1945, 1947; Graham, van Dorp & Arens, 1949; Dieterle & Robeson, 1954; Wald, Brown, Hubbard & Oroshnik, 1955; Robeson *et al.* 1955*b*). Their formulae are shown in Fig. 1. It is now known that some of these compounds occur naturally. Their presence can be detected by a comparison of chemical and biological assays of vitamin A extracts (Fisher, Kon & Thompson, 1952; Robeson *et al.* 1955*b*), this test being based on the finding that the *cis* isomers so far investigated have lower