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Studies in Detoxication

61. THE METABOLISM OF ALKYL BENZENES. *TERT*-BUTYLBENZENE

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In earlier papers in this series (Smith, Smithies & Williams, 1954b; Robinson, Smith & Williams, 1955) it was shown that ethylbenzene and isopropylbenzene were oxidized in rabbits at the activated carbon atom next to the benzene ring. In *tert*-butylbenzene (I) the carbon atom next to the benzene ring carries no replaceable hydrogen; furthermore, unlike ethyl and isopropylbenzene, it is resistant to autoxidation and is relatively stable to oxidation. *tert*-Butylbenzene is nevertheless extensively metabolized by rabbits, for Smith, Smithies & Williams (1954a) have shown that it causes the excretion of conjugated glucuronic acid corresponding to more than two-thirds of the dose. *tert*-Butylbenzene could undergo oxidation of its benzene ring or of its methyl groups. Nuclear oxidation would yield a phenol, which would be expected to be excreted in part as an ethereal sulphate. However, very little if any ethereal sulphate is excreted by rabbits receiving *tert*-butylbenzene (cf. Smith *et al.* 1954a). Oxidation of one of the methyl groups might be expected to yield 2:2-dimethyl-2-phenylethanol (II), the corresponding aldehyde (III) or acid (IV).

(I) (II)

(III) (IV)

Dimethylphenylacetic acid would be expected to yield a reducing ester glucuronide, but the urine from *tert*-butylbenzene is non-reducing (Smith *et al.* 1954a). Dimethylphenylethanol, on the other hand, would yield a non-reducing glucuronide. It will be shown here that 2:2-dimethyl-2-phenylethyl glucuronide appears to be the major if not the only metabolite of *tert*-butylbenzene in the rabbit.

EXPERIMENTAL

Materials

tert-Butylbenzene (British Drug Houses Ltd.) was redistilled, b.p. 166–167°.

αα-Dimethyl-*α*-phenylacetic acid. Phenylacetoneitrile (46.8 g.; Light and Co.) was dissolved in liquid NH₃ (300 ml.) containing 50 mg. anhydrous FeCl₃ and 32 g. of potassium was added in small pieces to the solution. During the addition of the potassium the solution turned first blue then slowly brownish red. When the potassium had dissolved, slightly more than the calculated amount of methyl iodide (125 g.) was added with shaking and the solution was then allowed to stand until the NH₃ had evaporated. The dark brown oily residue was washed with very dil. acid which contained sodium thiosulphate to remove I₂. The oil was then taken up in ether and the solution dried over anhydrous Na₂SO₄. After evaporation of the ether, the oily residue of dimethylphenylacetoneitrile was distilled under reduced pressure (yield 40 g.). The nitrile was mixed with 50 ml. of 80% (v/v) H₂SO₄ and heated under reflux for 3 hr. The product was poured on 200 g. ice and the mixture kept overnight. The solid dimethylphenylacetamide was collected, washed with saturated NaHCO₃ and recrystallized repeatedly from 50% aqueous ethanol (m.p. 160°; Haller & Bauer (1912) give m.p. 160°). The amide (20 g.) was dissolved in 80% (v/v) H₂SO₄ and the solution cooled in ice. The calculated amount of a 10% solution of NaNO₂ was carefully added below the surface of the solution, and the mixture then kept overnight at room temperature. On pouring on to ice, dimethylphenylacetic acid separated and was collected. It was dissolved in 2*N*-NaOH and the solution extracted with ether to remove impurities. It was then precipitated by acidification and recrystallized from water (yield 12.5 g., m.p. 76°; Haller & Bauer (1912) give m.p. 80–81°).

2:2-Dimethyl-2-phenylethanol. A solution of the above acid (12.5 g.) in 200 ml. dry ether was run slowly (0.5 hr.) into a suspension of lithium aluminium hydride (4.5 g.) in 250 ml. dry ether, the flask, which was fitted with a condenser, being kept in an ice bath. The mixture was allowed to

warm up to room temp. and then refluxed for 0.5 hr. After keeping overnight, water was carefully added to decompose excess hydride, and the white ppt. which formed was re-dissolved by adding 10% (v/v) H_2SO_4 . The ether layer was now separated and the aqueous layer extracted once with ether. The ether solutions were pooled, washed with 20% (w/v) NaOH and then with water, and finally dried over anhydrous Na_2SO_4 . After evaporation of the ether, the residue was distilled under reduced pressure to yield 12 g. of dimethylphenylethanol, b.p. 125° (at 14–20 mm.). The alcohol was characterized as the α -naphthylurethane, m.p. 92° (Whitmore, Weisgerber & Shabica (1943) give m.p. $91.5\text{--}92.5^\circ$.)

Methods

The glucuronic acid output of rabbits which had received oral doses of *tert.*-butylbenzene and 2:2-dimethyl-2-phenylethanol was determined by the naphthoresorcinol method as modified by Paul (1951) (for results see Table 1).

Isolation of metabolites

From 2:2-dimethyl-2-phenylethanol. A rabbit was given 1 g. of the alcohol by mouth. The 24 hr. urine was neutral and non-reducing, but gave an intense naphthoresorcinol reaction. It gave no colour with FeCl_3 . The glucuronide gum (1.5 g.), prepared by the basic lead acetate method, was soluble in methanol. On methylation and acetylation (cf. Kamil, Smith & Williams, 1951) it yielded 2 g. of triacetyl methyl ester, which on recrystallization from ethanol followed by isopropyl ether yielded colourless needles (0.5 g.) of methyl (2:2-dimethyl-2-phenylethyl tri-*O*-acetyl- β -D-glucosid)uronate m.p. 125° , $[\alpha]_D^{20} - 30^\circ$ in CHCl_3 (c, 1). (Found: C, 59.4; H, 6.7; OMe, 6.3. $\text{C}_{25}\text{H}_{30}\text{O}_{10}$ requires C, 59.2; H, 6.5; OMe, 6.65%.)

The above ester (0.7 g.) was deacetylated by quickly heating to boiling with 2.5N-NaOH. The solution was cooled and made 2N with respect to H_2SO_4 and heated under reflux for 3 hr. After cooling, the solution was extracted twice with 20 ml. of light petroleum (b.p. 120°). The extract was dried over anhydrous Na_2SO_4 and then refluxed with α -naphthylisocyanate (200 mg.) for 3 hr. After filtering and cooling, 2:2-dimethyl-2-phenylethyl- α -naphthylurethane separated (200 mg., 45%). This was recrystallized from light petroleum (b.p. 120°) and had m.p. 90° , and mixed m.p. $90\text{--}91^\circ$ with the authentic naphthylurethane.

From α -dimethylphenylacetic acid. This acid (1 g.) was fed to a rabbit and the 24 hr. urine collected. The urine readily reduced Fehling's and Benedict's solutions and gave a rapid, strong, positive naphthoresorcinol test characteristic of ester glucuronides. The glucuronide was obtained as a reducing gum (1 g.) which on methylation and acetylation

failed to yield a crystalline product. On hydrolysis the gum yielded α -dimethylphenylacetic acid, m.p. and mixed m.p. 76° , showing that this acid had formed an ester glucuronide.

*From *tert.*-Butylbenzene.* A total of 2.2 g. of the hydrocarbon was fed to three rabbits and the urine collected for 24 hr. The urine was neutral in reaction, non-reducing and gave no colour reaction with FeCl_3 . It gave no reaction with 2:4-dinitrophenylhydrazine (Brady's reagent), showing the absence of aldehydes. The glucuronide gum (2 g.) was prepared in the usual manner and was converted into the triacetyl methyl ester. After recrystallization 0.8 g. of pure methyl (2:2-dimethyl-2-phenylethyl tri-*O*-acetyl- β -D-glucosid)uronate, m.p. $126\text{--}127^\circ$ and $[\alpha]_D^{20} - 28^\circ$ in CHCl_3 (c, 1) was obtained. (Found: C, 58.9; H, 6.3. $\text{C}_{25}\text{H}_{30}\text{O}_{10}$ requires C, 59.2; H, 6.5%.) It was identical in all respects with the ester (mixed m.p. 126°) isolated after feeding 2:2-dimethyl-2-phenylethanol.

The high conjugation of dimethylphenylethanol and of *tert.*-butylbenzene (Table 1), together with the absence of any reducing power in the urine, suggests that only the glucuronide of 2:2-dimethyl-2-phenylethanol is excreted after feeding these compounds and that the α -dimethylphenylacetylglucuronide is not formed. However, this does not eliminate α -dimethylphenylacetic acid as a minor metabolite of *tert.*-butylbenzene. This acid could be formed and excreted as such or as a glycine conjugate. The basic lead acetate filtrate obtained during the preparation of the glucuronide from *tert.*-butylbenzene was therefore investigated by paper chromatography. When a spot of this filtrate was chromatographed on Whatman no. 1 paper using saturated *n*-butanol–water as solvent, three acid spots were detected on spraying the paper with iodide–iodate mixtures followed by starch solution. These spots had R_F values of 0.25, 0.6 and 0.9. That the second spot (R_F 0.6) was a glucuronide was revealed by spraying with naphthoresorcinol and heating. This was probably the dimethylphenylethyl glucuronide. The last spot, which was weak, corresponded to α -dimethylphenylacetic acid which has an R_F of 0.9 under similar conditions. This spot increased in intensity if the filtrate used was subjected to acid hydrolysis before chromatography, suggesting the presence of the glycine conjugate of dimethylphenylacetic acid. Since this acid appeared to be a very minor metabolite, its further identification was not pursued. The spot of R_F 0.25 had a long tail and appeared to be due to traces of free glucuronic acid and glucurone.

DISCUSSION

The present paper completes the study of the major metabolites formed in rabbits from alkylbenzenes containing a linear chain of two carbon atoms. The

Table 1. *Glucuronic acid conjugation of 2:2-dimethyl-2-phenylethanol and *tert.*-butylbenzene in rabbits*

Results on individual animals. Figures in parentheses are average values. The excretion of extra glucuronic acid was complete in one day after dosing.

Compound fed	Dose (mg./kg.)	Percentage of dose excreted as glucuronide
<i>tert.</i> -Butylbenzene	268 (2 m-moles) 500 (4 m-moles)	63, 85, 94 (81) (66)*
2:2-Dimethyl-2-phenylethanol	300 (2 m-moles) 150 (1 m-mole)	93, 94 (93.5) 92, 93, 100 (95)

* Quoted from Smith *et al.* (1954a)

Table 2. *The oxidation products of ethyl-, isopropyl- and tert.-butyl-benzene in rabbits*

Compound	Known oxidation products (% of dose)		
	($\pm$)Ph.CHOH.CH ₃ (32%)	Ph.CHOH.COOH (1-2%)*	Ph.COOH (30%)†
Ph.CH ₂ .CH ₃			
Ph.CH(CH ₃) ₂	Ph.COCH(CH ₃) ₂ (40%)	Ph.CH(CH ₃).CH ₂ OH‡ (25%)	Ph.CH(CH ₃).COOH‡ (25%)
Ph.C(CH ₃) ₃	Ph.C(CH ₃) ₂ .CH ₂ OH (95%)	Ph.C(CH ₃) ₂ .COOH (traces)	—

* Data from Thierfelder & Daiber (1923).

† Unpublished data (el Masry, Smith & Williams); cf. Thierfelder & Daiber (1923) who give 25%.

‡ Probably both optical forms.(cf. Robinson *et al.* 1955).

results are summarized in Table 2 from which it is clear that oxidation at the activated carbon atom next to the benzene ring takes place more readily than at the ω -carbon atom when such an oxidation is possible. In *tert*.-butylbenzene, ω -oxidation is the only possible reaction, and it is to be noted that this oxidation hardly goes beyond the primary alcohol stage. 2:2-Dimethyl-2-phenylethanol (Ph.C(CH₃)₂.CH₂OH) contains a primary alcohol group which is not readily oxidized *in vivo* and it should be compared with 2-phenylethanol (Ph.CH₂.CH₂OH) and 2-methyl-2-phenylethanol (hydratropyl alcohol, Ph.CH(CH₃).CH₂OH). 2-Phenylethanol is almost completely oxidized in the rabbit to phenylacetic acid (Smith *et al.* 1954*b*). 2-Methyl-2-phenylethanol is less readily oxidized, for it is excreted partly as the glucuronide of the unchanged alcohol and partly as α -methylphenylacetic acid (hydratropic acid) (Robinson *et al.* 1955). It appears, therefore, that the substitution of two methyl groups on the β -carbon atom of 2-phenylethanol almost completely inhibits the oxidation of the primary alcohol group *in vivo*. It is thus to be compared with di- and tri-chloroethanols, which are not readily oxidized in the rabbit (Smith & Williams, 1954).

SUMMARY

1. *tert*.-Butylbenzene is oxidized in rabbits mainly to 2:2-dimethyl-2-phenylethanol (up to

90% of the dose), which is excreted as a glucuronide, characterized as a triacetyl methyl ester. Traces of $\alpha\alpha$ -dimethylphenylacetic acid may also be formed.

2. 2:2-Dimethyl-2-phenylethanol is not oxidized in rabbits to any appreciable extent, and 90-100% of doses of 150-300 mg./kg. is excreted as 2:2-dimethyl-2-phenylethyl glucosiduronic acid, which is identical with the glucosiduronic acid excreted after feeding *tert*.-butylbenzene.

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