Studies in Detoxication

60. THE METABOLISM OF ALKYLBENZENES. *ISO*PROPYLBENZENE (CUMENE) AND DERIVATIVES OF HYDRATROPIC ACID

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*iso*Propylbenzene (cumene) is used as a solvent for cellulose lacquer, although not extensively. It has narcotic properties but no toxic effects in man have been reported (see Browning, 1953). When fed to rabbits it causes the excretion of considerable amounts of conjugated glucuronic acid and the urine reduces Benedict's reagent (Smith, Smithies & Williams, 1954*a*), but nothing definite is known concerning the nature of the metabolites of cumene. The earlier work of Nencki & Giacosa (1880) and of Thierfelder & Klenk (1924) showed that it did not give rise to hippuric acid or any other aromatic acid. However, the former authors found that the ratio of inorganic sulphate to ethereal sulphate in the urine of the dog was reduced after administering cumene, and on these grounds suggested that a phenol was formed (cf. Donahue & Harris, 1945), but Smith *et al.* (1954*a*) found that in the rabbit ethereal sulphate formation was very small.

Our work on ethylbenzene (Smith, Smithies & Williams, 1954*b*) has suggested that in this compound biological oxidation occurs initially mainly at the active methylene group next to the benzene ring. We were, therefore, particularly interested in whether a similar oxidation would occur with cumene giving rise to 2-phenylpropan-2-ol ($Ph.C(OH)(CH_3)_2$). We shall show that this is, in fact, true and that cumene gives rise also to hydratropic acid (α-phenylpropionic acid) and hydratropyl alcohol (2-phenylpropan-1-ol).

EXPERIMENTAL

Materials. The following compounds were purchased: cumene, b.p. 152–153°; (±)-hydratropic aldehyde, b.p. 205°; α-methylstyrene, b.p. 165°. 2-Phenylpropan-2-ol (dimethylphenylcarbinol), b.p. 202°, was prepared from bromobenzene and acetone according to Tissier & Grignard (1911). (±)-Hydratropic alcohol, b.p. 113–114°, was prepared in excellent yield by the reduction of hydratropic aldehyde with lithium aluminium hydride by standard methods (cf. Brown, 1951). (±)-Hydratropic acid, b.p. 265°, was prepared according to Arcus & Kenyon (1939) from the aldehyde.

Animals. Chinchilla rabbits kept on a constant diet were used. All compounds were administered suspended in water by stomach tube.

Table 1. *Glucuronic acid conjugation of hydratropic acid and related compounds by the rabbit*

Figures in parentheses are average values.

Compound fed	Dose (g./kg.)	Conjugation (% of dose)
(±)-Hydratropic alcohol	0·5	60, 78, 80 (73) 68*, 66* (67*)
(±)-Hydratropic aldehyde	0·3	51*, 52*, 54* (52*)
(±)-Hydratropic acid	0·3	63*, 72*, 100* (78*)
(+)-Hydratropic acid†	0·3	73*, 74*, 90* (79*)
(−)-Hydratropic acid‡	0·3	58*, 66*, 67* (64*)
2-Phenylpropan-2-ol	0·5	60, 97, 98 (85)
Cumene	0·45	68§

* Ester glucuronide as shown by titration with Benedict's reagent.
† 95% (+)-isomer.
‡ 77% (−)-isomer.
§ Figure quoted from Smith *et al.* (1954*a*).

Methods. Total glucuronic acid in urine was determined by the modified naphthoresorcinol method described by Paul (1951). Ester glucuronide was estimated by titrating in the usual way with Benedict's reagent standardized against hydratropoylglucuronide (see Table 1 for results).

EXPERIMENTS WITH HYDRATROPIC ACID AND ITS DERIVATIVES

Hydratropoylglucosiduronic acid and its derivatives

From (±)-hydratropic aldehyde. A total of 18 g. of the aldehyde was fed to six rabbits, and the urine, which reduced Benedict's and Fehling's solutions readily and gave a strong naphthoresorcinol test, was collected for 24 hr. The glucuronide gum was prepared through the basic lead salt (cf. Kamil, Smith & Williams, 1951). The gum was dissolved in a little methanol, and 250 ml. ether followed by 100 ml. of light petroleum (b.p. 40–60°) were added. This caused the separation of a gummy precipitate *A* which yielded no crystalline material on methylation and acetylation. The filtrate from *A* was treated with a further 100 ml. of light petroleum and the gum *B* which separated was methylated with ethereal diazomethane. On evaporation of the solvents and recrystallization of the residue from water *methyl (+)-hydratropoyl-β-D-glucosiduronate dihydrate (methyl (+)-α'-phenylpropionyl-β-D-glucosiduronate)*, 0·7 g., separated as colourless needles, m.p. 165–166° and $[\alpha]_D^{20} + 22°$ in $CHCl_3$ (*c*, 1). (Found: C, 51·3; H, 6·3; OMe, 8·3. $C_{16}H_{20}O_8$, $2H_2O$ requires C, 51·1; H, 6·4; OMe, 8·4%.) It reduced Fehling's and Benedict's reagents on warming. By adding

more light petroleum to the filtrate from gum *B*, more gum separated and this on methylation yielded a further 0·8 g. of the methyl ester (total yield, 1·5 g. or about 6% of dose). Acetylation of this ester with pyridine and acetic anhydride and then pouring the mixture into water yielded *methyl (+)-hydratropoyl-tri-O-acetyl-β-D-glucosiduronate* which formed needles from ethanol, m.p. 130–132° and $[\alpha]_D^{20} - 10°$ in $CHCl_3$ (*c*, 1). (Found: C, 56·7; H, 5·6. $C_{22}H_{26}O_{11}$ requires C, 56·6; H, 5·6%.) This compound also reduced Fehling's and Benedict's reagents on heating.

The above experiment was repeated with 6 g. of hydratropic aldehyde to the stage of removal of the gummy precipitate *A*. The filtrate from this was then treated with more light petroleum and the whole allowed to stand several days at 0°. A crystalline solid (50 mg.) separated and was crystallized from water. This was *(+)-hydratropoyl-glucosiduronic acid* (*(+)-α'-phenylpropionyl-β-D-glucosiduronic acid*), which formed colourless needles, m.p. 163–164° and $[\alpha]_D^{20} + 23°$ in ethanol (*c*, 1). (Found: C, 53·3, 53·2; H, 6·2, 6·1. $C_{15}H_{18}O_8$, 0·5 H_2O requires C, 53·7; H, 5·7%.) The glucuronide readily reduced Benedict's reagent, and on methylation and acetylation yielded the same triacetyl methyl ester (m.p. 132° and $[\alpha]_D^{20} - 10°$ in $CHCl_3$) as that described in the preceding paragraph.

The positive rotation of the hydratropoylglucosiduronic acid and its methyl ester suggested that we were dealing with the glucuronide of (+)-hydratropic acid, although the (+) and (−) forms could be expected from (±)-hydratropic aldehyde. Hydrolysis of the methyl ester (1 g.) with 2N-H_2SO_4 yielded, after ether extraction, evaporation and distillation, 250 mg. of an acidic oil (hydratropic acid) with $[\alpha]_D^{20} + 72·3°$ in ethanol (*c*, 1·5). In another experiment (+)-hydratropamide was isolated as follows. Methyl hydratropoylglucosiduronate, m.p. 165–166° (0·9 g.) in 2N-H_2SO_4 (5 ml.) was heated on a water bath for 4 hr. After cooling, the solution was extracted with ether, the extract evaporated and the residue distilled *in vacuo*. The acidic distillate (220 mg.) was converted into the amide (cf. Wild, 1947) which was recrystallized from benzene–*n*-hexane. The (+)-hydratropamide (80 mg.) had m.p. and mixed m.p. 97° and $[\alpha]_D + 52°$ in $CHCl_3$ (*c*, 1) and was identical with a sample of authentic (+)-hydratropamide, m.p. 97–98° and $(\alpha)_D + 50°$ in $CHCl_3$, prepared from (+)-hydratropic acid. (Mislow & Heffler (1952) give m.p. 100–101° and $[\alpha]_D + 59·5°$ in $CHCl_3$ for the (+)-amide, and Wild (1947) gives m.p. 92° for the (±)-amide.) Further proof of the stereochemical nature of the glucuronide was obtained by feeding (+)-hydratropic acid.

From (+)-hydratropic acid. (±)-Hydratropic acid was prepared from the aldehyde and resolved according to Arcus & Kenyon (1939). The (+)-hydratropic acid obtained had $[\alpha]_D + 73°$ in ethanol and thus contained about 95% of the (+)-isomer. (Raper (1923) gives $[\alpha]_D^{20} + 81·1°$ in ethanol.) A total of 2·9 g. of the acid was fed to three rabbits, and the 24 hr. urine worked up as described above. Methyl (+)-hydratropoylglucosiduronate dihydrate, m.p. 166° and $[\alpha]_D + 24°$ in 30% aqueous ethanol (*c*, 0·2), was obtained. (Found: C, 51·6; H, 6·4%). It was identical with the methyl ester isolated after feeding (±)-hydratropic aldehyde.

A partially resolved sample of (−)-hydratropic acid was also prepared. It had $[\alpha]_D^{20} - 44°$ and thus contained about 77% of the (−)-isomer. Rabbits when fed with this compound yielded a highly reducing urine, but the glucuronide gum from the urine could not be induced to crystallize nor could any crystalline derivative be prepared.

Hydratropyl glucosiduronic acid (2-phenyl-n-propyl glucosiduronic acid)

(±)-Hydratropic alcohol could be expected to yield in the rabbit at least four glucuronides, namely, (+)- and (−)-hydratropyl glucosiduronic acids as well as (+)- and (−)-hydratropoylglucosiduronic acids. Both types (i.e. ether and ester) of glucuronide are, in fact, formed, but the amount of one in relation to the other was very variable in different experiments. In most experiments large amounts of hydratropoylglucuronide and only small amounts of hydratropyl glucuronide appeared to be excreted. Occasionally appreciably more of the alcohol glucuronide was formed.

The following experiment gave a relatively good yield of hydratropyl glucuronide. (±)-Hydratropic alcohol (3 g.) was fed to two rabbits. The 24 hr. urine gave an intense naphthoresorcinol reaction and readily reduced Fehling's and Benedict's reagents. It was made 2N with respect to NaOH and kept at 100° for 30 min. in order to hydrolyse the hydratropoylglucuronide. After cooling, the urine was neutralized with glacial acetic acid and then worked up by the lead acetate procedure to yield the glucuronide gum. The latter was dissolved in 2 ml. ethanol and the solution poured into ether (200 ml.) to precipitate any free glucuronic acid. After standing a short while, the ether was filtered and ethereal diazomethane added. After evaporation of the solvents, the residue was acetylated and the product poured into water. The solid (4 g.) which separated was recrystallized twice from ethanol, yielding *methyl (hydratropyl tri-O-acetyl-β-D-glucosid)uronate* (*methyl (2-phenyl-n-propyl tri-O-acetyl-β-D-glucosid)uronate*), as white needles, m.p. 123–124° and $[\alpha]_D^{20} - 35°$ in $CHCl_3$ (*c*, 1). (Found: C, 58·1; H, 6·0. $C_{22}H_{28}O_{10}$ requires C, 58·4; H, 6·2%.) It did not reduce Fehling's or Benedict's reagents.

In another experiment hydrolysis of the ester glucuronide with alkali was omitted. The glucuronide gum was prepared, methylated and acetylated and the hydratropyl glucuronide triacetyl methyl ester, m.p. 123°, isolated as above. The mother liquors on allowing to stand deposited crystals of the reducing methyl (+)-hydratropoyl-tri-*O*-acetylglucosiduronate, which on recrystallization had m.p. 132° and did not depress the m.p. of the (+)-isomer of this triacetyl methyl ester prepared from (+)-hydratropic acid. In two quantitative experiments it was found that the ratio of ester glucuronide to ether glucuronide was roughly 6:1.

2-Phenylisopropyl glucosiduronic acid

A total of 7·75 g. of 2-phenylpropan-2-ol was fed to four rabbits. The neutral 24 hr. urine did not reduce Benedict's or Fehling's solutions, but gave a very rapid and intense naphthoresorcinol reaction. On warming with dilute acid, the urine became cloudy owing to separation of oily droplets. It thus contained a glucuronide labile in dilute acid. Part of the urine was worked up by the usual lead acetate procedure, but it was found that on concentrating the aqueous solution of the glucuronide obtained at the stage of removal of lead by H_2S, the glucuronide decomposed to give an oily emulsion. The rest of the urine was therefore worked up by the usual lead acetate procedure to the stage of removal of lead with H_2S. The aqueous solution thus obtained was not concentrated but extracted continuously with ether. The ether extract, as such, was then methylated with diazomethane and after removal of the ether by evaporation at low temperature the residue was acetylated in the usual way. On pouring the acetylated mixture into water, a

crystalline solid (130 mg.) was obtained which was recrystallized from ethanol. The *methyl (2-phenylisopropyl tri-O-acetyl-β-D-glucosid)uronate* formed colourless needles, m.p. 122–123° and $[\alpha]_D^{20} - 41{\cdot}5°$ in $CHCl_3$ (*c*, 1). (Found: C, 58·6; H, 6·1. $C_{22}H_{28}O_{11}$ requires C, 58·4; H, 6·2%.) The above ester (0·5 g.) was dissolved in a little ethanol and slowly titrated with ethanolic KOH so that the pH remained about 8. On keeping the solution *potassium 2-phenylisopropyl glucosiduronate* (100 mg.) separated as needles. It was recrystallized from ethanol and had m.p. 250° (decomp.) and $[\alpha]_D^{20} - 54°$ in water (*c*, 1·5). (Found: C, 48·4; H, 5·9. $C_{15}H_{19}O_7K$, H_2O requires C, 48·9; H, 5·8%.)

Experiments with cumene

The qualitative reactions of the urine of rabbits receiving cumene have already been described (Smith *et al.* 1954*a*). They indicated the presence of an alkali-labile ester glucuronide, and a glucuronide very sensitive to dilute acid. Using paper chromatography and countercurrent extraction it could be shown that at least three glucuronides were present in the urine.

Chromatography of the urine. A portion of the 24 hr. urine of rabbits which had received 2 ml. each of cumene was subjected to systematic lead acetate precipitation (cf. Kamil *et al.* 1951). The aqueous solution left after removal of the lead from the basic lead acetate precipitate with H_2S was used for chromatography. A spot of this solution was placed on the starting line using Whatman no. 1 paper and *n*-butanol saturated with water used as the solvent. The chromatogram was allowed to run overnight (16 hr.). The position of the glucuronides on the paper was detected by using two sprays separately. The glucuronides as acids were detected by the blue spots given when the paper was sprayed with an aqueous solution containing KI, KIO_3 and starch (1% each, w/v). Glucuronic acid was detected by spraying the paper with 20% (w/v) trichloroacetic acid in *n*-butanol containing 0·1% naphthoresorcinol and then heating the paper at 110° for 15 min. Blue spots develop readily when acid-labile glucuronides were present. The iodide–iodate–starch spray revealed three spots of R_F 0·1–0·15, 0·6–0·7 and 0·9–0·95. The naphthoresorcinol spray also revealed three spots of the same R_F, but whilst the two slower ones were intense, the fast moving spot was weak. This indicated that the fast moving spot was due to a relatively stable glucuronide probably present in small amount.

Countercurrent extraction. An attempt was made to separate these glucuronides on a preparative scale by countercurrent distribution using *n*-butanol and 4% (w/v) NaCl as solvents. In two experiments seven and twelve separating funnels respectively were used. The *n*-butanol layers were analysed spectroscopically for benzene-like absorption at 280 mμ. and the aqueous layers for glucuronic acid by the naphthoresorcinol method. Again three glucuronides were detected. The first was slow moving and tended to remain stationary in the first portion of aqueous solvent. The other two separated in the *n*-butanol. Attempts to isolate the glucuronides by working up the relevant fractions by the lead acetate method failed because of dilution and the presence of chloride, which gave rise eventually to acid conditions sufficient to decompose the glucuronides at the final stage of evaporation. However, sufficient information was obtained by these procedures to allow isolation of each glucuronide in separate experiments. Thus one glucuronide was unstable to alkali, the second was unstable to dilute acid and the third was relatively stable to both dilute acid and alkali.

Isolation of hydratropyl glucuronide. Six rabbits were each fed with 1 ml. of cumene. The 24 hr. urine was made 2N with respect to NaOH and then heated for 5 hr. on a boiling-water bath under reflux to decompose ester glucuronide. The mixture was acidified with HCl and extracted with ether. The ether extract yielded on evaporation 0·3 g. of hydratropic acid ($[\alpha]_D^{20} + 25°$ in ethanol) containing an excess of the (+)-isomer. The residual urine was worked up by the lead acetate procedure to the stage of removal of lead with H_2S. The aqueous filtrate after removal of PbS was continuously extracted with ether for 6 hr. This ether extract was then methylated with diazomethane and the gummy methyl ester acetylated with pyridine and acetic anhydride. On pouring the product into water, 0·12 g. of crude triacetyl methyl ester was obtained. This on recrystallization from ethanol yielded 60 mg. of pure methyl (hydratropyl tri-*O*-acetylglucosid)uronate m.p. 123° and $[\alpha]_D^{20} - 35°$ in $CHCl_3$ (*c*, 1). (Found: C, 58·5; H, 6·2%.) It was non-reducing and did not depress the m.p. of the authentic triacetyl methyl ester prepared biosynthetically from hydratropyl alcohol. It considerably depressed the m.p. of methyl (2-phenyl*iso*propyl tri-*O*-acetyl glucosid)-uronate, m.p. 122–123°.

Isolation of 2-phenylisopropyl glucosiduronic acid. A total of 18 g. of cumene was fed to six rabbits and the 24 hr. urine was worked up by the lead acetate method. The filtrate from PbS was freeze-dried in order to avoid decomposition of the acid-labile glucuronide. The resulting acidic glucuronide gum was taken up in 20 ml. 90% (v/v) aqueous ethanol and a large volume of ether added, sufficient to produce two layers. The top ether layer was methylated with ethereal diazomethane, and the solvent removed and the residue acetylated in the usual manner. On pouring the product into water, 8 g. of crude triacetyl methyl ester were obtained. The urine from the second 24 hr. after feeding was similarly treated and yielded 1 g. of triacetyl methyl ester (total 9 g., equivalent to 15% of the dose). On recrystallization from ethanol, 1·5 g. of pure methyl (2-phenyl*iso*propyl tri-*O*-acetyl glucosid)uronate, m.p. and mixed m.p. 123° and $[\alpha]_D^{20} - 42{\cdot}1°$ in $CHCl_3$ (*c*, 1) was obtained. (Found: C, 57·9; H, 6·0%.)

Isolation of hydratropoylglucuronide. Six rabbits were each fed 2 ml. cumene and the glucuronide gum was prepared from the 24 hr. urine in the usual way. The gum was dissolved in 10 ml. methanol and the solution filtered to remove a small amount of inorganic matter. The methanol solution was poured into 500 ml. ether and a ppt. separated. This ppt. was dissolved in a little aqueous methanol and methylated with ethereal diazomethane. The resulting solution was evaporated under reduced pressure to a syrup, which slowly crystallized. Recrystallization from water yielded the neutral methyl (+)-hydratropoylglucosiduronate (50 mg.), m.p. and mixed m.p. 167° and $[\alpha]_D^{25} + 24°$ in 30% aqueous ethanol (*c*, 0·25). The ester reduced Fehling's solution and was sparingly soluble in water and methanol but more soluble in mixtures of these solvents. On acetylation it yielded methyl (+)-hydratropoyl-tri-*O*-acetyl-glucosiduronate, m.p. and mixed m.p. 132°. (Found: C, 56·4; H, 5·7%.)

The action of dilute acid on cumene urine

Detection of a styrene derivative. When cumene urine is heated with dilute acid, oily drops separate and these were

suspected to contain α-methylstyrene (cf. Smith *et al.* 1954*a*). Styrene and its α- and β-methyl derivatives in ethanol show an absorption band at about 240–250 mμ. with an extinction in the region of 10 000. ($\lambda_{max.}$ for styrene is 244 mμ., for α-methylstyrene, 242 mμ. and for β-methylstyrene 246 mμ.; see Gillam & Stern, 1954; Magat & Maier, 1943.) If a styrene derivative is formed from cumene urine, it could arise from 2-phenylpropan-2-ol or its glucuronide and possibly from 2-phenylpropan-1-ol (hydratropyl alcohol) or its glucuronide. Both these alcohols have only a low absorption in the 240–250 mμ. region (Fig. 1).

Two rabbits were each fed with 2 ml. of cumene and the 24 hr. urine was treated with 0·1 vol. of conc. H_2SO_4. The mixture was heated under reflux for 2 hr. on the water bath and then steam-distilled. The distillate containing oily droplets which gave no colour for phenols with $FeCl_3$ was extracted with ether (200 ml.). The extract was washed with 2N-NaOH (2 × 10 ml.) to remove volatile acids (see below) and, after drying over anhydrous Na_2SO_4, was evaporated to yield a neutral oil (0·9 g. or 26% of the dose on the assumption that the oil is α-methylstyrene). In ethanol the oil showed a large band at 240 mμ. (Table 2) which suggested that it was in part a styrene derivative, although we failed to make a solid derivative in order finally to identify it. It was purified by distillation and had b.p. 169°/760 mm., n_D^{22} 1·5324 and $\lambda_{max.}$ 242–243 mμ. with log ε 4·01 in ethanol; α-methylstyrene had b.p. 161–162°, n_D^{21} 1·5333 and n_D^{24} 1·5311 and $\lambda_{max.}$ 242 mμ., log ε 4·05 (Magat & Maier, 1943). This material appears to arise from 2-phenylpropan-2-ol or its glucuronide, since this alcohol on treatment with dilute acid also yields a neutral oil with a high extinction at 240 mμ. The results of the action of acid and alkali upon the isomeric 2-phenylpropanols are shown in Table 2. It is to be noted that hydratropyl alcohol is relatively stable to acid and alkali.

Isolation of hydratropic acid. The NaOH washings of the ether extract described in the preceding paragraph were acidified and extracted with ether. Evaporation of the ether left a brownish oil (0·49 g. or 14% of the dose) which appeared to be mainly hydratropic acid. After distillation the colourless oil had $[\alpha]_D^{20}$ +25° in ethanol and thus appeared to contain an excess of (+)-isomer. On conversion of the material into the amide a small amount was obtained of what appeared to be (+)-hydratropamide, m.p. 95–97°, which did not depress the m.p. of authentic (+)-hydratropamide, m.p. 97–98°. (Found: C, 72·4; H, 7·6; N, 9·0. Calc. for $C_9H_{11}ON$: C, 72·4; H, 7·4; N, 9·4%.) The amount of the amide obtained was insufficient for a determination of the optical rotation. In another experiment 8 ml. cumene were fed and 300 ml. urine collected. By titration with Benedict's reagent, the urine contained 1 g. of hydratropic acid combined with glucuronic acid. This urine was mixed with 25 ml. concn. H_2SO_4 and heated for 4 hr. It was then extracted with ether in a separating funnel. The acids in the ether extract were transferred to NaOH, the alkaline extract acidified and the acids transferred to ether. The ether was evaporated and the resulting syrup treated with thionyl

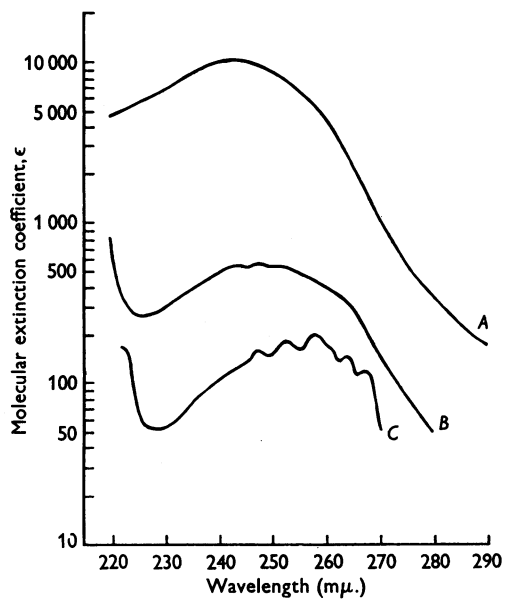


Fig. 1. The ultraviolet absorption spectra of α-methylstyrene (curve *A*), 2-phenylpropan-2-ol (*B*) and 2-phenylpropan-1-ol (hydratropic alcohol; *C*) in ethanol. (α-Methylstyrene, $\lambda_{max.}$ 242–243 mμ., ε 10 200; 2-phenylpropan-2-ol, $\lambda_{max.}$ 244, 247·5, 250 mμ., ε 545, 558, 552; 2-phenylpropan-1-ol, $\lambda_{max.}$ 248, 252, 258, 264, 267 mμ., ε 157, 190, 210, 149, 126.)

Table 2. *The effect of acid and alkali upon the isomeric 2-phenylpropanols*

Compound	Treatment	Extinction in ethanol at 240 mμ. ($E_{1\,cm.}^{1\%}$)
α-Methylstyrene	None	835·0
Neutral oil from acid hydrolysis of cumene urine	None (see text)	550·0
2-Phenylpropan-2-ol	None	55·5
	Steam distilled	57·5
	15 min. at 100° in 2N-HCl, then steam distilled	380·0
	3 hr. at 100° in 2·5N-NaOH, then steam distilled	51·5
2-Phenylpropan-1-ol (hydratropic alcohol)	None	11·0
	15 min. at 100° in 2N-HCl, then steam distilled	11·0
	1·5 hr. at 100° and 18 hr. at 20° in 2N-HCl, then steam distilled	19·4

chloride. The product was treated with NH_3 and the solution neutralized and exhaustively extracted with ether. On evaporation of the ether and recrystallization of the residue from *n*-hexane-benzene, hydratropamide (50 mg.), m.p. 91–94° and $(\alpha)_D$ +44° in $CHCl_3$ (*c*, 1), was obtained. (Found: C, 71·7; H, 7·4. Calc. for $C_9H_{11}ON$: C, 72·4; H, 7·4%.) This amide thus contained over 90% of the (+)-isomer, but its m.p. was a little higher than that of the (±)-amide, m.p. 91–92°. Mixtures of the (+) and (±)-amide were found to melt between 91 and 97°. The m.p. of the (±)-amide was not depressed but raised by admixture with the (+)-amide.

Approximate estimation of 2-phenylisopropyl glucosiduronic acid. When potassium 2-phenyl*iso*propyl glucosiduronate is heated with dilute acid it does not yield α-methylstyrene quantitatively. However, using constant conditions it yielded material with a maximum absorption at 244 mμ. in reproducible amounts to within ±10%. A calibration curve for the approximate estimation of this glucuronide was therefore constructed as follows. One ml. of a solution of the potassium salt of the glucuronide (0·05–0·5 mg./ml.) was heated with 1 ml. 2N-H_2SO_4 for 30 min. in a stoppered test tube at 110–120°. After cooling, 1 ml. of 20% (w/v) NaOH was added and the mixture shaken with 10 ml. of *cyclo*hexane (spectroscopic grade). The upper layer was filtered through a cotton wool plug and its optical density measured at 244 mμ. against a *cyclo*hexane blank in a Unicam spectrophotometer SP 500. The same procedure was used for rabbit urine, 1 ml. of urine diluted 25 times being substituted for 1 ml. of glucuronide solution. Recoveries of 0·3 mg. glucuronide added to 1 ml. diluted urine were within ±10%. This procedure was used to obtain the approximate amount of 2-phenylpropan-2-ol and its glucuronide present in cumene urine. In three animals which had each received 0·86 g. (1 ml.) cumene, it was estimated that 37, 38 and 40% of the cumene fed was converted into 2-phenylpropan-2-ol and its glucuronide. In two of these animals the hydratropoylglucuronide output was also estimated by titrating the urine with Benedict's reagent standardized against pure hydratropoylglucuronide. The values found were 24 and 25% of the dose. The total glucuronide excreted by these animals estimated by the naphthoresorcinol method was about 86% of the dose of cumene, so that by difference, i.e. total glucuronide—(hydratropoylglucuronide + 2-phenyl*iso*propyl glucuronide), the hydratropyl glucuronide is roughly 20–25%. By these approximate methods we conclude that cumene yields about 40% of 2-phenylpropan-2-ol and 25% each of hydratropic acid and alcohol.

DISCUSSION

The present work shows that the side chain of cumene (I) is oxidized in the rabbit at the ω and (ω-1) carbon atoms, yielding 2-phenylpropan-2-ol (II), hydratropic alcohol (III) and hydratropic acid (IV):

$$\underset{\text{(I)}}{\text{Ph.CH(CH}_3)_2} \rightarrow \underset{\text{(II)}}{\text{Ph.C(OH)(CH}_3)_2}$$

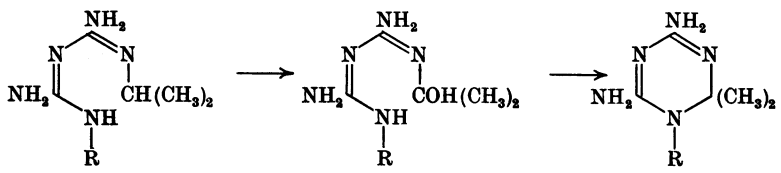
$$+\underset{\text{(III)}}{\text{Ph.CH(CH}_3)\text{.CH}_2\text{OH}} \rightarrow \underset{\text{(IV)}}{\text{Ph.CH(CH}_3)\text{.COOH.}}$$

All three oxidation products have been isolated as glucuronides and thereby characterized. Approximately 40% of the cumene is oxidized to (II) and about 25% to each of (III) and (IV), the total ω-oxidation being about 50%. It appears thus that oxidation takes place more readily at the carbon atom adjacent to the ring than at the ω-carbons since there are two of the latter. It is interesting to note that autoxidations of cumene, which are purely chemical reactions at the methyne carbon atom, proceed by free radical mechanisms, the radical $PhCMe_2$ being formed (cf. Waters, 1945; Melville & Richards, 1954).

The formation of a tertiary alcohol by the oxidation of the methyne carbon atom of an *iso*propyl group at the end of an *iso*amyl group has been reported to occur during the metabolism of 5-*iso*amyl-5-ethylbarbituric acid (Amytal) in the dog. The major metabolite of Amytal is 5-ethyl-5-(3′-hydroxy*iso*amyl)barbituric acid (Maynert, 1952). A similar reaction also occurs in man, for Fieser (1947) has shown that 2-hydroxy-3-*iso*amyl-1:4-naphthoquinone is metabolized to 2-hydroxy-3-(3′-hydroxy*iso*amyl)-1:4-naphthoquinone. We suggest that it is also a possible reaction in the conversion of the antimalarial, proguanil (V) into its active metabolite (VI) (Crowther & Levi, 1953) in man and the rabbit.

The conversion of cumene into hydratropic acid and alcohol involves the production of an asymmetric carbon atom next to the benzene ring. In the case of ethylbenzene (Smith *et al.* 1954*b*) it was shown that the biological oxidation produced both isomers of methylphenylcarbinol and in this case the hydroxylated carbon atom also became the asymmetric one. In the case of cumene the carbon atom hydroxylated is not the one which becomes asymmetric:

$$\text{Ph.CH}_2\text{.CH}_3 \rightarrow \text{Ph.CHOH.CH}_3;$$
$$\text{Ph.CH(CH}_3)_2 \rightarrow \text{Ph.CH(CH}_3)\text{.CH}_2\text{OH}$$



Both optical forms of hydratropic acid are formed when cumene is fed to rabbits, since a mixture of unequal quantities of the (+)- and (−)-isomers, with the (+)-isomer predominating, can be isolated from the urine. However, when hydratropoylglucuronide was isolated from cumene urine only the (+)-diastereoisomer was obtained in the crystalline state and this in small yields. Hydratropoylglucuronide is also formed when (±)-hydratropic aldehyde is fed to rabbits, but again only the (+)-diastereoisomer could be isolated in the crystalline state. It thus appears that (−)-hydratropoylglucuronide is difficult to obtain crystalline, although it is highly probable that it is formed, since both (±)- and (−)-hydratropic acids when fed to rabbits give high yields of reducing glucuronide (see Table 1). Kay & Raper (1922) have reported that when (±)-hydratropic acid is fed to dogs, about one-third of the dose is excreted combined with glycine, and here again the urinary product contained an excess of the (+)-isomer.

The glucuronide of hydratropyl alcohol isolated from cumene urine as the triacetyl methyl ester was identical with the glucuronide obtained by feeding (±)-hydratropyl alcohol. However, we have been unable to determine the optical form of this glucuronide which could be a derivative of (+)-, (−)- or (±)-hydratropyl alcohol. The glucuronide was only obtained in small yield and on hydrolysis it gave a liquid which appeared to be hydratropyl alcohol, but no satisfactory solid derivative could be made in quantities sufficient for an optical rotation. The rotation ($[\alpha]_D - 35°$ in $CHCl_3$) of the methyl (hydratropyl tri-*O*-acetylglucosid)uronate suggests that the aglycone is in the (±)-form, since its isomer methyl (2-phenyl*iso*propyl tri-*O*-acetylglucosid)uronate, which contains an optically inactive aglycone, has $[\alpha]_D - 41°$. However, since the optical forms of hydratropyl alcohol have low rotations (approx. ±15°; Cohen, Marshall & Woodman, 1915), the rotations of their glucuronides are not likely to differ by more than about 20° (cf. Kamil, Smith & Williams, 1953) and therefore the optical form of the hydratropyl glucuronide isolated in this work has to be left undecided.

It is to be noted that hydratropyl alcohol is a primary alcohol which is not completely oxidized *in vivo* to the corresponding acid (cf. chlorinated ethanols, Smith & Williams, 1954, and 2:2-dimethyl-2-phenylethanol, Robinson & Williams, 1955).

SUMMARY

1. *iso*Propylbenzene (cumene) when fed to rabbits is oxidized to 2-phenylpropan-2-ol, 2-phenylpropan-1-ol (hydratropyl alcohol) and α-phenylpropionic acid (hydratropic acid), which are excreted as conjugated glucuronic acids.

2. The conjugated glucuronic acids of cumene urine have been isolated and characterized as triacetyl methyl esters.

3. The feeding of (±)-hydratropic aldehyde to rabbits results in the excretion of hydratropoylglucosiduronic acid, but only the (+)-diastereoisomer could be isolated in crystalline form. This was identical with the reducing glucuronide isolated after feeding (+)-hydratropic acid or cumene.

4. Hydratropyl alcohol gives rise in rabbits to hydratropyl and hydratropoylglucuronides. The former, characterized as its triacetyl methyl ester, was identical with the non-reducing relatively stable glucuronide found in cumene urine. Only the (+)-diastereoisomer of hydratropoyl glucuronide was isolated.

5. 2-Phenylpropan-2-ol gives rise in rabbits to 2-phenyl*iso*propyl glucuronide, which was isolated and characterized as the triacetyl methyl ester and the potassium salt. This glucuronide is readily decomposed by dilute acid to yield α-methylstyrene and is identical with acid-labile glucuronide of cumene urine.

6. These results are discussed, and it appears that the α-carbon atom of cumene is more readily oxidized *in vivo* than the β-carbons, since the yield of 2-phenylpropan-2-ol is about 40% of the dose, whereas the yields of hydratropyl alcohol and hydratropic acid are 25% each, the total ω-oxidation being about 50%.

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Studies in Detoxication

61. THE METABOLISM OF ALKYLBENZENES. *TERT.*-BUTYLBENZENE

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In earlier papers in this series (Smith, Smithies & Williams, 1954*b*; Robinson, Smith & Williams, 1955) it was shown that ethylbenzene and *iso*propylbenzene 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 *iso*propyl-benzene, it is resistant to autoxidation and is relatively stable to oxidation. *tert.*-Butylbenzene is nevertheless extensively metabolized by rabbits, for Smith, Smithies & Williams (1954*a*) 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.* 1954*a*). 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).

$$\underset{\text{(I)}}{\mathrm{Ph.CMe_3}} \rightarrow \underset{\text{(II)}}{\mathrm{Ph.CMe_2.CH_2OH}} \rightarrow \underset{\text{(III)}}{\mathrm{Ph.CMe_2.CHO}} \rightarrow \underset{\text{(IV)}}{\mathrm{Ph.CMe_2.COOH}}.$$

Dimethylphenylacetic acid would be expected to yield a reducing ester glucuronide, but the urine from *tert.*-butylbenzene is non-reducing (Smith *et al.* 1954*a*). 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. Phenylacetonitrile (46·8 g.; Light and Co.) was dissolved in liquid NH_3 (300 ml.) containing 50 mg. anhydrous $FeCl_3$ 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 NH_3 had evaporated. The dark brown oily residue was washed with very dil. acid which contained sodium thiosulphate to remove I_2. The oil was then taken up in ether and the solution dried over anhydrous Na_2SO_4. After evaporation of the ether, the oily residue of dimethylphenylacetonitrile was distilled under reduced pressure (yield 40 g.). The nitrile was mixed with 50 ml. of 80% (v/v) H_2SO_4 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 $NaHCO_3$ 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) H_2SO_4 and the solution cooled in ice. The calculated amount of a 10% solution of $NaNO_2$ 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 2N-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