2. The phosphorylserine was found to be radioactive when derived from cerebral slices incubated with glucose in the presence of radioactive inorganic phosphate. The specific radioactivity of the phosphorylserine was increased when derived from slices which had been subjected to electrical pulses for a period of 10 sec.

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Studies in Detoxication

74. THE METABOLISM OF BENZHYDROL, BENZOPHENONE AND *p*-HYDROXYBENZOPHENONE*

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It is known that many mixed aliphatic aromatic ketones, such as acetophenone, are reduced in the animal body to the corresponding secondary alcohols (Smith, Smithies & Williams, 1954*a*, *b*), but there seems to be no information in the literature concerning the metabolic fate of a purely aromatic ketone, such as benzophenone. A study of the metabolism of this ketone, and its possible metabolites, benzhydrol and *p*-hydroxybenzophenone, was therefore undertaken. *p*-Hydroxybenzophenone is referred to by Schubenko (1893) as being excreted as such by animals, but this appears now to be incorrect for the rabbit. No previous study has been made of benzhydrol, a compound which it has been suggested is a metabolite of the antihistaminic drug, Benadryl (Glazko, McGinty, Dill, Wilson & Ward, 1949; Glazko & Dill, 1949).

Freedlander (1942) has shown that benzophenone and a number of its derivatives have tuberculostatic properties *in vitro*, the highest activity being shown by 2:4′-dichlorobenzophenone. Benzhydrol and *p*-hydroxybenzophenone were less active than benzophenone itself. Benzophenone also has some herbicidal properties (Erickson & Schlesinger, 1954).

* Part 73: El Masri, Smith & Williams (1958).

MATERIALS AND METHODS

Benzhydrol, m.p. 68°, benzophenone, m.p. 49–50°, and *p*-hydroxybenzophenone, m.p. 132°, were commercial samples which were purified. The compounds suspended in water were administered to rabbits by stomach tube. The urine of the animals was analysed for conjugated glucuronic acid and ethereal sulphates by the methods of Paul (1951) and Sperber (1948) respectively.

ISOLATION OF METABOLITES

The glucuronic acid derivatives described below had negative rotations, and by analogy with previously isolated metabolites (Bray, 1953; Teague, 1954) were supposed to be of the β configuration.

From benzhydrol. The urine of rabbits which had received 1 g. of benzhydrol was neutral in reaction and yellow-brown. It did not reduce Fehling's or Benedict's reagent, but when it was heated with dilute acid an oil separated rapidly which quickly turned red. The urine gave an intense naphtharesorcinol reaction for glucuronic acid which developed very rapidly. This latter test indicated the presence of a relatively labile glucuronide. Attempts to isolate this compound showed that it rapidly decomposed if temperatures were not kept low and if prolonged exposures to acid conditions were not avoided during its isolation.

Benzhydrol (1 g.) was fed to each of four rabbits and from the urine, collected for the succeeding 24 hr., the basic lead acetate fraction was prepared in the usual way

(Kamil, Smith & Williams, 1951). The lead was removed with H_2S, and the aqueous filtrate containing the glucuronide was freed from H_2S by aeration and freeze-dried. The resulting glucuronide gum was methylated in methanol with diazomethane and the product acetylated with pyridine and acetic anhydride. The acetylation mixture was diluted with water and the triacetyl methyl ester of the glucuronide separated as a gum which was dried *in vacuo* over H_2SO_4. After several recrystallizations from ethanol, *methyl (diphenylmethyl tri-O-acetyl-β-D-glucosid)uronate* (350 mg.), m.p. 149° and $[\alpha]_D^{21} - 108 \pm 2°$ in $CHCl_3$ (*c*, 0·75), was obtained as white needles (Found: C, 62·2; H, 5·8. $C_{26}H_{28}O_{10}$ requires C, 62·4; H, 5·7%).

In another experiment the amide of the glucuronide was prepared. The gummy triacetyl methyl ester, prepared as above, did not crystallize readily. The ester was therefore dissolved in dry methanol and the solution was saturated with dry NH_3 at 0°. After the solution had been kept at 0° overnight, it was concentrated to small bulk and the amide (0·3 g.) separated. On recrystallization from water *diphenylmethyl β-D-glucosiduronamide* formed very small white needles, m.p. 195° and $[\alpha]_D^{20} - 93·5 \pm 1°$ in 50% (v/v) aqueous ethanol (*c*, 0·6) (Found: C, 61·4; H, 5·8; N, 3·85. $C_{19}H_{21}O_6N,\frac{1}{2}H_2O$ requires C, 61·9; H, 6·0; N, 3·8%). This compound did not lose water at 110° (see Kamil *et al.* 1951). When the amide (0·15 g.) was boiled under reflux with 4 N-HCl for 2 hr., cooled and then extracted with ether, benzhydrol (30 mg.; m.p. and mixed m.p. 67°) was obtained.

From p-*hydroxybenzophenone.* The ketone (3·5 g.) was administered to two rabbits (dose 0·5 g./kg.). The 24 hr. urine was non-reducing and gave a weak naphtharesorcinol reaction due to the stability of the glucuronide. It also gave with Brady's reagent (2:4-dinitrophenylhydrazine in N-HCl) a precipitate which appeared to be a hydrazone of a glucuronide. This compound, however, did not melt sharply after several recrystallizations and further investigation of it was abandoned. The basic lead acetate fraction of the urine was therefore prepared. The lead was removed in the usual way with H_2S and a crystalline glucuronide (1·3 g.) separated in the filtrate from the PbS. A further yield of 0·75 g. of the crystals was obtained in the same way from the normal lead acetate precipitate of the urine. The glucuronide was purified by recrystallization from water, and p-*benzoylphenyl β-D-glucosiduronic acid trihydrate* was obtained as white needles, m.p. 156° and $[\alpha]_D^{20} - 67·5 \pm 1°$ in ethanol (*c*, 1) (Found: C, 53·4; H, 5·5; loss at 110°, 12·6. $C_{19}H_{18}O_8,3H_2O$ requires C, 53·5; H, 5·6; H_2O, 12·6%). This glucuronide was very soluble in ethanol, but sparingly soluble in water. On hydrolysis of 0·5 g. of the glucuronide with 4 N-H_2SO_4 (50 ml.) by boiling under reflux for 1·5 hr., cooling and extracting with ether, 0·25 g. of crude *p*-hydroxybenzophenone was obtained, which after recrystallization from aqueous ethanol had m.p. and mixed m.p. 134°.

Methylation of the glucuronide with ethereal diazomethane yielded *methyl* (p-*benzoylphenyl β-D-glucosid)uronate* as white needles after recrystallization from water and then from ethanol (m.p. 181°); $[\alpha]_D^{21} - 70·6 \pm 1°$ in ethanol (*c*, 0·6) (Found: C, 57·0; H, 5·7; loss at 110° *in vacuo*, 6·0. $C_{20}H_{20}O_8,2H_2O$ requires C, 56·6; H, 5·7; H_2O, 8·5%). The determination of the water content was rendered difficult by the hygroscopic nature of the dehydrated compound. On exposure to air overnight the compound completely regained its water. Acetylation of the glucuronide (0·1 g.) with acetic anhydride (0·5 ml.) and 1 drop of 60% $HClO_4$ yielded 0·1 g. of p-*benzoylphenyl tri-O-acetyl-β-D-glucosiduronic acid*, which crystallized from the acetylation mixture on cooling. On recrystallization from ethanol it formed white needles, m.p. 228° and $[\alpha]_D^{21} - 16·4 \pm 1°$ in $CHCl_3$ (*c*, 0·6) (Found: C, 59·9; H, 4·8. $C_{25}H_{24}O_{11}$ requires C, 60·0; H, 4·8%). Acetylation of the methyl ester with acetic anhydride and $HClO_4$, or methylation of the triacetyl acid with ethereal diazomethane, yielded the same *methyl* (p-*benzoylphenyl tri-O-acetyl-β-D-glucosid)uronate*, which formed white needles from ethanol, m.p. 173° and $[\alpha]_D^{21} - 25·7 \pm 1°$ in $CHCl_3$ (*c*, 0·5) (Found: C, 60·6; H, 5·1. $C_{26}H_{26}O_{11}$ requires C, 60·7; H, 5·1%).

From benzophenone. The 24 hr. urine of a rabbit which had been given 1 g. of benzophenone had properties similar to that after benzhydrol had been given. The urine was neutral and yellow-brown; it did not reduce Benedict's or Fehling's solution nor did it give a precipitate with Brady's reagent, but it gave a rapid and intense naphtharesorcinol reaction. When it was heated with dilute acid, an oil separated which rapidly turned red. The urine was made 4 N with respect to HCl and was heated for 2 hr. on the boiling-water bath. The cooled red urine was continuously extracted with ether for 5 hr. and the extract was dried and evaporated to dryness. The red residue was dissolved in ether and passed through an alumina column (2 cm. in diameter, 10 cm. long) and the column was eluted with light petroleum (b.p. 40–60°). The red pigment was left on the column. On evaporation of the clear eluate, benzhydrol (0·1 g.) was obtained, m.p. and mixed m.p. 67°. Paper chromatography of the urine in butanol–aq. NH_3 soln. (sp.gr. 0·88) (7:3, v/v) revealed one spot of R_F 0·5–0·6 reacting with naphtharesorcinol, suggesting that only one glucuronide was being extracted. This glucuronide was isolated and proved to be benzhydrol glucuronide. Paper chromatography in propanol–aq. NH_3 soln. (sp.gr. 0·88) (7:3, v/v) of the ether extract of hydrolysed benzophenone urine showed only benzhydrol, R_F 0·9, when viewed under u.v. light, and no 4-hydroxybenzophenone was detected. Alkaline extraction of this ether solution also yielded no 4-hydroxybenzophenone.

In two experiments, in one of which a total of 3 g. of benzophenone was fed to two rabbits, and in the other experiment 4 g., the glucuronide was isolated by systematic precipitation with lead acetate, in very poor yield [40 and 60 mg. as methyl (diphenylmethyl tri-*O*-acetyl-β-D-glucosid)uronate, m.p. and mixed m.p. 147°]. Better yields were obtained by preparing the amide. The glucuronide filtrate from the urine of four rabbits which had been fed with 2 g. each of benzophenone was prepared from the basic lead salt and neutralized with $NaHCO_3$. The solution was evaporated to dryness *in vacuo*. The dried product was dissolved in a little water and passed several times through a column of IR-100 H resin until the solution was strongly acidic. It was then evaporated *in vacuo* below 40° to a gum, which was methylated with ethereal diazomethane. After removal of the ether, the methyl ester was dissolved in dry methanol and the solution saturated at −20° with dry NH_3, and then kept at 0° overnight. Evaporation to small bulk, followed by addition of a little water, yielded 600 mg. of diphenylmethyl glucosiduronamide, m.p. and mixed m.p. 195° and $[\alpha]_D^{20} - 93 \pm 2°$ in 50% aqueous ethanol (*c*, 0·5).

RESULTS AND DISCUSSION

The quantitative aspects of the metabolism of benzophenone and the corresponding alcohol are shown in Table 1. The main route of metabolism of benzophenone is reduction of the keto group to yield benzhydrol, which is excreted in conjugation with glucuronic acid. Both benzophenone and benzhydrol yield the same labile glucuronide which is readily decomposed by dilute acid to yield a red solution (cf. the red colour obtained with benzhydrol and sulphuric acid). The hydroxyl group in benzhydrol is relatively acidic since it forms a sodium derivative. Neither of these compounds appears to be hydroxylated in the aromatic ring and their metabolism is mainly the following:

$$(C_6H_5)_2CO \rightarrow (C_6H_5)_2CH \cdot OH \rightarrow (C_6H_5)_2CH \cdot O \cdot C_6H_9O_6.$$

p-Hydroxybenzophenone, in contrast with benzophenone, is not reduced *in vivo*, but is directly conjugated with glucuronic acid:

$$C_6H_5 \cdot CO \cdot C_6H_4 \cdot OH \rightarrow C_6H_5 \cdot CO \cdot C_6H_4 \cdot O \cdot C_6H_9O_6.$$

The glucuronide is readily isolated and is relatively stable compared with the glucuronide of benzhydrol. It thus appears that in *p*-hydroxybenzophenone the keto group does not undergo reduction, possibly because the compound can be readily metabolized by the alternative reaction of direct conjugation. Although *p*-hydroxybenzophenone is a phenol, it does not undergo the sulphate conjugation which is a typical reaction *in vivo* of many phenols.

Table 1. *Conjugation of benzophenone and related compounds in the rabbit*

Dose: 2 m-moles/kg. Urine was collected for 48 hr. after dosage. Results are expressed as means for three animals with ranges in parentheses.

Compound	Percentage of dose excreted as: Glucuronide	Ethereal sulphate
Benzophenone	50 (46–61)	0
Benzhydrol	60 (48–77)	0
p-Hydroxybenzophenone	72 (47–92)	0

SUMMARY

1. Benzhydrol when given to rabbits is excreted as an acid labile glucuronide. The metabolite was isolated and characterized.

2. No reduction of the keto group takes place when *p*-hydroxybenzophenone is administered, and the compound is excreted as a stable glucuronic acid conjugate which was isolated.

3. Benzophenone is reduced in the rabbit to benzhydrol. The labile glucuronic acid conjugate of benzhydrol which is excreted was isolated, and production of phenolic derivatives by ring oxidation was not detected.

4. No ethereal sulphate conjugation was detected.

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