Studies in Detoxication

21. THE FATES OF QUINOL AND RESORCINOL IN THE RABBIT IN RELATION TO THE METABOLISM OF BENZENE

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The study of the metabolic fates of quinol and resorcinol was undertaken to elucidate certain aspects of the metabolism of benzene and phenol (see Porteous & Williams, 1949*a*, *b*). We have already reported on the fate of catechol (Garton & Williams, 1948), and a further study of the fate of phenol is in progress. Quinol is also of importance in industry as a photographic chemical and as a food preservative. Its position as an industrial hazard has been discussed recently by Sterner, Oglesby & Anderson (1947).

There is little exact information concerning the fate of these two phenols in the body. Baumann & Preusse (1879) found that a dog given 0·5 g. quinol excreted a quinol ethereal sulphate though no free quinol could be detected. von Mering (1876) also demonstrated the presence of a quinol ethereal sulphate in the urine of rabbits fed with arbutin (quinol monoglucoside). Külz (1890) found that quinol-fed rabbits excreted a glucuronide. Baumann (1878–9) reported that resorcinol raised the ethereal sulphate output in dogs, and Külz (1890) showed that it formed a glucuronide in rabbits and hares.

EXPERIMENTAL

Quantitative experiments.

The rabbits (giant chinchilla) used were kept on a diet of Lever's cubes (50 g./day) and water *ad lib.* Quinol (m.p. 169°) and resorcinol (m.p. 110°), dissolved in water, were fed by stomach tube. Doses up to 0·4 g./kg. of quinol and 0·5 g./kg. of resorcinol could be administered without apparent toxic effect. Doses of 0·45 g./kg. quinol and 0·6 g./kg. resorcinol caused temporary muscular twitching and an increased rate of respiration. In most of the present experiments doses of 0·1–0·2 g./kg. were used.

Glucuronic acid and *ethereal sulphate* excretions were determined as described in earlier papers in this series (Hanson, Mills & Williams, 1944; Williams, 1938). The results, given in Table 1, show that with quinol 30% of the dose is excreted as an ethereal sulphate and 43% as a monoglucuronide, and with resorcinol 13·5% is excreted as a sulphate and 52% as a monoglucuronide.

Table 1. *The excretion of glucuronic acid and ethereal sulphate by rabbits receiving quinol or resorcinol orally*

(The dose of dihydroxybenzene was 100 mg./kg. of body wt.)

			Average normal values		'Extra' excretion		Percentage of dose excreted		
Rabbit no.	Wt. (kg.)	Dose (mg.)	Ethereal sulphate (mg. SO_3/day)	Glucuronic acid (mg./day)	Ethereal sulphate (mg. SO_3/day)	Glucuronic acid (mg./day)	As mono-sulphate	As mono-glucuronide	Total conjugation
Quinol fed:									
122	3·15	630*	25·1	—	129·4	—	28·3	—	—
123	3·50	700*	14·7	—	200·3	—	39·3	—	—
124	3·05	710†	13·9	—	126·8	—	24·6	—	—
98	3·15	315	17·1	168	86·7	232	37·8	41·8	79·6
90	2·95	295	—	116	—	184	—	35·4	—
81	2·85	285	—	150	—	213	—	42·4	—
124	2·90	290	16·7	126	56·2	250	26·6	48·9	75·5
125	3·10	310	18·3	155	56·2	231	24·9	42·2	67·1
126	3·05	305	19·2	156	62·6	245	28·3	46·6	74·8
Resorcinol fed:									
124	2·85	285	13·8	106	27·8	272	13·4	54·1	67·5
125	3·25	325	15·8	105	31·2	295	13·2	51·5	64·7
126	3·10	310	15·2	107	39·7	277	14·0	50·6	64·6

* Dose 200 mg./kg. † Dose 230 mg./kg.

Isolation of metabolites

Metabolites of quinol

Quinol urine is normal in colour when freshly voided, but darkens considerably on standing. The slightly alkaline urine gives a dark green-brown colour with 2% $FeCl_3$. It reduces very slightly to Fehling's and Benedict's reagents and gives an intense Tollens test for glucuronic acid.

Isolation of free quinol. The 24 hr. urine (340 ml.), collected after feeding a total of 4 g. quinol to three rabbits, was filtered, treated with 0·1 g. $Na_2S_2O_4$ and extracted continuously for 6 hr. with peroxide-free ether. The extract was evaporated at 30° to a clear pale-yellow gum (90 mg.). The gum was dissolved in 5 ml. 2·5 N-NaOH and treated with 0·2 g. *p*-toluenesulphonyl chloride in 5 ml. acetone. After it had been shaken for 10 min. the mixture was poured into 50 ml. water. The crystals (10 mg., 0·065% of dose) which formed were collected and dried and identified as quinol di-*p*-toluenesulphonate, m.p. 155° alone and 157° mixed with an authentic specimen, m.p. 159°. A separate experiment showed that quinol added to normal urine could be recovered quantitatively by ether extraction after the addition of $Na_2S_2O_4$.

The glucuronide of quinol. Isolation of the quinol glucuronide gum. A 24 hr. urine (945 ml.) was collected after feeding a total of 12 g. quinol to twelve rabbits and the basic lead acetate precipitate prepared in the usual manner. The precipitate was well washed with water and Pb removed with H_2S. The Pb-free filtrate was aerated, treated with charcoal, filtered and evaporated to a gum (18 g.) *in vacuo* at 50–60°. The gum was dissolved in 10 ml. absolute ethanol and kept at 0° overnight. A precipitate (0·5 g.) of inorganic material was removed, and the solution was then evaporated to dryness. The gum, presumably *quinol glucuronide*, was purified by dissolving in ethanol, filtering and evaporating several times and yielded 16·5 g. of a clear brown gum. It was acid to litmus, gave a pale green colour with $FeCl_3$ which deepened on making slightly alkaline with $NaHCO_3$ and an intense Tollens naphthoresorcinol test. It was insoluble in ether, benzene and light petroleum.

Quinol monoglucuronide methyl ester. The gum (3 g.) was dissolved in 50 ml. ethanol and 200 ml. of a saturated solution of diazomethane in ether added. The mixture was kept overnight, filtered from a little sludge and evaporated to dryness. The treatment with diazomethane was repeated twice. Finally a clear yellow, neutral, ether-soluble gum (2·47 g.) was obtained which could not be induced to crystallize. The p-*hydroxyphenylglucuronide methyl ester* gave a blue colour with $FeCl_3$, indicating the presence of a free phenolic OH group. (Found: OCH_3, 10·2. $C_{13}H_{16}O_8$ requires OCH_3, 10·3%.)

Acetylation of quinol glucuronide methyl ester. The above methyl ester (2·47 g.) was dissolved in 10 ml. pyridine and 15 ml. acetic anhydride. After it had been kept overnight at room temperature the mixture was poured into 100 ml. of ice water. After the mixture had been stirred for some time at 0° crystallization occurred. The crystals (3·25 g.; m.p. 148°) were collected and recrystallized from ethanol. The p-*acetoxyphenyl-triacetyl-*D-*glucuronide methyl ester* formed long, colourless needles, m.p. 151°, $[\alpha]_D^{19°} - 21·8°$ (*c*, 1 in acetone). (Found: C, 53·7; H, 5·1; OCH_3, 6·9. $C_{21}H_{24}O_{12}$ requires C, 53·85; H, 5·2; OCH_3, 6·6%.) The compound was insoluble in water, sparingly soluble in ethanol, soluble in ether and readily soluble in acetone. It gave the naphthoresorcinol reaction on prolonged heating (5 min.) with the reagents.

Hydrolysis of acetylated quinol monoglucuronide methyl ester. *p*-Acetoxyphenyl-triacetylglucuronide methyl ester (1·5 g.) in 15 ml. ethanol and 100 ml. 4 N-HCl was heated under reflux for 4 hr. on the water bath. After cooling, the brown solution was extracted with 5 × 50 ml. peroxide-free ether. The extracts were evaporated to dryness *in vacuo* and the partially crystalline residue dissolved in 10 ml. 2·5 N-NaOH and treated with 0·5 g. *p*-toluenesulphonyl chloride. After shaking for 20 min. the mixture was poured into 150 ml. cold water. The crystalline precipitate (315 mg.) of quinol di-*p*-toluenesulphonate was recrystallized from absolute ethanol to give colourless prisms, m.p. and mixed m.p. 159°.

The ethereal sulphate fraction of quinol urine. If it is assumed that quinol forms a monosulphate, then, according to Table 1, the proportion of quinol conjugated with sulphuric acid is twice that of catechol (Garton & Williams, 1948) or resorcinol. It was, therefore, necessary to show whether quinol monosulphuric acid or quinol disulphuric acid is excreted by rabbits.

Attempts to isolate the ethereal sulphate and compare it with synthetic material (see below) were unsuccessful, but we proved it to be a monosulphate by the following procedure. The urine (1025 ml.), collected after feeding a total of 10 g. quinol to ten rabbits, was treated with 0·5 g. K_2CO_3 and reduced *in vacuo* at 40° to 250 ml. The resulting dark brown liquid was clarified with 0·25 g. $Na_2S_2O_4$ and saturated with $(NH_4)_2SO_4$; 250 ml. acetone were added and the mixture filtered at the pump, the precipitated $(NH_4)_2SO_4$ being washed with 150 ml. acetone. The combined filtrates were transferred to a separating funnel and the aqueous layer removed. The acetone solution was treated with 0·5 g. K_2CO_3 and concentrated *in vacuo* at 40° to 200 ml. This was now poured into 2 l. dry acetone. A dark brown viscous layer containing glucuronides separated and was discarded. The clear golden acetone layer was again reduced *in vacuo* at 40° to 150 ml. Glucuronides were still present and so the solution was poured into 1 l. dry acetone and the brown gum of glucuronide again removed. The acetone solution was again evaporated at 40° to a gummy crystalline mass. The product was practically free from glucuronides, and contained no inorganic sulphate. The crystals in it were identified as urea. This material contained the ethereal sulphates of quinol urine, but these could not be isolated. It was, therefore, dissolved in 100 ml. 50% aqueous ethanol, and its content of free and total quinol and ethereal sulphate estimated as follows.

Quinol was estimated by the iodometric method of Wieland (1910), as described by Neuberger (1947) for the determination of homogentisic acid in urine. Urea does not interfere with the determination. The above ethereal sulphate fraction contained 55 mg. of free quinol. For the estimation of total quinol, 5 ml. of the ethereal sulphate fraction were boiled under reflux with 10 ml. of 0·3 N-HCl for 30 min., cooled and the quinol estimated iodometrically. The combined quinol found was 1·045 g.

A gravimetric determination of ethereal sulphate showed that the fraction contained 0·8561 g. SO_3. Thus the ratio ethereal sulphate SO_3/combined quinol in the fraction is 0·8561/1·045 = 0·82. For quinol monosulphuric acid the calculated ratio is 0·73, whereas for quinol disulphuric acid

it is 1·45. These results indicate that the ethereal sulphate of quinol urine is quinol monosulphuric acid.

Search for quinol oxidation products. In an earlier paper (Garton & Williams, 1948) we showed that, in the rabbit, hydroxyquinol is an oxidation product of catechol. Since catechol, quinol and hydroxyquinol are metabolites of benzene (Porteous & Williams, 1949*b*), it is important to know whether quinol is also oxidized to hydroxyquinol. A careful search was made for this phenol in the urine collected 1, 2 and 3 days after feeding a total of 4 g. quinol to three rabbits. No trace of free or combined hydroxyquinol was found. The colour reactions used have been described in earlier papers (Garton & Williams, 1948; Porteous & Williams, 1949*b*).

Metabolites of resorcinol

Resorcinol urine is slightly darker than normal rabbit urine, but does not darken on standing as does quinol urine. The slightly alkaline urine is non-reducing, gives no colour with $FeCl_3$, but gives a strong naphthoresorcinol reaction. It contains free resorcinol, giving a purplish red colour in the 2:6-dichloroquinone chloroimide test of Porteous & Williams (1949*b*).

Isolation of free resorcinol. Resorcinol (1 g.) was fed to each of six rabbits. The slightly alkaline 24 hr. urine (625 ml.) was filtered through glass wool and then extracted continuously for 8 hr. with peroxide-free ether. Evaporation of the extract at 20° yielded a partially crystalline product (1·97 g.) from which 0·5 g. resorcinol (m.p. and mixed m.p. 110°, after crystallization from dry benzene) was obtained. The mother liquors, on benzoylation, yielded 0·255 g. of resorcinol dibenzoate (m.p. and mixed m.p. 117°). A further 8 hr. extraction removed all the free resorcinol from the urine and 0·27 g. of the dibenzoate was obtained. Thus a total of 11·4% of the resorcinol fed was recovered from the urine in the free state.

The glucuronide of resorcinol: isolation of the resorcinol glucuronide gum. The glucuronide gum (22 g.) was prepared by the usual basic lead acetate procedure from the 24 hr. urine (1·5 l.) of eight rabbits which had collectively received 12 g. of resorcinol. The gum was purified by dissolution in absolute ethanol and filtering. In this way 23·5 g. of a viscous brown gum were obtained consisting mainly of *resorcinol monoglucuronide.* This gum was acidic, non-reducing, gave an olive-green colour with $FeCl_3$ and an intense naphthoresorcinol reaction. It was readily soluble in water, ethanol and ethyl acetate. It could not be crystallized, or induced to give crystalline salts with organic bases.

Since resorcinol monoglucuronide is a phenol in which the position para to the phenolic OH group is unsubstituted, it should give a coloration with 2:6-dichloroquinone chloroimide. In fact the gum gives an intense purple colour with this reagent in slightly alkaline solution ($NaHCO_3$) and at all values of pH up to 10. This reaction in itself shows that the glucuronide of resorcinol is a monoglucuronide. Resorcinol itself gives a purplish red colour with 2:6-dichloroquinone chloroimide. The quinol monoglucuronide gum which has already been described gives no colour with 2:6-dichloroquinone chloroimide, a result to be expected since quinol glucuronide is a *p*-substituted phenol.

Resorcinol monoglucuronide methyl ester. The above gum (5 g.) was methylated in ethanol with ethereal diazomethane. m-*Hydroxyphenylglucuronide methyl ester* was obtained as an ether-soluble, neutral, clear brown gum (Found: OCH_3, 11·4. $C_{13}H_{16}O_8$ requires OCH_3, 10·3%), which gave a transient purple colour with $FeCl_3$.

Acetylation of resorcinol glucuronide methyl ester. The ester (3·6 g.) was acetylated at room temperature with 10 ml. pyridine and 15 ml. acetic anhydride. On pouring the mixture into water a yellow oil separated. The oil was taken up in 50 ml. $CHCl_3$ and the solution washed successively with 10% Na_2CO_3, 2N-HCl and water. After drying over anhydrous $CaCl_2$, the chloroform was evaporated, leaving 4 g. of a pale yellow gum which did not crystallize. The acetylation and extraction procedure was therefore repeated, but again a yellow gum was obtained. The gum was dissolved in 30 ml. ethanol and water was added dropwise with stirring. After vigorous scratching the precipitated material crystallized and eventually 3·16 g. of small colourless needles, m.p. 112°, were obtained. The m-*acetoxyphenyl-triacetyl-*D*-glucuronide methyl ester* was recrystallized from ethanol, forming small needles, m.p. 113–114°, $[\alpha]_D^{20°}$ −24·5° (*c*, 2 in acetone). (Found: C, 54·25; H, 5·2; OCH_3, 6·9; CH_3CO, 39·8. $C_{21}H_{24}O_{12}$ requires C, 53·85; H, 5·2; OCH_3, 6·6, CH_3CO, 36·8%.) The compound was soluble in ethanol, acetone and chloroform, but insoluble in water. It gave a strong naphthoresorcinol reaction when boiled for 2–3 min. with the reagents.

Hydrolysis of the acetylated resorcinol monoglucuronide methyl ester. The ester (3 g.) was refluxed on a water bath with 15 ml. ethanol and 100 ml. 4N-HCl for 2·5 hr. A red-brown solution was formed which, on cooling, deposited a reddish flocculent precipitate. This precipitate is presumably the result of the condensation of the free resorcinol with the free glucuronic acid formed during hydrolysis of the ester (cf. the naphthoresorcinol reaction). The solution was extracted with 4 × 75 ml. ether and the combined extracts dried over anhydrous Na_2SO_4. Removal of the ether at 30° left a pale brown gum (1·53 g.). On benzoylating the gum and pouring the product into water, an oil separated. This was dissolved in 30 ml. acetone which was then poured into 100 ml. water giving 350 mg. of small colourless plates of resorcinol dibenzoate, which, after recrystallization from aqueous ethanol, had m.p. and mixed m.p. 117° with authentic material.

The residual solution, after removal of resorcinol by ether, contained glucuronic acid as indicated by the Tollens test and by the reduction of Benedict's and Fehling's solutions.

Search for resorcinol oxidation products. Resorcinol urine was also carefully examined for hydroxyquinol, pyrogallol and phloroglucinol. No trace of any of these phenols was found in either the ethereal sulphate or glucuronide fraction of the urine collected 24 or 48 hr. after feeding a total of 6 g. resorcinol to six rabbits.

The colour tests used were those described by Porteous & Williams (1949*b*), together with a specific colour reaction for phloroglucinol. In this test traces of phloroglucinol give a red-orange colour with 0·25% aqueous quinol and 0·5N-KOH (cf. Porteous & Williams, 1949*a*).

DISCUSSION

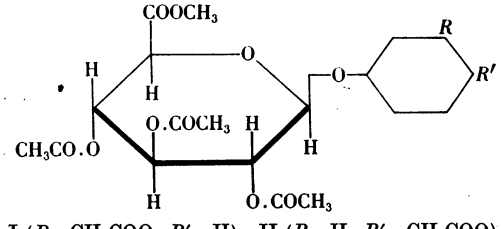
The quantitative aspects of the metabolic fates of the *o*-, *m*- and *p*-dihydroxybenzenes are summarized in Table 2, the values for catechol being quoted from Garton & Williams (1948). Quinol and catechol

Table 2. *The metabolism of the isomeric dihydroxybenzenes in the rabbit*

Compound	Conjugation (% of dose): As ethereal sulphate (*E*)	As glucuronide (*G*)	Total	*G*/*E*	Other metabolites detected
Catechol	18	70	88	3·9	Free catechol (2% isolated), traces of hydroxyquinol as an ethereal sulphate
Resorcinol	13·5	52	65·5	3·9	Free resorcinol (11·4% isolated) (combined + free = 77%)
Quinol	30	43	73	1·4	Free quinol in traces (0·065% isolated)

are excreted almost entirely conjugated and although resorcinol is also highly conjugated, appreciable amounts (11–12% by isolation) are excreted in the free state. The ratio glucuronide/ethereal sulphate is approximately 4 for both catechol and resorcinol, but only 1·4 for quinol, which is more highly conjugated with sulphate than its isomers. The proportion of the sulphate conjugation of quinol is twice that of its isomers and at first we suspected that quinol was being excreted as a disulphuric ester. The evidence presented, however, indicates that the ethereal sulphate is quinol monosulphuric acid. A consideration of the figures for the sulphate conjugation of resorcinol suggests that there is no reason to believe that resorcinol forms other than a monosulphuric ester.

The glucuronides of resorcinol and quinol have been shown to be monoconjugates by the isolation and characterization of the crystalline acetylated methyl esters (I) and (II) (*m*- and *p*-acetoxyphenyl-2:3:4-triacetyl-β-D-glucuronide methyl esters).



I (R=CH_3COO; R'=H) II (R=H; R'=CH_3COO)

Our proof of the structure of catechol monoglucuronide (Garton & Williams, 1948) depended on the fact that, on methylation with ethereal diazomethane, the glucuronide was converted to *o*-methoxyphenylglucuronide methyl ester. Hydrolysis of the crystalline triacetyl derivative of this ester yielded catechol monomethyl ether, thus proving that the original glucuronide contained one free phenolic hydroxyl group. In the present work, however, we found that when quinol and resorcinol glucuronides were methylated with diazomethane only the carboxyl groups were methylated. The non-crystalline methyl esters of quinol and resorcinol glucuronides obtained gave colours with ferric chloride, thus showing the presence of a free phenolic hydroxyl group. The proof that these glucuronides were monoconjugates, therefore, depends on the elementary analysis of the crystalline acetylated methyl esters (I) and (II) and on colour tests for free phenolic hydroxyl groups. The purified non-crystalline quinol monoglucuronide gave a pale green colour with ferric chloride, its methyl ester a blue colour, whereas non-crystalline resorcinol monoglucuronide gave an olive-green and its methyl ester a transient purple colour. Further evidence that resorcinol glucuronide contains a free phenolic group was obtained by the use of 2:6-dichloroquinone chloroimide (see p. 236).

Neither quinol nor resorcinol undergoes further oxidation *in vivo* as does catechol. We searched very carefully for trihydric phenols, but we found no indication of their presence. In an earlier paper (Porteous & Williams, 1949*b*) it was shown that phenol, catechol, quinol and hydroxyquinol were oxidation products of benzene in the rabbit. The present paper shows that hydroxyquinol is not a metabolite of quinol, though we have shown it to be formed from catechol (Garton & Williams, 1948). Thus the hydroxyquinol in benzene urine must be derived from catechol. A detailed study of the metabolism of phenol in the rabbit (Garton & Williams, unpublished) has shown that phenol gives rise to catechol, quinol and hydroxyquinol. Porteous & Williams (1949*b*) suggested that benzene was oxidized in the rabbit as follows:

benzene → phenol → catechol → hydroxyquinol.
↓
quinol

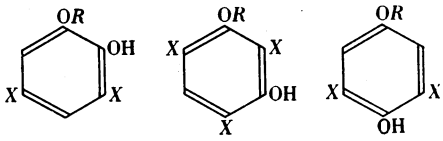
This scheme has now substantial experimental support.

The present work also raises the question, why is catechol oxidized to hydroxyquinol, whereas quinol and resorcinol are not? There are three possible answers to this question.

First, the enzymic systems which occur in animal tissues may oxidize only catechol. It is known, for example, that the tyrosinase system will oxidize catechol, but not quinol and resorcinol (Nelson & Dawson, 1944; Sumner & Somers, 1947). Nevertheless, Cadden & Dill (1942) have obtained from pig kidney a polyphenolase which oxidizes both

catechol and quinol. This enzyme resembles the plant enzyme laccase which oxidizes quinol and catechol to quinones, but not resorcinol (Yakushiji, 1940). Samisch (1937) claims that lemon leaves contain a metaphenolase which oxidizes resorcinol very slowly.

A second possible explanation may be derived from a consideration of the structures of the three phenols and their monoconjugates. It is well known that phenols are oxidized either *o*- or *p*- to the existing —OH group. In the formulae (III), (IV) and (V), the *o*- and *p*-positions are marked with *X*



(III) (IV) (V)

and it is to be noted that in the resorcinol (IV) and quinol (V) derivatives, the positions *X* are all ortho to an existing group. There may, therefore, be steric hindrance to oxidation in these positions. There is one position *X* in the catechol derivative (III) which is not subjected to steric hindrance by ortho substituents, and catechol is the only dihydric phenol which is metabolized to hydroxyquinol. More information is required, however, on the metabolism of hydroxyquinol before the plausibility of this explanation can be assessed. Preliminary work on hydroxyquinol is in hand.

A third explanation takes into account the time factor. If quinol and resorcinol were excreted more rapidly than catechol, then it is possible that catechol, because of its longer stay in the body, would have more chance of being oxidized than the others. We have no exact data on relative rates of excretion of these phenols, except that we observed that in the doses used in the present work all three were almost completely excreted within 24 hr. of dosing.

SUMMARY

1. The metabolism of quinol and resorcinol in the rabbit has been studied.

2. Quinol is excreted almost entirely as monoglucuronide (43 %) and monosulphate (30 %). Only traces of free quinol are excreted.

3. Resorcinol is excreted as monoglucuronide (52 %), monosulphate (13·5 %) and in the free state (11·4 %).

4. Quinol and resorcinol monoglucuronides were isolated and characterized as the crystalline methyl esters of the acetoxyphenyltriacetyl glucuronides.

5. Neither quinol nor resorcinol is oxidized *in vivo* to trihydric phenols or other substances.

6. The results have been discussed in relation to the metabolism of benzene, phenol and catechol.

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