Studies in Detoxication

65. THE METABOLISM OF QUINOLINE. NEW METABOLITES OF QUINOLINE, WITH OBSERVATIONS ON THE METABOLISM OF 3-, 5- AND 6-HYDROXYQUINOLINE AND 2:4-DIHYDROXYQUINOLINE*

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Scheunemann (1923) has shown that in rabbits quinoline gives rise to 6- and 8-hydroxyquinoline which were isolated in quantities corresponding to 1–1·5% of the dose. In dogs 30% of small intravenous doses (0·5 g./animal) are excreted as conjugated 3-hydroxyquinoline (Novack & Brodie, 1950). However, when quinoline is incubated with rabbit-liver extracts it yields 2-hydroxyquinoline (2-quinolone; carbostyril) (Knox, 1946). In some animals, e.g. the dog but not in rabbits, quinoline undergoes another reaction and yields *N*-methylquinolinium hydroxide (Tamura, 1924). Fühner (1906), from a study of the colour reactions of hydrolysed quinoline urine, concluded that 5:6-dihydroxyquinoline was formed, and Scheunemann (1923) isolated a small quantity of crystalline material which he claimed to be 4:6-dihydroxyquinoline, but the properties of this compound do not agree with those of authentic 4:6-dihydroxyquinoline. The metabolites of quinoline definitely identified as being formed in intact animals are thus 3-, 6- and 8-hydroxyquinolines and the *N*-methyl derivative. 2-Quinolone formed *in vitro* by the action of liver extracts on quinoline has not yet been detected *in vivo*.

A number of earlier workers (see Scheunemann, 1923) also showed that quinoline gave rise to conjugated glucuronic acids and ethereal sulphates, and recently Smith (1953) has shown that in rabbits conjugated glucuronic acid corresponding to 40% of the dose (0·25 g./kg.) and ethereal sulphates corresponding to 8% are excreted. Quinoline urine from rabbits also contains a compound which yields quinoline on heating with dilute acid (Tamura, 1924) but the nature of this compound has not been elucidated.

EXPERIMENTAL

Reference compounds. Quinolyl-6-glucuronide, m.p. 205° (decomp.), 2-quinolonyl-6-glucuronide, m.p. 250°, 4-quinolonyl-6-glucuronide, m.p. 208°, and 6-acetoxy-2-quinolone, m.p. 225–226° were specimens which had been previously prepared in this laboratory (Smith, 1953; Smith & Williams, 1954).

5:6-*Dihydroxyquinoline.* Mathëus (1888) treated 5-amino-6-hydroxyquinoline in dilute H_2SO_4 with a slight excess of $FeCl_3$ and eventually isolated a substance, as long red-yellow needles, which he described as quinoline-5:6-quinone hydrochloride. Only a nitrogen analysis was quoted for the compound. The substance does not melt below 350° (cf. Fühner, 1906). We have repeated Mathëus's experiments starting with 6-hydroxyquinoline, then making 5-nitroso- and 5-amino-6-hydroxyquinolines, and finally oxidizing the last with ferric chloride. The substance we isolated was 5:6-*dihydroxyquinoline hydrochloride*, which formed deep yellow needles on recrystallizing from 2N-HCl and did not melt on heating to 350°. (Found: C, 54·7; H, 4·0; N, 7·15; Cl, 17·8. $C_9H_7O_2N$, HCl requires C, 54·7; H, 4·1; N, 7·1; Cl, 17·95%; 5:6-dioxoquinoline hydrochloride, $C_9H_5O_2N$, HCl, requires C, 55·3; H, 3·1; N, 7·2; Cl, 18·1%.) The elementary analysis for hydrogen clearly shows the compound to be a dihydroxyquinoline and not a quinone. Further proof of this was obtained. Our hydrochloride in aqueous solution gave, with $FeCl_3$, a green colour which soon faded; it also immediately reduced $AgNO_3$ solutions. Furthermore, it yielded a ditoluene-*p*-sulphonate. The above hydrochloride (100 mg.) in saturated aqueous $NaHCO_3$ (10 ml.) was shaken with a slight excess of toluene-*p*-sulphonyl chloride in acetone (10 ml.). After the mixture had cooled a few drops of 40% NaOH was added to destroy excess of the chloride and a granular precipitate separated (50 mg.). On recrystallization from aqueous methanol, 5:6-*dihydroxyquinoline ditoluene*-p-*sulphonate* separated as colourless needles, m.p. 133°. (Found: C, 58·1; H, 4·0; N, 2·9. $C_{23}H_{19}O_6NS_2$ requires C, 58·8; H, 4·1; N, 3·0%.) Treatment of the hydrochloride with picric acid in aqueous solution yielded 5:6-dihydroxyquinoline picrate as yellow needles of the *monohydrate* which had m.p. 200–205° after recrystallization from water containing a little ethanol and still had the same m.p. after dehydration. (Found: C, 44·0; H, 3·2; N, 13·8; H_2O, 4·35. $C_{15}H_{10}O_9N_4$, H_2O requires C, 44·1; H, 3·0; N, 13·7; H_2O, 4·4%.) The synthesis of 5:6-dihydroxyquinoline (crystallized as a monohydrate which decomposes above 250°) from 5:6-dimethoxyquinoline-2:3-dicarboxylic acid, has recently been described by Ried & Schiller (1952). They also prepared the picrate (needles), m.p. 193–195°, which is formulated as being anhydrous but only an analysis for nitrogen was given and this would fit equally well for the anhydrous or the monohydrated picrate.

It appears therefore that the material described by Mathëus (1888) and later by Fühner (1906) as 5:6-dioxoquinoline was probably 5:6-dihydroxyquinoline which was first prepared authentically by Ried & Schiller (1952).

* Part 64 of this series: Parke, 1955, **59**, 422.

3-, 5- and 6-Hydroxyquinolines were prepared as previously described (Smith, 1953). 2:3-Dihydroxyquinoline, m.p. 260–265°, was prepared according to Ault, Hirst & Morton (1935); 2:4-dihydroxyquinoline was purchased (Light and Co.) and 4-methoxy-2-quinolone, m.p. 258°, was prepared according to Arndt, Ergener & Kutlu (1953).

Synthesis of 6-hydroxyquinolyl-5-sulphuric acid. 6-Hydroxyquinoline (10 g.) was added to a solution of potassium persulphate (20 g.) and KOH (20 g.) in water (400 ml.). The mixture was kept at room temperature overnight. It was then acidified with 2N-H_2SO_4 and the precipitate (mainly unchanged 6-hydroxyquinoline) removed by filtration. The filtrate was neutralized with K_2CO_3 and then evaporated to a small volume. Acetone was added to precipitate inorganic salts which were filtered and the filtrate was taken to dryness. The residue was extracted with boiling absolute ethanol to remove any 6-hydroxyquinoline and then crystallized from a small volume of water. *Potassium 6-hydroxyquinolyl-5-sulphate* (0·7 g.) was obtained as colourless needles which decomposed on heating to 110°. (Found: S, 10·5. $C_9H_6O_5NSK$, H_2O requires S, 10·8; $C_9H_6O_5NSK$ requires S, 11·5%.) The salt was dissolved in a little water and acidified with glacial acetic acid, when *6-hydroxyquinolyl-5-sulphuric acid* separated as pale yellow needles. (Found: C, 44·9; H, 3·3; S, 13·3. $C_9H_7O_5NS$ requires C, 44·8; H, 2·9; S, 13·3%.) It gave a red-purple colour with aqueous $FeCl_3$ and a green colour when treated with bromine water and ammonia (cf. Elderfield, 1952). It gave no colour with 2:6-dichloroquinonechloroimide (i.e. absence of a free phenolic group in position 5) or with the stabilized diazo compound, Brentamine Fast Blue B salt (see Table 3). On hydrolysis with dilute HCl, the solution turned yellow and gave the characteristic colour reactions of 5:6-dihydroxyquinoline which was isolated as the picrate, m.p. 205°. (Found: C, 43·8; H, 3·2; N, 13·1. Calc. for $C_{15}H_{10}O_9N_4$, H_2O, C, 44·1; H, 3·0; N, 13·7%.)

Animals. Large chinchilla rabbits (about 3 kg. weight) were used throughout this work. Quinoline (redistilled, b.p. 238°) and the hydroxyquinolines were administered by stomach tube with water.

Estimation of quinoline precursor. Quinoline in urine can be readily estimated by steam distillation of alkaline urine and measuring the quinoline in the distillate spectrophotometrically at 313 mμ. (ε 6100) after acidification to 0·1N with HCl. Beer's law was obeyed over the range 0–20 mg. quinoline/l. which was the concentration used in this work and the recoveries were almost quantitative. Steam distillation of normal rabbit urine showed that no steam volatile material was produced which absorbed at 313 mμ. The estimation of free and total quinoline was carried out as follows. Free quinoline: urine (1 ml.) was mixed with 1 ml. of 40% NaOH and the mixture steam distilled into 5 ml. of N-HCl until 50 ml. of distillate had been collected. The extinction of the solution at 313 mμ. was then determined. Total quinoline: the urine (1 ml.) was mixed with 4 ml. of 2·5N-HCl and heated under reflux for 1·5 hr. (the time required for maximum liberation of quinoline as shown in preliminary experiments). After cooling, 2 ml. of 40% NaOH was added and the steam distillation and measurement of the extinction of the distillate carried out as before. The spectrum of the distillate was determined over 230–330 mμ. as a check for quinoline.

Estimation of 5:6-dihydroxyquinoline. When solutions of 5:6-dihydroxyquinoline are shaken in air with ammonia (sp.gr. 0·88) they turn green or blue (Mathëus, 1888). The intensity of the colour which has a maximum absorption at 620 mμ. is proportional to the amount of 5:6-dihydroxyquinoline present, and can be used for the approximate determination of the compound. Solutions (2 ml.) containing 10–100 μg. of 5:6-dihydroxyquinoline in 0·1N-HCl were mixed with 1 ml. of ammonia (sp.gr. 0·88) in graduated flasks and the mixture was shaken for 1 min. The solutions were then diluted to 25 ml. with water and the extinction at 620 mμ. measured in a Unicam spectrophotometer (SP. 600). The calibration curve obtained was linear and recoveries were within ±5%. For urine the method was as follows: urine (10 ml.) was mixed with 10 ml. conc. HCl, heated under reflux for 4 hr., and then diluted to 25 ml. with water. Then 1 ml. of the hydrolysate was mixed with 1·5 ml. of ammonia (sp.gr. 0·88), shaken for 1 min., diluted to 25 ml. with water and the extinction of the solution measured.

Metabolism of quinoline

The results of the estimation of free and total quinoline excreted after administration of quinoline to three rabbits are given in Table 1. In two rabbits, which had each received 0·5 g. of quinoline, the amount of 5:6-dihydroxyquinoline excreted in 24 hr. after dosing was found to be 23 and 20·5 mg. or 3·7 and 3·3% of the dose respectively.

Table 1. *The excretion of quinoline precursor by rabbits receiving quinoline orally*

Values are for 24 hr. urine. Dose level 250 mg./kg.

	Quinoline excreted		Quinoline precursor	
Dose (mg.)	Free (mg.)	Total (mg.)	(mg. quinoline)	(% of dose)
820	7	98	91	11·0
820	5	60	55	6·7
690	3	60	57	8·9

Isolation of 6-hydroxyquinolyl-5-sulphuric acid. A total of 8 g. of quinoline was fed to sixteen rabbits and a 24 hr. urine collected. The glucuronide fraction was separated through the basic lead salt in the usual manner (cf. Kamil, Smith & Williams, 1951). Evaporation of the aqueous glucuronide fraction was continued to an aqueous syrup (20 ml.). This syrup contained ethereal sulphates as well as glucuronides. The sulphates were separated from the glucuronides by acetone fractionation (Smith & Williams, 1949). The syrup (20 ml.) was stirred with acetone (150 ml.) whereby the ethereal sulphates remained in the supernatant fluid and the glucuronides separated as an amorphous solid. The supernatant was evaporated *in vacuo* to a syrup and the acetone treatment repeated to remove a further small quantity of glucuronide. The supernatant was again evaporated to a syrup (5 ml.) which now gave a strong positive test for ethereal sulphate and a negative test (naphthoresorcinol) for glucuronides. The syrup was brought to pH 4–5 with glacial acetic acid and kept at 0°. Crystals of 6-hydroxyquinolyl-5-sulphuric acid (250 mg.) separated and were collected, washed with ethanol and ether, and were recrystallized from water as pale greenish needles. (Found: N, 5·5; S, 13·3. $C_9H_7O_5NS$ requires N, 5·8; S, 13·3%.) They were shown to be identical with a synthetic sample of this ethereal sulphate by

Table 2. *R_F values of some quinoline derivatives*

Whatman no. 1 or 4 paper was used and the chromatogram run until front had moved 12–15 in. from the origin. Solvent systems: *A*, *n*-Butanol–acetic acid–water, 4:1:5; *B*, saturated *n*-butanol–water; *C*, benzene–*n*-butanol–ammonia (sp.gr. 0·88), 2:5:2; *D*, ethyl methyl ketone saturated with water; *E*, ethyl methyl ketone–2N ammonia, 1:1; *F*, benzene–ethanol–water, 5:1:4; *G*, benzene–ethanol–water, 5:2:5.

	R_F in solvent system						
	A	*B*	*C*	*D*	*E*	*F*	*G*
Quinolyl-6-glucuronide	0·25	0·15	—	—	—	—	—
2-Quinolonyl-6-glucuronide	0·38	0·30	—	—	—	—	—
4-Quinolonyl-6-glucuronide	0·34	0·25	—	—	—	—	—
6-Hydroxyquinoline	0·86	0·87	0·88	—	—	—	—
2:6-Dihydroxyquinoline	0·78	0·80	0·59	—	—	—	—
4:6-Dihydroxyquinoline	0·78	0·80	0·41	—	—	—	—
6-Hydroxyquinolyl-5-sulphuric acid	0·53	0·33	—	0·68	0·20	—	—
3-Hydroxyquinoline	—	—	—	—	—	0·30	0·84
2:3-Dihydroxyquinoline	—	—	—	—	—	0·18	0·54
2:4-Dihydroxyquinoline	0·83	—	—	0·9	0·06	—	—

Table 3. *Colour reactions used to detect hydroxyquinolines and their derivatives*

The colours given below are those obtained with spots on Whatman no. 1 paper. Brentamine Fast Blue B salt was sprayed as a 0·1% aqueous solution and followed by saturated aqueous $NaHCO_3$.

		Fluorescence in ultraviolet light	
Compound	Brentamine Fast Blue B salt	Acid (HCl fumes)	Alkaline (NH_3 fumes)
5:6-Dihydroxyquinoline*	Red-purple	Blue	Lilac
6-Hydroxyquinolyl-5-sulphuric acid	None	None	Green
6-Hydroxyquinoline	Red-purple	Blue	Lilac
2:6-Dihydroxyquinoline	Red-purple	Blue	Green
4:6-Dihydroxyquinoline	Red-purple	Blue	Blue
3-Hydroxyquinoline	Red-purple	Blue	Blue
2:3-Dihydroxyquinoline*	Red-purple	Weak blue	Weak blue
2:4-Dihydroxyquinoline	Red-purple	Weak blue	Weak blue
2-Quinolonyl-4-glucuronide	None	Weak purple	Weak green

* These compounds reduce ammoniacal $AgNO_3$ and give a green colour with $FeCl_3$ in the test tube.

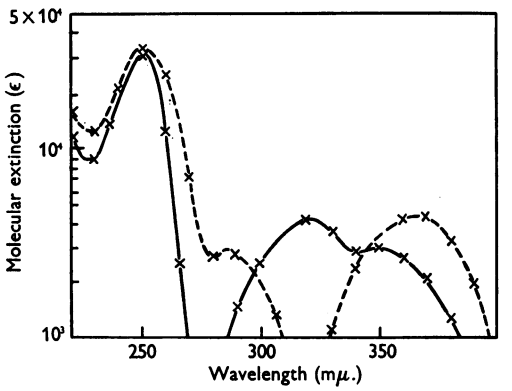


Fig. 1. The absorption spectrum of synthetic 6-hydroxyquinolyl-5-sulphuric acid; – – – in 0·1N-NaOH, —— in 0·1N-HCl. The crosses represent the spectra of the same compound isolated from the urine of rabbits dosed with quinoline. For the synthetic sample in 0·1N-NaOH, $\lambda_{max.}$ 252, 285 and 365 mμ. with $\epsilon_{max.}$ 33000, 3100 and 4500 respectively; in 0·1N-HCl, $\lambda_{max.}$ 250, 320 and 350 mμ. with $\epsilon_{max.}$ 31400, 4300 and 3100 respectively.

paper chromatography in four solvent systems and by absorption spectrum (see Table 2 and Fig. 1). On hydrolysis with dilute acid and treatment of the hydrolysate with aqueous picric acid, 5:6-dihydroxyquinoline picrate, m.p. and mixed m.p. 200–205°, was isolated.

Isolation of 3-hydroxy- and 2:6-dihydroxy-quinolines. These two hydroxylated quinolines were isolated from the glucuronide fraction of the urine of rabbits dosed with quinoline. This fraction on hydrolysis with 2N-acid yielded mainly a dark purple solid, insoluble in organic solvents but soluble in conc. H_2SO_4, which could partly be sublimed. This material has not been identified, but other methods of hydrolysis of the glucuronide fraction are being investigated.

The glucuronide gum from the urine of rabbits which had received 16 g. of quinoline was freed from ethereal sulphates by the acetone fractionation described above. The purified gum (10 g.) was dissolved in 0·5N-H_2SO_4 (500 ml.) and the solution boiled for 9 hr. It was then neutralized with $Ba(OH)_2$ and filtered, the solid residue being extracted with hot 80% (v/v) aqueous ethanol and the extracts added to the filtrate. On evaporation the filtrate yielded a syrup (3·6 g.) which was fractionated on a 'Celite' column (Hyflo Supercel; Johns-Manville and Co.) (5 cm. diam. and 300 g. Celite). The solvent system used was a 1:1 mixture of ethyl methyl ketone and ammonia solution (sp.gr. 0·88). The Celite

(300 g.) was rubbed into 300 ml. of the bottom layer of the solvent system so that a uniform dry powder was obtained. The powder was then packed dry into the column. The syrup was dissolved in a small amount of the top layer of the solvent system and carefully placed at the top of the column. Then the chromatogram was developed with top layer of solvent and illuminated with ultraviolet light. Five definite zones were obtained. These were, in order from the bottom of the column, *A*, a red band, non-fluorescent; *B*, a band with a green fluorescence; *C*, a narrow band with a purple fluorescence; *D*, a band with a pale yellow fluorescence (probably 2:6-dihydroxyquinoline); and *E*, a broad band with a blue fluorescence suggesting 3-hydroxyquinoline. This was followed by several very narrow fluorescent bands. Each zone was eluted from the column with the top layer of solvent, but most of the eluates on evaporation contained very little solid material. Only zones *D* and *E* contained enough material to identify. The residue from *D* was extracted with ether to remove impurities and then acetylated (pyridine and acetic anhydride) to yield 6-acetoxy-2-quinolone (5 mg.), m.p. and mixed m.p. 225–228°. (Found: C, 64·4; H, 4·8. Calc. for $C_{11}H_9O_3N$: C, 65·0; H, 4·5%.) The residue from *E* was dissolved in dilute HCl, treated with charcoal and filtered. Sodium bicarbonate solution was added to the filtrate to precipitate the hydroxyquinoline present. The solid obtained on treatment with hot aqueous picric acid solution yielded 3-hydroxyquinoline picrate (100 mg.) m.p. and mixed m.p. 249–250°. (Found: N, 15·2. Calc. for $C_{15}H_{10}O_8N_4$, N, 15·0%.)

The metabolism of 5- and 6-hydroxyquinolines

Knox (1946) has shown that rabbit liver extracts convert quinoline into 2-quinolone (carbostyril), and Smith & Williams (1954) have shown that in the intact rabbit 2-quinolone is metabolized to 6-hydroxy-2-quinolone. It seems likely therefore that in the intact rabbit the reactions

quinoline → 2-quinolone → 6-hydroxy-2-quinolone

take place, and that 2-quinolone is not detected in urine because it has become further hydroxylated and is excreted as 2-quinolonyl-6-glucuronide. However, since Scheunemann (1923) has shown that 6-hydroxyquinoline is a metabolite of quinoline in the rabbit, the reactions

quinoline → 6-hydroxyquinoline → 6-hydroxy-2-quinolone

are also possible. The metabolism of 6-hydroxyquinoline was therefore further studied. Smith (1953) has already shown that this compound is mainly excreted as the glucuronide (54% of the dose) with some ethereal sulphate (4%). We felt that besides conjugates of 6-hydroxyquinoline, those of 2:6-, 4:6- and 5:6-dihydroxyquinoline might be formed. The chromatography of these compounds was therefore studied. The separation of the glucuronides available is shown in Table 2. The ethereal sulphate conjugates are readily hydrolysed by dilute acid to hydroxyquinolines which are separable by paper chromatography and detectable by colour reactions or fluorescence in ultraviolet light (see Tables 2 and 3).

A rabbit was given 1 g. of 6-hydroxyquinoline by mouth, and the 24 hr. urine collected and acidified with glacial acetic acid. After keeping at 0° for 24 hr. the separated quinolyl-6-glucuronide (0·92 g. or 39% of the dose) was collected by filtration. By paper chromatography this glucuronide was shown to be free of the glucuronides of 2:6- and 4:6-dihydroxyquinolines. The filtrate from the glucuronide was now worked up by the lead acetate procedure, and a further 0·1 g. of quinolyl-6-glucuronide was separated from the glucuronide gum. The residual gum contained ethereal sulphate, and it was therefore separated into glucuronide and ethereal sulphate fractions with acetone. Chromatography of the glucuronide fraction showed that it contained largely quinolyl-6-glucuronide with only traces of 2-quinolonyl-6-glucuronide (detected by their fluorescence, see Smith, 1953). The ethereal sulphate fraction was hydrolysed by heating for a short time with dilute HCl. The hydrolysed solution gave colour tests for 5:6-dihydroxyquinoline and on paper chromatography of the solution, spots corresponding to 6-hydroxy- and 2:6-dihydroxyquinoline were found. 4:6-Dihydroxyquinoline was not found. From the size of the spots it appeared that 6-hydroxy- and 2:6-dihydroxy-quinoline were present in the ethereal sulphate fraction in roughly equal amounts. However, 2:6- and 5:6-dihydroxy-quinolines are very minor metabolites of 6-hydroxyquinoline the main bulk of which appears to be metabolized by direct conjugation.

The possibility that 5:6-dihydroxyquinoline could arise from 5-hydroxyquinoline had also to be considered. 5-Hydroxyquinoline is in part directly conjugated in rabbits (Smith, 1953). The urine of rabbits which had received 5-hydroxyquinoline was examined for 5:6-dihydroxyquinoline, but none was detected in any of the fractions. Other metabolites were not investigated.

The metabolism of 3-hydroxyquinoline

Since 3-hydroxyquinoline is a metabolite of quinoline both in the rabbit and in the dog, it was interesting to find out whether it is further hydroxylated. Smith (1953) has shown that it is excreted conjugated with glucuronic acid (40% of dose) and with sulphuric acid (5%) and he described the potassium salt of quinolyl-3-glucuronide.

A rabbit was dosed with 2 g. of 3-hydroxyquinoline and a 48 hr. urine (250 ml.) collected. The glucuronide gum, prepared through the basic lead salt, contained small amounts of ethereal sulphates which were separated by the acetone procedure. The acetone solution of the sulphates was evaporated to dryness to a gum which on acid hydrolysis yielded a solution which gave a weak green colour with $FeCl_3$ and reduced ammoniacal $AgNO_3$ slowly. These reactions could be due to 2:3- or 3:4-dihydroxyquinoline or both. The glucuronide gum from this separation solidified on standing and on recrystallization from methanol–ethanol (1:1) yielded the *ammonium salt* of quinolyl-3-glucuronide (1 g. of colourless needles), m.p. 180° (decomp.) and $[\alpha]_D^{20}$ – 103° in water (*c*, 1). (Found: C, 50·3; H, 5·6; N, 8·3. $C_{15}H_{18}O_7N_2$, H_2O requires C, 50·6; H, 5·7; N, 7·9%.) The

compound decomposed on heating to 110°, so that direct determination of water was not possible. The isolation of this ammonium salt rather than free quinolyl-3-glucuronide was unexpected, but ammonia solution is used during the separation of the basic lead salt of the glucuronide from the urine, and it is probable that this salt survived from that stage. That it was an ammonium salt was proved by the release of ammonia when it was heated with alkali and from the analytical figures. On hydrolysis with β-glucuronidase from locust crop liquor (Robinson, Smith & Williams, 1953) it yielded 3-hydroxyquinoline which was identified by paper chromatography. The combined mother liquors from the separation of the above glucuronide were hydrolysed by heating for 6 hr. in 2N-H_2SO_4. The hydrolysate was neutralized and the precipitate of 3-hydroxyquinoline (50 mg.; m.p. and mixed m.p. 195–197° decomp.) removed. The filtrate gave a green colour with $FeCl_3$ and this was shown to be due to very small amounts of 2:3-dihydroxyquinoline by measurement of R_F values in two solvents (see Table 2). The presence or absence of 3:4-dihydroxyquinoline was not proved.

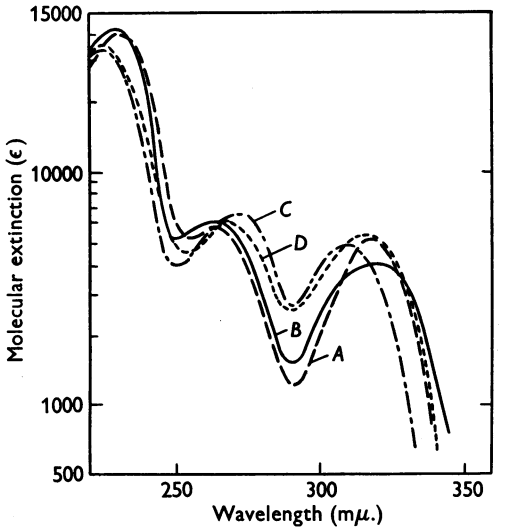


Fig. 2. The absorption spectra of 4-methoxy-2-quinolone (——, *B* in 0·1N-NaOH, and — · —, *C* in 0·1N-HCl) and of 2-quinolonyl-4-glucosiduronic acid (– – –, *A* in 0·1N-NaOH, and · · · · ·, *D* in 0·1N-HCl). 4-Methoxy-2-quinolone in 0·1N-NaOH has $\lambda_{max.}$ 230, 260 and 320 mμ. with $\epsilon_{max.}$ 43 000, 6100 and 4250 respectively and in 0·1N-HCl, $\lambda_{max.}$ 225, 275 and 310 mμ. with $\epsilon_{max.}$ 34 400, 6950 and 5000 respectively. 2-Quinolonyl-4-glucosiduronic acid in 0·1N-NaOH has $\lambda_{max.}$ 231, 260 and 315 mμ. with $\epsilon_{max.}$ 44 400, 5700 and 5350 respectively and in 0·1N-HCl, $\lambda_{max.}$ 225, 267 and 315 mμ. with $\epsilon_{max.}$ 36 800, 6430 and 5600 respectively.

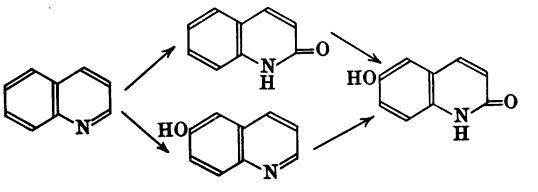
The metabolism of 2:4-*dihydroxyquinoline*

2:4-Dihydroxyquinoline was interesting from a theoretical point of view, since in the solid state it occurs as 4-hydroxy-2-quinolone, whereas in solution there is some formation of 2-hydroxy-4-quinolone. In the rabbit it was found to form a monoglucuronide which could be 2-quinolonyl-4-glucuronide or 4-quinolonyl-2-glucuronide. Determinations of the glucuronic acid and ethereal sulphate output of rabbits receiving oral doses of 0·25 g./kg. of 2:4-dihydroxyquinoline showed that an ethereal sulphate was not formed and that the glucuronide output in three animals was 17, 28 and 38 % of the dose.

The urine from the three animals was worked up by the lead acetate method and the glucuronide fraction prepared in the usual manner. The glucuronide separated in poor yield (0·32 g. from 2 g. of the sodium salt of 2:4-dihydroxyquinoline) as very long filaments. It formed gels with great ease from water. The hydrated *2-quinolonyl-4-β-D-glucosiduronic acid* crystallized in small colourless plates from water containing 10% ethanol, m.p. 186° (decomp.) and $[\alpha]_D^{25}$ – 103° in water (*c*, 0·08). (Found: C, 49·1; H, 5·1; N, 3·9. $C_{15}H_{15}O_8N$, 1·5 H_2O requires C, 49·45; H, 5·0; N, 3·85%.) The anhydrous compound was very hygroscopic. It gave a feeble yellow colour with $FeCl_3$ and reduced Fehling's solution on prolonged boiling but not Benedict's reagent. In the presence of alkali it fluoresced a feeble blue in ultraviolet light and in the presence of HCl a feeble purple. On hydrolysis with locust crop liquor β-glucuronidase, it yielded 2:4-dihydroxyquinoline which was detected in the hydrolysate by paper chromatography (see Table 2). Its spectra in acid and alkali were not very different and were very similar to those of 4-methoxy-2-quinolone in the same solvents, strongly suggesting that it is a 2-quinolone (see Fig. 2).

DISCUSSION

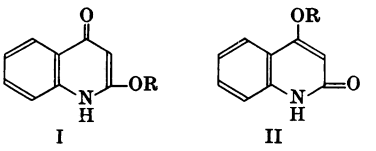
In addition to the 6- and 8-hydroxyquinolines of Scheunemann (1923), it has now been proved that rabbits excrete 3-hydroxy- and 2:6- and 5:6-dihydroxy-quinolines. It seems likely that 2:6-dihydroxyquinoline is produced via 2-quinolone, since the latter compound is the main product of the metabolism of quinoline in rabbit liver extracts (Knox, 1946) and in the intact rabbit it is largely converted into 2:6-dihydroxyquinoline (Smith & Williams, 1954). An alternative route to the 2:6-compound is via 6-hydroxyquinoline, since the latter is a metabolite of quinoline and is also converted into the 2:6-compound, albeit in small amounts. The very small conversion of administered 6-hydroxyquinoline into the 2:6-derivative may be due to the rapid competing reaction of conjugation which results in ionized conjugates which are readily excreted.



The structure of 6-hydroxyquinolyl-5-sulphuric acid. The formation of 5:6-dihydroxyquinoline is interesting because it is excreted as a monosulphuric acid, the sulphate being located in the 5-position. The structure of this ethereal sulphate was proved by synthesis from 6-hydroxyquinoline by the Elbs persulphate oxidation reaction. The action of alkaline persulphate on 6-hydroxyquinoline might be expected to yield either the 5- or the 7-sulphate, since sulphation in this reaction occurs *o*- and/or *p*- to the existing OH group (cf. Forrest & Petrow, 1950). The sulphate isolated, on hydrolysis, yielded 5:6-dihydroxyquinoline which is a known compound. Thus the action of persulphate on 6-hydroxyquinoline yielded 6-hydroxyquinolyl-5-sulphuric acid and this was identical with the ethereal sulphate from quinoline urine. 5:6-Dihydroxyquinoline is also a minor metabolite of 6- but not of 5-hydroxyquinoline, and since 6-hydroxyquinoline is a known metabolite of quinoline in the rabbit it could be an intermediate in the formation of the 5:6-derivative. The formation of a 5:6-dihydroxyquinoline or more correctly a 5:6-quinoline quinone also occurs during the metabolism of the antimalarial drug Pamaquin (8-(4′-diethylamino-1′-methylbutyl)amino-6-methoxyquinoline) in the hen. The metabolite, 8-(4′-diethylamino-1′-methylbutyl)amino-5:6-quinoline-quinone, appears to be the active form of Pamaquin which is itself relatively inactive *in vitro* but markedly plasmodicidal *in vivo* (Josephson, Greenberg, Taylor & Bami, 1951).

From a quantitative aspect very little of the quinoline fed has been accounted for in detail. Just under 50% of the quinoline fed is excreted as conjugates (ethereal sulphate and glucuronide) (Smith, 1953) presumably of hydroxyquinolines, but less than 5% of the dose has been identified, nearly 4% being 5:6-dihydroxyquinoline. The quantitative aspects of the excretion of the other hydroxyquinolines are now being investigated with [^{14}C]quinoline. About 10% of the quinoline fed is excreted as a compound which gives rise to quinoline on heating with acid. On analogy with naphthalene (cf. Bourne & Young, 1934; Boyland & Solomon, 1953) it is possible that this compound is a dihydromonohydroxyquinoline, several of which can be postulated. The formation of 5:6-dihydroxyquinoline *in vivo* suggests the possibility that the 5:6-double bond of quinoline may be involved in the formation of such compounds, since it is equivalent to the 1:2-double bond of naphthalene.

The structure of the glucosiduronic acid from 2:4-dihydroxyquinoline. The monoglucuronide formed in the rabbit from 2:4-dihydroxyquinoline could be the 2- or the 4-glucosiduronic acid (I or II, R = $-C_6H_9O_6$):



I II

4-Quinolones have different spectra from 2-quinolones. The former show a characteristic bifurcation in the absorption band occurring in the 320–360 mμ. region (Ewing & Steck, 1946; Steck, Ewing & Nachod, 1949) which is not found in the 2-quinolones. This bifurcation is, in fact, present in the spectrum of 4-quinolonyl-6-glucosiduronic acid (Smith & Williams, 1954). If the spectrum of the glucosiduronic acid shows this characteristic it would be expected to be I. The spectrum of the glucuronide, however, was similar to that of 2-quinolone and almost identical with that of 4-methoxy-2-quinolone (II, R = Me). It was therefore concluded that the glucuronide had structure II (R = $-C_6H_9O_6$) (see Fig. 2). 2:4-Dihydroxyquinoline in the solid state appears to be 4-hydroxy-2-quinolone, and partly isomerizes in solution to 2-hydroxy-4-quinolone, but there is no evidence for the existence of the dihydroxy form (see Walker, 1953). It is interesting to note that 2:4-dihydroxyquinoline did not form an ethereal sulphate in the rabbit. This suggests that neither hydroxyl group is sufficiently phenolic in character to undergo this conjugation, and furthermore that 2:4-dihydroxyquinoline is probably not hydroxylated elsewhere in the molecule, since such a hydroxylation would produce a phenolic OH group (i.e. positions 3-, 5-, 6-, 7- or 8-), which would be expected to form an ethereal sulphate.

SUMMARY

1. 3-Hydroxy-, 2:6- and 5:6-dihydroxy-quinolines have been isolated in small amounts from the urine of rabbits dosed with quinoline.

2. The 5:6-dihydroxyquinoline occurs in the urine as 6-hydroxyquinolyl-5-sulphuric acid, which was isolated. This ethereal sulphate was synthesized by an Elbs persulphate oxidation of 6-hydroxyquinoline. About 3–4% of quinoline (250 mg./kg.) was excreted as 5:6-dihydroxyquinoline.

3. Some 10% of the quinoline is excreted as a labile compound which yields quinoline on heating with acid.

4. 6-Hydroxyquinoline is converted to a very small extent into 2:6- and 5:6-dihydroxyquinoline by the rabbit. 5-Hydroxyquinoline is not oxidized to the 5:6-dihydroxy derivative.

5. 3-Hydroxyquinoline yields very small amounts of 2:3-dihydroxyquinoline. 3-, 5- and 6-Hydroxyquinolines are mainly metabolized by direct conjugation.

6. 2:4-Dihydroxyquinoline does not yield an ethereal sulphate in the rabbit but is conjugated (20–40 %) with glucuronic acid. The glucuronide was proved to be 2-quinolonyl-4-glucosiduronic acid.

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Studies in Immunochemistry

15. THE SPECIFIC POLYSACCHARIDE OF THE DOMINANT 'O' SOMATIC ANTIGEN OF *SHIGELLA DYSENTERIAE**

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The preparation and properties of a material possessing the immunological specificity and activities of the 'O' somatic antigen of *Shigella dysenteriae* have been described by Morgan (1937), Morgan & Partridge (1940, 1941*a*) and Davies, Morgan & Mosimann (1954), who showed it to be a protein-polysaccharide-phospholipid complex; this was the 'endotoxin' of earlier workers. The phospholipid component can be removed from the antigenic complex by the dissociating action of the highly polar solvent formamide and its elimination does not appear to affect any of the known immunological or toxic properties of the original antigen. Treatment of the antigenic material, after removal of the phospholipid component, with dilute acetic acid at 100° brings about the separation of the polysaccharide and protein components. The latter material is a lipoprotein (Davies & Morgan, 1953), and is identical with the 'conjugated protein' of Morgan & Partridge (1940, 1941*b*); the lipid component is insoluble in ether but soluble in chloroform whereas the phospholipid removed from the native antigenic complex by formamide is soluble in ether. These materials will be described in a later communication.

The polysaccharide component remains in solution after hydrolysis of the antigen with acetic acid and can be readily separated from the water-insoluble conjugated protein. The polysaccharide can be extracted from the whole organism with weak acetic acid at 100° (Morgan, 1931, 1936) and has been shown to contain glucosamine, galactose and rhamnose (Morgan, 1936, 1938; Partridge, 1948).

The antigenic complex yields an undegraded polysaccharide by repeated precipitation from solution in formamide with ethanol (Morgan & Partridge, 1940) or by treatment with cold dilute alkali or with 90 % (w/v) phenol (Morgan & Partridge, 1941*a*). The undegraded polysaccharide differs from the degraded form, which results from the use of mild acid, in certain of its properties; it is viscous and will recombine with the conjugated protein component to give an antigenic complex (Partridge & Morgan, 1940).

Lipopolysaccharides have been obtained from a number of species of bacteria; Goebel & Adams (1943) isolated an 'F' heterophile polsaccharide from Pneumococcus which only differed from the

* Part 14 of this series, Gibbons & Morgan, 1954, **57**, 283.