Studies in Detoxication

53. THE GLUCURONIC ACID CONJUGATION OF HYDROXYQUINOLINES AND HYDROXYPYRIDINES IN THE RABBIT

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(*Received* 18 *February* 1953)

Investigations in progress in this laboratory on the metabolism of quinoline necessitated the preparation, as reference compounds, of the glucuronides of all the isomeric monohydroxyquinolines. The possibility was also kept in mind that some of the glucuronides might also find use as substrates for the study of β-glucuronidase (cf. quinolyl-8-glucuronide; Friedenwald & Becker, 1948; Burton & Pearse, 1952; Robinson, Smith & Williams, 1953*a*).

Chemically, the seven monohydroxyquinolines fall into two groups. The 3-, 5-, 6-, 7- and 8-hydroxyquinolines behave like phenols whereas the 2- and 4-derivatives react mainly as quinolones (see Elderfield, 1952). In this paper, it will be shown that these differences in chemical behaviour are also evident during their metabolism in the rabbit, since the phenolic quinolinols are conjugated directly with glucuronic acid to form quinolylglucuronides, whereas the 2- and 4-quinolones are apparently partly hydroxylated, in an unknown position *x*, to hydroxyquinolones which are then excreted as quinolone glucuronides and ethereal sulphates.

The preparation of quinolyl-8-glucuronide has already been described (Brahm, 1899; Robinson *et al.* 1953*a*). That 3-quinolinol is excreted conjugated in the dog has been shown by Novack & Brodie (1950), but the conjugate was not isolated. Fenyvessy (1900) fed 2-quinolone (carbostyril) to rabbits and isolated a glucuronide which he described as carbostyril glucuronide, but it will be shown that this glucuronide is probably a derivative of a hydroxycarbostyril. The metabolism of the other hydroxyquinolines has not so far been studied. The 3- and 6-quinolinols, however, have received attention as possible therapeutic agents (Davin & Schlauch, 1932; von Jacksch, 1884) and 8-quinolinol (oxine) has been used as an antiseptic and fungicide (Albert, 1951; Frear, 1951). 8-Quinolinol is reported by Root & Chen (1952) to produce pancreatic diabetes occasionally in rabbits, but no ill effects were noted in the present experiments.

Since the chemical differences between the quinolinols and quinolones are analogous to those between 3-hydroxypyridine and the pyridones, it was of interest to study the conjugation of the hydroxypyridines in the rabbit. Anderton, Smith & Williams (1948) observed that only 3-hydroxypyridine formed appreciable amounts of ethereal sulphate in the rabbit and it will be shown that only this isomer is conjugated to any appreciable extent with glucuronic acid.

EXPERIMENTAL

Melting points are uncorrected and optical rotations are given in Table 5. Ultraviolet-absorption spectra were measured in a Unicam S.P. 500 spectrophotometer.

Table 1. *The excretion of glucuronic acid and ethereal sulphate by rabbits after oral doses of pyridine, quinoline and their monohydroxy derivatives*

(Dose 250 mg./kg. Each value gives average result from three rabbits, except those for 5-quinolinol on one animal only.)

	Percentage of dose excreted combined with	
	Glucuronic acid	Ethereal sulphate
Conjugated directly:		
3-Quinolinol	40	5
5-Quinolinol	19	12
6-Quinolinol	54	4
7-Quinolinol	55	6
8-Quinolinol	20	6
Hydroxylated and then conjugated:		
Quinoline	40	8
2-Quinolone	27	23
4-Quinolone	26	6
Pyridine	0	0
2-Pyridone	4	4
3-Pyridol	30	11
4-Pyridone	0·8	0·5

Preparation of compounds. 8-Quinolinol (m.p. 75°) and 2-quinolone (m.p. 200°) were purchased. The 3- and 5-quinolinols, prepared from the corresponding amino compounds by diazotization (Mills & Watson, 1910), had m.p. 197 and 225–230° respectively after crystallization from methanol. 4-Quinolinol, m.p. 200°, was prepared by decarboxylation of kynurenic acid (Albert & Magrath, 1947). The 6- and 7-quinolinols, prepared by Cohen's (1930) modification of the Skraup synthesis, had m.p. 193 and 238° (decomp.) respectively after crystallization from dioxan. 2-Pyridone, m.p. 110°, was purchased. 3-Hydroxypyridine,

m.p. 128°, was prepared by alkaline fusion of the 3-sulphonic acid (Weidel & Murmann, 1895). 4-Pyridone, m.p. 149°, was prepared from chelidamic acid (King & Ware, 1939).

Methods. Compounds were administered orally by stomach tube to rabbits kept as previously described (e.g. Kamil, Smith & Williams, 1951). The glucuronides were isolated from the urine by the methods of Kamil *et al.* (1951) unless they crystallized directly from the urine. Glucuronic acid was estimated by Mills & Paul's modification (cf. Paul, 1951) of the naphthoresorcinol reaction and ethereal sulphates by the method of Sperber (1948). The results are given in Table 1.

Isolation of the glucuronides

2-Quinolonyl-x-glucuronide. 2-Quinolone (2 g.) was fed to two rabbits. The urine was collected for 24 hr. (400 ml.), acidified with 5 ml. of glacial acetic acid and kept at 0° for 2 weeks. The crystalline deposit (1·3 g., 13% of dose) was collected and recrystallized from hot water. The same product was also obtained through the basic lead salt as described by Fenyvessy (1900). *β-2-Quinolonyl-x-D-glucuronide* formed short colourless needles, m.p. 250–252° (decomp.). (Fenyvessy's carbostyril glucuronide 'darkened at 220° and charred at 250–252°'.) Its solubility in water at room temperature was less than 0·1% (w/v) and it was insoluble in ethanol. (Found: C, 50·6; H, 4·8; N, 4·1; H_2O, 5·2. $C_{15}H_{15}O_8N$, H_2O requires C, 50·7; H, 4·8; N, 4·0; H_2O, 5·1%.) The glucuronide (0·2 g.) was treated with 2 ml. conc. HCl and gently warmed. On cooling, colourless needles of the *hydrochloride* separated, m.p. 190–200° (decomp.). (Found: C, 47·7; H, 4·8. $C_{15}H_{15}O_8N.HCl$ requires C, 48·2; H, 4·3%.) This compound was very sparingly soluble in cold water presumably due to hydrolysis. The potassium salt of the glucuronide crystallized as colourless plates from aqueous ethanol and decomposed at 250–260°. (Found: C, 39·5; H, 4·7. $C_{15}H_{14}O_8NK$, $4·5H_2O$ requires C, 39·5; H, 5·1%.) A K salt was also described by Fenyvessy (1900).

Quinolyl-3-glucuronide. Two rabbits were each fed 2 g. of 3-quinolinol, and 2 g. of crude glucuronide gum were obtained from the 24-hr. urine through the basic lead salt. This gum did not crystallize but on neutralization with K_2CO_3 in 10 ml. of water and cautious addition of ethanol a precipitate started to form, which on keeping at 0° gave a small crop of crystals mixed with some gum. Recrystallization from aqueous ethanol and then from aqueous dioxan yielded the *potassium salt* of *β-quinolyl-3-D-glucuronide* as clusters of needles very soluble in water and insoluble in ethanol. (Found: C, 47·3; H, 4·5; N, 3·6; K, 10·6; H_2O, 4·3. $C_{15}H_{14}O_7NK$, H_2O requires C, 47·7; H, 4·3; N, 3·7; K, 10·4; H_2O, 4·8%.)

4-Quinolonyl-x-glucuronide. The glucuronide gum (5 g.) was obtained through the basic lead salt from the 24-hr. urine of three rabbits which had collectively received 6 g. of 4-quinolone. This gum did not crystallize directly, but after neutralization with Na_2CO_3 in 10 ml. water and cautious addition of ethanol, a crystalline Na salt separated and was recrystallized from 50% (v/v) aqueous ethanol. (Found: C, 42·7; H, 4·9; N, 3·6; Na, 5·5; H_2O, 14·2. *Sodium salt* of *β-4-quinolonyl-x-D-glucuronide*, $C_{15}H_{14}O_8NNa$, $3·5H_2O$ requires C, 42·7; H, 5·0; N, 3·3; Na, 5·4; H_2O, 14·9%.) On drying at 110° it lost water but the anhydrous salt was very hygroscopic and rapidly rehydrated on standing in the air. Yield (three experiments) 0·5–1% of the dose fed. The Na salt (50 mg.) was dissolved in 0·1 ml. of warm water and a drop of glacial acetic acid added. On cooling, free *β-4-quinolonyl-x-D-glucuronide* separated as a mass of long needles, m.p. 208° (decomp.). (Found: C, 46·1; H, 5·5; N, 3·5. $C_{15}H_{15}O_8N$, $3H_2O$ requires C, 46·0; H, 5·4; N, 3·6%.) The ethanolic mother liquor from the above Na salt, when treated with an excess of absolute ethanol, yielded an amorphous precipitate which appeared to be the Na salt of another glucuronide which did not yield 4-quinolone on hydrolysis. The structures of these two glucuronides are being investigated.

Quinolyl-5-glucuronide. 5-Quinolinol (1·5 g.) was fed to a rabbit and the glucuronide isolated through its basic lead salt from the 24-hr. urine as a clear yellow syrup. This was dissolved in hot 50% aqueous ethanol and, on cooling, the partially crystalline, greenish yellow precipitate which formed was filtered off and converted into the K salt which crystallized from 50% ethanol as colourless plates (yield 200 mg. or 6% of the dose). This appeared to be the *potassium salt* of *β-quinolyl-5-D-glucuronide.* (Found: C, 47·2; H, 4·7; N, 3·5; K, 10·8. $C_{15}H_{14}O_7NK$, H_2O requires C, 47·7; H, 4·3; N, 3·7; K, 10·4%.)

Quinolyl-6-glucuronide. The 24-hr. urine (220 ml.) from two rabbits which each received 2 g. of 6-quinolinol was acidified with a few ml. of glacial acetic acid and left at 0° for 24 hr. The crop of colourless crystals was removed and recrystallized from 50% aqueous ethanol containing a trace of acetic acid (yield, 1·7 g. or 17% of the dose). *β-Quinolyl-6-D-glucuronide dihydrate* formed long colourless needles, sparingly soluble in ethanol and water, m.p. 205° (decomp.). (Found: C, 50·6; H, 5·5; N, 3·7. $C_{15}H_{15}O_7N$, $2H_2O$ requires C, 50·4; H, 5·4; N, 3·9%.)

Quinolyl-7-glucuronide. Urine from two rabbits which had each received 2 g. of 7-quinolinol yielded, through the basic lead salt, the glucuronide as a light-brown gel containing some crystals. This was dissolved in 20 ml. of warm 50% aqueous ethanol and on cooling slowly to 0° the solution set to a mass of crystals (yield, 5 g. or 50% of the dose). Recrystallized from water, *β-quinolyl-7-D-glucuronide* formed colourless needles, m.p. 225° (decomp.). (Found: C, 48·9; H, 5·2; N, 4·2; H_2O, 12·2. $C_{15}H_{15}O_7N$, $2·5H_2O$ requires C, 49·2; H, 5·5; N, 3·8; H_2O, 12·2%.) The glucuronide (1 g.) when dissolved in 4 ml. of warm conc. HCl and cooled, yielded the dihydrate of *quinolyl-7-glucuronide hydrochloride* as small colourless needles (0·5 g.). (Found: C, 44·8; H, 4·8; N, 3·6. $C_{15}H_{15}O_7N.HCl$, $2·5H_2O$ requires C, 44·7; H, 5·3; N, 3·5%.)

Quinolyl-8-glucuronide. Quinolyl-8-glucuronide was prepared according to Robinson *et al.* (1953*a*). The *hydrochloride* was prepared as for the 7-isomer. (Found: C, 45·5; H, 5·0; Cl, 9·5. $C_{15}H_{15}O_7N.HCl$, $2H_2O$ requires C, 45·7; H, 5·1; Cl, 9·0%.)

3-Pyridinol. The 24-hr. urine of a rabbit which had received 1·2 g. of 3-pyridinol yielded through the basic lead salt 1·4 g. of crude glucuronide gum. This was treated with 0·3 g. of K_2CO_3 in 3 ml. water, and ethanol was added until turbid. On keeping at 0°, the solution deposited crystals. The *potassium salt* of *β-pyridyl-3-D-glucuronide monohydrate* recrystallized from aqueous ethanol as colourless plates. (Found: C, 40·4; H, 4·4; N, 3·6; K, 12·3. $C_{11}H_{12}O_7NK$, H_2O requires C, 40·4; H, 4·3; N, 4·3; K, 12·0%.) It showed $\epsilon_{max.}$ 2900 at 270 mμ. in 0·1 N-NaOH and $\epsilon_{max.}$ 4880 at 274 mμ. in 0·1 N-HCl.

Table 2. *Colour reactions and R_F values of hydroxyquinolines*

(Solvent mixture: *A*, *n*-butanol (5 vol.), benzene (2 vol.), ammonia, sp.gr. 0·880 (2 vol.); *B*, *n*-butanol (4 vol.), acetic acid (1 vol.), water (5 vol.). Whatman no. 1 paper. Run until solvent front had moved 12 in. from starting line.)

	R_F value in solvent mixture		Colour with		Fluorescence in ultraviolet light in 0·1 N-HCl or 0·1 N-NaOH
	A	*B*	Brentamine Fast Blue + $NaHCO_3$	$FeCl_3$	
2-Quinolone	0·9	0·9	None	Orange	Purple
3-Quinolinol	0·6	0·9	Red	Red	Blue
4-Quinolone	0·9	0·8	None	Red	Pale purple
5-Quinolinol	0·8	0·8	Red-brown	Green	Pale yellow-green*
6-Quinolinol	0·8	0·8	Red	Brown	Pale yellow
7-Quinolinol	0·7	0·8	Purple	Brown	Bright green
8-Quinolinol	0·9	0·8	Purple	Green	None

* None in 0·1 N-NaOH.

Hydrolysis of the quinolylglucuronides and identification of the quinolinols by paper chromatography

The R_F values of the seven isomeric quinolinols in two solvent mixtures are given in Table 2. The quinolinols were located by their characteristic fluorescence in ultraviolet light, by their colour reaction with $FeCl_3$ and with Brentamine Fast Blue B salt (tetraazotized di-*o*-anisidine). The quinolylglucuronides do not give colours with $FeCl_3$ or Brentamine Fast Blue (Robinson *et al.* 1953*a*) and they fluoresce differently from the free quinolinols (Table 3).

Table 3. *Ultraviolet fluorescence of the quinolylglucuronides*

	Colour of fluorescence in	
β-Glucuronide	0·1 N-HCl	0·1 N-$NaHCO_3$
2-Quinolonyl-*x*-	Purple	Purple
Quinolyl-3-	Purple	Blue
4-Quinolonyl-*x*-	Purple	Purple
Quinolyl-5-	Pale green	None
Quinolyl-6-	Blue	None
Quinolyl-7-	Purple	Blue
Quinolyl-8-	Blue-green	None

A few mg. of each glucuronide were evaporated on a steam bath with 1 ml. of conc. HCl and the residue taken up in a few drops of water and chromatographed on paper. In the case of 3-, 5-, 6-, 7- and 8-quinolylglucuronides the corresponding quinolinols were identified in the hydrolysate. The same results were obtained when the glucuronides were hydrolysed by incubating with locust β-glucuronidase (Robinson *et al.* 1953*a*). The glucuronides isolated after feeding 2- and 4-quinolones, however, gave hydrolysis products differing in R_F and in colour reactions from the material administered. The structure of these glucuronides is being investigated.

DISCUSSION

All the phenolic quinolinols (I; OH at 3, 5, 6, 7 or 8) appear to conjugate directly with glucuronic and sulphuric acids (II; X = $C_6H_9O_6$ or SO_3H). The sum of these conjugations, however, does not account for more than 50–60 % of the dose (Table 1). In all cases the sulphate conjugation is low and phenolic quinolinols appear to be mainly excreted as glucuronides, all of which have been isolated and characterized. The 2- and 4-quinolones (III*a*, *b*; O at 2 or 4) behave differently from their phenolic isomers since they do not conjugate directly but are first hydroxylated elsewhere in the molecule, i.e. in positions 3, 5, 6, 7 or 8 and are excreted as conjugates of hydroxyquinolones (IV; position of OX arbitrary). This suggests that there is insufficient of the hydroxylated or lactim form (III*a*) present to permit conjugation and biologically the quinolones behave as if they were entirely in the keto or lactam form (III*b*). The pyridinols behave similarly (cf. Anderton *et al.* 1948). 3-Pyridinol (V), in agreement with its phenolic character, conjugates with both glucuronic and sulphuric acids (VI), whereas the non-phenolic 2- and 4-pyridones (VII*a*, *b*; O at 2 or 4) hardly conjugate at all. In fact, nothing is

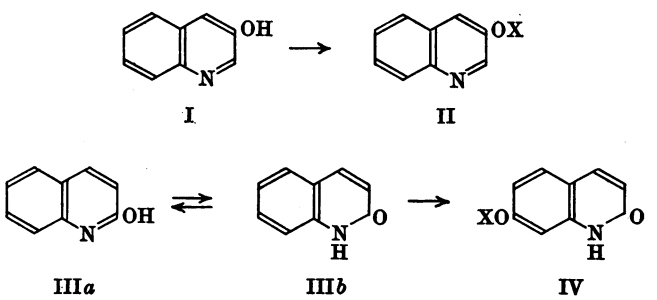
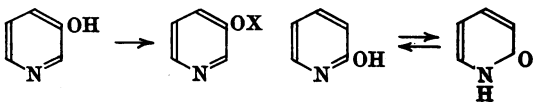


Table 4. *Maximum values from absorption spectra of quinolylglucuronides*

β-Glucuronide	In 0·1 N-HCl						In 0·1 N-NaOH					
	$\lambda_{max.}$ (mμ.)	$\epsilon \times 10^{-3}$	$\lambda_{max.}$ (mμ.)	$\epsilon \times 10^{-3}$	$\lambda_{max.}$ (mμ.)	$\epsilon \times 10^{-3}$	$\lambda_{max.}$ (mμ.)	$\epsilon \times 10^{-3}$	$\lambda_{max.}$ (mμ.)	$\epsilon \times 10^{-3}$	$\lambda_{max.}$ (mμ.)	$\epsilon \times 10^{-3}$
2-Quinolonyl-*x*-	—	—	270	9·2	336	6·9	232	54·7	—	—	340	6·5
Quinolyl-3-	240	26·4	315	5·3	—	—	232	31·7	280	3·0	315	3·5
4-Quinolonyl-*x*-	235	52·4	298	5·0	320	5·5	240	30·4	308	6·8	320	7·0
Quinolyl-5-	245	39·1	315	3·3	345	2·9	235	36·6	—	—	300	4·0
Quinolyl-6-	243	36·4	315	6·5	—	—	220–5	36·2	275	3·3	315	3·3
Quinolyl-7-	240	36·9	~310	4·8	330	7·0	228	39·0	265	2·9	315	4·2
Quinolyl-8-	245	46·6	313	3·8	335	2·5	234	44·1	~262	2·3	300	4·4

~ denotes an inflexion.

known about their metabolism. Pyridine itself does not stimulate the excretion of extra glucuronic acid and ethereal sulphate and if pyridine is oxidized in the rabbit, it may well form one or both pyridones. A pyridone is formed in man during the metabolism of nicotinamide (Knox & Grossman, 1947).



V VI VII*a* VII*b*

Quinoline, unlike pyridine, forms large amounts (40% of the dose) of glucuronide and a small amount (8%) of ethereal sulphate. This suggests that quinoline is at least oxidized in one or more of the positions giving rise to phenolic quinolinols, i.e. 3, 5, 6, 7 and 8 (cf. Novack & Brodie, 1950); it may also be oxidized in the 2- and 4-position, but this type of oxidation would not result in the excretion of extra glucuronic acid. In fact, Knox (1946) has shown that quinoline is oxidized *in vitro* by a rabbit liver preparation to 2-quinolone. The metabolism of quinoline in the intact rabbit is being further investigated.

The assignment of a glucuronosidoquinolone structure (IV) to the metabolites of 2- and 4-quinolones is supported by the nature of their absorption spectra (Table 4). The phenolic glucuronides show specific light absorption in the 230–240 and 290–330 mμ. regions and the spectra show the bathochromic shifts characteristic of quinolyl ethers in general on moving from alkaline or neutral to acid solution (cf. Ewing & Steck, 1946). The glucuronides from the quinolones, on the other hand, do not show this shift and the wavelength of maximum absorption is little different in acid and alkali (Table 4). According to Ewing & Steck (1946) this is characteristic of the intact quinolone structure and suggests that the glucuronic acid is attached through the newly introduced hydroxyl rather than through the oxygen atom at the 2- or 4-position. The glucuronide from 4-quinolone also exhibits the divided maximum at 300–320 mμ. noted by Ewing & Steck in several substituted 4-quinolones but which is not present in the 4-quinolyl ethers (Fig. 1). The quinolone structure is also supported by the values found for the basic ionization constants of the glucuronides from 2- and 4-quinolone. While the quinolyl glucuronides have basic pK_a values of

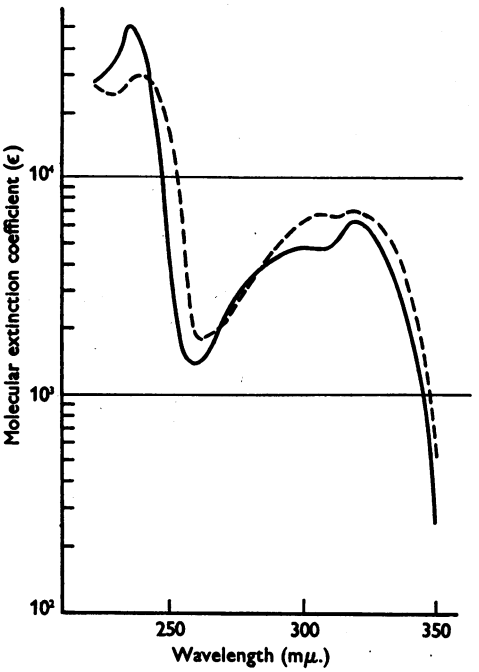


Fig. 1. Absorption spectra of 4-quinolonyl-*x*-glucuronide in 0·1 N-HCl (—)and 0·1 N-NaOH (– – –).

about 5 (Robinson, Smith & Williams, 1953*b*), near those of quinoline and quinolyl ethers (Veley, 1904; Keneford, Morley, Simpson & Wright, 1949), the glucuronosidoquinolones are weaker bases and have pK_a values of about 3·7, near to the value of 2·4 found by Keneford *et al.* for 4-quinolone, and 4·3 reported by Veley for 2-quinolone.

The main purpose of this work, however, was the preparation of the quinolyl glucuronides as reference compounds for work on the metabolism of quinoline.

Table 5. *The optical rotations of the quinolylglucuronides*

(Observations were made on 1% solutions unless the concentration is given in parentheses. The error in $[\alpha]_D$ when $c=1$ is $\pm 3°$.)

β-Glucuronide	In water		In 0·1 N-NaOH	
	$[\alpha]_D^{20}$ (°)	$[M]_D^{20}/100$ (°)	$[\alpha]_D^{20}$ (°)	$[M]_D^{20}/100$ (°)
2-Quinolonyl-*x*-	−99 (*c*, 0·05)	—	−97	−344
K salt of 2-quinolonyl-*x*-	−75 (*c*, 2·5)	—	—	—
Hydrochloride of 2-quinolonyl-*x*-	−66 (*c*, 0·1)	—	—	—
K salt of quinolyl-3-	−94	−354	−92	−348
4-Quinolonyl-*x*-	−128 (*c*, 0·015)	—	—	—
Na salt of 4-quinolonyl-*x*-	−111	−468	—	—
K salt of quinolyl-5-	−71	−268	−73	−276
Quinolyl-6-	−114 (*c*, 0·1)	—	−108	−348
Quinolyl-7-	−66·5	−243	−97	−346
Hydrochloride of quinolyl-7-	−90	−353	—	—
Quinolyl-8-	−75 (*c*, 0·1)	—	−88	−350
Hydrochloride of quinolyl-8-	−77·2 (*c*, 0·2)	—	—	—
K salt of pyridyl-3-	−81	−265	—	—

These compounds are easy to identify since they have a marked fluorescence in ultraviolet light which in many cases changes characteristically on moving from acid to alkaline solution (Table 3). This property may also be of use in the enzymic study of glucuronide synthesis since quinolyl-8-glucuronide can be detected readily by its fluorescence in the presence of excess 8-quinolinol which does not itself fluoresce. The easily prepared 6- and 7-quinolylglucuronides may also serve as alternative substrates to quinolyl-8-glucuronide in glucuronidase estimations (cf. Robinson *et al.* 1953*a*). The quinolinols, when liberated from the conjugates, all give intense colours with Brentamine Fast Blue (Table 2). The glucuronides do not give a colour with this reagent.

The specific and molecular optical rotations of the glucuronides prepared in this work are given in Table 5. The molecular rotations of the glucuronides (except that of 5-quinolinol) in 1% solution in 0·1 N-sodium hydroxide are of the same order, i.e. $[M]_D^{20}/100$ is about $-350°$. This means that the position of attachment of glucuronic acid to the quinoline nucleus (other than positions 2 and 4) has little influence on the molecular rotation.

SUMMARY

1. Measurements have been made of the excretion of glucuronic acid and ethereal sulphate by rabbits dosed with pyridine, quinoline, and each of the monohydroxy derivatives.

2. 3-Hydroxypyridine and 3-, 5-, 6-, 7- and 8-hydroxyquinolines conjugate directly to form glucuronides which have been isolated and characterized.

3. 2- and 4-Quinolones are hydroxylated by the rabbit and excreted as quinolonylglucuronides which have been isolated.

4. The ultraviolet-absorption spectra of the glucuronides and their characteristic fluorescence in ultraviolet light have been recorded.

The author wishes to thank Prof. R. T. Williams for his encouragement and interest in this work, Dr D. Robinson for the measurement of some of the spectra quoted, and Dr G. T. Mills and Dr J. Paul for details of their modification of the naphthoresorcinol method.

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