## REFERENCES

(1) D. Killander, A. Dohlwitz, L. Engstedt, S. Franzén, G. Gahrton, B. Gullbring, G. Holm, A. Holmberg, S. Höglund, A. Killander, D. Lockner, H. Mellstedt, P. J. Moe, J. Palmblad, P. Reizenstein, K. O. Skårberg, B. Swedberg, A.-M. Udén, B. Wadman, L. Wide, and L. Åhström, *Cancer*, **37**, 220 (1976).

(2) B. Ekman, C. Lofter, and I. Sjöholm, *Biochemistry*, **15**, 5115 (1976).

(3) B. Ekman and I. Sjöholm, *J. Pharm. Sci.*, **67**, 693 (1978).

(4) P. Edman and I. Sjöholm, *J. Pharmacol. Exp. Ther.*, **211**, 663 (1979).

(5) A. Kober, B. Ekman, and I. Sjöholm, *J. Pharm. Sci.*, **67**, 107 (1978).

(6) I. Sjöholm and P. Edman, *J. Pharmacol. Exp. Ther.*, **211**, 656 (1979).

(7) H. Hoppe, J. Koppe, and F. Winkler, *Plaste Kautsch.*, **24**, 105 (1977).

(8) S. Hjertén, *Arch. Biochem. Biophys. Suppl. 1*, 147 (1962).

(9) O. Lowry, N. Rosebrough, L. Farr, and R. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(10) Y. Pocker and J. Stone, *J. Am. Chem. Soc.*, **87**, 5497 (1965).

(11) I. Sjöholm, B. Ekman, A. Kober, I. Ljungstedt-Påhlman, B. Seiving, and T. Sjödin, *Mol. Pharmacol.*, **16**, 767 (1979).

(12) J. Marchalonis, *Biochem. J.*, **113**, 299 (1969).

(13) S. Lindskog and B. Malmström, *J. Biol. Chem.*, **237**, 1129 (1962).

(14) B. Meloun, L. Morávek, and V. Kostka, *FEBS Lett.*, **58**, 134 (1975).

(15) G. Edelman, B. Cunningham, W. Gall, P. Gottlieb, U. Rutishauser, and M. Waxdal, *Proc. Natl. Acad. Sci. USA*, **63**, 78 (1969).

(16) T. Samejima, W. McCabe, and J. Yang, *Arch. Biochem. Biophys.*, **127**, 354 (1968).



## ACKNOWLEDGMENTS

Supported by the Swedish Board for Technical Development and the I. F. Foundation for Pharmaceutical Research.

The authors thank Mr. Per Artursson and Miss Siv Larsson for technical assistance.


# Isolation, Identification, and Synthesis of 4-Amino-6,7-dimethoxy-3-quinolinol, the Major Metabolite of Amiquinsin Hydrochloride in Rats and Humans


**GEORGE C. WRIGHT *x, RONALD J. HERRETT ‡, JAMES P. HEOTIS ‡, and JAMES L. BUTTERFIELD ‡**



Received March 20, 1978, from the **Chemical Research Division and the* ‡*Biological Research Division, Norwich-Eaton Pharmaceuticals, Division of Morton-Norwich Products, Inc., Norwich, NY 13815.* Accepted for publication February 26, 1980.



**Abstract** □ The metabolism of amiquinsin hydrochloride (4-amino-6,7-dimethoxyquinoline hydrochloride monohydrate, I) was studied in rats and humans. The major metabolite isolated from human urine was identified through synthesis as 4-amino-6,7-dimethoxy-3-quinolinol hydrochloride hydrate (II). Acid hydrolysis of the major metabolite from rat urine also gave II. The structure of the rat metabolite subsequently was tentatively identified as the potassium salt of the 3-*O*-sulfate of II. The pharmacological and toxicological properties of I and II are discussed.



**Keyphrases** □ Amiquinsin hydrochloride—rat and human metabolites, isolation and identification □ Antihypertensive agents—amiquinsin hydrochloride, rat and human metabolites, isolation and identification □ 4-Amino-6,7-dimethoxy-3-quinolinol—isolation and identification as metabolite of amiquinsin hydrochloride, rats and humans


Wright *et al.* (1) reported that amiquinsin hydrochloride (4-amino-6,7-dimethoxyquinoline hydrochloride monohydrate, I) (Scheme I) demonstrated hypotensive activity. Early biological studies with I included the pharmacological mechanism of action (2) and the absorption, distribution, and elimination of I in dogs (3, 4).

In the present investigation, the metabolism of I was studied in rats and humans. Williams (5) reported that 4-aminoquinoline administered to rabbits was metabolized to 4-amino-3-hydroxyquinoline, which was excreted mainly as an ether sulfate, and that glucuronic acid conjugation was low. Although 4-amino-7-chloroquinoline also was believed to be hydroxylated in rabbits to the 3-hydroxy derivative (5), hydroxylation of this compound in humans was not observed (6).

## RESULTS AND DISCUSSION

**Isolation and Identification of Metabolites**—Metabolite studies with I were done initially in rats. When the rats were dosed orally with [3-$^{14}$C]I, the major excreted material was parent I; TLC followed by estimation of the activity of the radioactive materials showed the presence of I (61%) and substances at $R_f$ 0.6 (25%), 0.0 (12%), and 0.9 (2%). The materials at $R_f$ 0.0 and 0.9 were not identified.

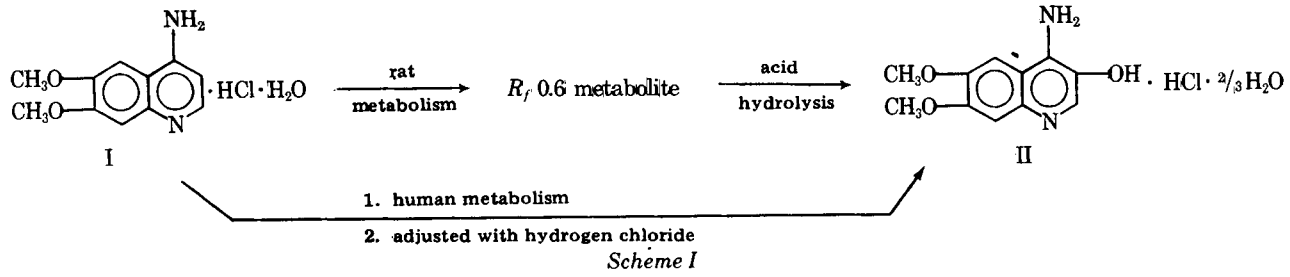
A preparative method was developed for the isolation of the major rat metabolite at $R_f$ 0.6 that involved separation and purification by gel filtration.

Treatment of the purified $R_f$ 0.6 metabolite with hot methanolic hydrogen chloride gave a hydrolyzed product (hydrochloride of $R_f$ 0.6 metabolite) with physical properties similar to those of the starting $R_f$ 0.6 compound but with significant differences in the NMR spectra and TLC values (Table I). However, a comparison of the physical properties of the hydrolyzed product with those of the synthetic 3-hydroxy derivative II (Table I) clearly demonstrates that the two compounds are identical with respect to their NMR and UV spectra and TLC values. Thus, the hydrochloride of the hydrolyzed $R_f$ 0.6 metabolite was identified as 4-amino-6,7-dimethoxy-3-quinolinol hydrochloride.

The $R_f$ 0.6 metabolite was characterized further through enzymatic hydrolysis with a combination of β-glucuronidase and aryl sulfatase. Isolation of the end-products of enzymatic treatment was attained by extraction and TLC, and each spot then was counted for carbon 14. In the absence of enzymes, or by treatment with β-glucuronidase alone, the structure of the $R_f$ 0.6 metabolite was not altered (Table II). However, treatment with aryl sulfatase and β-glucuronidase together converted the $^{14}$C-labeled $R_f$ 0.6 metabolite to [$^{14}$C]II. Thus, the enzymatic data indicate that the $R_f$ 0.6 metabolite may be the sulfate conjugate of II. The IR spectrum showed a strong S=O stretching band at 8.1 μm, also indicative of a sulfate group in the molecule. The NMR spectra showed that both the $R_f$ 0.6 metabolite and II are substituted in the 3-position. Considering the specificity of the enzymes used, the IR data, and the substitution pattern from the NMR spectra, the $R_f$ 0.6 metabolite probably



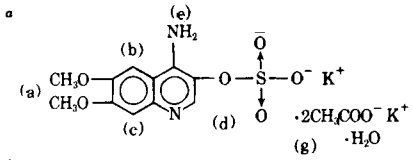

0022-3549/80/0700-0842$01.00/0




*Scheme I*

**Table I—Physical Data for the $R_f$ 0.6 Metabolite, Hydrochloride of Hydrolyzed $R_f$ 0.6 Metabolite, and Synthetic 3-Hydroxy Derivative**

| Test | $R_f$ 0.6 Metabolite[a] | Hydrochloride of Hydrolyzed $R_f$ 0.6 Metabolite | Synthetic 3-Hydroxy-Derivative (II) |
|---|---|---|---|
| NMR: proton peaks, δ ppm (integral) | a, 3.92 (6)<br>b, 7.2 (1)<br>or<br>c, 7.5 (1)<br>d, 8.28 (1)<br>e, 6.26 (2)[b]<br>f, —<br>g, 1.92 (2.5) | a, 3.89 (6)<br>b, 7.37 (1)<br>or<br>c, 7.85 (1)<br>d, 8.11 (1)<br>e, 8.03 (2)[b]<br>f, —[c]<br>g, —[c] | a, 3.92 (6)<br>b, 7.29 (1)<br>or<br>c, 7.77 (1)<br>d, 8.07 (1)<br>e, 8.03 (2)[b]<br>f, 10.6 (~1)[b]<br>g, 13.8 (~1)[b] |
| UV absorption (water) | Maxima: 250 and 321 nm<br>Minimum: 270–275 nm | Maxima: 250 and 316–320 nm<br>Minimum: 275 nm | Maxima: 250 and 316–320 nm<br>Minimum: 275 nm |
| TLC[d] | 0.60 | 0.25–0.30 | 0.30–0.35 |

[a] 


[b] Exchanged with deuterium oxide. [c] These peaks did not appear, probably because of relatively low concentration. [d] Eastman chromatographic sheet type K-301R (silica gel) developed with water-saturated 1-butanol.

is the 3-*O*-sulfate of II. Elemental analysis of the $R_f$ 0.6 metabolite suggested that the isolated form was a hydrated potassium salt, with potassium acetate cocrystallized with the metabolite. No further work on the structural elucidation of the $R_f$ 0.6 metabolite is planned.

Human volunteers were given a single oral dose of I, and the urine was analyzed for metabolites as described under *Experimental*. Although free I was isolated from human urine (the ether–chloroform extract), none of the $R_f$ 0.6 metabolite was present (Table III). However, the $R_f$ values and fluorescence color observed in isopentanol (or 1-butanol) extracts of urine corresponded to values observed for the synthetic 3-hydroxy derivative (II). The structure of the human metabolite was supported further by a comparison of the NMR, fluorescence, and UV absorption spectra of the human metabolite with those of the synthetic compound (Table III). Controls were run on the human urine to rule out the possibility that the $R_f$ 0.6 metabolite initially was present and subsequently was hydrolyzed to II during isolation. Therefore, the human metabolite was identified as 4-amino-6,7-dimethoxy-3-quinolinol hydrochloride hydrate (II).

**Pharmacology**—Studies were conducted to evaluate the cardiovascular activity of II in anesthetized dogs, the vasodepressor activities of I and II in anesthetized rats, and the $LD_{50}$ values of I and II in mice (see *Experimental*). Significant hypotensive activity in dogs was observed for II. The intensities of the hypotensive responses elicited by comparable intravenous injections of I and II were similar in rats; however, the duration of the hypotensive response in rats was considerably longer for I than for II. The $LD_{50}$ values for I and II administered intravenously to mice were comparable.

**Table II—TLC Data on Enzymatic Treatment of $R_f$ 0.6 Metabolite[a]**

| Compound | Enzyme | $R_f$ |
|---|---|---|
| $R_f$ 0.6 metabolite | β-Glucuronidase + aryl sulfatase | 0.46 |
| $R_f$ 0.6 metabolite | None | 0.96 |
| $R_f$ 0.6 metabolite | β-Glucuronidase | 0.93 |
| II | None | 0.50 |

[a] Eastman chromatographic sheet type K-301R2 developed with benzene–95% ethanol (water) (9:1).

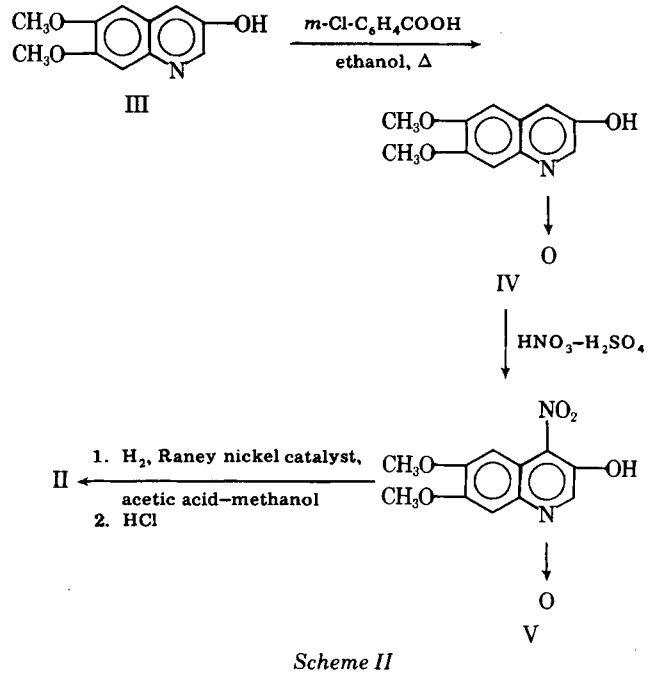
**Synthesis**—The synthesis of II from 6,7-dimethoxy-3-quinolinol (7) (III) was accomplished in a three-step sequence (Scheme II).

In the nitration of IV, it was assumed that the substitution was directed at position 4 to give V; this assumption was based primarily on the published results (8) of the nitration of quinoline 1-oxide, in which 4-nitroquinoline 1-oxide was obtained in 70% yield with only minor amounts of the 5- and 8-nitro isomers. Furthermore, the fact that the properties of synthetic II correspond to those of the metabolite (where the amino substituent must be in position 4) proves that the nitro substituent of V is in position 4.

## EXPERIMENTAL[1]

**Isolation of Rat Metabolites**—A solution of [3-$^{14}$C]I was administered orally to fasted rats at 10 mg/kg. The excretion of carbon 14 in urine was determined periodically for 24 hr after dosing; of the total [3-$^{14}$C]I dosage, 81 and 17% were excreted in the urine and feces, respectively. A 50–250-μl aliquot of the urine collected during each period was suspended in thixotropic gel (9) and counted in the liquid scintillation spectrometer.

TLC[2] was used for identification of the $^{14}$C-labeled urinary metabolites of [3-$^{14}$C]I. Several solvent systems were tested initially, but only butanol saturated with water was satisfactory in separating the metabolites of I. The chromatograms were scanned[3] for radioactivity.




*Scheme II*

[1] Melting points were determined on a Fisher-Johns hot-stage apparatus and are uncorrected. NMR spectra were determined in dimethylsulfoxide-$d_6$ with tetramethylsilane as an internal standard on a Varian A60A spectrometer. Fluorescence spectra were obtained on an Aminco-Bowman spectrophotofluorometer.

[2] Eastman Kodak type K301R sheets.

[3] Duo Scan thin-layer scanner.

**Table III—Comparison of Physical Data of Human Metabolite of I, $R_f$ 0.6 Metabolite, and II**

| | I | $R_f$ 0.6 Metabolite | Metabolite from Human Urine | Synthetic 3-Hydroxy Derivative (II) |
|---|---|---|---|---|
| TLC[a]: $R_f$ (color) | | | | |
| Water-saturated 1-butanol | 0.10 (dark purple) | 0.60 (dark blue) | 0.10[b] (dark purple)<br>0.33[c] (bright blue) | 0.30 (bright blue) |
| Chloroform–95% ethanol (water) | 0.35 (dark purple) | 0.80 | 0.20[c] (bright blue)<br>0.35[b] (dark purple) | 0.20 (bright blue) |
| Emission spectra (activation/emission) | 260/365 nm | 250 nm/— | 250/450 nm | 250/450 nm |
| UV absorption | Maximum: 335 nm | Maxima: 250 and 321 nm | Maxima: 250 and 316–320 nm | Maxima: 250 and 316–320 nm |
| | Minimum: 248–250 | Minimum: 270–275 nm | Minimum: 275 nm | Minimum: 275 nm |

[a] Commercially prepared silica gel F-254 plates. [b] Found in ether–chloroform or hexanol–heptane (4:1 v/v) extracts. [c] Found in isopentanol or the 1-butanol extracts.

A preparative method was developed for isolation of the major metabolite ($R_f$ 0.6). Forty milliliters of the composite urine sample was placed on a gel filtration column[4] (30 × 4 cm, previously equilibrated with water) and eluted with water (1200–1400 ml). Fractions of ~14 ml were collected with a fraction collector. The absorbance of each fraction was determined at 250 and 320 nm. The $R_f$ 0.6 metabolite fraction was concentrated, placed on a second column[4] (12 × 6 cm), and eluted with water; this procedure resulted in purification of the $R_f$ 0.6 metabolite, as shown by little change in the ratio of the absorbances at 250 and 320 nm, which was 2.9 for the first filtration and 2.8 for the second filtration. The purified $R_f$ 0.6 metabolite was obtained through concentration with an evaporator[5].

The $R_f$ 0.6 metabolite was converted to the hydrochloride of the hydrolyzed $R_f$ 0.6 metabolite by the following procedure. Fifty milligrams of the isolated metabolite was dissolved in 3 ml of absolute methanol, and the resulting solution was purged with hydrogen chloride gas followed by refluxing for 1 hr. Atmospheric moisture was kept from the reaction vessel by a silica gel drying tube. The cooled (tap water) solution was filtered to remove a small amount of insoluble material, and the filtrate was concentrated and filtered with the original precipitate. This procedure yielded 25 mg of dry material. The same procedure was followed with the synthetic 3-hydroxy derivative (II), except that 60 mg of the starting material was used; the yield of the synthetic hydrochloride was 37 mg. Physical data were obtained on these hydrochloride products (Table I).

The enzymatic hydrolysis of the $R_f$ 0.6 metabolite was carried out with β-glucuronidase–aryl sulfatase[6] and β-glucuronidase[7] of bovine origin. The glucuronidase–sulfatase enzyme mix (0.1 ml) was diluted to 5.5 ml with pH 4.5 acetate buffer. This dilution was followed by the addition of a neutral aqueous solution (0.5 ml) containing the $^{14}C$-labeled $R_f$ 0.6 metabolite or standard. The 0.5-ml standards were 200 μg of phenolphthalein glucuronide/ml for the β-glucuronidase activity and 200 μg of esterone sulfate/ml for the sulfatase activity. Test tubes containing no enzyme but with all other constituents were included as controls.

All samples were incubated at 37° for 18 hr. At that time, all of the samples of the $^{14}C$-labeled $R_f$ 0.6 metabolite and the controls were adjusted to pH 7.5 with 0.1 *N* NaOH and extracted twice with equal volumes of 1-butanol. The butanol extracts were set aside while enzyme activity was checked. This test was done by adding 0.5 ml of 30% trichloroacetic acid to precipitate the enzyme protein, followed by centrifugation and adjustment of the supernate to pH 10.4. Formation of a pink color indicated hydrolysis of the phenolphthalein–glucuronide conjugate to free phenolphthalein. The sulfatase activity was checked by adjusting the solution pH to 7.0 with 0.1 *N* NaOH, followed by extraction of the free estrogen with chloroform. The chloroform extract was reduced in volume *in vacuo*, spotted on thin-layer plates[2], and developed in benzene–95% ethanol (water) (9:1). The free estrone gave a blue spot against a yellow background when the chromatogram was sprayed with 10% phosphomolybdic acid in methanol and heated at 100° for several minutes. TLC[2] of the butanol extracts of samples containing the $^{14}C$-labeled $R_f$ 0.6 metabolite were performed with a chloroform–95% ethanol (water) (1:1) solvent system.

**Isolation of Human Metabolite**—Compound I in gelatin capsules was given to three male adult volunteers as a single oral 50-mg dose, equal to ~0.75 mg/kg. Urine was collected during 0–4 and 8–24 hr. These samples were refrigerated soon after collection and processed for metabolite separation within 1 or 2 weeks.

Solvent extraction of human urine was conducted as follows. The urine was adjusted to pH 10–11.5 with the addition of borate to glycine buffer at pH 10.5, and final adjustment was made with I or 5 *N* NaOH using a pH meter. Extraction with equal volumes of ether–chloroform or hexanol–heptane (4:1) was performed three times, and the extracts were pooled; free amiquinsin was removed in this process. The urines then were adjusted to pH 7–7.5 with 5 *N* HCl and extracted three times with either isopentanol or 1-butanol; free II was removed by this procedure.

Controls were run on the human urine to rule out the possibility that the $R_f$ 0.6 metabolite initially was present and subsequently was hydrolyzed to II during isolation. The $R_f$ 0.6 metabolite was not detected (by TLC) in isopentanol extracts of the human urine but definitely was observed in comparable isopentanol extracts of rat urine.

Additional evidence for the absence of the $R_f$ 0.6 metabolite in human urine was obtained through gel filtration. The urine sample was filtered through a gel filtration column[4] followed by elution with water. The $R_f$ 0.6 metabolite was not in the water eluates, which were examined for UV-absorbing material. However, when previously prepared $R_f$ 0.6 metabolite was added to control human urine and passed through the column[4], it was readily detected by UV absorption at a concentration as low as 1 μg/ml. Thus, the $R_f$ 0.6 metabolite apparently was stable under the conditions of the isolation procedure.

**Pharmacology**—*Cardiovascular Activity*—Compound II was evaluated for hypotensive activity in three anesthetized (pentobarbital sodium, 35 mg/kg iv) dogs. The compounds were administered intravenously as an aqueous solution, and the blood pressure was recorded from a cannulated femoral artery.

A dose of 1 mg of II/kg iv decreased the arterial blood pressure 48% below the control level within 1 min. After 45 min, the blood pressure returned to within 6% of the control value.

Five milligrams of II/kg given intravenously lowered the arterial blood pressure 68% in <1 min. The pressure gradually rose to a level only 7% below the control by ~120 min postinjection.

Two dogs died after receiving an intravenous 10-mg/kg dose of II.

*Vasodepressor Activity*—The vasodepressor activities of I and II were evaluated in anesthetized[8], adult male, albino rats[9]. Drug injections were made *via* the femoral vein, and the blood pressure was recorded from a cannulated carotid artery.

A relationship between the dose and the degree of hypotension elicited was not observed; both I and II exhibited a decrease in blood pressure, averaging 30–40% at the doses tested. For II, the time required for the blood pressure to return to 50% of the pretreatment value averaged 2, 3, and 6 min at dose levels of 0.5, 1.0, and 2.0 mg/kg, respectively. However, for I, the 50% return times averaged 8 min at 0.5 mg/kg, 4–55 min at 1.0 mg/kg, and >2 hr at 2.0 mg/kg.

*Acute Toxicity*—To compare the acute toxicities of I and II, groups of 10 male TFSW mice (19–24 g) were used. For each compound, two doses below and two above the $LD_{50}$ value were administered intravenously. Death within 30 min postinjection was considered as a positive response. The $LD_{50}$ values were calculated by a computerized probit–log dose regression analysis. The observed $LD_{50}$ values for I and II were 67.1 and 66.8 mg/kg, respectively.

[4] Sephadex G-10.
[5] Rinco evaporator.
[6] Calbiochem 34742.
[7] Calbiochem 34743.
[8] Dial-urethan, 0.7 ml/kg ip.
[9] Charles River.

**Chemistry**—*6,7-Dimethoxy-3-quinolinol 1-Oxide (IV)*—A solution of 6,7-dimethoxy-3-quinolinol (6) (III) (52 g, 0.26 mole) in 95% ethanol (methanol) (786 ml) was treated portionwise with *m*-chlorobenzoic acid (73 g, 0.42 mole) with mechanical stirring. The reaction solution was refluxed for 45 min, stirred for an additional 2 hr, stored in the refrigerator overnight, and filtered to remove a small amount of solid. The filtrate was concentrated to near dryness, and the residue was slurried in ether (500 ml) and stored overnight. The solid product was collected by filtration (50 g), mp 235°. Recrystallization from 95% ethanol (methanol) gave 29.6 g (52% yield) of IV, mp 243–245°.

An analytical sample was prepared by further recrystallization from 95% ethanol (methanol), mp 250–251°; NMR: $\delta$ 3.93 (s, 6H, 2 $OCH_3$), 7.17 (d, 1, aromatic, $J$ = 1.9 Hz), 7.30 (s, 1, aromatic), 7.78 (s, 1, heteroaromatic), 8.18 (d, 1, heteroaromatic, $J$ = 1.6 Hz), and 10.3 (s, 1, OH, exchanged with $D_2O$).

*Anal.*—Calc. for $C_{11}H_{11}NO_4$: C, 59.72; H, 5.01; N, 8.33. Found: C, 59.32; H, 4.85; N, 8.29.

*6,7-Dimethoxy-4-nitro-3-quinolinol 1-Oxide (V)*—To IV (5.0 g, 0.023 mole) was added a cold solution of concentrated nitric acid (7.5 ml) in water (4.0 ml). Concentrated sulfuric acid (15 ml) then was added to the mixture at 10–20° over 15 min with hand stirring. The reaction mixture was allowed to warm (exothermic) to 35° over 6–10 min, warmed to 45° over 3 min, maintained at 45–50° for 30 min, and left at 25° for 1 hr. (Since the heat of reaction was erratic and difficult to control, the sample size was limited to 5 g.) The reaction mixture was added to ice (70 ml), and the resultant yellow, crystalline solid was collected by filtration and washed with cold water (20 ml).

This process was repeated a total of four times, the combined product was treated with hot nitromethane (140 ml), and the insoluble product, assumed to be the sulfate salt of V, was collected by filtration. The cooled filtrate yielded 2.0 g of yellow, crystalline V, mp 232–234°. The nitromethane-insoluble product (5.8 g) was dissolved in 10% $Na_2CO_3$ (29 ml) with cooling in an ice bath and subsequently acidified with acetic acid (4.0 ml) with cooling. The product (V) was collected by filtration and washed with cold water (4 × 3 ml), cold 2-propanol (2 × 4 ml), and ether to yield 4.4 g, mp 228–232° dec. The total yield was 6.4 g (27%).

An analytical sample was prepared by recrystallization from nitromethane, mp 228–232° dec.; NMR: $\delta$ 3.95 and 3.98 [2s, 6, $(OCH_3)_2$], 7.10 and 7.74 (2s, 2, aromatic), and 8.32 (s, 1, heteroaromatic).

*Anal.*—Calc. for $C_{11}H_{10}N_2O_6$: C, 49.63; H, 3.79; N, 10.52. Found: C, 49.22; H, 3.87; N, 10.93.

*4-Amino-6,7-dimethoxy-3-quinolinol Hydrochloride Hydrate (II)*—A mixture of V (7.0 g, 0.026 mole), methanol (470 ml), acetic acid (5.8 ml), and Raney nickel catalyst (18 g, wet basis) was subjected to hydrogenation at 48 psig. A pressure drop of 90% (of theory) was observed over 4.0 hr. The reaction mixture was cooled in an ice bath, treated with charcoal, and filtered. The filtrate was treated with a hydrogen chloride–2-propanol solution to pH 3–4, concentrated to 80 ml under reduced pressure, and cooled in an ice bath. The product was collected by filtration and washed with cold methanol, 2-propanol, and ether to give 4.9 g (70% yield), mp 264–267° dec.

An analytical sample was prepared *via* three recrystallizations from methanol, mp 265–267° dec.

*Anal.*—Calc. for $C_{11}H_{12}N_2O_3 \cdot HCl \cdot 2/3\ H_2O$: C, 49.16; H, 5.37; N, 10.43. Found: C, 49.38; H, 5.74; N, 10.34.

*4-Amino-6,7-dimethoxy[3-$^{14}$C]quinoline Hydrochloride Hydrate ([3-$^{14}$C]I)*—The [3-$^{14}$C]I was prepared by the same basic procedure as that reported previously (1) for the preparation of I.

*Potassium Salt of 3-O-Sulfate of II (VI)*—*Anal.*—Calc. for $C_{11}H_{11}KN_2O_6S \cdot 2CH_3COO^-K^+ \cdot H_2O$: C, 32.60; H, 3.47; N, 5.07; S, 5.80. Found: C, 32.87; H, 3.74; N, 4.77; S, 5.52.

## REFERENCES


(1) G. C. Wright, E. J. Watson, F. F. Ebetino, G. Lougheed, B. F. Stevenson, A. Winterstein, R. K. Bickerton, R. P. Halliday, and D. T. Pals, *J. Med. Chem.*, **14**, 1060 (1971).

(2) B. S. Jandhyaia, G. J. Gregs, and J. P. Buckley, *Arch. Int. Pharmacodyn. Ther.*, **167**, 217 (1967).

(3) J. D. Conklin and R. D. Hollifield, *Eur. J. Pharmacol.*, **10**, 360 (1970).

(4) J. D. Conklin and R. D. Hollifield, *Life Sci.*, **9**, 577 (1970).

(5) R. T. Williams, "Detoxication Mechanisms," Wiley, New York, N.Y., 1959, p. 651.

(6) E. W. McChesney, W. D. Conway, W. F. Banks, Jr., J. E. Rodgers, and J. M. Shekosky, *J. Pharmacol. Exp. Ther.*, **151**, 482 (1966).

(7) British pat. 942,524 (1963); through *Chem. Abstr.*, **60**, P5469e (1964).

(8) R. C. Elderfield, "Heterocyclic Compounds," Wiley, New York, N.Y., 1952, p. 252. Ochiai, *Imp. Acad. (Tokyo)*, **19**, 307 (1943); through *Chem. Abstr.*, **41**, 5880d (1947).

(9) C. F. Gordon and A. L. Wolfe, *Anal. Chem.*, **32**, 574 (1960).


## ACKNOWLEDGMENTS


The authors thank Mr. George Klein for the synthesis of the [3-$^{14}$C]I, Mr. Charles Williams (deceased) for able assistance, and Dr. Donald Polin for advice in the metabolite identification studies.