Synthesis of 8-(3-Carboxy-1-methylpropylamino)-6-methoxyquinoline: A Newly Characterized Primaquine Metabolite

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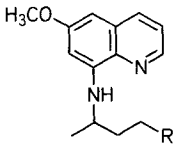
Abstract: Primaquine (I), a 6-methoxy-8-aminoquinoline derivative used for the treatment of malaria has previously been shown to be metabolized to 8-(3-carboxy-1-methylpropylamino)-6-methoxyquinoline by both micro-organisms and laboratory rats. Reported herein is the synthesis of this novel metabolite.

Primaquine (I) is used for the radical cure of vivax malaria and is used in combination with other antimalarial drugs such as chloroquine for prophylaxis in endemic areas (1). The major drawback of primaquine is its low therapeutic index. The drug is known to cause hemolytic lesions, particularly in patients deficient in glucose-6-phosphate dehydrogenase. Unfortunately, this enzyme deficiency is most common among the inhabitants of regions in which malaria is endemic.

Apart from a few relevant studies on pamaquine by early workers (2, 3, 4), and, more recently, some preliminary studies on primaquine (5–7), very little is known about the metabolism and excretion of the 8-aminoquinolines. Essentially nothing is known about the chemical basis of its toxic side effects. Studies on the effect of primaquine and its suspected metabolites on normal and glucose-6-phosphate deficient erythrocytes (4–6) have indicated that primaquine and 8-amino-6-methoxyquinoline have very little effect, whereas there is substantial oxidative activity in the suspected hydroxy metabolites. However, adequate characterization of these metabolites or their oxidation product, the quinoline quinone, from humans or animals has not been reported. It had been suggested earlier that the parent 8-aminoquinoline had very little antimalarial activity, but that *in vivo* degradation results in a more active substance (8). Whether the same metabolite is the active antimalarial agent and is responsible for the hemolytic toxicity of the 8-aminoquinolines is as yet totally unknown.



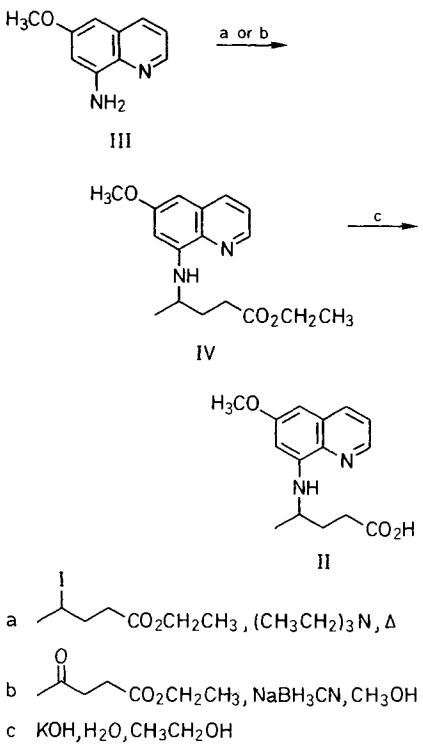
I R = CH_2NH_2, Primaquine
II R = CO_2H

In an attempt to gain information about primaquine metabolism and its role in the antimalarial activity of the drug, we have initiated studies of metabolism of primaquine in microorganisms (9) and in laboratory rats (10). A new metabolite of the drug is present in both systems; 8-(3-carboxy-1-methylpropylamino)-6-methoxyquinoline (II). To verify the structure of this new metabolite it has been prepared by total synthesis. Reported herein is the synthesis of this material.

Materials and Methods

Analytical procedures

TLC was done on Sil G-25 UV_{254} (Brinkmann Instruments, Inc.) plates using A) methanol: chloroform (15:85) and B) ethanol:benzene (8:92) as solvents. HPLC was done with a Waters Associates M6000A pump, U6-K injector and UV detector (254 nm) using a μBondapak C_{18} column. A mobile phase of 1.2 L distilled water:2.8 L methanol containing 2.2 g potassium dihydrogen phosphate and 3.3 g potassium hydrogen phosphate was used at a flow rate of 2 ml/min. Column chromatography was performed with E. Merck alumina, neutral, activity grade I, 70–230 mesh. GC was performed on a Beckman GC65 instrument using a 6 ft. × ¼ OD glass column of 3% OV-17 with deactivated support. NMR was recorded on a Varian E-390 instrument using tetramethylsilane as internal standard. MS was recorded on a Finnigan 3200 GC/MS/DS (INCOS) System.



Preparation of ethyl 4-iodovalerate

Ethyl levulinate (2.9 gm, 0.02 mole) was dissolved in methanol (10 ml). Sodium borohydride (0.95 gm, 0.025 mole) was added slowly at such a rate that the temperature of the reaction was kept between 25° and 30°C. The solution was stirred at room temperature for one hour and a small portion of the mixture was analyzed by gas chromatography. The starting keto ester was found to be absent. Methanol was removed under reduced pressure and the residue was

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treated with acetic acid (1 ml) and then extracted with ether. The ethereal layer was washed first with saturated brine solution, then dried over sodium sulfate and concentrated. The product was obtained as a colorless liquid (2.9 g, 90 %), which was used for the preparation of the iodide.

NMR: (deuterochloroform),
4.0 δ (m, 3, $CH_3CH(OH)CH_2$, $-CO_2CH_2CH_3$),
2.9 (s, 1, exchangeable, OH),
2.5 (m, 2, $CH_2CO_2C_2H_5$),
1.83 (m, 2, $-CHOHCH_2CH_2$),
1.3 (m, 6, $-CO_2CH_2CH_3$, CH_3CHOH).

The above hydroxy ester (1.5 g) was dried under vacuum for 8 hours. The dried material was dissolved in chloroform (20 ml). Iodotrimethylsilane (4.0 g, 0.02 mole) was added slowly and the reaction mixture was stirred at room temperature under nitrogen atmosphere for 16 hours. Methanol (1 ml) was added and the chloroform layer was washed successively with saturated sodium bisulfite, brine, dried and concentrated. The pale yellow liquid, 2.0 g, was homogenous by gas chromatography.

NMR: (deuterochloroform),
4.27 δ (m, 2, $-CO_2CH_2CH_3$),
2.63 (m, 4, $-CH_2CH_2$),
2.0 (m, 7, CH_3CH I-, $CO_2CH_2CH_3$).

Preparation of 8-(3-carboxy-1-methylpropylamino)-6-methoxyquinoline (II): Alkylation of 6-methoxy-8-aminoquinoline (III)

6-Methoxy-8-aminoquinoline (0.14 g, 0.8 mmole), ethyl 4-iodovalerate (0.39 g, 1.5 mmole) and triethylamine (0,15 ml, 1.1 mmole) were heated in nitrogen atmosphere at 80–90°C for 12 hours. Additional iodo compound (0.2 g) and triethylamine (0.1 ml) were added and heating was continued for an additional 6 hours. The residue was dissolved in chloroform (50 ml), and washed successively with 10% sodium hydroxide solution and then saturated brine. Removal of the solvent under reduced pressure gave a residue which was chromatographed on a column of neutral alumina. Chloroform eluted a colorless, viscous material (IV), 0.152 g (63 %), which was found to be homogenous by TLC (solvents A and B) and HPLC. NMR: (deuterochloroform) 8.5 δ (dd,1,C-2H), 7.9 (dd.1,C-4H), 7.27 (dd,1,C-3H), 6.3 (s,2,C-5H and C-7H), 6.0 (b, 1, exchangeable -NH), 4.1 (q,2,-$CO_2CH_2CH_3$), 3.86 (s,3,-OCH_3), 3.67 (m,1,-NH-$CH(CH_3)$-), 2.47 t,2,CH_2CO_2Et), 2.0 (m,2,-$CH_2CH_2CO_2Et$), 1.3 (t,3,-$COOCH_2CH_3$) 1.14 (d,3,CH_3CH-). MS (m/z): 302 (M^+, 3.7 %), 259 (5.2), 215 (29.0) 202 (15.7), 201 (100.0) 186 (12.8), 158 (13.3).

The above ester (IV) (0.1 g, 0.3 mmole) was dissolved in 10 % aqueous alcoholic potassium hydroxide (4 ml) and refluxed on a steam-bath for 2 hours. It was then cooled and diluted with water (15 ml). The clear aqueous layer was made acidic with glacial acetic acid (pH 6.0) which caused crystals to separate. The solid was filtered and recrystallized from methanol-water as colorless needles, (II) mp 154–56°C, yield – 70 mg (77 %). The compound was identical in every respect (IR (chloroform), TLC (solvents A and B), HPLC) to the metabolite of primaquine isolated earlier (9, 10). Mixture melting point determination gave no depression. MS (m/z): 274 (M^+, 6.9 %), 215 (22.2), 202 (10.0), 201 (100.0), 187.0 (15.8), 180 (26.1), 159 (38.0), 158 (21.8).

Reductive Alkylation of 6-methoxy-8-aminoquinoline (III)

Freshly distilled 6-methoxy-8-aminoquinoline (3.3 g, 0.02 mole) was dissolved in anhydrous methanol (50 ml). Ethyl levulinate (15.0 g, 0.1 mole), p-toluene-sulphonic acid (5 mg) and 3A molecular sieves (6 gm) were added, and the mixture was stirred at room temperature for 30 minutes. Sodium cyanoborohydride (6.5 g, 0.103 mole) was added and the reaction mixture was stirred at room temperature for 7 days. Additional amounts (0.5 g) of sodium cyanoborohydride were added at 24, 48, and 120 hours of reaction. At the end of seven days, the solution was filtered, the residue washed thoroughly with methanol; the filtrate and washings were combined and concentrated. The residue was carefully acidified with glacial acetic acid to pH 6.0 and extracted with ether. The ethereal layer was washed with saturated brine, dried over anhydrous sodium sulfate and the solvent removed under reduced pressure to yield an oily product which was chromatographed over neutral alumina. Chloroform elution gave 2.2 g (35 %) of the required 8-(3-carboethoxy-1-methylpropylamino)-6-methoxyquinoline. The amount of unreacted 6-methoxy-8-aminoquinoline recovered was 1.27 g. This ester was hydrolyzed as above to produce 8-(3-carboxy-1-methylpropylamino)-6-methoxyquinoline (II).

Results and Discussion

Early investigations of the metabolism of primaquine (2–6) suggested that hydroxylation of the quinoline ring portion of the drug predominates. To date we have not identified any metabolites resulting from aromatic hydroxylation (9, 10). Rather, the oxidative deamination product, 8-(3-carboxy-1-methylpropylamino)-6-methoxyquinoline (II), has been obtained. Verification of the structure of this metabolite has been accomplished by its synthesis. The synthesis was accomplished by two routes: 1) Alkylation of 6-methoxy-8-aminoquinoline with the secondary halide derivative, ethyl-4-iodopentanoate and subsequent ester hydrolysis; 2) Reductive alkylation of 6-methoxy-8-aminoquinoline with ethyl levulinate in the presence of sodium cyanoborohydride and subsequent ester hydrolysis. The material produced by synthesis was identical in all respects with that isolated from the metabolic studies [IR-(chloroform), TLC (2 solvent systems), HPLC, MS, and mixture melting point determination gave no depression]. Both routes proceeded satisfactorily although in modest yields due to the poor reactivity of 6-methoxy-8-aminoquinoline.

Efforts are continuing in our laboratory to gain a complete understanding of the metabolism of primaquine and the role of metabolism in the action of the drug.

Acknowledgements

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Metabolism and Distribution of Primaquine in Monkeys

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Abstract Rhesus monkeys were administered primaquine diphosphate (6.0 to 10.5 mg/kg, I.V.), and plasma samples were analyzed by high performance liquid chromatography for the presence of the unchanged drug and the major metabolite, 8-(3-carboxy-l-methylpropylamino)-6-methoxyquinoline (II). Primaquine had an unusually high affinity for tissue compartments which produced a rapid initial drop in plasma concentration. Within 15 minutes, the plasma concentration of II far exceeded that of primaquine. 35 to 83 % of the primaquine dose was converted to II; moreover, metabolite II possessed much lower affinity for the tissue compartments than primaquine itself.

Primaquine (I) is used for the prophylaxis of malaria in endemic areas. Though it probably is the most effective drug for this use, it has a fairly low therapeutic index, and its use is frequently associated with hemolytic anemia and methemoglobin formation. It is also thought that the toxicity of primaquine results from biotransformation of the drug. Only trace quantities of unchanged primaquine have been found in urine, and 6-methoxy-8-aminoquinoline was the only metabolite that has been positively identified. However, this metabolite represented only 4 % of the dose (2). More recently, it has been shown that the major metabolite of primaquine by fungi (3) and by rats (4, 5) is the oxidative deamination product (II), 8-(3-carboxy-l-methylpropylamino)-6-methoxyquinoline.

The objective of the present study was to determine the extent of conversion of primaquine diphosphate to II in Rhesus monkey as a common animal model for malaria studies.

These studies with Rhesus monkeys might more accurately reflect the pharmacokinetics and metabolism of primaquine in man than the previous studies with rats (4, 5).

Materials and Methods

Primaquine diphosphate was utilized as obtained from Aldrich (Milwaukee, WI), and the metabolite II was obtained from the fermentation of primaquine as previously reported (3). This material had spectral and chromatographic properties identical to synthetic II.

The analysis of the plasma samples for primaquine diphosphate and II was accomplished using high performance liquid chromatography with a C-18 reversed phase column and ultraviolet detection as previously reported (4). The identification of primaquine and II was based on comparisons of retention times and the absorbance ratios of 254 nm and 280 nm with a dual UV detector system of the test samples, the two reference standards, and plasma blanks.

In the metabolism studies, the Rhesus monkey *(M. Mulatta)* was first sedated with ketamine HCl (12 mg/kg); then an I.V. drip (30 drops/min) of lactated ringers solution was administered to the restrained animal. After I. V. administration of the test drug, 10 ml blood samples were collected at 5, 10, 15, 30 minutes and thereafter at 1, 2, 4, 8, and 24 hours using an indwelling catheter. The primaquine diphosphate was administered as an aqueous solution. Because of the low water solubility of II, this material was first converted to the water soluble salt by the addition of an equivalent amount of sodium hydroxide immediately before administration.

Results

Following I. V. administration of primaquine diphosphate, an extremely rapid fall in plasma concentration of the drug was observed in the three monkeys that were tested (Fig. 1). Furthermore, concentration of the metabolite in plasma after 15 minutes was far greater than that of the drug (Fig. 2). The concentration of the metabolite remained high and at a fairly constant level during the 2 to 8

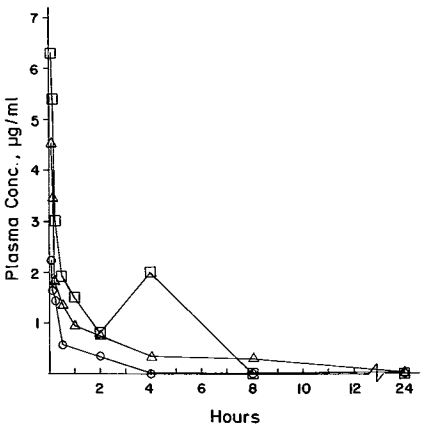

Figure 1: Plasma concentration of primaquine diphosphate following IV administration: O, monkey #183 given 10.5 mg/kg primaquine diphosphate; □ monkey #413 given 10.5 mg/kg primaquine diphosphate; △, monkey #331 given 6.0 mg/kg primaquine diphosphate.

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