Rapid Aromatic Hydrogen Exchange in the Antimalarial Primaquine

James D. McChesney[1] and Srinivasin Sarangan[1]

Received: September 8, 1983; accepted: January 25, 1984.

Abstract: Primaquine, an 8-aminoquinoline antimalarial, is shown to undergo unexpectedly rapid aromatic proton exchange with the medium. At a pH less than 4, the exchange of C-5 is so fast as to be unmeasurable by proton nmr methods. At a pH of 6–6.5 the half-time for exchange is 4 to 5 minutes. This unexpectedly high rate of exchange of a carbon-proton with medium may provide an important clue to the biological activity of primaquine.

Primaquine, 6-methoxy-8-(4-amino-l-methylbutyl)aminoquinoline, (**1**) is a radical curative agent for relapsing malaria. It is active against the exoerythrocytic forms of *Plasmodium vivax* and *P. ovale* and against the gametocytes of *P. falciparum*, thus inhibiting sporogony (1). However, primaquine has a low chemotherapeutic index, which limits its prophylactic and therapeutic applications. Although earlier workers suggested that a metabolite(s) of primaquine may be responsible for its toxicity and/or antimalarial activity, very little has actually been accomplished in identifying the metabolite(s) of primaquine. Therefore, we have undertaken a reinvestigation of the mammalian metabolism of the drug (2, 3). In this connection it was desirable to prepare some deuterium labeled primaquines (4). Five monodeutero derivatives were prepared in which each quinoline ring hydrogen was substituted in turn with deuterium. It was found that sodium borodeuteride reduction of the corresponding bromo-6-methoxy-8-aminoquinoline in the presence of palladium catalyst followed by alkylation produced the corresponding deuteroprimaquine derivative with greater then 95 % incorporation of deuterium (4). In the case of the 5-bromo derivative there were some unexpected difficulties. It was initially not possible to isolate a product of more than 60–65 % deuterium incorporation. This observation suggested an unusual reactivity of the 5-position of primaquine to proton exchange during work up.

This unusual reactivity of the 5-position of primaquine was further emphasized by the following observations. Primaquine undergoes biotransformation to produce two novel dimers, the directly 5,5″-linked biphenyl-like dimer (**2**) and the 5,5″-methylene linked dimer (**3**) (5, 6), after initial primary amine acetylation. Upon attempted derivatization with trifluoroacetic anhydride, primaquine gives an unexpected bis-derivative, 5-trifluoroacetyl-6-methoxy-8-(4-trifluoroacetamido-1-methylbutyl)aminoquinoline **4** (7). Hydrobromic acid hydrolysis of 5-bromo-6-methoxy-8-acetamidoquinoline produces 7-bromo-6-methoxy-8-aminoquinoline rather than the expected 5-bromo derivative (8, 9).

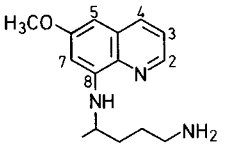
Herein is reported an examination of the chemical reactivity of primaquine toward proton exchange with the medium under various conditions of solvent and pH.

Experimental

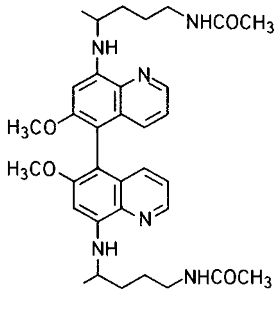
Primaquine diphosphate was purchased from Aldrich Chemical Company as were all the deuterated solvents. The ^{1}H-NMR spectra (90 MHz) were recorded on a Varian EM390 spectrometer (probe temperature 38 ± 2°C) using sodium-2,2-dimethyl-2-silapentane-5-sulfonate (DSS) (deuterium oxide as solvent) or tetramethylsilane (TMS) (deuterated organic solvents) as internal standards. The ^{13}C-NMR spectra (15.03 MHz) were recorded on a JEOL-FX60 FT spectrometer using DSS or TMS as internal standards. Mass spectra were obtained by solid probe inlet using a Finnigan 3200 GC/MS coupled to an INCOS data system.

Determination of Proton Exchange Rate

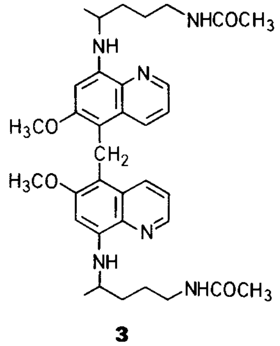
Primaquine diphosphate was weighed directly into an nmr sample tube and an appropriate volume of solvent (deuterium oxide or buffer solution) was added to bring the concentration to the desired level (Table I). The tube was shaken vigorously to speed dissolution, and the proton nmr spectrum was determined as quickly as possible. Typically three to four minutes elapsed before the first determination of peak intensities of C-5H and C-7H could be made. The intensities of these peaks were recorded at two minute intervals for 16 minutes, then after ½, 1, 2, 4, 8 hours and overnight (12–18 hours).



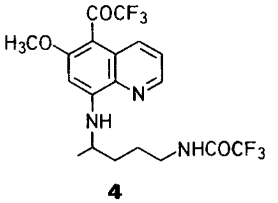
1



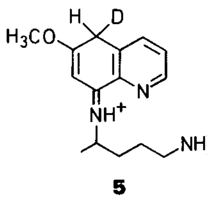
2



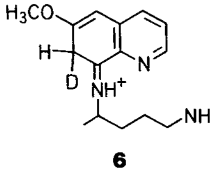
3



4



5



6

---

[1] The Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677

Table I. Apparent pH of solutions of primaquine diphosphate in deuterium oxide.

Unbuffered primaquine diphosphate, mg/ml	Apparent pH
10	3.05
20	2.95
50	2.80
100	2.80
Buffered (see experimental) primaquine diphosphate, mg/ml	Apparent pH
10	6.35
20	6.1
50	5.1
100	4.4

Preparation of Buffer Solutions

One molar stock solutions of potassium dihydrogen phosphate (solution 1) and sodium hydroxide (solution 2) were prepared in deuterium oxide. A mixture of 5.0 ml of solution 1 and 1.15 ml of solution 2 gave a buffered solution of an apparent pH of 6.6. Portions of the stock solutions were diluted 2 fold (stock solutions 1a and 2a) and 10 fold (stock solutions 1b and 2b). Mixture of 5.0 ml of solution 1a and 1.15 ml of solution 1a gave a buffer of apparent pH 6.6 as did a mixture of 5.0 ml of solution 1b and 1.15 ml of solution 2b.

Determination of Apparent pH

Solutions of appropriate concentration (Table I) of primaquine diphosphate in deuterium oxide or buffer were prepared and their apparent pH measured with a calibrated pH meter.

Results

Our inability to deuterate completely the 5-position of the 6-methoxy-8-aminoquinoline derivatives suggested an unusual reactivity of that proton. To assess the extent of this reacitvity, exchange reactions of primaquine with various deuterated reagents were studied. It was observed that there was no exchange of the C-5 proton of the quinoline nucleus when primaquine free base was refluxed in methanol-d_1. (It may also be mentioned here that the C-5 and C-7 protons could be seen as separate doublets using methanol-d_4 as solvent for ^{1}H-NMR determination. Such a separation of signals was not observed in either deuterochloroform ($CDCl_3$) or dimethyl sulfoxide-d_6 in which the two protons appeared as a broad 2 proton singlet.) Treatment of the free base with dilute acidic reagents, deuterotrifluoroacetic acid, deuterosulfuric acid or deuterophosphoric acid, at room temperature gave a product after work up whose ^{1}H-NMR (in $CDCl_3$) suggested that deuterium exchange had occurred at either C-5 or C-7 only. When the ^{1}H-NMR spectrum of commercial primaquine diphosphate was run in deuterotrifluoroacetic acid with tetramethylsilane as internal standard, the signal due to the protons at C-5 and C-7 began to disappear very rapidly and after only two hours the signal was completely absent. Thus it appeared that both the C-5 and the C-7 protons could be exchanged with deuterium from the medium rather easily. However, when the ^{1}H-NMR was taken after workup (neutralization with ammonium hydroxide followed by chloroform extraction), the recovered primaquine was found to give a sharp signal of one proton intensity at 6.3 δ which indicated that reexchange had taken place at one of the two positions during workup. Carbon-13 NMR clearly showed that it was the C-7 which was deuterated and the C-5 position which was now protonated. The signal at 96.8 ppm (C-7) had disappeared while the one at 91.9 ppm (C-5) was a doublet in the off-resonance spectrum. The complete assignment of carbon resonances for primaquine has been reported (10). These results demonstrate that the C-5 proton has some unusual reactivity.

It was not convenient to study the exchange reaction in deuterotrifluoroacetic acid or other strongly acidic media. The use of deuterium oxide as the exchange medium would be advantageous, since the pH can be varied with buffered systems and in addition the reaction conditions in aqueous phase may be considered more similar to those of physiological fluids. The ^{1}H-NMR spectrum of primaquine diphosphate in deuterium oxide with sodium-2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal standard was very similar to that of the free base in deuterochloroform. In Table I is given the variation of apparent pH of an unbuffered solution of primaquine diphosphate in deuterium oxide at different concentrations. It was observed that at a concentration of 100 mg/ml (apparent pH 2.8) there was a rapid exchange at both C-5 and C-7 positions. As quickly as the ^{1}H-NMR could be measured (3–5 minutes after addition of solvent), the broad singlet resonance of H-5 and H-7 was reduced in intensity to less than 1 proton. Repeated determination of the intensity of the resonance showed that the remaining proton was completely exchanged in about 90 minutes. No exchange took place at the C-2, C-3, and C-4 positions. In order to vary the pH of the medium it was necessary to make use of buffers. A mixture of potassium dihydrogen phosphate and sodium hydroxide solutions was used to form an initial pH of 6.6. Three different buffer concentrations were evaluated. Buffer solution of one molar strength resulted in very poor solubility of primaquine diphosphate. The solubility was so poor that it was not possible to obtain a ^{1}H-NMR spectrum. Dilution of the buffer by 10 fold gave a solution of insufficient buffering capacity to control the pH of primaquine diphosphate solutions, especially at higher concentrations of primaquine diphosphate. Solutions formed by only 2 fold dilution appeared to be suitable, especially at lower concentrations of primaquine diphosphate. Table I lists the variation of apparent pH with concentration in buffer solution. In this system, at 100 mg/ml concentration (apparent pH 4.4), the exchange at C-5 was still quite rapid, the proton signal was nearly absent with the first determination of the spectrum which took a maximum time of 3–4 minutes after the dissolution of the diphosphate. The exchange rate at C-7 was somewhat slower, the peak height due to the C-7 proton was reduced to almost one half in 65 minutes. When the solution was left at room temperature overnight and the spectrum was run (after 15 hours), it was found that the C-7 proton was also completely exchanged. No exchange at C-2, C-3, and C-4 was observed. The spectrum of the free base, obtained by careful neutralization with ammonium hydroxide while cooling and extraction with chloroform, showed that both the C-5 and C-7 protons had exchanged completely. This was further confirmed by ^{13}C-NMR determination (absence of peaks for C-5 and C-7) and the mass spectrum which showed M^+ at 261 with m/z 203 as the base peak. (Non-deuterated primaquine gives an M^+ of 259 and a base peak of m/z 201.)

At pH 5.1 (50 mg/ml) the exchange at C-5 was completed after 5 minutes while it took more than 15 hours for the C-7 position to become completely deuterated thus clearly demonstrating the difference in the reactivity of C-5 and C-7 positions. The difference in reactivity between the two positions was more striking as the pH approached a neutral value. At pH 6.1 (20 mg/ml) the exchange at C-5 could be followed by ^{1}H-NMR for up to 15 minutes and at pH 6.35 (10 mg/ml) the time taken for complete exchange at C-5 was a little more than 15 minutes. At pH 6.35 there was no deuterium exchange at any other position including C-7 even after 18 hours at room temperature.

The rate of exchange of proton for deuterium was also determined as a check of our results above using the diphosphate of the 7-deutero derivative of primaquine (4). When the spectrum was run in water it was found that the C-7 deuterium exchanged relatively slowly; it took a little more than 90 minutes for the exchange to reach completion at room temperature and a pH of 3.6.

Discussion

The previously observed reactivity of the C-5 position (5, 6, 7) is confirmed by the deuterium exchange studies. These exchange studies show that the C-5 position of primaquine is unusually reactive to protonation-deprotonation. The C-7 position is somewhat activated to proton exchange but much less so than C-5; an appreciable exchange rate requires a relatively acidic medium. A clue to the nature of this reactivity is provided by the ease with which primaquine-N-acetate undergoes reaction with formaldehyde ("dimerization") (6) and the observed isomerization of 5-bromo-6-methoxy-8-acetamidoquinoline to 7-bromo-6-methoxy-8-aminoquinoline upon hydrobromic acid hydrolysis (8, 9) as well as C-5 acylation during derivatization for GC analysis (7).

A reversible rapid deuteration (protonation) of primaquine at C-5 in the acidic medium (pH 2.8–6.35) would produce an iminium ion (**5**) which may loose a deuteron to reform primaquine or loose a proton to lead to formation of the exchanged primaquine. Similarly, protonation (deuteration) may proceed at C-7 via the isomeric iminium ion, (**6**). However, as C-7 is somewhat more sterically hindered and as *para* activation (C-5) is favored over *ortho* activation (C-7) in aromatic electrophilic substitution, it is not unexpected the C-7 exchange is slower and requires somewhat more acidic media than C-5 exchange. It is assumed that the protonation of primaquine is rapid and reversible providing a pseudo-first order reaction whose rate is dependent on the acidity of the medium. The half-time for exchange at C-5 as determined from a plot of natural logarithm of peak intensity versus time is 4.5 minutes at apparent pH 6.1 and 5.5 minutes at apparent pH 6.35. It is so rapid in media of higher acidity to preclude precise measurement with ^{1}H-NMR techniques.

This observed reactivity of the C-5 position of primaquine is of potential significance relative to the biological activity of the drug. The proton may be viewed as a specific example of the general class of electrophiles. The rapid exchange observed emphasizes the high reactivity of primaquine with electrophiles. This is further supported by the earlier observations of electrophilic substitution at C-5. It is likely that various biological electrophiles may undergo substitution of C-5 *in vivo*. This could provide a mechanism for inactivation of specific enzymes or nucleic acids or attachment to and modification of function of specific membranes. Any or all of these should produce a mechanism for primaquine to bring about its observed biological activities.

Acknowledgements

This investigation received the financial support of the UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases.

The authors also thank Dr. John K. Baker, Department of Medicinal Chemistry, School of Pharmacy, The University of Mississippi for many helpful suggestions.

References

(1) Schmidt, L. H. (1969) Ann. Rev. Microbiol. 23, 427–454.
(2) Baker, J. K., McChesney, J. D., Hufford, C. D., Clark, A. M.,(1982) J. Chromato 230, 69–77.
(3) Clark, A. M., Baker, J. K., McChesney, J. D. J. Pharm. Sci. in press.
(4) McChesney, J. D., Sarangan, S. (1983) J. Labelled Compds. 20, 53–62.
(5) Clark, A. M., Hufford, C. D., Puri, R. K., McChesney, J. D., (1984) Applied Environ. Micro. 47, 540–543.
(6) Clark, A. M., Hufford, C. D., Gupta, R. C., Puri, R. K., McChesney, J. D., (1984) Applied Environ. Micro. 47, 537–539.
(7) Clark, A. M., Evans, S. L., Hufford, C. D., McChesney, J. D. (1982) J. Nat. Products 45, 574–581.
(8) Lauer, W. M., Claus, C. J., Von Korff, R. W., Sundet, S. A. (1952) J. Amer. Chem. Soc. 74, 2080–2082.
(9) McChesney, J. D., Sarangan, S., Hufford, C. D. J. Pharm. Sci. in press.
(10) Clark, A. M., Hufford, C. D., McChesney, J. D. (1981) Antimicro. Agents Chemotherapy 19, 337–341.