SYNTHESIS OF SITE SPECIFICALLY DEUTERATED PRIMAQUINES I. QUINOLINE RING DEUTERATED PRIMAQUINES.

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SUMMARY

Site specific deuterated primaquines were prepared. The attempts to prepare these by deuterolysis of bromoprimaquines were unsuccessful because the required bromoprimaquines could not be produced satisfactorily by alkylation of the various bromo-6-methoxy-8-aminoquinolines. Instead primaquines deuterated at positions 2, 3 and 4 were obtained by alkylation of site specifically deuterated 6-methoxy-8-aminoquinoline. 5-Deuteroprimaaquine was obtained by deuterolysis of the corresponding 5-bromo compound and 7-deuteroprimaquine was obtained by acid catalyzed exchange.

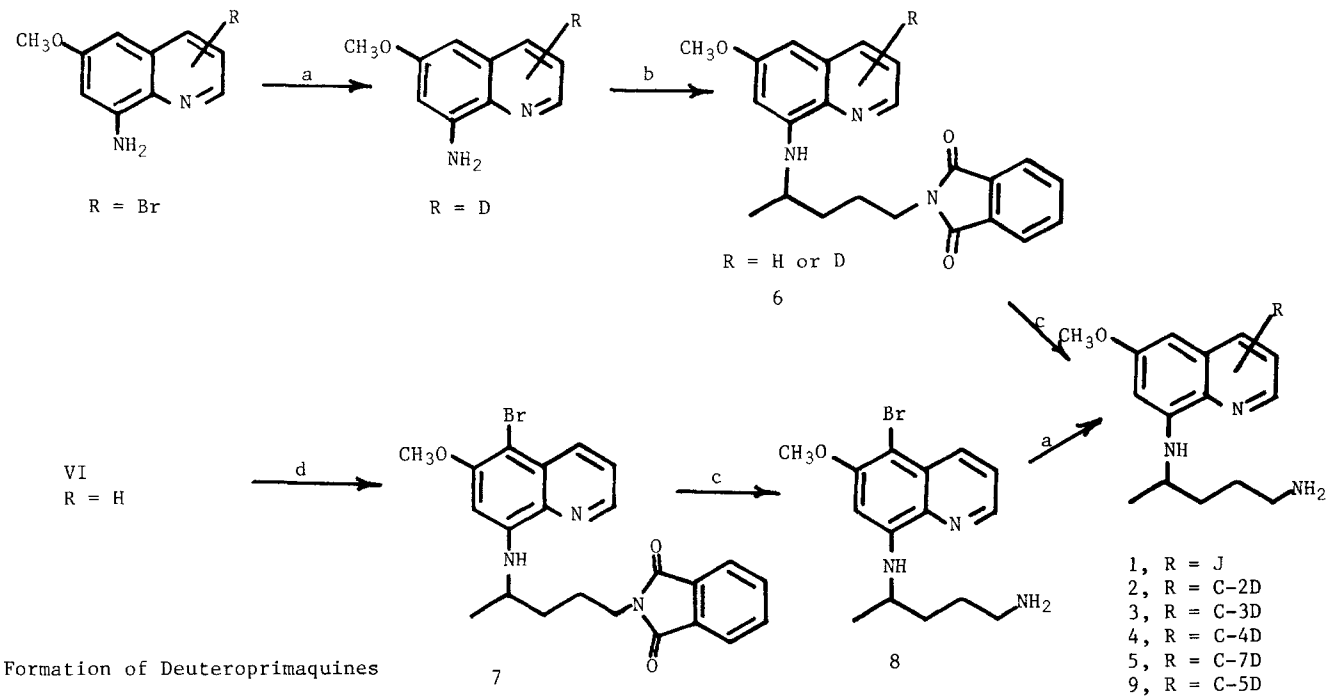
Key words: Primaquine, deuterium

INTRODUCTION

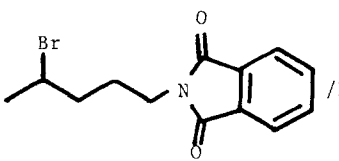
Primaquine [6-methoxy-8-(4-amino-1-methylbutyl)aminoquinoline], 1, is a radcal curative agent for relapsing malaria. It was first synthesized by Elderfield and co-workers. (1) Few studies have been published on the metabolism of this antimalarial drug. (2-4) As a result of these earlier studies quinoline ring hydroxylation has been proposed as the major route of primaquine metabolism. We have undertaken a reinvestigation of the mammalian metabolism as well as the microbiological transformation of this drug. (5,6) In this connection it was desirable to prepare some deuterium-labelled primaquines. There were two reasons for preparing these stable isotope derivatives. 1) In recent years deuterated drugs have been used extensively in drug metabolism studies (7) and these labelled compounds may be used as internal standards in mass fragmentographic studies; 2) further, deuterium labelling at the site of metabolism is known to

0362-4803/83/010053-10$01.00

Received April 8, 1982
Revised July 21, 1982



Scheme - Formation of Deuteroprimaquines

Reagents: a, $NaBD_4/PdCl_2/CH_3OD$; b,  /Et_3N; c, $NH_2NH_2 \cdot H_2O$/EtOH;

d, $C_5H_5N \cdot HBr \cdot Br_2$/HOAc.

influence the metabolic pathway of a drug. (8) If multiple pathways are possible, deuterium substitution can cause a shift in the amount of drug distributed among the competing pathways. Such a change in metabolic pathways may be helpful in understanding the toxic nature of the drug. With the substituents (at positions 6 and 8) present on the quinoline nucleus of the drug, it is reasonable to expect positions 2, 4, 5 and 7 to be possible sites of metabolism. It was therefore decided to deuterate these sites individually in connection with the metabolic study of primaquine. In the present paper we report the preparation of five site specifically deuterated primaquines.

RESULTS AND DISCUSSION

It is normally desirable to introduce the isotopic labelling step towards the end of a multi-step synthesis so as to maximize the overall incorporation yield. Deuterium can be introduced at a specific site of the molecule by a variety of methods. One of the more direct methods involves the hydrogenolysis (deuterolysis) of the appropriate halo compound.

Primaquine has been synthesized, (1,9), by first condensing 1-phthalimido-4-bromopentane with 6-methoxy-8-aminoquinoline and subsequent removal of the protective phthalimide group. The synthetic strategy in our case should then involve the preparation of the appropriate chloro or bromo substituted 6-methoxy-8-aminoquinoline derivatives which could be alkylated with 1-phthalimido-4-bromopentane to introduce the N-alkyl side chain. The resultant halo derivatives would serve as the substrates for deuterium introduction. The required monobromo (2, 3, 4, 5 and 7) substituted 6-methoxy-8-aminoquinoline derivatives were prepared in good yields and characterized by H-1 and C-13 nmr data. (10) However, N-alkylation of these weakly nucleophilic aminoquinolines under all reaction conditions attempted gave extremely poor yields of the desired products. The elimination reaction involving the secondary bromide of the reactant (1-phthalimido-4-bromopentane) was a significant accompanying reaction. It was therefore necessary to introduce deuterium into the molecule prior to alkylation of the amino group.

The deuterolysis of the various bromo-6-methoxy-8-aminoquinolines was accomplished under various conditions:

1) Iron in deuteroacetic acid or iron in deuterosulfuric acid
2) Catalytic reduction with deuterium gas in the presence of palladium and nickel catalysts
3) Formation of the lithium derivative followed by deuterooxide quenching
4) Sodium borodeuteride reduction in the presence of palladium chloride or palladium on charcoal as catalyst.

It was found that the sodium borodeuteride/palladium catalyst method (11) gave good results in the case of the 2, 3 and 4 bromo compounds. There was more than 90% deuterium incorporation as demonstrated by H-1 nmr and ms data. The resultant deutero-6-methoxy-8-aminoquinoline derivatives were alkylated in the usual way and subsequent removal of the phthalimide group gave the appropriate deuteroprimaquines, 2, 3, 4. All the final compounds were characterized by nmr and ms data.

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 proton reexchange during workup of the reaction.

The 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 reactivity, 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 H-1 nmr determination. Such a separation of signals was not observed in either deuterochloroform 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 whose proton nmr suggested that deuterium exchange had occurred at either C-5 or C-7 only. When the proton nmr spectrum was run in deuterotriflouroacetic acid with tetramethylsilane as internal standard, the signals due to the protons at C-5 and C-7 began to disappear very rapidly and

after only two hours both signals are absent. Thus it appeared that both the C-5 and the C-7 protons could be exchanged with deuterium rather easily. However, when the proton nmr was taken after workup (ammonium hydroxide neutralization followed by chloroform extraction) the recovered primaquine was found to give a sharp signal (one proton) 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. These results clearly demonstrate that the C-5 proton has some unusual reactivity. Exact determination of the rate of exchange of both positions will be reported elsewhere.

The 5-deutero compound was obtained by the following method. Bromination of the phthalimido derivative of primaquine with pyridinium hydrobromide perbromide in glacial acetic acid gave a very good yield of the monobromo derivative. Proton nmr analysis showed a singlet (one proton) at 6.4 δ indicating that bromination had occurred either at C-5 or C-7. Carbon 13 nmr data clearly showed that it is C-5 and not C-7 which was brominated. In the proton noise decoupled spectrum there were two signals, at 89.5 and 93.5 ppm respectively. In the off-resonance spectrum the former appeared as singlet while the latter was a doublet. In the proton-coupled spectrum the signal at 89.5 ppm appeared as a triplet (J=4.9 Hz). This is due to three bond coupling between C-5 and H-4 and C-5 and H-7. The signal at 93.5 ppm appeared as a doublet of doublets (J=165 Hz, 4.5 Hz), one bond coupling to H-7 and three bond coupling to C-8-NH. This latter coupling diappears with deuterium oxide exchange. Thus it was proved that bromination had taken place at C-5. Removal of the phthalimide group followed by sodium borodeuteride reduction gave the 5-deutero primaquine, 9, with more than 90% deuterium incorporation.

Work is in progress to deuterate specific sites on the N-alkyl chain.

EXPERIMENTAL[1]

General Procedure for Deuterolysis:

The specific bromo-6-methoxy-8-aminoquinoline was repeatedly dissolved in MeOD and the solvent was evaporated under reduced pressure so that exchangeable hydrogens would be exchanged. The bromo compound (ca. 1 mmole) was then dissolved in MeOD (10 ml) and $PdCl_2$ (ca. 3/4 w/w) was added. The addition of $NaBD_4$ (ca 3/2 w/w) was accomplished over a period of 15 minutes. The mixture was stirred at room temperature for about 2 hours (until TLC examination indicated completion of the reaction). The solvent was removed under reduced pressure and the residue was treated with 1 ml of ice-cold 1 N aqueous HCl and then extracted with $CHCl_3$. The organic phase was washed with saturated brine and then dried over anhydrous Na_2SO_4. Removal of the solvent gave a pale straw colored oil which was homogenous by TLC, GC and HPLC.

2-Deutero-6-methoxy-8-aminoquinoline:

2-Bromo-6-methoxy-8-aminoquinoline (0.51 g, 2 mmole), $PdCl_2$ (0.12 g) and $NaBD_4$ (0.48 g). Yield: 0.342 g (98%). ^{1}H NMR ($CDCl_3$): 7.87 δ (1H, d, J=8 Hz, C-3H), 7.23 (1H, d, J=8, C-4H), 6.55 (1H, d, J=2, C-7H), 6.41 (1H, d, J=2, C-5H) and 3.87 (3H, s, $-OCH_3$). MS (m/z): 175 (M^+, 100.0%), 174 (absent), 146 (69.8), 145 (48.8), 133 (12.2), 132 (68.5), 118 (31.2).

3-Deutero-6-methoxy-8-aminoquinoline:

3-Bromo-6-methoxy-8-aminoquinoline (0.26 g, 1 mmole), $PdCl_2$ (0.12 g) and and $NaBD_4$ (0.24 g). Yield: 0.16 g (92.0%). ^{1}H NMR ($CDCl_3$): 8.58 δ (1H, d, J=2 Hz, C-2H), 7.91 (1H, d, J=2 Hz, C-4 H), 6.58 (1H, d, J=2 Hz, C-7H), 6.43 (1H, d, J=2 Hz, C-5H) and 3.80 (3H, s, OCH_3). MS (m/z): 175 (M^+, 100.0%), 174 (absent), 146 (81.5), 145 (54.6), 144 (23.3), 133 (35.0), 132 (75.8), 118 (32.5).

[1] The ^{1}H-NMR spectra (90 MHz) were recorded on a Varian EM 390 spectrometer using tetramethylsilane as an internal standard. The ^{13}C-NMR spectra (15.03 MHz) were recorded on a JEOL-FX60 FT spectrometer using tetramethylsilane as internal standard. Mass spectra were obtained using a Finnigan 3200 GC/MS coupled to an INCOS data system. HPLC was accomplished on a μBondapak C_{18}-reverse phase column and a mobile phase of 2.2 g KH_2PO_4, 3.3 g K_2HPO_4, 1.2 L distilled H_2O and 2.8 L HPLC quality MeOH. TLC was accomplished on Silica G (254) precoated plates and GC was done on base deactivated 3% OV-17, 6 ft x ¼ in OD glass column, FID, on a Beckman GC-65 at a flow rate of 30 ml/min of N_2.

4-Deutero-6-methoxy-8-aminoquinoline:

4-Bromo-6-methoxy-8-aminoquinoline (0.52 g, 2 mmole); $PdCl_2$ (0.12 g) and $NaBD_4$ (0.48 g). Yield: 0.325 g (93.0%). ^1H NMR ($CDCl_3$): 8.5 δ (1H, d, J=5 Hz, C-2H), 7.2 (1H, d, J=5, C-3H), 6.53 (1H, d, J=2, C-7H), 6.4 (1H, d, J=2, C-5H) and 3.83 (3H, s, $-OCH_3$). MS (m/z): 175 (M^+, 100.0%), 174 (absent), 146 (85.3), 145 (55.0), 133 (23.7), 132 (75.7), 118 (36.0).

General Procedure for 6-methoxy-8-aminoquinoline alkylation:

The deutero-6-methoxy-8-aminoquinoline, 1-phthalimido-4-bromopentane, and trimethylamine were heated under nitrogen atmosphere at 120-130° for 24 hours. At the end of the first 12 hours, additional amounts of triethylamine (1.0 ml) and 1-phthalimido-4-bromopentane (1.5 g) were added. After 24 hours the mixture was cooled and diluted with $CHCl_3$ (200 ml). The solution was filtered and the clear filtrate washed sequentially with 5% aqueous KOH and saturated brine. The organic phase was separated, the solvent evaporated under reduced pressure and the residue dissolved in 50 ml of EtOH. To this solution was added 3.0 ml of $NH_2NH_2 \cdot H_2O$ and the mixture refluxed for 3 hours. The hydrazinolysis mixture was cooled, concentrated under reduced pressure and the solid phthalyhydrazide filtered. The resultant filtrate was further concentrated and the residue was adsorbed onto a column of neutral alumina (Act. II-III). Elution with $CHCl_3$ gave a pale yellow oil which was homogeneous by TLC, GC and HPLC.

2-Deutero-6-methoxy-8-(4-amino-1-methylbutyl)aminoquinoline, 2:

2-Deutero-6-methoxy-8-aminoquinoline (1.75 g), 1-phthalimido-4-bromopentane (4.5 g) and triethylamine (2.0 ml). Yield: 1.78 g (68.4%). ^1H NMR ($CDCl_3$): 7.86 δ (1H, d, J=8 Hz, C-3H), 7.23 (1H, d, J=8 Hz, C-4H), 6.31 (2H, s, C-5H and C-7H), 3.87 (3H, s, $-OCH_3$) 2.67 (2H, t), 1.57 (4H, m), 1.2 3H, d, J=7 Hz); ^{13}C NMR (PND): 159.7 (C-6), 145.3 (C-8), 134.7 (C-4), 130.0 (C-4a), 121.6 (C-3), 96.8 (C-7), 91.8 (C-5). MS (m/z): 260 (M^+, 10.1), 259 (1.8), 243 (M^+-NH_3, 10.1), 202 (100.0), 201 (25.9), 157 (27.0), 176 (52.3), 175 (26.3).

3-Deutero-6-methoxy-8-(4-amino-1-methylbutyl)aminoquinoline, 3:

3-Deutero-6-methoxy-8-aminoquinoline (1.63 g), 1-phthalimido-4-bromopentane

(4.5 g) and triethylamine (2.0 ml). Yield: 1.74 g (72.0%). ^{1}H NMR ($CDCl_3$): 8.50 δ (1H, d, J=2 Hz, C-2H), 7.84 (1H, d, J=2 Hz, C-4H), 6.21 (2H, bs, C-5H and C-7H), 3.86 (3H, s, OCH_3). ^{13}C NMR (PND): 159.6 (C-6), 145.2 (C-8), 144.2 (C-2), 135.5 (C-8a), 134.6 (C-4), 125.6 (C-4a), 96.8 (C-7), 91.9 (C-5). MS (m/z): 260 (M^+, 6.0%), 259 (absent), 243 (M^+-NH_3, 4.0), 203 (25.0), 202 (100.0), 201 (absent), 187 (24.2), 177 (27.2), 176 (33.0), 175 (30.3).

4-Deutero-6-methoxy-8-(4-amino-1-methylbutyl)aminoquinoline, 4:

4-Deutero-6-methoxy-8-aminoquinoline (0.9 g), 1-phthalimido-4-bromopentane (2.0 g) and triethylamine (1.5 ml). Yield: 0.72 g (55.0%). ^{1}H NMR ($CDCl_3$): 8.71 δ (1H, d, J=5 Hz, C-2H), 7.47 (1H, d, J=5 Hz, C-3H), 6.43 (2H, s, C-5H and C-7H), 3.98 (3H, s OCH_3). MS (m/z): 260 (M^+, 9.3), 259 (1.2), 243 (M^+-NH_3, 10.9), 202 (100.0), 201 (21.6), 187 (24.5), 176 (44.0), 175 (20.6).

5-Bromo-6-methoxy-8-(4-amino-1-methylbutyl)aminoquinoline, 8:

6-methoxy-8[(4-phthalimidoamino)-1-methylbutyl]aminoquinoline (0.3 g) was dissolved in glacial acetic acid (5 ml). Pyridinium hydrobromide perbromide (0.27 g) was added and the mixture was stirred at room temperature. After 1 hour the solution was made basic with ammonia and extracted with $CHCl_3$. The organic layer was washed with saturated brine and evaporated. The reddish-brown oily residue was homogeneous by TLC and HPLC. Yield - 0.3 g (81.0%). ^{1}H NMR ($CDCl_3$): 8.47 δ (1H, dd, J=5, 2 Hz, C-2 H), 8.30 (1H, dd, J=8, 2 Hz, C-4 H), 7.63 (4H, m, phthalimide aromatic), 7.30 (1H, dd, J=8, 5 Hz, C-3 H), 6.4 (1H, s, C-7H), 4.00 (3H, s, $-OCH_3$), 3.78 (3H, m, C-1' and C-4' H), 1.83 (4H, m, C-2' and C-3' H), 1.3 (3H, d, C-5'). ^{13}C NMR: 89.5 (s, SFORD, C-5), 93.5 (d, SFORD, C-7). The phthalimido group was removed by hydrazinolysis and the product was purified by column chromatography on neutral alumina as above. Yield: 0.18 g.

5-Deutero-6-methoxy-8-(4-amino-1-methylbutyl)aminoquinoline, 9:

5-Bromo-6-methoxy-8-(4-amino-1-methylbutyl)aminoquinoline (0.17 g) was reduced by $NaBD_4$ (0.1 g) and $PdCl_2$ (0.1 g) in MeOD. The product was worked up as above to produce a pale yellow oil which was homogeneous by HPLC and GC. Yield: 0.122 g (47.0%). ^{1}H NMR ($CDCl_3$): 8.50 δ (1H, dd, J=8, 2 Hz, C-2H), 7.87 (1H,

dd, J=5, 2 Hz, C-4H), 7.25 (1H, dd, J=8, 5 Hz, C-3H), 6.33 (1H, s, C-7H) and 3.98 3H, s, $-OCH_3$). ^{13}C NMR (PND): 159.7 (C-6), 145.3 (C-8), 144.3 (C-2), 135.6 (C-8a), 134.9 (C-4), 130.0 (C-4a), 121.8 (C-3), 96.8 (C-7), 55.1 (OCH_3), 48.1 (C-2'), 42.1 (C-5'), 34.2 (C-4'), 30.1 (C-3') and 20.6 (C-1'). MS (m/z): 260 (M^+, 4.7%), 259 (absent) 243 (M^+-NH_3, 4.0), 203 (13.0), 202 (100.0), 201 (absent), 187 (2.05),177 (30.4) and 176 (39.0).

7-Deutero-6-methoxy-8-(4-amino-1-methylbutyl)aminoquinolines, 5:

Primaquine (free base, 2.2 g) was first repeatedly treated with MeOD and dried under vacuum for 8 hours. It was then dissolved in 98% D_2SO_4 (6 ml) and the solution was stirred at 60-65°C under nitrogen atmosphere for 18 hours. Crushed ice was added and the solution was neutralized with excess NH_4OH and extracted with CH_2Cl_2. The organic layer was separated, washed with saturated brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to yield a pale yellow oil, 1.8 gm (69.0%). ^1H NMR ($CDCl_3$): 8.47 (1H, dd, J=5, 2 Hz, C-2H), 7.83 (1H, dd, J=8, 2 Hz, C-4H), 7.20 (1H, dd, J=5, 8 Hz, C-3H), 6.27 (1H, bs, C-5H), 3.86 (3H, s, OCH_3). ^{13}C NMR (PND): 159.6 (C-6), 145.2 (C-8), 144.2 (C-2), 135.6 (C-8a), 134.7 (C-4), 130.0 (C-4a), 121.8 (C-3), 91.9 (C-5), 55.1 (OCH_3), 48.1 (C-2'), 42.0 (C-5'), 34.2 (C-4'), 30.0 (C-3') and 20.5 (C-1'). MS (m/z): 260 (M^+, 6.5%), 259 (7.0), 243 (M^+-NH_3, 9.6), 203 (13.5), 202 (100.0), 201, (absent) 187 (16.7) 176 (32.9), 175 (37.4). Exchange with D_3PO_4 or CF_3CO_2D gave similar results.

ACKNOWLEDGMENTS

Supported by the UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases.

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