Metabolites of Chloroquine: Some Observations on Desethylchloroquine and *N*-Acetyldesethylchloroquine

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Abstract □ The major metabolite of chloroquine, (+)-desethylchloroquine, produced by stereoselective N-dealkylation of the drug, was obtained in 81.5% enantiomeric purity by resolution of racemic desethylchloroquine using an atropisomeric resolving agent and was shown by circular dichroism to have the absolute (*S*)-configuration. The minor metabolite *N*-acetyldesethylchloroquine was prepared in both the racemic and the (*S*)-(+)-form.

Chloroquine (Figure 1, **1a**) [7-chloro-4-[4′-(diethylamino)-1′-methylbutyl]aminoquinoline] has been the mainstay of malaria prophylaxis and treatment since 1946.[1] Early observations[2] reported the isolation of 40% of the dose of the drug given to man and identified 30% as unchanged drug and 10% as the major metabolite 7-chloro-4-[4′-(ethylamino)-1′-methylbutyl]-aminoquinoline (Figure 1, **1b**) (desethylchloroquine). This work has been repeated recently[3,4] using different analytical technology, with essentially the same results, accounting for ca. 55% of the administered dose and reporting 38.5–46% of unchanged drug and 12.0–12.65% of desethylchloroquine. The most remarkable observation[2] however was the finding of high optical activity ($[\alpha]_D$ +145°) in the metabolite. Since chloroquine is administered as the racemic diphosphate, this strongly suggests either the stereoselective interaction of the racemic drug at an asymmetric macromolecular receptor or a significant difference in the pharmacokinetics of the enantiomers.

Optical isomers of chloroquine[5,6] have been shown to differ in their binding to plasma, albumin, α_1-acid glycoprotein,[7] and DNA[8] and in their *in vivo* antimalarial activities against *Plasmodium berghei*[9] and *Plasmodium vinckei*.[8] In addition, the (+)-isomer showed lower toxicity compared with (−)-chloroquine.[9,10] The likelihood of stereoselective metabolism *in vivo* was further supported by the finding of stereoselective renal clearance.[11] Observations *in vitro* (where no metabolism occurs) showed that no significant differences existed between the activities of the enantiomers against both the chloroquine-sensitive and the resistant strain of *Plasmodium falciparum*.[10,12]

For a better understanding of the stereochemistry of chloroquine metabolism, it was desirable to establish the absolute configuration of the optically active major metabolite desethylchloroquine. It was also of interest to examine the properties of an additional metabolite of chloroquine recently[13] identified by chemical ionization mass spectrometry as *N*-acetyldesethylchloroquine (Figure 1, **1c**) but never isolated.

Attempts to resolve chloroquine using a wide range of acids have been uniformly unsuccessful.[5,14] Although bromocamphorsulfonic and was reported[14] to give enantiomers with equal and opposite rotations, it was later shown[5] that the optical purity of the material obtained was only 12%, explaining the observed identical antimalarial activity against *Plasmodium lophurae*[15] in mice. The atropisomeric acid 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate has been resolved by salt formation (1:1) with the quinine bases cinchonine or cinchonidine.[16] Since the synthetic antimalarial drugs are chemically and structurally very similar to the quinine alkaloids, the use of this acid as a resolving agent was investigated.

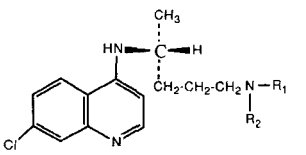

Figure 1—Chloroquine and its metabolites: (*R*)-(−)-**1a**, $R_1 = R_2 = C_2H_5$; (*R*)-(−)-**1b**, R_1 = H, $R_2 = C_2H_5$; (*R*)-(−)-**1c**, $R_1 = COCH_3$, $R_2 = C_2H_5$.

For chloroquine, the reaction of the base (1 mol) with 1 mol of the (+)-binaphthyl-2,2′-diyl phosphate was found[17] to give a highly stereoselective "kinetic resolution" resulting in 0.5 mol of the 2:1 (acid:base) diastereomeric salt of (−)-chloroquine, leaving in solution the opposite enantiomer (0.5 mol) of the base. By this means, both optical isomers of chloroquine could be obtained in 98–99% ee. The application of this method to desethylchloroquine was therefore of interest.

Results and Discussion

Resolution. Salt formation between racemic desethylchloroquine[18] and the (−)-binaphthyl phosphate[16] gave the same mixture of two diastereomeric salts whether a 2:1 (acid:base) or a 1:1 (acid:base) ratio was used. The less soluble diastereomer could be recrystallized to constant melting point and rotation, and only the (hydrated) 2:1 (acid:base) salt was formed. Liberation of the base gave (+)-desethylchloroquine of $[\alpha]_D$ +124 ± 1°. The more soluble diastereomeric salt afforded (−)-desethylchloroquine of $[\alpha]_D$ −124 ± 1°, which could also be obtained by employing the (+)-binaphthyl phosphate in exactly the same procedure. An HPLC assay with a chiral α_1-acid glycoprotein column, using a modification of the method of McLachlan, Tett, and Cutler,[19] showed that the base with $[\alpha]_D$ +122.5° had an enantiomeric ratio of 81.3% (+) and 18.7% (−).[20] The diastereomeric salt, the rotation of which remained unchanged after four additional crystallizations, was therefore most likely a crystal aggregate of constant composition containing an approximately 2:1 mixture of (*S*):(*RS*) isomers.[21] Formation of such aggregates has been reported in optical resolutions.[22]

A (+)-desethylchloroquine sample of $[\alpha]_D$ +125°, containing 81.5% of the (+)-enantiomer, was used to determinne the absolute configuration. Its circular dichroism (CD) spectrum was in good agreement with its UV absorption spectrum, and a comparison with the CD spectrum of (*S*)-(+)-chloroquine showed the two CD spectra to be superimposable (Figure 2). The absolute configuration of the major metabolite desethylchloroquine is therefore (*S*), suggesting that the optically active (+)-metabolite is formed from the (+)-enantiomer of the drug by enzymatic N-dealkylation. From the enantiomeric ratio found by HPLC of the sample of $[\alpha]_D$ +125°, the isolated optically active metabolite[2] of $[\alpha]_D$ +145° consisted of ca. 87% of the (+)-isomer and 13% of (−)-isomer.

The minor metabolite of chloroquine, *N*-acetyldesethylchloroquine, was prepared by acetylation of racemic desethylchlo-

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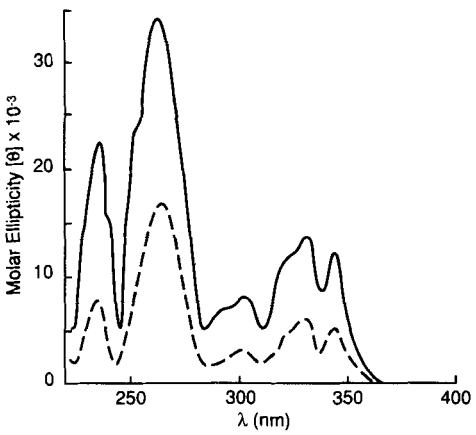

Figure 2—Circular dichroism spectra in water for (*S*)-(+)-chloroquine diphosphate (- -) and (*S*)-(+)-desethylchloroquine dihydrochloride (—).

roquine and had mp 135 °C. It was characterized by TLC and by UV, IR, and ^{1}H-NMR spectroscopy. It is probable that the minor metabolite[13] was also the (*S*)-isomer. Unfortunately, the analytical HPLC column used by Brown *et al.*[13] did not permit chiral separation, and the question therefore remains unanswered. However, (*S*)-(+)-*N*-acetyldesethylchloroquine was prepared by monoacetylation of resolved (*S*)-(+)-desethylchloroquine and had mp 155 °C and $[\alpha]_D$ +120.5°.

In conclusion, the major metabolite of chloroquine has been shown to have the absolute (*S*)-(+)-configuration and the minor metabolite *N*-acetyldesethylchloroquine has been prepared in both the racemic and the (*S*)-(+)-form.

Experimental Section

Analytical Procedures—Analytical thin-layer chromatography was performed using Analtech F-254 glass-backed thin-layer silica gel plates (250 μm). Melting points were obtained with a Kofler Hot Stage melting point apparatus and are corrected. Column chromatography was done using Fluka flash Florisil (200–300 mesh). Microanalyses were performed by the microanalytical laboratory, Department of Chemistry, University of California, Berkeley. Optical rotations were determined using a Perkin-Elmer Model 241 polarimeter. Infrared spectra were taken on a Nicolet Model 5DX FTIR spectrometer in chloroform solution. Circular dichroism spectra were recorded on a JASCO Model 500 spectropolarimeter at 25 °C and ultraviolet spectra on a Hewlett-Packard Model 8452-A diode-array spectrophotometer. Proton magnetic resonance spectra were measured in deuteriochloroform with tetramethylsilane as the internal standard on a General Electric QE-300 instrument.

(*S*)-(+)-7-Chloro-4-[4′-(ethylamino)-1′-methylbutyl]aminoquinoline—*A*—Racemic desethylchloroquine[18] (0.2915 g, 1 mmol) in 1 mL of methanol was treated with a solution of 0.348 g (1 mmol) of (*R*)-(−)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate[16] in 17 mL of hot methanol. The mixture was evaporated *in vacuo* and the residue crystallized from refluxing methanol:H_2O (1:1) to give the levorotatory salt (0.266 g, 53%). Several recrystallizations afforded colorless rhombic plates of constant melting point (mp 245–246 °C) and rotation [$[\alpha]^{25}_D$ −359° (*c* = 0.5, MeOH)] in a final yield of 17–23% based on a 2:1 (acid:base) salt. Anal. Calcd for $C_{56}H_{48}ClN_3O_8P_2{\cdot}6H_2O$: C, 61:34; H, 5.47; N, 3.83. Found: C, 61.53, 61.70; H, 5.23, 5.44; N, 3.54, 3.82%. Loss in weight on heating to 100 °C at 0.005 mm vacuum: 10.15%. Calculated for $6H_2O$: 9.86%. The salt was suspended in water, and the suspension was adjusted to pH 9 with aqueous NH_3 solution, saturated with NaCl, and extracted with CH_2Cl_2. The washed (NaCl solution) and dried (anhydrous Na_2SO_4) extracts were evaporated *in vacuo* to give (*S*)-(+)-desethylchloroquine, purified by flash chromatography on florisil ($CHCl_3$): $[\alpha]^{25}_D$ +122.5° (*c* = 0.6, MeOH); TLC, single spot, identical with an authentic racemic sample, R_f 0.36 (EtOAc:MeOH, 1:1 + 5% triethylamine); IR ($CHCl_3$) 3684, 3613 cm^{-1} (NH); ^{1}H NMR δ 1.13 (t, 3H, CH_3), 1.31 (d, 3H, CH_3), 1.43 (bs, ^{1}H, NH, exchanged w, D_2O), 1.72 (m, 4H, CH_2CH_2), 2.65 (m, 4H, NCH_2), 3.70 (q, 1H, CH), 5.59 (d, 1H, NH, exchanged w, D_2O), 6.4–8.5 (m, 5H, aromatic CH).

The enantiomeric composition was determined using a 5-μm chiral α_1-acid glycoprotein column (150 × 4 mm, Regis Chemical Co.). The mobile phase consisted of 20 mM KH_2PO_4 containing 12% (v/v) ethanol and 0.08% *N,N*-dimethyloctylamine, adjusted to pH 7.1 with 1 M KOH. Injection volume was 3 μL and the flow rate 0.8 mL/min. The (*R*)-isomer eluted first (24.5 min) followed by the (*S*)-isomer (27.5 min). The enantiomeric ratio was 18.7% (*R*):81.3% (*S*).

B—Repetition of the above experiment (A) using 2 equiv of the same resolving acid afforded the same salt (57% initial yield, 17% final yield) and gave (*S*)-(+)-desethylchloroquine having $[\alpha]^{25}_D$ + 123.3° (*c* = 0.2, MeOH); the dihydrochloride salt (obtained by addition of 10% ethanolic HCl) had $[\alpha]^{25}_D$ +132.4° (*c* = 0.2, MeOH): UV λ_{max}, nm (H_2O) (ε, M^{-1} cm^{-1}) 342 (18 500), 336 (min.) (16 000), 330 (18 000), 282 (min.) (2000), 256 (16 500), 244 (15 000), 236 (19 500), 220 (29 000), 202 (min.) (12 000). CD max (*c* = 0.0715, H_2O) [nm (θ)] 344 (12 170), 331 (14 200), 300 (8110), 263 (35 490), 236 (23 325). The mother liquors from the initial salt were freed of methanol *in vacuo*, and the residue was dissolved in water. Liberation of the base as described in part A gave (*R*)-(−)-desethylchloroquine [$[\alpha]^{25}_D$ +122.7° (*c* = 0.3, MeOH)] identical (TLC, IR) with an authentic racemic sample.

(*R*)-(−)-7-Chloro-4-[4′-(ethylamino)-1′-methylbutyl]aminoquinoline (Figure 1, 1b)—Repetition of experiment B using 2 equiv of the opposite acid, (*S*)-(+)-1,1′-binaphthyl-2,2′-diyl hydrogen phosphate, gave the enantiomeric 2:1 (acid:base) salt (mp 246–247 °C) as rhombic plates: $[\alpha]_D$ +364.1° (*c* = 0.4, MeOH) (final yield 23%) as the hydrate. Anal. Calcd for $C_{56}H_{48}ClN_3O_8P_2{\cdot}6H_2O$: C, 61.34; N, 5.47; N, 3.83. Found: C, 61.49, 61.78; H, 5.49, 5.41; N, 3.81, 3.81. Liberation of the base gave (*R*)-(−)-desethylchloroquine [$[\alpha]^{25}_D$ −125° (*c* = 0.2, MeOH)] identical (TLC and IR) with authentic racemic material.

7-Chloro-4-[4′-(*N*-acetyl-*N*-ethylamino)-1′-methylbutyl]aminoquinoline—Racemic desethylchloroquine (0.030 g, 0.0103 mmol) in dry dichloromethane was stirred under N_2 and treated with triethylamine (0.020 g) and acetyl chloride (0.010 g). After stirring for 1 h, TLC (EtOAc:MeOH, 2:1 + 5% TEA) showed the absence of starting material. After removal of solvent, the residue was dissolved in ether, washed ($NaHCO_3$, H_2O, NaCl solution), and dried (Na_2SO_4). Removal of solvent left the acetyl derivative (0.022 g, 65%), that crystallized from benzene: petroleum ether as white crystals: mp 135 °C; TLC, single spot, R_f 0.66; UV λ_{max} (ε) 342 (18 500), 330 (18 000), 282 (min) (2000), 256 (16 500), 220 (29 000), 202 (12 000); IR (KBr) 3381, 3333 (NH), 1637.5 ($NCOCH_3$) cm^{-1}; ^{1}H NMR δ 1.10 (t, 3H, CH_3), 1.21 (d, 3H, CH_3), 1.58 (m, 4H, CH_2-CH_2), 2.05 (s, 3H, $COCH_3$), 3.23 (m, 4H, NCH_2), 3.49 (q, 1H, CH), 5.76 (d, 1H, NH, exchanged with D_2O), 6.33–8.42 (m, 5H, aromatic CH). Anal. Calcd for $C_{18}H_{24}ClN_3O$: C, 64.77; H, 7.26; N, 12.59. Found: C, 64.38; H, 7.25; N, 12.52.

(*S*)-(+)-7-Chloro-4-[4′-(*N*-acetyl-*N*-ethylamino)-1′-methylbutyl]aminoquinoline—(*S*)-(+)-Desethylchloroquine (6 mg) in dry dichloromethane was acetylated by the method used for the racemic compound (above). The acetyl derivative (6 mg, 85%) was obtained as colorless prismatic needles: mp 155 °C; $[\alpha]_D$ +120.5° (*c* = 0.1, EtOH); TLC, single spot, R_f 0.66. IR and UV, identical with those of the racemic compound. Anal. Calcd for $C_{18}H_{24}{}^{35}ClN_3O$ 333.16079 and $C_{18}H_{24}{}^{37}ClN_3O$ 335.15784, found 333.16000 and 335.15745 by HR-MS (EI).

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