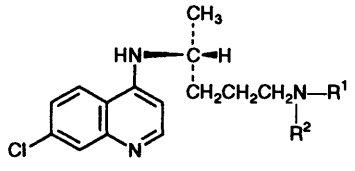
Kinetic Resolution of 7-Chloro-4-{4-[ethyl(2-hydroxyethyl)amino]-1-methylbutylamino}quinoline (Hydroxychloroquine) by an Atropisomeric Resolving Agent

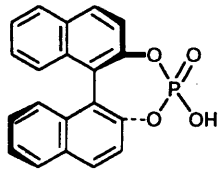
Aslam M. Ansari and J. Cymerman Craig*
Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143-0446, USA

The enantiomers of 7-chloro-4-{4-[ethyl(2-hydroxyethyl)amino]-1-methylbutylamino}quinoline (hydroxychloroquine) are not available by classical resolution methods. A procedure for the kinetic resolution of this compound is described in which the atropisomeric 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate stereoselectively transforms one enantiomer of the base into the diastereoisomeric salt, leaving the other enantiomer as the unchanged base. The products are easily separated, and both enantiomers of hydroxychloroquine are thus accessible in high optical purity. The (+)-enantiomer has been assigned the absolute *S*-configuration by the use of circular dichroism. The optical purity of the diastereomeric salts is obtained from their NMR spectra.

Hydroxychloroquine **1** is widely used in its racemic form as an antimalarial and in the treatment of rheumatoid arthritis[1] and lupus erythematosus.[2] Recent observations suggest that the metabolism of hydroxychloroquine is highly stereoselective,[3] and that the enantiomers show different pharmacokinetic parameters.[4] However, it has not been possible to prepare the enantiomers of hydroxychloroquine by classical resolution methods involving the separation of two diastereoisomeric products.[5,6] Since the atropisomeric† acid 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate **2** was recently[8] resolved by (1:1) salt formation with cinchonine, or cinchonidine, the structural similarities between the quinine bases and hydroxychloroquine led us to investigate the use of this novel acid as a resolving agent.



(–)-(*R*)-**1**; $R^1 = CH_2CH_3$, $R^2 = CH_2CH_2OH$
(–)-(*R*)-**3**; $R^1 = R^2 = CH_2CH_3$



(–)-(*R*)-**2**

Reaction of 1 mol of racemic hydroxychloroquine and 1 mol of the (*S*)-(+)-binaphthylphosphoric acid unexpectedly gave kinetic resolution,‡ affording the (+)-binaphthyl phosphate salt of (–)-hydroxychloroquine as a hydrated 2:1 (acid:base) compound, leaving in solution the (+)-isomer of the base which could be directly extracted from the above salt in >60% optical purity and transformed into the mirror image diastereoisomeric salt with 2 mol of the (*R*)-(–)-binaphthylphosphoric acid. This is analogous to the recently reported kinetic resolution of β-hydroxy amines (such as *N*-methylephedrine) by selective oxidation (using a chiral catalyst) of one enantiomer to the *N*-oxide, leaving the other enantiomer as the unchanged amine.[10]

The two diastereomeric salts, (–)-hydroxychloroquine (+)-binaphthyl phosphate and (+)-hydroxychloroquine (–)-binaphthyl phosphate, gave identical ^1H NMR spectra (as expected since they are enantiomers),[11] both showing only one signal at 1.26 ppm (split into a doublet, $J = 6$, by the C-H) for the protons of the methyl group attached to the asymmetric centre. However, the salt made from one mol of the *racemic* base and 2 mol of the (+)-binaphthylphosphoric acid consists of two diastereoisomers which are not mirror images, and each methyl group gave its own characteristic doublet ($J = 6$) at different chemical shifts (1.22 and 1.26 ppm) in a 50:50 ratio of intensities (Fig. 1). These assignments were verified by the preparation of the salt from (–)-hydroxychloroquine and the (–)-binaphthylphosphoric acid, which gave only the upfield doublet ($J = 6$) at 1.22 ppm.

When the ^1H NMR spectrum of the (–)-base (+)-binaphthyl phosphate salt was run on a 500 MHz instrument (Fig. 2), the missing upfield doublet at 1.22 ppm could be detected and calculation of the integrated peak intensities gave an enantiomer ratio of 98.5 : 1.5 (97% ee). Monitoring the ratio of the two doublets at 1.22 and 1.26 ppm in the resolved diastereoisomeric binaphthyl phosphates thus allows a rapid determination of the optical purity of the optically active free bases which will be obtained from them.

The respective bases could be isolated (ammonia–chloroform) from the pure diastereoisomeric salts, and the resolving acid recovered from the aqueous alkaline layer. The bases could also be obtained by filtration through a column of alkaline alumina (pH 10) without the use of water. The oily bases were transformed into their diphosphate salts by reaction with phosphoric acid in ethanol, and lyophilized to give microcrystalline solids.

The CD spectrum of (+)-hydroxychloroquine diphosphate (Fig. 3) was found to be superimposable on that of (*S*)-(+)-chloroquine[12] **3** (Fig. 1) which has been[13] correlated with L-glutamic acid. The absolute configuration of (+)-hydroxychloroquine is thereby established as *S*.

† Atropisomerism refers to chirality due to restricted rotation about a single bond (in this case dissymmetry rather than asymmetry).[7]
‡ Kinetic resolution is defined as a process in which one of the enantiomeric constituents of a racemic mixture is more readily transformed into a product than is the other.[9]

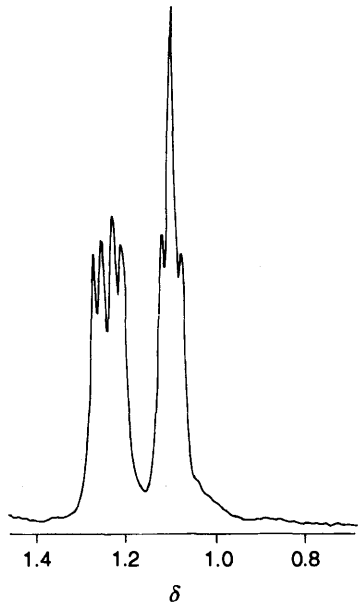


Fig. 1 ^{1}H NMR spectrum ([$^2H_6$]Me_2SO) of racemic hydroxychloroquine (+)-binaphthyl phosphate (300 MHz)

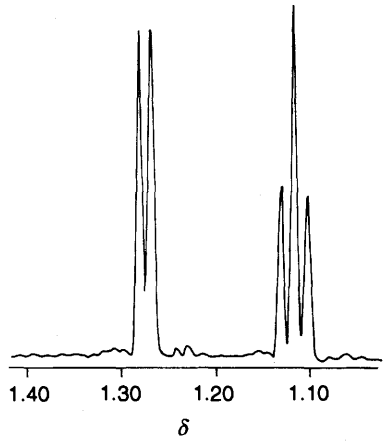


Fig. 2 ^{1}H NMR spectrum ([$^2H_6$]Me_2SO) of (−)-hydroxychloroquine (+)-binaphthyl phosphate (500 MHz)

Experimental

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, and CD spectra on a JASCO model 500 spectropolarimeter at 25 °C. UV spectra were obtained on a Hewlett-Packard model 8452-A diode array spectrophotometer, and mass spectra on a VG-7070 instrument by the Mass Spectrometry Research Resource, University of California, San Francisco. Proton magnetic resonance spectra were recorded in deuteriochloroform, using tetramethylsilane as the internal standard, on a General Electric QE-300 instrument at 300 MHz. Coupling constants were obtained from the printouts.

(R)-(−)-7-*Chloro*-4-{4-[*ethyl*(2-*hydroxyethyl*)*amino*]-1-*methylbutylamino*}*quinoline* [(R)-(−)-*Hydroxychloroquine*].—(*a*) Racemic hydroxychloroquine (1.01 g, 3 mmol, m.p. 91–92 °C) [lit.,[14] m.p. 89–91 °C] and (*S*)-(+)-binaphthylphosphoric acid[8] (1.05 g, 3 mmol) were dissolved in 20 cm^3 of hot methanol. The mixture was evaporated *in vacuo* and the residue was extracted with benzene at 80 °C. (The benzene extracts were set aside to prepare the other enantiomer.) The residue insoluble in benzene was recrystallized from methanol–water (80:20) to give colourless rhombic plates (1.152 g, 74% yield) of the (+)-*binaphthyl phosphate salt* of constant m.p. 224–225 °C and constant rotation $[\alpha]_D^{25}$ + 354 ± 6 (*c* = 0.6, MeOH) (Found: C, 63.1; H, 5.45; N, 3.85. Calc. for $C_{58}H_{52}ClN_3O_9P_2 \cdot 4H_2O$: C, 63.07; H, 5.44; N, 3.81%).

(*b*) The benzene extracts set aside from the preparation of the (−)-binaphthyl phosphate salt of hydroxychloroquine (see below) were evaporated *in vacuo*. The residue (0.335 g, 67%) consisted of the enriched (−)-base, $[\alpha]_D^{25}$ −57.4 (*c* = 2.5, MeOH), giving a single spot on TLC identical with a sample of the racemic base (R_f 0.29, $CHCl_3$–MeOH, 60:40). The enriched (−)-base (0.335 g, 1 mmol) was reacted with a solution of (*S*)-(+)-binaphthylphosphoric acid (0.696 g, 2 mmol) in hot methanol to give the (+)-*binaphthyl phosphate salt* (0.524 g, 49%), isolated as above as rhombic plates, m.p. 224–225 °C, $[\alpha]_D^{25}$ + 360 (*c* = 0.8, MeOH) (Found: C, 63.3; H, 5.6; N, 3.85). Calc. for $C_{58}H_{52}ClN_3O_9P_2 \cdot 4H_2O$: C, 63.07; H, 5.44; N, 3.81%).

(*c*) The (+)-binaphthyl phosphate salt (0.331 g, 0.3 mmol) $[\alpha]_D^{25}$ +360 was powdered, suspended in ammonia solution (*d* 0.880) and extracted with chloroform.* The extracts were dried ($MgSO_4$) and evaporated *in vacuo* to give an oil which was purified by flash chromatography on Florisil ($CHCl_3$) to give (*R*)-(−)-hydroxychloroquine (0.77 g, 76%), $[\alpha]_D^{25}$ −90.3 (*c* = 1.19, MeOH) (92% ee).† The base gave a single spot on TLC (R_f 0.29, $CHCl_3$–MeOH, 60:40) identical with an authentic sample (Found: M^+, 337.174 617. Calc. for $C_{18}H_{26}{}^{37}ClN_3O$: *M*, 337.173 490; Found: M^+, 335.176 054. Calc. for $C_{18}H_{26}{}^{35}ClN_3O$: *M*, 335.176 440). λ_{max}(EtOH)/nm (ε/dm^3 mol^{-1} cm^{-1}) at 332 (5400), 256 (8800) and 218 (19 000); the IR spectrum ($CHCl_3$) was identical with that of the racemic base (3683, 3613, 3444 cm^{-1}, OH and NH); δ_H 1.01 (6 H, t, CH_3),

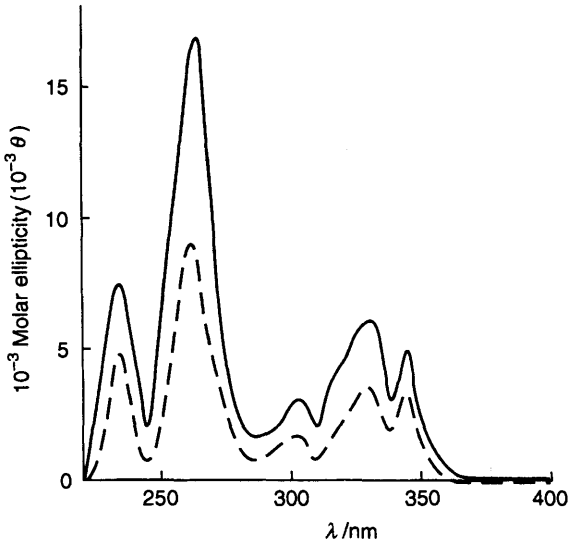


Fig. 3 Circular dichroism spectra in water for (*S*)-(+)-chloroquine diphosphate (——) and (*S*)-(+)-hydroxychloroquine diphosphate (- - - -)

* The aqueous layer from the extraction deposited a flocculent precipitate which when filtered, dissolved in hot water and then acidified (10 mol dm^{-3} HCl) gave the (−)-binaphthylphosphoric acid used.

† Based on $[\alpha]_D$ − 97 (EtOH) for the (−)-base, 99% ee (Sterling-Winthrop, personal communication).

1.31 (3 H, d, J = 6, CH_3CH), 1.6–1.8 (4 H, m, CH_2), 2.4–2.6 (6 H, m, CH_2N), 3.69 (1 H, q, J = 6, CH), 3.1 (1 H, br s, OH, exchanged with D_2O), 3.55 (2 H, t, CH_2OH), 5.1 (1 H, d, NH) and 6.4–8.5 (5 H, m, ArH).

(*d*) A solution of the (+)-binaphthyl phosphate salt $[\alpha]_D^{25}$ +358 (0.22 g, 0.2 mmol) in the minimum of methanol was poured onto a column of alkaline alumina (10 g, Brockmann activity 1, pH 10, 150 mesh) made with chloroform–methanol (9:1), and allowed to percolate down the column. Elution with the same solvent mixture (50 cm^3) gave 0.67 g (90%) of (−)-hydroxychloroquine. After flash chromatography on Florisil ($CHCl_3$), the base had $[\alpha]_D^{25}$ −98 (c = 5.3, MeOH) (>99% ee).* UV and IR spectra were identical with those of the racemic base. TLC gave a single spot (R_f 0.29) identical with an authentic sample. Extraction of the alumina with aqueous ammonia solution (5 mol dm^{-3}), filtration and acidification (10 mol dm^{-3} HCl) gave recovered (+)-binaphthylphosphoric acid.

(S)-(+)-7-Chloro-4-{4-[ethyl(2-hydroxyethyl)amino]-1-methylbutylamino}quinoline [(S)-(+)-*Hydroxychloroquine*].—(*a*). The benzene extracts set aside in the preparation of the (+)-binaphthyl phosphate salt (above) were evaporated *in vacuo* to give 0.382 g (76.4%) of the enriched (+)-base, $[\alpha]_D^{25}$ +64.7 (c = 9.55, EtOH). It showed a single spot on TLC identical with that of a sample of the racemic base (R_f 0.29; $CHCl_3$–MeOH 60:40). The enriched (+)-base (0.382 g, 1.14 mmol) was dissolved in methanol and treated with a hot methanolic solution of 0.794 g (2.28 mmol) of (*R*)-(−)-binaphthylphosphoric acid. After evaporation *in vacuo*, the residual (−)-binaphthyl phosphate salt (0.870 g, 74%) was recrystallized to constant m.p. 224–225 °C and constant rotation $[\alpha]_D^{25}$ −348 ± 4 (c = 0.5, MeOH) (Found: C, 63.4; H, 5.6; N, 3.85. Calc. for $C_{58}H_{52}ClN_3O_9P_2$·4 H_2O: C, 63.07; H, 5.44; N, 3.81%).

(*b*) Racemic hydroxychloroquine (1.01 g, 3 mmol) and (*R*)-(−)-binaphthylphosphoric acid (1.05 g, 3 mmol) were reacted as described above for the (*S*)-(+)-isomer. After benzene extraction of the (−)-base, the residue crystallized from aqueous methanol as plates (0.947 g, 61% yield) of the (−)-*binaphthyl phosphate salt* with constant rotation $[\alpha]_D^{25}$ −345 (c = 0.6, MeOH) and m.p. 224–225 °C.

(*c*) The (−)-binaphthyl phosphate salt (0.221 g, 0.2 mmol) was powdered, suspended in ammonia solution (*d* 0.880) and extracted with dichloromethane. The extracts were dried ($MgSO_4$) and evaporated *in vacuo* to give an oil which was purified by flash chromatography on Florisil ($CHCl_3$) to give (S)-(+)-*hydroxychloroquine* (0.0565 g, 84%), $[\alpha]_D^{25}$ +93.1 (c = 1.28, MeOH) (97.5% ee).* It gave a single spot on TLC, identical with that of a sample of the racemic base (R_f 0.29, $CHCl_3$–MeOH 60:40) (Found: M^+, 337.172 136. Calc. for $C_{18}H_{26}{}^{37}ClN_3O$: M, 337.173 490; Found: M^+, 335.177 274. Calc. for $C_{18}H_{26}{}^{35}ClN_3O$: M, 335.176 440).

(*d*) A solution of 0.280 g of the (−)-binaphthyl phosphate salt, $[\alpha]_D^{25}$ −350, in the minimum of methanol was poured onto a column of alkaline alumina (10 g, Brockmann activity 1, pH 10, 150 mesh) made with chloroform–methanol (9:1), and allowed to percolate down the column. Elution with the same solvent mixture (50 cm^3) gave (+)-hydroxychloroquine (0.086 g, 94%). TLC gave a single spot (R_f 0.29; $CHCl_3$–MeOH 60:40) identical with that of a sample of racemic base. The base was purified by flash chromatography on Florisil and had $[\alpha]_D^{25}$ +94.4 (c = 4.2, MeOH) (98.1% ee).* UV and IR spectra were identical with those of the racemic base.

(R)-(−)-7-*Chloro*-4-{4-[*ethyl*(2-*hydroxyethyl*)*amino*]-1-*methylbutylamino*}*quinoline Diphosphate* [(R)-(−)-*Hydroxychloroquine diphosphate*].—A solution of (−)-hydroxychloroquine $[\alpha]_D^{25}$ −98 (0.100 g, 0.3 mmol) in ethanol (1 cm^3) was treated with phosphoric acid (0.068 g, 0.6 mmol, 85%). A white precipitate formed immediately and was washed with cold ethanol, dried and dissolved in the minimum of water. The solution was lyophilized overnight, leaving a microcrystalline white powder (0.145 g, 91.5%) of the *diphosphate* m.p. 198–200 °C (decomp.), $[\alpha]_D^{25}$ −76 (c = 0.6, H_2O) (>99% ee).† (Found: C, 35.6; H, 6.1; N, 6.55. Calc. for $C_{18}H_{32}ClN_3O_9P_2$·4H_2O: C, 35.79; H, 6.53; N, 6.91%). $\lambda_{max}(H_2O)$/nm had (ε/dm^3 mol^{-1} cm^{-1}), 344 (13 250), 330 (15 000), 256 (18 000) and 220 (34 700) nm. The IR spectrum (KBr) was identical with that of the racemic diphosphate (997 and 1103 cm^{-1}, PO_4^-). The CD spectrum (c = 0.109, H_2O) showed λ_{max}/nm ([θ]) 342 (−3000), 330 (−3200), 302 (−1800), 264 (−8200) and 234 (−6000).

(S)-(+)-7-*Chloro*-4-{4-[*ethyl*(2-*hydroxyethyl*)*amino*]-1-*methylbutylamino*}*quinoline diphosphate* [(S)-(+)-*Hydroxychloroquine diphosphate*].—A solution of (+)-hydroxychloroquine $[\alpha]_D^{25}$ +93 (0.067 g, 0.2 mmol) in absolute ethanol (1 cm^3) was treated with phosphoric acid (0.050 g, 0.44 mmol, 85%) in 1 cm^3 of absolute ethanol. The white precipitate was washed with ethanol and refluxed repeatedly with ethanol and finally with benzene. Solvents were distilled off *in vacuo* and the residue dissolved in water and lyophilized, giving the (+)-diphosphate as a microcrystalline powder (0.090 g, 85%), m.p. 198–200 °C (decomp.), $[\alpha]_D^{25}$ +72.2 (c = 0.216, H_2O) (97% ee).† (Found: C, 39.8; H, 6.4, N, 7.2. Calc. for $C_{18}H_{32}ClN_3O_9P_2$·H_2O: C, 39.31; H, 6.19; N, 7.64%). The UV and IR spectra were identical with those of the (−)-enantiomer. The CD spectrum (c = 0.0795, H_2O) showed λ_{max}/nm ([θ]) 343 (3275), 330 (3350), 303 (1600), 264 (8000) and 234 (4700).

Acknowledgements

The authors would like to thank Sterling-Winthrop, Inc. for their support of this project.

References

1 H. E. Howard-Lock, C. J. L. Lock, A. Mewa and W. F. Kean, *Semin. Arthritis Rheum.*, 1986, **15**, 261.
2 A. H. Mackenzie and A. L. Scherbel, *Clin. Rheum. Dis.*, 1980, **6**, 545.
3 A. J. McLachlan, S. E. Tett and D. J. Cutler, *J. Chromatogr.*, 1991, **570**, 119.
4 J. Iredale and I. W. Wainer, *J. Chromatogr.*, 1992, **573**, 253.
5 P. Newman, *Optical Resolution Procedures for Chemical Compounds*, Optical Resolution Information Center, Riverdale, New York, 1981.
6 S. H. Wilen, *Tables of Resolving Agents and Optical Resolutions*, ed. E. L. Eliel, University of Notre Dame Press, Notre Dame, 1972.
7 M. Oki, *Top. Stereochem.*, 1983, **14**, 1.
8 J. Jacques and C. Fouquey, *Org. Synth.*, 1989, **67**, 1.
9 H. B. Kagan and J. C. Fiaud, *Top. Stereochem.* 1988, **18**, 249.
10 S. Miyano, L. D. L. Lu, M. Viti and K. B. Sharpless, *J. Org. Chem.*, 1985, **50**, 4350.
11 S. Yamaguchi, in *Asymmetric Synthesis*, ed. J. D. Morrison, Academic Press, New York, NY, 1983, vol. 1, p. 125.
12 J. C. Craig, B. LaBelle and U. Ohnsorge, *Chirality*, 1991, **3**, 436.
13 J. C. Craig, H. N. Bhargava, E. T. Everhart, B. LaBelle, U. Ohnsorge and R. V. Webster, *J. Org. Chem.*, 1988, **53**, 1167.
14 A. R. Surrey and H. F. Hammer, *J. Am. Chem. Soc.*, 1950, **72**, 1814.

* Based on $[\alpha]_D$ − 97 (EtOH) for the (−)-base, 99% ee (Sterling-Winthrop, personal communication).
† Based on $[\alpha]_D$ − 74 (c 0.75, H_2O) for the (−)-diphosphate, 99% ee by HPLC analysis[3] using an α_1-acid glycoprotein column to separate the enantiomers (Sterling-Winthrop, personal communication).

Paper 4/02647K
Received 25*th February* 1994
Accepted 15*th April* 1994