Resolution of Antimalarial Agents via Complex Formation with α-(2,4,5,7-Tetranitro-9-fluorenylideneaminooxy)propionic Acid

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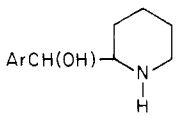
Received September 23, 1977

The resolution of several antimalarial agents via π-complex formation with α-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy)propionic acid (TAPA) is reported. Since this represents the first use of this agent for the resolution of amines, some details of the separations are presented. The method proved successful for resolving weakly alkaline amines that did not form stable salts with common resolving acids, highly insoluble amines that did not form soluble salts with usual resolving acids, and amines that did not form crystalline salts with commonly available resolving acids. The optical isomers of several antimalarial agents were evaluated against *Plasmodium berghei* in the mouse. None of the optically active forms showed any significant differences. The curative activity of (+)- and (−)-primaquine against *Plasmodium cynomolgi* in the rhesus monkey was essentially identical; however, significant differences in toxicity were noted.

During the past 15 years, the U.S. Army Medical Research and Development Command has been supporting a drug development program. The program involved both reinvestigation of some effective antimalarial agents prepared on an earlier program and the development of new agents active against resistant strains of malaria. As a result of this program, several arylcarbinolamines and 2,4-diaminopyrimidines were selected as promising antimalarial agents. In an earlier report we described the preparation of the optical isomers of several aryl-2-piperidylmethanol antimalarial agents (1).[1,2] As a continuation of this work, we now report on the resolution of several additional antimalarial agents.



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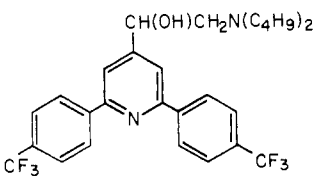
Resolution. We initially attempted to resolve α-(di-*n*-butylaminomethyl)-2,6-bis(4-trifluoromethylphenyl)-4-pyridinemethanol (**2**),[3] 6-bromo-α-diheptylaminomethyl-9-phenanthrenemethanol (**3**),[4-7] and α-(di-*n*-butylaminomethyl)-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinolinemethanol (**4**)[5,6,8,9] via diastereoisomeric salt formation. We were able to resolve **2** via its (−)- and (+)-*O,O*-di-*p*-toluoyltartrate salts; however, the salts of **3** and **4** with commonly available resolving acids[10] led to regeneration of the free base on attempted recrystallization. This dissociation probably occurred owing to the weakly alkaline nature of **3** and **4**. In the case of **3**, Pearson and Rosenberg[11] were able to accomplish the resolution of a small sample, employing a very large excess of *d*-tartaric acid used as resolving agent. Pearson and Rosenberg call this separation a resolution from chiral solution.[11] We found that both **3** and **4** were easily resolved using optically active α-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy)propionic acid (TAPA),[12-14] a resolving agent capable of forming both crystalline molecular complexes and salts with racemic Lewis bases. TAPA has been used for the resolution of several polycyclic aromatic compounds which do not possess functional groups but had not apparently been used for the resolution of amines.[12,13,15] We also found that TAPA was an excellent resolving agent for the antimalarial drug primaquine (**5**) and its 4-methyl analogue, 8-(4′-amino-1′-methylbutylamino)-6-methoxy-4-methylquinoline (**6**),[16] both of which did not form crystalline salts with commonly used resolving acids,[10] and 2,4-diamino-6-(β-naphthylsulfonyl)-5,6,7,8-tetrahydroquinazoline (**7**), a highly insoluble compound.



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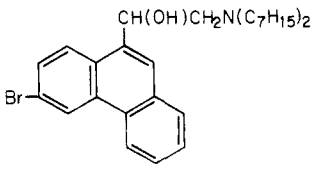

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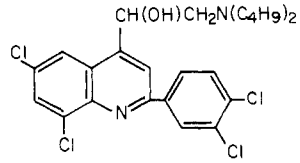

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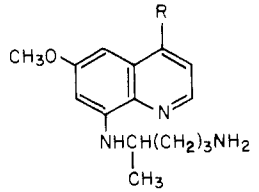
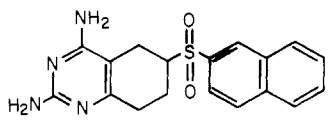


5, R = H
6, R = CH_3



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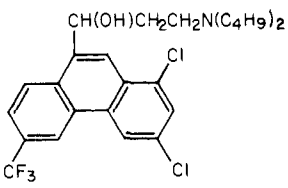

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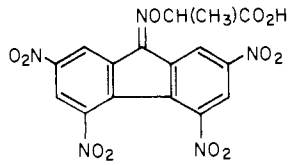
In a series of probe experiments using (+)- and (−)-TAPA, we determined that the highest degree of resolution of **3** and **4** was obtained by treating 1 equiv of the free base with 0.5 equiv of optically active TAPA. Using this procedure, pure (+)-**3**-(+)-TAPA was obtained as an orange complex from acetone. The partially resolved free base **3** isolated from the mother liquor of the complex was treated with (−)-TAPA to give (−)-**3**-(−)-TAPA. Optically pure (+)- and (−)-**3** were obtained by treatment of the complexes with dilute sodium hydroxide. In the case of **4**, tetrahydrofuran proved to be the best solvent and the (−) and (+) isomers of **4** formed complexes with (+)- and (−)-TAPA, respectively. The complexes were decomposed with dilute sodium carbonate in this case to give (−)- and (+)-**4**.

Primaquine (**5**) and its 4-methyl analogue **6** are viscous oils that did not give crystalline salts with commonly available resolving acids. However, both **5** and **6** gave dark green complexes with TAPA. Fractional crystallization of **5**-(+)-TAPA complexes from THF resulted in only



TAPA

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partial resolution. Samples of the complexes were initially converted to the free bases by alkali treatment and chloroform extraction for rotation analysis. These experiments indicated two disadvantages of the resolution method. Due to low solubility of the 5–TAPA complexes, the volume of solvent required for recrystallization was excessive, and the yield and purity of the free base of 5 recovered by the alkaline decomposition of the complexes were low. These difficulties were overcome by using a solvent mixture of THF and water for the fractional crystallization of the complexes and by using phosphoric acid in place of alkali to decompose the complexes. Due to the low solubility of the resulting diphosphate salts of (+)- and (−)-5 in organic solvents, the recovery of (+)- and (−)-5 was greater, and the purity of the products was increased. An added advantage was that the acid workup allowed the recovery of a major portion of the resolving agent. It is interesting to note that the diphosphates of (+)- and (−)-5, as well as solutions of these isomers in water, are bright yellow, whereas the diphosphate of racemic 5 and its solution in water are orange. Recrystallization of the partially resolved diphosphate of 5 led to a mixture of orange and yellow crystals which separated side by side. Even small contamination of one optical isomer with racemic 5 could be recognized.

Compound 6 was resolved by a procedure using 0.5 equiv of TAPA per equivalent of 6, THF–water as the solvent for fractional crystallization, and phosphoric acid to decompose the (−)-6–(+)-TAPA and (+)-6–(−)-TAPA complexes obtained. One peculiarity of (+)- and (−)-6 phosphate salts was their stoichiometry. Elemental analysis indicated that the phosphates of the optically active isomers of 6 contained 1.5 mol of phosphoric acid per mole of 6, whereas racemic 6 was a diphosphate. The chemical composition was confirmed in the following manner. Combining equal parts of (+) and (−) salts in ethanol–water gave crystals of yellow racemic 6 diphosphate. From the mother liquor 6 monophosphate was isolated as tan crystals. The same tan product was obtained by combining molar equivalents of racemic 6 (free base) and phosphoric acid.

Of all the antimalarial drugs dealt with in this and our previous paper,[1] 7 exhibited the lowest solubility in organic solvents. Initially this represented a grave difficulty, in particular concerning the resolution of gram quantities of 7. In small-scale experiments dioxane was found to be the most suitable solvent. Complexes of 7 with (+)-TAPA could be obtained in good yield on a 0.5-mmol scale. Alkali treatment of the complex yielded partially resolved 7. In these experiments it was also recognized that the 7–TAPA complex was significantly more soluble in dioxane than 7 itself. This observation led to the following modified procedure. The resolving agent TAPA was first combined with 7 in acetic acid in which the base was readily soluble. After removal of the acetic acid by freeze drying, the 7–TAPA obtained was fractionated by recrystallization from dioxane and dioxane–ethanol in which it was fairly soluble. The pure (+)-7–(+)-TAPA and (−)-7–(−)-TAPA obtained in two separate experiments were decomposed with hydrochloric acid in dioxane. This method was preferred to alkali workup since the hydrochloride could be recovered in higher yield and better purity, and, in addition, any possibility of racemization of 7 by alkali was excluded. The free bases of (+)- and (−)-7 could be obtained by mild neutralization of the hydrochlorides with aqueous methanolic sodium carbonate solution.

The only antimalarial agent investigated which resisted resolution attempts using (+)- and (−)-TAPA was α-(di-*n*-butylaminoethyl)-1,3-dichloro-6-trifluoromethyl-9-phenanthrenemethanol (8),[17] which possesses an additional methylene group between the asymmetric center and the amine moiety. Both 8 and the *O*-acetyl derivative of 8 gave racemic complexes which could not be separated. Complexes with 8 and the methyl ester of TAPA also gave no resolution. Compound 8 was resolved by fractional crystallization of the *d*-camphoric acid salts from a hexane–THF solvent mixture.

Optical Purity. In a previous report[1] we described a method whereby the optical purity of aryl-2-piperidylmethanols could be determined using chiral lanthanide shift reagents. The analysis involved first conversion of the amino alcohol to its *O*- or *N*-acetyl derivative, followed by NMR analysis in the presence of the shift reagent. The acetate derivatives of (±)-, (+)-, and (−)-3 were prepared and subjected to NMR analysis after the addition of tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium(III). The acetate methyl group gave two separate resonances in the case of the racemic mixture, whereas only one signal each was observed for the (+) and (−) isomer at δ 3.51 and 3.73 ppm, respectively. Since a contamination of either optical isomer with the other of 2% could be distinguished by this method, (+)- and (−)-3 are at least 98% optically pure. We were unable to use this method for 4 and 8 since we found that the optical isomers of these compounds undergo racemization upon esterification. The NMR spectra of 2, 5, and 6 or their acetyl derivatives containing varying amounts of shift reagent showed no shift differences useful for establishing optical purity. Compound 7 was too insoluble in nonpolar solvents for NMR analysis.

The fact that the complexes or diastereoisomeric salts of the optical isomers of 2–8 were recrystallized to constant optical rotation suggests that the optical isomers are optically pure in each case.

Biological Testing. The antimalarial activity for the (+), (−), and (±) isomers of compounds 2–4, 7, and 8 against *Plasmodium berghei* in mice is shown in Table I and was determined as previously described.[18,19] None of the optically active forms of the arylcarbinolamines reported in this or our previous paper[1] showed any significant differences in antimalarial activity against *P. berghei* in rodents. However, the (−) and (±) form of the tetrahydroquinazoline 7 was more active and more toxic than the (+) isomer.

Dr. L. H. Schmidt, Southern Research Institute, Birmingham, Ala., tested (+)-, (−)-, and (±)-primaquine (5) for prophylactic antimalarial activity against *Plasmodium cynomolgi* in rhesus monkeys.[20] He found that radical curative activities of (+)- and (−)-5 were essentially identical and were similar, if not identical, to the curative activity of racemic (±)-5 (see Table II).[21] However, Dr. Schmidt also found that (−)-5 was three to five times as toxic as (+)-5 and at least twice as toxic as racemic (±)-5 in rhesus monkeys and suggested that a critical comparison of (+)-5 and (±)-5 in human volunteers is indicated.[21]

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus. IR spectra were measured with a Perkin-Elmer Model 467 grating infrared spectrophotometer. NMR spectra were recorded on a Varian Model HA-100 spectrometer with tetramethylsilane as an internal standard. All observed rotations were determined with a Perkin-Elmer Model 141 polarimeter (1-dm cell). Microanalyses were carried out by Micro-Tech Laboratories, Skokie, Ill. Where analyses were indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Compounds 2·HCl, 3·HCl, 4·HCl, 5·2H_3PO_4, 6·2H_3PO_4,

Table I. Comparison of Antimalarial Activity against *P. berghei* in Rodents[a]

	ΔMST, C or T,[b] dose (mg/kg)									
Compd	1.25	2.5	5	10	20	40	80	160	320	640
(+)-2				3.9	10.7	18.1	25.6 (2C)	5C	5C	
(−)-2				1.3	3.1	10.5	13.1	19.9	5C	
(±)-2[c]				5.5	8.6	2C	5C	5C	5C	
(+)-3					0.5	1.7	4.1	6.7	9.3	14.9
(−)-3					0.3	2.1	3.9	5.3	6.1	11.1
(±)-3[d]					2.9	6.9	11.3	15.3	25.9 (4C)	5C
(+)-4	0.5	0.5	3.5	6.9	10.0	11.8	5C	5C	5C	5C
(−)-4					3.8	7.8	9.0	12.8 (3C)	5C	5C
(±)-4[e]	0.3	4.1	6.1	10.7	14.9	11.9 (3C)	22.9 (4C)	5C	5C	
(+)-7					0.3	1.7	4.5	6.9	9.1	5C
(−)-7	1.3	3.1	4.9	7.1	11.3	5C	5C	2C, 3T	1C, 4T	5T
(±)-7[d]	4.9	7.5	10.7	11.9 (3C)	5C	5C	5C	5C	4C (1T)	2C (3T)
(+)-8	0.5	3.1	7.5	10.3	11.1	14.5	16.9 (3C)	5C	5C	5C
(−)-8	0.1	0.3	1.5	5.1	8.9	12.5	13.2 (3C)	5C	5C	5C
(±)-8[f]			9.3	14.9 (1C)	5C	5C	5C	5C		

[a] Tests were carried out by the Rane Laboratory, University of Miami.[18] [b] ΔMST is the mean survival time over controls (6.2 ± 0.5 days); C is the number of cures (mice surviving 60 days); T is the number of toxic deaths occurring on days 2-5 after infection. [c] Taken from ref 3. [d] Data supplied through the courtesy of Dr. E. A. Steck. [e] Taken from ref 6. [f] Taken from ref 17.

Table II. Comparison of Antimalarial Activities of (+)-Primaquine [(+)-5], (−)-Primaquine [(−)-5], and Racemic (±)-Primaquine [(±)-5] against *P. cynomolgi* in Rhesus Monkeys[a]

Compd[b]	Dose, mg/kg[c]	No. of animals	Cures[d]	Relapses[e]
(+)-5	0.375	3	1	2 (10)
	0.5	2	1	1 (28)
	0.75	3	3	
(−)-5	0.375	3	1	1 (21), 1 (11)
	0.5	2	1	1 (16)
	0.75	3	3	
(±)-5	0.375	1	0	1 (11)
	0.5	1	1	
	0.75	1	1	

[a] Data taken from ref 21 and supplied through the courtesy of Dr. E. A. Steck. [b] Compounds were tested as phosphate salts. [c] Dose indicated (as free base) was administered once daily for 7 days along with 2.5 mg of chloroquine per kilogram of body weight. [d] Infections were categorized as "cured" when thick films were parasite negative for 105 days or more after the last dose of drug had been delivered. [e] The number in parentheses is the days between the end of treatment and relapse.

and 8·HCl were converted to the free bases by a standard procedure.[22]

Resolution of 6-Bromo-α-diheptylaminomethyl-9-phenanthrenemethanol (3). A mixture of 20.5 g (0.04 mol) of **3** in 50 mL of acetone was mixed with 9.5 g (0.021 mol) of (+)-TAPA[14] dissolved in 50 mL of acetone. The resulting dark-orange solution was concentrated to 70 mL and cooled to −10 °C overnight, which resulted in the precipitation of the orange complex, (+)-**3**-(+)-TAPA (14 g), which was purified by recrystallization from 130 mL of acetone: mp 115–118 °C; $[\alpha]^{23}_D$ −17.4° (*c* 1.0, $CHCl_3$). By stepwise concentration of the filtrate and partial substitution of the solvent with ethanol, a total of 12.5 g of crude (−)-**3** base was recovered. This was dissolved in chloroform–ethanol and the mixture cooled to −15 °C to give 9.3 g of (−)-**3**. A solution of this product (0.018 mol) in 50 mL of acetone was mixed with a solution of 8.3 g (0.019 mol) of (−)-TAPA[14] in 50 mL of acetone. At −15 °C the orange complex, (−)-**3**-(−)-TAPA, crystallized (11.3 g): mp 108–113 °C; $[\alpha]^{23}_D$ +16° (*c* 1.0, $CHCl_3$).

The final complex fractions were treated with a small excess of 0.5 N sodium hydroxide solution and extracted with chloroform. The extracts were washed with water, evaporated to syrups, and dissolved in benzene. The brown solutions were treated with Norit A on a steam bath for removal of colored contaminants. The residue, after evaporation of the benzene, was recrystallized from ethanol. The (+) and (−) isomers were recovered as a light-tan crystalline solid. The isomer (+)-**3** (7.9 g, 68%) had mp 53–56 °C; $[\alpha]^{23}_D$ +42.2° [*c* 1.0, $(CH_3)_2CHOH$]; $[\alpha]^{23}_D$ +87° (*c* 1.0, $CHCl_3$) [lit.[11] mp 51.5–56 °C; $[\alpha]_D$ +43.6° [*c* 10, $(CH_3)_2CHOH$]]. The (−)-**3** isomer (5.4 g, 64%) had mp 52–56 °C; $[\alpha]^{23}_D$ −38.5° [*c* 1.0, $(CH_3)_2CHOH$]; $[\alpha]^{23}_D$ −81° (*c* 1.0, $CHCl_3$) [lit.[11] mp 55–58 °C; $[\alpha]_D$ −41° [*c* 10, $(CH_3)_2CHOH$]].

The hydrochloride salts of (+)- and (−)-**3** were prepared using THF–C_2H_5OH solvent and drying of the crystals obtained at 50 °C under vacuum. The (+)-**3**·HCl had mp 184–186 °C; $[\alpha]^{23}_D$ +37.8° (*c* 1.0, CH_3OH). Anal. ($C_{30}H_{43}BrClNO$) C, H, N. The (−)-**3**·HCl had mp 180–183 °C; $[\alpha]^{23}_D$ −37.3° (*c* 1.0, CH_3OH). Anal. ($C_{30}H_{43}BrClNO$) C, H, N.

Resolution of α-(Di-*n*-butylaminomethyl)-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinolinemethanol (4). Racemic **4** (20.5 g) was dissolved in THF (130 mL) and mixed with 9.5 g of (+)-TAPA dissolved in 30 mL of THF. The (−)-**4**-(+)-TAPA complex (16.5 g) separated at room temperature as bright yellow crystals which did not possess a characteristic melting point but decomposed gradually upon heating between 130 and 170 °C. After evaporation of the filtrate, the remaining solid was crystallized from chloroform–ethanol. This gave impure (+)-**4** as a light-brown material (8.1 g), $[\alpha]^{22}_D$ +35° (*c* 1, chloroform), which was treated with (−)-TAPA to give the (+)-**4**-(−)-TAPA complex. The two complexes were recrystallized from THF, decomposed with sodium carbonate solution, and extracted with chloroform. The extracts were washed with water, dried with sodium sulfate, and concentrated to syrups. Recrystallization twice from an ethanol and chloroform mixture gave (+)-**4** [mp 98–101 °C; $[\alpha]^{23}_D$ +50.9° (*c* 1.0, $CHCl_3$)] and (−)-**4** [mp 96–100 °C; $[\alpha]^{23}_D$ −50.7° (*c* 1.0, $CHCl_3$)]. The free bases (+)- and (−)-**4** were dissolved each in 50 mL of THF. An excess (6 mL) of 5 N hydrochloric acid was added and the resulting mixture diluted with 300 mL of water. After concentration to 250 mL, the precipitated hydrochlorides were separated by filtration, washed with water, and dried at 70 °C in vacuo. The (+)-**4**·HCl (5.7 g, 28%) had mp 195–198 °C dec; $[\alpha]^{23}_D$ +33.7° (*c* 1.0, CH_3OH). Anal. ($C_{25}H_{29}N_2OCl_5$) C, H, Cl, N. The (−)-**4**·HCl (5.0 g, 24%) had mp 197–199 °C dec; $[\alpha]^{23}_D$ −33.4° (*c* 1.0, CH_3OH). Anal. ($C_{25}H_{29}N_2OCl_5$) C, H, Cl, N.

Resolution of Primaquine (5). Primaquine (21 g) and (+)-TAPA (36 g) were separately dissolved in THF, combined, and brought to a total volume of 900 mL by addition of THF and 35 mL of water. A dark green solution resulted immediately. The crystallization of a green complex started after 5 min. After storing at room temperature overnight, the crystalline material was collected on a large filter and dried. The product did not show a sharp melting point but decomposed gradually between 160 and 180 °C. Fractional crystallization of the complex from THF–H_2O gave 21.2 g of (−)-**5**-(+)-TAPA and 16.5 g of (+)-**5**-(+)-TAPA.[23] The (−)-**5**-(+)-TAPA complex was dissolved in 500 mL of THF and 75 mL of water, filtered, and decomposed with 20 mL of 85%

phosphoric acid. An orange solid A had separated after cooling of the mixture for 2 days. The decanted solution was concentrated to 60 mL, diluted with 50 mL of water, and filtered to yield a yellow-brown precipitate and filtrate B. The precipitate was recrystallized from 50% acetic acid to afford 10.8 g (83%) of pure (+)-TAPA: $[\alpha]^{22}_D$ −96° (c 1, $CHCl_3$). Filtrate B was evaporated to a syrup and taken up in 150 mL of ethanol and 300 mL of THF. An oily precipitate separated at 5 °C and was combined with solid A. A solution of the products in 40 mL of water was filtered and diluted with 700 mL of ethanol. The crude diphosphate which crystallized at 5 °C was collected and recrystallized from a mixture of 50 mL of water, 450 mL of ethanol, and 200 mL of THF. The hot solution was filtered through a hardened filter for removal of a greenish impurity. The filtrate was allowed to crystallize overnight at room temperature. For a final purification the diphosphate was recrystallized from 50 mL of water and 700 mL of ethanol. Pure yellow (−)-primaquine diphosphate [(−)-**5**·$2H_3PO_4$] was thus obtained in a yield of 6.2 g (34%): mp 179–181 °C dec; $[\alpha]^{22}_D$ −27.8° (c 1, H_2O). Anal. ($C_{15}H_{27}N_3O_9P_2$) C, H, N.

For the preparation of the (+)-primaquine diphosphate the (+)-**5**–(+)-TAPA complex was dissolved in 25 mL of H_2O and 225 mL of THF and filtered. The solution was acidified with 8 mL of 85% phosphoric acid. The oily precipitate of crude diphosphate C was collected. The decanted mother liquor was concentrated to a small volume which led to the separation of crude (+)-TAPA. The aqueous filtrate D was kept for further workup. The crude (+)-TAPA, after recrystallization from 50% acetic acid, weighed 8.2 g (80%). It was pure enough for utilization in further resolution experiments. The aqueous filtrate D, after concentration to a small volume, was diluted with 40 mL of ethanol which resulted in the precipitation of crude diphosphate. This was combined with the diphosphate C and dissolved in 20 mL of water. After standing at room temperature overnight, a dark green precipitate was removed by filtration. The filtrate was concentrated to 10 mL and diluted with 500 mL of ethanol. The solution was allowed to crystallize at room temperature for several hours and then at 5 °C overnight. The diphosphate obtained was recrystallized from a mixture of 30 mL of water and 450 mL of ethanol. The resulting pure yellow (+)-primaquine diphosphate [(+)-**5**·$2H_3PO_4$] weighed 7.1 g (39%) and had mp 180–181 °C dec; $[\alpha]^{22}_D$ +28.7° (c 1, H_2O). Anal. ($C_{15}H_{27}N_3O_9P_2$) C, H, N.

Resolution of 8-(4′-Amino-1′-methylbutylamino)-6-methoxy-4-methylquinoline (6). Racemic **6** (36 g) was dissolved in 500 mL of THF and a solution of 30 g (0.5 equiv) of (+)-TAPA in 125 mL of THF was added. A dark-green solution resulted immediately. After cooling to 0 °C overnight, the complex had separated as green crystals. The crystals were separated to yield the complex and filtrate A. The complex was washed with THF and air-dried to give 33 g of (−)-**6**–(+)-TAPA. The complex, after one recrystallization from THF–H_2O, was dissolved in THF–6% water (400 mL) and diluted with 400 mL of ethanol which contained 12 mL of phosphoric acid. The precipitated phosphate was dissolved in 70 mL of water. After 1 day of standing at room temperature, this solution deposited a dark precipitate which was separated by filtration. Ethanol (60 mL) was carefully added to the filtrate until a precipitation started. The precipitate was redissolved and the crystallization triggered with a seed crystal. After washing with ethanol and vacuum drying at 70 °C, 10 g of (−)-**6** phosphate salt was obtained as yellow prisms which had mp 112–114 °C; $[\alpha]^{24}_D$ −25.8° (c 1, H_2O). Anal. ($C_{16}H_{23}N_3O$·$1.5H_3PO_4$) C, H, N, P.

Filtrate A was combined with a solution of (−)-TAPA in 100 mL of THF to give 32 g of (+)-**6**–(−)-TAPA. The (+)-**6**–(−)-TAPA complex was recrystallized once from a THF and H_2O mixture and dissolved in 700 mL of THF containing 7% water. Dropwise addition of 12 mL of phosphoric acid with stirring to the solution precipitated an amorphous phosphate which was separated and dissolved in 60 mL of water. After standing at ambient temperature overnight, a small amount of a dark precipitate was removed by filtration, and the filtrate was diluted with ethanol to precipitate the phosphate. Recrystallization from ethanol–water (60:40) followed by drying at 70 °C gave 9.2 g of (+)-**6**·$1.5H_3PO_4$: mp 112–114 °C; $[\alpha]^{24}_D$ +25.1° (c 1.0, H_2O). Anal. ($C_{16}H_{23}N_3O$·$1.5H_3PO_4$) C, H, N, P.

Resolution of 2,4-Diamino-6-(β-naphthylsulfonyl)-5,6,7,8-tetrahydroquinazoline (7).[21] A mixture of 11.4 g of **7** in 200 mL of acetic acid and a solution of 14.7 g of (+)-TAPA in 30 mL of acetic acid were combined at room temperature and lyophilized to leave 28.2 g of the **7**–(+)-TAPA complex. The product was dissolved in dioxane (300 mL) and cooled to 5 °C overnight. After filtration of the separated crystalline (+)-**7**–(+)-TAPA, the mother liquor was concentrated to half its volume and diluted with an equal portion of ethanol. Upon cooling, (−)-**7**–(+)-TAPA crystallized and was separated by filtration. Further purification of the complexes was achieved by two recrystallizations from dioxane–ethanol (1:1). A second preparative scale resolution of **7** in an exactly corresponding manner was carried out.

The combined (+)-**7**–(+)-TAPA complexes from two large-scale resolutions (18.7 g) were dissolved in 350 mL of hot dioxane, cooled to room temperature, and treated with 40 mL of 5 N hydrochloric acid. After stirring for 30 min, 400 mL of chloroform was added and the precipitated dihydrochloride separated by filtration and air-dried. The white product had the rotation $[\alpha]^{23}_D$ +40.7° (c 1, MeOH) and weighed 10.8 g. The filtrate of the hydrochloride was kept for recovery of the resolving agent.

The final complexes of (−)-**7** from two preparative scale runs were combined (13.9 g) and treated in the same manner as above. The yield of (−)-**7**·2HCl was 7.6 g. It appeared to contain a red-brown by-product. The optical rotation of the crude hydrochloride was $[\alpha]^{23}_D$ −33.0° (c 1, MeOH). The filtrate from this material was combined with the corresponding solution from the (+)-**7**·HCl. After adding 800 mL of water, the chloroform phase of the mixture was separated, washed with water, and evaporated to dryness. The residue (18.7 g) was recrystallized from 50% acetic acid–water and subsequently from 60 mL of acetic acid. The yellow (+)-TAPA thus recovered weighed 10.8 g (37% yield) and had $[\alpha]^{23}_D$ +94.9° (c 1, $CHCl_3$).

Treatment of a methanol solution (450 mL) of (−)-**7**·2HCl (7.5 g) with a mixture of 15 g of sodium carbonate in 500 mL of water gave a slurry of crude (−)-**7**. The mixture was stirred vigorously for 30 min, filtered, and washed with methanol–water (1:1). The dry material weighed 4.2 g, and its rotation was $[\alpha]^{23}_D$ −40.5° (c 1, pyridine). Since the product appeared red brown in color, it was dissolved in hot dioxane and heated with 0.5 g of Norit A for 10 min. This operation removed part of the color. The crystalline pink material weighed 3.5 g. It was dissolved in 75 mL of dioxane and diluted with 500 mL of ethanol. Heating with 0.5 g of Norit A for 15 min on a steam bath and filtration gave a clear, colorless solution. Upon concentration, 2.7 g of white (−)-**7** was recovered. One further recrystallization from water–ethanol (1:1, 250 mL) yielded 1.9 g (17%) of a product with mp 277–280 °C; $[\alpha]^{23}_D$ −51.5° (c 1, pyridine). Anal. ($C_{18}H_{18}N_4O_2S$·H_2O) C, H, N, S.

Similar treatment of (+)-**7**·2HCl (7.8 g) with Norit A (0.5 g) in dioxane–ethanol solution as above gave 4.24 g (37%) of a colorless product which had mp 279–280 °C; $[\alpha]^{23}_D$ +57.2° (c 1, pyridine). Anal. ($C_{18}H_{18}H_4O_2S$·$0.5H_2O$) C, H, N, S.

Resolution of α-(Di-*n*-butylaminoethyl)-1,3-dichloro-6-trifluoromethyl-9-phenanthrenemethanol (8). To a mixture of 20 g of **8** and 8 g of *d*-camphoric acid in 60 mL of hot THF was added 250 mL of hot hexane. The resulting mixture was allowed to cool gradually to room temperature. Subsequent cooling to −10 °C for 2 days gave a white crystalline precipitate of **8** camphorate (20.7 g) which showed $[\alpha]^{23}_D$ +2.5° (c 1, MeOH). This solid was dissolved in hot methanol–ethyl acetate (2:1). On cooling to room temperature crude (+)-**8** camphorate (12.5 g) crystallized which had $[\alpha]^{23}_D$ +12.1° (c 1, MeOH). The mother liquor was concentrated to a small volume and diluted with benzene which precipitated crude (−)-**8** camphorate (3.5 g) with $[\alpha]^{23}_D$ −1.8° (c 1, MeOH). The mother liquor of this fraction was further concentrated to a syrup. Upon addition of acetone, more (−)-**8** camphorate crystallized (2.5 g), $[\alpha]^{23}_D$ −11.8° (c 1, MeOH). Two recrystallizations of (+)-**8** camphorate gave a salt which retained its optical rotation unchanged upon further recrystallization. The mother liquors from the first recrystallization of the (+) isomer camphorate were kept for workup of the (−) salt. The combined (−)-**8** camphorate fractions after two recrystallizations from ethyl acetate–acetone (1:2) weighed 8.7 g and had $[\alpha]^{23}_D$ −8.6° (c 1, MeOH). For further purification this material (8.7 g) was dissolved in methanol and, after addition of an equal volume of ethyl acetate, allowed to crystallize at room temperature. The mother liquor was decanted from the crystals as soon as an

estimated 1.5 g of product had separated. The crystals actually weighed 1.7 g, $[\alpha]^{23}_D$ +3.5° (c 1, MeOH). The decanted mother liquor was evaporated and treated with acetone. This led to the crystallization of (−)-8 camphorate: 6.8 g; $[\alpha]^{23}_D$ −14.7° (c 1, MeOH). Two repetitions of this operation gave finally 3.5 g of the (−) salt, $[\alpha]^{23}_D$ −24.3° (c 1, MeOH).

A solution of (+)-8 camphorate in benzene (15 mL/g) was treated with a dry steam of hydrogen chloride gas for 1 min. A white crystalline mixture separated. This was recrystallized from benzene with addition of a few milliliters of methanol. The recovered material (6.5 g) was treated with sodium bicarbonate solution and extracted with chloroform. The dried (Na_2SO_4) extract was evaporated to dryness, dissolved in benzene, and treated with hydrogen chloride gas. The crystalline hydrochloride of (+)-8 was separated by filtration and rinsed thoroughly with benzene. It was recrystallized from benzene–methanol. The final product weighed 2.76 g (26%) and had mp 212–215 °C; $[\alpha]^{23}_D$ +41.3° (c 1, MeOH). Anal. ($C_{26}H_{31}Cl_3F_3NO$) C, H, N, Cl.

The camphorate of the (−) isomer worked up by an analogous procedure weighed 1.88 g (17%) and had mp 211–214 °C; $[\alpha]^{23}_D$ −41.9° (c 1, MeOH). Anal. ($C_{26}H_{31}Cl_3F_3NO$) C, H, N, Cl.

Resolution of α-(Di-*n*-butylaminomethyl)-2,6-bis(4-trifluoromethylphenyl)-4-pyridinemethanol (2). To a solution of 89.7 g (0.167 mol) of **2** in 890 mL of methanol was added 66.5 g (0.17 mol) of (−)-*O,O*-di-*p*-toluoyltartaric acid. The crystals which separated were isolated by filtration, and the filtrate was retained for further examination. The salt was recrystallized three times from methanol to give 27.6 g of (+)-α-(di-*n*-butylaminomethyl)-2,6-bis(4-trifluoromethylphenyl)-4-pyridinemethanol (−)-*O,O*-di-*p*-toluoyltartrate: mp 158–159 °C; $[\alpha]^{27}_D$ −21.3° (c 0.376, CH_3OH). Anal. Calcd for $C_{49}H_{50}F_6N_2O_9$: C, 63.63; H, 5.78; N, 3.03. Found: C, 63.64; H, 5.76; N, 3.80.

The salt (27.5 g) was suspended in a mixture of chloroform (300 mL) and water (2200 mL) and treated with 25 g of potassium carbonate. The chloroform layer was washed with water, dried (Na_2SO_4), and concentrated to give an oil. The oil was converted to the hydrochloride by the standard procedure. Recrystallization from an acetonitrile and methylene chloride mixture gave 16 g of (+)-**2**·HCl: mp 230–231 °C; $[\alpha]^{24}_D$ +36.56° (c 0.238, CH_3OH), +32.58° (c 0.347, C_2H_5OH). Anal. ($C_{29}H_{33}N_2ClF_6$) C, H, Cl, F, N.

The filtrate retained from the preparation of (+)-α-(di-*n*-butylaminomethyl)-2,6-bis(4-trifluoromethylphenyl)-4-pyridinemethanol (−)-*O,O*-di-*p*-toluoyltartrate was neutralized to give 54.9 g of partially resolved (−)-**2**. This sample was dissolved in 600 mL of methanol and 39.4 g (0.10 mol) of (+)-*O,O*-di-*p*-toluoyltartaric acid was added. The crystals which separated on the addition of water were recrystallized twice from a methanol and water mixture to give 27.4 g of (−)-α-di-*n*-butylaminomethyl-2,6-bis(4-trifluoromethylphenyl)-4-pyridinemethanol (+)-*O,O*-di-*p*-toluoyltartrate: $[\alpha]^{27}_D$ +21.0° (c 0.328, CH_3OH). Anal. Calcd for $C_{49}H_{50}F_6N_2O_9$: C, 63.63; H, 5.78; N, 3.03. Found: C, 63.85; H, 5.73; N, 3.79.

This salt was converted to the (−)-hydrochloride salt as described for the (+)-optical antipode. Recrystallization from an acetonitrile and methylene chloride mixture gave 16.1 g of (−)-**2**·HCl: mp 228–229 °C; $[\alpha]^{29}_D$ −36.40° (c 0.366, CH_3OH), −34.41° (c 0.340, C_2H_5OH). Anal. ($C_{29}H_{33}N_2ClF_6O$) C, H, Cl, F, N.

Acknowledgment. The authors wish to express their gratitude to Drs. E. A. Steck and R. E. Strube for many helpful suggestions and discussions during the course of this work. This work was supported by the U.S. Army Medical Research and Development Command under Research Contracts DADA-17-71-C-1012, DADA-17-73-C-3128, and DADA-17-74-C-4107. This is Contribution No. 1478 to the Army Research Program on Malaria.

References and Notes

(1) F. I. Carroll and J. T. Blackwell, *J. Med. Chem.*, **17**, 210 (1974).
(2) F. I. Carroll and J. T. Blackwell, *Chem. Ind. (London)*, 574 (1972).
(3) A. Markovac, M. P. LaMontagne, P. Blumbergs, A. B. Ash, and C. L. Stevens, *J. Med. Chem.*, **15**, 918 (1972).
(4) E. L. May and E. Mosettig, *J. Org. Chem.*, 11, 1 (1946).
(5) C. J. Canfield and R. S. Rozman, *Bull. W.H.O.*, **50**, 203 (1974).
(6) R. E. strube, *J. Trop. Med. Hyg.*, **78**, 171 (1975).
(7) J. D. Arnold, D. C. Martin, P. E. Carson, K. H. Rieckmann, D. Willerson, Jr., D. F. Clyde, and R. M. Miller, *Antimicrob. Agents Chemother.*, **3**, 207 (1973).
(8) L. E. Lutz, P. S. Bailey, M. T. Clark, J. F. Codington, A. J. Deinet, J. A. Freek, G. H. Harnest, N. H. Leake, T. A. Martin, R. J. Rowlett, J. M. Sakbury, N. H. Shearer, Jr., J. D. Smith, and J. W. Wilson, III, *J. Am. Chem. Soc.*, **68**, 1813 (1946).
(9) D. C. Martin, J. D. Arnold, D. F. Clyde, M. Al. Ibrahim, P. E. Carson, K. H. Rieckmann, and D. Willerson, Jr., *Antimicrob. Agents Chemother.*, **3**, 214 (1973).
(10) Camphor-10-sulfonic acid, 3-bromo-8-camphorsulfonic acid, *O,O*-di-*p*-toluoyl-*d*-tartaric acid, *d*-tartaric acid, and *d*-camphoric acid were among the resolving acids tried.
(11) D. E. Pearson and A. A. Rosenberg, *J. Med. Chem.*, **18**, 523 (1975).
(12) P. H. Boyle, *Q. Rev., Chem. Soc.*, **25**, 323 (1971).
(13) S. H. Wilen, *Top. Stereochem.*, **6**, 107 (1971).
(14) P. Block, Jr., and M. S. Newman, "Organic Syntheses", Collect. Vol. V, Wiley, New York, N.Y., 1973, p 1031.
(15) A preliminary report on our work appeared in *Chem. Ind. (London)*, 477 (1975).
(16) R. C. Elderfield, H. E. Mertel, R. T. Mitch, I. M. Wempen, and E. Werble, *J. Am. Chem. Soc.*, **77**, 4816 (1955).
(17) W. T. Colwell, V. Brown, P. Christie, J. Lange, C. Reece, K. Yamamoto, and D. W. Henry, *J. Med. Chem.*, **15**, 771 (1972).
(18) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).
(19) The test results were supplied through the courtesy of Drs. R. E. Strube and E. A. Steck of the Walter Reed Army Institute of Research, Washington, D.C.
(20) The test procedure used by Dr. L. H. Schmidt is described in a World Health Organization report (1972b), WHO/MAL/72763 (cyclostyled report), WHO, Geneva.
(21) L. H. Schmidt, S. Alexander, L. Allen, and J. Rasco, *Antimicrob. Agents Chemother.*, **12**, 51 (1977).
(22) Samples of the salts of **3**–**6** and **8** and the free base of **7** were supplied by Dr. R. E. Strube of the Walter Reed Army Institute of Research, Washington, D.C.
(23) The optical purity of each fraction of the fractional crystallization was tested by converting a small sample to the 5 diphosphate and measuring its rotation. For this purpose the complex was dissolved in THF (2 mL); an equal volume of ethanol and 2 drops of 85% phosphoric acid were added. The reaction mixture changed its color immediately from dark green to orange. The **5** diphosphate crystallized overnight, was separated by filtration, washed with THF and ethanol, and dried.