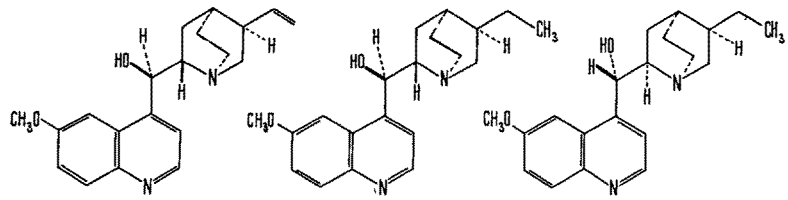
Antimalarial Activity of Natural, Racemic and Unnatural Dihydroquinine, Dihydroquinidine and Their Various Racemic Analogs in Mice Infected with *Plasmodium berghei*

The relationship between chemical structure and antimalarial activity in the series of Cinchona alkaloids has thus far dealt only with materials obtained from natural sources and their modified derivatives[1]. Recent efforts have resulted in much improved total syntheses[2,3] and quantities of Cinchona alkaloids with unnatural configuration became available for the first time. In this paper we would like to report the antimalarial activity of both enantiomers[4] and the racemate[5] of dihydroquinine (II)[6] and dihydroquinidine (III)[6] in mice infected with *Plasmodium berghei* and tested in form of their water soluble hemisulfates. The comparison has been carried out with the classical antimalarial quinine (I) as its hydrochloride.

The racemates of dihydroquinine and dihydroquinidine[5] were prepared in analogy to the method described for the total synthesis of quinine and quinidine[2]. Optical resolution using (–)-tartaric acid as resolving agent gave in each case the pure unnatural enantiomer[4].

Biological method. The *P. berghei* strain has been kept in the laboratory for 9 years through weekly blood passages in mice. In the described experiments, male albino mice weighing 18–20 g were inoculated, by the i.p. route, with 10 million infected red blood cells. The percentage of infected cells was determined by counting 300–400 unselected red cells in blood smears stained by the Giemsa method and the number of red cells per cubic millimeter

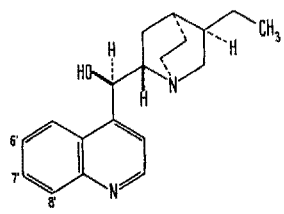


I II III

Table I. Antimalarial activity and acute toxicity of racemic dihydroquinine and racemic dihydroquinidine and their enantiomers

Compound	LD_{50} i.p.[7] in mice (mg/kg)	Activity against *Plasmodium berghei* p.o. in mice (MED mg/kg)
Quinine hydrochloride (I)	210 ± 8	200
Dihydroquinine hemisulfate (II)	210 ± 12	200
Enantiomer of II hemisulfate	210 ± 5	200
Racemate of II hemisulfate	225 ± 5	200
Dihydroquinidine hemisulfate (III)	192 ± 2	50
Enantiomer of III hemisulfate	94 ± 5	50
Racemate of III hemisulfate	128 ± 3	50

Table II. (±)-Dihydroquinine analogs



$R_{6'}$	$R_{7'}$	$R_{8'}$	Salt	LD_{50}, i.p. in mice (mg/kg)	Activity in mice vs. *Plasmodium berghei* MED (mg/kg)
CH_3O			H_2SO_4	225 ± 5	200
	CH_3O		2HCl	192 ± 7	200
CH_3O	CH_3O		2HCl	240 ± 5	100
CH_3			2HCl	196 ± 9	not active
Cl			2HCl	186 ± 5	100
	Cl		2HCl	195 ± 10	50
Cl		Cl	2HCl	310 ± 30	200
Quinine			2HCl	210 ± 8	200

in the blood pool determined by means of a Neubauer chamber.

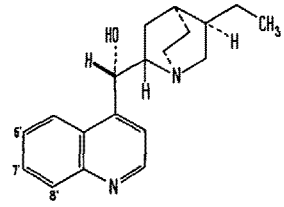
The inoculated mice were divided in groups of 8 animals and received 4 consecutive daily oral doses of the drugs in the different desired concentrations. Treatment started on the day following inoculation. On the 5th day of infection smears were performed, stained by the Giemsa method and, then, 50 microscopical fields were examined with a $\times 8$ eyepiece and $\times 100$ oil immersion objective. The minimum effective dose (MED) has been considered the lowest dose that keeps the blood of the inoculated animals free of parasites on the 5th day of inoculation. At least 2 series of tests were performed for each compound and untreated controls were normally used in all experiments. The results obtained with the compounds are listed in Table I.

Natural, unnatural and racemic dihydroquinine are equally active against *P. berghei* and at levels comparable to that of quinine. The corresponding enantiomeric forms of dihydroquinidine also show identical activities which are approximately four times higher than those of dihydroquinine. However, in contrast to the equal toxicities of enantiomeric dihydroquinines, unnatural dihydroquinidine is approximately twice as toxic as the natural enantiomer. The non-specificity of the two Cinchona alkaloids in this antimalarial screening is remarkable. Other alkaloids thus far, including the classical amebicide natural emetine, have shown high stereoselectivity with regard to their absolute configuration[8].

We have now extended our investigation to include dihydroquinine and dihydroquinidine analogs with varied substitution patterns in the aromatic ring. The racemates of these compounds are readily available by total synthesis[2] and the validity of their investigation for antimalarial activity is clearly indicated from our demonstration that the absolute configurations of the parent compounds are not a factor in the *Plasmodium berghei* test. Consequently, water soluble salts of the racemates of these derivatives[9] were tested by the above method, and the results obtained with the (±)-dihydroquinine and the (±)-dihydroquinidine analogs are listed in Tables II and III respectively.

Conclusions. Of the various (±)-dihydroquinine and dihydroquinidine derivatives prepared, the two 6′-demethoxy-7′-chloro analogs were most active in the *Plasmodium berghei* test, showing 4 times the antimalarial activity of natural quinine. The potential of (±)-6′-demethoxy-7′-chloro-dihydroquinine as an antimalarial and the possible cardiac activity of the corresponding dihydroquinidine isomer are being studied.

Table III. (±)-Dihydroquinidine analogs



$R_{6'}$	$R_{7'}$	$R_{8'}$	Salt	LD_{50}, i.p. in mice (mg/kg)	Activity in mice vs. *Plasmodium berghei* MED (mg/kg)
CH_3O			H_2SO_4	128 ± 3	50
	CH_3O		2HCl	195 ± 6	100
CH_3O	CH_3O		2HCl	220 ± 5	100
CH_3			2HCl	174 ± 11	200
Cl			2HCl	132 ± 4	100
	Cl		2HCl	240 ± 9	50
Cl		Cl	2HCl	260 ± 15	200
Quinine			2HCl	210 ± 8	200

Zusammenfassung. Racemisches Dihydrochinin und die beiden optischen Antipoden zeigen die gleiche Aktivität gegen *Plasmodium berghei* wie Chinin. Hingegen waren die entsprechenden Dihydrochinidine sowie die 6′-Desmethoxy-7′-chlor-Analogen von Dihydrochinin und Dihydrochinidin etwa viermal wirksamer.

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Chemical Research Division, Hoffmann-La Roche Inc., Nutley (New Jersey 07110, USA) and Instituto Nacional de Endemias Rurais, C.P. 1743, and Department of Parasitology, University of Minas Gerais, Belo Horizonte (Brazil), 3 March 1971.

[1] P. B. Russell in *Medicinal Chemistry*, 2nd Edition (Ed. A. Burger, Interscience Publishers Inc., New York 1960), p. 822.

[2] M. Uskoković, J. Gutzwiller and T. Henderson, J. Am. chem. Soc. *92*, 203 (1970); J. Gutzwiller and M. Uskoković, J. Am. chem. Soc. *92*, 204 (1970).

[3] M. Gates, B. Sugavanam and W. L. Schreiber, J. Am. chem. Soc. *92*, 205 (1970).

[4] The unnatural enantiomers of dihydroquinine and dihydroquinidine were first prepared by P. Rabe and A. Schultze, Ber. dt. chem. Ges. *66*, 120 (1933), but results on their antimalarial activity are not available.

[5] The racemates of dihydroquinine and dihydroquinidine were first prepared by P. Rabe, W. Huntenberg, A. Schultze and G. Volger, Ber. dt. chem. Ges. *64*, 2487 (1931); the antimalarial activity of racemates are also not reported.

[6] Formulae of the unnatural alkaloids correspond to the mirror images of the structures shown.

[7] Determination of LD_{50} was performed by Dr. W. Pool, Department of Pharmacology, Hoffmann-La Roche Inc., Nutley, New Jersey, USA.

[8] A. Brossi, Z. Brener, J. Pellegrino, H. Stohler and J. R. Frey, Experientia *16*, 64 (1960).

[9] The syntheses of the compounds listed in Tables II and III will be reported elsewhere.