# Thiosulfuric Acid Analog of Quinine as a Potential Antimalarial Agent

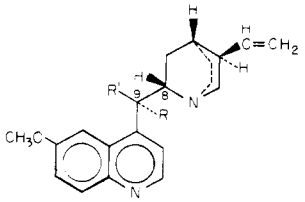
Daniel L. Klayman,* T. Scott Griffin, J. David Bower, and Samuel W. Page

*Division of Medicinal Chemistry, Walter Reed Army Institute of Research, Washington, D. C. 20012.* *Received April 30, 1973*

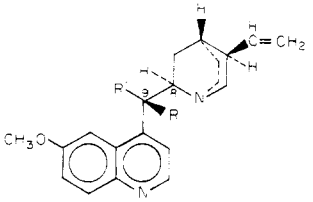
A comparison of the activity of antimalarial carbinolamine compounds, such as quinine, with their thiol analogs is of interest in the development of more efficient drugs. The substitution of sulfur for oxygen in the auxotherapeutic hydroxyl group[1] could cause useful changes in such factors as drug absorption, toxicity, and activity against drug-resistant strains of *Plasmodia.*

The conversion of quinine to a compound described as bis(quinyl) sulfide through the use of phosphorus pentasulfide has been reported earlier;[2] however, recent attempts to repeat this work have not been successful.† Since the direct displacement of chloride from 9-deoxy-9-chloroquinine (**1**) utilizing aqueous sodium hydrogen sulfide could not be readily accomplished, we approached the synthesis of the thiol analog *via* the 9-thiosulfuric acid (Bunte salt) derivative.

Quinine hydrochloride was treated with $SOCl_2$ to afford the corresponding chloro derivative **1** as an HCl salt.[3] On the basis of ORD and optical rotation studies which had previously been reported,[4] this displacement apparently occurs with inversion of the configuration at C-9. Therefore, **1** is shown with the same configuration at C-9 as that of epiquinine (**3**).



quinine, R = H; R′ = OH
**1**, R = Cl; R′ = H
**2**, R = $SSO_3H$; R′ = H
**3**, R = OH; R′ = H



quinidine, R = H; R′ = OH
**4**, R = H; R′ = H
**5**, R, R′ = O=
**6**, R = OH; R′ = $CH_3$ or R = $CH_3$; R′ = OH

The reaction of **1**·HCl with sodium thiosulfate in $H_2O$–MeOH gave 9-deoxyepiquinine-9-thiosulfuric acid (**2**) as the hemithiosulfuric acid salt in yields of 18–25%. The poor yields obtained are attributed to the difficulty in displacing the sterically hindered 9-chloro group. The specific rotation $[\alpha]D$ of $2 \cdot 0.5H_2S_2O_3$ was determined to be +288°, which is in the same direction as the $[\alpha]D$ +43° for epiquinine **3**·2HCl and in the opposite direction of the $[\alpha]D$ −121° reported for quinine hydrochloride.[4] In addition, ORD measurements in the same solvent system showed large Cotton effects of opposite sign for $2 \cdot 0.5H_2S_2O_3$ ($[\alpha]_{378}$ +1810°) and for quinine hydrochloride ($[\alpha]_{364}$ −1650°). Hence, $2 \cdot 0.5H_2S_2O_3$ is proposed to have the epiquinine (**3**) configuration at C-9.

Compound $2 \cdot 0.5H_2S_2O_3$ is slightly soluble in water, methanol, and dilute base and moderately soluble in dilute acid. A solution of $2 \cdot 0.5H_2S_2O_3$ in $H_2SO_4$ exhibited the blue fluorescence under ultraviolet light characteristic of the cinchona alkaloids.[5] Just as quinine carbonate and ethyl carbonate,[6] made by the esterification of quinine with phosgene and ethyl chlorocarbonate, respectively, lack the intensely bitter taste of quinine, compound $2 \cdot 0.5H_2SO_3$ was similarly found to be devoid of taste.

**Antimalarial Activity.** All previously reported alterations at C-9 in quinine or in quinidine, other than esterification of the carbinol function, have resulted in a decrease in or loss of antimalarial activity. For example, the 9-deoxy-9-chloro derivatives of quinine and quinidine were found to be inactive against *Plasmodium relictum* as was the hydrochloride salt of quinene (8,9-dehydroquinine).[3] In addition, the 9-deoxy-9-chloro and the 9-deoxy-9-hydro derivatives of quinine and quinidine (**4**) were reported to be inactive against *P. gallinaceum* in chicks.[7]

As indicated in Table I, compounds in which C-9 of quinine and quinidine have been modified, with the exception of $2 \cdot 0.5H_2S_2O_3$, showed decreased or no activity against *P. berghei* in mice.‡ The thiosulfate analog, $2 \cdot 0.5H_2S_2O_3$ exhibited greater activity than did quinine in its free base or salt forms including *N*-quininium-*S*-thiosulfuric acid sesquithiosulfate salt.[9]

Since $2 \cdot 0.5H_2S_2O_3$ most probably has the same configuration at C-9 as that of epiquinine (**3**), it would be of interest to compare its antimalarial activity with that of the latter compound. Test results for **3** against *P. berghei* have not been reported, but **3** was found to be slightly more active ($Q < 1.5$) than quinine ($Q = 1.0$) against *P. gallinaceum* in chicks and much less active ($Q < 0.06$) than quinine against *P. lophurae* in ducks.[10]

The thiosulfate analog $2 \cdot 0.5H_2S_2O_3$ was tested orally (gavage) in mice against a drug-sensitive parent strain and a chloroquine-resistant strain of *P. berghei.* Against the parent strain, $2 \cdot 0.5H_2S_2O_3$ had the same order of activity as quinine. Against the chloroquine resistant strain, $2 \cdot 0.5H_2S_2O_3$ showed greatly diminished activity which is similar to that observed for quinine.§

In addition, $2 \cdot 0.5H_2S_2O_3$ was tested *in vitro* for its effectiveness in inhibiting maturation of schizonts in human red blood cells of a drug-resistant Vietnam (Marks) strain and a drug-sensitive Uganda I strain of *P. falciparum.* Against both strains $2 \cdot 0.5H_2S_2O_3$ was equally effective at 1000 μg/l. of blood required for inhibition of <50% of the parasites and at 2500 μg/l. of blood required for inhibition of >90% of the parasites relative to the control sample. Quinine is also equally effective against both strains, 1000 μg/l. of blood being required for inhibition of >90% of the parasites.#

The above data indicate that replacement of the hydroxyl group in quinine with a thiosulfuric acid group does not appear to lessen its antimalarial activity. Work is now in progress on the conversion of $2 \cdot 0.5H_2S_2O_3$ to the corresponding thiol and disulfide derivatives.

## Experimental Section

Infrared spectra (KBr) were determined on a Beckman IR-5 spectrophotometer, ultraviolet spectra on a Cary Model 14 spectrophotometer, optical rotation measurements on a Perkin-Elmer 141 polarimeter, ORD spectra on a Jasco Model 5 spectropolarimeter, and melting points on a Fisher-Johns melting point apparatus (uncorrected). Microanalyses were performed by Mr. Joseph F. Alicino, New Hope, Pa. ORD spectra were measured at 30° in a 1:5 (v/v) solution of 10% aqueous HCl and MeOH.

†C. C. Cheng, Midwest Research Institute, Kansas City, Mo., personal communication.

‡Biological tests were performed by Dr. Leo Rane of the University of Miami. For the testing procedure, see ref 8.

§Tests were performed by Dr. Paul E. Thompson of the University of Georgia. For the procedure, see ref 11.

#The testing was done by Dr. Karl H. Rieckmann of the University of Chicago. For the procedure, see ref 12.

Table I. Comparison of Antimalarial Activities of Quinine, Quinidine, and Their Derivatives[a]

| | Dose, mg/kg (test minus control)[b] | | | | | |
|---|---|---|---|---|---|---|
| | 640 | 320 | 160 | 80 | 40 | 20 |
| 9-Deoxyepiquinine-9-thiosulfuric acid $0.5H_2S_2O_3$ (2) | 22.4 (*c*, 3) | 15.9 (*c*, 3) | 12.7 (*a*) | 3.9 | 0.9 | 0.5 |
| *N*-Quininium-*S*-thiosulfate · $1.5H_2S_2O_3$[c] | 13.4 (*c*, 3) | 12.7 (*a*) | 10.3 (*a*) | 4.9 | 0.5 | 0.5 |
| Quinine · $0.5SO_4^{2-}$[d] | 7.1 (*a*) | 6.1 | 4.7 | 2.3 | 1.5 | 0.5 |
| Quinidine · $0.5SO_4^{2-}$[e] | 7.7 (*t*, 1) | 4.0 | 2.4 | 2.2 | 1.2 | |
| 9-Deoxy-9-chloroquinine (1) | 0.5 | 0.5 | 0.3 | 0.3 | 0.3 | 0.1 |
| 9-Deoxy-9-hydroquinidine (4)[f] | | 0.3 | | 0.1 | | 0.1 |
| Quininone (5)[g,h] | 0.9 | | 0.7 | | 0.7 | |
| Quinidinone (5)[g,h] | 0.2 | | 0.2 | | 0.0 | |
| 9-Methylquinidine (6)[i] | 0.2 | | 0.2 | | 0.2 | |

[a]The compounds were evaluated against *Plasmodium berghei* KBG 173 malaria by administration to five male mice per dilution in a single subcutaneous dose 72 hr after infection.[8] [b]The mean survival time of the control animals was 6.1 days. Deaths from days 2–5 after drug administration are attributed to toxicity (*t*, number of dead mice). Compounds are classified as active (*a*) when the mean survival time of the treated mice is twice that of the controls, *i.e.*, test − control > 6.1 days, and curative (*c*, number of surviving mice) when one or more test animals live 60 days postinfection.[8] [c]This derivative was reported as a *N*-quininium-*S*-thiosulfate zwitterion[9] but was obtained by us as the sesquithiosulfate salt. [d]Source: S. B. Penick and Co., New York, N. Y. [e]Source: K and K Laboratories, Jamaica, N. Y. [f]Source: Dr. V. I. Stenberg, University of North Dakota. [g]Both samples are shown as structure **5** due to the probable epimerization at C-8 of quininone to form quinidinone as reported by Lyle and Gaffield.[4] The antimalarial activities of the two samples are not considered to be significantly different. [h]Source: Dr. G. R. Pettit, Arizona State University. [i]Source: Dr. D. E. Pearson, Vanderbilt University.

**9-Deoxy-9-chloroquinine (1).** To a mixture of 100 g (0.13 mol) of quinine · $SO_4^{2-}$ · $2H_2O$ and 200 ml of $CHCl_3$ was added a solution of 10 g (0.25 mol) of NaOH in 100 ml of $H_2O$. This mixture was stirred at room temperature for 3 hr. The $CHCl_3$ layer containing the quinine free base was then separated, and the aqueous layer was extracted with additional $CHCl_3$. The combined $CHCl_3$ extracts were washed with $H_2O$ and dried. Anhydrous HCl was then bubbled into the solution to form quinine dihydrochloride. Thionyl chloride (132 g, 1.1 mol) was added to the mixture, which was then heated under reflux for 6 hr and cooled to room temperature. Water (30 ml) was added to decompose the excess $SOCl_2$, and the mixture containing the crude 1 · HCl in $CHCl_3$ was neutralized with 50 ml of 5 *N* NaOH solution. Compound 1 was extracted into the $CHCl_3$ layer which was washed with $H_2O$ and dried. The resulting yellow solution was chromatographed on a 4 × 50 cm column of alumina (activity II–III) using $CHCl_3$–$C_6H_6$ (1:1) as the eluent. The product was recrystallized from EtOAc to yield 79 g (89%) of 1 as a white crystalline solid: mp 155–157° (lit.[3] 151°); $[\alpha]^{20}D$ +62° (*c* 0.5 in MeOH) (lit.[3] $[\alpha]^{20}D$ +62.0° (*c* 1.0 in EtOH)). *Anal.* ($C_{20}H_{23}ClN_2O$) C, H, N, Cl.

**Monohydrochloride of 1.** Concentrated HCl was added dropwise to a solution of 50 g (0.15 mol) of 1 in 150 ml of MeOH until pH 5 was reached. An additional 150 ml of MeOH was added, and the mixture was heated to dissolve the precipitated HCl salt. The hot solution was diluted with 300 ml of EtOAc and cooled to room temperature causing crystallization of the product. The yield of 9-deoxy-9-chloroquinine · HCl (from MeOH–EtOAc) was 43 g (78%): mp 218–219° dec (lit.[3] 219° dec); $[\alpha]^{20}D$ +10.3° (*c* 0.7 in $H_2O$) (lit.[3] $[\alpha]^{20}D$ +9.75° (*c* 1.0 in $H_2O$)). *Anal.* ($C_{20}H_{24}Cl_2N_2O$) C, H, N, Cl.

**9-Deoxyepiquinine-9-thiosulfuric Acid (2) Hemithiosulfate.** To 10 g (0.026 mol) of 1 · HCl in 60 ml of $H_2O$–MeOH (1:2) was added a solution of 6.5 g (0.026 mol) of $Na_2S_2O_3 \cdot 5H_2O$ in 20 ml of $H_2O$. This resulting solution was heated on a steam bath for 6 hr, and the precipitate which formed was collected and triturated with $CHCl_3$ to remove 3.6 g of unreacted 1. The resulting light yellow solid was recrystallized from MeOH–$H_2O$ (1:1) to yield 2.4 g (19%) of **2** as the hemithiosulfate: mp 197–199° dec; $[\alpha]^{20}D$ +288° [*c* 0.016 (4.6 (v/v) 10% aqueous HCl–MeOH)]; uv [4.2 × $10^{-5}$ *M*, 1:5 (v/v) 10% aqueous HCl–MeOH] $\lambda_{max}$ 359 nm (*e* 5540), sh 330 (4100), 256 (21,100); ORD (*c* 0.01) $[\alpha]_{378}$ +1810, $[\alpha]_{332}$ −2050, $[\alpha]_{298}$ +39, $[\alpha]_{270}$ −130°; $\nu_{max}$ (KBr) 3448, 1230, and 1022 $cm^{-1}$ *Anal.* ($C_{20}H_{24}O_4N_2S_2 \cdot 0.5H_2S_2O_3$) C, H, N, S.

**ORD of quinine · HCl · $2H_2O$**** (*c* 0.0077) had $[\alpha]D$ −52°; $[\alpha]_{364}$ −1650, $[\alpha]_{320}$ +1290, $[\alpha]_{272}$ sh −520, $[\alpha]_{270}$ −723°.

**Acknowledgment.** We thank Col. W. E. Rothe and Drs. T. R. Sweeney and T. S. Woods for their interest in this project and for helpful suggestions. We also thank Lt. Col. K. E. Kinnamon for review of the biological portions of the manuscript.

**Source, Merck and Co., Rahway, N. J.

## References

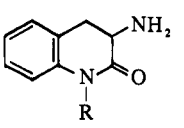
(1) R. M. Pinder, "Medicinal Chemistry," 3rd ed, A. Burger, Ed., Wiley-Interscience, New York, N. Y., 1970, p 505.
(2) E. Comanducci and L. Pescitelli, *Gazz. Chim. Ital.*, **36**, 781 (1906).
(3) A. Cohen and H. King, *Proc. Roy. Soc., Ser. B*, **125**, 49 (1938).
(4) G. G. Lyle and W. Gaffield, *Tetrahedron*, **23**, 51 (1967).
(5) P. Rabe and O. Marshall, *Justus Liebigs Ann. Chem.*, **382**, 360 (1911).
(6) P. C. Stecher, Ed., Merck Index, 8th ed, Merck and Co., Rahway, N. J., 1968, p 904.
(7) D. K. de Jongh and E. G. van Proosdij-Hartzema, *Arch. Int. Pharmacodyn.*, **98**, 270 (1954).
(8) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, 10, 431 (1967).
(9) J. T. Edward and J. Whiting, *Can. J. Chem.*, **48**, 337 (1970).
(10) F. Y. Wiselogle, "A Survey of Antimalarial Drugs, 1941–1945," Vol. II, part 2, J. W. Edwards, Ed., Ann Arbor, Mich., 1946, p 1097.
(11) P. E. Thompson, B. Olszewski, A. Bayles, and J. A. Waitz, *Amer. J. Trop. Med. Hyg.*, **16**, 133 (1967).
(12) K. H. Rieckmann, J. V. McNamara, H. Frischer, T. A. Stockert, P. E. Carson, and R. D. Powell, *ibid.*, **17**, 661 (1968).

# Synthesis and Microbiological Properties of 3-Amino-1-hydroxy-2-indolinone and Related Compounds

Alvie L. Davis,* David R. Smith,† and Tommy J. McCord

*Department of Chemistry, Abilene Christian College, Abilene, Texas 79601.* *Received March 14, 1973*

In our first study of the structure-activity relationships of hydroxamic acids in heterocyclic systems, the synthesis and antibacterial properties of 3-amino-3,4-dihydro-1-hydroxycarbostyril (**1**) were described.[1] Following this report, the 7-methoxy and 7-hydroxy derivatives of **1** were prepared and shown to have antibacterial activities equally



**1**, R = OH
**3**, R = H

†Taken in part from the M.S. Thesis of D. R. Smith, Abilene Christian College, Abilene, Texas, May 1971.