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THE QUININE–HAEMIN INTERACTION AND ITS RELATIONSHIP TO ANTIMALARIAL ACTIVITY

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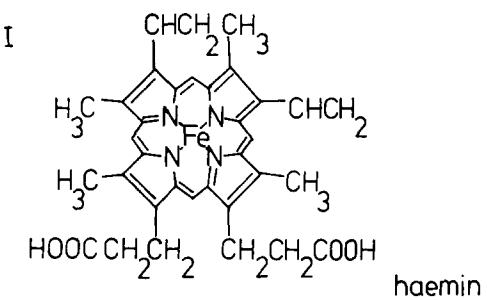
Abstract—Benzene-soluble complexes of quinine and related antimalarial drugs with haemin (ferriprotoporphyrin IX) were examined spectrophotometrically and gave evidence for coordination of haemin iron with the nitrogen atom of the quinuclidine or piperidine group. The antimalarially inactive 9-epimer of quinine did not show evidence of coordination with haemin. A model of the interaction is presented.

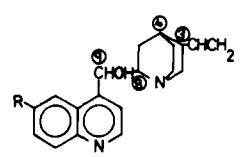
The solubility of haemin chloride (ferriprotoporphyrin IX —Cl) (I) in solutions of the antimalarially active cinchona alkaloid quinine (II) in organic solvents was first reported in 1937 [1]. Spectrophotometric studies in aqueous solution were carried out by Cohen and colleagues [2, 3] who found that complex formation took place between quinine and haemin (ferriprotoporphyrin IX —OH), leading to the loss of the typical ultraviolet absorption peak of quinine, and a reduction in intensity of the Soret (γ) peak of the iron porphyrin. Fitch and colleagues [4] have carried out studies on isolation of the common receptor for quinine and chloroquine from *Plasmodium berghei* and have brought forward strong evidence that haemin may be an essential component.

I



haemin

II, III



quinine, epiquinine, quinidine: R = -OCH_3—
cinchonidine, cinchonine : R = -H

The present work examines spectrophotometrically the interaction of haemin with quinine, with its antimalarially inactive 9-epimer, epiquinine, and with other drugs which are thought to have a quinine-like mode of action [5, 6].

* Public Health Laboratory Service.

MATERIALS AND METHODS

Table 1 shows details of the drugs used. Haemin chloride was obtained from Sigma Chemical Co. (London, U.K.). Benzene-soluble complexes of haemin with antimalarials were prepared as follows. The drugs were dissolved at a concentration of 4×10^{-5} M in 5 ml pH 7.4 potassium phosphate buffer, 0.07 M, in glass-stoppered test tubes. One mg haemin chloride was added and then 5 ml A.R. benzene. The stoppered tubes, including controls without drugs and without haemin were gently mixed on a roller-tube machine for 24 hr at 37°. Benzene and aqueous layers were examined visually, diluted with solvent where necessary, and spectra were determined with solvent as reference, using a Unicam SP1700 instrument.

RESULTS

Haemin alone. No dissolved haemin could be detected in aqueous or benzene phase.

Quinine alone. The characteristic absorption peak of quinine base (335 nm) could be detected in the benzene layer.

Quinine/haemin. The benzene layer showed a greenish-brown colouration, and on spectrophotometry no quinine absorption peak could be detected. The spectrum showed a large γ peak at 408 nm (indicative of haemin iron in a high-spin state) and clearly defined β and α peaks at 490 and 602 nm respectively, indicating the formation of a quinine hemichrome coordination complex (Fig. 1, Table 2). No quinine or dissolved haemin could be detected in the aqueous phase.

9-Epiquinine alone. The characteristic absorption peak (335 nm) was detected in the benzene layer.

9-Epiquinine/haemin. A faint colour was detected in the aqueous layer, which had the spectral characteristics of haemin. The benzene layer showed a faint colour and the absorption showed a 335 nm peak and a weak peak at 408 nm, indicating the presence of uncomplexed epiquinine and some solubilised haemin. There was no evidence of hemichrome formation.

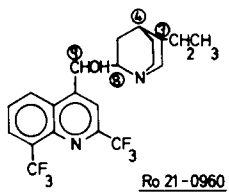
Table 1. Drugs used in this study

Name*	Chemical name	Enantiomer	Salt	Relative stereochemistry	Configuration
(1) Quinine	3-vinyl-6′-methoxyruban-9-ol	−	2HCl	8,9 *erythro*	9R,8S 4S,3R
(2) Cinchonine	3-vinyl-ruban-9-ol	+	base	8,9 *erythro*	9S,8R 4S,3R
(3) RO 21,0960	2′,8′-bis(trifluoromethyl) dihydrocinchonine	±	base	8,9 *erythro*	9RS,8SR 4RS,3SR
(4) 9-Epiquinine	3-vinyl-6′-methoxyruban-9-ol	+	2HCl	8,9 *threo*	9S,8S 4S,3R
(5) Mefloquine	α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinoline methanol	±	HCl	1′,2′ *erythro*	1′RS,2′SR
(6) WR 177,602	α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinoline methanol	±	HCl	1′,2′ *threo*	1′RS,2′RS
(7) WR 122,455	3,6-bis(trifluoromethyl) α-(2-piperidyl)-9-phenanthrene methanol	±	HCl	85:15 . 1′,2′ *erythro:threo* mixture	1′RS,2′SR 1′RS,2′RS

* Sources of drugs: (1) BDH Ltd. (Poole, U.K.); (2) Dr. J. Williamson; (3) Hoffmann la Roche (Basel, Switzerland); (4) Hoffmann la Roche; (5) U.S. Army Research and Development Command (Washington, DC); (6) U.S. Army (synthesised by Dr. R. E. Olsen, Cordova Chemical Co.); (7) U.S. Army Research and Development Command.

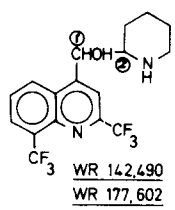
Cinchonine (III). This is *des*methoxyquinidine, an antimalarially active dextrorotatory cinchona alkaloid. Results here were very similar to those for quinine: a hemichrome was found in the benzene layer (see Table 2).

IV



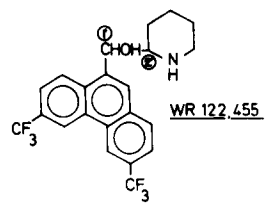
RO 21,0960 (IV). This is a synthetic quinoline methanol with a quinuclidine side chain. It gave similar results to cinchonine and quinine.

V



Mefloquine (V). This is a synthetic quinoline methanol, like RO 21,0960 but with a piperidine side chain. It has given promising results in clinical trials and is likely to replace quinine in the therapy of chloroquine-resistant *P. falciparum* malaria [6]. A hemichrome similar to that of quinine was again evident in the benzene layer, with α and β peaks at slightly longer wavelengths than was the case for the drugs tested which had quinuclidine side chains. The 1′, 2′ *threo* derivative of mefloquine, WR 177,602 gave similar results (see Table 2).

VI



WR 122,455 (VI). This antimalarially active phenanthrene methanol has a piperidine side chain. A hemichrome soluble in benzene was produced on

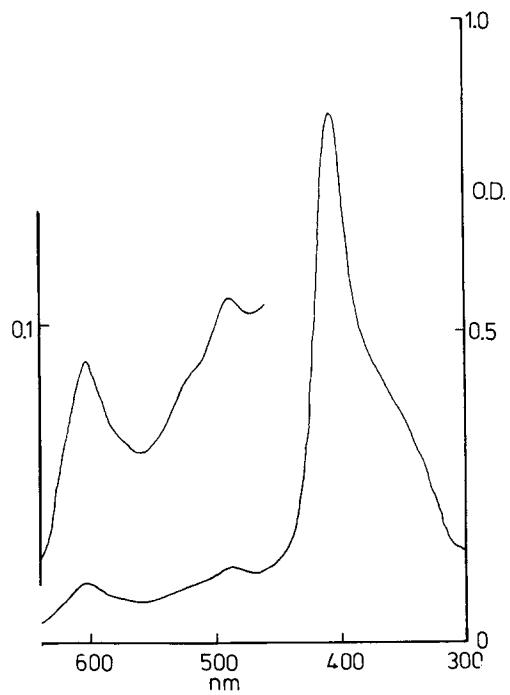
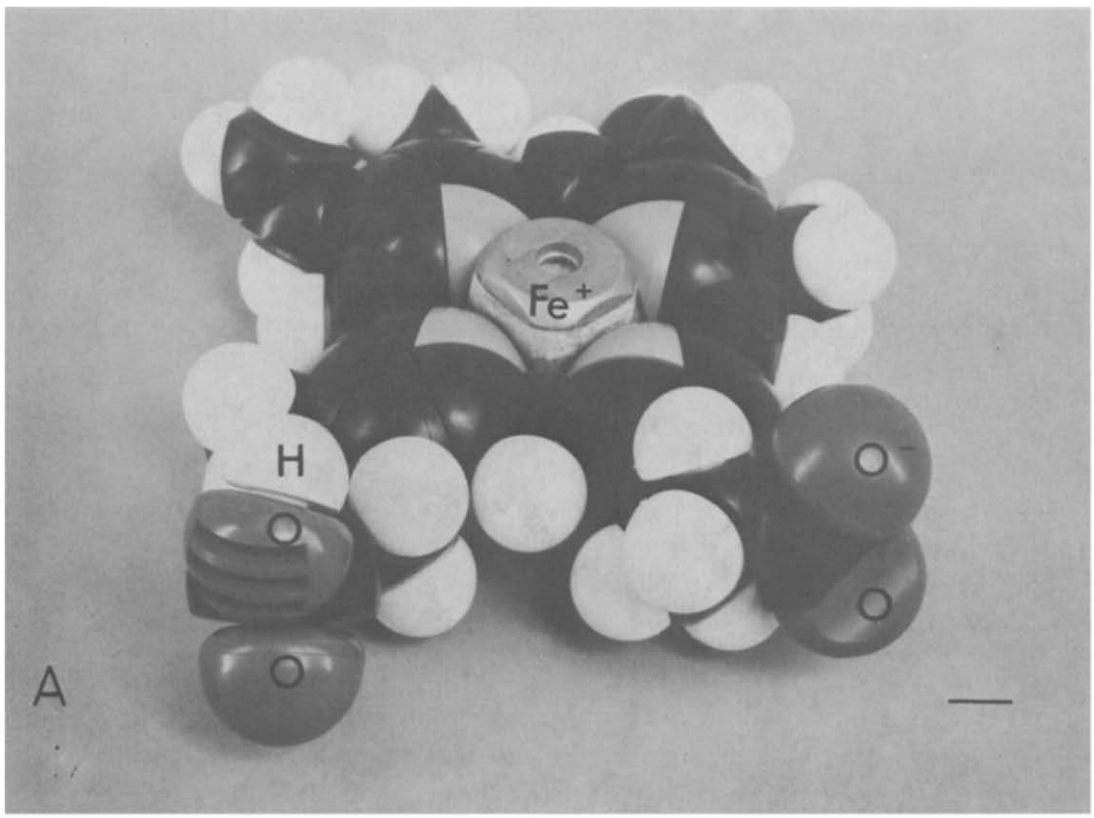


Fig. 1. Absorption spectrum (path length 1 cm) of a diluted benzene solution of quinine–haemin complex.



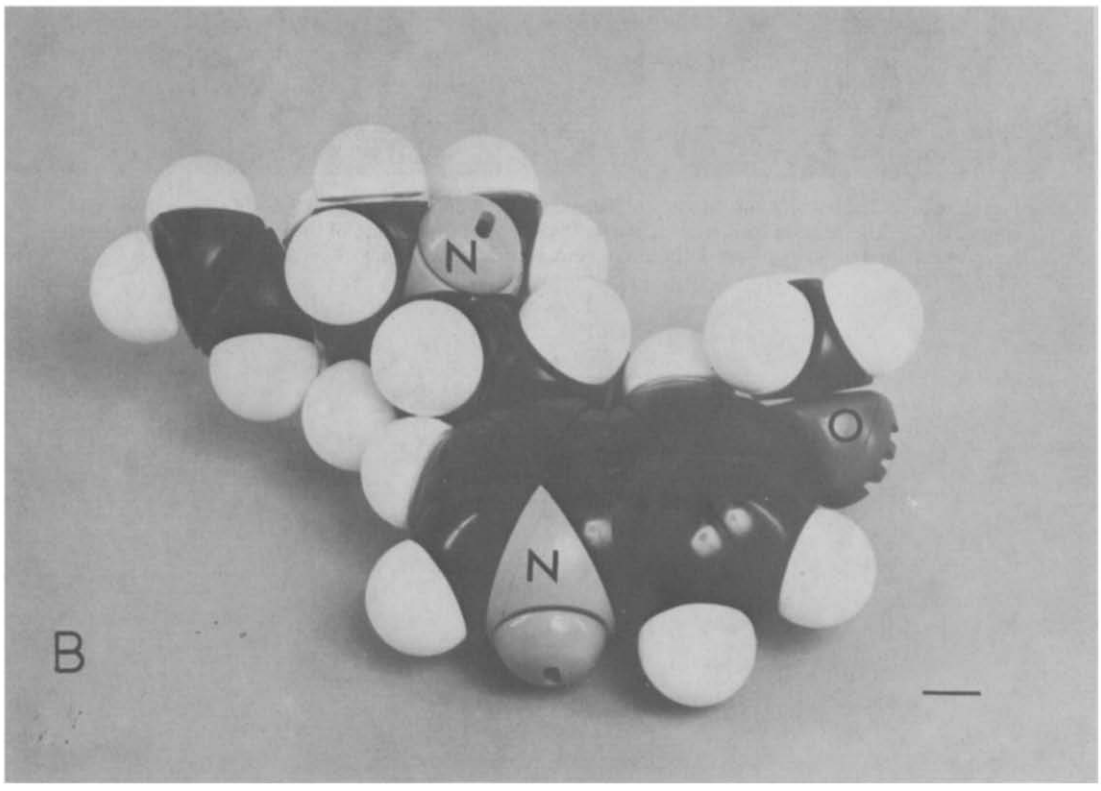


Fig. 2 (A and B). See legend on p. 3326.

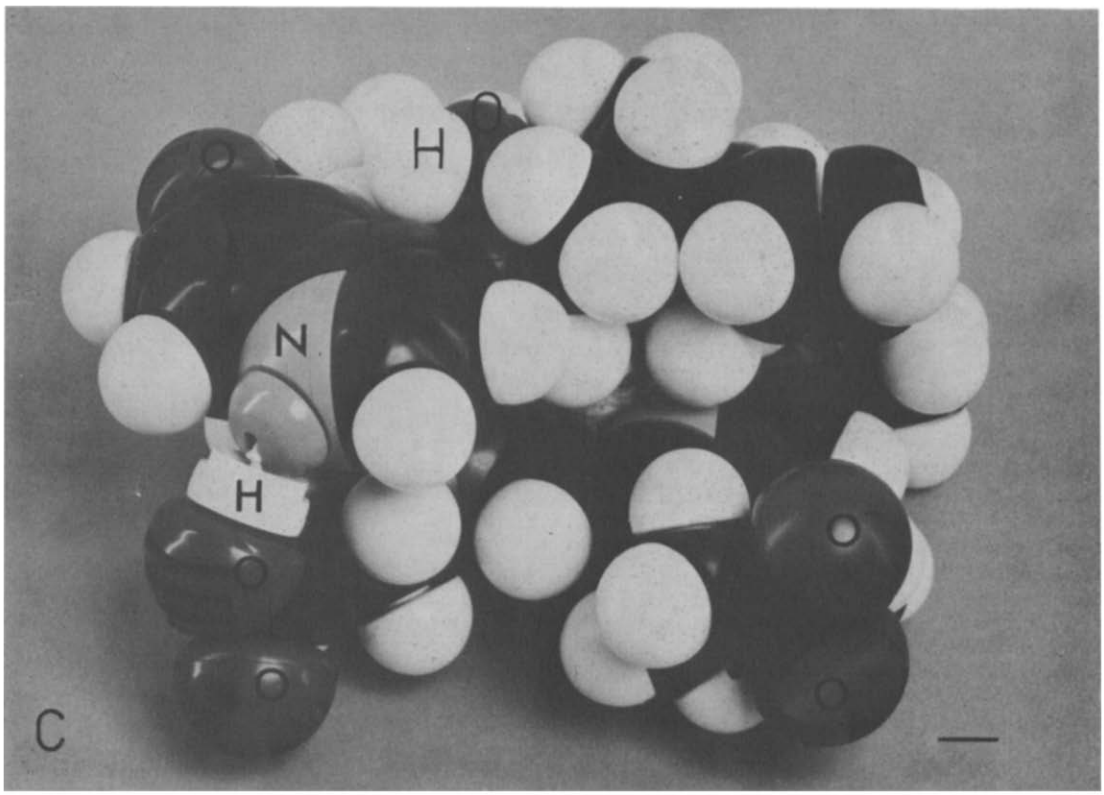


Fig. 2. Corey–Pauling–Koltun models of haemin (A), quinine (B) and a possible haemin–quinine complex (C). The haemin iron atom is shown 0.45 Å out of the plane of the porphyrin ring [13] and is retained in its high-spin form in the pentacoordinate complex. The bar represents 1 Å.

Table 2. Benzene-soluble complexes of antimalarials with haemin

Drug	Wavelength of peak (nm)			O.D.	E(mM)*			[Drug]
	α	β	γ	γ peak	α	β	γ	[Haemin]
Quinine	602	490	408	4.1	7.9	9.6	79.0	0.8
Cinchonine	605	490	409	2.9	9.6	11.3	92.6	1.3
RO 21,0960	605	492	408	2.0	5.8	6.4	58.4	1.2
Epiquinine	—	—	408	0.1	—	—	—	—
Mefloquine	608	495	408	3.2	9.8	11.7	102.0	1.3
WR 177,602	611	496	410	2.8	8.4	10.3	80.8	1.1
WR 122,455	608	495	410	2.7	5.5	6.3	52.1	0.8

* As haemin. Haemin was measured as the pyridine haemochrome [14].

interaction with haemin, and the α and β peaks were similar to those of mefloquine (Table 2).

DISCUSSION

Hemichrome coordination complexes were detected with all the drugs tested except the 9-epimer of quinine. From the structures it can be inferred that the atom coordinating with haemin iron is the side-chain quinuclidine or piperidine nitrogen. This is supported by the differences observed in the wavelengths of the hemichrome absorption peaks for quinuclidine and piperidine-containing compounds. It appears that the aromatic nitrogen atom of the quinoline ring plays a relatively unimportant role in the complex, since it is absent in the phenanthrene methanol WR 122,455.

The major importance of the 8, 9 *erythro* configuration for antimalarial activity of the cinchona alkaloids [7] is reflected in the ability of quinine and cinchonine (both 8, 9 *erythro*) to form hemichromes whilst 9-epiquinine (8, 9 *threo*) did not.

In the case of 1′, 2′ *threo* and *erythro* mefloquine, both configurations are antimalarially active [6] and both form a hemichrome.

Loss of the quinoline-related absorption peak and depression of the γ porphyrin peak in the haemin–quinine complex indicate that ring–ring interactions are taking place. For coordination therefore, the quinuclidine nitrogen atom needs to be in the same plane as the quinoline ring. Models of quinine and cinchonine allow this, but in 9-epiquinine the 9-OH group prevents contact of the nitrogen atom with the haemin iron. X-ray diffraction studies and least energy calculations [8] have shown that the 8,9 *erythro* cinchona alkaloids have *trans* and *gauche* conformers, whilst the 8, 9 *threo* alkaloids have only *gauche*. This would accord well with the present observations, because the conformer binding to haemin would be *trans*. In the case of the aryl methanols with piperidine side chain studied here, the piperidine ring is markedly less bulky than the quinuclidine ring, and *trans* conformers would appear to be possible for both the *erythro* and *threo* configurations.

The role of the aromatic nitrogen atom of the quinoline ring in quinine–haemin interaction is difficult to decide, since the phenanthrene methanols with an extra aromatic ring formed a hemichrome without its help. It is possible, however, that a hydrogen bond could be formed between this group and one of the carboxyl groups of haemin. Such an interaction seems however to be relatively unimportant compared with the coordination and ring–ring interactions. Spacefilling models of quinine, haemin, and the proposed coordination complex are shown in Fig. 2.

These observations support the findings of Fitch and colleagues [4] and may also explain the partial dissolution of malaria pigment, a complex of haemin with two proteins [9] after quinine or mefloquine treatment of intraerythrocytic plasmodia [10, 11]. The toxic effects of quinine and similar drugs on malaria parasites may relate to the lipid solubility of the coordination complexes, and the damaging effects of haemin upon membranes [12].

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