XCVIII. THE ACTION OF THE CINCHONA AND CERTAIN OTHER ALKALOIDS IN BIRD MALARIA.

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ALTHOUGH cinchona bark first appeared in Europe about 1639 and the first cinchona alkaloid, quinine, was isolated from it in 1820 and has been manufactured and used in medicine ever since, there are still many unsolved problems in connection with the use of cinchona alkaloids in malaria.

Some of these questions are of great practical importance. Thus it has been assumed that for the treatment of malaria the best of the four principal cinchona alkaloids is quinine, and over 90 % of the bark now produced from cinchona plantations is bark cultivated to give high yields of quinine.

In the last few years misgivings have arisen as to whether this is the best policy to adopt, and it has even been suggested that it might be advisable to go back to the plan adopted in British India and Ceylon in the early days of cinchona planting and to cultivate the hardy *Cinchona succirubra* species, which yields a mixture of alkaloids, in which quinine and cinchonidine predominate, and to prepare this mixture as "quinetum" for the large scale treatment of malaria [Gage, 1925]. As a basis for the serious discussion of such questions it is desirable that the relative therapeutic values of the principal crystalline alkaloids found in cinchona bark, viz. quinine, quinidine, cinchonidine and cinchonine, should be determined. Hitherto, the only method of making such a comparison has been by clinical trials. The difficulties of making such comparative trials are explained in a report by the Medical Research Council [1925] dealing with the relative values of quinine and quinidine. Probably the difficulties experienced in the case of these two bases have so far precluded extension of the investigation to a similar comparison between quinine and the other readily procurable cinchona alkaloids, cinchonidine, cinchonine, and the reduction product of quinine, hydroquinine. If, in such a comparative trial, cinchonidine proved to be as useful as quinine, there would be a *prima facie* case for the use of "quinetum" in place of quinine.

The relative values of the four alkaloids are also of importance in connection with the use of "cinchona febrifuge," which consists of the total

residual alkaloids of cinchona bark, left after the removal of as much of the crystallisable alkaloids as manufacturers normally require to meet commercial demands. Large quantities of this material are now used in malarial countries and it is, as might be expected, of varying quality [Howard, 1925]. Its use has numerous advocates, but before this can be placed on a scientific basis, a satisfactory method of analysis for cinchona febrifuge is required so that definite standards can be set up for it [Goodson and Henry, 1930]. These standards ought to take into account the relative therapeutic values of the components as well as industrial convenience.

It is also sometimes suggested that the special value attributed by some authorities to the antimalarial activity of cinchona febrifuge is due to the presence of some highly active and so far undiscovered component [Prain, 1925].

Apart from these practical problems, there are also questions of purely scientific interest. Much discussion has taken place as to the particular part of the complex structure of the quinine molecule which determines its anti-malarial activity, and certain tentative conclusions have been reached based partly on *in vitro* tests with protozoa and partly on limited clinical trials, but there is still no certainty about these conclusions, though they may have an important bearing on the synthesis of new antimalarial drugs [Kaufmann, 1926; Giemsa, Weise and Tropp, 1926; Shaw, 1928].

Further, in the course of the last 10 years many "modified cinchona alkaloids" have been prepared in the laboratory and for some of these claims have been made for increased action in malaria.

It seemed to us that the technique standardised by Roehl [1926] for testing the efficacy of drugs in bird malaria might be used with advantage to investigate some of these questions since it was by this means that the efficacy of "plasmoquin" in malaria was discovered, though the case of "dimeplasmin" [Green, 1929], which was investigated in the first instance by the same method, indicates that positive results in bird malaria may not always indicate that a drug will be efficacious in human malaria.

Among the "modified cinchona alkaloids" the series of "quitenines" obtained by the gentle oxidation of the four principal cinchona alkaloids, whereby the $\text{CH}:\text{CH}_2$ group in the quinuclidine nucleus (R' in the generalised formula, p. 876) is oxidised to a carboxyl group, occurred to us as a promising basis for a homologous series of products, the "alkylquitenines," in which the value of Roehl's technique for dealing with questions of this kind could be tested. It is, for example, well known that in *in vitro* tests of bactericidal agents action increases as a homologous series is ascended up to a maximum and then diminishes. In the series of 4-*n*-alkylresorcinols, maximal effect is reached at hexylresorcinol [Johnson and Lane, 1921; Dohme, Cox and Miller, 1926], in the *p*-*n*-alkylphenols at *n*-amylphenol [Coulthard, Marshall and Pyman, 1930] and in the alkyl dihydrocupreines at octyldihydrocupreine [Schäffer, 1917; Bieling, 1918]. But this principle, though it is traceable in the results

of a number of investigations [*e.g.* Morgenroth and Tugendreich, 1916], has not been established with the same certainty in *in vivo* therapeutic tests. It has, however, already been shown that in a short series of cupreine ethers the following changes in activity in bird malaria occur. The figures in brackets give the retardation of attack in days [Giemsa, Weise and Tropp, 1926]:

Cupreine (0-4).

Cupreine ethers:

Methyl: quinine (12-13); quinidine (12).

Ethyl: quinethyline (8-40).

Propyl: quinpropyline (4-10).

Amyl: quinamyline (7-11).

With regard to quitenine itself it has been clearly established that this substance is inactive in both human and bird malaria, but that activity is recovered in the ethyl ester [Giemsa, 1926]; investigation in this series does not seem to have been carried beyond this single observation.

Finally, there is a considerable number of natural drugs other than cinchona bark and its constituent alkaloids which have local reputations as remedies for malaria or as febrifuges. Some of these, such as the *Alstonia* barks, have been recognised in Pharmacopoeias, but the evidence for their therapeutic value is slender. The discovery of a highly active substance among them might provide a new starting-point for the synthesis of antimalarial drugs, and it seemed reasonable to suppose that Roehl's method might at least enable any promising product to be picked out of this group for further examination. The present paper records the results of attempts to apply this method to the solution of some of these problems.

General formula for cinchona alkaloids.

As it will frequently be necessary to refer to derivatives of the various cinchona alkaloids, it may be convenient at this stage to give the following generalised formula for the principal cinchona alkaloids:

$R = H$ in cinchonine, cinchotenine, dihydrocinchonine, cinchonidine, cinchotenidine and dihydrocinchonidine: in all other cases it is $\cdot OCH_3$, except in cupreine where it is $\cdot OH$ and the cupreine alkyl ethers, quinethyline ($\cdot OC_2H_5$); quinpropyline ($\cdot OC_3H_7$); quinamyline ($\cdot OC_5H_{11}$).

$R' = \cdot CH:CH_2$ in cinchonine, cinchonidine, quinine and quinidine
 $= \cdot CH_2.CH_3$ in the dihydro-reduction products of these four alkaloids
 $= COOAlk.$ for the alkyl-quiténines, quitenidines, cinchotenines and cinchotenidines; $Alk. =$ methyl, ethyl, propyl, etc.

Carbon atoms 1, 2, 3, 4 are all dextrorotatory in cinchonine and its derivatives and in quinidine and its derivatives: carbon atoms 1 and 2 are dextrorotatory and 3 and 4 laevorotatory in cinchonidine and quinine and their derivatives [King, 1922].

PREPARATION OF THE DRUGS.

The cinchona alkaloids used were laboratory stock, which had been used for other investigations and were of satisfactory purity. The hydrogenated alkaloids were prepared from them by reduction with hydrogen in presence of palladium as a catalyst. The esters of quitenine and of the analogous acids derived from cinchonidine, quinidine and cinchonine were prepared by the general method described below. Many of them are new and consequently it is desirable to include a brief record of their chemical characteristics; this is given below, the names of the new substances being printed in italics.

The *isoquinoline* alkaloids referred to on p. 888 were laboratory specimens prepared mainly by Dr F. L. Pyman, F.R.S., in the course of former investigations carried out in one of these laboratories.

The other preparations referred to on pp. 887, 888 are mainly derived from chemical investigations now in progress. The various "total alkaloids as hydrochlorides" were prepared by extracting the total alkaloids of the plant specified and neutralising these with hydrochloric acid to form a neutral solution of known strength. This rule of using all the drugs as neutral hydrochlorides was only diverged from when the neutral hydrochloride was too sparingly soluble to enable a dose completely in solution in 0.5 cc. to be given, and in such cases either the acid hydrochloride or a still more soluble salt, such as the acetate or the ethylsulphonate, was used.

Esters of quitenine, quitenidine, cinchotenine, cinchotenidine.

The esterifications were effected with good yields, in the manner described for methylquitenine, unless otherwise stated.

Methylquitenine. Dry hydrogen chloride was passed into a mixture of anhydrous quitenine (10 g.) and methyl alcohol (50 cc.) heated on the water-bath for 2 hours. After removal of methyl alcohol by distillation, the aqueous solution of the residue was made alkaline with sodium carbonate solution, and

the methyl ester extracted by chloroform, the small amount of unchanged quitenine, which separated, being removed by filtration.

The base was not obtained crystalline. It softened at 165° (corr.), melted at 170° (corr.) and had $[\alpha]_D^{20} - 130^{\circ}$ ($c = 2.24$ in chloroform). (Found: C, 67.1; H, 6.9. $C_{20}H_{28}O_4N_2$ requires C, 67.4; H, 6.8 %.) *Methylquitenine monohydrochloride* was prepared by neutralising a solution of the base in alcohol with dilute hydrochloric acid. It crystallised in plates (frothing at 85°) with difficulty from methyl alcohol on adding dry ether, and once crystallised it could readily be recrystallised from benzene in needles, frothing at 96° . The anhydrous salt commenced to change at 153° (corr.) and gradually frothed. It had $[\alpha]_D^{21} - 189^{\circ}$ ($c = 0.994$ in water) and was very soluble in water. (Found in air-dried crystals from methyl alcohol-ether: loss at 105° ¹ in a vacuum, 7.4. $C_{20}H_{24}O_4N_2$, HCl, $2H_2O$ requires H_2O , 8.4 %. Found in dry salt: C, 60.9; H, 6.8; Cl, 8.8; MeO, 15.6. $C_{20}H_{24}O_4N_2$, HCl requires C, 61.1; H, 6.4; Cl, 9.0; 2MeO, 15.8 %.)

Ethylquitenine crystallises from wet ether or alcohol, or better from a mixture of chloroform and ethyl acetate, in anhydrous needles, m.p. 201° (corr.), and has $[\alpha]_D^{20} - 141^{\circ}$ ($c = 1.5935$ in chloroform). (Found: C, 67.9; H, 7.6. $C_{21}H_{26}O_4N_2$ requires C, 68.1; H, 7.1 %.) Ethylquitenine monohydrochloride was recrystallised from acetone. The dry salt sintered at 158° (corr.), melted at 162° (corr.) and had $[\alpha]_D^{16} - 206^{\circ}$ ($c = 0.988$ in water). It is very soluble in water. (Found: Cl, 8.6. $C_{21}H_{26}O_4N_2$, HCl requires Cl, 8.7 %.) Ethylquitenine dihydrochloride crystallises from alcohol. It is hygroscopic and very soluble in water. (Found in dry salt: Cl, 15.7. $C_{21}H_{26}O_4N_2$, 2HCl requires Cl, 16.0 %.)

n-Propylquitenine separated from acetone in anhydrous crystals, apparently tetrahedra, melting at 170° (corr.) and having $[\alpha]_D^{17} - 143^{\circ}$ ($c = 2.048$ in chloroform). (Found: C, 68.8; H, 7.6. $C_{22}H_{28}O_4N_2$ requires C, 68.7; H, 7.3 %.) *n-Propylquitenine monohydrochloride* crystallised from benzene in needles, frothing at 89° . (Found in air-dried salt: loss at 100° in a vacuum, 8.4. $C_{22}H_{28}O_4N_2$, HCl, $2H_2O$ requires H_2O , 7.9 %.) The anhydrous salt sintered at 125° (corr.), melted at 187° (corr.) and had $[\alpha]_D^{20} - 225^{\circ}$ ($c = 1.01$ in water). Solubility 1 in 7 of water at 23° . (Found: Cl, 8.3. $C_{22}H_{28}O_4N_2$, HCl requires Cl, 8.4 %.)

isoPropylquitenine crystallised from benzene in anhydrous clusters of plates, sintering at 184° (corr.), melting at 189° (corr.) and having $[\alpha]_D^{22} - 142^{\circ}$ ($c = 2.011$ in chloroform). (Found: C, 68.8; H, 7.6. $C_{22}H_{28}O_4N_2$ requires C, 68.7; H, 7.3 %.) *isoPropylquitenine monohydrochloride* crystallised from benzene in needles, frothing at 88° (corr.). (Found in air-dried salt: loss at 105°

¹ When the air-dried substances described in this paper underwent change below 105° , they were dried first at a low temperature and finally at the temperature recorded. In a number of cases the salts crystallised with benzene, which was partly replaced by water on exposure to air, the products becoming sticky. In these cases the loss on drying does not correspond with any definite number of molecules of either solvent.

in a vacuum, 11.5 %.) The anhydrous salt sintered at 126° and gradually melted. It had $[\alpha]_D^{20} - 231^\circ$ ($c = 1.008$ in water). Solubility in water 1 in 19 at 20°. (Found: Cl, 8.4. $C_{22}H_{28}O_4N_2$, HCl requires Cl, 8.4 %.)

n-Butylquitenine crystallised from a mixture of benzene and light petroleum (B.P. 60–80°) in anhydrous silky needles, sintering at 137° (corr.), melting at 142° (corr.) and having $[\alpha]_D^{22} - 143^\circ$ ($c = 2.00$ in chloroform). (Found: C, 68.9; H, 7.8. $C_{23}H_{30}O_4N_2$ requires C, 69.3; H, 7.6 %.) *n-Butylquitenine monohydrochloride* crystallised from benzene in needles, frothing at 88°. (Found in air-dried salt: loss at 105° in a vacuum, 11.0 %.) The anhydrous salt sintered at 110° and gradually melted. It had $[\alpha]_D^{21} - 227^\circ$ ($c = 0.631$ in water) and solubility 1 in 123 of water at 20°. (Found: Cl, 8.2. $C_{23}H_{30}O_4N_2$, HCl requires Cl, 8.2 %.) *n-Butylquitenine dihydrochloride* was not obtained crystalline. The dried substance, which softened at 168° (corr.) and frothed at 178° (corr.), was hygroscopic and had $[\alpha]_D^{18} - 250^\circ$ ($c = 2.045$ in water). (Found: Cl, 14.8. $C_{23}H_{30}O_4N_2$, 2HCl requires Cl, 15.0 %.)

isoButylquitenine crystallised from benzene in matted needles, softening at 147° (corr.), melting at 154° (corr.) and having $[\alpha]_D^{20} - 144^\circ$ ($c = 2.02$ in chloroform). (Found: C, 68.9; H, 7.8. $C_{23}H_{30}O_4N_2$ requires C, 69.3; H, 7.6 %.) *isoButylquitenine monohydrochloride* crystallised from benzene in needles, frothing at 93° (corr.). (Found in air-dried salt: loss at 100° in a vacuum, 9.2. $C_{23}H_{30}O_4N_2$, HCl, 2H₂O requires H₂O, 7.7 %.) The anhydrous salt sintered at 125°, melted at 248° (corr.) and had $[\alpha]_D^{20} - 223^\circ$ ($c = 1.52$ in water). Solubility 1 in 66 of water at 20°. (Found: Cl, 8.1. $C_{23}H_{30}O_4N_2$, HCl requires Cl, 8.2 %.)

n-Amylquitenine crystallised from a mixture of acetone and ligroin in needles, softening at 129° (corr.), melting at 135° (corr.) and having $[\alpha]_D^{22} - 135^\circ$ ($c = 2.00$ in chloroform). (Found: C, 70.0; H, 8.1. $C_{24}H_{32}O_4N_2$ requires C, 69.9; H, 7.8 %.) *n-Amylquitenine monohydrochloride* crystallised from benzene in minute needles, softening at about 40° and melting at 194° (corr.). (Found in air-dried salt: loss at 100° in a vacuum, 7.6 %. $C_{24}H_{32}O_4N_2$, HCl, 2H₂O requires H₂O, 7.4 %.) The anhydrous salt softened at about 115°, resolidified and melted at 195° (corr.). It had $[\alpha]_D^{20} - 217^\circ$ ($c = 0.92$ in water) and solubility 1 in 107 of water at 20°. (Found: Cl, 7.9. $C_{24}H_{32}O_4N_2$, HCl requires Cl, 7.9 %.)

isoAmylquitenine crystallised from benzene in matted needles, softening at 154° (corr.), melting at 160° (corr.) and having $[\alpha]_D^{21} - 136^\circ$ ($c = 1.989$ in chloroform). (Found: C, 69.0; H, 8.0. $C_{24}H_{32}O_4N_2$ requires C, 69.9; H, 7.8 %.) *isoAmylquitenine monohydrochloride* crystallised well from benzene. The air-dried substance frothed at 90°. (Found in air-dried salt: loss at 105° in a vacuum, 19.8 %.) The anhydrous salt softened at 122° (corr.) and gradually decomposed. It had $[\alpha]_D^{17} - 222^\circ$ ($c = 0.804$ in water) and solubility 1 in 112 of water at 18°. (Found: Cl, 7.9. $C_{24}H_{32}O_4N_2$, HCl requires Cl, 7.9 %.)

Octylquitenine was obtained in about 10 % yield only, although the proportion of octyl alcohol (methyl-*n*-hexylcarbinol) to quitenine was increased

to 50 cc. of alcohol to 5 g. of quitenine, and the esterification was carried out at 110° for 6 hours. The base was not obtained crystalline. *Octylquitenine monohydrochloride* crystallised from benzene in needles, softening at 166° (corr.) and melting at 181° (corr.). (Found in air-dried salt: loss at 105° in a vacuum, 3.6 %. $\text{C}_{27}\text{H}_{38}\text{O}_4\text{N}_2$, HCl, H_2O requires H_2O , 3.5 %.) The anhydrous salt had $[\alpha]_D^{21} - 191^{\circ}$ ($c = 0.244$ in water) and solubility of 1 in 410 of water at 21° . (Found: Cl, 7.2. $\text{C}_{27}\text{H}_{38}\text{O}_4\text{N}_2$, HCl requires Cl, 7.2 %.) *Octylquitenine dihydrochloride* was not obtained crystalline. It softened at 136° (corr.) and melted at 164° (corr.). A 1 % aqueous solution could be prepared. (Found: Cl, 13.2. $\text{C}_{27}\text{H}_{38}\text{O}_4\text{N}_2$, 2HCl requires Cl, 13.4 %.)

Benzylquitenine was obtained in a yield of about 20 %. After the esterification, water was added and the unchanged benzyl alcohol and benzyl chloride extracted by chloroform. The chloroform solution was washed with water and the washings added to the main acid aqueous solution of the base and unchanged quitenine. The base was liberated by sodium carbonate, extracted by chloroform and recrystallised from a mixture of benzene and light petroleum. The base usually separates as a jelly which changes into anhydrous needles, sintering at 157° (corr.) and melting at 161° (corr.). It had $[\alpha]_D^{24} - 128^{\circ}$ ($c = 2.002$ in chloroform). (Found: C, 71.7; H, 6.9. $\text{C}_{26}\text{H}_{28}\text{O}_4\text{N}_2$ requires C, 72.2; H, 6.5 %.) *Benzylquitenine monohydrochloride* crystallised from alcohol in anhydrous prisms, melting at 233° (corr.), frothing at 238° (corr.), having $[\alpha]_D^{20} - 175^{\circ}$ ($c = 0.4$ in water) and solubility 1 in 250 of water at 20° . (Found: C, 66.1; H, 6.3; Cl, 7.4. $\text{C}_{26}\text{H}_{28}\text{O}_4\text{N}_2$, HCl requires C, 66.6; H, 6.2; Cl, 7.6 %.) *Benzylquitenine dihydrochloride* was not obtained crystalline. It softened at about 160° and melted with frothing at about 200° (corr.). A 10 % aqueous solution could be prepared. (Found: Cl, 13.9. $\text{C}_{26}\text{H}_{28}\text{O}_4\text{N}_2$, 2HCl requires Cl, 14.0 %.)

Ethylquitenidine crystallised from acetone in six-sided prisms, sintering at 98° and frothing at 107° (corr.). (Found in air-dried crystals: loss at 120° in a vacuum, 7.4 %. $\text{C}_{21}\text{H}_{26}\text{O}_4\text{N}_2$, 2 H_2O requires H_2O , 8.9 %.) The anhydrous base softened at 131° (corr.), melted at 139° (corr.) and had $[\alpha]_D^{20} + 163^{\circ}$ ($c = 2.143$ in chloroform). (Found: C, 67.9; H, 7.2. $\text{C}_{21}\text{H}_{26}\text{O}_4\text{N}_2$ requires C, 68.1; H, 7.1 %.) *Ethylquitenidine monohydrochloride* crystallised from acetone in anhydrous, apparently cubical, crystals, melting at 242° (corr.), having $[\alpha]_D^{17} + 151^{\circ}$ ($c = 1.0296$ in water) and solubility 1 in 23 of water at 23° . (Found: Cl, 8.7. $\text{C}_{21}\text{H}_{26}\text{O}_4\text{N}_2$, HCl requires Cl, 8.7 %.)

isoAmylquitenidine monohydrochloride crystallised from benzene. (Found in air-dried salt: loss at 105° in a vacuum, 2.1 %.) The anhydrous salt melted at 245° (corr.), had $[\alpha]_D^{22} + 132^{\circ}$ ($c = 0.958$ in water) and solubility in water of 1 in 104 at 22° . (Found: Cl, 7.9. $\text{C}_{24}\text{H}_{32}\text{O}_4\text{N}_2$, HCl requires Cl, 7.9 %.)

Ethylcinchotenidine was obtained in 50 % yield under the usual conditions of esterification. It crystallised from acetone in anhydrous rhombic plates, softening at 177° (corr.), melting at 180° (corr.) and having $[\alpha]_D^{20} - 115^{\circ}$ ($c = 2.081$ in chloroform). (Found: C, 70.3; H, 7.2. $\text{C}_{20}\text{H}_{24}\text{O}_3\text{N}_2$ requires

C, 70.5; H, 7.1 %.) *Ethylcinchotenidine monohydrochloride* was not obtained crystalline. It softened at 120°, frothed at 180° (corr.) and had $[\alpha]_D^{20} - 165^\circ$ ($c = 1.3408$ in water). It is very soluble in water. (Found: Cl, 9.6. $C_{20}H_{24}O_3N_2$, HCl requires Cl, 9.4 %.)

isoAmylcinchotenidine monohydrochloride was not obtained crystalline. It melted at about 152°, had $[\alpha]_D^{21} - 151^\circ$ ($c = 3.788$ in water) and solubility 1 in 26 of water at 21°. (Found: Cl, 8.5. $C_{23}H_{30}O_3N_2$, HCl requires Cl, 8.5 %.)

Ethylcinchotenine crystallised from chloroform in anhydrous needles, melting at 213–214° (corr.) and having $[\alpha]_D^{23} + 151^\circ$ ($c = 2.012$ in chloroform). (Found: C, 70.2; H, 7.5. $C_{20}H_{24}O_3N_2$ requires C, 70.5; H, 7.1 %.) *Ethylcinchotenine monohydrochloride* crystallised from alcohol in sheaves of plates, frothing at 245° (corr.). (Found in air-dried salt: loss at 105° in a vacuum, 4.6 %. $C_{20}H_{24}O_3N_2$, HCl, H₂O requires H₂O, 4.6 %.) The anhydrous salt frothed at 251° (corr.), had $[\alpha]_D^{17} + 130^\circ$ ($c = 0.982$ in water) and solubility 1 in 16 of water at 17°. (Found: C, 63.7; H, 6.8; N, 7.6; Cl, 9.4. $C_{20}H_{24}O_3N_2$, HCl requires C, 63.7; H, 6.7; N, 7.4; Cl, 9.4 %.)

isoAmylcinchotenine crystallised from chloroform or benzene in anhydrous needles, melting at 147–149° (corr.). *isoAmylcinchotenine monohydrochloride* was not obtained crystalline. It softened at 129° (corr.), melted at 145° (corr.), had $[\alpha]_D^{20} + 117^\circ$ ($c = 1.3224$ in water) and solubility 1 in 10 of water at 20°. (Found: Cl, 8.6. $C_{23}H_{30}O_3N_2$, HCl requires Cl, 8.5 %.)

The solubilities of anhydrous quitenine, quitenidine, cinchotenidine and cinchotenine in water and their optical rotation in 5 % solution in $N H_2SO_4$ were determined.

	Solubility	Specific rotation
Quitenine	1 in 8300 at 18°	$[\alpha]_D^{25} - 298^\circ$
Quitenidine	1 in 200 at 23°	$[\alpha]_D^{23} + 258^\circ$
Cinchotenidine	1 in 277 at 21°	$[\alpha]_D^{25} - 207^\circ$
Cinchotenine	1 in 233 at 19°	$[\alpha]_D^{26} + 209^\circ$

The 5-nitrodihydroquinine and 5-aminodihydroquinine were prepared by the methods of Jacobs and Heidelberg [1920].

BIOLOGICAL TESTS.

The biological tests¹ were made in a manner similar to that standardised by Roehl. Canaries were infected with malaria by intraperitoneal inoculation and were subsequently treated daily for 6 days with the compound to be tested. The strain of malaria used was kindly sent to us by Roehl from Elberfeld.

¹ The biological tests were made at the London School of Hygiene and Tropical Medicine, where one of us (M.) was engaged in investigations on bird malaria on behalf of the Medical Research Council's Chemotherapy Committee, with the aid of grants from the Milner Research Fund and from the Medical Research Council. Our sincere thanks are due to the Director, Sir Andrew Balfour, and to Dr J. G. Thomson for the hospitality of their laboratory.

Thanks to the courtesy of Dr J. G. Thomson we have been able to examine named specimens (sent from Baltimore by Prof. R. Hegner) of the three species of bird-malaria parasite separated by Hartman [1927] and to compare them with the strain obtained from Roehl. The latter corresponded to the type named by Hartman *Plasmodium inconstans*. The doses of the compounds to be tested, dissolved in 0.5 cc. water, were administered to the canaries by means of an oesophageal tube passed into the stomach, and were proportional to the weight of the bird, a bird of 20 g. weight being taken as the standard. The first dose was given 4 hours after the malaria inoculation. As a rule only one bird was treated with each dose of the compound to be tested, and the first tests were made with the largest dose tolerated for 6 consecutive days. Evidence of malaria infection was sought by the examination of stained blood films daily or on alternate days.

In each experiment several birds, usually 20 %, were not treated and were regarded as controls. In the blood of such controls malaria parasites appeared for the first time usually on the 7th or 8th day of the experiment. The virulence of the parasite was somewhat variable, but during 2 years no seasonal variation and no progressive change in virulence was observed. In the birds used as controls during this time the incubation period varied from 3 to 11 days, and averaged 7.3 days; in 49 % the parasites appeared first on the 7th or 8th day, and in 83 % between the 6th and 9th days. Making allowance for this degree of variability in the incubation period, we have adopted an arbitrary standard for estimating the antimalarial action of the compounds tested. A delay in the appearance of parasites as compared with the controls of only 1 or 2 days has been considered negligible, a delay greater than this but not exceeding 7 days has been construed as indicating a "slight" action, and a delay of over 7 days a "moderate" action. The canaries were ordinarily kept under observation for 3 or 4 weeks. If at the end of this time they had shown no evidence of malaria, they were re-inoculated so as to determine whether they had actually been cured by the treatment or were the subjects of a latent infection. If the birds were actually cured, that is malaria-free, the effect of re-inoculation was to produce a normally acute infection, whereas if they were still infected, although not showing parasites in the blood, re-inoculation was without effect. This test presupposes that it is impossible to super-infect with *P. inconstans*, a fact which has been abundantly demonstrated both in the course of this work and by numerous previous investigators. It also assumes that in the course of the 20 days or so intervening between the end of treatment and the day of re-inoculation the drug being tested will have been eliminated from the bodies of the birds. It will be noted that on one or two occasions this assumption was probably incorrect because the first re-inoculation was without effect, whereas re-inoculation at a later date produced a normal acute infection.

The test as outlined above is clearly not a delicate one. It is probably sufficiently trustworthy to distinguish broadly between active and inactive compounds, but for the differentiation of compounds not having widely

dissimilar actions, or for the grading of a series of substances with actions of the same order, experiments would have to be made with very much larger numbers of birds.

Table I. *Cinchona alkaloids*.

Drug	Dose in mg. No. of daily doses given	Day on which parasites ap- peared in blood		Antimalarial action
		Control birds	Treated birds	
Quinine hydrochloride	10.0 × 6	6	—	Cured*
	5.0 × 6	6-8	11-13	Slight
	2.0 × 6	6	14-15	Moderate
	1.0 × 6	7-9	16-17	Moderate
	1.0 × 6	—	12	Slight
	1.0 × 6	6	5	None
	0.5 × 6	5	10	Slight
	0.5 × 6	7-8	9	None
	0.5 × 6	7-9	5	None
	0.5 × 6	7-9	5	None
Hydroquinine dihydrochloride	5.0 × 6	6-8	—	Cured†
	5.0 × 6	7-8	—	Cured‡
	2.5 × 6	7-8	—	Cured§
	1.0 × 6	7-8	—	Cured‡
	1.0 × 6	5-9	16	Moderate
	1.0 × 6	6	14-15	Moderate
	0.5 × 6	6	11-12	Slight
	0.5 × 6	6	17	Moderate
5-Aminohydroquinine hydrochloride	5.0 × 6	7-8	—	Cured‡
	5.0 × 6	7-8	11-12	Slight
	5.0 × 6	6	12-13	Slight
	1.0 × 6	7-8	12-13	Slight
	0.5 × 6	6-8	7-8	None
Quinidine hydrochloride	10.0 × 6	7-9	22-26	Moderate
	5.0 × 6	7-8	26	— ¶
	5.0 × 6	6-8	13	— ¶
	5.0 × 6	5-9	19-20	Moderate
Dihydroquinidine dihydrochloride	5.0 × 6	6-8	14-16	Moderate
Cinchonine hydrochloride	5.0 × 6	7-8	11-12	Slight
Dihydrocinchonine dihydrochloride	5.0 × 6	6-8	11-13	Slight
Cinchonidine hydrochloride	5.0 × 4 : 2.5 × 2	7-8	16-17	Moderate**
Dihydrocinchonidine hydrochloride	5.0 × 6	7-8	14-16	Moderate

* Re-inoculation on 24th day without effect. Re-inoculation on 46th day produced normal infection, indicating that bird had been cured.

† Re-inoculation on 30th day without effect. Re-inoculation on 40th day produced normal infection, indicating that bird had been cured.

‡ Re-inoculation on 26th day produced normal infection, indicating that bird had been cured.

§ Re-inoculation on 26th day without effect. Re-inoculation on 36th day produced normal infection, indicating that bird had been cured.

¶ Bird died on 26th day without showing evidence of infection.

¶ Bird died on 13th day without showing evidence of infection.

** Dose reduced owing to toxicity.

Cinchona alkaloids (Table I).

Five of these alkaloids have been compared in bird malaria by Giemsa, Weise and Tropp [1926] and the results of the two comparisons may be summarised as follows:

Drug	Giemsa, Weise and Tropp		Present authors	
	Dose (mg.)	Retardation of attack (days)	Dose (mg.)	Retardation of attack (days)
Quinine hydrochloride	2.5-3.0 × 4	12-13	2.0 × 6	8-9
Hydroquinine dihydrochloride	2.5-3.0 × 4	11	2.5 × 6	Cured
" "	—	—	1.0 × 6	7-11 or cured
" "	—	—	0.5 × 6	5-11
5-Aminohydroquinine (base)	2.5-3.0 × 4	11-12	—	—
5-Aminohydroquinine hydrochloride	—	—	5.0 × 6	4-7 or cured
" "	—	—	1.0 × 6	5
Quinidine hydrochloride	1.25-1.5 × 4	12	5.0 × 6	14 or cured
Cinchonine hydrochloride	2.5-3.0 × 4	0-5	5.0 × 6	4-5

Giemsa, Weise and Tropp's results indicate that cinchonine is definitely inferior to any of the other four, which are all of about equal value, whilst our results show that hydroquinine is definitely superior to any of the other four, among which there is little to choose except that there are some signs that quinidine and aminohydroquinine, which both produced cures at a dose of 5 mg., may be better than quinine or cinchonine. From our results cinchonidine also appears to be as good as quinine. The only other investigation which need be mentioned on this point is that of Hegner, Shaw and Manwell [1928], who arrange four of these alkaloids in the following descending order as regards absorption by red blood-corpuscles, which they regard as an indication of efficiency against malaria. The figures are partition coefficients (concentration in corpuscles over concentration in serum) for chicken blood. Quinine, 5.0; quinidine, 4.4; cinchonine, 4.3; cinchonidine, 3.8.

The results of such comparative clinical trials as have been made with the principal cinchona alkaloids are equally confusing.

MacGilchrist [1915-16] arranges them in the following descending order:

Cinchonine
 Hydroquinine → Quinine → Cinchonidine
 Quinidine

It is remarkable that notwithstanding MacGilchrist's results hydroquinine has not been given a more extended trial in human malaria. The results of our tests on birds show clearly that hydroquinine is much more active than quinine, and suggest that unless its action on human malaria is very different from its action on bird malaria hydroquinine should prove to be of great value in the treatment of the former disease.

Fletcher [1923] says that in doses of 10 grains, twice a day, quinine, quinidine, cinchonine and cinchonidine appear to be of equal value in bringing about the disappearance of malaria parasites, but that in small doses of 5 grains, twice a day, cinchonine did not appear quite so potent as quinine and quinidine, whilst cinchonidine was definitely inferior to the other three.

A comparison of quinine and quinidine made under the auspices of the

Medical Research Council [1925] resulted in the establishment of the practical equivalence of quinine and quinidine, and this view is accepted by the Malaria Commission of the League of Nations [1927], who state that quinine and quinidine are practically of equal value in doses of 1 g. and add that cinchonine is only equally effective in doses of 1.5 g.

Sinton and Bird [1929] on the contrary regard quinine, cinchonine and cinchonidine as of almost equal value in benign tertian malaria and quinidine as inferior to these three.

So far as other alkaloids in Table I are concerned, the results indicate that 5-aminohydroquinine is no better than hydroquinine and that whilst hydrogenation of quinine produces the more active hydroquinine, the hydrogenation of quinidine, cinchonidine and cinchonine does not enhance their antimalarial value. This last observation confirms clinical results recorded by Giemsa and Werner [1914].

It must, however, be pointed out that, so far as bird malaria trials are concerned, the simple technique employed in which a drug is tested on one bird is inadequate. This point is better brought out in Table II, but is also evident in the results recorded in Table I. Thus 5 mg. of quinine hydrochloride gave a retardation of only 5 days in the single bird to which it was given, a less powerful action than that sometimes produced by the smaller doses. A similar variability appears even when, as in Giemsa, Weise and Tropp's experiments [1926], three birds are used. In human malaria the response of patients to treatment with quinine is notoriously variable, a fact usually attributed to variable absorption of the drug from the intestine, and it is reasonable to suppose that birds also would show this variability. Less variable results might be obtained if the drugs could be administered by intramuscular or intravenous inoculation, but this procedure is not practicable for repeated doses of considerable amounts to small and delicate birds. Our experience shows that, using the technique adopted, larger numbers of birds must be used than is at present the custom if trustworthy results are to be obtained.

So far as comparative clinical trials are concerned, there is one point which calls for comment. With the exception of the comparison of quinine and quinidine, made under the auspices of the Medical Research Council, no particular pains seem to have been taken to ensure purity of the alkaloids used. Dawson and Garbade [1930] have recently called attention to this matter in another connection, and if the information they provide as to the extent of the contamination of commercial supplies of individual cinchona alkaloids is accurate, it may account for some of the discrepant results obtained by different observers. Thus, if cinchonidine is, as Dawson and Garbade state, liable to contain up to 10 % of quinine with some hydrocinchonidine, it is conceivable that variation in the amount of these impurities may account for the varying estimates of the antimalarial value of this alkaloid formed by different observers.

Table II. *Esters of quitenine.*

Drug	Dose in mg. No. of daily doses given	Day on which parasites ap- peared in blood		Anti- malarial action
		Control birds	Treated birds	
Methylquitenine monohydrochloride	10.0 × 6	6-8	11	Slight
	5.0 × 6	7-8	14-15	Slight
	5.0 × 6	6-8	7-8	None
Ethylquitenine monohydrochloride	10.0 × 6	6-8	7-8	None
	5.0 × 6	7-8	9	None
Ethylquitenine dihydrochloride	10.0 × 6	7-9	14-15	Slight
	5.0 × 6	7-8	6	None
<i>n</i> -Propylquitenine monohydrochloride	5.0 × 6	7-8	7-8	None
<i>iso</i> Propylquitenine monohydrochloride	10.0 × 6	6-8	12	Slight
	5.0 × 6	7-8	9	None
<i>n</i> -Butylquitenine monohydrochloride	4.1 × 6*	6-8	5	None
<i>n</i> -Butylquitenine dihydrochloride	10.0 × 6	6-8	9-10	Slight
	5.0 × 6	6-8	6	None
	5.0 × 6	6-8	9	Slight
<i>n</i> -Butylquitenine monoacetate	5.0 × 6	7-9	—	Cured†
	5.0 × 6	7-8	14-15	Moderate
	5.0 × 6	7-8	11-12	Slight
	1.0 × 6	7-9	7-8	None
<i>iso</i> Butylquitenine dihydrochloride	5.0 × 6	7-8	7-8	None
	5.0 × 6	7-8	12-13	Slight
<i>n</i> -Amylquitenine dihydrochloride	5.0 × 6	7-8	16-17	Moderate
	1.0 × 6	6-11	6	None
<i>iso</i> Amylquitenine monohydrochloride	5.0 × 6	7-9	—	Cured†
	5.0 × 6	7-8	10-11	Slight
	5.0 × 6	7-8	9-10	Slight
	1.0 × 6	7-9	10	Slight
<i>iso</i> Amylquitenine ethylsulphonate	5.0 × 6	5-9	12-15	Slight
Benzylquitenine monohydrochloride	2.5 × 6*	6	7-8	None
Benzylquitenine dihydrochloride	5.0 × 6	7-8	14-16	Moderate
Benzylquitenine ethylsulphonate	5.0 × 6	6-8	14-15	Moderate
<i>n</i> -Octylquitenine dihydrochloride	2.5 × 6	7-8	7-8	None

Esters of cinchotenidine.

Ethylcinchotenidine monohydrochloride	5.0 × 6	7-8	12-13	Slight
	1.0 × 6	7-8	5	None
<i>iso</i> Amylcinchotenidine monohydrochloride	5.0 × 6	7-8	7-8	None
	5.0 × 6	7-8	14-15	Moderate

Esters of quitenidine.

Ethylquitenidine monohydrochloride	10.0 × 6	7-9	9	None
<i>iso</i> Amylquitenidine dihydrochloride	5.0 × 6	7-8	10	Slight

Esters of cinchotenine.

Ethylcinchotenine monohydrochloride	10.0 × 6	6	10	Slight
<i>iso</i> Amylcinchotenine monohydrochloride	5.0 × 6	7-8	7-8	None
<i>iso</i> Amylcinchotenine dihydrochloride	5.0 × 6	7-8	6	None
	5.0 × 6	7-8	7-8	None

* Maximum possible dose owing to low solubility.

† Re-inoculation on 26th day caused normal infection, indicating that bird had been cured.

Quitenine series (Table II).

It has been clearly established already that the free acid itself—quitenine—is inactive both in bird malaria and in human malaria [Stephens *et al.*, 1919], and the object of examining the series of esters of quitenine and the analogous derivatives of the other three cinchona alkaloids dealt with in Table II was to confirm the observation of Giemsa, Weise and Tropp [1926] that the ethyl ester of quitenine is active, and to ascertain whether, by the use of higher alcohols, more active alkylquitenines could be obtained.

The results recorded in Table II make it clear that quitenine does regain activity on esterification and that, on the whole, activity increases as the homologous series is ascended, reaching its maximum at butyl or amyl, at which point cures begin to appear. But, apart from these occasional cures, none of these esters appears to approach hydroquinine or even quinine and quinidine in activity, and the simple quitenine esters do not appear to hold out any hope of a considerable improvement on quinine.

It has been pointed out already by Schnitzer [1926] that esters of quitenine act as local anaesthetics. Dr J. Trevan has kindly tested this point for us. He finds that the esters do act as local anaesthetics, the benzyl ester being markedly active, but that they are too irritating to be of practical value.

We have also examined the esters of the carboxylic acids analogous to quitenine derived from the other three cinchona alkaloids, viz. cinchotenidine from cinchonidine, quitenidine from quinidine and cinchotenine from cinchonine. In these three cases only the ethyl and *iso*amyl esters were used. Here also it is clear, though at most two birds only were used in the trial of each substance, that the esters are active, the best of them being *iso*amyl-cinchotenidine.

OTHER DRUGS WITH A REPUTATION FOR ANTIMALARIAL ACTION.

Alstonia species. Two of the authors have at present under chemical examination the following species of *Alstonia*:

<i>A. congensis</i>	West Africa.
<i>A. scholaris</i>	India and Philippine Islands.
<i>A. macrophylla</i>	Philippine Islands.
<i>A. constricta</i>	Australia.

The first two contain the alkaloid echitamine [Goodson and Henry, 1925]. The third and fourth contain no echitamine, but are rich in alkaloids, which are still under examination. The total alkaloids of *A. scholaris* showed perhaps a slight action in doses of 2.5 mg., which was the highest dose tolerated, and those of *A. constricta* in doses of 10 mg., but not in doses of 5 mg. Echitamine sulphate was slightly active at a dosage of 5 mg. The total alkaloids of *A. macrophylla* and *A. congensis* were inactive.

Picralima klaineana. The seeds of this plant are regarded by natives in Northern and Western Africa as a cure for malaria. The principal alkaloid present, akuammine [Henry and Sharp, 1927], and the total alkaloids, used as hydrochlorides, proved to have no action in bird malaria.

Nectandra rodioei bark. This material (greenheart bark) is used in British Guiana and elsewhere as a remedy for malaria. The total alkaloids, used as hydrochlorides, had no action in bird malaria.

Indole alkaloids. The alkaloids, echitamine and akuammine, referred to above, are probably both indole derivatives; the former is slightly active. Harmine and harmaline were considered by Gunn and Marshall [1920] to be active in human malaria. Both were tried in this series of experiments and found to be inactive in bird malaria in doses of 2.5 or 5.0 mg.

isoQuinoline derivatives.

Among the alkaloids, which have at one time or another been used for the treatment of malaria, is narcotine, which belongs to the *isoquinoline* group. It seemed worth while, therefore, to try a series of *isoquinoline* alkaloids in bird malaria and the following were used.

Papaverine hydrochloride	<i>N</i> -Methylpavine methochloride
Tetrahydropapaverine hydrochloride	Laudanosine hydrochloride
Pavine hydrochloride	Aminolaudanosine hydrochloride
<i>N</i> -Ethyltetrahydropapaverine hydrochloride	Berberine phosphate
<i>N</i> -Methylpavine hydrochloride	Anhydromethylcanadine

None of these had any traceable preventive or curative action. It has been suggested by Waldorp [1926] that berberine is a useful drug for provoking latent malaria into activity, but no such effect was observed in these experiments.

Miscellaneous drugs.

Hegner, Shaw and Manwell [1928] have already tried a large number of miscellaneous drugs in bird malaria without finding anything outside the cinchona alkaloid series which seemed promising, with the possible exception of one organic mercury compound.

In spite of these discouraging results, it was considered worth while to try a number of compounds, which we had available. These were: pseudoaconine, conessine, daphnandrine, acriflavine, proflavine, sparteine.

There was no reason to expect protozoocidal activity in the case of pseudoaconine and daphnandrine, but conessine is now known to be very toxic to *Entamoeba histolytica*, and sparteine has long been supposed to resemble the cinchona alkaloids in possessing a quinuclidine structure. None of these six substances showed any activity in bird malaria.

SUMMARY.

1. Out of an extensive series of alkaloids tested on canaries infected with *Plasmodium inconstans* only the alkaloids belonging to, or derived from, the cinchona series showed marked remedial action.

2. Of the natural cinchona alkaloids the most active was hydroquinine followed by quinidine, quinine, cinchonidine and cinchonine in descending order, though there is little to choose among the last four. Hydrogenation of quinidine, cinchonidine and cinchonine does not appear to lead to increased activity, as it does in the case of quinine.

3. Activity is restored to quitenine and the analogous carboxylic acids derived from quinidine, cinchonidine and cinchonine, by esterification, and there are clear indications of a rise in activity as the molecular weight of the alcohol used for esterification is increased, which reaches a maximum at butyl- or amyl-quitenine.

4. Among other alkaloidal drugs having some reputation as remedies for malaria, activity was observed only in two *Alstonia* species, viz. *A. scholaris* and *A. constricta*, and there it was slight. The former species no doubt owes its activity to echitamine, which exhibited slight action in doses of 5 mg.

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