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

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IN the first paper [Goodson *et al.* 1930] of this series, it was shown that in a homologous series of alkylquitenines there is a slow and irregular rise in anti-malarial activity as the series is ascended, to a maximum in the neighbourhood of *n*-butyl- or *n*-amyl-quitenine and after that a fall. As the alkylquitenines are easily hydrolysed, it was suspected that some of the irregularities were due to this factor, and it was considered desirable to investigate a homologous series of more stable derivatives of the cinchona alkaloids. For that purpose the alkyl ethers of *apoquinine* and *apoquinidine* were selected. These *apo*-bases proved not to be simple substances, but complex mixtures of quinine and quinidine transformation products of unknown constitution. The results of their chemical investigation have been dealt with elsewhere [Henry *et al.* 1934, 1935, 1937], and a list of the substances isolated in the course of this work is given in Table I with references to the formulae (II to V) printed below, by which they are represented and which will be sufficient to indicate their relationships to each other and to the parent substances, quinine and quinidine, which are stereoisomerides represented by (I: R=Me). The original objective of the investigation has been realized by the preparation of an extensive series of ethers of the principal phenolic base, *apoquinine* (II: R=H), obtained, and of its reduction product dihydrocupreine (VI: R=H), and the opportunity has been taken to test biologically (a) all the products mentioned in Table I, which could be obtained in

Table I

Name of substance	Formula	Name of substance	Formula
<i>apo</i> Quinine	II: R=H	α - <i>iso</i> Quinidine†	V: R=Me
β - <i>iso</i> Quinine	II: R=Me	γ - <i>iso</i> Quinidine†	V: R=Me
<i>isoapo</i> Quinine	II: R=H	<i>apo</i> Quinidine	II: R=H
α - <i>iso</i> Quinine	II: R=Me	<i>apo</i> Quinidine methyl ether	II: R=Me
α -Hydroxydihydro <i>apo</i> quinine	III: R=H	<i>neoapo</i> Quinidine	II: R=H
α -Hydroxydihydroquinine	III: R=Me	<i>neiso</i> Quinidine	II: R=Me
β -Hydroxydihydro <i>apo</i> quinine*	III: R=H	α -Hydroxydihydro <i>apo</i> quinidine	III: R=H
ψ -Quinidine†	IV: R=Me	α -Hydroxydihydroquinidine	III: R=Me
<i>isoapo</i> Quinidine	V: R=H	β -Hydroxydihydro <i>apo</i> quinidine	III: R=H
β - <i>iso</i> Quinidine	V: R=Me	β -Hydroxydihydroquinidine	III: R=Me

* Corresponding methyl ether unknown.

† Corresponding phenolic base unknown.

sufficient quantity, and (b) certain interesting by-products arising out of this work, of which the following brief description may be given to indicate their

relationship to the main enquiry. It will be clear that formula II requires the existence of two geometrical isomerides represented by *apoquinine* (methyl ether= β -*isoquinine*) and *isoapoquinine* (methyl ether= α -*isoquinine*) in the

case of quinine. On hydrogenation of either α - or β -*isoquinine* there will be produced dihydroquinine (VI: R=Me) and its epimeride about carbon atom 3, which may be named *epi*-C³-dihydroquinine. This substance and the analogous *epi*-C³-dihydroquinidine have been isolated in a pure state [Henry *et al.* 1937] and tested biologically.

In certain of the methods used for the preparation of the crude mixture until recently known as "*apoquinine*" there is always formed a minute amount of niquine. This base was first obtained forty-five years ago by Skraup [1892], as a well-defined, crystalline substance, but it is still uncertain whether it should be represented by the formula C₁₉H₂₄O₂N₂, as proposed by Skraup [1892], by C₂₀H₂₆O₂N₂, as suggested by Léger [1937] or by C₂₀H₂₄O₂N₂ as adopted by Reyman and Suszko [1935]. Léger has suggested that niquine should be represented by formula (VII) and as a substance of that formula in which the

bridged ring has been converted into a substituted piperidine ring is of obvious biological interest, in the present connexion, specimens of niquine and its reduction product, dihydroniquine, have been made and tested. The corresponding quinidine product, niquidine, first obtained by Domanski & Suszko [1935] and for which these authors appear to prefer the formula C₂₀H₂₄O₂N₂, though their analytical results are in better agreement with C₁₉H₂₄O₂N₂, has also been prepared together with a new isomeride, which it is proposed to call *isoniquidine*. Niquidine and *isoniquidine* yield the same dihydro-base and, there being no doubt of their chemical individualities, have also been tested biologically (Table IV).

apoQuinine ethers (II: R = Alkyl)

β -*isoQuinine* was first shown to be *apoquinine* methyl ether by Suszko [1925]. *apoQuinine* ethyl ether has been described elsewhere [Henry *et al.* 1934; 1935] and attention has been directed to its pneumococcicidal action by various workers [Miura and Okamoto, 1930; Gundel and Seitz, 1933; Maclachlan *et al.* 1934]. The propyl-, *isopropyl*-, butyl-, *isobutyl*-, *isoamyl*-, hexyl-, octyl- and *isooctyl*-ethers were made in Japan or in Germany for bactericidal tests [Ishizaka *et al.* 1933; Liebetrueth *et al.* 1935], but were not fully characterized, the German workers describing the bases as amorphous, whereas the present authors found most of them to be well-crystallized substances.¹ In the descriptions given below the names of ethers not previously prepared are marked *. The names of new derivatives of known ethers are printed in italics. The following methods of preparation have been used. Equimolecular quantities of *apoquinine* purified as described [Henry & Solomon, 1934] and KOH were dissolved in alcohol (3 parts), or alcohol and ether when necessary, the solution cooled to 0° and treated with 1 mol. of the alkylating agent. The mixture was kept, at first in a refrigerator, and later at room temperature, until inorganic salt ceased to deposit, usually in 1 to 4 weeks. The brown liquid was decanted, evaporated to dryness in a vacuum desiccator, the residue dissolved in dilute mineral acid, the solution washed with ether and the base recovered by making alkaline and extracting with ether. The higher alkyl ethers were insoluble in dilute mineral acids and in these cases the solid residues had to be washed with dilute acid and ether, and finally with alkali and ether. The ethereal extracts were dried over anhydrous Na₂SO₄, decolored by boiling with charcoal and evaporated, leaving a residue, usually crystalline, which was purified by recrystallization as the base or as a salt. The yields varied from about 30 to 48% except for undecyl and cetyl ethers where they rose to 66 and 68% respectively.

The ethers are very soluble in alcohol, readily so in ether, less so in acetone and sparingly soluble or insoluble in petroleum. The higher members tend to soften and melt indefinitely; their neutral salts with mineral acids are insoluble or sparingly soluble in water, but readily soluble in alcohol; the acid salts are more soluble in water except in the case of the highest members, where the ethanesulphonates had to be used for polarimetric work and for the biological tests.

In the following descriptions the specific rotations are recorded for salts in *M*/40 solution in water, and for the anhydrous base in *M*/40 solution in *N*/10 H₂SO₄, except in the cases where *N*/10-ethanesulphonic acid is specified for the reason already given, and in other instances where the special conditions are stated. The analytical results are recorded for the substance dried in a vacuum, usually at 110°, but at a suitable lower temperature for low-melting products.

n-Propyl ether. Amorphous, colourless, precipitate, $[\alpha]_D^{15^\circ} - 286.8^\circ$; *hydrochloride*, colourless needles from acetone, m.p. 249–251° (*decomp.*) with some previous sintering, $[\alpha]_D^{15^\circ} - 172^\circ$. (Found: Cl, 9.15%. C₂₂H₃₈O₂N₂.HCl requires Cl, 9.1%.)

iso Propyl ether. Colourless, amorphous precipitate or jelly from solvents, $[\alpha]_D^{15^\circ} - 288^\circ$; *hydrochloride*, colourless needles from acetone, sintering at 240°, m.p. 254–257° (*decomp.*). (Found: Cl, 9.20%. C₂₂H₃₈O₂N₂.HCl requires Cl, 9.1%.)

n-Butyl ether. Minute glistening, colourless needles from ether, m.p. 162–164°, $[\alpha]_D^{15^\circ} - 280.7^\circ$. (Found: C, 75.4; H, 8.35; N, 7.7%. C₂₂H₃₀O₂N₂ requires C, 75.4; H, 8.25; N, 7.65%); *hydro-*

¹ While this paper was in the press Buttle *et al.* [1937] published descriptions of a few of the lower members of the series.

chloride, glistening, colourless, felted needles, sintering at 192° , m.p. 219° (*decomp.*). (Found: Cl, 8.9%. $C_{23}H_{30}O_2N_2 \cdot HCl$ requires Cl, 8.8%.)

*n-Amyl ether**. Glistening, colourless rosettes of needles from ether, m.p. $143\text{--}145.5^{\circ}$, $[\alpha]_D^{15} - 268.1^{\circ}$; *hydrochloride*, minute, colourless needles from water, m.p. $234.5\text{--}236.5^{\circ}$ (*decomp.*). (Found: loss on drying, 3.1; Cl, 8.17%. $C_{24}H_{32}O_2N_2 \cdot HCl$ requires Cl, 8.51%.)

iso Amyl ether. Colourless needles, from a mixture of benzene and light petroleum, sintering from 168° , m.p. 185° ; $[\alpha]_D^{15} - 269.7^{\circ}$; *hydrochloride*, rosettes of minute needles from acetone, m.p. $201\text{--}205^{\circ}$. (Found: Cl, 8.5%. $C_{24}H_{32}O_2N_2 \cdot HCl$ requires Cl, 8.5%.)

n-Hexyl ether. Felted mass of glistening short needles from acetone, m.p. $135\text{--}137^{\circ}$, $[\alpha]_D^{15} - 273.4^{\circ}$ (0.1 N $EtSO_3H$); *hydrochloride*, colourless, concentrically grouped needles, m.p. $223\text{--}225^{\circ}$. (Found: Cl, 7.9%. $C_{25}H_{34}O_2N_2 \cdot HCl$ requires Cl, 8.2%.)

*n-Heptyl ether**. Long silky needles from acetone, m.p. $137\text{--}140^{\circ}$, $[\alpha]_D - 263.5^{\circ}$ (0.1 N $EtSO_3H$); *hydrochloride*, crystalline mass from acetone, m.p. $172\text{--}176^{\circ}$. (Found: loss on drying, 4.6; Cl, 8.1%. $C_{26}H_{32}O_2N_2 \cdot HCl$, H_2O requires for $1H_2O$, 3.9, and for dried salt Cl, 8.0%.)

n-Octyl ether. Rosettes of colourless needles from ether, m.p. $141\text{--}143^{\circ}$, $[\alpha]_D^{15} - 232.5^{\circ}$ (0.1 N $EtSO_3H$). (Found: C, 76.7; H, 9.1; N, 6.7%. $C_{27}H_{38}O_2N_2$ requires C, 76.7; H, 9.1; N, 6.6%); *hydrochloride*, minute, hard granules, melts indefinitely $90\text{--}145^{\circ}$. (Found: Cl, 7.2%. B.HCl requires Cl, 7.7%.)

*sec-Octyl ether**. Amorphous, $[\alpha]_D^{15} - 225.6^{\circ}$ (0.1 N $EtSO_3H$). (Found: C, 76.3; H, 8.9; N, 6.7%. $C_{27}H_{38}O_2N_2$ requires C, 76.7; H, 9.1; N, 6.6%). It yields a well-defined, but abnormal *acid oxalate*, crystallizing in short cream-coloured needles, softening at 70° and frothing at 120° . (Found: C, 62.7; H, 7.26; N, 5.0%. $B.2H_2C_2O_4$ requires C, 61.8; H, 7.0; N, 4.6 and $B_4.7H_2C_2O_4$ requires C, 62.4; H, 7.1; N, 4.7%.)

*n-Nonyl ether**. Colourless, concentrically grouped needles, m.p. $138\text{--}141^{\circ}$, $[\alpha]_D - 211.6^{\circ}$ (0.1 N $EtSO_3H$) from ether. The *hydrochloride* is a nearly white powder consisting of minute needles from a mixture of benzene and petroleum, m.p. $100\text{--}120^{\circ}$. (Found: N, 5.9; Cl, 8.0%. $C_{28}H_{40}O_2N_2 \cdot HCl$ requires N, 5.9; Cl, 7.5%.)

*n-Decyl ether**. Glistening mass of rosettes of needles from ether, m.p. $124\text{--}127^{\circ}$, with some sintering at 120° , $[\alpha]_D^{15} - 177.6^{\circ}$ (0.1 N $EtSO_3H$); *hydrochloride*, minute, colourless needles from a mixture of benzene and light petroleum, m.p. $90\text{--}115^{\circ}$. (Found: C, 71.0; H, 8.6; N, 5.8; Cl, 7.4%. $C_{29}H_{42}O_2N_2 \cdot HCl$ requires C, 71.5; H, 8.9; N, 5.8; Cl, 7.3%.)

*n-Undecyl ether**. Colourless, silky mass of rosettes of needles from ether, m.p. $124\text{--}129^{\circ}$, $[\alpha]_D^{15} - 164.6^{\circ}$ (0.1 N $EtSO_3H$); *hydrochloride*, colourless, short needles from a mixture of benzene and petroleum, m.p. $100\text{--}120^{\circ}$. (Found: C, 71.1; H, 8.9; N, 5.6; Cl, 7.1%. $C_{30}H_{44}O_2N_2 \cdot HCl$ requires C, 71.9; H, 9.1; N, 5.6; Cl, 7.1%.)

*Cetyl ether**. Rosettes of colourless needles, m.p. $103\text{--}106^{\circ}$, $[\alpha]_D^{15} - 133.7^{\circ}$ (0.1 N $EtSO_3H$) from ether; *hydrochloride*, translucent, waxy masses of minute rosettes of needles, m.p. $90\text{--}110^{\circ}$, from a mixture of benzene and petroleum. (Found: C, 73.6; H, 9.75; N, 4.8; Cl, 6.3%. $C_{35}H_{64}O_2N_2 \cdot HCl$ requires C, 73.55; H, 9.7; N, 4.9; Cl, 6.2%.)

isoapoQuinidine alkyl ethers ($V: R = Alkyl$). Of the three *apoquinidines* of this type only *isoapoquinidine* was available in quantity and from this a series of ethers was prepared by the general method already described. The yields ranged from 20 to 27%: the products were usually amorphous, but yielded crystalline salts, of which the acid sulphate, $B.H_2SO_4$, was generally the most convenient for purification. The methyl ether is the well-known β -*isoquinidine*.

*Ethyl ether**. Amorphous. The *neutral sulphate* crystallizes from water in prisms, m.p. 84° or $140\text{--}145^{\circ}$ (dry), $[\alpha]_D^{15} - 43.4^{\circ}$. (Found: loss on drying, 15.3; C, 64.7; H, 7.35; N, 7.2; S, 4.0; EtO, 11.6%. Calc. for $10H_2O$: loss on drying 15.7%; ($C_{21}H_{26}O_2N_2 \cdot H_2SO_4$ requires C, 65.1; H, 7.0; N, 7.2; S, 4.1; EtO, 11.6%.)

*n-Amyl ether**. Amorphous. The *acid sulphate* forms needles from 80% alcohol, m.p. 173° , $[\alpha]_D^{20} + 26.2^{\circ}$. (Found: loss on drying, 3.8; C, 60.45; H, 7.3; N, 5.6; S, 6.3%. Calc. for $1H_2O$, loss on drying, 3.6%. $C_{24}H_{32}O_2N_2 \cdot H_2SO_4$ requires C, 60.2; H, 7.2; N, 5.85; S, 6.7%.)

*isoAmyl ether**. Amorphous. The *neutral sulphate* separated from 50% alcohol in needles, sintering at 100°, m.p. 130° (*decomp.*), $[\alpha]_D^{20} + 31.7^\circ$ (0.1 N H_2SO_4). (Found: loss on drying, 9.1; C, 67.2; H, 7.7; N, 6.7; S, 4.0%. Calc. for $5\text{H}_2\text{O}$ loss on drying, 9.2%. $(\text{C}_{24}\text{H}_{32}\text{O}_2\text{N}_2)_2 \cdot \text{H}_2\text{SO}_4$ requires C, 67.1; H, 7.7; N, 6.5; S, 3.7%.)

*n-Hexyl ether**. Amorphous. The *acid sulphate* crystallized from 80% alcohol in needles, m.p. 175°, $[\alpha]_D^{20} + 24.9^\circ$ (0.1 N HCl). (Found: loss on drying, 4.0; C, 59.5; H, 7.6; N, 5.8; S, 6.8%. Calc. for $1\text{H}_2\text{O}$, loss on drying, 3.5%. $\text{C}_{25}\text{H}_{34}\text{O}_2\text{N}_2 \cdot \text{H}_2\text{SO}_4$ requires C, 60.9; H, 7.4; N, 5.7; S, 6.5%.) This salt gave consistently low figures for C ranging from 59.4 to 59.6%.

*n-Heptyl ether**. Amorphous. The *acid sulphate* separated from 85% alcohol in needles, m.p. 170°, $[\alpha]_D^{20} + 25.3^\circ$ (0.1 N HCl). (Found: loss on drying, 3.4; C, 61.1; H, 7.5; N, 5.5; S, 6.1. Calc. for $1\text{H}_2\text{O}$, loss on drying, 3.4%. $\text{C}_{26}\text{H}_{36}\text{O}_2\text{N}_2 \cdot \text{H}_2\text{SO}_4$ requires C, 61.6; H, 7.6; N, 5.5; S, 6.3%.)

*n-Octyl ether**. Amorphous. The *acid sulphate* formed needles from 95% alcohol, m.p. 161°, $[\alpha]_D^{20} - 5.1^\circ$ (0.1 N HCl). (Found: loss on drying, 3.7; C, 61.9; H, 7.75; N, 5.3; S, 6.2%. Calc. for $1\text{H}_2\text{O}$, loss on drying, 3.3; $\text{C}_{27}\text{H}_{38}\text{O}_2\text{N}_2 \cdot \text{H}_2\text{SO}_4$ requires C, 62.3; H, 7.75; N, 5.4; S, 6.2%.)

Alkyl ethers of dihydrocupreine and dihydrocupreidine (VI: R = Alkyl).

When dihydroquinine or dihydroquinidine (VI: R = Me) is demethylated no intramolecular change occurs, such as takes place in the demethylation of quinine or quinidine, and the corresponding phenolic base, dihydrocupreine or dihydrocupreidine is formed. The former is one of the hydrogenation products of *apoquinine* or its geometrical isomeride, *isoapoquinine* (II: R = H) due to saturation of the ethylenic linkage between C³ and C¹⁰, and the same relation holds for the hydrogenation of *apoquinidine* (II: R = H) to dihydrocupreidine (VI: R = H). In view of the quantitative change in antimalarial activity induced by the hydrogenation of quinine to dihydroquinine and of quinidine to dihydroquinidine [Buttle *et al.* 1934], it was of interest to compare the antimalarial activities of the alkyl ethers of the *apo*-bases with alkyl ethers of their hydrogenation products—the alkyl dihydro-cupreines and -cupreidines. The whole series of alkyl ethers of dihydrocupreine from C₂ to C₁₆ has been prepared and used for bactericidal, trypanocidal and other biological tests [Morgenroth (with Halberstaedter, 1910; 1911); (with Levy, 1911); (with Tugendreich, 1916): Schaeffer, 1917; (with Braun, 1917)], but only a few members of the series have been characterized and described. The ethyl and propyl ethers were patented [Zimmer, 1912] and the former was described by Giemsa & Halberkann [1918] and by Heidelberger & Jacobs [1919], who also prepared the *sec*-octyl ether. These three, together with the *n*-butyl, *iso*amyl and hexyl ethers were also prepared by Slotta & Behnisch [1933]. The others, described below, are new so far as chemical literature is concerned.

*isoPropyl ether**. Amorphous, $[\alpha]_D - 226.0^\circ$; *hydrochloride*, colourless, hemispherical rosettes of needles from acetone, m.p. 227–230°, with previous sintering. (Found: C, 67.9; H, 8.0; N, 6.8; Cl, 8.6%. $\text{C}_{22}\text{H}_{30}\text{O}_2\text{N}_2 \cdot \text{HCl}$ requires C, 67.6; H, 8.0; N, 7.2; Cl, 9.1%.)

*n-Amyl ether**. Colourless, felted masses of minute needles from acetone, sintering at 117°, m.p. 130–132°, $[\alpha]_D^{15} - 210.2^\circ$; *dihydrochloride*, needles from water, sinters at 233°, m.p. 240–243° (*decomp.*), $[\alpha]_D^{15} - 177.9^\circ$. (Found: loss on drying, 4.05; Cl, 15.7%. Calc. for $1\text{H}_2\text{O}$, loss on drying, 3.8%. $\text{C}_{24}\text{H}_{34}\text{O}_2\text{N}_2 \cdot 2\text{HCl}$ requires Cl, 15.6%.)

*n-Heptyl ether**. Colourless needles with $1\text{H}_2\text{O}$ from acetone, m.p. 130–132°; $[\alpha]_D^{15} - 198.9^\circ$ (0.1 N EtSO_3H); *dihydrochloride*, sheaves of felted needles, m.p. 140–160° from water. (Found: loss on drying, 8.9; Cl, 14.7%. Calc. for $2.5\text{H}_2\text{O}$, loss on drying, 8.5%. $\text{C}_{26}\text{H}_{38}\text{O}_2\text{N}_2 \cdot 2\text{HCl}$ requires Cl, 14.7%.)

*n-Octyl ether**. Rosettes of colourless needles from acetone, m.p. 119°, $[\alpha]_D^{15} - 186.3^\circ$ (0.1 N EtSO_3H). (Found: loss on drying, 3.3; C, 76.1; H, 9.4; N, 6.7%. Calc. for $0.75\text{H}_2\text{O}$, loss on drying, 3.1%. $\text{C}_{27}\text{H}_{40}\text{O}_2\text{N}_2$ requires C, 76.35; H, 9.5; N, 6.6%); *dihydrochloride*, rosettes of needles from

water, m.p. indefinite, sintering from 135°. (Found: loss on drying, 9.4; Cl, 13.7%. Calc. for $3\text{H}_2\text{O}$, loss on drying, 9.8%. $\text{C}_{27}\text{H}_{40}\text{O}_2\text{N}_2 \cdot 2\text{HCl}$ requires Cl, 13.7%.)

*n-Nonyl ether**. Feathery crystals from light petroleum, sintering at 96°, translucent at 102–104°, clear oil at 130°, $[\alpha]_D^{15} - 146^\circ$ (0.1 N EtSO_3H). (Found: C, 76.8; H, 9.6; N, 6.4%. $\text{C}_{28}\text{H}_{44}\text{O}_2\text{N}_2$ requires C, 76.6; H, 9.6; N, 6.4%); *dihydrochloride*, rosettes or sheaves of colourless needles from water, softens at 120°, m.p. 180°.

*n-Decyl ether**. Separates as a jelly from light petroleum but on standing changes to felted needles, m.p. 111–113°, with some sintering at 107°, $[\alpha]_D^{15} - 120.8^\circ$ (0.1 N EtSO_3H); *dihydrochloride*, granules made up of minute needles, m.p. 135–155°. (Found: loss on drying, 7.8; C, 65.8; H, 8.7; N, 5.6; Cl, 12.9%. Calc. for $2.5\text{H}_2\text{O}$, loss on drying, 7.9%. $\text{C}_{28}\text{H}_{44}\text{O}_2\text{N}_2 \cdot 2\text{HCl}$ requires C, 66.2; H, 8.8; N, 5.3; Cl, 13.5%.)

*Undecyl ether**. Silky needles from a mixture of ether and light petroleum, sintering at 105°, m.p. 107–109°, $[\alpha]_D^{15} - 108.7^\circ$ (0.1 N EtSO_3H). (Found: C, 77.1; H, 9.9; N, 6.1%. $\text{C}_{30}\text{H}_{46}\text{O}_2\text{N}_2$ requires C, 77.2; H, 9.9; N, 6.0%); *dihydrochloride*, minute cream-tinted rosettes of needles, m.p. 130–150°. (Found: Cl, 12.6%. B. 2HCl requires Cl, 13.2%.)

As no alkyldihydrocupreidine ethers appear to have been subjected to antimalarial tests, four were made for this purpose. The ethyl ether had m.p. 199–201°, $[\alpha]_D + 286.9^\circ$ [cf. Heidelberger & Jacobs, 1919]. The other three already prepared by Ghosh & Chatterjee [1931; 1932] had the following constants: *isobutyl*, m.p. 178°, $[\alpha]_D^{20} + 271.6^\circ$; *amyl*, m.p. 161°, $[\alpha]_D^{21} + 253.1^\circ$; *isoamyl*, m.p. 169°, $[\alpha]_D^{20} + 246.8^\circ$.

Results of antimalarial tests.†

The anti-malarial tests were made by Roehl's method in canaries in the manner already described [Buttle *et al.* 1934]. This is now a standard method for

Fig. 1. Action of various doses of quinine on bird malaria: abscissae, time in days after infection; ordinates, serial number of individual birds.

The black areas represent the duration of time during which no parasites were seen for each individual bird; the cross-hatched areas the time during which parasites were seen in less than 1% of the corpuscles. When parasites appeared in 1% or more of the corpuscles the blood examination was discontinued. * One bird on 2.5 mg. was cured.

■ No parasites seen.

▨ Parasites in less than 1/100 R.B.C.

□ Parasites in more than 1/100 R.B.C.

the investigation of antimalarial drugs, and as quinine has been used throughout this investigation as a standard drug for comparison, it was considered advisable

† G. A. H. Buttle and J. W. Trevan are responsible for all the experiments on animals.

to determine the point at which antimalarial action can be detected for this alkaloid. The results are shown in Fig. 1, which shows the effects of the administration of a series of doses to groups of six canaries. Each dose was repeated daily for 6 days on and after the day of injection. The black columns represent the time in days from the infection to the appearance of an occasional parasite in the blood. The cross-hatched portion represents the further time elapsing till the parasites were present in 1 % or more of the red cells. The average time of appearance of parasites is given against each dose. Where none appeared in 30 days of observation the "time of appearance" is taken as 30.

The curative effect of a given dose varies from experiment to experiment, not always in proportion to the intensity of the infection as judged by the time of appearance of parasites in the control birds. In this particular experiment the action of quinine is rather more pronounced than in the average.

A trace of effect first appears with 0.02 mg. of quinine; the mean time of appearance is 4.8 days instead of 4.2 for the control but the difference is probably not statistically significant. With 0.1 mg. doses the difference from the control is obvious, and doses of 0.5 mg. produce large increases in the time of appearance of parasites. Above this again the discrimination falls off. Between 0.1 and 0.5 mg. in this experiment doses in the ratio of 2:1 could probably be just distinguished. In other experiments the range of doses of quinine over which this degree of discrimination could be made would probably be slightly higher.

It would have been necessary to set up some such experiment as this with each drug, together with a corresponding group on various doses of quinine, to have established a quantitative ratio of activity for each drug. But as we were interested primarily in the order of activity and not in the establishment of the actual quantitative ratio to quinine, we adopted a simpler procedure, which involved the use of fewer birds. Constant doses of 2.5 mg. of quinine in one group of 4-6 birds and 2.5 mg. of the drug in another group were employed, and the average time of appearance of parasites was observed. These are recorded in columns 3 and 4 of Tables II, III and IV. In column 2 is given the average time of appearance of parasites in a control group of birds. Column 5 headed Quinine ratio is the ratio of the increase in the time of appearance of parasites in the drug-treated birds to the increase in time of appearance in the birds treated with quinine. It is obtained by dividing the difference between the figures in columns 3 and 2 by the difference between the figures in columns 4 and 2; where there is no difference between columns 3 and 2 the drugs are labelled Inactive. This means, if we consider the first experiment, that the activity is probably less than 1/25 of that of quinine. When the ratio is greater than 1 although the experiment indicates that the drug is more active than quinine we can give no sort of estimate of the ratio of activity except that the drugs with the longer retardation times relative to quinine are the more active. The Tables also include the number of birds cured as determined by re-infection experiments. This phenomenon is so erratic in appearance that very large numbers of birds would have to be used before any conclusions could be based on the proportion of cures obtained.

Table II. *apoQuinine and dihydrocupreine ethers*

Name of drug	Average time of appearance of parasites in peripheral blood			Quinine ratio	Birds cured	
	Controls days	Drug days	Quinine days		Drug	Quinine
<i>apo</i> Quinine (II: R=H)	5.5	18.4	18.5	0.98	—	1
<i>apo</i> Quinine ethers (II: R=Alkyl):						
Methyl (β - <i>iso</i> quinine)	5.0	16.4	21.5	0.69	—	—
Ethyl	5.5	21.0	18.6	1.18	1	1
Propyl	5.5	21.6	18.6	1.23	1	1
<i>iso</i> Propyl	5.3	10.0	14.5	0.51	—	1
<i>n</i> -Butyl	4.2	22.2	20.75	1.1	—	1
<i>n</i> -Amyl	4.2	28.6	20.75	1.47	1	1
<i>iso</i> Amyl	5.3	17.0	14.5	1.27	—	1
<i>n</i> -Hexyl	4.3	21.0	14.0	1.72	—	—
<i>n</i> -Heptyl	4.3	16.0	14.0	1.21	—	—
<i>n</i> -Octyl	4.3	22.0	14.0	1.82	Av. 2	—
	4.4	19.5	15.4	1.36		
<i>sec</i> -Octyl	4.4	20.4	15.4	1.44	1	—
	5.0	18.0	25.5	0.65	Av. 0.98	—
	5.0	20.8	26.2	0.745		
	7.9	29.0	27.5	1.08	3	3
<i>n</i> -Nonyl	4.2	20.3	20.75	0.958	0.98	1
	4.0	24.0	24.0	1.0		
<i>n</i> -Decyl	4.2	21.8	20.75	1.32	1.18	—
	4.0	25.0	24.0	1.05		
<i>n</i> -Undecyl	4.0	19.0	24.0	0.75	—	—
Cetyl	7.7	17.0	27.5	0.47	Av. 1	3
	5.0	19.3	26.2	0.69		
<i>isoapo</i> Quinine (II: R=H)	4.0	12.0	13.0	0.9	—	—
Methyl ether (α - <i>iso</i> quinine)	5.0	18.1	26.2	0.62	—	—
<i>Dihydrocupreine</i> (VI: R=H)						
	5.0	23.0	21.5	1.109	Av. 0.92	—
	4.0	12.0	14.7	0.75		
<i>Dihydrocupreine ethers</i> (VI: R=Alkyl):						
Methyl (<i>dihydro</i> quinine)	7.2	26.2	21.0	1.35	—	1
Methyl (<i>epi</i> -C ³ - <i>dihydro</i> - quinine)	4.0	21.0	21.3	1.0	—	1
Ethyl	5.5	19.3	18.6	1.05	—	1
Propyl	5.3	19.0	14.5	1.49	—	1
<i>iso</i> Propyl	5.5	23.3	18.6	1.37	2	1
<i>n</i> -Butyl	5.3	22.5	14.5	1.87	1	1
<i>n</i> -Amyl	5.5	19.3	18.6	1.33	2	1
<i>iso</i> Amyl	5.3	22.0	14.5	1.83	—	1
<i>n</i> -Hexyl	4.3	16.5	14.0	1.26	Av. 1	—
	4.3	21.0	14.0	1.73		
<i>n</i> -Heptyl	4.0	>30.0	24.0	>1.3	Av. 1.70	—
	4.3	22.0	13.0	2.04		
<i>n</i> -Octyl	4.3	19.4	14.0	1.56	Av. 1.43	—
	4.0	23.0	15.4	1.67		
	4.0	20.4	19.2	1.08		
<i>sec</i> -Octyl	4.3	24.5	16.0	1.53	1	—
<i>n</i> -Nonyl	4.3	18.0	16.0	1.12	1	—
<i>n</i> -Decyl	4.3	25.7	16.0	1.6	2	—
<i>n</i> -Undecyl	4.3	20.0	16.0	1.25	—	—

Table III. *apoQuinidine and dihydrocupreidine ethers*

Name of drug	Average time of appearance of parasites in peripheral blood			Quinine ratio	Birds cured	
	Controls days	Drug days	Quinine days		Drug	Quinine
<i>apo</i> Quinidine (II: R = H)	5.0	11.6	21.5	0.4	—	—
Methyl ether	4.0	13.0	13.0	1.0	—	—
<i>neoapo</i> Quinidine (II: R = H)	7.7	16.6	27.5	0.465	—	3
ψ -Quinidine (IV: R = Me)	3.0	17.0	20.6	0.79	—	—
<i>isoapo</i> Quinidine (V: R = H)	5.5	7.0	18.6	0.11	—	1
<i>isoapoQuinidine ethers</i> (V: R = Alkyl):						
Methyl (β - <i>iso</i> quinidine)	5.0	5.6	21.5	0.1	—	—
Ethyl	4.3	7.0	14.0	0.28	Av. 0.25	—
	4.0	6.0	13.0	0.22		—
<i>n</i> -Amyl	4.0	6.0	13.0	0.22		—
<i>iso</i> Amyl, <i>n</i> -hexyl, <i>n</i> -heptyl, <i>n</i> -octyl	4.0-4.3	4.0-5.0	13.0-14.0	Inactive	—	—
α - <i>iso</i> Quinidine (V: R = Me)	7.7	9.0	27.5	0.065	—	3
γ - <i>iso</i> Quinidine (V: R = Me)	4.0	<6.0	21.3	Inactive	—	1
Dihydrocupreidine (VI: R = H)	5.0	16.2	21.5	0.68	—	—
<i>Dihydrocupreidine ethers</i> (VI: R = Alkyl):						
Methyl (dihydroquinidine)	3.0	17.4	20.6	0.81	—	—
Ethyl	5.0	24.0	25.0	0.95	Av. 0.98	1
	5.0	18.0	25.0	0.65		—
	5.0	27.2	21.5	1.35		—
<i>iso</i> Butyl	4.0	20.0	24.0	0.8	—	—
<i>n</i> -Amyl	4.0	12.0	24.0	0.4	—	—
<i>iso</i> Amyl	4.0	17.0	24.0	0.65	—	—

Table IV. *Various quinine and quinidine transformation products*

Name of drug	Average time of appearance of parasites in peripheral blood			Quinine ratio	Birds cured	
	Controls days	Drug days	Quinine days		Drug	Quinine
<i>Hydroxy-bases</i> (III: R = H or Me):						
α -Hydroxydihydroapoquinine	5.3	6.0	14.5	0.076	—	1
Methyl ether (hydroxydi- hydroquinine)	5.0	16.4	26.2	0.542	—	—
β -Hydroxydihydroapoquinine	5.0	5.6	21.5	0.036	—	—
<i>epi-Bases</i> :						
<i>epi</i> -C ⁹ -Quinine (I: R = Me)	4.5	6.5	24.7	0.099	} Av. 0.1	—
	5.0	<7.0	25.0	0.1		—
<i>epi</i> -C ⁹ -Quinidine (I: R = Me)	4.5	6.2	24.7	0.084	} Av. 0.13	—
	3.0	<7.0	25.0	0.181		—
<i>epi</i> -C ⁸ -Dihydroquinine (VI: R = Me)	4.0	21.0	21.3	1.0	—	1
<i>epi</i> -C ⁸ -Dihydroquinidine (VI: R = Me)	4.0	16.0	20.7	0.72	—	—
Niquine (VII, Léger)	4.2	23.7	26.2	0.86	—	1
Dihydroniquine	3.0	14.2	20.6	0.63	—	—
Niquidine	4.0	22.0	16.4	1.45	1	—
<i>iso</i> Niquidine	4.0	17.0	16.4	1.05	—	—
Dihydroniquidine	4.0	22.4	21.3	1.06	—	—
Quinine dihydrochloride zinci- chloride, B. 2HCl. ZnCl ₂	7.7	28.5	27.5	1.05	} Av. 0.93	3
	4.0	17.2	21.3	0.81		—

DISCUSSION OF BIOLOGICAL RESULTS

It is remarkable that whilst epimerization at C⁹ reduces the activity of quinine from 1 to 0.1 in *epi*-C⁹-quinine and that of quinidine from 0.5 to 0.1 in *epi*-C⁹-quinidine [cf. Dirscherl & Thron, 1935] and that whilst dihydroquinine has a quinine ratio of 1.35, *epi*-C³-dihydroquinine has the same activity as quinine, there is no obvious relationship traceable between optical activity and antimalarial action in the cinchona alkaloids; thus while it is true that the *l*-bases, quinine and cinchonidine, are more active than the *d*-bases, quinidine and cinchonine [Buttle *et al.* 1934], *apo*quinidine methyl ether (+334.2°) is more active (quinine ratio 1.0) than either of its *l*-isomerides α - or β -isoquinine (quinine ratios 0.62 and 0.69 respectively). It is also of interest in this connexion to note that, whilst conversion of the phenolic *l*-bases *apo*quinine and *isoapo*quinine into their methyl ethers β - and α -isoquinines results in a reduction of antimalarial action, the converse is the case when the *d*-phenolic base, *apo*quinidine, is methylated.

It is not surprising that the ether oxide type of structural isomeride (V: R=Me) represented by α -, β - and γ -isoquinidines should prove to be almost inactive, since it is well known that any modification of the structure of the —CHOH— group connecting the two halves of the cinchona alkaloids reduces antimalarial activity.

The most interesting results are to be found in the homologous series of *apo*quinine and dihydrocupreine ethers, as will be seen from Table V, in which

Table V

Quinine ratio		<i>apo</i> Quinine ethers Formula	Name of ether	Dihydrocupreine ethers Formula	Quinine ratio	
Even	Odd				Odd	Even
0.69	—	C ₂₀ H ₂₄ O ₂ N ₂	Methyl	C ₂₀ H ₂₆ O ₂ N ₂	—	1.35
—	1.18	C ₂₁ H ₂₆ O ₂ N ₂	Ethyl	C ₂₁ H ₂₈ O ₂ N ₂	1.05	—
1.23	—	C ₂₂ H ₂₈ O ₂ N ₂	<i>n</i> -Propyl	C ₂₂ H ₃₀ O ₂ N ₂	—	1.49
—	1.10	C ₂₃ H ₃₀ O ₂ N ₂	<i>n</i> -Butyl	C ₂₃ H ₃₂ O ₂ N ₂	1.87	—
1.47	—	C ₂₄ H ₃₂ O ₂ N ₂	<i>n</i> -Amyl	C ₂₄ H ₃₄ O ₂ N ₂	—	1.33
—	1.72	C ₂₅ H ₃₄ O ₂ N ₂	<i>n</i> -Hexyl	C ₂₅ H ₃₆ O ₂ N ₂	1.50	—
1.21	—	C ₂₆ H ₃₆ O ₂ N ₂	<i>n</i> -Heptyl	C ₂₆ H ₃₈ O ₂ N ₂	—	1.70
—	1.6	C ₂₇ H ₃₈ O ₂ N ₂	<i>n</i> -Octyl	C ₂₇ H ₄₀ O ₂ N ₂	1.43	—
0.98	—	C ₂₈ H ₄₀ O ₂ N ₂	<i>n</i> -Nonyl	C ₂₈ H ₄₂ O ₂ N ₂	—	1.12
—	1.18	C ₂₉ H ₄₂ O ₂ N ₂	<i>n</i> -Decyl	C ₂₉ H ₄₄ O ₂ N ₂	1.6	—
0.75	—	C ₃₀ H ₄₄ O ₂ N ₂	<i>n</i> -Undecyl	C ₃₀ H ₄₆ O ₂ N ₂	—	1.25
—	0.58	C ₃₅ H ₅₄ O ₂ N ₂	Cetyl	Not prepared	—	—

the quinine ratios are assembled. If each homologous series is considered as a whole there appears to be no regularity in rise or fall of the quinine ratio, but if each series is divided into members containing odd and even numbers of carbon atoms, it will be seen that, with some anomalies, there is a definite tendency in the four series so produced for the quinine ratio to rise to a maximum and then fall as the series is ascended.

This is not the first instance of this curious alternation in the parasiticide activity of members of a homologous series; it was first noticed by Magidson [Magidson & Strukow, 1933; Magidson *et al.* 1935] in testing the antimalarial activity in infected siskins of a series of 6-methoxyquinolines substituted in position 8 by the chain —NH—[CH₂]_{*n*}—NEt₂. These authors found the following chemotherapeutic indices (*i*) for different values of *n*.

<i>n</i>	2	3	4	5	6	7	8	9	10	11
<i>i</i>	—	26	—	25	—	34	—	40	—	5
<i>i</i>	6	—	11	—	13	—	—	—	—	—

This alternation was confirmed by Bovet *et al.* [1934], who tested the same series on Java sparrows, but in this instance it was observed that as the series was ascended the drug acquired schizonticidal action in addition to its original gametocidal character.

Alternation is not, however, always shown in such tests with homologous series. Magidson & Strukow [1933] found that when the chain in position 8 was kept constant at $\text{—NH—[CH}_2\text{]}_2\text{—NEt}_2$ and the group in 6 varied from HO— to $\text{C}_5\text{H}_{11}\text{O—}$, there was a continuous but irregular fall in the chemotherapeutic index from 13 to 0. Similarly in a short series of substituted acridines with methoxyl at position 7, chlorine at position 3 and at position 5 the chain $\text{—NH—[CH}_2\text{]}_n\text{—NEt}_2$, where $n=2$ to 6, the chemotherapeutic index began at 8, rose to 20 at $n=4$ and fell to 6 and 5 at $n=5$ and 6 respectively [Magidson & Grigorovski, 1936]. These series are very short and the rise or fall is much more abrupt than in the instances in which the alternating effect is shown, but as they stand there is no reason to suppose that an alternating effect has been obscured by the particular experimental conditions. Comparatively few investigations have been made of the change in action *in vivo* exhibited by a homologous series of parasitocidal drugs, though the results of numerous *in vitro* investigations of this kind are available. In the latter the results generally show a rise to a maximum followed by a fall as the series is ascended and may be explained as resulting from the action of two opposing factors, e.g. parasitocidal action conditioned by the solubility or some other physical property of the drug used. It has been generally assumed that results obtained *in vivo* would show the same tendency, but the results of the Russian workers, and those now recorded, seem to indicate that when such investigations are conducted *in vivo* some new factor may come into play which leads to the alternation in the effect of length of chain on activity.

The short series of dihydrocupreidine ethers (Table III) is mainly of interest as showing that in the dextrorotatory bases a rise in activity also occurs as the side-chain is increased in length.

The results given by the hydroxy *apo*-bases (Table IV) are perhaps to be expected since the presence of a phenolic hydroxyl group in the quinoline nucleus, and an additional hydroxyl group attached to carbon atom 10, reduces the basic character, which seems to be essential for antimalarial action and it is noticeable that the activity is largely increased by methylation of the phenolic hydroxyl group.

The activities of niquine and the two niquidines are surprising, niquidine (quinine ratio 1.45) being much the most active of the dextrorotatory cinchona alkaloids yet known. The zincichlorides have proved so useful as a means of isolating transformation products of the cinchona alkaloids, being sometimes the only crystallizable derivatives obtainable, that it seemed desirable to ascertain whether in case of necessity they could be used for antimalarial tests. In the single case tried, quinine dihydrochloride zincichloride (Table IV) the complex salt seemed to behave like any other salt of quinine.

It is not yet possible to draw any precise general conclusions as to the mode of action of the cinchona alkaloids as antimalarial drugs. It seems to be clearly established that they act directly on the parasites and that any modification in structure which results in the formation of a weaker base, as in the conversion of quinine into quitenine, reduces or extinguishes their activity. That inactivity ensues from even a spatial distribution change as well as from small structural changes in the connecting —CHOH— group seems to imply that the essential condition for activity is a close "fit" between the drug and the protein molecules

of the parasite, and that view seems to be supported by the alternation in activity shown by successive members of homologous series.

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