STRUCTURE AND ANTIPNEUMOCOCCIC ACTIVITY IN THE CINCHONA SERIES

ALICE G. RENFREW AND LEONARD H. CRETCHER

Department of Research in Pure Chemistry, Mellon Institute of Industrial Research, Pittsburgh, Pennsylvania

Received October 14, 1941

The present survey gives particular emphasis to recent studies of the antipneumococcic action of some cinchona derivatives. A brief historical survey is presented and reference is made to speculations concerning the mode of action of cinchonas in the animal body. Antipneumococcic action is analyzed with regard to isomeric and structural modifications of the cinchona molecule. A summary of recent data on bacteriostasis, toxicity, animal protection, pharmacologic testing, and clinical studies is given. Synthetic analogs are discussed in relation to significant structural factors.

Cinchona alkaloids have been used as antimalarials since 1630, but evidence of the specific antipneumococcic action of these compounds dates only from 1911. Morgenroth's (84) study of cinchonas as antipneumococcic agents has been described as "the first striking instance of the successful application of a chemical to the treatment of a bacterial infection in a laboratory animal" (53). Because Morgenroth had been studying quinine in relation to trypanosome infections in animals and was acquainted with the capsule-formation characteristic of trypanosomes, spirilla, and the pneumococcus, he was led to investigate the action of the alkaloid in pneumococcic septicemia in mice. There followed an extensive study of hydrocupreine ethers with the synthesis of the well-known optochin, vuzin, and eucupin. Between 1919 and 1924 Heidelberger and Jacobs (42, 54) at the Rockefeller Institute and Giemsa (32, 33) in Germany published the results of further studies in the hydrocupreine series. These results have been reviewed by von Oettingen (111) and by Houben (50), and the clinical findings have been discussed by Moore and Chesney (87), by Kolmer (63), and by Solis-Cohen (107).

The remarkable antipneumococcic action of optochin has been a spur to reinvestigation of this field. In again studying cinchona derivatives the search was not so much for a compound of greater power but rather for a compound free from deleterious side-effects. The last decade has brought extensive synthesis in the field of apocinchonas. In the early 1930's methylapocupreine[1] [or

[1] At the time the term apocupreine (8) was suggested, its use was based firstly on the similarities of apocupreine (formula II) and cupreine (formula I), which differ chemically only in the 3-substituent of ethylidene or vinyl, respectively, and secondly on the established usage of Léger (59, 66) in applying the terms apocinchonine and apocinchonidine (characterized by the ethylidene side chain) to the corresponding substances derived from cinchonine and cinchonidine. In the case of quinine, demethylation is accomplished by boiling with 60 per cent sulfuric acid; a shift of the double bond also occurs. The product, called apoquinine by Hesse (49), consists of substances of different toxicities and antipneumococcic powers and these give derivatives of various biological properties. The name

isoquinine (39)] and ethylapocupreine[1] or [ethylapoquinine (13, 14, 22, 45, 52, 56, 72, 73, 76, 79, 80, 81, 89, 90)] were reported by investigators in the United States, Germany, and Japan to have protective action in experimental pneumonias. Since 1934 Maclachlan *et al.* have published results of the experimental testing of alkyl and hydroxylated alkyl ethers of apocupreine in lower animals and of the use of hydroxyethylapocupreine in the treatment of pneumonia in man. In clinical use, this latter compound has been very effective and not a single instance of eye damage has been encountered. The recent Japanese work has consisted largely of experimental studies of antipneumococcic action, and the English investigators have reported antimalarial studies of apocinchonas and similar compounds.

Hegner (41) has reported hydroxyethylapocupreine and quinine as approximately equally effective against *P. lophurae* in ducks, *P. relictum* in pigeons, and *P. cathemerium* in canaries. The low toxicity of hydroxyethylapocupreine gives particular interest to these findings.

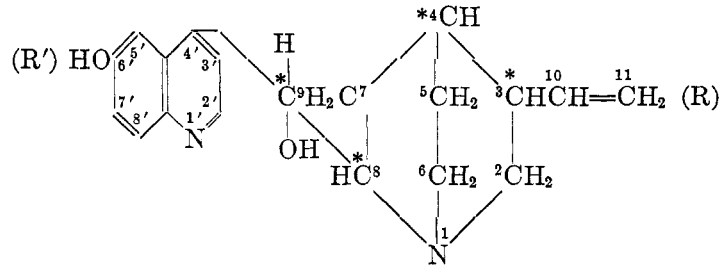
Morgenroth (84) pointed out the importance of systematic variation of the component groups in the cinchona molecule in relation to an ultimate understanding of the mode of antimalarial action or the basis of bacterial specificity of this class of alkaloids. In attempting to correlate chemical structure and bactericidal power the results of the experimental testing of a large number of compounds are considered. It must be remembered that comparison of the data from different workers involves many variables: differences in type and virulence of organism, method of determination of power *in vitro*, age and sex of animals used, method of inducing infection and of administration of the drug, —whether oral, intravenous, or intraperitoneal. Even seasonal factors may influence the numerical results. Marshall (75) has cited the difficulties and has contributed in great measure to the standardization of such biological studies. In the cinchona series a general relationship between the bacteriostatic action *in vitro* and the protective action *in vivo* seems to exist (25, page 771; 83a; 10). Hence the preliminary testing of the antipneumococcic action of this type of drug has included three determinations: bacteriostasis *in vitro*, toxicity to mice, and protective action against pneumococcal septicemia in mice.

From the chemical point of view the cinchona molecule is a comparatively large and complex one and is susceptible to many types of modification. Nature has provided but few of the many possible variants. The best-known naturally occurring alkaloids are the stereoisomeric 6′-methoxy compounds, quinine and quinidine, and the corresponding methoxyl-free compounds, cinchonidine and cinchonine. These have little antipneumococcic action (73, 10).

Formula I represents cupreine, and the corresponding methyl ether is quinine. The quinoline and quinuclidine nuclei linked through the secondary alcoholic

---

apocupreine has been applied to the main component of the reaction product (13) and corresponds to the terms apoquinine (45, 46), as used by Henry, and β-isocupreine (55), as used by Suszko.



I
Cupreine

group are characteristic of the naturally occurring alkaloids (quinine, quinidine, cupreine, cinchonidine, and cinchonine, and their dihydro derivatives). It is perhaps permissible to consider this rubanol nucleus of quinoline and quinuclidine joined through the secondary alcohol as the power center (contributing structural arrangement, electronic energies, basicity, oxidation potential, or some such fundamental function) and the substituent groups of R at position 3 in the quinuclidine ring and R′ at 6′ in the quinoline ring as having directive influences. At least, any marked change in the rubanol structure usually lessens or destroys bactericidal action in general, whereas changes in R and R′ often serve to modify toxicity and bacterial specificity. The properties of recent synthetic analogs of quinine have emphasized some of the limits in the simplification of structure and have indicated some promising modifications (1, 19, 31, 61).

Among the general factors which have been variously emphasized in considering the mode of action of cinchonas are molecular weight (61, 106, 114), greater effectiveness at a somewhat alkaline pH (62, 78), and surface tension, which is lower in neutral or alkaline solution (17, 78). Ostromislensky (93) stressed the importance of the colloidal nature of a medicinal and thought that the quinoline residue gives colloidal properties and bacterial specificity. Domagk (24) considers the biologically functioning group to be the quinuclidine nucleus, or the nitrogen of this nucleus. Henry (43, page 441) and Findlay (26, page 115) call attention to the decrease in antimalarial action in the case of changes which lessen the basicity of the molecule. The correlation of fluorescence with the antimalarial action of the cinchonas and of atabrin has been noted by Oesterlin (92). Berkenheim (3) has suggested an electronic interpretation of the action of quinoline derivatives.

The mechanism of the action against the pneumococcus is not understood, but this action must differ from that of members of the sulfanilamide group. In contrast with the effect of sulfonamides, the typing characteristics of the organism are not altered when the cinchonas are used in treating clinical cases of pneumonia; no observable change occurs in the bacterial capsule during studies *in vitro*. An interesting observation by Felton and Dougherty (25, page 771) was a so-called "therapeutic zone phenomenon" in the treatment of pneumo-

coccic septicemia in mice. In the range of non-toxic concentrations of the cinchona there was an optimum dose for protective action, and with the use of an amount larger or smaller than the optimum, mice died of septicemia.

A study of cinchonas in relation to biological activity must consider both isomeric and structural modifications. The following main headings with some subdivisions have been used:

I. Stereoisomerism
II. Variations in the quinoline nucleus
  A. Simple substituted quinolines
  B. Modifications of the 6′-substituent (R′) in quinoline, as H, HO, RO in the cinchonas
  C. Substitution in the quinoline ring of the cinchona: nitro, amino, and azo derivatives
  D. Reduction of the quinoline nucleus
III. Variations of the secondary alcohol bridge
IV. Variations in the quinuclidine nucleus
  A. Modification of the 3-substituent (R)
  B. Quaternary salts
  C. Nitrogen-oxide derivatives
  D. Fission of the quinuclidine ring at the 1,8-position (cupreicine derivatives)
    Fission at the 1,2-position (niquine, niquidine, cinchonhydrines)
V. Detailed study of variations in the ether grouping
VI. Synthetic analogs

I. STEREOISOMERISM

Bactericidal action and physiological properties are found to be markedly influenced by the optical configuration of the asymmetric carbon atoms. For instance, some individuals are allergic to quinine but can be successfully treated for malaria with quinidine without unpleasant side reactions, as shown by Dawson and Newman (23) and by Sanders (103). Dawson and Garbade (23) in studying an individual with a quinine idiosyncrasy, demonstrable by Boerner's test, found the subject allergic to the levorotatory drugs quinine, dihydroquinine, cinchonidine, dihydrocinchonidine, ethyldihydrocupreine (optochin), dihydrocupreine, and ethylquitenine but not allergic to the dextrorotatory isomers quinidine, dihydroquinidine, cinchonine, dihydrocinchonine, ethyldihydrocupreidine, dihydrocupreidine, or ethylquitenidine.

The asymmetric centers in formula I are indicated by asterisks on carbon atoms 3, 4, 8, and 9. (The numbering introduced by Rabe (97) has been adopted in most publications, although it is not in strict conformity with the rules established by the International Commission (94)). C_4 has a levo rotation, as observed by Solomon (108a) in methylapocupreicine and in other apocupreicine ethers (99), and the combined rotational value of carbon atoms 3 and 4, in the naturally occurring cinchona alkaloids, indicates a dextro configuration

for carbon atom 3 (43, page 423). The steric arrangement of carbon atoms 8 and 9 in the four epimeric modifications was established by Rabe (97) and is shown below:

QUININE CINCHONIDINE		QUINIDINE CINCHONINE		EPIQUININE EPICINCHONIDINE		EPIQUINIDINE EPICINCHONINE	
9	8	9	8	9	8	9	8
−	−	+	+	+	−	−	+

For the typical cinchona structure, the levorotatory quinine derivatives are more effective than the dextro isomers against both the malarial parasite and the pneumococcus, as evidenced by dihydroquinine and optochin in contrast with the dextro forms. The comparative antimalarial values of the dextro and levo series of different types of ethers have been presented by Buttle (14). Where an asymmetric center has been modified in structure, no predictions can be made concerning the more effective optical isomer. Both in the "apo" series (formula II) with the double bond at C_3 and in the niquine series where the quinuclidine nucleus has been changed as shown in formula V, the dextro forms show somewhat greater antimalarial action than the corresponding levo forms; the antipneumococcic power of these derivatives in comparison with the levo isomers is not known.

Epimerization at C_8 and C_9 takes place when a naturally occurring alkaloid such as quinine is refluxed with potassium hydroxide in amyl alcohol and the four theoretically possible isomers are obtained: quinine, quinidine, epiquinine, and epiquinidine. Of these four, epiquinidine is the most effective against the pneumococcus, although all are low in power (73). Another asymmetric center is C_{10} when unsymmetrical additions have been made to the double bond of the vinyl side chain; there is little difference in the antimalarial power of the α-isomer as compared with the β-isomer of chlorodihydroquinine (35) or in the antipneumococcic power of the α- and β-hydroxydihydroquinines (10).

II. VARIATIONS IN THE QUINOLINE NUCLEUS

A. Simple substituted quinolines

It should be noted that some antipneumococcic action can be demonstrated for quinolines, even for those with structures much simpler than the cinchona structure.

In the action of simple quinolines on the pneumococcus, 8-hydroxyquinoline (quinisol) has definite antipneumococcic power but considerable toxicity (11, 111). However, the ethyl, propyl, and hydroxylated ethyl and propyl ethers of 8-hydroxyquinoline had no significant antipneumococcal action (11) and 6-hydroxy or 6-ethoxy derivatives of 2-hydroxyquinolines were ineffective.[2] Bührmann (4) claimed antipneumococcic action at 1:10,000 for a number of quinolines, such as the propyl ester of 6-hydroxyquinoline-4-car-

[2] Unpublished work.

boxylic acid or isobutyl 2-phenyl-4-quinolylaminoacetate. In a study of substituted 4-aminoquinolines, Iensch (51) reported a benzthiazole derivative which had prophylactic action in experimental mouse pneumonias but failed in clinical testing.

Attempts to combine the sulfanilamide molecule and an aminoquinoline have not been productive (88, 99) in the simple derivatives, but in the testing of N^4-alkylaminoacetylsulfanilamidoquinolines Juneja (58) has claimed results "comparing favorably with sulfapyridine."

Synthetic derivatives of 6-methoxyquinoline in which a pyrryl (60) or a piperidyl (1) nucleus or a β-dialkylaminomethyl group (61) has been substituted for the quinuclidine portion of the cinchonas have shown action on paramecia or antimalarial power, but no study of the antipneumococcic action has been reported. The preparation and testing of such relatively simple types of synthetic compounds opens an interesting field for future research.

B. Modifications of the 6′-substituent (R′) in quinoline

In the study of antimalarials and in testing the bacteriostatic action of compounds against pneumococcus, streptococcus, or staphylococcus, the 6′-substituent in quinoline has been extensively varied. R′ may be hydrogen, a phenolic hydroxyl, or an ether group. Cinchonas with an unsubstituted hydrogen in the 6′-position have little antipneumococcic value (73).

Phenolic derivatives are obtained when the 6′-methoxydihydrocinchonas undergo demethylation with boiling halogen acids or with 60 per cent sulfuric acid. In the vinyl series a shift of the double bond toward the quinuclidine nucleus takes place, so that the resulting phenolic substances have the ethylidene side chain of the apocinchonas (formula II). Apocupreine had no therapeutic action in cases of clinical pneumonia (72).

In the alkylation of the phenolic cinchonas alkyl, alkoxyalkyl, aroxyalkyl, hydroxyalkyl, and (alkylamino)alkyl ethers have been studied. The remarkable antipneumococcic specificity of ethyldihydrocupreine (optochin) marks a maximal point in the dihydrocupreine series (83a). However, the ethoxyl group is not the only factor necessary, as shown by the fact that the dextrorotatory isomer ethyldihydrocupreidine (prepared by Heidelberger and Jacobs (42a)) had little antipneumococcic action (10), and that ethylcupreine (with the vinyl side chain) was only one-fourth as effective as optochin (50, page 1003). High antipneumococcic action does mark ethylapocupreine (with an ethylidene side chain) (13, 14, 22, 45, 52, 56, 72, 73, 76, 79, 80, 81, 89, 90).

The 2-carbon and 3-carbon ethers of hydrocupreine and apocupreine have been the most effective against the pneumococcus, as will be apparent in later tables; higher homologous ethers showed a decreasing antipneumococcic power but indicated an optimal specificity against other pathogens in relation to the length of the ether chain. Because a rather extensive and systematic study has been made of variations of the ether grouping, the more detailed discussion is presented later.

C. Substitution in the quinoline ring of the cinchona: nitro, amino, and azo derivatives

The quinoline nucleus of cinchonas should be subject to typical substitution reactions in the aromatic nucleus. The only modification of this sort as yet reported to give increased bactericidal power has been the introduction of aromatic azo groups, and most of these compounds have marked toxicity. Heidelberger and Jacobs prepared the 5′-nitro- and 5′-amino derivatives of dihydroquinine, optochin, and dihydroquinidine. Using 5′-aminodihydroquinine as a starting point, these investigators obtained 5′-amino-8′-phenylazo-, 5′-amino-8′-(*p*-sulfophenylazo)-, and 5′-hydroxy-8′-phenylazodihydroquinines (54c) as well as 5′, 8′-diamino derivatives (54d). Both 5′-amino-8′-phenylazooptochin and its dextro isomer were prepared. In coupling diazotized aromatic amines with the phenolic dihydrocupreine and dihydrocupreidine these investigators obtained cinchona dyestuffs of which "many were highly bactericidal *in vitro*" (42b). Boyd found these azo dye derivatives toxic for canaries (5); he reports studies of toxicity, hemolytic action, and antimalarial action for a number of cinchona derivatives. Giemsa and Halberkann reported similar coupling reactions of aromatic diazo compounds with cupreine and dihydrocupreine (32) and in 1933 presented an extensive chemical and chemotherapeutic report on compounds prepared and tested against malaria (33; 26, page 109). Buttle found 5′-sulfamidophenylazoapocupreine (16) of little value against the pneumococcus. At dilutions greater than 1:100,000, neither phenylazoapocupreine nor its ethyl ether showed power against the pneumococcus.[3] Miura found no protective action with small doses of 5′-amino-8′-(sulfamidophenylazo)-ethylhydrocupreine (80). The 5′-amino group of aminodihydroquinine and aminoöptochin has recently been used for the preparation of mustard oils (115); these derivatives have no antimalarial action.

D. Reduction of the quinoline nucleus

Jacobs and Heidelberger (54e) prepared hexahydrocinchonas by reduction of the quinoline ring with sodium and amyl alcohol or with zinc and hydrochloric acid. Boyd (5) found the products toxic and of lower antimalarial power than the unreduced substances.

III. VARIATIONS OF THE SECONDARY ALCOHOL BRIDGE

Many modifications of the secondary alcohol bridge at position 9 have been studied, but in general the changes are dystherapeutic. With regard to antimalarial action, Henry (43, page 443) says that "every alteration so far made in that group has resulted in destruction of antimalarial action," although simple esters such as eucupin are effective *in vivo*. Acetyl and other acyl esters have been made. The Japanese workers found 9-acetylethylapocupreine one-twentieth as effective *in vitro* as ethylapocupreine against the pneumococcus (52),

[3] Unpublished work.

and Maclachlan[4] found that diacetylapocupreine has less inhibiting action on the pneumococcus than apocupreine.

The alcoholic hydroxyl group may be replaced by chlorine, on treatment with phosphorus pentachloride. Chlorine, in turn, may be replaced by hydrogen. Heidelberger and Jacobs studied the 9-chloro and 9-desoxy derivatives of optochin and its dextro isomer, but found these compounds "less bactericidal for the pneumococcus than the corresponding parent alkaloids" (42c). The 9-aminoquinine was prepared by Fränkel (28) and 9-alkylaminoquinines by Altman (2) but were tested only against malaria.

With chromic acid oxidation the alcohol group is converted to a ketone and, surprisingly, Cretcher and Renfrew (100) found that the cinchonas were converted to the corresponding ketones in fair yield during reaction with sodium amide. Dihydroquininone has little action against the pneumococcus (10) and no antimalarial action (43, page 443), but Morgenroth and Bumke (83) claim small antipneumococcic action *in vitro* for 9-ketoöptochin.

Cupreicine derivatives (formula IV) in which 1, 8 rupture of the quinuclidine ring has given 4′-quinolyl β-(4-piperidyl)ethyl ketones are discussed under modifications of the quinuclidine nucleus.

IV. VARIATIONS IN THE QUINUCLIDINE NUCLEUS

A. Modification of the 3-substituent (R)

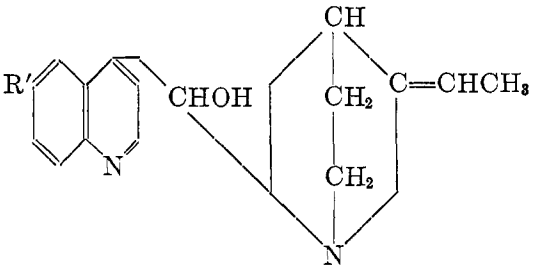
In relation to antipneumococcic action both 3-ethyl and 3-ethylidene structures are effective. To consider first the saturated side chain:—in the levo series the 3-ethyl substituent is much more effective than the vinyl group, as shown in the comparison of dihydroquinine and quinine (84), and the fact that ethyldihydrocupreine is about four times as effective as ethylcupreine (10, 73; 50, page 1003). The dihydrocinchonas are obtained in limited amount from the cinchona bark and may be readily prepared by reduction of the unsaturated alkaloids. On reduction of the ethylidene group of the apo structures (48), both the known dihydroalkaloid and a new epi-C_3-dihydrocinchona are formed, but no test of such a derived epi-C_3-dihydrocinchona against the pneumococcus has been reported.

Addition of water (10), hydrogen chloride (35), or hydrogen iodide (101, 102) gives rise to C_{10} isomers, as noted in section I. Very low action *in vitro* has been reported for hydroxydihydroquinine (10). Halogen in the side chain slightly lessens the antipneumococcic action, as observed by the Japanese workers (81, 82).

On oxidation of the vinyl group there is loss of the terminal carbon atom and formation of a carboxylic acid. The quitenine structure, ineffective of itself, regains antimalarial power on esterification of the carboxyl, as shown in a series of homologous esters tested by Goodson (36). Ethylquitenine is without power against the pneumococcus (10, 73).

[4] Unpublished work.

The 3-ethylidene group of the apocinchonas (formula II) seems to enhance

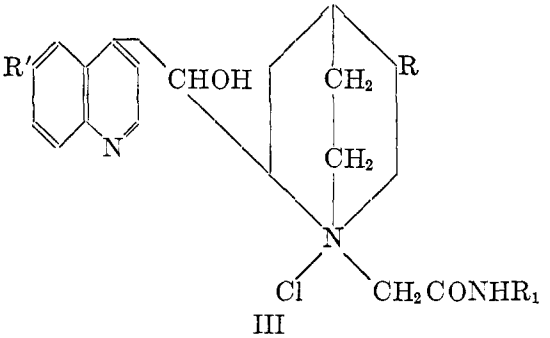


II

antipneumococcic action and to lessen toxicity. Methylapocupreine (isoquinine) is more effective than quinine or dihydroquinine (39, 89; also unpublished work); ethylapocupreine has about the same antipneumococcic action as optochin (13, 80, 89). The apocupreine (apoquinine) ethers have lower toxicity than the corresponding ethers of the hydrocupreine or cupreine series, as is apparent in the data cited by Maclachlan (56, 72, 73), Butler (10, 13), and Gundel and Seitz (39). Conversion of the vinyl group to an ethylidene group introduces the possibility of geometric isomerism. Of the two isomeric series only the ethers of apocupreine (6–13; 22, 38, 56, 68–73, 99, 100; 39, 67; 52, 76, 79, 80, 81, 89, 90) have been tested in pneumonia studies, except for some incomplete reports by the Japanese workers (80).

B. Quaternary salts

The lowered bactericidal power of most quaternary salts in this series and of the *N*-oxide derivatives of cinchonas is interpreted as emphasizing the rôle of tertiary nitrogen (86, 91) in the bactericidal action in cinchonas.



III

The rubanol complex has two basic nitrogen atoms which make possible neutral and acid salts (57) and also serve for the formation of quaternary salts. The quinuclidine nitrogen is the first to be converted to the quinquevalent state. Heidelberger and Jacobs studied a great variety of cinchona quaternary salts, including a series of addition products of dihydroquinine with chloroacetanilide,

chloroacetylaminophenol, chloroacetyl-*p*-anisidine, chloroacetylaminopyrocatechol, etc. (54a). Although the rather low protective action of dihydroquinine against the pneumococcus is enhanced in the hydroquinine quaternary salts (25, 86), it is apparent from table 1 that the bacteriostatic action against this organism remains at a low level whether the cinchona nucleus of itself is characterized by much or by little antipneumococcic power. *p*-Hydroxyacetanilide cinchonidinium chloride hydrochloride is bacteriostatic at $1:1 \times 10^4$.

Ishizaka (52) found ethylapocupreine bacteriostatic for pneumococcus at 1:20,000, whereas the methochloride and the methoiodide were ineffective at 1:1000; on the other hand, these quaternary salts retained the initial moderate effectiveness of the cinchona against streptococcus and staphylococcus (bacteriostatic at 1:2000).

TABLE 1

Bacteriostatic activity of quaternary compounds of cinchonas against Pneumococcus II

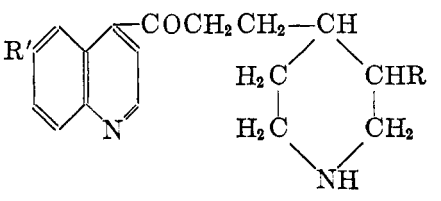
QUATERNARY DERIVATIVE AS A HYDROCHLORIDE	ACTIVE *in vitro* AT CONCENTRATION OF	INVESTIGATOR
p-Chloroacetylaminophenol salt of:		
Dihydroquinine	$1:4 \times 10^4$	Felton (25)
	$1:5 \times 10^4$	Maclachlan *et al.* (18)
Optochin	$1:4 \times 10^4$	Morgenroth (86)
Hydroxyethylapocupreine*	$1:5 \times 10^4$	Maclachlan *et al.* (18)
p-Chloroacetanilide salt of:		
Dihydroquinine	$1:12 \times 10^4$	Felton (25)
	$1:8 \times 10^4$	Morgenroth (86)
Optochin	$1:8 \times 10^4$	Morgenroth (86)
Hydroxyethylapocupreine	$1:1 \times 10^4$	Maclachlan *et al.* (18)

* *p*-Hydroxyacetanilide hydroxyethylapocupreinium chloride hydrochloride.

C. Nitrogen-oxide derivatives

In view of the theories associating bactericidal action with oxidation potential or intermediate oxidation products (105), the *N*-oxides of the cinchonas (formed on the quinuclidine nitrogen) should be of interest. However, the *N*-oxides show less power than the unmodified bases against the malarial parasite (91) or against pneumococci.[5]

D. Fission of the quinuclidine ring at the 1,8-position (cupreicine derivatives)

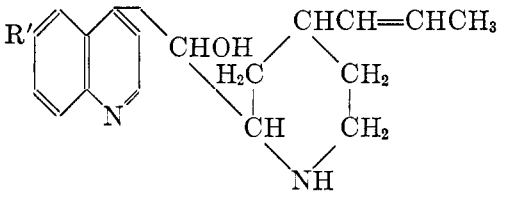


IV

[5] Unpublished work.

Hydrolysis of the 1,8-linkage of the quinuclidine ring leads to compounds of low bacteriostatic power and considerable toxicity (99). Neither reduction of the keto group to give an asymmetric secondary alcohol (42e), nor restoration of a tertiary character to the nitrogen by replacement of the hydrogen of the piperidyl NH group with an alkyl group (42d, 42e) produces active compounds. The introduction of the sulfanilyl radical on the piperidine nitrogen gave compounds without value against the pneumococcus (99).[6] In considering the significance of the 1,8-fission with regard to the correlation of structure and antipneumococcic action, it is important to remember that conversion of the quinuclidine to a piperidine ring is not invariably dystherapeutic, as shown by the antimalarial effectiveness of niquine and niquidine (31, 108b) and of 4′-(6′-methoxyquinolyl)-2-piperidylcarbinol (synthesized by Ainley and King (1)).

Fission of the 1,2-linkage (niquine, niquidine, cinchonhydrines): The fission of the 1,2-linkage to form niquine or niquidine has been formulated clearly only recently. As early as 1890 Skraup observed that when the vinyl side chain of quinine had been saturated by the addition of hydrogen iodide or hydrogen bromide, subsequent removal of the halogen acid gave "isoquinine" (formula II) and niquine (formula V). Léger (66) has studied these transformations especially in the cinchonidine–cinchonine series, where the niquine analogs are called cinchonhydrines; and very recently Gibbs and Henry (31) have shown that the formation of niquine or niquidine involves splitting of the 1,2-linkage with loss of a methylene group as formaldehyde. The resultant structure is shown in formula V.



V

Buttle *et al.* (14) report that niquidine has the highest antimalarial action of any of the *d*-compounds. Although niquine has no effective action against the pneumococcus, nothing is known as yet of the action of the stereoisomers or of the action of higher homologous ethers. Niquidine, like the cupreicines, has a piperidine ring, but the short carbinol bridge to the α-position of this ring is similar to the cinchona linkage.

V. DETAILED STUDY OF VARIATIONS IN THE ETHER GROUPING

Inasmuch as the most useful variations in the bacteriostatic action of the cinchona molecule have been associated with changes of R′ and R, it has seemed desirable to discuss these groups in some detail. Tables 2, 3, and 4 present

[6] Crystalline addition compounds of cinchona alkaloids with sulfanilamide were not more effective than the individual components (109).

some of the comparative evaluations of the action *in vitro* and of the toxicity of apocupreine ethers and certain related compounds. To minimize the effect of differences in the methods of obtaining biological data, the numerical values have been taken only from studies made by Maclachlan and his coworkers, but references to other investigators are included and, in general, the findings have been similar.

TABLE 2

Bacteriostasis against Pneumococcus II

COMPOUND*	FORMULA	CONCENTRATION OF DRUG	REFERENCE
δ-Hydroxy-*n*-butyl-	*n*-HOC_4H_8—		
β-Hydroxy-*n*-propyl-	$CH_3CHOHCH_2$—	1:0.5 to 2	
β-Keto-*n*-propyl-	CH_3COCH_2—	× 10^5	
β-Methyl-β-hydroxy-*n*-propyl-	$(CH_3)_2C(OH)CH_2$—		
Methyl-	CH_3—		
Butoxyethyl-	$C_4H_9OC_2H_4$—		(39)
8-Alkoxyquinolines			
β-Hydroxyethyl-	$HOCH_2CH_2$—		
β,β′-Dihydroxyisopropyl-	$(HOCH_2)_2CH$—	1:3 × 10^5	
Phenoxyethyl-	$C_6H_5OC_2H_4$—		
α-Methyl-β-hydroxy-*n*-propyl-	$CH_3CHOH(CH_3)CH$—		
α-Methylol-*n*-propyl-	$CH_3CH_2(CH_2OH)CH$—	1:4 × 10^5	
n-Butyl-	*n*-C_4H_9—		(14, 67)
Amyl- (four isomers)	C_5H_{11}—		(14, 52, 67, 81, 90)
8-Hydroxyquinoline			(11, 37; 111, p. 33)
Ethyl-	C_2H_5—		(13, 14, 22, 45, 52, 56, 72, 73, 76, 79, 80, 81, 89, 90)
β-Chloroethyl-	ClC_2H_4—		
γ-Hydroxy-*n*-propyl-	HOC_3H_6—		
sec-Butyl-	$CH_3CH_2(CH_3)CH$—	1:8 × 10^5	
n-Amyl-	*n*-C_5H_{11}—		(14, 67)
α-Methyl-β-hydroxyethyl-	$HOCH_2(CH_3)CH$—		
n-Propyl-	*n*-C_3H_7—		(14, 67)
Isopropyl-	$(CH_3)_2CH$—	1:16 × 10^5	(14, 52, 67)
Isobutyl-	$(CH_3)_2CHCH_2$—		(52)

* The compounds listed are ethers of apocupreine dihydrochloride except as indicated and are entered according to the maximum dilution effecting complete bacteriostasis *in vitro*.

Bacteriostasis (table 2)

Most of the compounds indicated in table 2 are apocupreine ethers. These compounds are listed according to bacteriostatic action *in vitro*. The dilution of the drug is given as 1 part in $n \times 10^5$. In the cinchona series the protective value of a compound seems roughly to parallel the antipneumococcic action

in vitro. Both experimental and clinical experience show that the bacteriostatic action is effective against all types of pneumococcus—with more variation with reference to different strains than to difference in type (6, 56).

It is apparent from table 2 that of the ethyl, propyl, butyl, and amyl ethers tested, the greatest antipneumococcic power is associated with 2-carbon and 3-carbon ethers. This corresponds with Morgenroth's finding the maximal action against the pneumococcus shown by ethyldihydrocupreine in the dihydrocupreine series. There is as yet no apparent correlation of effectiveness with the position at which branching occurs in the higher alkyl ether groupings. The apocupreine series thus far prepared is too brief to allow comparison with the antimalarial studies of Buttle *et al.* (14) and Magidson and Strukov (74), who observed a rise and fall of antimalarial action characterizing the *n*-alkyl ethers in separate series of odd- and even-numbered carbon chains. The higher alkyl apocupreine ethers have some action against streptococcus and staphylococcus (52, 81; also unpublished work), and it will be remembered that Morgenroth (85) found the highest bactericidal action for streptococcus in isoöctyldihydrocupreine (vuzin) and for staphylococcus in isoamyldihydrocupreine (eucupin). *In vitro* action of hydroxyethylapocupreine against streptococcus has been reported (113). Ishizaka (52) found isoöctylapocupreine only one-sixteenth as powerful as ethylapocupreine against the pneumococcus.

Modified alkyl ethers were prepared by Slotta and Behnisch (106), who alkylated dihydrocupreine with dialkylaminoalkyl halides to introduce a basic ether group similar to the side chain of plasmoquin and atabrin. Growth of pneumococci was inhibited for 8 hr. by γ-dimethylaminoisoamyldihydrocupreine at a concentration of 1:200,000 with partial inhibition for 24 hr.; other alkylaminoalkyl ethers were less effective. Alkoxyalkyl ethers[7] and aroxyalkyl ethers are represented in the table by butoxyethyl-, phenoxyethyl-, and benzyloxyethyl-apocupreines, which were not found effective against the pneumococcus, although other modifications of the alkyl ether were more successful. Halogen substitution in the alkyl group did not greatly alter the properties of the compound; this is still under investigation. There has not been enough study to allow generalizations with regard to varying the position of the hydroxyl or ketone group.

The study of hydroxyalkyl ethers was undertaken in an attempt so to modify cinchona derivatives that the antipneumococcic power would be retained but host toxicity lessened. When tested in the barbituric acid series, the replacement of alkyl groups by hydroxyalkyl groups had greatly lowered toxicity (21). It seemed a plausible speculation that modification of the hydrocarbon character of the ether groupings of cinchonas might alter the lipoid–water distribution in the direction of water solubility and in this manner avoid the tendency to eye damage which has prevented generalized use of optochin. That hydroxylation did lower animal toxicity is apparent from table 3, in the comparison of hydroxyalkyl ethers with the parent alkyl ethers. The absence of eye damage in

[7] The report of a study of alkylthioalkyl apocupreine ethers by Tipson and Cretcher is in press.

dogs receiving massive doses (22) and the complete absence of any eye symptoms in a large group of clinical cases treated with hydroxyethylapocupreine (71) are evidence of the definite modification in physiological properties which may accompany a small change in chemical structure. That this change also brought somewhat lowered antipneumococcic action is evident in the data of table 2.

Toxicity (tables 3 and 4)

The toxicity data given in table 3 are for intraperitoneal tests. The doses reported as L.D. 50 are milligrams of drug lethal to 50 per cent of the animals (20-g. mice). As indicated for hydroxyethylapocupreine, the L.D. 50 on an oral basis may be six times as large as the intraperitoneal value. It will be noted that the higher homologous alkyl ethers (butyl and amyl) show greater toxicity. The effect of hydroxylation on toxicity and action *in vitro* is summarized in table 4 for the ethyl and isopropyl ethers of apocupreine, to emphasize the remarkable changes in selectivity brought about by slight changes in a large cinchona molecule.

Animal protection

Ethylhydrocupreine, ethylapocupreine, hydroxyethylhydrocupreine, and hydroxyethylapocupreine have protective power when tested in experimental mouse pneumonias (56, 73). β-Isoquinine gave some protection *in vivo* (39), although considerably less than hydroxyethylapocupreine.[8] The action of ethylapocupreine in mouse protection has been reported by various investigators (39, 56, 73, 76, 80, 89); Liebetruth (67) found power *in vivo* noticeably decreased in the higher *n*-alkyl apocupreine ethers. In 1940 Bracken (6) found comparable protection against virulent pneumococci with hydroxyethylapocupreine or sulfapyridine in equal doses; in mouse protection experiments the use of either chemical enhanced the protective action of the other.

Pharmacology

The pharmacology of the cinchonas has been reviewed in considerable detail by von Oettingen (111) and other authors (27, 50, 63, 65, 87, 107). Kruse (64, 69) found that hydroxyethylapocupreine is less toxic to the circulation and respiration of the dog than are quinine or quinidine. Dawson *et al.* (22) reported the ophthalmoscopic examination and histologic study of the eyes of dogs subcutaneously injected with large doses of cinchona ethers. They observed that in massive doses quinine and the alkyl ethers caused a destruction of cells in the ganglionic layer; comparable dosage with hydroxyethylapocupreine caused no demonstrable damage.

Clinical studies

Interesting observations have been made in the course of the clinical study of apocupreine, ethylapocupreine, and hydroxyethylapocupreine. Although in some tests apocupreine had shown bacteriostasis *in vitro* and protective action

[8] Unpublished work.

for mice, it has no therapeutic action in clinical trial. Ethylapocupreine was not acceptable clinically because of temporary eye damage to some individuals;

TABLE 3

Intraperitoneal toxicity

COMPOUND*	APPROXIMATE L.D. 50†
	milligrams
n-C_3H_7—	2–3
C_4H_9— (three isomers)	2–3
C_5H_{11}— (five isomers)	2–3
Ethylapocupreicine	2–3
8-Hydroxyquinoline	2–3
$C_6H_5OC_2H_4$—	2–3
Ethylhydrocupreine	4–5
ClC_2H_4—	4–5
$(CH_3)_2CH$—	4–5
C_2H_5—	4–5
Quinine	5
$(CH_3)_2C(OH)CH_2$—	5+
$CH_3CHOH(CH_3)CH$—	5+
n-HOC_4H_8—	5+
n-HOC_3H_6—	6–8
$CH_3CH_2(CH_2OH)CH$—	6–8
CH_3COCH_2—	6–8
$HOCH_2CH_2$—‡	6–8
$HOCH_2(CH_3)CH$—	6–8
$CH_3CHOHCH_2$—	6–8

* Ethers of apocupreine dihydrochloride except as indicated.

† Dose in milligrams for a 20-g. mouse.

‡ Oral toxicity: L.D. 50 ≈ 40 mg.

TABLE 4

Effect of hydroxylation on toxicity and action in vitro

ETHERS OF APOCUPREINE	TOXICITY APPROX. L.D. 50	BACTERIOSTASIS CONCENTRATION OF DRUG
	milligrams	
C_2H_5—	4	$1:8 \times 10^5$
HOC_2H_4—	7	$1:3 \times 10^5$
$(CH_3)_2CH$—	4	$1:16 \times 10^5$
$HOCH_2CH(CH_3)$—	7	$1:16 \times 10^5$
$(HOCH_2)_2CH$—	9	$1:3 \times 10^5$

hence other derivatives were sought. The clinical use of hydroxyethylapocupreine in over six hundred cases of pneumococcic pneumonia with good results has been reported by Maclachlan (71) and his associates. The cases included

many types of pneumococcus (6). The use of the drug does not interfere with the typing of the pneumococcus and no visual disturbance has been observed with any patient; there is "no evidence that the leucocytes are depressed, and ear symptoms do not occur." In direct topical application, hydroxyethylapocupreine monohydrochloride has been found highly effective in treating pneumococcic conjunctivitis (69) and very satisfactory results have been obtained in routine use of an aqueous solution of the dihydrochloride in treating pharyngitis.[9]

VI. SYNTHETIC ANALOGS

In developing methods for the synthesis of aza-bicyclic compounds, Prelog (95) has synthesized the simple quinuclidine. Clemo and Metcalfe (19a) synthesized 3-ketoquinuclidine. No biological testing of these simple units has been reported. Clemo and Hoggarth (19b) found 7-ketoruban to be without antimalarial action, but this compound lacks the secondary alcohol group and the methoxyl substituent in the quinoline nucleus. Both Rabe (98) and Prelog (96) have recently synthesized isomeric 6′-methoxyruban-9-ols, but Rabe reports absence of antimalarial action in any of the four isomers, whereas Prelog reports power similar to quinine in the treatment of bird malaria with the 6′-methoxyrubanol.

That cyclic units other than quinuclidine may serve as the "second half" of cinchona analogs possessing therapeutic properties is apparent from the properties of niquidine (formula V), which is a substituted quinolyl-2-piperidylcarbinol (31, 108b). Ainley and King (1) found that the synthetic 4′-(6′-methoxyquinolyl)-2-piperidylcarbinol had considerable antimalarial action in bird malaria and suggest "that the piperidine ring system should be as near the quinoline ring system as possible, consistent with retention of the carbinol group." The effectiveness of these α-piperidine derivatives is in sharp contrast to the absence of antimalarial power in cupreicine ethers (formula IV). In 1917 Karrer (60) synthesized 4′-(6′-methoxyquinolyl)-2-pyrrylcarbinol and described it as having action of the same order as quinine on paramecia. In studying even simpler compounds which had an ethanol bridge linking 6′-methoxyquinoline with tertiary amines, King and Work (61) found that these structures had small antimalarial power.

CONCLUSION

Thus far the alkoxyquinoline and alkyl-substituted quinuclidine nuclei, bound together by a secondary alcohol bridge, appear to be a necessary basis for antipneumococcic activity in the cinchona series. Maximum activity seems to be shown by the ethyl and isopropyl alkyl ethers. Some types of substitution in the alkyl ether group modify, without wholly changing, the bactericidal and physiological properties. The quinuclidine side chain may be ethyl (e.g., of hydrocupreine) or ethylidene (e.g., of apocupreine); greater antipneumococcic action appears to be associated with the ethylidene group.

[9] Unpublished work.

The steric configuration of the molecule is a factor, and it is probable that linkage at the α-carbon atom of the quinuclidine nucleus is important.

The fact that certain recently prepared cinchona analogs possess antimalarial action suggests that the quinuclidine nucleus may be replaced by other cyclic nitrogen components. The short secondary alcohol bridge between the two nitrogen units and the spatial arrangement of the linkage at the α-carbon atom of the non-aromatic nitrogen group appear to be important factors in the properties of these compounds.

The authors desire to express their appreciation to Dr. R. Stuart Tipson for helpful suggestions and criticism in the preparation of this manuscript.

REFERENCES

(1) AINLEY, A. D., AND KING, H.: Proc. Roy. Soc. (London) **B125,** 60 (1938).
(2) ALTMAN, R. F. A.: Rec. trav. chim. **57,** 941 (1938).
(3) BERKENHEIM, A. M.: J. Gen. Chem. (U.S.S.R.) **6,** 1039 (1936); Chem. Abstracts **31,** 1779 (1937).
(4) BÜHRMANN, I.: Z. Immunitäts. **84,** 300 (1935).
(5) BOYD, G. H.: Am. J. Hyg. **6,** 173 (1926).
(6) BRACKEN, M. M., JOHNSTON, J. M., CRUM, G. E., PATRICK, D. R., PERMAR, H. H., AND MACLACHLAN, W. W. G.: J. Pharmacol. **68,** 259 (1940).
(7) BUTLER, C. L., AND CRETCHER, L. H.: J. Am. Pharm. Assoc. **22,** 414 (1933).
(8) BUTLER, C. L., AND CRETCHER, L. H.: J. Am. Chem. Soc. **57,** 1083 (1935).
(9) BUTLER, C. L., HOSTLER, M., AND CRETCHER, L. H.: J. Am. Chem. Soc. **59,** 2354 (1937).
(10) BUTLER, C. L., NELSON, W. L., RENFREW, A. G., AND CRETCHER, L. H.: J. Am. Chem. Soc. **57,** 575 (1935).
(11) BUTLER, C. L., AND RENFREW, A. G.: J. Am. Chem. Soc. **60,** 1473, 1582 (1938).
(12) BUTLER, C. L., RENFREW, A. G., AND CLAPP, M. A.: J. Am. Chem. Soc. **60,** 1472 (1938).
(13) BUTLER, C. L., RENFREW, A. G., CRETCHER, L. H., AND SOUTHER, B. L.: J. Am. Chem. Soc. **59,** 227 (1937).
(14) BUTTLE, G. A. H., HENRY, I. A., SOLOMON, W., TREVAN, J. W., AND GIBBS, E. M.: Biochem. J. **32,** 47 (1938).
(15) BUTTLE, G. A. H., HENRY, T. A., AND TREVAN, J. W.: Biochem. J. **28,** 426 (1934).
(16) BUTTLE, G. A. H., GRAY, W. H., AND STEPHENSON, D.: Lancet **230,** 1286 (1936).
(17) CHOPRA, R. N., AND CHOUDHURY, S. G.: Indian J. Med. Research **17,** 360 (1929); reference 111, pp. 142, 210.
(18) CLAPP, M. A., RENFREW, A. G., AND CRETCHER, L. H.: J. Am. Chem. Soc. **63,** 2169 (1941).
(19) (a) CLEMO, G. R., AND METCALFE, T. P.: J. Chem. Soc. **1937,** 1989.
(b) CLEMO, G. R., AND HOGGARTH, E.: J. Chem. Soc. **1939,** 1241; **1941,** 476.
(20) COHEN, A., AND KING, H.: Proc. Roy. Soc. (London) **B125,** 49 (1938).
(21) CRETCHER, L. H., KOCH, J. A., AND PITTENGER, W. H.: J. Am. Chem. Soc. **47,** 3083 (1925).
CRETCHER, L. H., AND PITTENGER, W. H.: J. Am. Chem. Soc. **46,** 1503 (1924); **47,** 2560 (1925).
(22) DAWSON, W. T., PERMAR, H. H., JOHNSTON, J. M., AND MACLACHLAN, W. W. G.: Am. J. Med. Sci. **193,** 543 (1937).
(23) DAWSON, W. T., AND GARBADE, F. A.: J. Am. Med. Assoc. **94,** 704 (1930).
DAWSON, W. T., AND NEWMAN, S. P.: J. Am. Med. Assoc. **97,** 930 (1931).
(24) DOMAGK, G.: Angew. Chem. **48,** 657 (1935).

(25) FELTON, L. D., AND DOUGHERTY, K. M.: J. Exptl. Med. **35**, 761 (1922); **36**, 163 (1922).
(26) FINDLAY, G. M.: *Recent Advances in Chemotherapy*. The Blakiston Company, Philadelphia, Pennsylvania (1939).
(27) FISCHL, V., AND SCHLOSSBERGER, H.: *Handbook of Chemotherapy*, English translation. H. G. Roebuck and Son, Baltimore (1933).
(28) FRÄNKEL, S., TRITT, C., MEHRER, M., AND HERSCHMANN, O.: Ber. **58**, 544 (1925).
(29) GANAPATHI, K.: Indian J. Med. Research **27**, 947 (1940).
(30) GHOSH, S., AND CHATTERJEE, W. R.: J. Indian Chem. Soc. **8**, 257 (1931).
(31) GIBBS, E. M., AND HENRY, T. A.: J. Chem. Soc. **1939**, 240, 1294.
(32) GIEMSA, G., AND HALBERKANN, J.: Ber. **52**, 906 (1919).
(33) GIEMSA, G., AND OESTERLIN, M.: Arch. Schiffs-Tropen-Hyg. **37**, 217 (1933).
(34) GOODMAN, L., AND GILMAN, A.: *The Pharmacological Basis of Therapeutics*. The Macmillan Company, New York (1941).
(35) GOODSON, J. A.: J. Chem. Soc. **1935**, 1094.
(36) GOODSON, J. A., HENRY, T. A., AND MACFIE, J. W. S.: Biochem. J. **24**, 874 (1930).
(37) GRAY, W. H.: J. Chem. Soc. **1939**, 1202.
(38) GREEN, M. H., RENFREW, A. G., AND BUTLER, C. L.: J. Am. Chem. Soc. **61**, 1783 (1939).
(39) GUNDEL, M., AND SEITZ, L.: Z. Immunitäts. **80**, 240 (1933).
(40) HEFFRON, R.: *Pneumonia*. The Commonwealth Fund, New York (1939).
(41) HEGNER, R., WEST, E., RAY, M., AND DOBLER, M.: Am. J. Hyg. **33**, 101 (1941).
HEGNER, R., WEST, E., AND DOBLER, M.: Am. J. Hyg. **34**, 132 (1941).
(42) HEIDELBERGER, M., AND JACOBS, W. A.: J. Am. Chem. Soc. (a) **41**, 817 (1919); (b) **41**, 2131 (1919); (c) **42**, 1489 (1920); (d) **44**, 1091 (1922); (e) **44**, 1098 (1922).
(43) HENRY, T. A.: *Plant Alkaloids*, 3rd edition. The Blakiston Company, Philadelphia, Pennsylvania (1939).
(44) HENRY, T. A.: Chemistry & Industry **55**, 111 (1936).
(45) HENRY, T. A., AND SOLOMON, W.: J. Chem. Soc. **1934**, 1923.
(46) HENRY, T. A., AND SOLOMON, W.: Chemistry & Industry **54**, 641 (1935).
(47) HENRY, T. A., SOLOMON, W., AND GIBBS, E. M.: J. Chem. Soc. **1935**, 966.
(48) HENRY, T. A., SOLOMON, W., AND GIBBS, E. M.: J. Chem. Soc. **1937**, 592.
(49) HESSE, O.: Ann. **205**, 314 (1880).
(50) HOUBEN, J.: *Fortschritte der Heilstoffchemie*, zweite Abteilung, Band III, Houben und Pfankuch, "Die Heterocyklischen Verbindungen," pp. 744–1033. Walter de Gruyter, Berlin (1939).
(51) IENSCH, H.: Angew. Chem. **50**, 891 (1937).
(52) ISHIZAKA, N., OKAMOTO, H., MIURA, K., MATSUDA, S., AND SHAKO, T.: Japan J. Med. Sci. IV. Pharmacol., Proc. **7**, 42 (1933).
(53) JACOBS, W. A.: *The Harvey Lectures*, 1923–24. J. B. Lippincott, Philadelphia, Pennsylvania.
(54) JACOBS, W. A., AND HEIDELBERGER, M.: J. Am. Chem. Soc. (a) **41**, 2090 (1919); (b) **42**, 1481 (1920); (c) **42**, 2278 (1920); (d) **44**, 1073 (1922); (e) **44**, 1079 (1922).
(55) JARZÝNSKI, L., LUDWICZAKÓWNA, R., AND SUSZKO, J.: Rec. trav. chim. **52**, 839 (1933).
(56) JOHNSTON, J. M., BURCHELL, H. B., PERMAR, H. H., AND MACLACHLAN, W. W. G.: J. Pharmacol. **61**, 364 (1937).
(57) JOHNSON, F. F.: J. Am. Pharm. Assoc. **26**, 1227 (1937).
(58) JUNEJA, G. L., NARANG, K. S., AND RAY, J. N.: J. Indian Chem. Soc. **17**, 495 (1940).
(59) JUNGFLEISCH, E., AND LÉGER, E.: Ann. chim. [9] **14**, 59 (1920).
(60) KARRER, P.: Ber. **50**, 1499 (1917).
(61) KING, H., AND WORK, T. S.: J. Chem. Soc. **1940**, 1307.
(62) KOHLTOFF, J. M.: Biochem. Z. **162**, 289 (1925).
(63) KOLMER, J. A.: *Principles and Practice of Chemotherapy*. W. B. Saunders, Philadelphia, Pennsylvania (1926).
(64) KRUSE, K. T.: J. Pharmacol. **66**, 20 (1939).

(65) LAQUEUR, E.: *Die neuren chemotherapeutischen Präparate aus der Chininreihe und aus der Akridinreihe.* Julius Springer, Berlin (1923).
(66) LÉGER, E.: Bull. soc. chim. [4] **23,** 133, 142, 240, 328 (1918).
LÉGER, E.: J. pharm. chim. [8] **23,** 558 (1936).
(67) LIEBETRUTH, E.: Z. Immunitäts. **84,** 445 (1935).
(68) MACLACHLAN, W. W. G.: International Clinics IV, 127 (1937); Series 47.
(69) MACLACHLAN, W. W. G.: Penn. Med. J., February, 1940.
(70) MACLACHLAN, W. W. G., JOHNSTON, J. M., BRACKEN, M. M., AND CRUM, G. E.: Am. J. Med. Sci. **197,** 31 (1939).
(71) MACLACHLAN, W. W. G., JOHNSTON, J. M., BRACKEN, M. M., AND PIERCE, L. S.: Am. J. Med. Sci. **201,** 367 (1941).
(72) MACLACHLAN, W. W. G., PERMAR, H. H., JOHNSTON, J. M., AND BURCHELL, H. B.: Am. J. Med. Sci. **194,** 474 (1937).
(73) MACLACHLAN, W. W. G., PERMAR, H. H., JOHNSTON, J. M., AND KENNEY, J. R.: Am. J. Med. Sci. **188,** 699 (1934).
(74) MAGIDSON, O. YU., AND STRUKOV, I. T.: Arch. Pharm. **271,** 359 569 (1933).
(75) MARSHALL, E. K.: Bull. N. Y. Acad. Med. **16,** 723 (1940).
(76) MATSUDA, S.: Japan J. Med. Sci. IV. Pharmacol., Proc. **7,** 45 (1933); Proc. **8,** 30 (1934).
(77) MAY, P., AND DYSON, G. M.: *The Chemistry of Synthetic Drugs.* Longmans, Green and Company, London (1939).
(78) MICHAELIS, L., AND DERNBY, K. G.: Z. Immunitäts. **34,** 194 (1922).
(79) MIURA, K.: Japan J. Med. Sci. IV. Pharmacol. **11** (Trans. of the 12th Annual Meeting), 150 (1938).
(80) MIURA, K.: Japan J. Med. Sci. IV. Pharmacol. **12,** 209 (1940).
(81) MIURA, K., AND OKAMOTO, H.: Japan J. Med. Sci. IV. Pharmacol., Proc. **5,** 1 (1930).
(82) MIURA, K., AND SOGEN, Y.: Japan. J. Med. Sci. IV. Pharmacol., Proc. **5,** 41 (1931).
(83) MORGENROTH, J., AND BUMKE, E.: Deut. med. Wochschr. (a) **40,** 538 (1914); (b) **44,** 729 (1918).
(84) (a) MORGENROTH, J., AND LEVY, R.: Berlin klin. Wochschr. **48,** 1560, 1979 (1911).
(b) MORGENROTH, J.: Berlin. klin. Wochschr. **54,** 55 (1917).
(85) MORGENROTH, J., AND TUGENDREICH, J.: Berlin. klin. Wochschr. **53,** 794 (1916); Biochem. Z. **79,** 257 (1917).
(86) MORGENROTH, J., AND SCHNITZER, R.: Z. Hyg. Infektionskrankh. **103,** 441 (1924); reference 50, p. 1019.
(87) MOORE, H. F., AND CHESNEY, A. M.: Arch. Internal Med. **19,** 611 (1917); **21,** 659 (1918).
(88) NORTHEY, E. H.: Chem. Rev. **27,** 85 (1940).
(89) OKAMOTO, H.: Japan. J. Med. Sci. IV. Pharmacol., Proc. **5,** 36 (1931).
(90) OKAMOTO, H., AND SOGEN, Y.: Japan. J. Med. Sci. IV. Pharmacol., Proc. **5,** 4 (1930).
(91) OESTERLIN, M.: Klin. Wochschr. **15,** 957 (1936); Squibb Abstract Bull. **9,** 1855 (1936).
(92) OESTERLIN, M.: Z. Hyg. Infektionskrankh. **118,** 263 (1936); Chem. Abstracts **30,** 7209 (1936).
(93) OSTROMISLENSKY, I. I.: *The Scientific Basis of Chemotherapy.* Inter-American Medical Publishing Company, New York (1926).
(94) PATTERSON, A. M.: J. Am. Chem. Soc. **47,** 543 (1925).
(95) PRELOG, V.: Ann. **532,** 69 (1937).
(96) PRELOG, V., SEIWERTH, R., HEIMBACH-JUHASZ, S., AND STERN, P.: Ber. **74,** 647 (1941).
(97) RABE, P.: Ber. **55,** 522 (1922); Ann. **492,** 242 (1932).
(98) RABE, P., AND HAGEN, G.: Ber. **74,** 636 (1941).
(99) RENFREW, A. G., AND BUTLER, C. L.: J. Am. Chem. Soc. **62,** 3304 (1940).
(100) RENFREW, A. G., AND CRETCHER, L. H.: J. Am. Chem. Soc. **57,** 738 (1935).

(101) REYMAN, J., AND SUSZKO, J.: Bull. Acad. Polonaise **1935A,** 360.
(102) ROSENMUND, K. W., AND KITTLER, C.: Arch. Pharm. **262,** 18 (1924).
(103) SANDERS, J. P.: J. Am. Med. Assoc. **97,** 850 (1931).
SANDERS, J. P., AND DAWSON, W. T.: J. Am. Med. Assoc. **99,** 1773 (1932).
(104) SCHMIDT, L. H., AND SESLER, C. L.: J. Pharmacol. **72,** 311 (1941).
(105) SHAFFER, P. A.: Science **89,** 547 (1939).
(106) SLOTTA, K. H., AND BEHNISCH, R.: Ber. **68,** 754 (1935).
(107) SOLIS-COHEN, S., AND GITHENS, T. S.: *Pharmacotherapeutics.* D. Appleton, New York (1928).
(108) SOLOMON, W.: (a) J. Chem. Soc. **1938,** 6; (b) J. Chem. Soc. **1941,** 77.
(109) STUART, E. H., POWELL, H. M., ROSE, C. L., AND BIBBINS, F. E.: J. Am. Pharm. Assoc. **28,** 90 (1939).
(110) SUSZKO, J., *et al.:* Chem. Abstracts **24,** (1930) and ff.
(111) VON OETTINGEN, W. F.: *The Therapeutic Agents of the Quinoline Group.* The Chemical Catalog Company, New York (1933).
(112) WHITE, B.: *Biology of the Pneumococcus.* The Commonwealth Fund, New York (1938).
(113) WHITE, H. J., BRATTON, A. C., LITCHFIELD, J. T., AND MARSHALL, E. K.: J. Pharmacol. **72,** 112 (1941).
(114) WORK, T. S.: J. Chem. Soc. **1940,** 1315.
(115) ZETZSCHE, F., AND FREDRICH, A.: Ber. **73,** 1420 (1940).