

# SOME RECENT RESULTS ON THE CHEMOTHERAPY OF AMEBIASIS, COCCIDIOSIS, AND MALARIA

A. BROSSI

*Chemical Research Department, Hoffmann-La Roche Inc.,  
Nutley, N.J., U.S.A.*

## INTRODUCTION

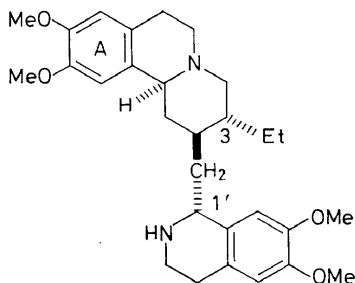
The biology of these three diseases and the pharmacology and clinical aspects of the so-called classical drugs used for their treatment have been adequately covered by excellent review articles. Consequently, the following discussion will emphasize some of the newer findings. Since coccidiosis is included in this review, the importance of this disease in domestic animals is demonstrated and will, therefore, be discussed.

## AMEBIASIS

Amebiasis is an acute or chronic, enteric disease caused by the protozoan, *Endamoeba histolytica*. An excellent review article by Woolfe<sup>1</sup> covers its chemotherapy up to 1965. The following discussion will update this review with special emphasis given to chemical aspects.

### Emetine and related compounds

The powdered root of Ipecacuanha has long been used for the treatment of amebic dysentery. Not until 1912 was it discovered that the antiamebic activity was primarily due to one of its major constituents, the alkaloid emetine. As a consequence of the establishment of the absolute configuration of natural or (-)-emetine (I) and its total synthesis, many derivatives

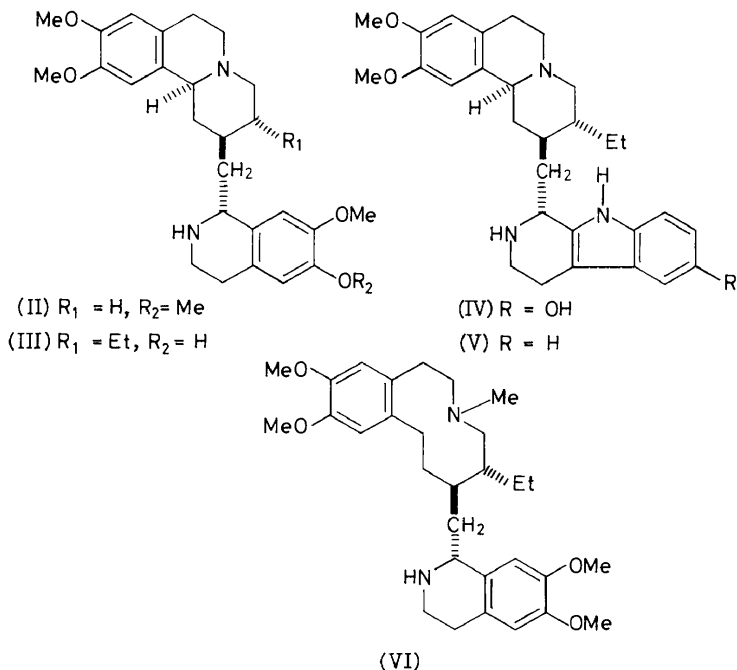


(I)

Natural or (-) - Emetine

became available for testing. Certain structure-activity relationships made during these studies have been summarized<sup>1</sup>. A few interesting points are worthy of discussion.

*Importance of the substitution at C-3.* It has been reported that ( $\pm$ )-3-desethyl-emetine (II), prepared in various laboratories, has practically no activity *in vivo*<sup>2</sup>. Various racemic emetine derivatives with alkyl substituents at the 3-position, other than an ethyl group, prepared in our laboratories were also found to be of no interest. The configuration of these racemic compounds, however, was never established with certainty, and the conclusion that the



The structures shown represent one of the two enantiomers. All compounds with the exception of (II) and (VI) are natural products.

alkyl group at the 3-position must be ethyl is, therefore, not proven. The synthesis of racemic (II) with the correct relative configuration at the three asymmetric centres has recently been achieved at Burroughs Wellcome & Co. in England. It has been shown that this compound exerts modest antiamebic activity *in vivo*<sup>3</sup>. The finding, that the nature of the substituent at the 3-position is not as critical as had originally been supposed, is also supported by the fact that ( $\pm$ )-3-nordehydroemetine, a compound to be discussed later, is an active ameicide *in vivo*.

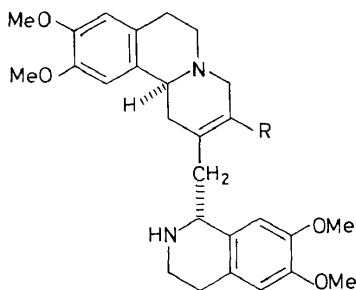
*Other changes in the emetine molecule.* Other changes, which are permissible without considerable loss of antiamebic activity, are variations in the substitution pattern in the aromatic portion of the isoquinoline moiety or replacement of the isoquinoline system by a carboline system. Cephaeline (III), a minor Ipecac alkaloid, tubulosine (IV)<sup>4</sup> and deoxytubulosine (V)<sup>5</sup>

are amebicides<sup>6</sup>. It must be emphasized that the absolute configuration of these alkaloids is identical with that of natural emetine (I). It can also be anticipated that further changes, such as replacing one of the methoxy groups in ring A of emetine by a hydroxy group or replacing the tricyclic benzoquinolizidine moiety in emetine by an indoloquinolizidine moiety, could yield amebicides<sup>7</sup>.

An interesting modification is the *N*-methyl-1',2'-secoemetine (VI) prepared from natural emetine in the Wyeth laboratories<sup>8</sup>. The *N*-acetyl derivative of this compound, tested unfortunately *in vitro* only, seemed to be less active than emetine.

### (±)-2,3-Dehydroemetine and (±)-3-nor-2,3-dehydroemetine

Another compound found during studies carried out in our laboratories, which warranted extensive investigation and which has now been successfully introduced into the market, is (±)-2,3-dehydroemetine (VII). The discovery of this compound was actually the result of an unsuccessful



(VII) R = Et

(VIII) R = Me

The compounds discussed are racemates. The structures shown represent one of the two enantiomers.

attempt to reduce an unsaturated intermediate. When the final compound, which had an additional double bond at the 2,3-position, was tested *in vivo*, it showed activity similar to emetine, but seemed to be less toxic. Extensive clinical investigations initiated by Herrero<sup>9</sup> and Blanc<sup>10</sup> and later extended world-wide<sup>1</sup>, showed it to be equal to emetine as an amebicide. It seemed, furthermore, to be better tolerated and faster excreted than emetine<sup>11</sup>. The nor-compound (VIII) prepared later was tolerated even better than (VII) but was also less active and, therefore, seemed to offer no advantage as an amebicide.

### Mode of action of emetine and (±)-2,3-dehydroemetine

It is of interest to note that Grollman, who studied the mechanism of action of emetine and related compounds, found that all the active

compounds, including (VII), inhibit protein synthesis in certain mammalian and other cells<sup>12,13</sup>. This provides valuable information, which may account for the therapeutic and toxic properties of these substances. Since Grollman's results quite accurately parallel the *in vivo* screening results reported by various investigators, his *in vitro* test might be a valuable additional tool for the evaluation of compounds structurally related to emetine.

### Technical synthesis of (–)-emetine and (±)-2,3-dehydroemetine

The total synthesis of (–)-emetine (I), identical with natural emetine, is now being performed on a large scale at Burroughs Wellcome & Co. in England. (±)-2,3-Dehydroemetine (VII) is presently being manufactured at Hoffmann-La Roche in Switzerland. The synthesis of any molecule as complex as emetine is a remarkable achievement. Emetine can now be made at any time and in any quantity. Furthermore, material produced by total synthesis has the advantage of being superior in quality, since the alkaloid from natural sources is always contaminated with impurities which are difficult to remove. Since (±)-2,3-dehydroemetine has two asymmetric centres less than (–)-emetine (I) and an optical resolution is not involved in its production, it is more readily available than synthetic (–)-emetine.

### Clinical application of emetine and (±)-2,3-dehydroemetine

For treatment of severe acute amebic intestinal or extra-intestinal infections emetine (I), possibly replaceable by (±)-2,3-dehydroemetine (VII) which is more readily available, is still the drug of choice. It is recommended that its use be followed by the administration of other drugs which are more effective against the subacute disease<sup>1</sup>.

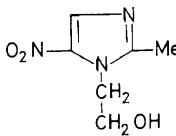
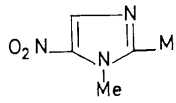
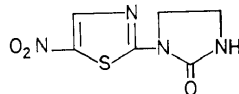
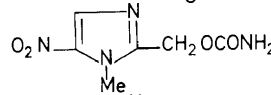
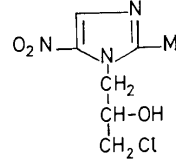
### Nitroheterocyclic compounds

Various investigators have detected high antiprotozoal activity among nitro-substituted heterocyclic compounds. Examples (given in *Table I*) are the trichomonacide metronidazole (IX)<sup>14</sup>, the orally effective schistosomacide niridazole (XI)<sup>15</sup>, and the highly effective histomonastat dimetridazole (X)<sup>14</sup>. Recent clinical studies have shown that both (IX)<sup>16</sup> and (XI)<sup>17</sup> are also effective in the treatment of amebic dysentery and amebic liver abscesses. These findings have stimulated others working in this area. The Merck nitroimidazole (XII), for example, which has been reported to be a coccidiostat and a histomonastat<sup>18</sup>, is also an effective amebicide. The Roche compound (XIII, Ro 7-0207), a highly active amebicide is presently being prepared for extensive clinical studies. LD<sub>50</sub> and CD<sub>50</sub> values for these compounds in comparison with (±)-2,3-dehydroemetine dihydrochloride (VII. 2HCl), are given in *Table I*<sup>19</sup>.

Extensive clinical studies with the above-mentioned nitroheterocycles will show whether these drugs approach the criteria set for a so-called ideal amebicide—one which, when given orally in completely non-toxic doses, would eradicate amoeba from the gut, lumen, and tissues.

GHEMOTHERAPY OF AMEBIASIS, COCCIDIOSIS, AND MALARIA

Table 1. Antiprotozoal activity among nitro-substituted heterocyclic compounds

Compound	LD <sub>50</sub> (mg/kg)			CD <sub>50</sub> (mg/kg po) <i>E. histolytica</i>	
	po	ip	sc	Intracecal	Hepatic
(IX) 	>4000	>4000	>4000	49	6.4
(X) 	>500	>500	>500	22	8.8
(XI) 	707	—	—	1.3	29
(XII) 	>2000	1320	—	7.7	16
(XIII) 	>2000	>2000	>2000	10	21
(VII).2HCl	71	35	35	2.3-6.5	3.5

COCCIDIOSIS

Coccidia are parasitic protozoa which infect vertebrates and invertebrates. Coccidiosis of chickens is practically universal and must be considered to be a serious economic threat to the poultry industry with its annual output of 2.8 billion broilers, 500 million layers and 120 million turkeys in the U.S.A. alone.

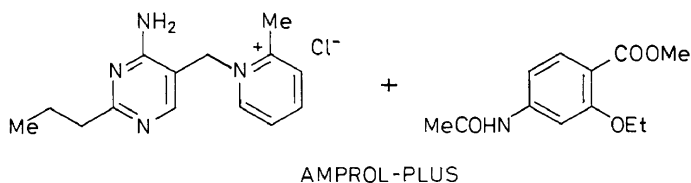
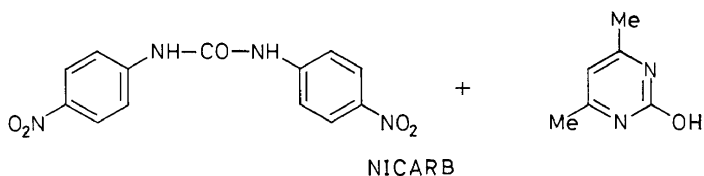
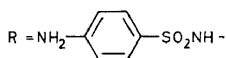
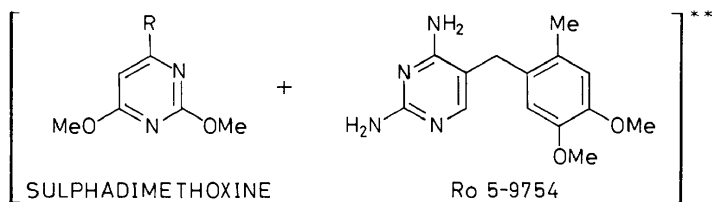
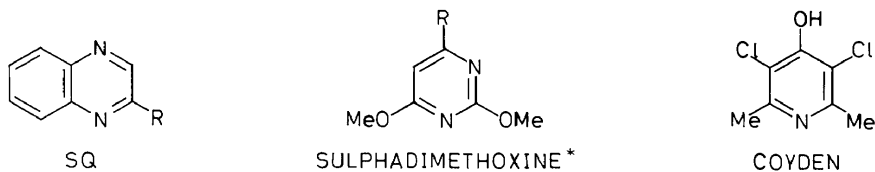
Mortality of chickens is caused mainly by infections with *Eimeria tenella*, although *E. acervulina*, *E. necatrix*, *E. maxima* and *E. brunetti* contribute. Turkeys are susceptible primarily to *Eimeria gallopavonis*, *E. meleagritidis* and *E. adenoides*.

A variety of agents have been used for the prevention and treatment of coccidiosis in poultry. In the following discussion, those coccidiostats currently in use or now being developed will be mentioned. More detailed reviews may be found in the literature<sup>20,21</sup>.

Prevention

Avian coccidiosis can be prevented by effective chemicals, called coccidiostats, administered via feed or drinking water. Sulphaquinoxaline (SQ) was

one of the first known poultry coccidiostats, and its success stimulated the development of new and better-tolerated agents. During the past two decades, many new coccidiostats have been introduced. Most of these are single chemical entities, while others are combinations of up to four different substances. The chemical structures of some of the more interesting representatives are shown below.



\* Sulphadimethoxine is the active ingredient in AGRIBON®.

\*\* Sulphadimethoxine and Ro 5-9754 are the active ingredients in ROFENAID™.

The anticoccidial activity of sulpha drugs is antagonized by *p*-aminobenzoic acid, indicating that the sulpha drugs interfere with the *p*-aminobenzoic acid-folic acid metabolic sequence. 2,4-Diaminopyrimidines also interfere with this sequence, but at a different stage; thus, 2,4-diaminopyrimidines synergize or potentiate the anticoccidial activity of sulpha drugs. This property is the basis for the development of combinations such as sulphadimethoxine and Ro 5-9754. This synergism of sulpha drugs by

2,4-diaminopyrimidines reduces the effective anticoccidial concentration of the pure sulpha drug. Data pertinent to several coccidiostats commercially available in the U.S.A. as well as others currently being developed are given in *Table 3*. The data compiled therein are those released by the manufacturers, reported in the literature or obtained in our own laboratory<sup>22</sup>. Only SQ, NICARB, CO-BAN, Ro 2-2985, the combination of sulphadimethoxine with Ro 5-9754, and STATYL are fully effective against five pathogenic species of *Eimeria* in chickens. In turkeys, only SQ, AMPROL, the combination of sulphadimethoxine with Ro 5-9754, and Ro 2-2985, evaluated thus far, appear to be fully effective against three pathogenic species of *Eimeria*.

*Table 3* also shows that the combination of sulphadimethoxine with Ro 5-9754, at the suggested coccidiostatic use levels, exerts in addition, high antibacterial activity against four major bacterial diseases in poultry, namely fowl cholera, infectious coryza, colibacillosis-CRD and salmonellosis.

### Therapy

In spite of successful prophylactic coccidiosis control by extensive and continuous use of coccidiostats in the feed, sporadic outbreaks of coccidiosis do occur in flocks of poultry. Such outbreaks are best controlled by the administration of selected drugs in drinking water for short time intervals. This therapeutic concept of avian coccidiosis control introduced by Levine<sup>23</sup> led to the commercialization of several agents over the past two decades, most of which are sulpha drugs. Renewed interest in the use of sulpha drugs has developed since the discovery of the so-called long-acting sulpha drugs. The anticoccidial and antibacterial activity of sulphadimethoxine, the active ingredient in the product, recently introduced under the trademark AGRIBON<sup>®</sup>, for therapeutic coccidiosis control is a result of this trend<sup>24,25</sup>. A good therapeutic response can be expected from AGRIBON<sup>®</sup>, since it is well tolerated, possesses good tissue retention, and has a relatively low excretion rate.

*Table 2.* Comparative anticoccidial and antibacterial efficacy of the commercially most established coccidiosis therapeutics versus those newly developed at manufacturers' suggested dosages

Product	Suggested use levels (% active in drinking water)	Anticoccidial activity*		Antibacterial activity†	
		Chickens	Turkeys	Chickens	Turkeys
SULMET	0.05-0.1	+	+	+	+
SQ	0.025-0.05	+	+	+	+
WHITSYN-S	0.0065	±	±	±	±
AMPROL	0.0125-0.025	+	+	-	-
ESB <sub>3</sub> ‡	0.03	+	+	+	+
SULPHADIMETHOXINE§	0.025-0.05	+	+	+	+

\* All pathogenic species of *Eimeria*

† Fowl cholera and infectious coryza

+ High activity

± Moderate activity

- Inactive

‡ N<sup>1</sup>-(6-chloro-3-pyridazinyl)sulphanilamide

§ Active ingredient in the product introduced as AGRIBON<sup>®</sup> in 1968 by Hoffmann-La Roche Inc.

Table 3. Comparative efficacy of the commercially most established coccidiostats versus those newly developed at manufacturers' suggested dosages

Coccidiostats	Suggested use levels (% active in feed)	Anticoccidial activity					Antibacterial activity						
		A	B	C	D	E	A <sub>1</sub>	B <sub>1</sub>	C <sub>1</sub>	A <sub>3</sub>	B <sub>2</sub>	C <sub>2</sub>	D <sub>2</sub>
SQ	0-0125	+	+	+	+	+	+	+	+	+	+	+	+
NICARB	0-0125	+	+	+	+	+	+	+	+	+	+	+	+
UNISTAT	0-060	+	+	+	-	-	-	-	-	-	-	-	-
ZOAMIX	0-0125	+	+	±	±	+	±	±	±	±	±	±	±
AMPROL	0-0125	+	+	+	+	-	+	+	+	+	+	+	+
AMPROL PLUS	0-0129	+	+	+	+	+	+	+	+	+	+	+	+
BONARD	0-00875	+	+	+	-	-	-	-	-	-	-	-	-
COYDEN <sup>1</sup>	0-0125	+	+	+	+	+	+	+	+	+	+	+	+
CO-BAN	0-011	+	+	+	+	+	+	+	+	+	+	+	+
SULPHADIMETHOXINE													
+ RO 5-9754 <sup>1,2</sup>	0-02-0-01	+	+	+	+	+	+	+	+	+	+	+	+
STATYL <sup>1</sup>	0-001	+	+	+	+	+	+	+	+	+	+	+	+
RO 2-2985/1 <sup>1</sup>	0-00312-0-00625	+	+	+	+	+	+	+	+	+	+	+	+

+ High activity; + Moderate activity; ± Slight activity; - Inactive; Blank space no activity claim.

<sup>1</sup> Not commercially available<sup>2</sup> Active ingredients in ROFENAID<sup>TM</sup>

A = *E. tenella*  
 B = *E. necatrix*  
 C = *E. acroculina*  
 D = *E. maxima*  
 E = *E. brunetti*

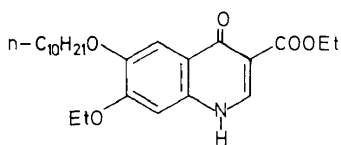
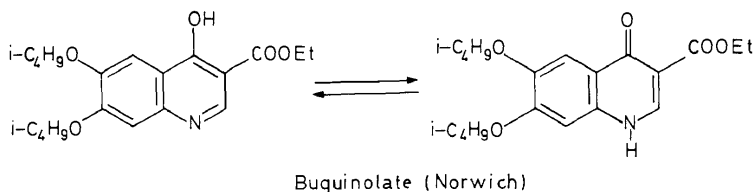
A<sub>1</sub> = *E. gallinarius*  
 B<sub>1</sub> = *E. meleagridis*  
 C<sub>1</sub> = *E. acroculina*

A<sub>2</sub> = *Posteurella multocida*  
 B<sub>2</sub> = *Escherichia coli*  
 C<sub>2</sub> = *Hemophilus gallinarum*  
 D<sub>2</sub> = *Salmonella typhimurium*

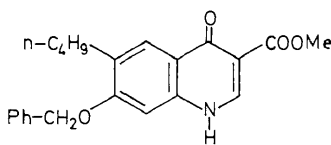


**Future developments**

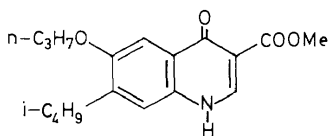
*Substituted 4-hydroxyquinoline-3-carboxylates.* Many 6,7-disubstituted 4-hydroxyquinoline-3-carboxylates, tautomeric with their corresponding quinolone structures, have been found to exert a high level of anticoccidial activity in chicks. The anticipated introduction of buquinolate<sup>26</sup>, the appearance of publications on STATYL<sup>27</sup>, and M & B 15497<sup>28</sup>, and a Merck compound<sup>29</sup> structurally closely related to buquinolate suggests that more compounds of this type are presently being developed.



M and B 15497 (May and Baker)

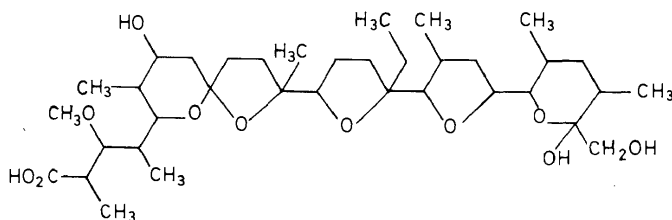


STATYL (ICI)

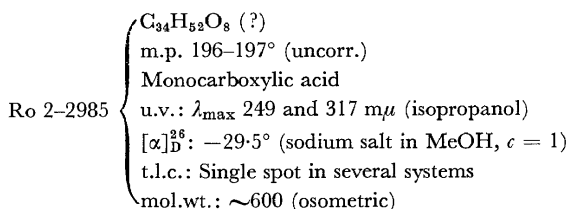


(Merck)

*Antibiotics.* Several antibiotics isolated from cultures of unidentified strains of streptomycetes several years ago<sup>30</sup> have shown broad-spectrum anticoccidial activity. The most interesting one, originally identified as X-537A and now termed Ro 2-2985, has excellent activity against all 5 important *Eimeria* species. It is characterized below. Another antibiotic (monensic acid, Eli Lilly & Co.) has also been shown to be an effective coccidiostat. The structure of monensic acid has been elaborated by x-ray crystallographic analysis of its silver salt<sup>31</sup>, and details on its biological properties have been reported



Monensic acid (CO-BAN)



recently<sup>32</sup>. Monensic acid and Ro 2-2985 are different antibiotics but, based on accumulated data, they could be chemically related to each other.

## Conclusions

The search for new coccidiostats will continue. One reason is the necessity of replacing drugs to which coccidia have developed resistance. Such compounds are more likely to be found in chemicals which act by a different mechanism. Another reason is the desirability of having broad spectrum coccidiostats which have a combined effect against the various pathogenic *Eimeria* species, other protozoa and bacteria as well. This is a major task because—even for an ideal drug—the cost will determine whether or not it will be marketable. In other words, in this field of endeavour, the biological spectrum is only one of the factors used to determine the utility of a drug.

## MALARIA

Malaria, a disease caused by parasitic protozoa of the genus plasmodium, is still an unsolved international problem. Whereas the existence of this disease is dramatized by the current military operations in Southeast Asia, it is the emergence of drug-resistant strains of the malaria parasites which highlights the extreme gravity of the problem. Malaria and its chemotherapy have recently been reviewed in depth<sup>33-35</sup>. This summary will, therefore, be restricted to some chemical aspects of some of the currently more important antimalarials.

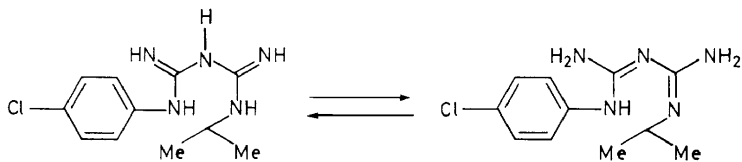
### Classical antimalarials

In the two decades between World Wars I and II, there was an uninterrupted intensive search for synthetic quinine substitutes. These efforts led to the discovery of several highly active compounds, mainly alkylaminoalkyl derivatives of aminoacridines such as quinacrine, and 4- and 8-aminoquinolines such as chloroquine and pamaquine. This important phase in the chemotherapy of malaria is well documented and will not be discussed. Many of these compounds, particularly chloroquine, have, unfortunately, developed resistant strains of malarial parasites.

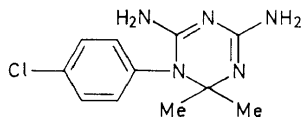
*Biguanides and related compounds.* The discovery of chloroguanide or paludrine is of special interest. In the body, chloroguanide is metabolized to a very active dihydrotriazine called cycloguanil. Cycloguanil is excreted too

rapidly in humans to be useful, but it forms an insoluble salt with pamoic acid, called CAMOLAR, which serves as a depot for cycloguanil<sup>35</sup>. This repository form exhibits long-term prophylactic protection against certain susceptible malarial parasites, and its discovery, therefore, represents a significant theoretical advance in the chemotherapy of malaria.

*Sulphonamides and sulphones.* That certain sulphonamides such as sulphadiazine and sulphones such as DAPSONE show activity against malarial



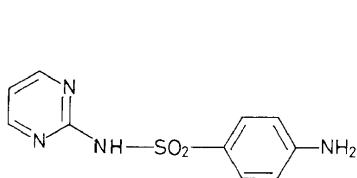
Chloroguanide = Paludrine



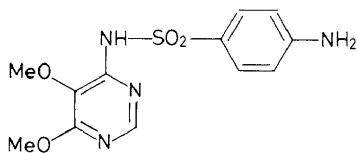
Cycloguanil

parasites has long been recognized<sup>33,34</sup>. The appearance of strains of parasites which are resistant to the classical antimalarials and the development of long-acting sulpha drugs such as sulphadoxine<sup>35,36</sup> have revived interest in the use of these compounds for the suppression of drug-resistant Plasmodia<sup>34,35</sup>. Administration of pyrimethamine, trimethoprim, or chloroguanide in combination with a sulpha drug or a sulphone potentiates the effects of these drugs, and this combination therapy is presently undergoing intensive study<sup>37</sup>.

*2,4-Diaminopyrimidines.* The fact that the pyrimidine structure is an integral part of nucleoproteins, certain vitamins and some sulphonamides prompted an intensive effort to incorporate this moiety into potential antimalarials. This work resulted in the development of DARAPRIM (pyrimethamine) and trimethoprim<sup>33,34,38</sup>. Both of these compounds, as do the sulpha drugs, interfere with folic acid metabolism as indicated in the section on

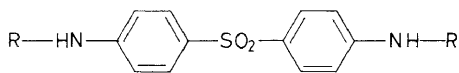


Sulphadiazine

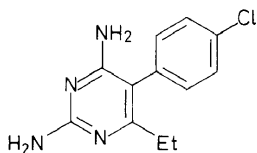


Sulphadoxine

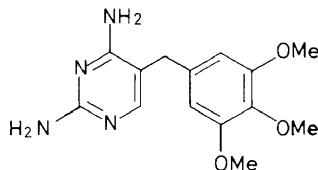
## A. BROSSI



Dapsone R=H



Pyrimethamine



Trimethoprim

coccidiosis. Especially in combination with other drugs, they have proven to be valuable therapeutic agents<sup>37</sup>.

### Cinchona alkaloids

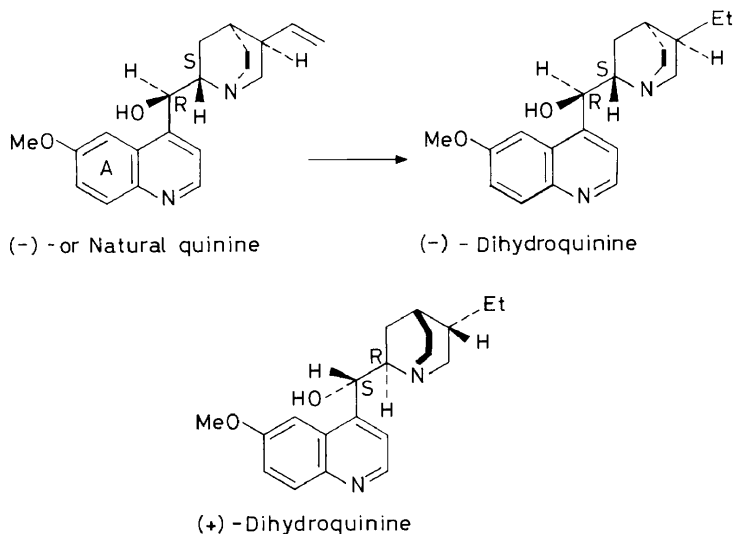
Resistance of malaria parasites to all of the drugs mentioned, particularly to the 4-aminoquinolines, is steadily increasing. As a consequence, the use of the classical drug quinine is becoming increasingly more important. The correlation of structure with activity of some major Cinchona alkaloids against avian malaria has been reported, but little is known of the validity of these data with respect to human malaria. With the total synthesis of quinine by Woodward and Doering<sup>39</sup>, work on the chemistry of Cinchona alkaloids practically came to a standstill, and only a few significant papers appeared after 1950<sup>40</sup>. Therefore, we decided to reinvestigate the Cinchona alkaloids—quinine in particular, which is currently not readily available. The recent successful technical syntheses of the reserpine and emetine molecules, which are structurally as complex as quinine, encouraged us to attempt a technical synthesis of quinine and related compounds and in this way to participate in the search for better antimalarials.

### Recent progress in the field of Cinchona alkaloids

It gives me great pleasure to report that a new synthesis of natural quinine has been achieved in our laboratories at Roche in Nutley (U.S.A). We have also improved the synthesis of (–)-dihydroquinine, an active antimalarial easily obtained from natural quinine by hydrogenation. This compound was first synthesized by Rabe<sup>41</sup>. We are at a stage where our synthesis of (–)-dihydroquinine can almost be considered to be practical. Clearly the synthesis of (–)-dihydroquinine, which has an ethyl group in place of the vinyl group present in natural quinine, is much easier to achieve. Details of our chemical work will soon be reported by Uskoković, who was in charge of this programme from its inception<sup>42</sup>.

The question concerning the absolute configuration of natural quinine and closely related alkaloids as it relates to their antimalarial activity has

never been answered. In connection with our project it was important to know whether synthetic racemic quinine could be substituted for the natural alkaloid or whether an optical resolution would be necessary. Our most recent findings on this point are based only on a few experiments carried out by Brener<sup>43</sup> in mice infected with *P. berghei*. The results obtained by comparing synthetic ( $\pm$ )-dihydroquinine<sup>44</sup> with natural (—)-dihydroquinine, both as the sulphate salts, seem to indicate that the racemic mixture



is as active and possibly not more toxic than the natural (—)-enantiomer<sup>43,45</sup>. Unequivocal proof of this important question will soon be obtained by testing the unnatural (+)-enantiomer of dihydroquinine<sup>44</sup>. The results of these studies will influence the ultimate design of our project.

## Conclusion

The development of new and better antimalarial drugs must continue. Fundamental work must be done on the mechanism of drug action. Intensive studies concerning the development of resistance by malarial parasites and other aspects of the host-drug-parasite relationship must be pursued. Metabolic studies of important antimalarial drugs, such as quinine, are also necessary, because through knowledge gained from these studies the medicinal chemist will be in a better position to achieve a possible breakthrough in the chemotherapy of malaria.

## CLOSING REMARKS

With reference to each of the three topics—amebiasis, coccidiosis, and malaria—which I have discussed from the chemist's point of view, it can be stated that, while considerable progress has been made, much remains to be

done. In closing I wish to acknowledge the cooperation of Dr. A. Rachlin in preparing this lecture and the advice and support of many of my colleagues at Roche in Nutley (U.S.A.) and Basel (Switzerland) who are actively involved in studying several aspects of these three major protozoal diseases.

## References

- <sup>1</sup> G. Woolfe. *Prog. Drug Res.* **8**, 11 (1965).
- <sup>2</sup> M. Barash and J. M. Osbond. *J. Chem. Soc.* 2157 (1959).
- <sup>3</sup> Personal communication by Drs. A. Openshaw and N. Whittaker, Burroughs Wellcome & Co., England. See also *J. Chem. Soc. (C)* 101 (1969).
- <sup>4</sup> H. T. Openshaw and N. Whittaker. *Chem. Commun.* 131 (1966).
- <sup>5</sup> A. R. Battersby, J. R. Merchant, E. A. Ruveda and S. S. Salgar. *Chem. Commun.* 315 (1965).
- <sup>6</sup> A. P. Grollman. *Science* **157**, 84 (1967).
- <sup>7</sup> Two Ipecac alkaloids with free hydroxy groups in Ring A have recently been described by S. C. Pakrashi and E. Ali, *Tetrahedron Lett.* 2143 (1967).
- <sup>8</sup> J. P. Yardley, R. W. Rees and H. Smith. *J. Med. Chem.* **10**, 1088 (1967).
- <sup>9</sup> J. Herrero, A. Brossi, M. Faust and J. R. Frey. *Ann. Biochem. Exptl. Med.* **20** (Suppl.), 475 (1960).
- <sup>10</sup> F. Blanc, Y. Nosny, M. Armengaud, M. Sankale, M. Martin and G. Charmot. *Presse Med.* **69**, 1548 (1961).
- <sup>11</sup> D. E. Schwartz and J. Herrero. *Am. J. Trop. Med. Hyg.* **14**, 78 (1965).
- <sup>12</sup> A. P. Grollman. *Proc. Natl. Acad. Sci., Wash.* **56**, 1867 (1966).
- <sup>13</sup> A. P. Grollman. *J. Biol. Chem.* **243**, 4089 (1968).
- <sup>14</sup> C. Cosar, C. Crisan, R. Horclois, R. M. Jacob, J. Robert, S. Tchelitcheff and R. Vaupre. *Arzneimittel-Forsch.* **16**, 23 (1966).
- <sup>15</sup> M. Wilhelm, F.-H. Marquardt, Kd. Meier and P. Schmidt. *Helv. Chim. Acta* **49**, 2443 (1966).
- <sup>16</sup> S. J. Powell, I. MacLeod, A. J. Wilmot and R. Elsdon-Dew. *Lancet* **2**, 1329 (1966).
- <sup>17</sup> S. J. Powell, I. MacLeod, A. J. Wilmot and R. Elsdon-Dew. *Lancet* **2**, 20 (1966).
- <sup>18</sup> D. R. Hoff. *Proc. Intern. Symp. on Drug Research, Montreal*, June 12-14, 1967, p. 100.
- <sup>19</sup> Data accumulated in the Chemotherapy Department, Hoffmann-La Roche Inc., Nutley, N.J. (Director, Dr. E. Grunberg).
- <sup>20</sup> L. Goldman in *Medicinal Chemistry*, 2nd ed., p. 997; A. Burger, Ed., Interscience Publishers Inc., New York, 1960.
- <sup>21</sup> L. P. Joyner, S. F. M. Davies and S. B. Kendall. *Exptl. Chemother.* **4**, 440 (1966). Suppl. to Vol. **1**, 445-486 (1963).
- <sup>22</sup> Data elaborated by M. Mitrovic *et al.* in the laboratories of the Animal Health Department, Research Division, Hoffmann-La Roche Inc., Nutley, N.J.
- <sup>23</sup> P. P. Levine. *Cornell Vet.* **29**, 309 (1939).
- <sup>24</sup> M. Mitrovic and J. C. Bauernfeind. *Poultry Sci.* **46**, 402 (1967).
- <sup>25</sup> M. Mitrovic. *Poultry Sci.* **46**, 1153 (1967).
- <sup>26</sup> G. F. Spencer, A. Engle, C.-N. Yu, R. C. Finch, E. J. Watson, F. F. Ebetino and C. A. Johnson. *J. Med. Chem.* **9**, 934 (1966).
- <sup>27</sup> R. A. Bowie, J. P. Cairns, M. S. Grant, A. Hayes, W. G. M. Jones and J. F. Ryley. *Nature* **214**, 1349 (1967).
- <sup>28</sup> S. J. Ball, M. Davis, J. N. Hodgson, J. M. S. Lucas, E. W. Parnell, B. W. Sharp and D. Warburton. *Chem. Ind. (London)* 56 (1968).
- <sup>29</sup> Netherlands Patent 6709074; January 2, 1968.
- <sup>30</sup> J. Berger, A. I. Rachlin, W. E. Scott, L. H. Sternbach and M. W. Goldberg. *J. Am. Chem. Soc.* **73**, 5295 (1951).
- <sup>31</sup> A. Agtarap, J. W. Chamberlin, M. Pinkerton and L. Steinrauf. *J. Am. Chem. Soc.* **89**, 5737 (1967).
- <sup>32</sup> Abstracts of Papers presented at the *Interscience Conference on Antimicrobial Agents and Chemotherapy*, 7th, Am. Soc. Microbiol., Chicago, Illinois, October 25-27, 1967, pp. 17 and 40.
- <sup>33</sup> R. D. Powell. *Clin. Pharmacol. Therap.* **7**, 48 (1966).

## CHEMOTHERAPY OF AMEBIASIS, COCCIDIOSIS, AND MALARIA

- <sup>34</sup> D. G. Davy and J. Hill. *Exptl. Chemother.* **1**, 487 (1963); **4**, 448 (1966).
- <sup>35</sup> E. F. Elslager. In *Annual Reports in Medicinal Chemistry*, 1966, C. K. Gain, Ed., Academic Press, New York, 131 (1967).
- <sup>36</sup> E. Herrero. *Revista Soc. Brasileira de Med. Trop.* **1**, No. 3, 103 (1967).
- <sup>37</sup> World Health Organization Technical Report Series, Geneva, Switzerland, No. 375 (1967).
- <sup>38</sup> D. C. Martin and J. D. Arnold. *J. Clin. Pharmacol. J. New Drugs* **7**, 336 (1967).
- <sup>39</sup> R. B. Woodward and W. E. Doering. *J. Am. Chem. Soc.* **67**, 860 (1945).
- <sup>40</sup> R. B. Turner and R. B. Woodward. In *The Alkaloids*, Vol. III, pp. 1-63; A. Manske, Ed., Academic Press Inc., New York, 1953.
- <sup>41</sup> P. Rabe, W. Huntenburg, A. Schultze and G. Volger. *Chem. Ber.* **64**, 2487 (1931).
- <sup>42</sup> M. Uskoković, Chemical Research Division, Hoffmann-La Roche Inc., Nutley, N.J. Scheduled for presentation at the Gordon Research Conferences, Section on Steroids and Other Natural Products, New Hampton, New Hampshire, 1968.
- <sup>43</sup> Personal communication by Z. Brener, Instituto Nacional de Endemias Rurais, Belo Horizonte, Brazil.
- <sup>44</sup> P. Rabe and A. Schultze. *Chem. Ber.* **66**, 120 (1933). These authors have prepared these compounds, but no biological results have been reported.
- <sup>45</sup> The acute toxicity data after various forms of application have been accumulated in the Chemotherapy Department of Hoffmann-La Roche Inc., Nutley, N.J. (Director, Dr. E. Grunberg).