ANTIPROTOZOAL AGENTS FROM HIGHER PLANTS

David J. PHILLIPSON and C. W. WRIGHT

Department of Pharmacognosy, The School of Pharmacy, University of London
29-39 Brunswick Square, London WC1N 1AX, U.K.

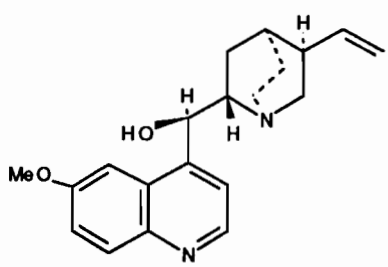
Résumé : *la malaria, les amibiases, leishmanioses et trypanosomiases sont des maladies tropicales importantes provoquées par des protozoaires. De nouveaux médicaments sont nécessaires pour soigner ces maladies, et il est essentiel d'évaluer l'efficacité et la toxicité des plantes utilisées en médecine traditionnelle pour le traitement de ces infections. Pour de telles recherches, des méthodes d'essais biologiques in vitro existent afin de tester et guider le fractionnement chimique des extraits de ces plantes. Quelques uns des produits naturels de plantes supérieures possédant une activité anti-protozoaire sont revus ici.*

Abstract : *malaria, amoebiasis, leishmaniasis and trypanosomiasis are major tropical diseases which are caused by protozoal infections. New drugs are needed to treat these diseases and it is essential that plants which are used in traditional medicine for the treatment of protozoal infections be evaluated for their efficacy and toxicity. In vitro test methods are available for bioassay guided fractionation of plant extracts for such investigations. Some of the higher plant natural products which have antiprotozoal activity are reviewed.*

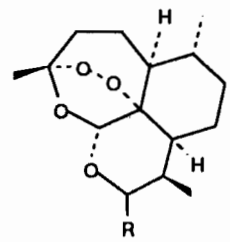
Introduction

During the 1950s it was believed that malaria would be eradicated because of the clinical effectiveness of drugs such as chloroquine against malaria parasites and because insecticides such as DDT were highly active against vector mosquitoes. The resistance of *Plasmodium falciparum*, in particular to chloroquine, and the resistance of mosquitoes to DDT coupled with restrictions imposed on the use of insecticides, have resulted in today's world-wide epidemic of malaria (1,2). It has been reported that there are 42 millions cases of amoebiasis each year due to infections by *Entamoeba histolytica* (3). The drug of choice is metronidazole which is not well tolerated by many patients. *Leishmania* species infect millions of people world-wide, *L. mexicana* and *L. tropica* causing the cutaneous form of the disease and *L. donovani* causing visceral leishmaniasis (4). There are no satisfactory drug treatments for severs forms of the disease. Trypanosomiasis in S. America is caused by *T. cruzi* and is known as Chagas's disease while in Africa *T. brucei* results in sleeping sickness. It is estimated in Brazil alone, that some 20 million people are infected with *T. brucei* (5) and adequate drug therapy is not available (6).

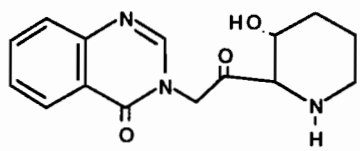
It is necessary that new drugs are developed for the treatment of these major life-threatening diseases and the question arises as to whether plants may serve as sources of new antiprotozoal drugs. In the past, higher plants have yielded three major types of antiprotozoal drugs which have proved effective clinically. Quinine (Figure 1), the alkaloid obtained from S. American species of *Cinchona* was the major antimalarial drug for many years. Gradually it became replaced by synthetic drugs but many of these owed their origin to a knowledge of the chemical structure of quinine which was use as a template to furnish the 8-aminoquinolines such as mepacrine, primaquine and also the 4-aminoquinolines such as chloroquine and amodiaquine. More recently, the sesquiterpene lactone artemisinin (Figure 1) has been introduced into antimalarial chemotherapy by the Chinese (7).



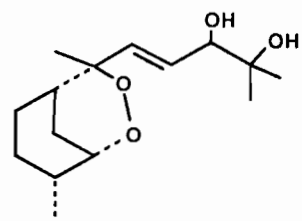
quinine



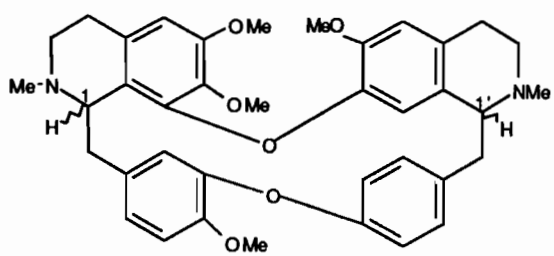
artemisinin R = =O
artemether R = OMe
artesunate R = $OCOCH_2CH_2COONa$



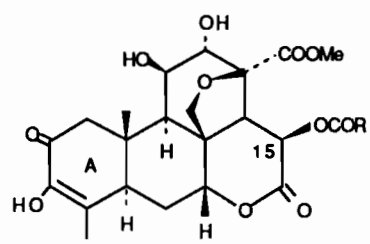
febrifugine



yingzhaosu A



	C-1H	C-1'H
phaeanthine	R	R
isotetrandrine	R	S



brusatol R = $CH=CMe_2$
bruceine C R = $CH=C(Me)C(OH)Me_2$

Figure 1 : examples of natural products with antimalarial activity

Artemisinin, the active principle from the Chinese medicinal herb *Artemisia annua*, is unusual chemically because it contains an endoperoxide moiety which is an essential requirement for its antiprotozoal activity. The natural product has poor solubility characteristics and modifications to the molecule have been made by reduction of the lactone carbonyl and derivitisation of the resulting alcohol into ethers (e.g. artemether) and esters (e.g. sodium artesunate) (Figure 1) which have enhanced pharmacokinetic characteristics. The isoquinoline alkaloid, emetine (Figure 2) obtained from the S. American *Cephaelis ipecacuanha* and related species has served for many years as an amoebicidal drug. It has been mainly superseded in clinical practice although it continues to be used in the initial treatment of severe cases of amoebic abscesses. Higher plants have yielded three major groups of antiprotozoal drug and this leads us to ask whether there are other potentially useful clinical agents awaiting discovery from the plant kingdom.

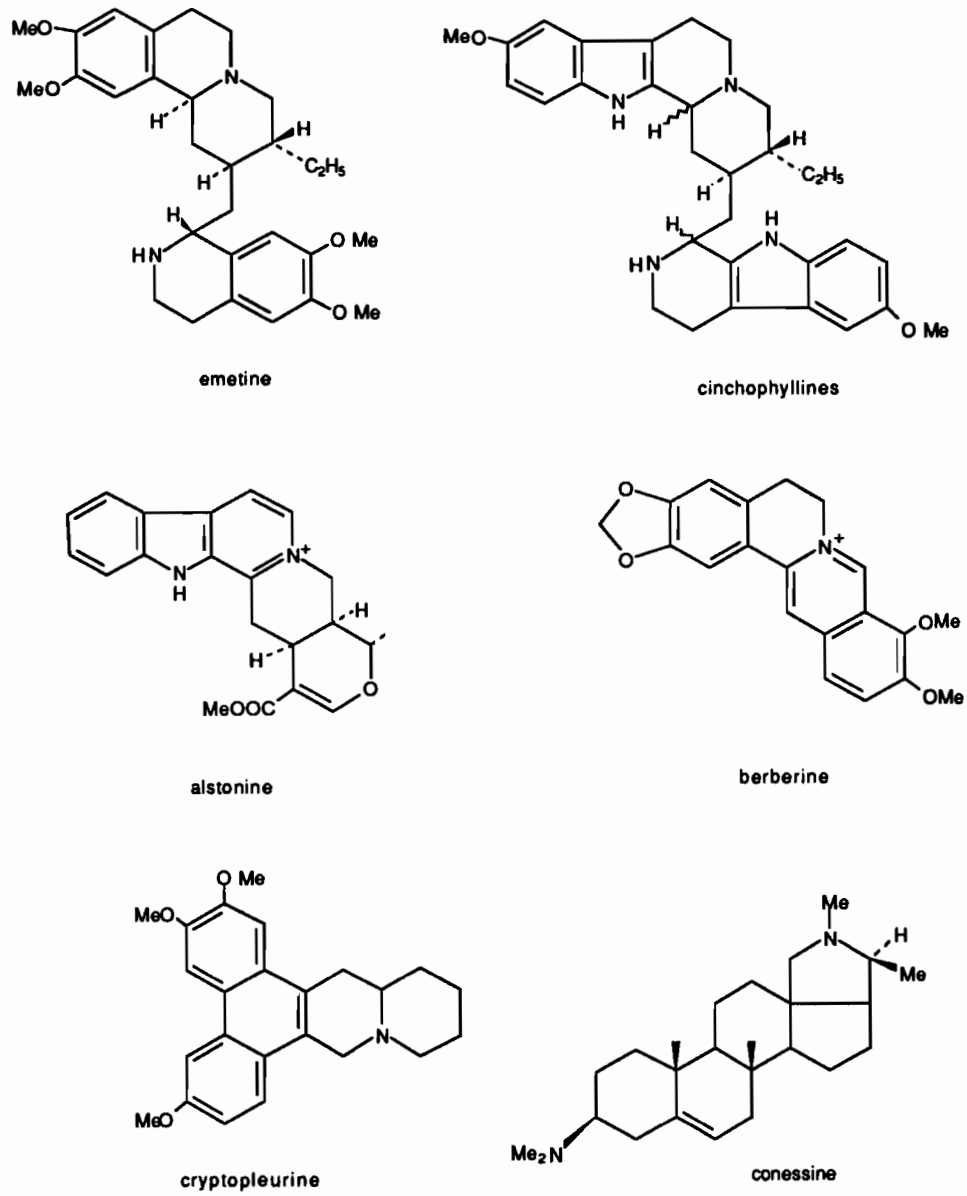


Figure 2 : examples of natural products with antiamoebic activity

Antiprotozoal testing of plant extracts

In order to screen extracts, prepared either from plants used in traditional medicine or selected at random for antiprotozoal activity, it is essential to have reliable test procedures. The most extensive investigation of higher plants for antiprotozoal activity was published in 1947 (8). As a result of studies undertaken during World War II, it was reported that some 600 species of higher plant had been tested for antimalarial activity. The test methods of the day relied on avian malarias and *in vivo* tests were carried our using *P. gallinaceum* in chicks and *P. cathemerium* and *P. lophurae* in ducklings. The predictive value of such avian malarias to select active compounds for the treatment of human malarias was not known at the time.

Furthermore, in the 1940s there were no extensive chromatographic separation techniques for fractionation of active extracts and there was not the array of spectroscopic techniques for structure determination which are in routine use today. It is not surprising, therefore, that the results of this antimalarial screening programme lay dormant for many years. In 1976, it was reported that *Plasmodium falciparum*, the cause of human malignant tertiary

malaria could be cultured *in vitro* in human blood (9) and by 1979 a microtitre plate assay procedure was described (10). The applicability of this *in vitro* test procedure using multi-drug resistant *P. falciparum* for bioassay guided fractionation of plant extracts has been described (11,12). *In vitro* tests are not necessarily predictive of *in vivo* activity and *P. berghei* infections in mice are used for *in vivo* evaluations. The applicability of *in vitro* and *in vivo* antiplasmodial tests for the evaluation of plant extracts has been reviewed (12).

A microtitre well test has been developed in our laboratories for bioassay guided fractionation of plant extracts with activity against *Entamoeba histolytica* (13). *In vitro* tests for antileishmanial activity using mice peritoneal macrophages infected with amastigotes of *L. donovani* (14) and antitrypanosomal *in vitro* tests (15) are available. Thus *in vitro* tests are available for bioassay guided fractionation of plant extracts for the isolation of antisplasmodial, antiamoebic, antileishmanial and antitrypanosomal compounds.

Traditional medicines for the treatment of protozoal diseases

It has been estimated that some 75-80% of the worl'ds population does not have access to western pharmaceuticals. Every country has its own indigenous system of traditional medicine which relies mainly on the use of plants and there are some 20,000 medicinal plants in use on a world-wide basis. It is the tropical countries, in particular, which have extensive lists of plants recommended for the treatment of protozoal infection. References to the literature for Africa, the Americas and Asia have been cited in a recent review (16). The NAPRALERT database, for example, lists some 152 genera which are used for the treatment of malaria and some 139 genera which have yielded extracts with antiamoebic effects (N.R. Farnsworth, personal communication).

The following account describes some of the natural products from higher plants which have activity against one or more species of *Plasmodium, Entamoeba, Leishmania* and *Trypanosoma*. For more detailed accounts, the reader is referred to review articles (16,17,18,19,20,21).

Higher plant natural products with antiprotozoal activities

Malaria: Some examples of antimalarial natural products are shown in Figure 1. Quinine, artemisinin and its derivates artemether and artesunate are in current clinical use. *Dichroea febrifuga* (Saxifragaceae) is a Chinese medicinal plant which is used for the treatment of several protozoal diseases including malaria. Clinical trials using thousands of patients have been carried out in China for the evaluation of extracts against tertian malaria (22). The active principles are alkaloids including febrifugine (β-dichroine, Figure 1) and isofebrifugine (α-dichroine). It has been reported that febrifugine is hepatotoxic (23) but the plant extract is apparently still used in China. There appears to be a lack of detailed scientific information on the *in vitro* and *in vivo* antiplasmodial activities of the individual alkaloids, and on their toxicities and mechanisms of action.

There is no doubt that the discovery of artemisinin as the antimalarial principle of *Artemisia annua* has given considerable impetus to the search for other antimalarial natural products. The endoperoxide moiety of artemisinin (Figure 1) is rare in natural products and it is interesting to note that there is another group of endoperoxide terpenoids which have antiplasmodial activity. Yingzhaosu A (Figure 1) and related terpenoids have been isolated from the Chinese medicinal herb *Artabotrys unciatus* (Annonaceae). Yingzhaosu A has *in vitro* activity against *P. falciparum*.

We have extracted some eighteen species of plant which are used in Sierra Leone for the treatment of malaria and fevers. The extracts were assessed for their activity against *P. falciparum* (K1, multi-drug resistant strain) and the most active extracts were those of the wood and bark of *Triclisia patens* (Menispermaceae). Bioassay guided fractionation led to the conclusion that the active principles were bisbenzylisoquinoline alkaloids (BBIQs). One of these alkaloids, phaeanthine (Figure 1) was found to be twice as potent *in vitro* against

chloroquine- resistant *P. falciparum* (K1) (IC_{50} value 366 nM) as againt a chloroquine-sensitive strain (T-96) (IC_{50} value 705 nM) (24). Experiments with chloroquine/phaeanthine combinations have shown that their effects were additive against K1 strain whereas they showed antagonism in T-96. Phaeanthine showed no toxicity against a mammalian cell line (KB) indicating some specificity of action against plasmodia. We have tested some 25 BBIQs for *in vitro* activity against *P. falciparum* and their IC_{50} values range from 0.1 to 45 mg ml^{-1}. Other workers have isolated BBIQs as the active principles of *Tiliacora triandra* which is used as an antimalarial drug in Thailand (25). The IC_{50} values of alkaloids of the tiliacorinine-type ranged from 0.5 to 3.5 μg ml^{-1} against five different isolated of *P. falciparum* from malarial patients.

In the 1947 screen for antimalarial activity from higher plants (8), the family Simaroubaceae proved to yield highly active extracts against avian malarias. In the mid 1980s, we selected five species from as far apart as Asia, India and C. America for further investigation - namely *Brucea javanica, Eurycoma longifolia, Ailanthus altissima, Simarouba amara* and *Picramnia antidesma*. Each plant was subjected to sequential extraction with hexane, chloroform and methanol, and the extracts assessed for their *in vitro* activity against *P. falciparum* (K1, multi-drug resistant strain). The chloroform extracts proved to be the most active and subsequent fractionation using bioassay guided fractionation has led to the isolation of a series of quassinoids (bitter, degraded triterpenoids) as the active principles. The methodology of the testing has been described in a series of publications (11, 16, 17, 18, 19, 26, 27, 28, 29). Concurrent investigations by other researchers have shown that quassinoids have potent antimalarial activity (30, 31, 32).

Ten of the quassinoids tested by us have *in vitro* IC_{50} activities against *P. falciparum* (K1) of less than 0.02μg ml^{-1} and are ten times more active than chloroquine diphosphate (IC_{50}0.21 μg ml^{-1}). Brusatol and bruceine V(Figure 1) have IC_{50} values of 0.003 and 0.005 μg ml^{-1}, respectively, against *P. falciparum* (K1) (28). Changes in the nature of the esterifying acid at C-15 result in changes of antiplasmodial potency (16, 26). Quassinoids occur as complex mixtures of closely related compounds within specific genera of the Simaroubaceae. Each genus is characterised by the type of quassinoid which it contains. It has been shown that the major factors which influence antiplasmodial activity are the nature of the carbon skeleton, the state of oxidation and of substitution of ring A, the presence of a C-8 methyleneoxy bridge to either C-11 or C-13 and the type of the substituent at C-15 (16). We have established that quassinoids are potent inhibitors of protein synthesis in *P. falciparum* and have little effect on glycolysis (33). Some quassinoids are potent cytotoxic agents but we have shown that cytotoxicity does not necessarily parallel antiplasmodial activity (16). Only limited *in vivo* studies have been undertaken with quassinoids ; brusatol (Figure 1), for example, has an ED_{90} value of 3.03 mg kg^{-1} day^{-1} against *P. berghei* in mice (28). The quassinoids represent a group of potent antimalarial compounds and more detailed investigations of structure-activity relationships, toxicities, *in vivo* activities, semi-synthetic modifications and synthetic analogies are required. Quassinoids continue to be taken in plant extract form in traditional medicines for the treatment of malaria in a number of pantropical countries.

Other higher plant natural products with antiplasmodial activity include isoquinoline alkaloids (e.g. berberine-type alkaloids), indole alkaloids (e.g. 1-vinyl-β-carbolines, usambarensine), acridones (e.g. atalaphillinine) ; sesquiterpenes (e.g. parthenin, bisaboloxide), diterpenes (e.g. taxol, phorbols), triterpenes (e.g. pristimerin, nimbolide, gedunin), quinones (e.g. lapachol) and flavonoids (e.g. artemetin, casticin) (16, 17, 18, 19).

Amoebiasis : Emetine (Figure 2) is derived biosynthetically from one molecule of secologanin and two molecules of dopamine in species of *Cephaelis* (Rubiaceae). In the related genus

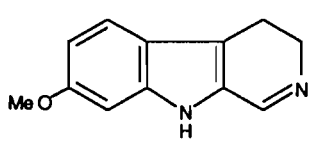
Cinchona, some species produce cinchophylline-type alkaloids (Figure 2) in their leaves. These alkaloids, which are derived from one molecule of secologanin and two molecules of tryptamine, can be considered to be indole analogues of emetine. A series of eighteen cinchophylline-type alkaloids have been tested in our laboratories for *in vitro* activity against *Entamoeba histolytica* (NIH 200 strain). 3a, 17b-cinchophylline, which has the same overall stereochemistry as emetine, proved to be some fourteen times less active *in vitro* than emetine. However 3a,17b-cinchophylline proved to be highly cytotoxic to guinea-pig keratinocytes (34). Other indole alkaloids with amoebicidal activity include the pentacyclic alkaloid alstonine (Figure 2).

The quaternary alkaloid berberine (Figure 2), which shows some structural similarity to alstonine, is reportedly active *in vivo* against *E. histolytica* but in our laboratories, it proved to have only weak *in vitro* activity (35). It is possible that berberine undergoes activation metabolically. The phenanthrene-indolizidine alkaloid cryptopleurine (Figure 2) reportedly binds to a ribosomal receptor in a similar manner to emetine. It has been postulated that emetine adopts a conformation in which the two aromatic rings are planar and it becomes structurally similar to cryptopleurine (36). *Holarrhena floribunda* has a traditional reputation for the treatment of amoebiasis and the active principles have been identified as steroidal alkaloids such as conessine (Figure 2) (20).

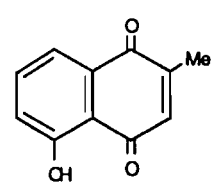
Other higher plant natural products which have antiamoebic activity include indole alkaloids (e.g. borrerine), piperidine alkaloids (e.g. carpaine), quinolizidine alkaloids (e.g. matrine, cytisine), terpenoids (e.g. bruceantin, simalikalactone D), chalcones (e.g. uvaretin, diuvaretin), coumarins (e.g. marmelosin) (16,20).

For many years, the major problem in assessing plant extracts for amoebicidal activity has been the lack of an *in vitro* test system. The development of a sensitive microtitre well assay in our laboratories has enabled bioassay guided fractionation to be used for the isolation of amoebicidal active principles (13).

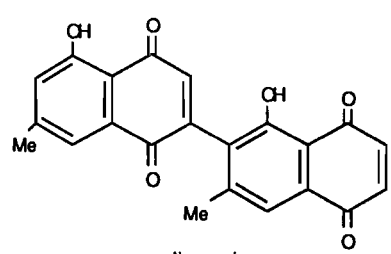
Leishmaniasis : The dihydro-β-carboline alkaloid, harmaline (Figure 3) from *Peganum harmala*, is active against *Leishmania amazonensis* amastigotes with an IC_{50} value of 24 μg ml^{-1}.



harmaline



plumbagin



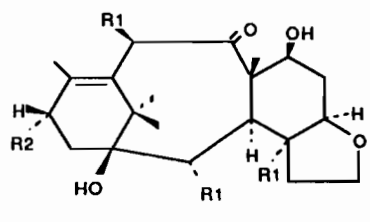
diospyrin

Figure 3 : examples of natural products with antileishmanial activity

Extracts of *Plumbago* species are used traditionally in Africa for the treatment of skin diseases. One of the active constituents is the naphthoquinone plumbagin (Figure 3) which is

active against *L. donovani* and *L. amazonensis* amastigotes *in vitro* (37). Plumbagin is also active *in vivo* when applied topically to leishmanial lesions. The bis-naphthoquinone diospyrin (Figure 3) from *Diospyros montana* inhibits the growth of *L. donovani* promastigotes (38). Other natural products with activity against species of *Leishmania* include berberine, the bisbenzyl-isoquinoline alkaloids gyrocarpine, daphnandrine and obaberine and the phorbol ester 12-0-tetradecanoyphorbol-13-acetate (18).

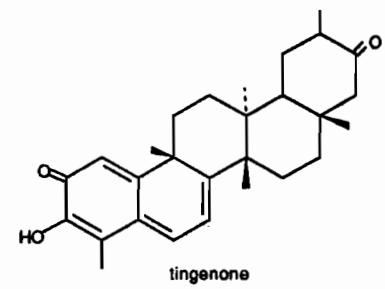
Trypanosomiasis : Taxol (Figure 4), a diterpene from *Taxus brevifolia* is active against *Trypanosoma cruzi in vitro* (39). The triterpenoid tingenone (Figure 4), obtained from several species of Celastraceae, is highly active against *T. cruzi* epimastigotes (40). Gossypol (Figure 4) is a polyphenolic compound from cotton seed oil. It is active against *T. cruzi* inhibiting the oxido.reductase enzymes α-hydroxyacid dehydrogenase and malate dehydrogenase (41). Several naphthoquinones are active *in vitro* against *T. cruzi* epimastigotes but β-lapachone and allyl-β-lapachone are potently active (16). The bisindole antitumour alkaloid vinblastine and the terpenoid vismione B have antitrypanosomal activity *in vitro*.



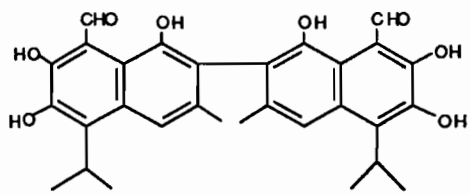
taxol R1 = OCOMe

R2 = OCOCH-CHNHCOC$_6$H$_5$

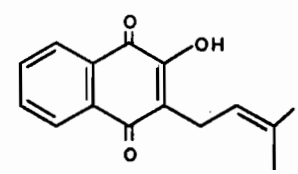
OH C$_6$H$_5$



tingenone



gossypol



lapachol

Figure 4 : examples of natural products with antitrypanosomal activity

Conclusions

A number of alkaloids, terpenoids, quinones and phenolic compounds from higher plants are known to have antiprotozoal activity. The results obtained to date are mainly from *in vitro* test systems and it is necessary that more *in vivo* and toxicity studies be undertaken. Many of the active compound which have been reported in the literature have been assessed against only one genus and they require testing against other protozoa. It is apparent to date that the possible modes of action of these natural products against protozoa include intercalation with DNA (e.g. alstonine, berberine), inhibition of protein synthesis (e.g. emetine, quassinoids), alkylation (e.g. quinones) or oxidant stress (e.g. artemisinin, quinones) (18).

Further leads to novel compounds and novel modes of activity may well be obtained from investigations of plants used in traditional medicine for the treatment of protozoal

infections. This offers one logical approach to the search for new drugs. *In vitro* tests are available for the four genera of protozoa discussed in this paper and these assays can be used for bioassay guided fractionation of active principles from plant extracts. In addition to searching for new drugs for antiprotozoal chemotherapy, it is also vital than plants which are used in traditional medicine are carefully investigated for their efficacy and toxicity. It is possible to obtain some measure of specificity of antiprotozoal action by comparison of *in vitro* antiprotozoal activity with cytotoxicity using mammalian cells. There is much to be done in this field of research which is a fruitful area for collaboration between phytochemists and protozoologists.

***Acknowledgements** : CWW gratefully acknowledges receipt of a Maplethorpe Fellowship. Financial support is gratefully acknowledged from the Medical Research Council, the Wellcome Trust, the European Economic Community, the Sir Halley Stewart Trust, The Royal Pharmaceutical Society of Great Britain and the School of Pharmacy, University of London. We acknowledge the active collaboration of colleagues from the London School of Hygiene and Tropical Medicine and in particular Doctors D.C. Warhurst and S.L. Croft.*

References

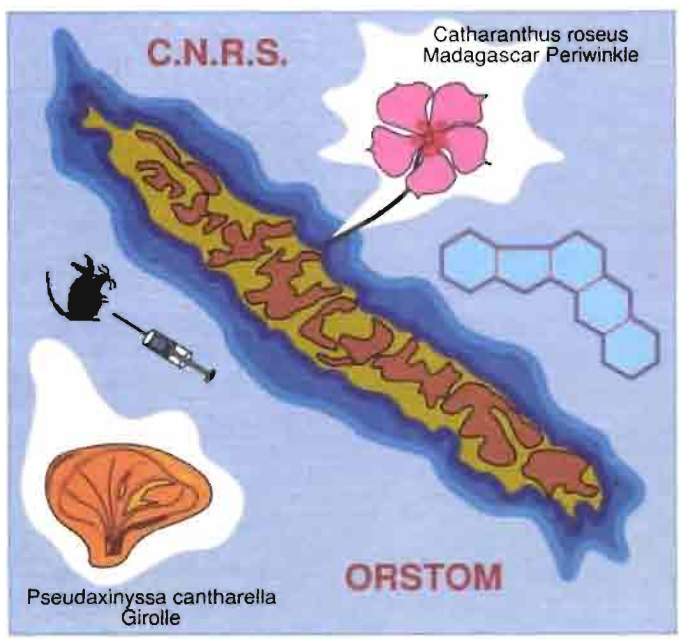
1. Anon , World Health Organisation, *Weekly Epidemiological Record, Geneva*, 1988.
2. Walsh J.A., Warren K.S., *New England Med. J.* **30**, 967-974 (1979)
3. Guerrant R.L., *Rev. Inf. Dis.* **8**, 218-227 (1986)
4. Barker D.C., *Parasit. Today* **3**, 177-184 (1987)
5. Goodwin L.G., *Brit. Med. Bull.* **41**, 103-104 (1985)
6. GutteridgeW.E., *Brit. Med. Bull.* **41**, 162-168, (1985)
7. Trigg P.I. *in : Economic and Medicinal Plant Research, Vol.3* (Wagner H., Hikino H., Farnsworth N.R., eds), pp. 20-56, Academic Press, London (1989)
8. Spencer C.F., Koniuszy F.R., Rogers E.F., Shavel Jr. J., Easton N.R., Kacczka E.A., Kuehl Jr. F.A., Phillips R.F., Walti J., Folkers K., Malanga C., Seeler A.O., *Lloydia* **10**, 145-174 (1947)
9. Trager W., Jensen J.B., *Science* **193**, 673-675 (1976)
10. Desjardins R.E., Canfield C.J., Haynes J.D., Chulay J.D., *Antimicrob. Agents Chemother.* **16**, 710-718 (1979)
11. O'Neill M.J., Bray D.H., Boardman P., Phillipson J.D., Warhurst D.C., *Planta Medica* 394-397 (1985)
12. Phillipson J.D. *in : Methods in Plant Biochemistry, Vol. 6, Biological Techniques* (Hostettmann K. ed.) 135-152, Academic Press, London (1990)
13. Wright C.W., O'Neill M.J., Philippson J.D., Warhurst D.C., *Antimicrob. Agents Chemother* **32**, 1725-1729 (1988)
14. Neal R.A., Croft S.L., *J. Antimicrob. Chemother.* **14**, 463-475 (1984)
15. Lopes J.N., Cruz F.S., Docampo R., Vasconcellas M.E., Sampaio M.C.R., Pinto A.V., Gilbert B., *Ann. Trop. Med. and Parasitol.* **72**, 523-531 (1978)
16. Phillipson J.D., Wright C.W., *Planta Med.*, in press (1991)
17. Phillipson J.D., Wright C.W., *Trans. Roy. Soc. Trop. Med. Hyg.* **85**, 18-21 (1991)
18. Wright C.W., Phillipson J.D., *Phytother. Res.* **4**, 127-139 (1990)
19. Phillipson J.D., *J. Ethnopharm.* **32**, 155-165 (1991)
20. Phillipson J.D., O'Neill M.J. *in : Biologically Active Natural Products* (Hoestettmann K., Lea P.J. eds.), 49-64, Clarendon Press, Oxford (1987)
21. Phillipson J.D., O'Neill M.J., *Acta Pharm. Nord.* **1**, 131-143 (1989)
22. Chang H.M., But P.P., *Pharmacology and Applications of Chinese Materia Medica*, Vol. 2, World Scientific, Singapore (1987)
23. Steck E.A., *The Chemotherapy of Protozoan Diseases, Walter Reed Army Institute of Research, Washington* (1972)
24. Ekong R., Partridge S.J., Anderson N.M., Kirby G.C., Warhurst D.C., Russel P.F., Phillipson J.D., *Ann. Trop. Med. Parasitol.* **85**, 205-213 (1991)

25. Pavanand K. Webster K.H., Yongvanitchit K., Dechatiwongse T., *Phytother. Res.* **3**, 215-217 (1989)
26. O'Neill M.J., Bray D.H., Boardman P., Phillipson J.D., Warhurst D.C., Peters W., Suffness M., *Antimicrob. Agents Chemother* **30**, 101-104 (1986)
27. Chan K.L., O'Neill M.J., Phillipson J.D., Warhurst D.C., *Planta Medica* **52**, 105-107 (1986)
28. O'Neill M.J., Bray D.H., Boardman P., Wright C.W., Phillipson J.D., Warhurst D.C., Peters W., *J. Nat. Prod.* **50**, 41-48 (1987)
29. O'Neill M.J., Bray D.H., Boardman P., Wright C.W., Phillipson J.D., Warhurst D.C., Gupta M.P., Correya M., Solis P., *J. Ethnopharm.* **22**, 183-190 (1988)
30. Trager W., Polonsky J., *Am. J. Trop. Med. Hyg.* **30**, 531-537 (1981)
31. Fandeur T., Moretti C., Polonsky J., *Planta Medica* **51**, 20-23 (1985)
32. Pavanand K., Nutakul W., Dechatiwongse T., Yoshira K., Yongvanitchit K., Scovil J.P., Flippen-Anderson J.L., Gilardi R., George C., Kangchanagee P., Webster H.K., *Planta Medica* 108-111 (1986)
33. Kirby G.C., O'Neill M.J., Phillipson J.D., Warhurst D.C., *Biochem. Pharm.* **38**, 4367-4374 (1989)
34. Keene A.T., Phillipson J.D., Warhurst D.C., Koch M., Seguin E., *Planta Medica* **53**, 201-206 (1987)
35. Wright C.W., *Antiamoebic and Antimalarial Natural Products, Ph.D. Thesis, University of London* (1989)
36. Gupta R.S. *in : Antibiotics*, Vol. 6 (Hahn F.E., ed.) Springer Verlag, Berlin (1983)
37. Croft S.L., Evans A.T., Neal R.A., *Ann. Trop. Med. Parasit.* **79**, 651-653 (1985)
38. Hazra B., Saha A.K., Ratamala R., Roy D.K., Sur P., Banerjee A., *Trans. Roy. Soc. Trop. Med. Hyg.* **81**, 738-741 (1987)
39. Baum S.G., Wittner M., Nadler P., Horwitz S.B., Dennis J.E., Tanowitz H.B., *Proc. Natl. Acad. Sci. USA* **78**, 4571-4575 (1981)
40. Goijman S.G., Turrens J.F., Marini-Bettolo G.B., Stoppani A.O.M., *Experientia* **41**, 646-648 (1985)
41. Gonzales-Garza M.T., Said-Fernandez S., *Exp. Parasit.* **66**, 253-255 (1988)

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie

Nouméa, Nouvelle-Calédonie, 26-30 Août 1991

ACTES



Editeurs : Cécile DEBITUS, Philippe AMADE,
Dominique LAURENT, Jean-Pierre COSSON