ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1994, p. 96–103
0066-4804/94/$04.00+0

Vol. 38, No. 1

In Vitro Antiplasmodial, Antiamoebic, and Cytotoxic Activities of a Series of Bisbenzylisoquinoline Alkaloids

SARAH J. MARSHALL,[1]† PETER F. RUSSELL,[1] COLIN W. WRIGHT,[2] M. MARIE ANDERSON,[2] J. DAVID PHILLIPSON,[2]* GEOFFREY C. KIRBY,[3] DAVID C. WARHURST,[3] AND PAUL L. SCHIFF, JR.[4]

Pharmaceutical Sciences Research Group, Department of Pharmacy, University of Brighton, Brighton BN2 4GJ,[1] Department of Pharmacognosy, The School of Pharmacy, London WC1N 1AX,[2] and Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London WC1E 7HT,[3] United Kingdom, and Department of Pharmacognosy, The School of Pharmacy, University of Pittsburgh, Pittsburgh, Pennsylvania 15261[4]

Received 1 December 1992/10 February 1993/Accepted 12 April 1993

Twenty-four bisbenzylisoquinoline alkaloids were screened for antiplasmodial, antiamoebic, and cytotoxic activities by use of in vitro microtests. Eight of the alkaloids had antiplasmodial activity, with a 50% inhibitory concentration (IC_{50}) of less than 1 μM against a multidrug-resistant strain of *Plasmodium falciparum* (chloroquine had an IC_{50} of 0.2 μM). The three alkaloids most active against *Entamoeba histolytica*, aromoline, isotrilobine, and insularine, had IC_{50}s of 5 to 11.1 μM (metronidazole had an IC_{50} of 1.87 μM). None of the 24 bisbenzylisoquinoline alkaloids exhibited significant cytotoxicity against the KB cell line, the most toxic being berbamine, with an IC_{50} of 17.8 μM (the IC_{50} of podophyllotoxin was 0.008 μM). Bisbenzylisoquinoline alkaloids merit further investigation as potential novel antimalarial agents.

It is estimated that about 500 million cases of malaria occur annually (19), with between one and two million deaths being caused by *Plasmodium falciparum* (2). It is believed that 75 to 80% of the world's population relies on traditional remedies to treat the disease (10); plants thus represent the only antimalarial therapy for a large proportion of the world's population. The reputation of plants used as febrifuges and in the treatment of malaria is increasingly being investigated, since plants may yield novel antimalarial agents. As part of a continuing investigation of plants used in traditional medicine (4, 13–16, 24), we have screened for in vitro antimalarial activity 12 plants which have reputations as either antimalarial agents or febrifuges in Sierra Leone (11, 15). The most active extract was that from the wood of a menispermaceous climber, *Triclisia patens*, a plant known to contain bisbenzylisoquinoline (BBIQ) alkaloids (3, 20). Screening of the related species *Triclisia dictyophylla* revealed that this plant also has potent antiplasmodial properties in vitro. Constituent alkaloids, such as phaeanthine, pycnamine, aromoline, isotetrandrine, obamegine, and gilletine, from both species showed antiplasmodial activity in vitro (11, 15, 16), and it is thought that these alkaloids are responsible, at least in part, for the antimalarial activity of these two menispermaceous species. Independent investigations into the antimalarial activity of *Tiliacora triandra* (Menispermaceae) from Thailand have shown that the BBIQ alkaloids tiliacorine, tiliacorinine, and nortiliacorinine A are the active principles (17). The antimalarial activity of eight BBIQ alkaloids is the subject of a patent (22).

Plants containing BBIQ alkaloids are also used in traditional medicine for the treatment of a number of diseases, including amoebic dysentery, leishmaniasis, bacterial infections, and cancer (6, 8–10, 12). The BBIQ alkaloid tetrandrine has been used clinically in China for the treatment of amoebic dysentery, lung cancer, and leukemia (9). To further our understanding of some of these activities, we decided to investigate the in vitro action of 24 BBIQ alkaloids against *P. falciparum*, *Entamoeba histolytica*, and KB cells (carcinoma of the human nasopharynx). The aims of this investigation were to ascertain some of the structural requirements for activity, to learn whether these alkaloids have any selectivity of action, and to provide further insight into the active principles of plants which are used in traditional medicine.

MATERIALS AND METHODS

BBIQ samples were available in one of our laboratories (that of P.L.S.). Chloroquine diphosphate, emetine dihydrochloride, and podophyllotoxin were obtained from Sigma, and metronidazole was obtained from May & Baker. The methods used for the in vitro screening of the alkaloids for antiplasmodial, antiamoebic, and cytotoxic activities have been described in detail elsewhere (1, 13, 14, 24) and will only be outlined here.

In vitro microtest for detecting antiplasmodial activity. The screening procedure for antiplasmodial activity compared the inhibition of the incorporation of tritiated hypoxanthine into drug-treated *P. falciparum*-infected erythrocytes, untreated infected erythrocytes, and uninfected controls. A multidrug-resistant strain of *P. falciparum* (K1) was maintained according to the methods of Trager and Jensen (21) and Fairlamb et al. (5) in human A^+ erythrocytes suspended in RPMI 1640 medium supplemented with A^+ serum and D-glucose. Prior to testing, cultures (predominantly ring stages) were diluted to 1% parasitemia. Alkaloids were

* Corresponding author. Mailing address: Department of Pharmacognosy, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom. Phone: 010 44 71 753 5847. Fax: 010 44 71 753 5909.
† Née Partridge.

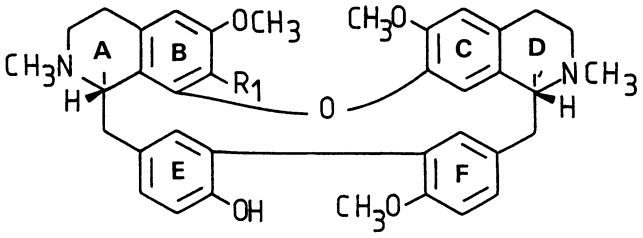
TABLE 1. In vitro antiplasmodial, cytotoxic, and antiamoebic activities of BBIQ alkaloids

Drug	Antiplasmodial activity		Cytotoxic activity		Antiamoebic activity	
	IC_{50} (μM)	SEM (*n*)	IC_{50} (μM)	SEM (*n*)	IC_{50} (μM)	95% Confidence interval
BBIQ alkaloid type						
IV						
Funiferine	0.63	0.15 (3)	108	25.7 (4)	45.5	30.7–67.3
Tiliageine	6.32	1.76 (6)	>411		31.6	20.4–48.8
VI						
Daphnoline	0.96	0.26 (4)	46.4	6.21 (4)	>86.2	
Aromoline	1.36	0.42 (4)	105	—[a] (2)	≈5.05	
Homoaromoline	3.46	0.29 (4)	>82.2		17.3	15.0–20.1
Oxyacanthine HCl	1.06	0.19 (4)	74.4	8.04 (4)	32.3	25.1–41.4
VII (thalisopidine)	0.09	— (2)	41.2	4.67 (3)	≈80.1	
VIII						
Phaeanthine	1.46	0.32 (4)	43.6	5.95 (4)	17.4	15.1–19.8
Tetrandrine	0.57	0.17 (4)	NT		16.9	12.0–23.0
Isotetrandrine	0.16	0.09 (4)	NT		22.2	15.7–31.3
Tetrandrine methiodide	>65.4	— (4)	>32.7	— (2)	39.5	33.0–47.8
Pycnamine	0.83	0.39 (4)	31.9	6.83 (4)	NT	
Fangchinoline	1.43	0.64 (4)	104	4.42 (4)	24.2	21.4–27.5
Berbamine	0.45	0.31 (4)	17.8	0.82 (4)	36.8	29.6–45.7
Obamegine	0.74	0.27 (4)	55.4	6.54 (4)	30.8	24.9–36.0
XVIII (dinklacorine)	3.92	1.53 (4)	54.8	10.8 (4)	34.4	25.3–46.5
XX (isochondodendrine)	22.0	1.87 (4)	>421	— (2)	17.7	14.8–21.0
XIII						
Trigilletimine	42.1	— (2)	>448	— (2)	41.7	31.0–56.1
Cocsoline	1.16	— (2)	NT		18.0	16.8–19.3
Cocsuline	≈88.9	— (2)	>222	— (2)	23.5	15.3–37.7
Isotrilobine	2.06	0.41 (4)	18.8	0.86 (4)	10.8	13.8–95.6
Cocsuline methiodide	>17.8	— (2)	>355	— (2)	36.2	13.8–95.6
XXIV (gilletine)	1.81	0.50 (4)	38.3	3.47 (4)	20.9	15.6–28.2
XXVI (insularine picrate)	2.07	0.29 (4)	>294		11.1	6.63–18.6
Standards						
Chloroquine diphosphate	0.20	0.03 (8)				
Podophyllotoxin			0.008	0.003 (4)		
Emetine dihydrochloride					2.23	1.99–2.46
Metronidazole					1.87	1.85–1.90

[a] —, SEM not calculated for two determinations.

dissolved or micronized in ethanol and further diluted with RPMI 1640 medium (the final ethanol concentration at testing did not exceed 0.5%, shown to have no deleterious effects on parasite growth). Alkaloids were tested twice in duplicate at 12 two- or fourfold dilutions in 96-well microtiter plates, beginning at 50 or 500 μg/ml. Alkaloids at these concentrations were incubated at 37°C with infected erythrocytes for 24 h, after which 5 μl of tritiated hypoxanthine (40 μCi/ml; Amersham) was added. After a further 18 to 24 h of incubation, cells were harvested onto glass fiber filters and, after the addition of scintillant (Ecoscint; National Diagnostics), vials were counted for tritium activity in a Tri-Carb scintillation spectrometer (Packard model 574). Chloroquine was also included as a standard antimalarial control. Values for counts per minute obtained were converted into percent inhibition of incorporation of radiolabel, and 50% inhibitory concentrations (IC_{50}s) were derived from the resulting sigmoidal curves following linear regression analysis.

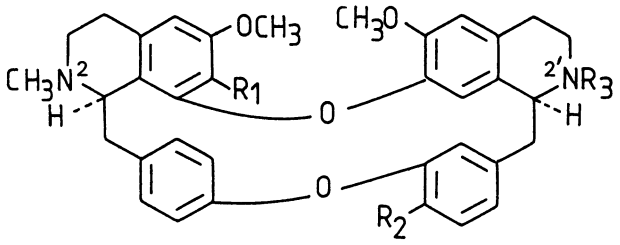
In vitro microtest for assessing antiamoebic activity. The screening procedure for antiamoebic activity (24) used *E. histolytica* (NIH 200) cultured at 37°C in Diamond's TPS-1 medium, with casein-digested peptone replacing the Trypticase (24). Alkaloids were dissolved or micronized in ethanol, further diluted in culture medium (the maximum ethanol concentration at testing was 0.25%, not inhibitory to amoebal growth), and incubated at eight concentrations with *E. histolytica* at 37°C for 72 h. Plates were quickly inverted to remove medium and rinsed with normal saline at 37°C. After the plates were dried, the amoebae were fixed with metha-



Type IV

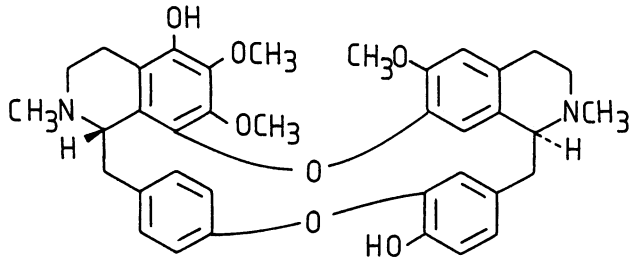
1 Funiferine S,R R_1=OCH_3

2 Tiliageine S,R R_1=OH



Type VI

3 Daphnoline	R,S	R_1=OH	R_2=OH	R_3=H
4 Aromoline	R,S	R_1=OH	R_2=OH	R_3=CH_3
5 Homoaromoline	R,S	R_1=OH	R_2=OCH_3	R_3=CH_3
6 Oxyacanthine	R,S	R_1=OCH_3	R_2=OH	R_3=CH_3



Type VII

7 Thalisopidine S,S

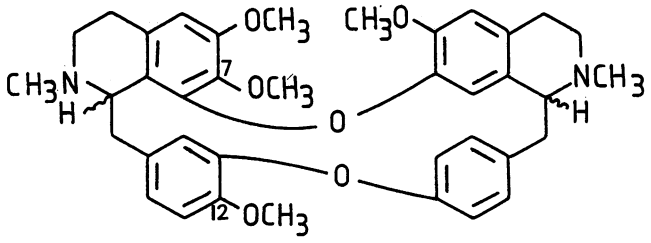
FIG. 1. Structures and stereochemistry of BBIQ alkaloids.

nol, the plates were dried once more, and the amoebae were stained with eosin. Sodium hydroxide was added to each stained well to dissolve the eosin-protein complex, and the absorbance of each well was read with a microplate reader (Minireader II; Dynatech Laboratories). Optical density has been shown to be proportional to the number of cells; hence, it is possible to measure the percent inhibition of growth and to determine IC_{50}s. Alkaloids were tested in duplicate at twofold dilutions, beginning at 50 μg/ml. Emetine and metronidazole were used as controls.

In vitro microtest for assessing cytotoxicity. The cytotoxicity test was developed by Anderson and colleagues (1) and, like the antiamoebic activity screening procedure, used eosin as a stain for cellular protein and optical density as a measure of cell number. The KB cell line originated from a nasopharyngeal carcinoma and was maintained for this study in Eagle's modified minimum essential medium with Earle's salts and sodium bicarbonate. The medium was further supplemented with bovine serum, glutamine, and nonessential amino acids. Alkaloids were dissolved or micronized in ethanol (the maximum ethanol concentration was 2.5%, previously determined to have no effect on cell growth) and tested in a 96-well microtiter plate at 12 twofold dilutions, beginning at 125 or 250 μg/ml. After 48 h of incubation at 37°C, the medium was removed and eosin was used to assess cell growth as described for the antiamoebic activity screening procedure. IC_{50}s were also derived as described above. Podophyllotoxin was tested as a control.

RESULTS

The in vitro IC_{50}s of the 24 BBIQ alkaloids screened against *P. falciparum*, *E. histolytica*, and the KB cell line are

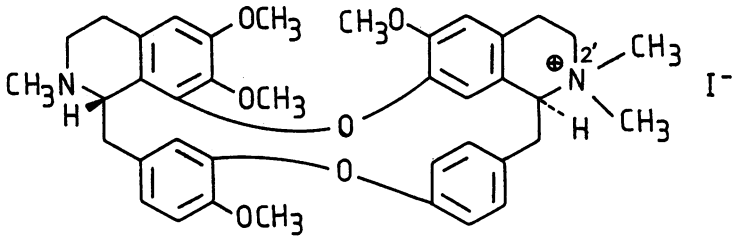


Type VIII

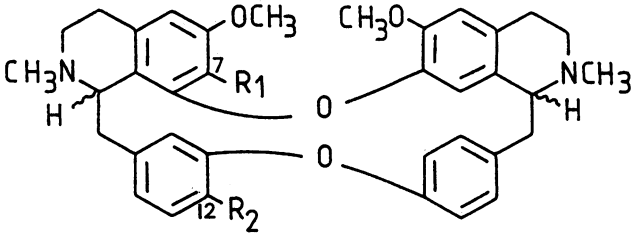
8 Phaeanthine R,R

9 Tetrandrine S,S

10 Isotetrandrine R,S



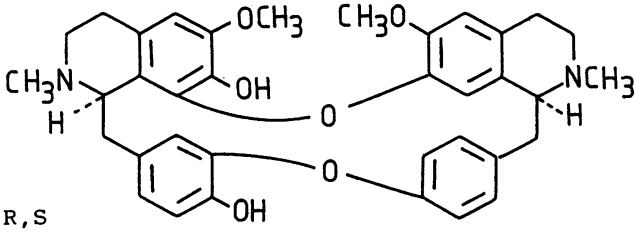
11 Tetrandrine methiodide S,S



12 Pycnamine R,R R_1=OCH_3 R_2=OH

13 Fangchinoline S,S R_1=OH R_2=OCH_3

14 Berbamine R,S R_1=OCH_3 R_2=OH



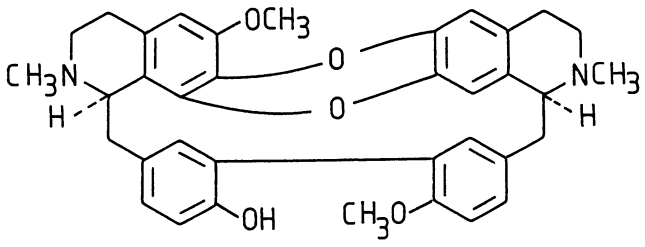
15 Obamegine R,S

FIG. 1—*Continued.*

given in Table 1. The IC_{50}s in the antimalarial activity and cytotoxicity tests are the means of several determinations, whereas those in the antiamoebic activity test represent duplicate results obtained in the same determination, computed together to yield a single IC_{50} value with 95% confidence limits for that value.

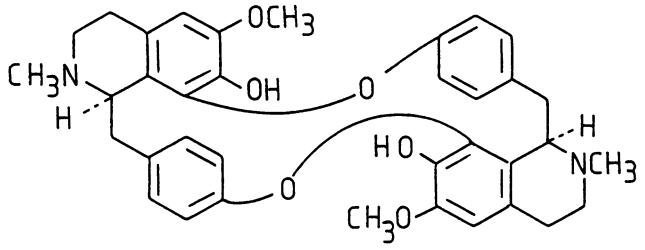
Table 1 shows that, of the 24 alkaloids screened for in vitro antiplasmodial activity, 19 had IC_{50}s of less than 10 μM; 8 of these had IC_{50}s of less than 1 μM. The IC_{50} of chloroquine against the same multidrug-resistant strain of *P. falciparum* was 0.2 μM.

In contrast, none of the alkaloids tested in the KB cell screen showed activity comparable to that of the standard, podophyllotoxin, which had an IC_{50} of 0.008 μM. The most



Type XVIII

16 Dinklacorine R,S



Type XX

17 Isochondodendrine R,R

FIG. 1—*Continued.*

cytotoxic alkaloid, berbamine, was more than 2,000-fold less active (IC_{50}, 17.8 μM) than the control drug.

Significant antiamoebic activity, comparable to those of the standards emetine (IC_{50}, 2.23 μM, when tested as the dihydrochloride) and metronidazole (IC_{50}, 1.87 μM), was demonstrated by aromoline, isotrilobine, and insularine.

DISCUSSION

BBIQ alkaloids comprise two isoquinoline moieties ("head" portions) linked to two benzyl moieties ("tail" portions). They have been classified into 26 structural types (denoted by Roman numerals) according to the number, position, and type of bridges linking the two monomers (18). The alkaloids investigated in the present work are representative of nine of the known structural types, namely, types IV, VI, VII, VIII, XVIII, XX, XXIII, XXIV, and XXVI (Fig. 1). Alkaloids within each group differ from one another by their stereochemistry at C-1 and C-1′ and/or their substituents and nitrogen functionalities (Fig. 1).

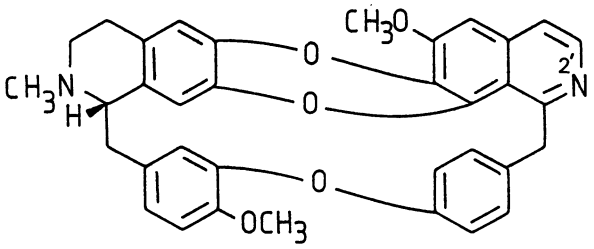
The results shown in Table 1 revealed that eight of the BBIQ alkaloids tested had IC_{50}s of less than 1 μM against multidrug-resistant *P. falciparum* in vitro. A further 11 had IC_{50}s between 1 and 10 μM. Under the same test conditions, chloroquine had an IC_{50} of 0.2 μM. The most potent antimalarial BBIQ alkaloids were found to be mainly of type VIII (Table 1). The IC_{50}s determined by other workers (22) for phaeanthine, pycnamine, isotetrandrine, berbamine, obamegine, and tetrandrine against chloroquine-resistant *P. falciparum* (W2 strain) are in good agreement with those obtained by us against the K1 strain (Table 1). We reported previously that phaeanthine (8) is twice as potent against chloroquine-resistant *P. falciparum* (K1) in vitro as against a chloroquine-susceptible clone (T9-96) (4).

In assessing structure-activity relationships, it can be seen that the 19 BBIQ alkaloids with in vitro antimalarial activities represented by IC_{50}s of less than 10 μM (Table 1) have a range of different structures (Fig. 1). Dimers of the head-to-head and head-to-tail types, e.g., phaeanthine and insularine, are active. The other bridges between the monomeric units may be two or three in number (e.g., tetrandrine and isotrilobine) and may be attached at several different positions on the monomers. It is not necessary for activity that there be an ether linkage between the two monomeric units; e.g., funiferine contains a direct carbon-carbon linkage. The status of the nitrogen atoms is fundamental to antiplasmodial activity, as evidenced by trigilletimine, with an aromatic N-2′, being 20-fold less active than isotrilobine, which is the corresponding D ring-saturated, N-2′-methylated analog. Quaternization of N-2′ results in the loss of antiplasmodial activity, as indicated by a comparison of tetrandrine methiodide and tetrandrine (Table 1), and has also been observed to reduce the cytotoxicity of BBIQ alkaloids for HeLa cells (8). Daphnoline and its N-2′-methylated analog aromoline have IC_{50}s of similar magnitudes, indicating that there is little difference in activity between secondary amine and tertiary N-methyl functions in ring D.

The substituents present in each monomeric half of the molecule may influence antiplasmodial activity. A comparison of phenols and their corresponding methyl ethers indicates that a phenolic hydroxyl may result in less activity; tiliageine is 10 times less active than funiferine, and cocsuline is 40 times less active than isotrilobine (Table 1). In contrast, some phenolic substituents result in an increase in activity in comparison with their corresponding methyl ethers, as exemplified by aromoline, which is twice as active as homoaromoline. A comparison of the antiplasmodial activities of aromoline and oxyacanthine, phaeanthine and pycnamine, and tetrandrine and fangchinoline (Table 1) shows that for these pairs of alkaloids, there is little difference in activity between alkaloids possessing particular phenolic substituents and those possessing their corresponding methyl ethers.

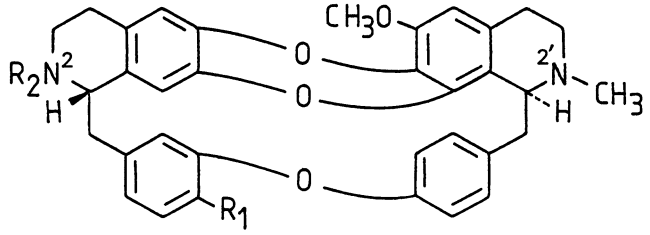
Antiplasmodial activity was observed for alkaloids possessing *R,R*, *S,S*, *R,S*, and *S,R* configurations at C-1 and C-1′. A direct comparison of three alkaloids with identical ether linkages and substituents and differing only at C-1 and C-1′ could be made for phaeanthine (*R,R*), tetrandrine (*S,S*), and isotetrandrine (*R,S*), which had IC_{50}s of 1.46, 0.57, and 0.16 μM, respectively. There were, however, no significant differences between the activities of phaeanthine and tetrandrine or tetrandrine and isotetrandrine, albeit isotetrandrine was significantly more active than phaeanthine.

None of the 24 alkaloids tested showed significant cytotoxic activity against KB cells, the most active being berbamine, with an IC_{50} of 17.8 μM (Table 1). In assessing selectivity of action against *P. falciparum* versus mammalian cells, we reported previously that the ratio of IC_{50}s in vitro for chloroquine diphosphate is 347 (IC_{50} for KB cells/IC_{50} for *P. falciparum* Kl) (1). In the present investigation, the least cytotoxic alkaloid, thalisopidine, had a cytotoxic activity/antiplasmodial activity ratio of 455, which was more favorable than that of the more cytotoxic berbamine, which had a corresponding ratio of 39.5. These ratios, which are indicators of selectivity, should be regarded with some caution. The IC_{50}s of the BBIQ alkaloids given in Table 1 were the result of a 48-h incubation period, whereas the IC_{50}s obtained by the National Cancer Institute (7) were the result of 72 h of incubation. The IC_{50}s of daphnoline were similar for both incubation periods, whereas the National Cancer Insti-

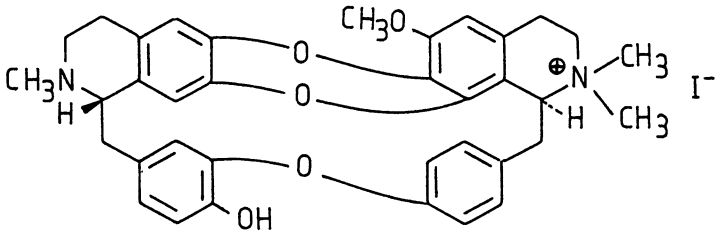


Type XXIII

18 Trigilletimine



19 Cocsoline	S,S	R_1=OH	R_2=H
20 Cocsuline	S,S	R_1=OH	R_2=CH_3
21 Isotrilobine	S,S	R_1=OCH_3	R_2=CH_3



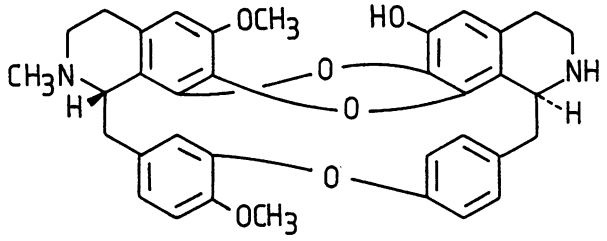
22 Cocsuline methiodide S,S

FIG. 1—*Continued.*

tute IC_{50}s of tetrandrine, isotetrandrine, fangchinoline, berbamine, and obamegine were between 0.1 and 8 μM (7) (see Table 1 for a comparison).

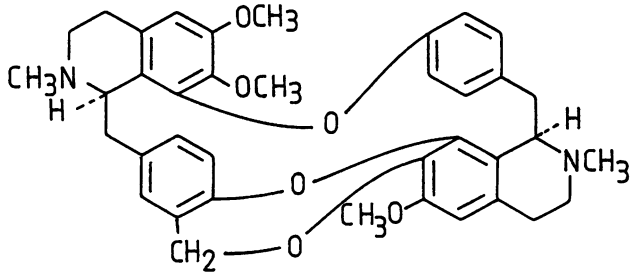
The most active BBIQ alkaloids against *E. histolytica* in vitro were aromoline, isotrilobine, and insularine, which had IC_{50}s in the range of 5 to 11 μM (Table 1). The IC_{50}s of the control antiamoebic drugs emetine hydrochloride and metronidazole were 2.2 and 1.87 μM, respectively (Table 1). The in vitro activity of the BBIQ alkaloids tested was markedly lower against *E. histolytica* than against *P. falciparum*, indicating that these alkaloids show some selectivity in antiprotozoal action.

The results given in Table 1 for the activities of BBIQ alkaloids against *P. falciparum*, *E. histolytica*, and KB cells in vitro provide some justification for the use of menispermaceous plants in traditional medicine as treatments for malaria and amoebic dysentery. Other factors besides direct chemotherapeutic activity may be involved, and it has been reported that some BBIQ alkaloids have immunostimulant activity (23). The most pronounced activity observed in the present investigation was against *P. falciparum*. Some of the BBIQ alkaloids have greater activity against chloroquine-resistant strains of *P. falciparum* than against chloroquine-susceptible strains and may be of use in combination with chloroquine in preventing resistance (4, 22, 25). Tetrandrine reportedly reverses the resistance of *P. falciparum* to chloroquine and has a long-acting synergistic effect on the antimalarial action of artemisinin (22). BBIQ alkaloids merit further investigation as potential novel antimalarial drugs.



Type XXIV

23 Gilletine S,R



Type XXVI

24 Insularine R,R

FIG. 1—*Continued.*

ACKNOWLEDGMENTS

The Royal Pharmaceutical Society of Great Britain, the Medical Research Council, the Science and Engineering Research Council, and the Wellcome Trust are gratefully acknowledged for their generous financial support. C. W. Wright held a Maplethorpe postdoctoral fellowship from the University of London, and D. C. Warhurst is supported by the Public Health Laboratory Service.

REFERENCES

1. **Anderson, M. M., M. J. O'Neill, J. D. Phillipson, and D. C. Warhurst.** 1991. *In vitro* cytotoxicity of a series of quassinoids from *Brucea javanica* fruits against KB cells. Planta Med. **57**:62–64.
2. **Björkman, A., and P. A. Phillips-Howard.** 1990. Drug-resistant malaria: mechanisms of development and inferences for malaria control. Trans. R. Soc. Trop. Med. Hyg. **84**:323–324.
3. **Dwuma-Badu, D., J. S. K. Ayim, A. N. Tackie, M. A. El Sohly, J. E. Knapp, D. J. Slatkin, and P. L. Schiff.** 1975. Trigilletimine: a new bisbenzylisoquinoline alkaloid from *Triclisia* species. Experientia **31**:1251–1252.
4. **Ekong, R., S. J. Partridge, M. M. Anderson, G. C. Kirby, D. C. Warhurst, P. F. Russell, and J. D. Phillipson.** 1991. *Plasmodium falciparum*: effects of phaeanthine, a naturally-occurring bisbenzylisoquinoline alkaloid, on chloroquine-resistant and -sensitive parasites *in vitro*, and its influence on chloroquine activity. Ann. Trop. Med. Parasitol. **85**:205–213.
5. **Fairlamb, A. H., D. C. Warhurst, and W. Peters.** 1985. An improved technique for the cultivation of *Plasmodium falciparum in vitro* without daily medium change. Ann. Trop. Med. Parasitol. **79**:379–384.
6. **Fournet, A., V. Muñoz, A. M. Manjon, A. Angelo, R. Hocquemiller, D. Cortes, A. Cave, and J. Bruneton.** 1988. Activité antiparasitaire d'alcaloïdes bisbenzylisoquinolinoleiques. a. Activité *in vitro* sur des promastigotes de trois souches de Leishmania. J. Ethnopharmacol. **24**:327–335.
7. **Hartwell, J. L., and B. J. Abbott.** 1969. Antineoplastic principles in plants: recent developments in the field. Adv. Pharmacol. Chemother. **7**:117–209.
8. **Kuroda, H., S. Nakazawa, K. Katagiri, O. Shiratori, M. Kozuka, K. Fujitani, and M. Tomita.** 1976. Antitumour effect of bisbenzylisoquinoline alkaloids. Chem. Pharm. Bull. (Tokyo) **24**:2413–2420.
9. **Liu, C., and P. Xiao.** 1983. Biological activities and domestic resources of bis(benzylisoquinoline) alkaloids. Yaoxue Tongbao **18**:287–292.
10. **Marini-Bettolo, G. B.** 1980. Present aspects of the use of plants in traditional medicine. J. Ethnopharmacol. **2**:5–7.
11. **Marshall, S. J.** 1991. Ph.D. thesis. Brighton Polytechnic, Brighton, United Kingdom.
12. **Oliver-Bever, B.** 1986. Medicinal plants in tropical West Africa. Cambridge University Press, Cambridge.
13. **O'Neill, M. J., D. H. Bray, P. Boardman, J. D. Phillipson, and D. C. Warhurst.** 1985. Plants as sources of antimalarial drugs. Part 1. *In vitro* test method for the evaluation of crude extracts from plants. Planta Med. **61**:394–398.
14. **O'Neill, M. J., D. H. Bray, P. Boardman, J. D. Phillipson, D. C. Warhurst, W. Peters, and M. Suffness.** 1986. Plants as sources of antimalarial drugs: in vitro antimalarial activities of some quassinoids. Antimicrob. Agents Chemother. **30**:101–104.
15. **Partridge, S. J., P. F. Russell, G. C. Kirby, D. H. Bray, D. C. Warhurst, J. D. Phillipson, M. J. O'Neill, and P. L. Schiff.** 1988. *In vitro* antimalarial activity of *Triclisia patens* and some of its constituent alkaloids. J. Pharm. Pharmacol. **40**(Suppl.):53.
16. **Partridge, S. J., P. F. Russell, G. C. Kirby, D. C. Warhurst, J. D. Phillipson, M. J. O'Neill, and P. L. Schiff.** 1989. *In vitro* antimalarial activity of *Triclisia dictyophylla* and some of its constituent alkaloids. J. Pharm. Pharmacol. **41**(Suppl.):92.
17. **Pavanand, K., H. K. Webster, K. Yongvanitchit, and T. Dechati-**

wongse. 1981. Antimalarial activity of *Tiliacora triandra* Diels. against *Plasmodium falciparum in vitro*. Phytother. Res. **3:**215–217.

18. **Shamma, M., and J. L. Moniot.** 1976. The systematic classification of bisbenzylisoquinolines. Heterocycles **4:**1817–1824.
19. **Sturchler, D.** 1989. How much malaria is there world-wide? Parasitol. Today **5**(2)**:**39–40.
20. **Tackie, A. N., D. Dwuma-Badu, T. Okarter, J. E. Knapp, D. J. Slatkin, and P. L. Schiff.** 1974. Constituents of West African medicinal plants. II. The isolation of alkaloids from selected *Triclisia* species. Lloydia **37:**1–5.
21. **Trager, W., and J. B. Jensen.** 1976. Human malaria parasites in continuous culture. Science **163:**673–675.
22. **Van Dyke, K.** June 1991. U.S. patent 5,025,020.
23. **Wagner, H., and A. Proksch.** 1985. Immunostimulatory drugs of fungi and higher plants, p. 113–153. *In* H. Wagner, H. Hikino, and N. R. Farnsworth (ed.), Economic and medicinal plant research, vol. 1. Academic Press, London.
24. **Wright, C. W., M. J. O'Neill, J. D. Phillipson, and D. C. Warhurst.** 1988. Use of microdilution to assess in vitro antiamoebic activities of *Brucea javanica* fruits, *Simarouba amara* stem, and a number of quassinoids. Antimicrob. Agents Chemother. **32:**242–248.
25. **Ye, Z., K. Van Dyke, and V. Castranova.** 1989. The potentiating action of tetrandrine in combination with chloroquine or qinghaosu against chloroquine-sensitive and -resistant falciparum malaria. Biochem. Biophys. Res. Commun. **165:**758–765.