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Warhurst, D. C. ; Quetin-Leclercq, Joëlle ; Angenot, Luc

ABSTRACT

Eight naturally occurring anhydronium bases and the synthetic quaternary compound Nb-methylharmalane were tested against Plasmodium falciparun (strain K1) in vitro. Cryptolepine was found to have similar activity to that of chloroquine but alstonine, 5,6-dihydroflavopereirine, matadine, Nb-methylharmalane, melinonine F, normelinonine F, strychnoxanthine and serpentine were found to have little activity. Cryptolepine, given orally to mice infected with Plasmodium berghei berghei was found to have moderate antimalarial activity; parasitemia was suppressed by 80% at 50 mg/kg/day.

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SHORT COMMUNICATION

Antimalarial Activity of Cryptolepine and Some Other Anhydronium Bases

C. W. Wright*

Postgraduate Studies in Pharmaceutical Technology, The School of Pharmacy, University of Bradford, Bradford, West Yorkshire BD7 1DP, UK

J. D. Phillipson

Department of Pharmacognosy, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, UK

S. O. Awe

Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria

G. C. Kirby and D. C. Warhurst

Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

J. Quetin-Leclercq and L. Angenot

Service de Pharmacognosie, Institut de Pharmacie, Université de Liège, Rue Fusch, 5, B-4000 Liège, Belgium

Eight naturally occurring anhydronium bases and the synthetic quaternary compound N*b*-methylharmalane were tested against *Plasmodium falciparum* (strain K1) *in vitro*. Cryptolepine was found to have similar activity to that of chloroquine but alstonine, 5,6-dihydroflavopereirine, matadine, N*b*-methylharmalane, melinonine F, normelinonine F, strychnoxanthine and serpentine were found to have little activity. Cryptolepine, given orally to mice infected with *Plasmodium berghei berghei* was found to have moderate antimalarial activity; parasitaemia was suppressed by 80% at 50 mg/kg/day.

Keywords: alkaloids; cryptolepine; *Plasmodium* spp.; *Alstonia* spp.; *Cryptolepis sanguinolenta*; *Strychnos* spp.

INTRODUCTION

A number of plant species used traditionally for the treatment of malaria contain indole alkaloids which are anhydronium bases. One of these, the West African shrub *Cryptolepis sanguinolenta* contains cryptolepine, and extracts of the roots of this species are used clinically in Ghana for the treatment of malaria (Boye and Oku-Ampofo, 1983). The barks of several species of *Alstonia* are used widely in Asia as well as in West Africa for malaria treatment and contain many constituent alkaloids including the anhydronium bases alstonine and alstoniline. However, despite the wide use of the above species there is little convincing evidence that they are clinically effective antimalarials (Kirby *et al.*, 1995; Wright *et al.*, 1993). In this paper we report the activities of cryptolepine and a number of other anhydronium bases against *Plasmodium falciparum in vitro* and the effects of cryptolepine given orally to mice infected with *Plasmodium berghei berghei*. The results are discussed in the context of previously published data.

MATERIALS AND METHODS

Sources of alkaloids. Strychnoxanthine was isolated from *Strychnos gossweileri* root bark as previously described (Coune *et al.*, 1984). *S. gossweileri* was also the source of matadine (Quetin-Leclercq *et al.*, 1991) and of alstonine. Powdered bark was macerated for 24 h with ethanol:water:acetic acid 16:3:1 and then extracted with the same mixture. The extract was concentrated under reduced pressure, basified to pH 8 with sodium hydroxide (10%) and then extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulphate, concentrated and then partitioned between the two phases of chloroform:methanol:water 4:4:3. The aqueous phase was evaporated and the residue fractionated on a reversed-phase Lobar column (LichroPrep RP-8) using acetone:water 1:9. Fractions containing alstonine were chromatographed on a medium pressure column (Superformance) filled with silica 60 eluted with ether:methanol:diethylamine 70:30:1. Alstonine containing fractions were quickly evaporated (to avoid decomposition of alstonine) and finally purified using Fractogel TSK HW 40S eluted with ethanol. The acetate was prepared by addition of acetic acid and precipitation with ether.

Dried, powdered root of *Cryptolepis sanguinolenta* from Togo was moistened with ammonia and extracted with chloroform. The concentrated extract was chromatographed over aluminium oxide eluted with chloroform:methanol 95:5 to obtain the major alkaloid base, cryptolepine which was crystallized as the hydrochloride using methanol/water as the solvent. Spectroscopic techniques, (MS, NMR, UV, IR) were employed to confirm the identity of cryptolepine.

Melinonine F, normelinonine F and 5,6-dihydroflavopereirine were obtained from *Strychnos usambarensis* as

* Author to whom correspondence should be addressed.

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previously described (Caprasse *et al.*, 1983) while N*b*-methylharmalane was prepared synthetically (Spath and Lederer, 1930).

Serpentine hydrogenotartrate was purchased from Fluka.

***In vitro* testing against *Plasmodium falciparum*.** This was carried out using a microdilution technique as described previously (Ekong *et al.*, 1990). The test relies upon the inhibition of incorporation of [G $-^3$H]-hypoxanthine into drug-treated infected red blood cells compared with untreated infected and uninfected red blood cells. The *P. falciparum* isolate used was the chloroquine and pyrimethamine resistant Thai strain K1. Dose-response curves were plotted and linear regression analysis was used to determine IC_{50} values, i.e. the concentration of drugs required to cause 50% inhibition of incorporation of [G $-^3$H]-hypoxanthine.

***In vivo* antimalarial testing.** Cryptolepine was assessed using the 4-day suppressive test against *P. berghei* infection in mice (Peters *et al.*, 1975). Albino Swiss mice (Wistar strain) weight 18–22 g, were inoculated with *P. berghei berghei* (each mouse received 1×10^7 infected erythrocytes by intraperitoneal injection) on the first day of the experiment. Groups of five mice were dosed daily by mouth for 4 consecutive days. On day 5 of the test a blood smear was taken and the animals were killed. The % suppression of parasitaemia was calculated for each dose level by comparing the parasitaemias present in infected controls with those of test animals. Chloroquine diphosphate was used as a positive control.

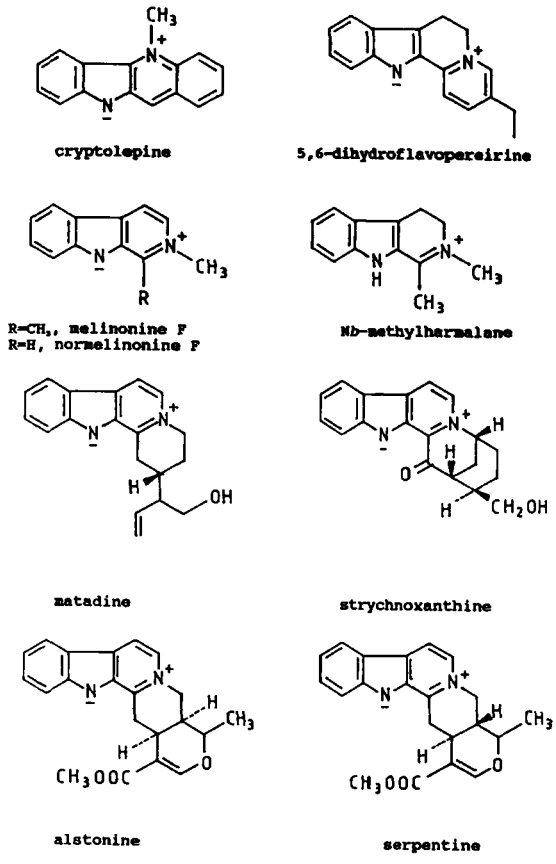

Figure 1. Structures of anhydronium bases and N*b*-methylharmalane.

RESULTS AND DISCUSSION

The structures and *in vitro* antiplasmodial activities of the anhydronium bases are shown in Fig. 1 and Table 1. Cryptolepine was the only compound found to have potent activity compared with chloroquine diphosphate (IC_{50} values 0.114 and 0.20 μM respectively) and this is consistent with previously published results (Kirby *et al.*, 1995). Melinonine F, normelinonine F, 5,6-dihydroflavopereirine serpentine and strychnoxanthine all exhibited very weak antiplasmodial activities while alstonine (tested as base, acetate and sulphate), matadine and N*b*-methylharmalane may be considered to be inactive. Interestingly, serpentine which is epimeric with alstonine was found to be several-fold more active than the latter. As alstonine is unstable, its degradation product was also tested but this was found to be inactive (IC_{50}=34.0 μg/mL).

It has been suggested that the target of cryptolepine is parasite DNA since experimental evidence and a structural similarity to the intercalator 9-aminoacridine supports this (Kirby *et al.*, 1995). Cryptolepine also has structural similarity to the DNA intercalator ellipticine (Kohn *et al.*, 1975). 9-aminoacridine has poor antiplasmodial activity, so that access of drug to the DNA target may be important. Similarly, in the present study the DNA-binding drugs melinonine F, normelinonine F and 5,6-dihydroflavopereirine (Caprasse and Houssier, 1984) show low antiplasmodial activity.

Table 2 shows the results obtianed from the *in vivo* investigation of cryptolepine against *P. berghei* in mice. Although the dose response curve obtained was rather flat, at the highest dose tested (50 mg/kg/day) parasitaemia was

Table 1. *In vitro* antiplasmodial activities of anhydronium bases, N*b*-methylharmalane and chloroquine diphosphate

Compound	IC_{50} μM±SD(*n*)
Alstonine acetate	30.2 (1)
Alstonine base	40.2 (1)
Alstonine sulphate	35.0 (1)
Cryptolepine hydrochloride	0.114±0.064 (3)
5,6-dihydroflavopereirine chloride	3.02±2.34 (2)
Matadine chloride	106 (1)
N*b*-methylharmalane iodide	79.7±6.13 (2)
Melinonine F chloride	5.13±1.02 (2)
Normelinonine F chloride	13.6±9.87 (2)
Strychnoxanthine chloride	8.43 (1)
Serpentine hydrogenotartrate	5.74±6.02 (2)
Chloroquine diphosphate	0.20±0.071 (2)

n=number of determinations

Table 2. *In vivo* antiplasmodial activities of cryptolepine hydrochloride and chloroquine diphosphate

Dose (mg/kg/day)	Parasitaemia (% red cells infected±SD)	Chemosuppression (%)
Control	34.4±3.5	0
Cryptolepine HCl		
12.5	11.6±1.27	66.3
25	10.5±2.17	69.6
50	6.74±0.23	80.5
Chloroquine diphosphate		
5	2.23±0.14	93.5

suppressed by 80.5% of that of the untreated control animals. However, with chloroquine diphosphate at 5 mg/kg/day parasitaemia was suppressed by 93.5%. Although relatively weak compared with chloroquine, the activity of cryptolepine found in this study is in marked contrast to that reported by Kirby *et al.* (1995) in which cryptolepine failed to produce a significant reduction in parasitaemia at a maximum dose of 113 mg/kg/day. The explanation for the different results obtained may lie in the routes of administration used; in the present study cryptolepine was administered orally while Kirby *et al.* (1995) used the subcutaneous route; further work will be needed in order to account for these observations.

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REFERENCES

Boye, G. L., and Oku-Ampofo (1983). Clinical uses of *Cryptolepis sanguinolenta*. In, *Proceedings of the First International Seminar on Cryptolepine.* 27–30 July 1983, University of Science and Technology, Kumasi, Ghana, p. 37–40.

Caprasse, M., Coune, C., and Angenot, L. (1983). Isolement et structure de trois bases anhydronium du *Strychnos usambarensis* du Rwanda. *J. Pharm. Belg.* **38**, 135–139.

Caprasse, M., and Houssier, C. (1984). Physico-chemical investigation of the mode of binding of the alkaloids 5,6-dihydroflavopereirine and sempervirine with DNA. *Biochimie* **66**, 31–41.

Coune, C., Tavernier, D., Caprasse, M., and Angenot, L. (1984). La strychnoxanthine, un alcaloide monoterpénique d'un nouveau type isolé à partir du *Strychnos gossweileri. Planta Med.* **50**, 93–95.

Ekong, R. M., Kirby, G. C., Patel, G., Warhurst, D. C., and Phillipson, J. D. (1990). Comparison of the *in vitro* activities of quassinoids with activity against *Plasmodium falciparum*, anisomycin and some other inhibitors of eukaryotic protein synthesis. *Biochem. Pharm.* **40**, 297–301.

Kirby, G. C., Noamese, B. K., Paine, A., Warhurst, D. C., and Phillipson, J. D. (1995) *In vitro* and *in vivo* antimalarial activity of cryptolepine, a plant-derived indoloquinoline. *Phytother. Res.* **9**, 359–363.

Kohn, K. W., Waring, M. J., Glaubiger, D., and Friedman, C. A. (1975). Intercalative binding of ellipticine to DNA. *Cancer Res.* **35**, 71–76.

Peters, W., Portus, J. H., and Robinson, B. L. (1975). The chemotherapy of rodent malaria XXII. The value of drug resistant strains of *Plasmodium berghei* in screening for blood schizonticidal activity. *Ann. Trop. Med. Parasitol.* **69**, 155–171.

Quetin-Leclercq, J., Coucke, P., Delaude, R., Warin, R., Bassleer, R., and Angenot, L. (1991). Matadine, a cytotoxic alkaloid from *Strychnos gossweileri. Phytochemistry* **30**, 1697–1700.

Spath, E., and Lederer, E. (1930). Synthese der Harmalaakaloide: harmalin, harmin und harman. *Chem. Ber.* **63**, 2102–2107.

Wright, C. W., Allen, D., Phillipson, J. D. *et al.* (1993). *Alstonia* species: are they effective in malaria treatment? *J. Ethnopharmacol.* **40**, 41–45.