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THE FIRST PROTOBERBERINE ALKALOID ANALOGUE WITH *IN VIVO* ANTIMALARIAL ACTIVITY

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Abstract
2,3,11,12-Tetramethoxyberbinium chloride at very low doses causes a marked reduction of parasitaemia produced by *Plasmodium chabaudi* infection in mice. The 2,3,10,11 and 13-amino-2,3,10,11-tetramethoxy analogues are inactive in this test system at the same dose levels, despite all three compounds having marked *in vitro* activity against *P. falciparum*. This is the first time that *in vivo* antimalarial activity of a protoberberine alkaloid analogue has been demonstrated. The parent alkaloid berberine also has activity *in vivo* against *P. chabaudi*, in contrast to its reported lack of activity against *P. berghei* in mice.

Despite persistent reports of the use of plant extracts containing protoberberine alkaloids in the folk treatment of malaria[1,2], and the demonstration of the potent antimalarial activity *in vitro* of a number of isolated compounds of this type[3], nobody has been able to demonstrate *in vivo* activity until now. The clearest and most systematic demonstration of the difference between *in vitro* and *in vivo* activity in this class of compounds was that conducted by Vennerstrom and Klayman at the Walter Reed Army Institute[4], who found *in vitro* potency against *Plasmodium falciparum* comparable to that of quinine, but a complete lack of effect in mice infected with *P. berghei*.

We have been developing a novel protoberberine synthesis, based on cyclisation of 1-cyano-2-benzyl-1,2,3,4-tetrahydroisoquinolines in anhydrous hydrogen fluoride. This method follows earlier work[5,7] on the synthesis of simpler isoquinolines and has proved to be effective, efficient and versatile, with yields on most steps in excess of 80%. A noted feature of this route is the intermediacy of a spiro-cyclised cation[8,9] following *ipso* attack of the protonated nitrile (1) on the benzyl substituent. The rearrangement which follows can thus give rise to 10,11-dimethoxy substitution (2b), from a 3,4-dimethoxybenzyl intermediate, or an 11,12-dimethoxy protoberberine (2a) from a 2,3-dimethoxybenzyl intermediate (Scheme 1). Previous studies, as for example

those of Vennerstrom and Klayman, have concentrated on compounds with the naturally occurring 9,10-oxygenation pattern.

The synthesis which we have developed gives rise to a 13-imino derivative (2) as the first-formed product after rearrangement. This intermediate is unstable and will either eliminate NH_3 (or hydrolyse[10] and eliminate H_2O) on attempted crystallisation (Scheme 2), giving directly and very conveniently the desired end-products (3a) or (3b). If allowed to stand in alkaline solution (2b) will oxidise to give the 13-amino salt (4). All three compounds ((3a), (3b) and (4)) have been tested against *P. falciparum* K1 multi-drug resistant strain *in vitro* and have similar IC_{50} values of 0.6 - 0.8 μg/ml, compared to the value for berberine[3] of 0.36 μg/ml.

In preliminary *in vivo* experiments neither (3b) nor (4) was active at the chosen dose level of 0.25 mg/kg. against *P. chabaudi* (AS strain) in BALB/c mice. The compound in buffered saline was given as a single intravenous dose one day after intraperitoneal infection with 10^6 parasitised red blood cells. However using a similar treatment regime compound (3a) not only markedly delayed the onset of a patent parasitaemia but significantly reduced ($p < 0.025$, unpaired Student's t test) the peak parasitaemia compared with untreated control animals (Fig 1).

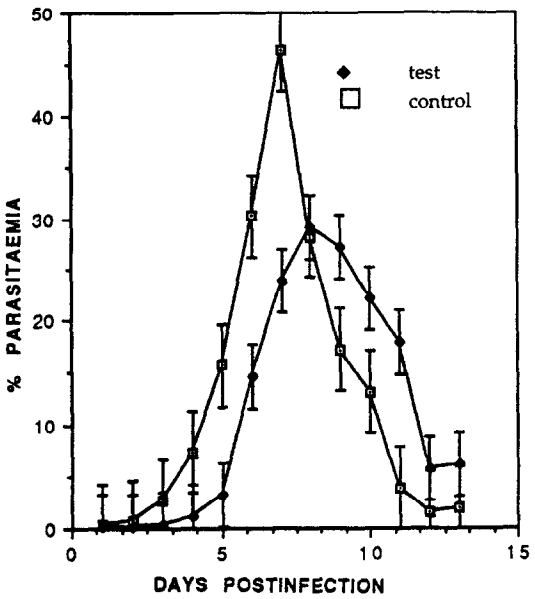


Fig 1. In vivo antimalarial activity of compound (3a) against P. chabaudi in BALB-c mice after a single i/v dose of 0.25 mg/kg: 5 mice in each group.

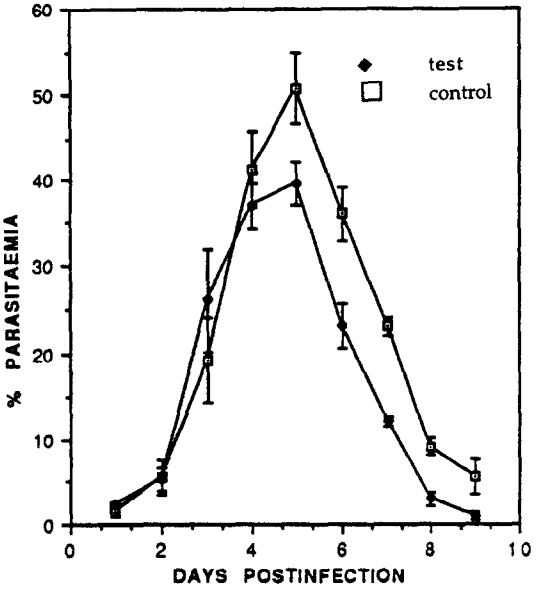
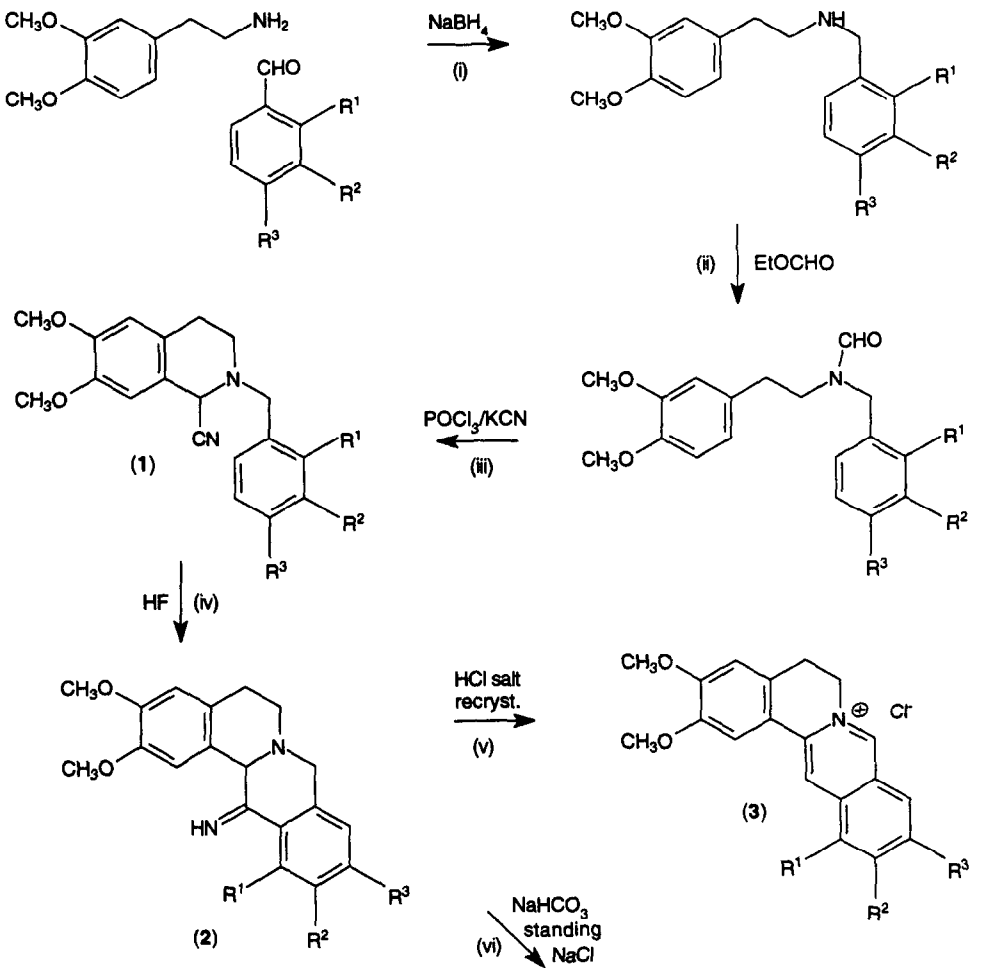


Fig 2. In vivo antimalarial activity of berberine against P. chabaudi in BALB-c mice after a single i/v dose of 0.25 mg/kg: 5 mice in each group.



(a) $R^1 = R^2 = OMe$, $R^3 = H$

Yields (%), m.p.°C (i) 92,oil (ii) 98,oil (iii) 83, 122-5 (iv) 100[a] (v) 48[b], >330

(b) $R^1 = H$, $R^2 = R^3 = OMe$

Yields (%), m.p.°C (i) 100,85 (ii) 91,85 (iii) 67, 132-3 (iv) 100[a] (v) 34[b], 221[c] (vi) 30[b], 268

[a]Base unstable, HCl salt gives (3) on recrystallisation.

[b]After several recrystallisations for biological tests.

[c] Lit.[11] 212-3°C from chloroform. Lit.[12] 207-8°C from methanol.

Scheme 1

This experiment has now been carried out five times, with reproducible results. In a similar experiment, berberine (0.25mg/kg) also produced a significant reduction in parasitaemia (Fig. 2).

These results show that substitution pattern is important, but may also indicate that *P. berghei* infections in mice are not an appropriate model for other *Plasmodium* infections in humans. The present results with berberine are in direct contrast to the results reported for *P.berghei* in mice[4]. It is not yet possible to predict whether differences between *in vitro* data against *P. falciparum* and *in vivo* data against *P. chabaudi* will reflect the differences for the same compounds against *P. falciparum in vitro* and *in vivo*.

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