Synthesis and *In Vivo* Antimalarial Evaluation of Isopropyl [(4-Chlorophenyl)amino]iminomethylcarbamimidate

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Abstract □ Isopropyl [(4-chlorophenyl)amino]iminomethylcarbamimidate was synthesized as a potential antimalarial agent. Biological activity was evaluated against *Plasmodium lophurae* in turkeys. The activity of this compound was compared to its nitrogen isostere, chloroguanide.

Keyphrases □ Carbamimidate ester, substituted—synthesized, screened for antimalarial activity, turkeys □ Antimalarial activity—screened in substituted carbamimidate ester, turkeys □ Structure–activity relationships—substituted carbamimidate ester screened for antimalarial activity, turkeys

During an *in vitro* study of the antiplaque activity of N^1-(4-chlorophenyl)-N^5-alkylbiguanides (I), it became necessary to prepare larger quantities of these compounds (1). To optimize yields, the ratio of alkylamine to N^1-(4-chlorophenyl)-N^3-cyanoguanidine (II) in ethanol was changed from 4:1 to 1:1. The product obtained from this reaction was the ethyl ester of [(4-chlorophenyl)amino]-iminomethylcarbamimidic acid (III) rather than the expected alkylbiguanide. The similarity between this compound and chloroguanide (IV) prompted the synthesis of the isopropyl derivative (V) as a potential antimalarial agent.

Reaction of II with isopropyl alcohol in the presence of triethylamine and copper acetate (Scheme I) yielded isopropyl [(4-chlorophenyl)amino]iminomethylcarbamimidate (V). This compound and chloroguanide were tested as their acetate salts against turkeys infected with *Plasmodium lophurae*.

EXPERIMENTAL

Chemistry[1]—*N*[1]-*(4-Chlorophenyl)*-N[3]-*cyanoguanidine (II)*—This compound was prepared from 4-chloroaniline hydrochloride and sodium dicyanamide, as previously reported (2), mp 202–203° [lit. (2) mp 204–205°].

Isopropyl [(4-Chlorophenyl)amino]iminomethylcarbamimidate (V)—Compound II (4.86 g, 0.025 mole), copper acetate monohydrate (2.5 g, 0.0125 mole), triethylamine (10 ml), and isopropyl alcohol (100 ml) were refluxed with stirring for 12 hr. The purple reaction mixture was evaporated *in vacuo* to yield a semisolid. Water (100 ml) was added, and the remaining alcohol was removed by azeotropic distillation. An additional 100 ml of water was then added, and the insoluble copper complex was destroyed with acetic acid (25 ml).

Gaseous hydrogen sulfide was bubbled through the mixture, and the resulting copper sulfide was removed by filtration. The filtrate was adjusted to pH 7 with concentrated ammonium hydroxide and allowed to stand at 4° for 12 hr, which resulted in the precipitation of the acetate salt. The acetate salt was then recrystallized using methanol to yield 2.88 g (36.2%) of a white solid, mp 181–182°.

Anal.—Calc. for $C_{13}H_{19}ClN_4O_3$: C, 49.60; H, 6.08; N, 17.80. Found: C, 49.65; H, 6.15; N, 17.85.

Antimalarial Activity—Turkey poults (3–4 weeks old) from the same brood were used. The turkeys were given food and water *ad libitum*. The strain[2] of *P. lophurae* used was maintained by weekly serial blood passage from an infected donor turkey to an uninfected poult. The turkeys used in both the Davey test (eight turkeys) and the Patency test (six turkeys) were infected intravenously *via* the wing vein with an inoculum (50 million parasitized red blood cells in 0.2 ml) taken from the same donor turkey.

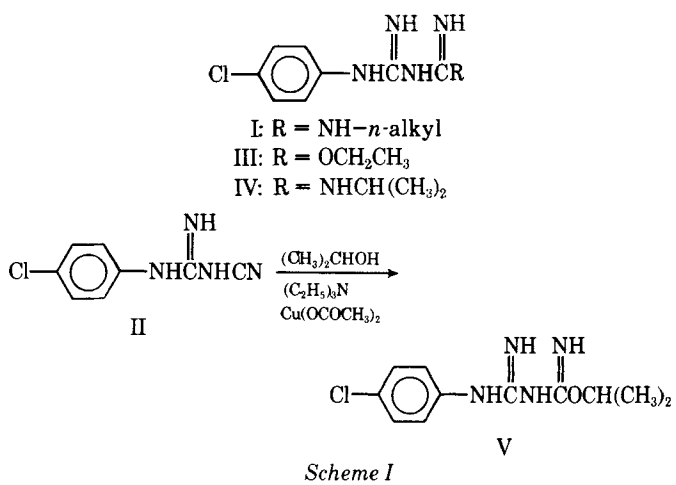
Modified Davey Test—This test was modified as suggested by Peters (3). The day of infection was termed D 0, and the following day was designated D + 1. The drugs were given as aqueous solutions by oral intubation. The first dose of the drug (10 mg/kg) was given 4 hr prior to infection. Doses of 5 mg/kg were then administered twice daily for 3 days for a total of seven doses (40 mg/kg). Blood smears were taken daily, and the percent parasitemia was determined by counting the parasites in 500 red blood cells.

Patency Test[3]—This test involved the measurement of the schizonticidal activity of the test compounds. Percent parasitemia levels of the untreated, infected turkeys were determined daily. When the levels were above 10%, a large, single oral dose (100 mg/kg) was administered.

RESULTS AND DISCUSSION

The results of the modified Davey test are summarized in Table I. Chloroguanide displayed suppressive activity against the blood forms of *P. lophurae* 7 days after infection. The carbamimidate ester suppressed parasitemia for only 1 day. At D + 8, the controls and carbamimidate ester-treated turkeys died, but the chloroguanide-treated animals showed only a trace of parasitemia. According to the Davey test criterion (percent parasitemia on D + 4), the carbamimidate ester-treated turkeys did not show a significant difference of suppression from that of the controls.

From these results, it may be concluded that the carbamimidate ester has no suppressive activity at a total dose of 40 mg/kg. Conceivably, the carbamimidate ester may display suppressive activity at a higher dose,



Scheme I

[1] Melting points were determined on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Microanalysis was performed by Midwest Microlab, Ltd., Indianapolis, Ind. A Varian Associates model T-60 NMR spectrometer and a Perkin-Elmer model 700 IR spectrometer were used. Spectral data were in accord with assigned structures.

[2] Donated by Dr. Richard Beaudoin, Malaria Division, Naval Medical Research Institute, Bethesda, Md.

[3] R. Beaudoin, Malaria Division, Naval Medical Research Institute, Bethesda, Md., personal communication.

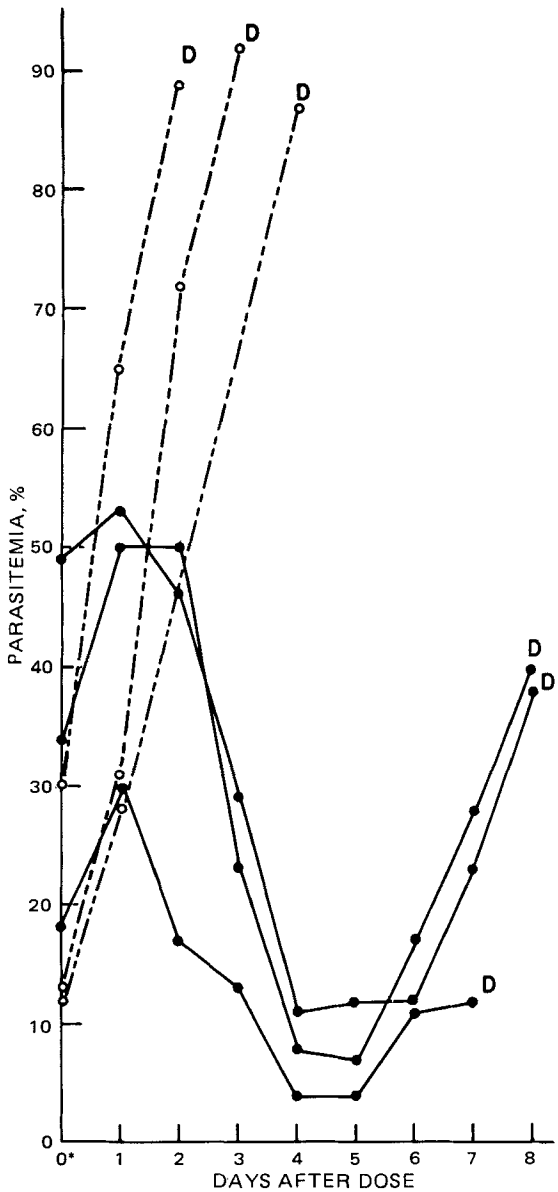


Figure 1—*Schizonticidal activity of single oral dose (100 mg/kg) of the test compounds. Key: *, birds given oral dose; O, carbamimidate ester; ●, chloroguanide; and D, bird died.*

Table I—Results of the Davey Test[a]

Time	Control	Chloroguanide[b]	Carbamimidate[b]
D + 1	1 ± 1	0	0
D + 2	1.1 ± 0.3	0	1
D + 3	3.5 ± 2.5	0	3.1 ± 2.1
D + 4	22.3 ± 13.3	0	15.5 ± 10.5
D + 5	65.3 ± 25.3	0	36.0 ± 17
D + 6	89.6 ± 9.4	0	58.0 ± 25
D + 7	97[c]	0	82[d]
D + 8	Death	1	Death
D + 9		6 ± 4	
D + 10		13.3 ± 5.3	
D + 11		54.3 ± 17.7	
D + 12		83.0 ± 3[d]	
D + 13		Death	

[a] Blood smears compared on D + 4 are used as Davey test criteria; there were three turkeys in each group. [b] Percent parasitemia is expressed as mean ± range. [c] Death of two birds. [d] Death of one bird.

but results from the Patency test (Fig. 1) indicate that this effect is not too likely. The blood smears taken at daily intervals show that chloroguanide possesses potent schizonticidal activity, whereas the carbamimidate ester displays no schizonticidal activity at the same dose (100 mg/kg).

The apparent lack of antimalarial activity for the carbamimidate ester may be due to: (*a*) an inability to form some biologically active metabolite [chloroguanide must cyclize prior to displaying antimalarial activity (4)] or (*b*) nonspecific esterase hydrolysis of the isopropyl group, yielding a product that could rearrange to a urea derivative.

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