11, 1:2) and shaken vigorously with 10 ml of either ether or ethyl acetate for 10 min followed by centrifugation to separate the layers. The standard and control solutions were subjected to the same conditions. After removal of the propranolol, propranolol glucuronide (7) as well as the acidic metabolites of propranolol (6, 8, 9) remained in the aqueous layer. Therefore, the plasma propranolol concentration may be expressed as the difference in the fluorescence intensity of the aqueous layer before and after basic extraction (Fig. 1).

The concentration of propranolol free base extracted into each nonaqueous layer was verified at 1, 3, and 5 hr on curve 4 (Fig. 1) as follows. Five milliliters of ether was evaporated to dryness, and the fluorescence intensity of the residue taken up in methanol was compared to that of the standards prepared in methanol. For samples extracted with ethyl acetate, the fluorescence intensity of the latter was compared to that of the standards prepared immediately in this solvent.

The results in Fig. 1 corroborate recent reports that the plasma propranolol concentration is much less than that of its glucuronide and acidic metabolites (6–9).

This analytical method is two-to-20-fold less sensitive than established GLC, high-pressure liquid chromatographic (HPLC), and GLC–mass spectrometric procedures (3, 10–12). However, it is more rapid than either GLC or GLC–mass spectrometric procedures and comparable in speed to HPLC. The ready availability and relatively low cost of fluorescence instrumentation make it a useful alternative to chromatographic techniques.

A comparison of the reported fluorometric method with the one currently in use indicates an improvement in methodology since the sample volume and workup time are reduced (5). Excitation at 317 nm rather than at 287 nm eliminates fluorescence background from the plasma but reduces sensitivity due to the diminished intensity at the vibrational feature maximum, 317 nm. Therefore, the two procedures are comparable with regard to their limiting detectable concentrations.

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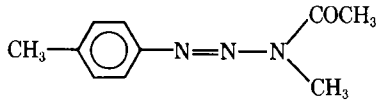
1-(*p*-Tolyl)-3-acetyl-3-methyltriazene: A Compound with Activity against African Trypanosomiasis

Keyphrases □ Antiprotozoal agents—1-(*p*-tolyl)-3-acetyl-3-methyltriazene, activity against African trypanosomiasis, mice □ 1-(*p*-Tolyl)-3-acetyl-3-methyltriazene—evaluation of activity against African trypanosomiasis, mice

To the Editor:

We wish to report our recent finding that 1-(*p*-tolyl)-3-acetyl-3-methyltriazene (I) is active against *Trypanosoma rhodesiense* and *T. rhodesiense* EATRO 1989 (mildly virulent strain) infection in the mouse. The triazene was synthesized according to the procedure of Dimroth (1). 1-(*p*-Tolyl)-3-methyltriazene was prepared from the diazocation of *p*-toluidine. The diazocation was treated with methylamine to give the monomethyltriazene. This compound was acetylated with acetic anhydride in pyridine. The yield of the triazene was 73% after recrystallization from hexane, mp 53° [lit. (1) mp 54–56°]; IR (KBr): 1716 cm^{-1}; mass spectrum (70 ev): *m/z* (relative intensity) 191 (8), 149 (5), 119 (8), 91 (100), and 43 (45).



I

Compound I was tested initially against *T. rhodesiense* (Wellcome CT strain) using a mouse model (2). Test and control mice (ICR/HA Swiss) were 6 weeks old and weighed 28–30 g. No differences in response between sexes have been reported. Each mouse was infected by intraperitoneal injection of 0.05 ml of a 1:50,000 dilution of heparinized heart blood drawn from donor mice infected 3 days earlier.

Drugs were administered subcutaneously or orally in peanut oil 2 hr after infection. Untreated mice died between 4.2 and 4.5 days postinfection. Surviving animals

Table I—Activity of I against *T. rhodesiense* (Wellcome CT Strain) Infection in Mice

Route of Administration	Dose, mg/kg					
	424	212	106	53	26.5	13.3
Subcutaneous	5/5[a]	—	0/5	—	0/5	—
Subcutaneous	5/5	3/5	0/5	0/5	0/5	0/5
Oral	5/5	2/5	0/5	0/5	0/5	0/5

[a] Number of mice cured/number of mice treated.

Table II—Activity of I against *T. rhodesiense* EATRO 1989 Infection in Mice

Dose[a], mg/kg ip	Days 5–6	7	8–9	10–12	19	30	39	50	60
1600	5/5[b]	4/5	0/5	0/5	0/2	0/2	0/2	0/1	0/1
800	5/5	4/5	0/5	0/4	0/3	0/3	0/3	0/3	0/3
400	5/5	5/5	0/5	0/5	0/5	2/5	3/5	2/4	1/3
200	5/5	5/5	0/5	0/5	2/5	3/5	5/5	4/4	3/3
100	5/5	5/5	0/5	0/5	5/5	5/5	5/5	3/3	1/1
50	5/5	4/5	0/5	0/5	5/5	5/5	5/5	2/2	Dead
25	3/5	5/5	0/5	3/5	5/5	5/5	2/2	Dead	
12.5	5/5	5/5	1/5	4/4	4/4	3/3	Dead		
6.25	5/5	5/5	5/5	4/5	4/4	4/4	1/1	Dead	
3.125	5/5	5/5	5/5	3/5	5/5	5/5	2/2	Dead	
1.56	5/5	5/5	5/5	4/5	5/5	5/5	2/2	Dead	
0.78	5/5	5/5	5/5	4/5	5/5	5/5	2/2	Dead	
Control (untreated)	5/5	5/5	5/5	4/5	5/5	5/5	2/2	Dead	

[a] The compound was dissolved in methanol, and the mice were treated on Day 5 only. [b] Number of parasitemic mice/number of mice treated and still alive on various days.

Table III—Activity of I against *T. rhodesiense* EATRO 1989 Infection in Mice

Dose[a], mg/kg ip	Days 4–6	8	10	12–14	15–16	19	20	30	40	50	60
1600	5/5[b]	0/5	0/5	1/5	0/5	0/5	2/5	3/5	3/5	3/5	2/4
800	5/5	0/5	0/5	0/5	0/5	4/5	4/5	4/5	4/5	3/4	3/3
400	5/5	0/5	0/5	2/5	2/5	2/4	2/4	3/4	2/3	1/2	0/1
200	5/5	1/5	0/5	3/5	4/5	4/4	3/4	4/4	3/3	2/2	1/1
100	5/5	2/5	5/5	5/5	5/5	5/5	5/5	5/5	3/3	1/1	Dead
50	5/5	5/5	5/5	5/5	5/5	5/5	5/5	4/4	1/1	Dead	
25	5/5	5/5	5/5	5/5	5/5	5/5	5/5	4/4	1/1	Dead	
Control (untreated)	5/5	5/5	5/5	2/2	2/2	2/2	1/1	Dead			

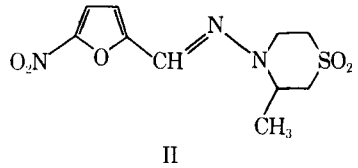
[a] The compound was dissolved in ethanol, and the mice were treated on Day 4 only. [b] Number of parasitemic mice/number of mice treated and still alive on various days.

were observed for 30 days. The mice surviving 30 days were considered cured. The test data are given in Table I.

To determine if I had activity against mildly virulent strains of the parasite, it was tested in a mouse model against *T. rhodesiense* EATRO 1989. Male albino mice were infected with the parasites on Day 0. The triazene was administered intraperitoneally as both ethanolic and methanolic solutions (total volume of 0.1 ml/injection) on Days 4 and 5, respectively. The mice then were observed until Day 60 postinfection; during the test period, the blood of the surviving mice was microscopically examined for parasites each day. The results of these tests are given in Tables II and III.

An examination of the data in Tables I–III indicates that I has activity against *T. rhodesiense* and its mildly virulent strain. The compound is curative when administered subcutaneously or orally at a dose of 424 mg/kg. Against the mildly virulent strain at a dose of 800 mg/kg in methanol, three of the five mice treated were alive and parasite free after 60 days; at 400 mg/kg, three survived and two were parasite free after 60 days.

Under the same conditions, (3-methyl-4,4′-nitrofurfurylidineamino)tetrahydro-4*H*-1,4-thiazene-1,1-dioxide



II

(II), a standard drug, only suppressed the parasite. At doses of ≥150 mg/kg ip, II cleared parasitemia for 9 days; when given orally at 400 mg/kg for 5 consecutive days, II cleared parasitemia temporarily from Days 6 to 9.

The results reported here indicate that I has sufficient activity to justify a thorough investigation of its chemical and pharmacological profiles using both drug-sensitive and drug-resistant strains. These studies are underway, and a more detailed report of the findings will follow.

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