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Antitrichomonal activity of 1-[(2, 4-dinitropyrryl)-ethyl]-2-methyl-5-nitroimidazole and related compounds

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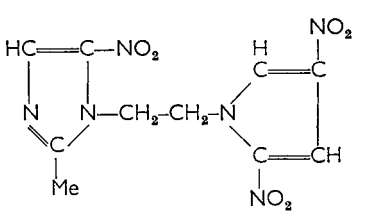
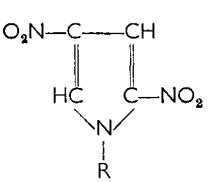
A series of 1-substituted dinitropyrroles was tested for trichomonacidal activity. The shorter the substituent the greater was the *in vitro* activity. No relationship was found between *in vivo* activity and substituent length. A comparison of the trichomonacidal activities of 1-[(2,4-dinitropyrryl)-ethyl]-2-methyl-5-nitroimidazole (ORF-2245) and 1-(2-hydroxymethyl)-2-methyl-5-nitroimidazole (8823 R.P.) was made. ORF-2245 exhibited activity equal to or greater than 8823 R.P. except in the treatment of established infections where its limited activity was attributed to poor absorption from the gut. Absorption, however, was apparently sufficient to combat or prevent the low-grade infections.

THE trichomonacidal activity of 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (8823 R.P.) in mice experimentally infected with *Trichomonas vaginalis* was reported by Cosar & Julou (1959). They compared it with the antibiotic azomycin (Horie, 1956), and 2-acetamido-5-nitrothiazole (acinitrazole). Cuckler, Kupferberg & Millman (1955) had observed earlier that acinitrazole has therapeutic activity when administered orally to monkeys and mice.

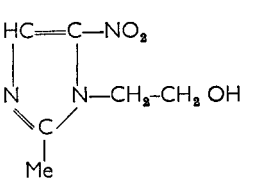
Recently we evaluated a series of nitro-derivatives of imidazoles and pyrroles particularly 1-[(2,4-dinitropyrryl)-ethyl]-2-methyl-5-nitroimidazole (ORF-2245) (I), the activity of which was compared with that of some other 2,4-dinitropyrrole derivatives (II) and with the compound 8823 R.P. (III) studied by Cosar & Julou.



ORF-2245 (I)



2,4-Dinitropyrroles (II)



8823 R.P. (III)

From the Ortho Research Foundation, Raritan, New Jersey, U.S.A.

Experimental

METHODS

Determination of *in vitro* trichomonacidal activity were made by testing the inhibitory effect of different concentrations of the compound against *Trichomonas foetus* (BrM_1) in simplified trypticase serum medium, pH 7·2, according to the procedure of Kupferberg, Johnson & Sprince (1948). Stock solutions of the drugs were made in acetone (no test tube contained more than 2% acetone). Inocula were prepared by incubating *T. foetus* (BrM_1) in simplified trypticase serum medium for 40 hr at 37·5°. Aliquots of 0·1 ml of these cultures were used to inoculate each test culture. The trichomonacidal level of the drug was the lowest concentration in which no viable cells were found after 3 days of incubation at 37·5°.*

For the determination of the PD50, white male mice (Barckman IS-32 strain), 18–22 g, were used. Cultures of *T. foetus* (BrM_1) grown for 40 hr in simplified trypticase serum medium at pH 7·2 and 37·5° were pooled and the number of the trichomonads per ml was determined with a Neubauer haemocytometer. The number of organisms was adjusted to approximately 10^6 ml by dilution with fresh serum medium and each test animal was injected intraperitoneally with 1 ml of the adjusted suspension.

For routine screening, the drug was suspended in a 0·25% sterile agar solution and administered orally on the day of infection. The drug concentration was adjusted so that no animal received more than 1 ml or less than 0·3 ml. Three groups of ten animals were each given varying doses of the drug on a mg/kg basis and ten untreated animals served as controls. Only tests in which the latter showed a mortality of at least 80% 7 to 10 days after infection were considered for analysis. The 50% end point was calculated according to the method of Reed & Muench (1938).

For the determination of therapeutic activity, the same procedure was used except that the drug was given 2 or 3 days after infection. Animals receiving multiple doses were given the drug on 3 successive days. Animals receiving only a single dose were treated on the second day after infection.

To determine drug activity against *T. vaginalis*, the drug was given orally and the organisms were injected subcutaneously. Several tubes of *T. vaginalis* (strain No.1†) were incubated at 37·5° in the serum medium (pH 6·0) for 40 hr and pooled. The number of organisms was adjusted to $1{\cdot}5 \times 10^6$/ml and mice were infected subcutaneously (dorsally) with 1 ml of this suspension. The drugs were given by mouth within 1 hr of infection. All animals were killed 10 days after infection and examined for abscesses at the site of injection. Usually, 9 out of 10 untreated infected controls developed large abscesses during this period, and the presence of trichomonads was confirmed by inoculating Trichosel broth‡ with fluids from the abscesses.

**T. vaginalis* No. 2, simplified trypticase medium at pH 6·0 and an inoculum of 0·05 ml.

†*T. vaginalis* No. 1 is more pathogenic for mice than strain No. 2.

‡Baltimore Biological Laboratories.

For the determination of serum and urine levels the drug was administered orally to 10 mice at 500 mg/kg. After 5 hr the animals were killed with ether and blood samples were collected from the heart immediately. The blood was allowed to clot and was stored overnight at 10°. Urine samples were also collected from the bladder directly after the animals were killed.

Both serum and urine were assayed for trichomonacidal activity in 10 × 75 mm tubes using Trichosel broth (pH 7·2) and *T. foetus* as the test organism. The serially diluted urine and serum were inoculated with 0·02 ml of a 48 hr culture of *T. foetus*. Control tubes contained normal mouse serum or urine at concentrations of 1 : 10, 1 : 20 and 1 : 40.

For chromatography the urine, serum and faecal samples from 20 mice* were extracted with ethyl acetate; urine and serum were extracted with 2 volumes, faeces with 500 ml of ethyl acetate. The ethyl acetate was evaporated to dryness and reconstituted with 2 ml of acetone. This material was then used for chromatographic analysis. One to 4 μl of the acetone solution were applied to a thin layer chromatographic plate† containing alumina G and developed with isopropanol : hexane (6 : 4) for 50 min. The spots were observed at λ260 mμ.

Results and discussion

ACTIVITY OF 1-SUBSTITUTED 2,4-DINITROPYRROLES

This group of compounds was examined to determine possible relationships between the length of the substituted alkane group and the trichomonacidal activity of the compounds. Table 1 lists the comparative *in vitro* and *in vivo* activities of ten 1-substituted 2,4-dinitropyrroles against *T. foetus*. All the compounds exhibited either *in vitro* or *in vivo* activity but it was of interest that the compound with the greatest *in vitro* activity (ORF-1300) was without *in vivo* activity at the levels tested and two of the compounds with activity *in vivo* (ORF-1460 and ORF-1509) were inactive *in vitro*.

The results suggest a relationship between the number of carbon atoms (0–4) in the alkyl group and the *in vitro* activity of the compounds. When the dinitropyrrole substituent (R) was hydrogen, the activity was maximal. When the substituent group contained 4 carbon atoms, essentially all the activity was lost. When R was increased to C_5 or C_6 some activity was restored (see Table 1). No difference in activity was found between the normal or the iso configurations. Whereas the LD50 of the compound increased with the increase in chain length, there was no correlation between therapeutic activity *in vivo* and the length of the substituted group.

COMPARATIVE STUDIES ON ORF-2245 AND 8823 R.P.

In vitro. The *in vitro* experiments were conducted as described above except that both *T. vaginalis* and *T. foetus* were used as test organisms and 0·1 ml of the *T. foetus* culture was used in inoculating the Trichosel

*Swiss Webster mice.
†Custom Service Chemicals, Wilmington, Delaware.

TABLE 1. TRICHOMONACIDAL ACTIVITY OF 1-SUBSTITUTED 2,4-DINITROPYRROLES

ORF No.	R	*In vitro** vs. *T. foetus*	*In vivo* (mg/kg) orally vs. *T. foetus*	
			LD50	PD50
1300	H	1:800,000	20	Inactive at 16·5
1350	CH_3	1:200,000	220	67
1452	C_2H_5	1:100,000	68	Inactive at 53
1454	C_3H_7	1:50,000	900	30
1518	iso-C_3H_7	1:50,000	400	170
1460	n-C_4H_9	<1:10,000	1,200	53
1509	iso-C_4H_9	<1:10,000	1,200	75
1706	n-C_5H_{11}	1:10,000	1,500	50
1517	iso-C_5H_{11}	1:10,000	1,000	5·25
1516	n-C_6H_{13}	1:50,000	2,150	140

*Tube Dilution.

broth tubes that contained the serially diluted drugs. Both compounds had the same activity against *T. foetus*, i.e. 1:500,000. *T. vaginalis* was more sensitive to the action of these compounds, showing a greater susceptibility to ORF-2245 than to 8823 R.P. ($1:2 \times 10^6$ against $1:1 \times 10^6$).

Massive doses of the drugs were given in an attempt to determine the LD50. No end-point was reached with either drug although the animals were given as much as 4 g/kg in a single dose.

PD50 *T. foetus*. Table 2 shows the dose response of the two drugs when given in a single dose at the time of infection. The activity of ORF-2245 was significantly greater than that of 8823 R.P.

TABLE 2. THE RESPONSE OF MICE INFECTED I.P. WITH *Trichomonas foetus* AND TREATED WITH A SINGLE ORAL DOSE OF 8823 R.P. OR ORF-2245 ON THE DAY OF INFECTION

Dosage (mg/kg)	Mortality	Cumulative totals*		% Mortality
		survivors	dead	
8823 RP†				
105	2/9	19	2	9·4
87	3/9	12	5	29·4
72·9	7/9	6	12	66·6
60·7	8/9	4	20	83·3
50·6	7/9	3	27	90·0
42·1	8/9	1	35	97·2
Controls	10/10			100·0
ORF-2245‡				
67	1/10	29	1	3·3
33·5	0/10	20	1	5·0
16·75	4/10	10	5	33·3
8·37	7/10	4	12	75·0
4·18	9/10	1	21	95·0
2·09	10/10	0	31	100·0
Controls	10/10			100·0

* According to the procedure of Reed & Muench.
† PD50 = 78·7 mg/kg.
‡ PD50 = 12·6 mg/kg.

PD50 *T. vaginalis*. In combating abscess formation both drugs had approximately the same oral PD50, but the amount of drug required to prevent abscess formation differed markedly. For ORF-2245 in doses of 33·5; 21·0; 10·5 mg/kg the numbers of mice with abscesses were respectively 0/10; 0/10; 5/10. For 8823 R.P. corresponding figures were 135·0;

75·0; 42·0; 33·5; 10·5 giving 0/10; 1/10; 1/10; 2/10; 5/10 abscesses. Controls had 9/10 abscesses.

Therapeutic activity. Twenty-one days after infecting the animals intraperitonally with *T. foetus*, the survivors were killed and examined for lesions. When the drugs were given as 100 mg/kg in a single dose two days after infection, more than 66% of the animals treated with 8823 R.P. failed to survive (Table 3). At 300 mg/kg the mortality rates for 8823

TABLE 3. THERAPEUTIC EFFECT OF ORF-2245 AND 8823 R.P. IN MICE INFECTED INTRAPERITONEALLY WITH *T. foetus*

Compound	No. of mice	Dosage (mg/kg/day)	Doses*	Day of start of treatment	% Mortality	No. of survivors infected
ORF-2245	20	100	3	3rd	35	1/13
8823 R.P.	20	100	3	3rd	15	0/17
ORF-2245	20	100	3	2nd	35	1/13
8823 R.P.	10	100	3	2nd	10	0/9
ORF-2245	30	100	1	2nd	66·6	3/11
8823 R.P.	30	100	1	2nd	13·3	0/26
ORF-2245	20	300	1	2nd	35	0/13
8823 R.P.	20	300	1	2nd	15	0/17

* Given on successive days.

R.P. and ORF-2245 respectively were 15 and 35%. The response to the drugs was about the same whether given as 300 mg/kg in a single dose on the second day of infection or given in three equal daily doses beginning on the second or third day of infection. The infecting organism was recovered from the survivors only when ORF-2245 was administered in a single dose (100 mg/kg) on the second day of infection.

Since the therapeutic activity of ORF-2245 did not parallel the PD50 results, an experiment was designed to test directly the effect of the drug on the trichomonad while resident in the host. Four groups of 10 animals were infected intraperitoneally with 1 to 1·5 $\times$ 10^6 *T. foetus* organisms, as in the above studies. On the third day of the infection each of ten mice were given 6 mg orally (approximately 300 mg/kg) of either ORF-2245 or 8823 R.P. The fourth group was not treated and was retained as a control. The animals were killed with ether 24 hr after administration of the drugs and the peritoneal fluids were collected and pooled and a count made of the motile and non-motile organisms.

The untreated group had a viable count of more than 300 $\times$ 10^6 organisms/ml. ORF-2245 showed no lytic activity against the trichomonads. All organisms were actively motile. In the groups of animals treated with 8823 R.P. no viable organisms were observed. The mice treated with this drug had a count of 50 $\times$ 10^6 trichomonads per ml, all of which were non-motile.

Serum and urine levels in drug-exposed mice. A trichomonacidal urine level was reached after 500 mg/kg by mouth. Within 5 hr the 8823 R.P. produced urine levels substantially higher than those produced by ORF-2245. The level of activity of 8823 R.P. was greater than 1:2,560 in urine while that of ORF-2245 was 1:160 (Table 4). ORF-2245 showed no detectable trichomonacidal level in the serum while 8823 R.P. produced complete inhibition at 1:40.

TABLE 4. TRICHOMONACIDAL ACTIVITY OF URINE AND SERUM FROM MICE 5 HR AFTER ORF-2245 AND 8823 R.P. AT 500 MG/KG* ORALLY

Compound	Inhibitory activity of urine								
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560
ORF-2245	—	—	—	—	—	+	4+	4+	4+
8823 R.P.	—	—	—	—	—	—	—	—	—
Control (normal urine) ..	4+	4+	4+						
	Inhibitory activity of serum								
ORF-2245	4+	4+	4+	4+	4+	4+	4+	4+	4+
8823 R.P.	—	—	—	+	2+	4+	4+	4+	4+
Control (normal serum) ..	4+	4+	4+						

* The urine and serum were serially diluted using Trichosel broth and *T. foetus* as the test organism.
4+ Normal (2·5 to 3·5 million organisms/ml).
3+ Growth less than control.
2+ Sparse growth.
\+ No growth but inoculum not lysed.
— No organisms present.

Drug absorption. Because of the apparent disparity between protective and therapeutic activity of ORF-2245 either the material was being poorly absorbed or it was being degraded.

8823 R.P. and ORF-2245 were administered to two groups of 20 mice each. The urine and faeces were extracted and concentrates of the extracts were chromatographed on alumina thin-layer plates. No 8823 R.P. was isolated from the faeces but it was found unchanged in high concentration in the urine. ORF-2245 was not recovered in the urine but unchanged drug was found in large amounts in the faeces. These observations suggest that little ORF-2245 is absorbed.

ORF-2245 was dissolved in 99·5% dimethyl sulfoxide in an attempt to enhance absorption when given orally to mice at 500 mg/kg. There was no increase in the trichomonacidal level of the urine and the serum remained inactive.

Thus a relationship was found to exist between trichomonacidal activity and the length of the substituent in the 1 position of the dinitropyrrole. The shorter the alkyl grouping the greater was the *in vitro* trichomonacidal activity, the longer the length of the substituent, the lower the animal toxicity. There was no relationship between chain length and *in vivo* activity.

ORF-2245 appeared to be the drug with the greater trichomonacidal activity but almost complete inability to cope with an established infection greatly restricts its therapeutic value. The explanation of the relative therapeutic ineffectiveness may lie in the fact that the drug seems to be poorly absorbed by the gut.

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