α-Chloromethyl-2-methyl-5-nitro-1-imidazoleethanol (Ro 7-0207), a Substance Exhibiting Antiparasitic Activity against Amebae, Trichomonads, and Pinworms (34503)

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The results of chemotherapeutic experiments with a series of derivatives of 2-nitroimidazole have been described in an earlier publication (1). Among these compounds, 1- (2-nitro-1-imidazolyl)-3-methoxy-2-propanol (Ro 7-0582) was of special interest because of its low acute toxicity, marked antitrichomonad activity, and good activity against *Endamoeba histolytica* in both intracecal and hepatic infections. We have since studied the chemotherapeutic properties of derivatives of 5-nitroimidazole, and have found that one among them, α-chloromethyl-2-methyl-5-nitro-1-imidazoleethanol RO 7-0207)[1], exhibited especially pronounced antiprotozoan effects. The present report summarizes the *in vivo* data obtained with this substance in a variety of experimental models as well as the results of experiments against both anaerobic and aerobic microorganisms since substances of this type have been shown to exert a selective effect against obligate anaerobes (2).

Materials and Methods. Antiamebic activity in vivo. The intracecal infection with *E. histolytica* and evaluation of activity against this infection was carried out as previously reported (3), with the exception that the weanling rats undergoing laparotomy were anesthetized with 30 mg of sodium pentobarbital per kg intraperitoneally instead of with ether. Hepatic infections with *E. histolytica* were carried out in hamsters; the technique and method of evaluation of chemotherapeutic activity have been described elsewhere (1). The substance was administered orally in the intracecal infection of rats and by both the oral and subcutaneous routes for the hepatic infection of hamsters.

Antitrichomonad activity in vivo. The essential procedure was to evaluate the substance both systemically (per os) and locally (infiltration) against a subcutaneous infection of mice with *Trichomonas vaginalis* and orally against a lethal intraperitoneal infection of mice with *Trichomonas foetus*. These tests have been described in detail in an earlier report (4).

Anthelmintic activity in vivo. A natural infection of mice with *Syphacia obvelata* and experimentally induced infections of mice with *Hymenolepis nana* and *Nematospiroides dubius* were employed. The methods of infection, oral treatment, and chemotherapeutic evaluation have been described in detail (3).

Other infections in vivo. Procedures described by Grunberg *et al.* (3, 5) were used for evaluation of the test substance against transplanted tumors, bacteria, fungi, and viruses in mice.

In vitro *tests*. Conventional serial broth dilution tests were performed using trypticase soy or thioglycollate, Sabouraud's, Dubos, STS, and CPLM broths for aerobic and anaerobic bacteria, fungi, *Mycobacterium tuberculosis* H37Rv, *T. vaginalis*, and *T. foetus* respectively. Detailed descriptions of these methods appear elsewhere (2, 4, 5).

Results and Discussion. Table I gives the basic features of the experimental models employed for the protozoan and parasitic infections and shows the chemotherapeutic responses obtained with α-chloromethyl-2-methyl-5-nitro-1-imidazoleethanol.

[1] Synthesized by Dr. M. Hoffer, Chemical Research Department, Hoffmann-La Roche, Inc., Nutley, New Jersey.

TABLE I. Antiprotozoan and Anthelmintic Activity of α-Chloromethyl-2-methyl-5-nitro-1-imidazoleethanol.

Organism	Infection[a] and host	Observation	CD_{50}[b] mg/kg po	mg/kg sc	μg/ml sc
Endamoeba histolytica	Intracecal, rat	Microscopic, presence of organisms	10[c]	—	—
Endamoeba histolytica	Hepatic, hamster	Gross lesions; microscopic, presence of organisms	21	17	—
Trichomonas vaginalis	Subcutaneous, mouse	Gross lesions; microscopic, presence of organisms	37[c]	—	86
Trichomonas foetus	Intraperitoneal, mouse	Peritonitis and death	3[c]	—	—
Syphacia obvelata	Natural, mouse	Adult worms, cecum	66	—	—
Hymenolepis nana	Eggs per os, mouse	Adult worms, small intestine	>1000	—	—
Nematospiroides dubius	Larvae per os, mouse	Adult worms, small intestine	>1000	—	—

[a] Treatment regimens were as follows: *E. histolytica,* 1 ml po 24 hr after infection (rat) and 1 ml po or sbc once daily for 3 consecutive days starting day after infection (hamster); *T. vaginalis,* 1 ml sbc 2 and 24 hr after infection or po 1, 24, 48, and 72 hr after infection; *T. foetus,* 1 ml po 1, 24, and 48 hr after infection; *S. obvelata, H. nana,* and *N. dubius* all 1 ml po once daily for 3 consecutive days.

[b] Determined by the method of Reed and Muench (6); the oral LD_{50} in mg/kg was 1780 and 1420 for the rat and mouse, respectively.

[c] 2-Methyl-5-nitroimidazole-1-ethanol (metronidazole) gave CD_{50} values of 49, 16, and 17 mg/kg po against the *E. histolytica, T. vaginalis,* and *T. foetus* infections, respectively (2).

From the data in Table I it can be seen that α-chloromethyl-2-methyl-5-nitro-1-imidazoleethanol exerted a marked chemotherapeutic effect against *E. histolytica* in both the intracecal infection of rats and the hepatic infection of hamsters. The substance also displayed excellent activity against *T. vaginalis* and *T. foetus* in all experimental models employed. From a comparative point of view, it should be pointed out that this 5-nitroimidazole derivative was superior to the previously reported 2-nitroimidazole substance (1) against the intracecal and hepatic amebic infections and, furthermore, displayed a similar antitrichomonad effect. With regard to 2-methyl-5-nitroimidazole-1-ethanol (metronidazole) the data presented here and elsewhere (2) suggest that the substance presently under investigation is, within the limits of the experimental methodology employed, equivalent as a trichomonacide and superior as an antiamebic agent *in vivo.*

Anthelmintic activity was restricted to a rather pronounced effect against the pinworm *S. obvelata.*

With respect to other *in vivo* tests, α-chloromethyl-2-methyl-5-nitro-1-imidazoleethanol was inactive at the doses tested against representative gram-positive and gram-negative bacteria, fungi, and viruses. It produced a slight effect against the sarcoma-180 tumor but not the Ehrlich solid tumor in mice. The substance was without effect in altering the immune response of mice immunized with sheep red blood cells as determined by the method described by Cleeland and Grunberg (7).

Table II shows the results of experiments in which the substance was tested *in vitro* against aerobic, facultative, and anaerobic microorganisms.

It is clear from the data in Table II that α-chloromethyl-2-methyl-5-nitro-1-imidazoleethanol is a potent inhibitor of anaerobic protozoa and bacteria but not of their facultative or aerobic counterparts. The substance was inactive against *M. tuberculosis,* dermatophytes, and *C. albicans.* The activity reported here against anaerobic microorganisms is superior to that described for 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol and similar to that described for the 5-nitroimidazole derivative, metronidazole (2).

TABLE II. *In Vitro* Activity of α-Chloromethyl-2-methyl-5-nitro-1-imidazoleethanol.

Anaerobic MIC (μg/ml)		Aerobic MIC (μg/ml)	
Trichomonas vaginalis	3.9	*Tetrahymena geleii*	500
Trichomonas foetus	7.8	*Lactobacillus acidophilus*	2000
Endamoeba histolytica	15.0	*Lactobacillus arabinosus*	>2000
Clostridium tetani	0.01	*Staphylococcus aureus*	1000
Clostridium septicum	0.5	*Streptococcus pyogenes*	1000
Fusobacterium polymorphum	0.03	*Diplococcus pneumoniae*	500
Veillonella alcalescens	0.06	*Escherichia coli*	500
		Pseudomonas aeruginosa	>5000
		Proteus vulgaris	1000

Overall, α-chloromethyl-2-methyl-5-nitro-1-imidazoleethanol can be considered to be a potentially potent antiparasitic substance whose range of usefulness may extend from helminthiasis, trichomoniasis, and amebiasis, in their several forms, to possibly other infections produced by obligately anaerobic microorganisms.

Summary. α-Chloromethyl-2-methyl-5-nitro-1-imidazoleethanol was found to exert marked chemotherapeutic effects against experimentally induced infections of rodents with *Endamoeba histolytica, Trichomonas vaginalis, Trichomonas foetus,* and against a natural pinworm infection of mice produced by *Syphacia obvelata.* The substance was also highly active *in vitro* against anaerobic bacteria but not against facultative or aerobic microorganisms. Except for a slight effect noted in the case of the sarcoma-180 tumor, this 5-nitroimidazole derivative was inactive in all other experimental models tested *in vivo* including bacteria, fungi, and viruses.

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