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THE ACTION OF 5-BROMO-5-NITROTETRAHYDRO-1,3-OXAZINE DERIVATIVES ON *TRICHOMONAS VAGINALIS* *

by

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Results of studies on the protozoocidal action of three new derivatives of nitrotetrahydro-1,3-oxazine are reported. The studied compounds killed Trichomonas vaginalis cells in very low concentrations, and this effect was much stronger than that of oxytetracycline and trichomycin. One of the compounds exhibited relatively low acute toxicity.

Our previous studies showed that some oxazine derivatives possess interesting and specific biologic properties. For instance, a number of derivatives of 1,3-tetrahydrooxazine are cytotoxic for tumor cells, often selectively [4, 5]. Some of the compounds inhibited growth of transplantable tumors and showed relatively low toxicity [1].

The work reported herein was concerned with the action of three derivatives of nitrotetrahydro-1,3-oxazine on *Trichomonas vaginalis*. The studied compounds are new and have not been described hitherto. The chemical structure of the studied derivatives of nitrotetrahydro-1,3-oxazine is shown in Table 1.

MATERIAL AND METHODS

Antiprotozoal activity was determined by the method proposed by KURNATOWSKA [3], consisting in estimation of DL_{50} of the compound for a standard strain of *Trichomonas vaginalis*. Values of DL_{50} were calculated by means of the equation used by KADŁUBOWSKI [2]. A strain of *Trichomonas vaginalis* from the collection of the Department of Biosynthesis of the Institute of Immunology and Experimental Therapy served as standard strain.

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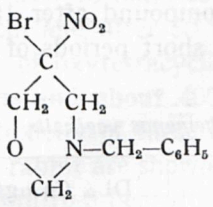
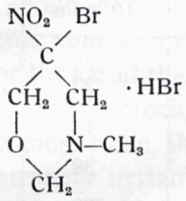
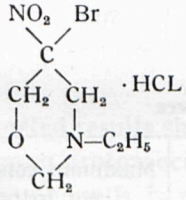
Table 1. Oxazine derivatives

Chemical formula	Preparation
	5-bromo-5-nitro-3-benzyltetrahydro-1,3-oxazine
	5-bromo-5-nitro-3-methyltetrahydro-1,3-oxazine hydrobromide
	5-bromo-5-nitro-3-ethyltetrahydro-1,3-oxazine hydrochloride

RESULTS

The results of the experiments are summarized in Table 2. For comparison, the table contains results of the action on *Trichomonas* of two antibiotics, trichomycin and oxytetracycline, which were studied under the same conditions.

Trichomycin, a polyene antibiotic with antiprotozoal and antifungal properties, under our experimental conditions killed 50% of the protozoal cells after 10 minutes' incubation in the concentration 630 μg/ml. Prolongation of incubation to 30 minutes only slightly increased the protozoocidal activity of trichomycin; after 30 minutes DL_{50} was 610 μg/ml. Oxytetracycline, which was also used for reference, exhibited much weaker protozoocidal activity. DL_{50} of oxytetracycline after 10 minutes' incubation was 7000 μg/ml, diminishing after incubation, to about 5200 μg/ml after 30 minutes.

Compared with the standard antibiotics, oxazines exhibited much stronger protozoocidal activity, especially the 5-bromo-5-nitro-3-methyltetrahydro-1,3-oxazine hydrobromide, which in the concentration of 12 μg/ml killed 50% of the protozoa after 10 minutes' incubation; after 30 minutes' incubation concen-

trations one-half smaller gave the same effect. Compared with oxytetracycline, the action of this compound after 10 minutes' incubation was about 580 times stronger, and after 30 minutes about 870 times.

The compound 5-bromo-5-nitro-3-benzyltetrahydro-1,3-oxazine proved equally active protozoocidally. The LD_{50} of this compound after 10 minutes was 22 μg/ml, and after 30 minutes 4 μg/ml. After short periods of exposure,

Table 2. DL_{50} of oxazine derivatives for *Trichomonas vaginalis*

Preparation	DL_{50} (in μg/ml) after	
	10 mins	30 mins
5-bromo-5-nitro-3-benzyltetrahydro-1,3-oxazine	22	4
5-bromo-5-nitro-3-methyltetrahydro-1,3-oxazine hydrobromide	12	6
5-bromo-5-nitro-3-ethyltetrahydro-1,3-oxazine hydrochloride	38	11
Trichomycin	630	610
Oxytetracycline	7000	5245

Table 3. Toxicity of oxazine derivatives

Preparation	DL_{50} * (mg/kg body weight)	Maximum concentrations not irritant for	
		rabbit ear	rabbit eye
5-bromo-5-nitro-3-benzyltetrahydro-1,3-oxazine	381	0·125	2·0
5-bromo-5-nitro-3-methyltetrahydro-1,3-oxazine hydrobromide	48	0·03	0·25
5-bromo-5-nitro-3-ethyl-tetrahydro-1,3-oxazine hydrochloride	24	0·03	0·25

* The preparations were injected intraperitoneally in white mice weighing about 20 g; calculations were made after 14 days' observation.

the effect of this compound was weaker than that of the preceding one, but when the incubation period was prolonged it exhibited protozoocidal activity in even lower concentrations. Compared with trichomycin, the action of this compound was about 30 times stronger after 10 minutes' incubation, and about 150 times after a further 20 minutes. After 10 minutes its effect was about 300 times stronger than that of oxytetracycline, and after 30 minutes even about 1300 times stronger.

The antiprotozoal properties of the third compound studied were only slightly weaker than those of the two preceding. The hydrochloride of 5-bromo-5-nitro-3-ethyltetrahydro-1,3-oxazine killed 50% of the protozoa after 10 minutes' incubation in concentrations of 38 μg/ml, and after 30 minutes at 11 μg/ml. Its effect after 10 minutes' exposure was about 17 times stronger than that of trichomycin and after 30 minutes about 55 times. Comparison of its activity with that of oxytetracycline showed activity about 180 times stronger after 10 minutes and about 400 times after longer exposure.

Acute toxicity of these compounds and their irritant action on the eye and ear in the rabbit are shown in Table 3. The least toxic of the three oxazines was 5-bromo-5-nitro-3-benzyltetrahydro-1,3-oxazine, the DL_{50} of which for mice weighing about 20 g was 380 mg/kg when injected intraperitoneally. In 2% solution this compound was not irritant for the rabbit eye, and in 0·125% solution for the ear of the rabbit. The hydrobromide of 5-bromo-5-nitro-3-methyltetrahydro- and hydrochloride of 5-bromo-5-nitro-3-ethyltetrahydro-1,3-oxazine were much more toxic, their DL_{50} being 48 and 24 mg/kg respectively. Moreover, they are strongly irritant; the lowest concentrations which did not irritate the rabbit ear were 0·03%, and 0·25% for the eye.

CONCLUSIONS

The reported results show that some of the nitrotetrahydrooxazine derivatives possess strong protozoocidal properties, but moderate toxicity. A further study of these compounds is planned.

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