EXPERIMENTAL STUDY OF THE NEW ANTITUBERCULOSIS DRUG DIAMBUTOL

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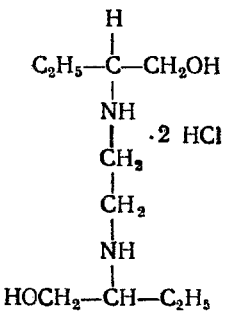
An original method of synthesizing the new antituberculosis drug diambutol (ethambutol) and its analogs and homologs has been developed in the organic synthesis division of this Institute [1].

Diambutol is 1 + 1 − 1',2'-bis(L-iminobutanol-1)ethane dihydrochloride. It is a white crystalline powder with a melting point of 197-198.5°, readily soluble in water. Its structural formula is:



EXPERIMENTAL METHOD AND RESULTS

The antibacterial activity of diambutol and its stereoisomers (meso forms, racemate and D-forms) was studied in a liquid nutrient medium with the addition of 10% human plasma by the method of double serial dilutions. The bacterial loading for all species and 250,000 bacterial cells/ml medium.

The compound was shown to be inactive against Gram-positive (Staphylococcus aureus, Streptococcus pyogenes, Sarcina lutea) and Gram-negative microorganisms (Escherichia coli, Proteus OX_{19}, Pseudomonas pyocyaneus, Salmonella typhi, Salm. paratyphi A, Salm. paratyphi B, Shigella flexneri, Shig. sonnei) even in concentrations of 200 μg/ml.

The action of diambutol on Mycobacterium tuberculosis was studied in a liquid synthetic medium (Shkol'nikova) with the addition of asparagine and, as in the case of determination of the activity of the compound against the other species of bacteria, 10% human blood plasma. The following strains of M. tuberculosis were used in the experiments: bovine strain B. Bovinus 8, and human strains "Akademiya", $H_{37}RV$, strain No. 123 resistant to streptomycin (100 units), phthivazid (10 μg) and PAS (50 μg/ml medium), and also strain No. 1445 resistant to streptomycin (100 units/ml) and phthivazid (20 μg/ml). Five experiments were carried out with each of the above strains, with a loading of 50 million bacterial cells/ml medium.

The experimental results were read every 7 days, starting from the end of the first week when abundant growth of the control culture was present. The results obtained on the 14th day of incubation are shown in Table 1.

The D-isomer and the meso form of diambutol were for practical purposes inactive against M. tuberculosis. The results show that the L-isomer possessed high activity (0.75-1.5 μg/ml) against different strains of M. tuberculosis,

TABLE 1. Minimal Bacteriostatic Concentration of Diambutol (in μg/ml) in Relation to Different Strains of Mycobacterium tuberculosis

Preparation	Strains				
	B. bovinus 8	"Akademiya"	$H_{37}RV$	No. 123 (resistant)	No. 1445 (resistant)
Racemate	3.0	3.0	3.0	4.2	3.0
L-isomer	1.5	0.75	0.75	1.5	1.5

TABLE 2. Results of Trials of the Chemotherapeutic Activity of Diambutol in Experimental Tuberculosis of Mice

Group of animals	Daily dose of compound	Number of mice	Mean change in weight of mice during experiment	Lungs		
				index of macroscopic changes	mean weight (in mg)	index of number of tubercle bacilli in smears
First	1	57	+ 1.75	0.28	235	0
Second	1.5	59	+ 2.55	0.19	220	0
Third	2.0	56	+ 2.65	0.09	225	0
Control	0	58	− 3.8	4.0	825	4.0

including those resistant to the action of other antituberculosis drugs. The activity of the racemate of diambutol in the same experimental conditions was only half that of the L-isomer, to which its activity was attributable.

The chemotherapeutic activity of diambutol hydrochloride (L-form) was studied in experimental hematogenous tuberculosis in albino mice. The mice used in the experiments weighed 18-20 g and the animals were inoculated intravenously with 0.1 mg of a culture of B. bovinus 8 to each mouse. Diambutol therapy began the day after inoculation. Each group of animals received an appropriate dose. Diambutol was given once daily by mouth for 32 days. The control group (untreated) and each experimental group consisted of about 60 mice. The experimental results were evaluated by a four-point system as suggested by G. N. Pershin.

It will be seen from Table 2 that the compound proved highly effective in the treatment of hematogenous tuberculosis in albino mice, as demonstrated by the indices of the macroscopic changes. In the animals of the experimental groups the weight of the lungs showed no change from that of the healthy animals, and no tubercle bacilli were found in smears from the lungs. During the experiment the mice gained in weight on the average by 1.75-2.55 g, whereas the animals of the control group lost weight, on the average by 3.8 g.

The animals of the experimental groups survived until the end of the experiment, whereas 72% of the mice of the control group died between the 27th and 31st days. It should be noted that, despite the remarkable therapeutic effect, when the mice received a dose of 2 mg per animal some degree of flabbiness and yellow discoloration of the liver was observed, and consequently this dose was not recommended for future use.

Hence experiments in vitro and the experimental treatment of animals infected with tuberculosis demonstrated the marked antituberculosis activity of diambutol. These findings agree with the observations of other workers [2, 3] who have made an experimental study of the action of this compound. The chemotherapeutic investigation of this compound is being continued in other species of animals and its pharmacological properties and its action on the host animal are being studied.

SUMMARY

Studies in vitro of diambutol (ethambutol), a new antituberculosis preparation, and of its analogs and homologs demonstrated that only the L-isomer of diambutol possessed high antituberculosis activity against various strains of Mycobact. tuberculosis both sensitive and resistant to the action of antituberculosis preparations.

A study of diambutol (L-form) on a model of hematogenic tuberculosis of albino mice demonstrated its high chemotherapeutic activity. Chemotherapeutic trails of the preparation being continued on other species of animals; further studies in its pharmacological properties and its effect on the macroorganism are also being made.

LITERATURE CITED

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
