N, N^1-MALONYL-BIS-ETHYLENIMINE (MEI) AND SOME OF ITS BIOLOGICAL PROPERTIES

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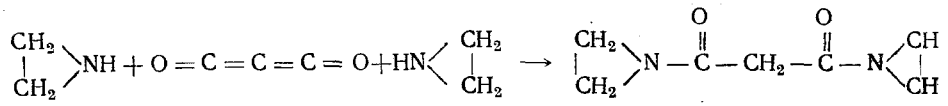
Compounds possessing the ethylenimine ring are potential bearers of antitumor properties. Thus, the synthesis of these preparations has attracted increasing attention from investigators. In the last few years, a rather large number of preparations have been obtained containing ethylenimine groupings, of which several have been taken as official, antitumor, therapeutic agents (TEF, ThioTEF, DIPIN, et al.).

Obtaining different derivatives of ethylenimine is accompanied by a number of experimental diffculties, involving the exceptional lability of the three-membered ethylenimine ring. Direct alkylation or acylation of ethylenimine, using the usual agents, is often excluded, since in the process of the reaction the ethylenimine ring is broken up. In connection with this, derivatives of ethylenimine are usually obtained by indirect means, through the cyclization reaction of C-substituted β-haloid-ethylamine. Nonetheless, this does not exclude the pathway of direct acylization. There are data in the literature [5] showing that N-acylethylenimine can be obtained by the direct acylation of ethylenimine with ketenes.

Occupying ourselves with a study of the chemistry of carbon suboxide, we obtained [4] N,N^1-malonyl-bis-chlor (brom) ethylamine (MEI)—a substance of interest both from the chemical and biological points of view.

Continuing the experiments, we decied to study the reaction of carbon suboxide with ethylenimine. It was shown that gaseous carbon suboxide at -20° reacts with ethylenimine in a medium of absolute diethyl ether, yielding a quantitative output of MEI according to the schema:



The obtained compound was identified with the aid of chemical and spectral analysis. The infrared spectrum of the material confirmed the presence of the ethylenimine ring (deformation waves of the ring according to our data: 1210 cm^{-1} and 857 cm^{-1}; according to the data in the literature for ethylenimine [8]: 1218 cm^{-1} and 852 cm^{-1}). All this made it possible to attribute to the synthesized compound the molecular structure of MEI. It should be noted that our proposed method of direct malonation of ethylenimine with the help of carbon suboxide is, at present, the only possible technique, and appears to be prospective, since it is possible, following the indicated schema, to malonate other C-substituted ethylenimines. Information on the obtaining and investigation of the latter will be presented in special reports.

EXPERIMENTAL METHOD AND RESULTS

Synthesis of MEI. Gaseous carbon suboxide was obtained by the method which we developed [3,4] and was taken directly from the pyrolysis oven and placed in the reaction mixture. Ethylenimine was obtained according to the technique described in the literature, from the chlorhydrate of β-chlorethylamine. For the reaction we used freshly distilled ethylenimine, with a boiling temperature of 56°. After chilling to minus 20—minus 15°, a solution of 4 grams of ethylenimine in 11 ml of absolute diethyl ether was passed through a small surplus of dry carbon suboxide. After a certain period of time fine white crystals began to settle out. Following conclusion of the reaction, the ether was evaporated in a vacuum and the material was washed several times in absolute ether. The melting point was 42—44° (out of the ether), and the output quantitative.

The material presented as white, acicular or lamellar crystals, soluble in chloroform, dichlorethane, acetone, and less soluble in ethanol, ether and water.

Real (in %): H 6.68; C 54.39; 18.11
Calculated (in %): H 6.54; C 54.53; 18.17

Biological Activity. The biological activity of MEI was studied on different biological subjects. For preliminary appraisal of the antitumor properties of the preparation, we used tissue cultures of tumor and normal cells and a bacteriophage model.

In test tubes containing earlier grown cells from a tissue culture of uterine fibrocarcinoma (strain HeLa) and from a single-layer trypsinized culture of tissue from human embryo, we added various concentrations of MEI.

Dilutions of the preparation were prepared in a medium consisting of Henk's solution plus antibiotics (90%) and aminopeptide (10%).

MEI in a concentration of 60 micrograms/ml and higher caused death of the HeLa cells, which peeled from the walls of the test tube on the following day after addition of the preparation. With use of smaller doses (15–30 micrograms/ml) we observed formation of large cells, with clearly contoured nuclei, which died by the 2nd–3rd day. With still lower concentrations of the preparation (5–10 micrograms/ml) the cells retained their viability, but were significantly larger than in the control. The same changes took place with the action of the preparation on the cells of the tissue culture of human embryo.

The antiphage activity of MEI was studied on bacteriophages previously selected by us as test subjects for a comparative appraisal of derivatives of di-β-chlorethylamine [1]. They were the coli-dysentery phages of the T-system (T 1, T 2, T 3, T 6r and T 7) and the coli-phages 0 26 and 0111, lysing enteropathogenic intestinal bacilli of the corresponding serotypes.

In a test tube containing a determined concentration of the preparation in 0.9 ml of physiological saline, we added 10^7 particles of phage, contained in a volume of 0.1 ml. After a 15 minute exposure we determined the number of active phage particles by the method of agar layers, according to Gratia [7]. In the control, instead of a solution of the preparation we used the same volume of physiological saline, into which we also placed 0.1 ml of phage. The percent activity of the phage was calculated by means of comparing the number of phage colonies in the control (taken as 100%) with the number of colonies obtained after treatment of the phage with the preparation.

TABLE 1. Antiphage Activity of MEI

Concentration of MEI (μg/ml)	Number of active particles of bacteriophage						
	T 1	T 3	T 7	0 26	0 111	T 2	T 6r
100	0,0007	28	0,0004	23	0,9	0,003	0,3
50	0,002	30	0,0007	23	2	0,07	0,3
25	0,03	36	0,009	25	31	0,009	0,45
12,5	0,09	46	0,05	32	42	0,1	0,8

Table 1 shows that MEI is an inhibitor of even and odd numbered phages of the T-system. Bacteriophages which contain cytosine (T 1, T 3, and T 7) and 5-oxymethylocytosine (T 2 and T 6) in the composition of their DNA were observed to be highly sensitive to MEI. It should be noted the Embichin 7, in these same concentrations, showed almost no inhibitory action on the phages containing the unusual nitrogenous bases–5-oxymethylcytosine [1].

T 3 was comparatively the most resistant of the T-phages, the composition of its DNA differing from the other T-phages in the relationship of the nucleic bases. It is known that for phages T 2, T 4 and T 6 the ratio: $\frac{\text{adenine + thymine}}{\text{guanine + cytosine}}$ is equal to 1.79 – 1.93, for phage T1–1.37, and for phage T 3–1 [2]. In connection with this, it is probable that the equal index of the relationship of nucleic bases in phage T 3 and its host gives the former a higher resistance than the other phages against MEI, which, like other ethylenimine derivatives, possesses DNA-tropic activity.

The antivirus activity of the preparation was investigated against viruses containing DNA and RNA: the Herpes virus, adenoviruses of the 2nd and 12th types, vaccine strain of type 2 poliomyelitis virus, and the grippe virus A2.

TABLE 2. Antibacterial Spectrum of MEI

Bacteria studied	Concentration of preparation (μg/ml), showing	
	bacteriostatic activity	bacteriocidal activity
Staphylococcus aureus	250	500
Streptococcus haemoliticus	125—250 and up	500
Bac. anthracoides	500	—
Bact. prodigiosum	500	—
Bact. pyocyaneum	250	500
Bact. proteus vulgaris	250	500
E. coli B	250	500
E. coli O26 O111	250	500
Bact. typhi abdominalis	125—250	250

To the virus, containing between 100 and 1000 infectious doses in 0.9 ml, we added various concentrations of the preparation, in a volume of 0.1 ml. After a 60 minute contact, the viruses were diluted 10 or 100 times respectively, and used to inoculate 4-day-old cultures of HeLa cells. The test tubes were inspected daily, and the final results were considered with the presence of a cytopathogenic effect in the virus control, and with the absence of degenerative changes in the cells of the preparation control. After treatment with the preparation at the indicated exposure, the grippe virus was injected into the allantoic cavity of 9—11 day old chick embryos, using a volume of 0.2 ml. The presence of virus was determined after 48 hours, using the reaction of hemagglutination.

MEI in a concentration of 250 micrograms/ml did not exert an inhibitory effect on the poliomyelitis virus, the adenoviruses or the grippe virus. It showed a marked inhibitory action on the Herpes virus in a concentration of 200 micrograms/ml. This was judged from the absence of the virus's cytopathogenic effect on the HeLa cells. With a concentration of 100 micrograms/ml, we noted a partial cytopathogenic effect, and with 50 micrograms/ml we did not observe any inhibitory action of the preparation on the virus.

The antibacterial properties of MEI were studied in relation to gram positive and gram negative bacteria, using the method of serial dilutions with subsequent seeding to nutritive agar in Petri dishes for evaluation of its bacteriocidal activity.

MEI showed a manifest inhibitory action on the bacterial cells. Table 2 shows that concentrations of the preparation of 250—500 micrograms/ml caused complete death of the bacteria studied. Partial elimination of the bacteria was observed with exposure to somewhat lower doses of the preparation. In this case, MEI did not show a selective action on certain species of the bacteria studied.

MEI is a representative of compounds which are unique in chemical structure and have not been described in the literature (malonated C-substituted ethylenimines). It has been established that it retains the ethylenimine three-membered ring, which imparts to it chemical properties characteristic of substituted ethylenimines.

MEI does not possess a selective activity, expressed only against tumor tissue. Like ThioTEF, and in approximately the same doses, it depresses the development of both normal and tumor cells in tissue cultures. The inhibitory activity of MEI on bacteriophages is considerably stronger than in other antitumor agents, which may be evidence of more manifest DNA-tropic properties. This arouses definite interest in the further study of the compound's antitumor activity.

SUMMARY

The work describes the synthesis of N N_1—malonyl-bis-ethylenimine (a representative of a class of chemical compounds uninvestigated as yet) by condensation of carbon suboxide with ethylenimine.

A study of biological activity of the given compound demonstrated that the latter possessed a marked cytostatic action in relation to the normal and tumor cells in the tissue cultures. The preparation is an inhibitor of various bacteriophages both resistant and sensitive to the derivatives of di-B-chlorethylamine. Besides, in comparatively high concentrations it possessed bacteriostatic and bactericidal properties in respect to Gram-positive and Gram-negative bacilli. N N_1-malonyl-bis-ethylenimine presents a definite interest for further study of its antitumor properties.

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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.
