MASS-SPECTROMETRIC STUDY OF THE STRUCTURES OF THE METABOLITES OF BIS(1-AZIRIDINYL)-2-PYRIMIDYLAMINOPHOSPHINE OXIDE (PHOSPHEMIDE)

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The principal pathways of fragmentation of bis(1-aziridinyl)-2-pyrimidylaminophosphine oxide and its metabolites under the influence of electron impact that are characteristic for this group of compounds were established. Bis(2-chloroethylamino)-2-pyrimidylaminophosphine oxide and 1-aziridinyl-2-chloroethylamino-2-pyrimidylaminophosphine oxide structures were assigned to the isolated metabolites.

It has been shown [1, 2] that when rats are injected intravenously with the antitumorigenic preparation phosphemide (I), the unchanged preparation and two of its metabolites are detected in the urine of the intact animals. The present paper is devoted to the establishment of the structures of these metabolites by means of mass spectrometry.

For the interpretation of the spectra of the isolated metabolites we initially studied the spectra of phosphemide itself and its deutero analog (Fig. 1), which enabled us to propose the scheme of the fragmentation of the side compounds.

The phosphemide molecule has low stability with respect to electron impact (the intensity of the molecular ion peak is 7% of the integral intensity); this is explained by the presence of a rather long unconjugated side chain. The principal pathway of fragmentation is stepwise detachment of two ethyleneimine groups

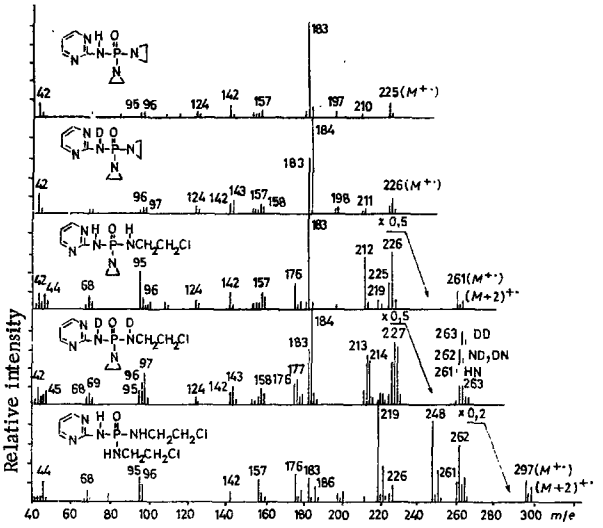

Fig. 1. Mass spectra of phosphemide and its deutero analog.

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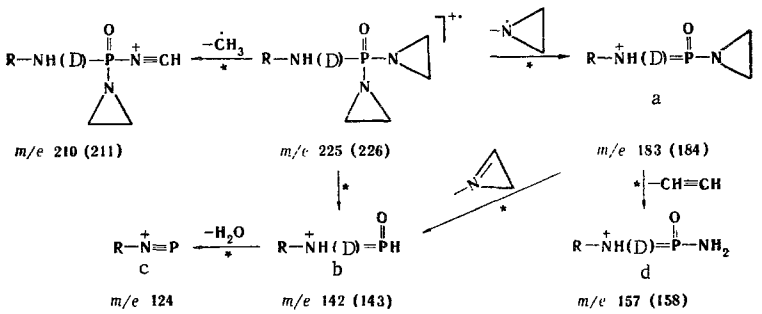
in the form of an ethyleneimine radical and a neutral aziridine particle with subsequent elimination of a neutral water molecule (Scheme 1). As a result of this chain of transformations, stable ion c, the phosphine group of which is in conjugation with the pyrimidine ring, is formed.

Scheme 1



R= 2-pyrimidyl

The maximum peak in the spectrum of phosphemide is the peak of fragment **a** with m/e 183, which is formed as a result of ejection from the molecular ion of an ethyleneimine radical. In addition to principal ion **b**, upon disintegration fragment **a** gives yet another minor fragment **d** (m/e 157), which is formed as a consequence of ejection of a neutral acetylene molecule.

Thus the principal pathways of fragmentation of phosphemide under the influence of electron impact are associated with disintegration of the side chain of the molecule, as a result of which characteristic ions with m/e 183, 157, 142, and 124 are formed. It should be noted that the ions characteristic for pyrimidine (m/e 79, 80) and aminopyrimidine (m/e 95, 96) in the spectrum of phosphemide have low intensities.

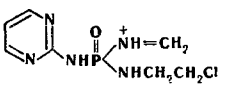
These data were used in the analysis of the mass spectra of metabolites II and III and their deutero analogs.

It follows from the mass spectrum of II that its molecular weight is 297. Thus the molecule contains an odd number of nitrogen atoms. The ratio of the principal isotope peaks in the molecular ions (I_{m+2}/I_m = 2/3) attests to the presence of two chlorine atoms in the molecule. Ions (m/e 183, 157, 142, 96, and 95) characteristic for the mass spectrum of phosphemide are observed in the spectrum of II.

The presence of ion peaks with m/e 95 and 96 attests to retention in the molecule of the investigated compound of a pyrimidine ring with an amino group in the side chain. At the same time, the spectrum does not contain fragments with m/e 94+35, 95 + 35, 93 + 35 + 35, and it is therefore logical to assume that the chlorine atoms are not bonded to the pyrimidine ring. The presence of ions with m/e 142, 157, 183, and 225 indicates retention of the phosphamide and, possibly, ethyleneimine groups, whereas the presence of a peak with m/e 226 constitutes evidence that a minimum of one other proton has appeared in II as compared with the starting phosphemide molecule.

Thus II is apparently the product of addition of two molecules of hydrogen chloride to the ethyleneimine portion of the phosphemide molecule (the difference in the molecular weights of II and phosphemide is 72). Inasmuch as ethyleneimine groups are usually opened at the C–N bond, it might be assumed that metabolite II is bis(2-chloroethylamino)-2-pyrimidylaminophosphine oxide.

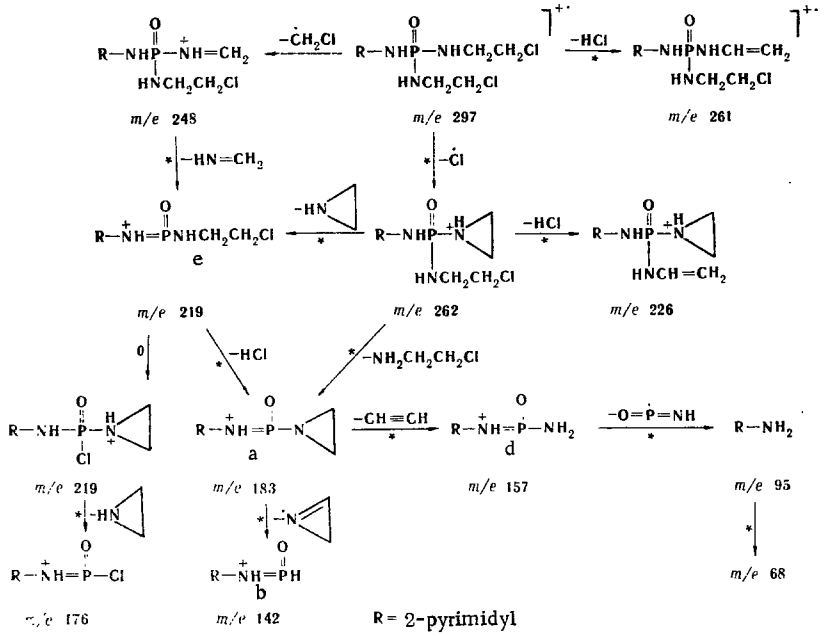
In fact, elimination of a $\dot{C}H_2Cl$ radical to give an intense ion peak with m/e 248 (87% of the maximum peak) is observed in the spectrum. This fragment should have high stability because of the appearance of amine stabilization:



m/e 248

The mass spectrum of the deutero analog of II, obtained by exchange of the labile protons by deuterium by recrystallization from deuteromethanol, also provides evidence in favor of proposed structure II. It follows from decomposition of the molecular ion into components as a function of the number of deuterium isotopes in the molecule that the compound under discussion contains three labile hydrogen atoms.

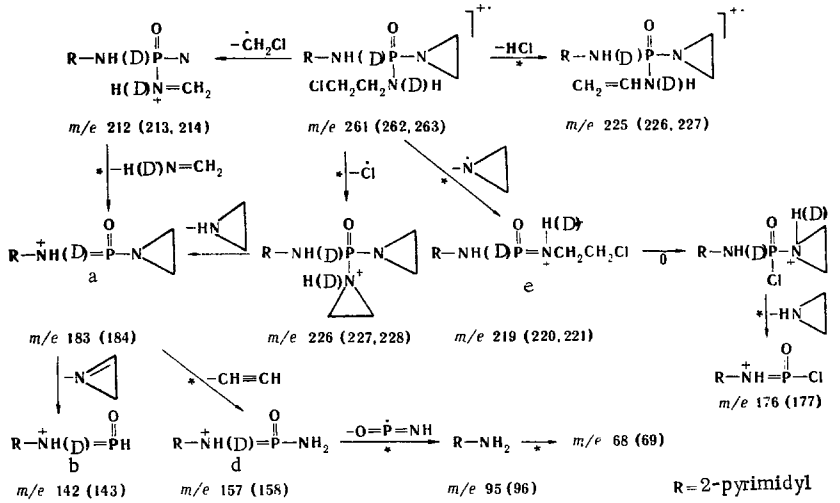
Scheme 2



The sequence of fragmentation of II presented in Scheme 2 completely confirms the structure proposed for it. Compound II was obtained synthetically and, according to its mass spectrum, proved to be identical to the isolated metabolite.

It follows from the mass spectrum of III that its molecular weight is 261 (thus the molecule retains an odd number of nitrogen atoms). It follows from the ratio of principal isotope peaks in the molecular ion ($I_{M+2}/I_M = 1/3$) that the molecule contains one chlorine atom. Ion peaks with m/e 226, 225, 219, 212, 183, 176, 157, 95, and 68 with metastable ions corresponding to the transitions between them, which are characteristic for the spectra of phosphemide and II, are observed in the mass spectrum of III. In addition, analysis of the mass spectrum of the deutero analog of III showed that the investigated compound contains two labile hydrogen atoms. Proceeding from these data we assumed that III is 1-aziridinyl-2-chloroethylamino-2-pyrimidylaminophosphine oxide. An analysis of the sequence of fragmentation of III and its deutero analog completely confirmed our assumption (Scheme 3).

Scheme 3



Thus the formation of a maximum ion with m/e 183 from the molecular ion as a result of stepwise elimination of a β-chloroethylamino fragment ($NHCH_2CH_2Cl$) via two paths occurs just as in the case of dichloro derivative II, and this indicates that this group is present in the molecule. Just as in the same process in the scheme of the disintegration of phosphemide, the elimination of a particle with mass 43 from the molecular ion to give fragment e attests to the presence of an ethyleneimine group in the starting molecule. The subsequent fragmentation of ions **a** and **e** is in complete agreement with that described in scheme 2.

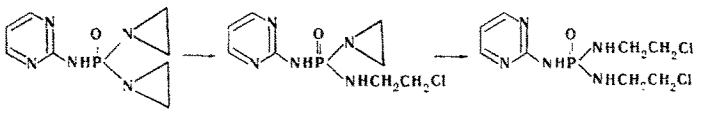
The following remarks touching upon the mass spectra of phosphemide and II and III must be made. Positively charged fragments that have a labile electron pair are formed during the fragmentation of the investigated substances, as a consequence of which the charge may be localized on different atoms of the fragment, for example:

m/e 183

However, in order to avoid encumbering on the schemes we present only one resonance structure for each ion.

As a rule, there are corresponding metastable ions for all of the processes (as indicated by the asterisks by the arrows in the fragmentation schemes). The proposed structures of the ions formed in the disintegration were confirmed by the spectra of the deutero analogs, the data for which are presented in parentheses.

Thus one of the pathways of biotransformation of phosphemide in the organism of the rat is successive opening of the ethyleneimine rings with the "formal" addition of hydrogen chloride molecules:



I III II

EXPERIMENTAL

The mass spectra were obtained with an MKh-1303 mass spectrometer with direct introduction of the samples into the ion source at 30 eV. The deutero derivatives were obtained by exchange of the labile protons by recrystallization from deutero methanol.

LITERATURE CITED

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2. L. F. Linberg, V. P. Pakhomov, N. S. Kurytov, G. F. Presnova, V. A. Chernov, and T. S. Safonova, Fourth International Symposium on Advances and Utilization of Chromatography, Bratislava (1973).