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ANALYSIS OF ORGANOPHOSPHATES. THE DETERMINATION OF AMIDOPHOSPHATES

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- CZECHOSLOVAKIA -

F O R E W O R D

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ANALYSIS OF ORGANOPHOSPHATES. THE DETERMINATION OF AMIDOPHOSPHATES

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Introduction

The application of organophosphates as insecticides has greatly expanded during the last few years. Thanks primarily to Schrader, a large variety of these compounds, which are highly effective especially against sucking insects, was prepared. Their biggest disadvantage was a strong toxicity for warm-blooded animals. Recently, however, there have been produced compounds which solve this inconvenience in a satisfactory way. An advantage of organophosphates is that they do not bring about resistance of insects (as is the case with chlorated insecticides).

A significant step forward was the discovery of systemically active organophosphates, although their toxicity is presently much higher than that of certain contact organophosphates.

The widest application among this group was noted with the so-called OMPA (octamethylpyrophosphoramide) or Schradan (I)

Pure OMPA is a viscose, colorless liquid with a melting point of 20° C, boiling point 142° C/2 mm Hg, density d_4^{25} 1.1314. It is soluble in water and organic solvents, with the exception of petroleum ether.

It is very stable in slightly acid or alkaline solutions. For spraying, it is used in a .1 - .3% water solution of technical preparations. In the same concentration, it is also used in seed treatment (saturation). The safety period is rather long and depends on the season of the harvest and the meteorological conditions. For crops harvested in May this period is four weeks; it has an increasing

tendency up to eight weeks for crops harvested in November. Its toxicity is rather high. According to accessible sources, there is a known LD_{50} for rats in oral application in doses between 10 - 20 mg/kg. The amount dangerous to men is estimated at 300-600 mg per os. In mammals the symptoms of poisoning arrive only after a certain latent period. Probably only the metabolites of Schradan act as inhibitors of cholinestherasis. Schradan was used even in this country against the poppy aphid. Schradan poisoning of forest animals was observed in this connection. It was necessary to test a method for detecting small amounts of Schradan and methods of isolation from biological material.

In technical products, OMPA is determined after a selective hydrolytic extraction in an alkaline media and titration of the free dimethylamine. In vegetable material, there were used in the detection and quantitative determination the phosphoric acid colorimetric method (in which H_3PO_4 is freed by acid hydrolysis), or the colorimetric method of determination of a secondary amine.

In our work we used both procedures. The latter was complemented by new methods of detection of secondary amines.

Experimental part

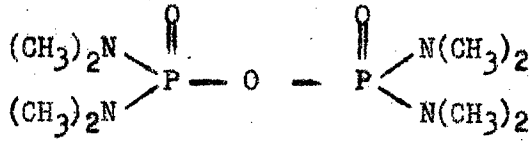
1) Appliances and chemicals used:

50 ml and 100 ml decomposition retorts with reversible cooling apparatuses.
250 ml and 100 ml measuring retorts.
Vasak-Sedivec arsenic determination apparatus with a test-tube provided with a side outlet.
Parnass-Wagner distillation apparatus.
Glycerine bath with a temperature control and mixer.
Lange-Universal colorimeter.
Brussels Sytam -- a technical product with 54% active substances.
1N H_2SO_4 , .02 N HCl , .02 N NaOH , .02 N $AgNO_3$; 30% NaOH, 20% soda.
.1% solution of hydrochloride dimethylamine freshly crystallized from alcohol.
10% volume solution of acetal in 1% nitroprussiate.
.1% solution $CuSO_4 \cdot 5H_2O$ in concentrated ammonia.
.5% solution $CoCl_2 \cdot 6H_2O$ in concentrated ammonia.
.5% solution $NiCl_2 \cdot 6H_2O$ in concentrated ammonia.
.5% solution $AgNO_3$ in distilled water.
Purest chloroform, refined pyridine, purest carbonic disulphide.
Arsenic trioxide.
Metal zinc.

2) Hydrolysis

In the hydrolysis of a molecule of OMPA with a mineral acid, there are formed 4 molecules of dimethylamine salt and 2 molecules

of phosphoric acid.



$$= 4\ /(CH_3)_2NH_2/^+ + 2H_3PO_4.$$

The conditions under which OMPA is quantitatively hydrolyzed have been examined. For that purpose a 1% (approximate) solution of active substances of the technical Brussels Sytam was prepared. The development of the hydrolysis was observed colorimetrically according to the contents of phosphoric acid in the hydrolyzed sample. In this process the phosphomolybdate blue method was used, and the intensity of coloring was measured by a Lange-Universal colorimeter with a yellow filter. The following procedure was observed.

A 1% sample (approx) of OMPA was taken with a 1 ml gauged pipette into a 50 ml decomposition retort, mixed with 20 ml of a mineral acid and heated under the reversible heating apparatus in a glycerine bath provided with a thermostat. After that, the sample was taken out of the bath, the cooling apparatus carefully rinsed with water, and the contents of the decomposition retort transferred into a 250 ml measuring retort, then filled up to the mark with distilled water. 10 ml of the measuring retort was sucked by a pipette and placed in a 100 ml measuring retort, dilluted approximately to 50 ml by distilled water, colored, filled up to the mark, and measured by a colorimeter. The results are given in Table I.

Table I.

Sample	Temperature °C	Length of hydrolysis in hours	Acid used	$mgPO_4^{-3}$a
1	25	¼	.08N H_2SO_4 b	.00
1	25	24	.08N H_2SO_4 b	.75
1	25	48	.08N H_2SO_4 b	1.25
2	100	1	1N H_2SO_4	6.65
3	100	2	1N H_2SO_4	6.95
4	100	3	1N H_2SO_4	6.90
5	110	1	.1N HCl	6.33
6	110	1	.1N H_2SO_4	5.45
7	110	1	1N HCl	7.00
8	110	1	1N H_2SO_4	6.80
9	110	2	1N H_2SO_4	7.00
10	110	4	1N H_2SO_4	6.90
11	110	6	1N H_2SO_4	7.10
12	110	8	1N H_2SO_4	7.00

"a" indicates the value denoting the number of mg of PO_4^{-3} ion in 1 ml of approximately 1% Sytam.

"b" indicates 1 ml of approximately 1% Sytam dilluted in a 250 ml measuring retort by .08N H_2SO_4 up to the mark and left at 25°C. 7.00 mg PO_4^{-3} is equivalent to 1.055% solution of Sytam (converted into OMPA for calculation purposes).

3) Proper detection

After hydrolysis, a proof for both products of the hydrolysis is made. The phosphoric acid is proved by conversion to phosphomolybden blue. For the detection of dimethylamine, a number of methods can be used, each of a different sensitivity.

A .1% solution of hydrochloride dimethylamine was used as a standard solution. The content of the active constituent was determined by a distillation method (for dimethylamine) and argentometrically (for chloride).

(a) The nitroprussiate - acetal reaction

This reaction is positive, even at a great dillution (positive even with a 1 ml sample mentioned in the preceding paragraph in phosphorus colorimetry). In greatly-dilluted solutions the method adapted by us proved to be better. 1 ml of activator and drops of a 20% solution of soda are added to a 1 ml sample until the originally vague color of the liquid reaches a deeper tone. In the presence of a secondary amine, there will appear gradually a red-purple to blue-purple coloring (according to the quantity of amine). The control test, which should be made at all times, gives an orange-red coloring.

(b) The carbonic disulphide-metal salts reaction

Secondary amines form in alkaline media with carbonic disulphide dialkyldithiocarbaminanes, which give, when combined with ions of certain metals, salts insoluble in water which can be transferred into organic solvents as color complexes. The proof was made currently with a copper salt, but we used also even cobalt and nickel salts.

First, 5 drops of a 30% NaOH, 1 ml ammonium complex of the respective metal, and 5-10 ml of .5% carbonic disulphide solution in chloroform are added to 1 ml of the sample and vigorously agitated. If a cobalt activator is used, the sensitivity at higher pH values decreases. The reaction is therefore made either without the addition of lye, or with a water solution of a cobalt salt in a mildly alkaline solution.

With a copper salt, there is found in the chloroform layer a yellow to ochre coloring; with a cobalt salt, a green one; with a nickel salt, a yellow-green one.

Our proof for dimethylamine by the reverse reaction of Vasak -- Sedivec belongs to this group.

1 ml of carbonic disulphide emulsion, dilluted in 100 ml of water, and 5 drops of 30% NaOH are added to 1 ml of the sample. After agitation it is neutralized by 15% H_2SO_4. A few drops of $AgNO_3$ solution (not too much!) are added and mixed in a test-tube with a 5 ml mixture of chloroform and pyridin (ratio of 4:1). A tube leading from a source of arsenic hydride is placed into the test-tube. The gas is brought inside for several minutes. The original clear or yellowish chloroform layer changes its color from pink through organge to carmine. In a small quantity of dimethylamine, it is necessary to separate the lower chloroform layer, dry it up with sodium sulphate; only then may arsenic hydride be introduced.

(c) The nitrous acid reaction

Secondary amines, when combined with nitrous acid, form nitrosamines, which are proved after distillation by the Liebermann reaction.

4) Isolation of biological material

The biological material is extracted in a Soxhlet apparatus by chloroform. Chloroform was transferred into a 100 ml decomposition retort, covered by 20 ml of 1N H_2SO_4, and after connection with the reversible cooling apparatus, the retort was put into a glycerine bath. By means of a gradual increase of temperature, chloroform is slowly vaporized (no water passes through the cooling apparatus). After that, the water was allowed to pass through the cooling apparatus, and the temperature of the bath increased to 110°C. At this temperature the sample was hydrolyzed for two hours. Then the sample was removed, the cooling apparatus rinsed with water, the hydrolyzed product filtrated, and the filtrate tested for the presence of phosphoric acid and dimethylamine. If the hydrolyzed product was not pure, it was reduced by vaporization to a smaller volume, and dimethylamin isolated by distillation in a Parnass-Wagner microapparatus.

Results and Discussion

It is evident from Table I that for a quantitative hydrolysis of Schradan, a two-hour heating with a normal acid at 100°C is sufficient. Further, it is also clear that hydrolysis by means of hydrochloric acid develops much better than hydrolysis by means of sulphuric acid of the same concentration. In spite of this fact, we used sulphuric acid; chiefly to prevent precipitation of silver chloride in proving dimethylamine by the silver dimethyldithiocarbamine method. At laboratory temperature, the hydrolysis develops even at a relatively low pH very slowly. At approximately 1.5 pH, the sample hydrolyzed only by 18% after two days.

The large variety of proofs for dimethylamine makes it possible to realize a semi-quantitative estimation of Schradan in the respective

sample. The mentioned reactions, as a matter of fact, have various degrees of sensitivity. When using the adapted nitroprussiate - acetal proof, we may detect as little as 5μg of the substance; with copper salt proof, 10μg; with the cobalt salt, 20-30μg; with nickel salt, approximately 100μg can be detected. In the silver salt - arsenic hydride reaction, 40μg of the substance will be sufficient, but a deep carmine color is obtained only after the content of dimethylamine hydrochloride reaches several tenths of mg. The nitrous acid reaction is positive on a centigram scale. It is therefore possible to estimate the quantity of dimethylamine in a sample according to which quantitative reactions are positive and which are negative.

In the isolation from biological material, it is a great advantage that OMPA is soluble both in water and in organic solvents. In its isolation it is possible to get rid of anorganic phosphates on one side (and thereby support the proof for phosphoric acid), and organic impurities on the other.

The most advantageous method of OMPA isolation and its quantitative determination will be presented in the following report.

Conclusion

The methods of proofs for Schradan, which can be used also for other aminophosphates, have been collected, extended, and supplemented. The recommended method is based on the acid hydrolysis of Schradan and proofs for both components of the hydrolysis: phosphoric acid and secondary amines. The report compares the sensitivity and advantages of methods used in the determination of secondary amines. Finally, it describes the isolation of the hydrolysis products from biological material.

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