INTERESTING GROUP OF HIGH-TOXIC ORGANOPHOSPHORUS COMPOUNDS

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Abstract

In the second half of the last century, a piece of information appeared that the U.S. planned to introduce a new supertoxic intermediate volatility agent (IVA) into the Armed Forces with the intent to gradually replace sarin (GB) and VX agent. The aim of this change should have been the elimination of disadvantages, low persistence of sarin in the field in summer conditions and, as for VX agent low efficiency in overcoming any protective barrier. The introduction of this substance has been closely connected with a new technology related to munitions called binary munitions. Binary technology is based on charging munitions using two independent harmless substances which are separated in two tanks. Both substances mix during the grenade or rocket flight and form a toxic compound. This mixing can be achieved by e.g. the rupture of a membrane between the tanks due to inertial forces, and in case of artillery rockets their rotation is employed. The binary munitions mostly contained sarin, VX and mustard gas. The "-2" character was then added to the code and so e.g. the charge the binary sarin was marked as GB-2. As to the new intermediate volatility agent, the principle of binary munitions had not been described until the beginning of the research. This information was an impulse for commencement and realization of research into the properties of a new agent or, if need be, a group of agents owing to defence and protection, for obvious reasons. The targeted research was initiated in 1983.

Key words
Intermediate volatility agent, organophosphorus agents, chemical warfare agents, supertoxic.

1 Introduction

In the modern history, the former Czechoslovakia was forced to pay attention to the protection against chemical weapons owing to its exposed strategic position. The research of new agents was the indispensable part of this effort, even in the times when the formulas of new agents were not known. The group of research workers conducted the primary research with the aim to synthetize and characterise (from physicochemical and toxicological point of view) relevant compounds and to support the small-scale production to enable the secondary research. The main goal of the secondary research was to determine the impact of relevant compound(s) on the already operational methods and means of the Czechoslovak Army and Civil Defence for physical and medical protection, i.e. on the set of protective clothing of all kinds, methods for field detection, monitoring and field analysis, means and methods of decontamination of all kinds, means and methods for first aid and medical treatment. The secondary research took place in all relevant departments of Research Institute 070 in Brno, the corresponding medical part at the Military Medical Academy in Hradec Kralove.

The research was considered as top secret in the very primary phases. Therefore, we marked the group of compounds under research arbitrarily and only for the internal communication by the GV code (due to the similarity with both G and V agents). We decided not to use both names, i. e. the code and chemical name or chemical formula together. The reason was obvious. First of all, using parts of the US army code could have brought future

problems in international communication because our designation was considered not to correspond to the potential (or actual) US Army code. This expectation has been proven as correct, actual code used in the US for our compound(s) is GP. Secondary, it was necessary to avoid any suspicion that Czechoslovakia had offensive chemical weapons assets. This was very serious in the time of the last rise of the East-West confrontation (synchronic with the beginning of the Star Wars era, of the deployment of nuclear forces in Europe and preparation for starting the binary weapons production program in the US). Especially, the latter issue and its justification were connected with compilation of various lists of chemical weapons possessors by CIA, containing, contrary to the truth, even the former Czechoslovakia. As the well-known, even outstanding authors, as J. P. Robinson and H. G. Brauch, in their review reports considered Czechoslovakia to be the owner of chemical weapons till the end of the 1980s and even later, obviously using the CIA database. (At various international forums Jiri Matousek protested repeatedly against the accusation that Czechoslovakia was stockpiling chemical weapons and the USSR deployed or stored chemical weapons on our territory). It was also decided not to publish the results prematurely.

It seems that nowadays is a correct momentum to reveal the principal results of the primary research to point out to the international community that the real danger of introducing new supertoxic lethal organophosphorus compounds exists.

From all possible models onto consideration a group of organophosphorus compounds corresponding to the general formula, see Figure 1.

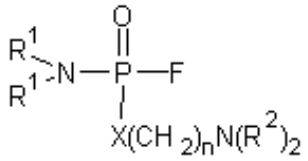
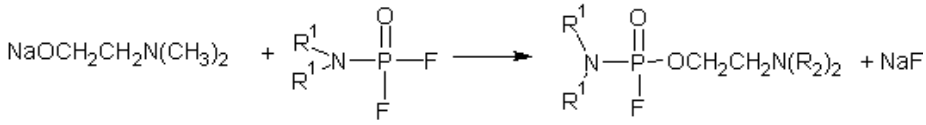


Fig. 1
General formula O-Dialkyalminoalkyl(dialkylamido)fluorophosphate

2 Results and Discussion

2. 1 Synthesis of O-Dialkylaminoalkyl(dialkylamido)fluorophosphate

The elaborated method for the O-dimethylaminoethyl(dimethylamido)fluorophosphate DMAEDMAFP synthesis, when difluoride(dialkylamido)phosphoric acid and sodium 2-dimethylaminoetanolate were used as the last step of the synthesis, have confirmed the feasibility of the conclusions resulting from the literature search focused on published general and special methods for the preparation of substituted alkylfluorphosphates.



(1)

The theoretical yield of the DMAEDMAFP synthesis obtained trough the above mentioned method is comparable with the literature data and ranged from 75 to 85 % (determined by the combined fluorine content). It proved that the DMAEDMAFP preparation using 2-dimethylaminoethanol is not suitable.

Table 1
Summary of physical properties of O-dialkyalminoalkyl(dialkylamido)fluorophosphate

Name	R^1	R^2	n	Index n_D^{20}	Density [g/cm^3]	M. p. [°C]	B. p. [°C/Pa]
DMAEDMAFP	$-CH_3$	$-CH_3$	2	1,4198	1,1096	- 110,2	39,0 /2,0
DMAEDEAFP	$-CH_2CH_3$	$-CH_3$	2	1,4099	1,0722	- 95,3	56,7 /6,5
DEAEDMAFP	$-CH_3$	$-CH_2CH_3$	2	1,4267	1,0463	- 84,1	53,0 /0,3
DEAEDEAFP	$-CH_2CH_3$	$-CH_2CH_3$	2	1,4308	1,0184	- 91,0	56,0 /0,1
DMAPDMAFP	$-CH_3$	$-CH_3$	3	1,4250	1,0370	- 82,1	56,0 /0,7
DMAPDEAFP	$-CH_2CH_3$	$-CH_3$	3	1,4282	1,0190	- 85,6	68,0/4,5

Note: Compare the name with the general formula, which is shown in the Figure 1.

The measured refractive indexes of individual compounds are in good conformity with the calculated values of total binding constants. The difference between the experimentally determined and calculated values has the range of ± 0.74 %. In addition to DMAEDEAFP, which showed a smaller refractive index than the theory, all the other experimental agents had higher experimental values. The confidence interval for averages was calculated based on 5 measurements for significance level of $\alpha = 0.05$. For the measured refractive indexes it was ± 0.0002 and for the specific density it was from ± 0.0033 up to 0.0051 for individual analogs. DMAEDMAFP showed the highest specific density. The specific density of individual analogs decreases based on the increasing molecular weight. When evaluating the relationship of the molar liquid volume (at a given temperature) and the molecular weight, the linear relationship was determined. In the experimental determination of melting points, the individual analogs super cooled to a temperature of -160 °C using liquid nitrogen, and after their freezing the course of melting was visually observed.

DMAEDMAFP showed the lowest melting point and DMAPDMAFP showed the highest melting point. All the studied compounds in the vicinity of the melting point showed the mesomorphic state. They became cloudy and had the consistency of honey. Upon further heating the liquid became clear and vivid and didn´t show higher viscosity.

When exposed to heat, all the studied compounds underwent decomposition to form solid products. Only in case DMAPDMAFP and DMAPDEAFP with extended alkoxy groups, the observable browning and thickening of the liquid occurred without the precipitation of solid products from the liquid. The boiling point of these compounds is measurable only in case of reduced pressure. The lowest boiling point was determined in DMAEDMAFP and the highest boiling point was determined in DMAPDEAFP. The vapor pressure was experimentally determined using the dynamic method in DMAEDMAFP only because a sizeable amount of the compound was needed for this determination from the viewpoint of time demand as well.

The calculated A, B constants August equation from experimental data for log p:

$$A = 12.802 \pm 3.322B = 3612 \pm 1022$$

The calculated boiling point at a pressure of 101.3 kPa:

$$t = 190.5 \pm 53.3\ °C$$

The calculated value of the molar heat of vaporization:

$$H_V = (4.86 \pm 1.36).10^3\ J.mol^{-1}$$

The standard deviation s_{yx} = 0.0536

The correlation factor R = - 0.9835

Proportional vapor density = 6.87

Solubility:
- easy soluble – water, alcohols, acetone, chlorinated hydrocarbons (chloroform, 1,2-dichloroethane),
- sparingly soluble – aliphatic and aromatic hydrocarbons.

2. 2 Molecular Distillation of Synthesized Compounds

0-dialkylaminoalkyl(dialkylamido)fluorophosphates prepared trough synthesis was brown, clear liquids. An active ingredient with a minimum content of 70 % was determined in then. However, this content was inadequate for further work and it was necessary to develop a new separation method that would provide the desired compounds with the content of an active ingredient at least 95 %. Common separation methods are not applicable for this group of compounds for the two following reasons:

1) any increase in temperature accelerated the decomposition of various analogs to form solid and liquid products while reducing inhibitory activity; and
2) Atmospheric moisture also adversely affected the separation to form the hydrolysis products. Individual compounds also manifested themselves as hygroscopic compounds capable of binding atmospheric moisture.

For these reasons a separation method that would eliminate both negative factors was sought.

Molecular distillation provided the only possibility for obtaining compounds with required purity.

It enabled the separation of a compound under defined conditions, i.e. sufficiently high temperature gradient of 60 to 100 °C between the surface of the distilled liquid and the surface of the cooler, and sufficiently high vacuum < 1 Pa and a short distance of 0.5 to 2 cm between the surface of the distilled liquid and the surface of the cooler. DMAEDMAFP and its analogs exhibited the content of active ingredient ≥ 95 % in all cases.

This fact was confirmed using the elemental analysis and gas chromatography when one peak appeared on the chromatogram. After the distillation it was necessary to temper the glass apparatus up to the laboratory temperature and to perform the transfer of the separated substance very quickly to limit the influence of air moisture. The active ingredient contents of the individual compounds are given in Table 2.

Table 2
Identification of the substance O-dialkylaminoalkyl(dialkylamido)fluorophosphates

Name	Distillation yield [%]	Assay of the active component in accordance with sec. N [%]	Assay of the active component bound by **F**
DMAEDMAFP	70 - 80	96.08 ± 0.67	97.04 ± 1.07
DMAEDEAFP	70 - 80	95.46 ± 0.77	95.23 ± 0.96
DEAEDMAFP	60 - 70	95.73 ± 0.97	96.34 ± 0.84
DEAEDEAFP	60 - 70	97.01 ± 0.82	96.57 ± 0.89
DMAPDMAFP	60 – 70	96.28 ± 0.88	95.86 ± 0.77
DMAPDEAFP	60 – 70	96.12 ± 0.93	96.30 ± 0.97

2. 3 Infrared, ^{1}H-NMR and Mass Spectra

2.3.1 Infrared spectra

No significant differences among spectra of the compounds were observed. Individual vibration maxima correspond to following bonds:

When compared with literature data it is possible to assign the characteristic vibrations to individual groups:

- bond vibration P = 0 for the wave number of 1270-1280 cm^{-1} *(the theoretical value for the measured group of substances is 1290 cm^{-1})*,
- bond vibration P-F for the wave number of 835-860 cm^{-1},
- bond vibration P -OC for the wave number of 835-860 cm^{-1} (coincide with P-F vibration),
- bond vibration PO- C for the wave number of 1020-1045 cm^{-1},
- bond vibration P-N for the wave number of 685 to 710 cm^{-1},
- bond vibration PN- C for the wave number of 965-995 cm^{-1}, 1310-1320 cm^{-1} (strain), 1075-1080 cm^{-1},
- bond vibration C-H for the wave number of 2960 cm^{-1}, 1460 cm^{-1} (deformation, asymmetric), 2870-2880 cm^{-1}, 1370-1380 cm^{-1} (deformation, symmetric),
- bond vibration $(R)_2N$-C for the wave number of 2930 cm^{-1}, 2800-2830 cm^{-1}.

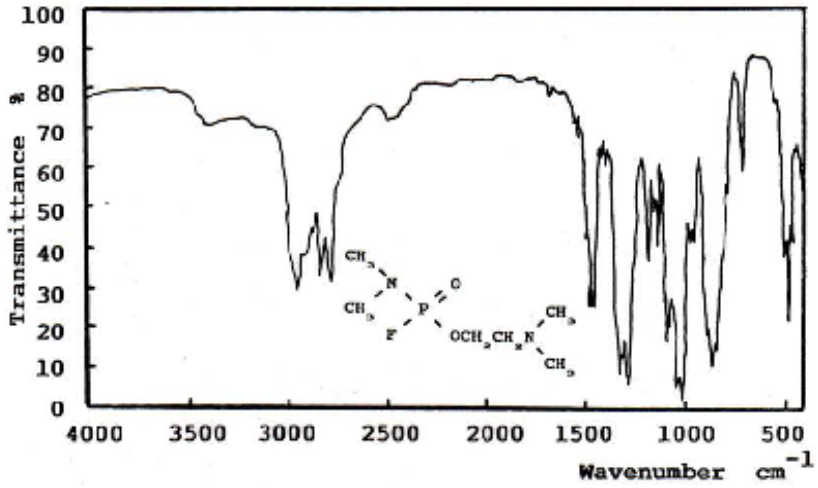


Fig. 2
IR spectrum O-dimethylaminoethyl(dimethylamido)fluorophosphate

2.3.2 ^{1}H-NMR Spectroscopy

The values of chemicals shifts of protons depend on their vicinity, i.e. electronegativity of the neighbouring atoms. Chemical shifts of protons were established in the range of 1.03 to 4.16 ppm.

Table 3
Values of chemical shifts δ [ppm] 0-dialkylaminoalkyl(dialkylamido)fluorophosphate

Name	**-CH_3**	**-CH_2-**	**-OCH_2-**	**-CH_2-**	**-CH_2N=**	**=NCH_2-**	**-CH_3**	**-OH**
DMAEDMAFP	2.75	-	4.14	-	2.65	-	2.30	-
DMAEDEAFP	1.,14	3.14	4.16	-	2.63	-	2.29	-
DEAEDMAFP	2.76	-	4.11	-	2.77	2.59	1.04	-
DEAEDEAFP	1.14	3.16	4.10	-	2.77	2.59	1.03	-
DMAPDMAFP	2.75	-	4.14	1.87	2.39	-	2.23	-
DMAPDEAFP	1.14	3.12	4.14	1.90	2.46	-	2.28	-

Table 4
Values of experimentally determined coupling constants

Coupling constants	**Dialkylamido - groups**	**Dialkylaminoalkoxy - groups**
$^3I_{HH}$	7.1 až 7.2	5.9 up to 6.3; 7.1up to 7.3
$^3I_{HP}$	8.6 až 12.3	7.3up to 7.,8
$^4I_{HF}$	1.6 až 1.9	was not measured

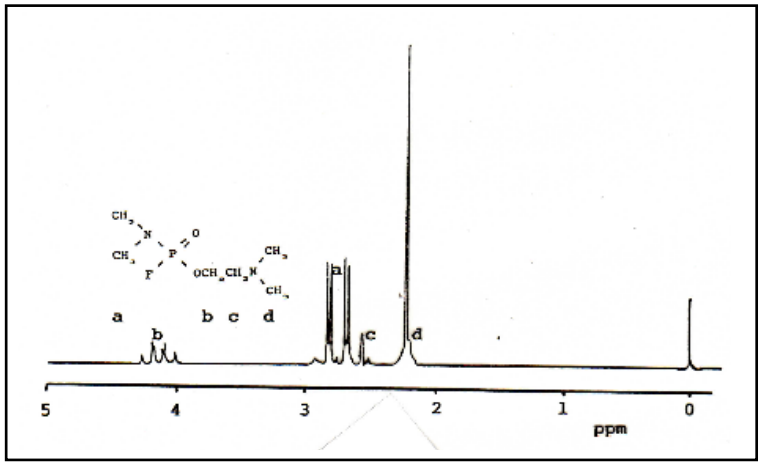


Fig. 3
^{1}H-NMR spectrum of O-dimethylaminoethyl(dimethylamido)fluorophosphate

Using the shifting agents the existence of two enantiomers was proved. ^{19}F, ^{31}P and ^{13}C NMR spectra were also determined.

2. 3. 3 Mass Spectrometry

The basic peak is due to the fragment $C_3H_8N^+$ with the value 58,1, except for dietylaminoethyl dimethylamidofluorophosphate where the basic peak is due to the fragment $C_2H_6N^+$ with the value of 44,1. The molecular peak was found in the case of compounds with

n = 3. Its represantion was up to 2 per cent. In the compound where R^1, R^2 = -CH_3 and n = 2, a fragment was found, corresponding with its mass to M-1^+. The splitting of the dialkylaminoalkoxy group proceeds at the α-carbon atom, similarly as in the case of the V-compounds.

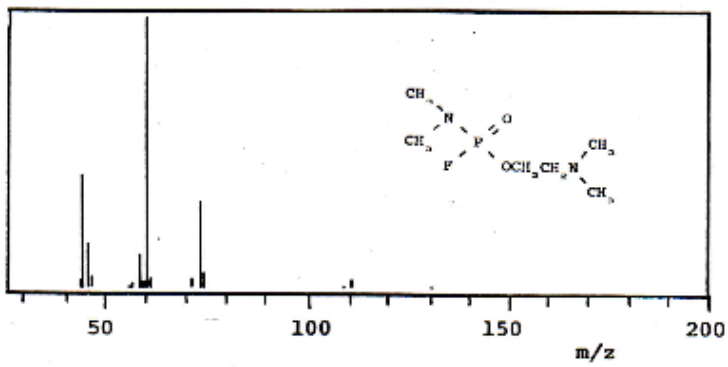


Fig. 4
Mass spectrum of O-dimethylaminoethyl(dimethylamido)fluorofosfate

Table 5
Values of main peaks and the weight of the largest peak of O-dialkylamonoalkyl (dialkylamido) fluorophosphate

Name	Basic peak		The largest peak	
	Value	Composition	Value	Composition
DMAEDMAFP	58.1	$C_3H_8N^+$	197.1 (154.1)	M-1^+, (M-$C_2H_6N^+$)
DMAEDEAFP	58.0	$C_3H_8N^+$	154.0	M- $C_4H_{10}N^+$
DEAEDMAFP	44.1	$C_2H_6N^+$	137.1	M- $C_5H_{14}N^+$
DEAEDEAFP	58.3	$C_3H_8N^+$	137.2	M- $C_6H_{15}NO^+$, (M-$C_7H_{19}N^+$)
DMAPDMAFP	58.0	$C_3H_8N^+$	212.1 (213.1)	M^+, M+1^+
DMAPDEAFP	58.2	$C_3H_8N^+$	240.1 (241.1)	M^+, M+1^+

2. 4 Inhibitory Activity

In case of synthesized compounds, the inhibitory activity was experimentally verified using the visual colorimetric biochemical method and its Ellman's modification. The inhibitory activity of decomposition products of various analogs was also verified.

It was at least by two orders of magnitude lower when compared with the initial substances. The biochemical method has been and continues to be one of the few available and applicable methods for the rapid and reliable determination of the active ingredient of O-dialkylaminoalky (dialkylamido) fluorophosphate in a normally equipped chemical laboratory without the need for the prior separation of decomposition products and precursors.

Table 6
I_{50} determination [mmol /ml]

DMAEDMAFP	DMAEDEAFP	DEAEDMAFP	DEAEDEAFP	DMAPDMAFP	DMEPDEAFP
$2.91.10^{-9}$	$2.80.10^{-9}$	$2.36.10^{-9}$	$1.50.10^{-9}$	$1.14.10^{-9}$	$1.76.10^{-9}$

When comparing both methods for determining the inhibition activity, the Ellman`s reagent method was less sensitive and, therefore, less suitable for the quantitative analysis. However, it has the advantage for the direct identification of separated spots using paper or thin-layer chromatography. It is also applicable for evaluating a larger number of samples.

2. 5 Toxicity of Synthesized Compounds

Table 7
Toxicity of O-dialkylaminoalkyl(dialkylamido)fluorophosphates

Compound	LD_{50} [μg/kg] at P = 0.95; i. m. application		Inhibition of Butyrylcholinesterase I_{50} [mmol/ml]
	Mice	Rats	
DMAEDMAFP	30.5 (28 - 55)	17 (15.5 - 23.6)	$2.91.10^{-9}$
DMAEDEAFP	191 (180 - 203)	35.5 (33 - 38)	$2.80.10^{-9}$
DEAEDMAFP	162 (150 - 175)	94 (87 - 101)	$2.36.10^{-9}$
DEAEDEAFP	409 (378 - 441)	261 (238 - 286)	$1.50.10^{-9}$
DMAPDMAFP	105 (94 - 118)	59 (52 - 67)	$1.14.10^{-9}$
DMAPDEAFP	1222 (1118 - 1336)	261 (238 - 286)	$3.76.10^{-9}$

Table 8
Toxicity of O-dimethylaminoethyl(dimethylamido)fluorophosphate

Application	LD_{50} [μg/kg] at P = 0.95	
	Mice	Rats
Intravenous	27.6 (25.6 - 29.4)	11 (8.5 - 17,.6)
Intramuscular	30.5 (28 - 55)	17 (15.5 - 23.6)
Subcutaneous	32 (29 - 53)	21 (18 - 26)
Oral	222 (194 - 255)	90 (81 - 272)
Percutaneous		1366 (881 - 3138)

Note: The agent of O-dimethylaminoethyl(dimethylamido)fluorophosphates had the highest toxicity from all mentioned agents. All others agents were syntetized for comparison of chemical and physical properties.

2. 6 Kinetics of the P-F Bond Cleavage of 2-dimethylaminoethyl (dimethylamido) fluorophosphate

The aim of experiments was to establish the kinetics of the P-F cleavage depending on pH and temperature. The fluoride ions liberated were determined continuously using the fluoride ion-selective electrode. The influence of the kind of buffer solution and ionic strength were established as well. No significant differences were found. From equation

of the potential response to the concentration of corresponding ion and from the kinetics equation of the 1st order, the equation for calculating the potential in the time **t** was determined depending on the rate constant, time and initial potential value $\mathbf{E_o}$.

$$E_t = E_o - b.\log(1 - e^{-k.t})\ [mV] \quad (2)$$

The data found experimentally were elaborated using the optimized program on the PC. From the measured values of the observed constant of the 1st order at pH = 9 and various temperatures in the range 25 - 45 °C the following data were calculated:

Activation energy	$E_a = 64.10 \pm 20.28$	$[kJ.mol^{-1}]$
Activation Gibbs's energy	$\Delta G^{\ddagger} = 93.76 \pm 29.67$	$[kJ.mol^{-1}]$
Activation entropy	$\Delta S^{\ddagger} = - 107.85 \pm 34.13$	$[J.mol^{-1}.K^{-1}]$
Activation enthalpy	$\Delta H^{\ddagger} = 61.66 \pm 19.51$	$[kJ.mol^{-1}]$
Frequency factor	$\ln A = 17.49 \pm 5.53$	

We have found that in the pH range 5 - 11 the dependence of the observed reaction rate constant of the 1st order at the constant temperature is linear.

3 Experimental Section

3.1 Method for the Synthesis of 0-dialkylaminoalkyl(dialkylamido)fluorophosphate

The chemicals have a minimum purity of more than 97% of the active ingredient. Our prepared and used intermediates have a minimum purity of 93.7%.

3.1.1 Preparation with 10 % Excess Difluoride (dialkylamido) phosphoric Acid and the Corresponding Alcoholate

DMAEDMAFP and its analogs were prepared by treating the corresponding difluoro (dialkylamido) phosphoric acid and sodium alkoholate at temperatures ranging from 20 to 25 °C.

3. 2 Molecular Distillation of Synthesized Compounds

The molecular distillation synthesized compounds was carried out in a glass apparatus of our own design which was attached to the VS - 35 high vacuum pump set, and the achieved vacuum was continuously monitored using Mac Leod´s vacuum gauge which was connected alongside the glass apparatus. The glass apparatus was cooled using a mixture of acetone and solid carbon dioxide at a temperature of - 86 °C. The vacuum in the apparatus was maintained in the range of 0.09 to 6 Pa for individual analogs.

3.3 Physical Properties of the Synthesized Compounds

The physical properties of synthesized compounds were determined by commonly used methods. The refractive index n_D was determined using an Abbe refractometer at a temperature of 20 °C. Specific density ρ^{25} was determined by pycnometry at a temperature 25 °C. The melting point was determined visually at freezing to a temperature of -160 °C by immersing the test tube with the liquid in liquid nitrogen and reading out the temperature at which the substance was dissolved. The boiling point was determined visually by reading out the temperature and pressure in the distillation of the synthesized compounds. The saturated

vapour pressure of DMAEDMAFP was determined by the dynamic saturation method. The vapour density, the maximum saturated vapour concentration, the boiling point under normal pressure, the molar heat of vaporization, and the August equation constants were determined by calculation.

3.4 Spectral Methods

3.4.1 Infrared Spectrophotometry

Used Equipment and Chemicals:

- SPECORD 75 IR spectrophotometer, C. Zeiss, Jena, Germany

Procedure:

Samples of purified and synthesized compounds were measured in a concentrated state using the l-film technique, without a solvent, in KBr demountable cells. The instrument itself was calibrated for polystyrene.

For the measurement the following parameters were selected:

- Measuring range 400-4 000 cm^{-1}
- Registration scale 7.5 mm/100 cm
- Measuring time 2.2 minutes
- Slot 3
- Gain 2
- Time constant 1

3.4.2 ^{1}H - NMR Spectroscopy

Used equipment and chemicals :

- Bruker WP 80 Sy spectrometer, Bruker, Germany
- Deuterated chloroform (99.96 %), Aldrich
- Deuterated water (99.96 %), Aldrich
- TMS (99 %), Aldrich
- Tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dionates) europium 99 %, Aldrich, hereinafter referred to as $Eu(fod)_3$
- Tris(3-trifluoroacetyl-d-cymporato)europium 99 %, Aldrich, hereinafter referred to as $Eu(tfc)_3$

Procedure:

For the measurement of ^{1}H-NMR spectra of synthesized compounds, the solution of these compounds in deuterochloroform with a concentration of 15 $mmol/dm^3$ was prepared. The solution of 0.5 % tetramethylsilane (TMS) was used as an internal standard. The operating frequency of the instrument 80.13 MHz, spectral width 1200 Hz, digital resolution of 0.15 Hz. The measurements were performed in a cylindrical tube with a diameter of 5 mm. To prove the existence of the enantiomers, the achiral shift reagent $Eu(fod)_3$ was added to another tube with the same solution, and the optically active shift reagent $Eu(tfc)_3$ was added to the third tube in such an amount that the molar ratio was in the range 1.8 to 2.5 (tested agent: shift reagent).

3.4.3 Mass Spectrometry

Used equipment and chemicals :

- Hewlett Packard 5880 A gas chromatograph with MS 5995 A mass sspectrometer, Hewlett Packard. The chemicals have a minimum purity of more than 97 % of the active ingredient.

Procedure:

For the GC-MS analysis pure synthesized O-dimethylaminoethyl (dimethylamido) fluorophosphate was used. The infill of about 10 mg of the substance was dissolved in 10 ml of n-hexane. 5 ml of the sample was used for the analysis.

Working conditions :

a) Gas chromatograph:
   - Temperature: - injection chamber to 200 °C.
   - Column - program 100-200 °C, halt at 100 °C for 1 minute, a gradient of 5 °C/min, halt at 200 °C for 5 min.
   - FSOT capillary column 16 m long, inner diameter of 0.2 mm, stationary phase of 5 % phenyl- methylsilicone, layer 0.11 μm.
   - Carrier gas - helium 99,99 %, flow rate 1 ml/min .

b) Mass detector:
   - Electron impact ionization - 70 eV
   - Electron multiplier - 2000 V
   - Working pressure - 1.10^{-5} Pa
   - Resolution - 1 000

3.5 Determination of Inhibitory Activity

Visual Determination Using the Colorimetric Biochemical Method.

The chemicals :

- Reagent No. 10 (lyophilized plasma), Imuna Sarisske Michalany, SR.
- Clean butyrylcholinjodid, Lachema Brno.
- Other chemicals of minimum purity p.a.

Procedure:

a) Preparation of reagents :
   - Buffer solution - to dissolve 4.4 g of sodium tetraborate decahydrate and 3.4 g of boric acid in 500 ml of distilled water.
   - A solution - to dissolve about 7 mg phenol red in 25 ml of buffer solution and to add distilled water (without carbon dioxide) to 500 ml.
   - B solution - to disolve 160 mg of reagent No. 10 in 53,3 ml of solution; the solution is kept in the refrigerator.
   - C solution - to disolve 0,1 g butyrylcholiniodide in 10 ml of distilled water.

b) Determination :

1 ml of analyte diluted in distilled water was pipetted into a 10 ml test tube and 1 ml of B solutions was added.

1.5 ml of distilled water was pipetted into the third test tube and 1 ml of B solution was added. Diluted acetic acid was added to the orange solution. The solutions in prepared test tubes were allowed to incubate in the water bath at a temperature of 37 °C for 15 minutes. After this time, 0.5 ml of C solution was added into the first and the second test tube and the time of discoloration of initially red solutions to the orange color (the same as in the third test tube) was monitored. The % of inhibition of the analyte was calculated from the readout times of solution discoloration in the first and the second test tube. The particular concentration of the analyte were read out from the calibration curve for a given % inhibition.

% inhibition (% I) was calculated as follows:

$$\%\, I = \left(1 - \frac{A}{A_0}\right).100\ [\%] \qquad (3)$$

A_0 – absorbance of the blank sample,
A – absorbance of the sample analyzed.

3.6 Kinetics of the P-F Bond Cleavage of O-dimethylaminoethyl(dimethylamido) fluorophosphate

The volume of 35 ml of phosphate buffer solution with a desired pH value (containing sodium fluoride 1.10^{-3} $M.dm^{-3}$) was poured into the tempered titration bottle (volume 10 ml), and sodium chloride for arranging ionic strength to the 0.2 was added. The electrodes were inserted into the water bath during constant stirring and tempering. After establishing the electrode potential, 0.05 ml of DMAEDMAFP was added and the concentration change of fluoride ions was continuously followed. The achieved values were registered with the plotter with the frequency of 8 values per minute.

4 Conclusions

Pretense of experimental work was to synthesize and to characterize a listed ingredient organophosphorus O-dimethylaminoethyl(dimethylamido)fluorophosphate, which is referred to as DMAEDMAFP, known as an intermediate volatility agent IVA and we also referred to as GV agent. It was important to simultaneously synthesize and characterize a group of substances of the general formula O-dialkylaminoalkyl(dialkylamido)fluorophosphate and make the comparison with the aforementioned first substance. It was conducted confusion alkyl chains on individual stifling, respectively alkoxy chain extension. In experimental work has shown that the properties of this group of substances are similar. Due to the large scope of experimental work was subsequently paid attention to only one specifically mentioned compound above.

As the results of our research confirmed, these compounds have limited stability and are usable for military use in binary systems only, for which the suitable methods of final synthesis were verified , see Scheme 1. From a series of synthesized compounds when R^1, R^2 = $-CH_3$, $-CH_2CH_3$, and $-CH(CH_3)_2$, X = O, S, n = 2-3 have been experimentally verified the simplest homolog with R^1, R^2 = $-CH_3$, X = O, n = 2 showed the highest inhibitory activity, see Scheme 3.

This compound exhibited intramuscular toxicity LD_{50} i. m. 0.017 mg/kg for rats (comparable with the substance VX) and percutaneous toxicity of LD_{50} p. c. 1.37 mg/kg for rats.

In total, 6 novel compounds were synthesized. The separation method was developed (molecular distillation), which allowed the preparation of compounds having a purity of $\geq$ 95 %. Conditions for storage in the concentrated and diluted state were verified. The methods of detection and determination, including determination in low concentrations were devised.

Acknowledgments
This paper authors devote his teacher prof. Jiri Matousek. Many thanks to the staff of the former Research Institute 070 in Brno and Department of Toxicology Military Medical Academy in Hradec Kralove.

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