THE ION ASSOCIATES OF METHYL-, ETHYL- AND ISOPROPYL DERIVATIVES OF DIALKYLAMINOETHYL DIALKYLAMIDOFLUOROPHOSPHATE WITH BROMOPHENOL BLUE

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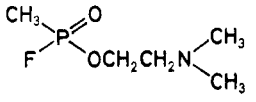
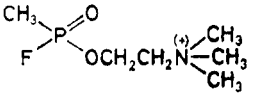
Nine new Tammelin esters were studied on the basis of the chloroform extracts of their ion associates with bromophenol blue. A study was made of the effect of the alkyl on the amino and amido groups of dialkylaminoethyl dialkylamidofluorophosphate and on the extraction efficiency of the ion pair. An increase in the number of carbon atoms on the amide group leads to an increase in the extraction efficiency of the ion pairs as a conseequnce of the increasing hydrophobicity. A further contribution to the increase in the extraction efficiency with increasing number of carbon atoms in the alkyls of the amino nitrogen is clearly retarded by the increasing basicitiy of the amino group. An extraction spectrophotometric determination of the test derivatives of dialkylaminoethyl dialkylamidofluorophosphate was developed and the interferences from precursors in the synthesis were examined.

In the nineteen fifties, in a search for toxic cholinergic phospho-organic substances, the Swedish team under the leadership of Tammelin[1,2] synthesized a number of substances including a group of derivatives of O-(N,N-dialkylaminoalkyl)methyl fluorophosphate (called Tammelin esters[3]), with a structure similar to that of acetyl choline. The substances were prepared[1,4] from the methylfluorochlorophosphate and 2-dialkylaminoethanol[1,4]. Special attention was paid to 2-dimethylaminoethyl methylfluorophosphate (*I*) and its choline derivative (*II*).



I



II

Freshly prepared dialkylaminoalkyl methylfluorophosphates are transparent, colourless liquids with high boiling points, preventing their distillation at normal pressures Compounds of type (*I*) are very unstable liquids readily undergoing conversions It has been suggested[5] that, on prolonged standing, the unstable cyclic compound

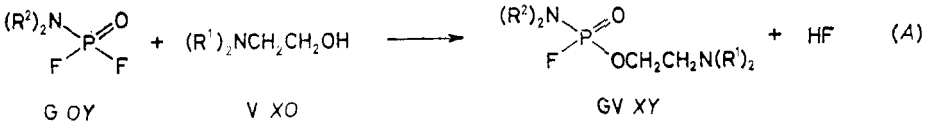
is formed spontaneously[6]; its structure has been studied by IR spectroscopy[7]. Bases (*I*, *II*) cannot be stored for prolonged periods under normal conditions as they are gradually converted to solid substances with high melting points. In spite of their low stability as a consequence of hydrolysis, choline esters of type (*II*) are about 3-times more toxic than isopropyl methylfluorophosphonate[2,8,9]. These esters phosphorylate enzymes through the release of a fluorine atom[9–11] and cannot be reactivated by oximes in vitro[12]; however, fast, spontaneous reactivation of the inhibited enzyme has been observed[13].

This work describes a study of the ability of selected dialkylaminoethyl dialkylamidofluorophosphates of the corresponding precursors – dialkylaminoethanol, dialkylamidodifluorophosphates and the decomposition hydrolytic products of dialkylaminoethyl dialkylamidofluorophosphates and dialkylamidodifluorophosphates to form ion associates with bromophenol blue that can be extracted into chloroform. This work was carried out in order to determine the possibility of determining the synthesized dialkylaminoethyl dialkylamidofluorophosphates by extraction spectrophotometry.

EXPERIMENTAL

Chemicals and Instruments

The Tammelin esters were synthesized by the reaction of the corresponding precursors dialkylamidodifluorophosphate (G *OY*) and dialkylaminoethanol (V *OX*) according to the general equation:



where *X* is the number of carbons in alkyl R^1 and *Y* is the number of carbons in alkyl R^2.

Table I gives a survey of the studied derivatives (manufactured by VOZ 072, Zemianské Kostolany) and precursors (Fluka). The purity of bromophenol blue (3,3',5,5'-tetrabromosulfophthalein) was controlled spectrophotometrically[14] and on Silufol UV plates (Kavalier, Sázava) using acetic acid–butyl acetate–H_2O (2:2:1) mobile phase; the dye was found to be 97·6% pure. Citrate and phosphate buffers were prepared from pH 2·0 to 7·0 in 0·5 pH steps (ref.[15]). Chloroform for extraction of the ion associates was shaken three times with distilled water and then distilled as the azeotropic mixture at 60°C. The remaining chemicals were at least of p.a. purity (Lachema, Brno).

The pH values of the buffer solutions were controlled using an MV-870 Präcitronic pH Meter (Dresden) with a type GA 50 N glass electrode and reference type SE 20 calomel electrode (Meinberg). The absorbance values were measured using a Spekol 11 single-beam spectrophotometer (C. Zeiss, Jena).

Procedures

The effect of the pH of the reaction medium on the formation and extraction of the ion associates of the test substances with the anionic dye was studied by determining the functional dependence $A = f(\mathrm{pH})$ at 410 nm (Figs 1, 2). Amounts of 2 ml of buffer with pH 2·0 to 7·0 in steps of 0·5 pH units were pipetted into test tubes along with 50 μl of a solution of the sample substance GV *XY* [*XY*, ($c_{\mathrm{GV}\ XY}$, μmol l^{-1}): 11 (39), 12 (54), 13 (25), 21 (100), 22 (23), 23 (22), 31 (43), 32 (29), 33 (18)] or precursor V *XO* [*XO*, ($c_{\mathrm{V}\ XO}$, mmol l^{-1}): 10 (1·26), 20 (0·32), 30 (0·06)] and 0·1 ml of a methanol solution of the dye bromophenol blue ($c_{\mathrm{L}} = 9$ mmol l^{-1}). The extraction was carried out by shaking into 2 ml of chloroform for a period of 2 min, drawing off of the aqueous phase and measuring the absorbance of the extract at 410 nm.

The composition of the associate between the test substance and the dye during the reaction was found from the functional dependence $A = f(x_{\mathrm{L}})$, where x_{L} is the mole fraction of the dye. An amount of 2 ml of the buffer with pH 3·5, 10 to 100 μl of methanol solution of the test substance ($c_{\mathrm{GV}\ XY} = 50$ mmol l^{-1}), transferred by micropipette in steps of 10 μl and 10 to 90 μl of a methanol solution of the dye ($c_{\mathrm{L}} = 50$ mmol l^{-1}) were extracted into 2 ml of chloroform and the absorbance of the ion associate was measured.

The molar absorption coefficient of the ion associate was determined after quantitative extraction of the dye as the ion associate into chloroform. An amount of 0 to 1 ml of the dye solution with $c_{\mathrm{L}} = 50$ μmol l^{-1} in buffer of pH 3·5 was diluted to a volume of 1 ml with buffer solution and was mixed with 1 ml of buffered solution of the test substance with a concentration of 20 mmol l^{-1} with the same pH. The extraction was carried out by shaking with 2 ml of chloroform. The quantitativeness of the extraction of the dye was checked spectrophotometrically.

TABLE I

Survey of the studied dialkylaminoethyl dialkylamidofluorophosphate, dialkylamidodifluorophosphates and dialkylaminoethanols

R^1	R^2	Code	Purity %
CH_3	CH_3	GV 11	48·6
CH_3	C_2H_5	GV 12	98·0
CH_3	i-C_3H_7	GV 13	97·0
C_2H_5	CH_3	GV 21	69·5
C_2H_5	C_2H_5	GV 22	77·4
C_2H_5	i-C_3H_7	GV 23	81·0
i-C_3H_7	CH_3	GV 31	99·5
i-C_3H_7	C_2H_5	GV 32	97·6
i-C_3H_7	i-C_3H_7	GV 33	90·6
—	CH_3	G 01	97·8
—	C_2H_5	G 02	98·3
—	i-C_3H_7	G 03	98·8
CH_3	—	V 10	98·0
C_2H_5	—	V 20	99·0
i-C_3H_7	—	V 30	98·0

After extraction of the ion associate, 2 ml of a solution of sodium hydroxide with a concentration of 0·1 mol l^{-1} was added to 1 ml of the aqueous phase and the absorbance was measured at 590 nm.

The dependence of the absorption at $\lambda = 410$ nm on the base concentration was determined by pipetting 2 ml of buffer of pH 3·5 into a set of test tubes, followed by 0·1 ml of methanol solution of the dye with $c_L = 9$ mmol l^{-1}, and micropipetting of 0 to 100 μl in steps of 10 μl of a methanol solution of the test substance. After shaking for 2 min with 2 ml of chloroform, the absorbance of the ion associate in the organic phase was measured at $\lambda = 410$ nm against pure solvent.

Chromatographic control of the ion associates of the test bases with bromophenol blue was carried out on commercial Silufol plates with a methanol–pyridine–formamide 80:15:5 (vol. %) mobile phase. An amount of 20 μl of the chloroform extract of the ion associated of the test dialkylaminoethyl dialkylamidofluorosphophate, methanol solutions of the technical products of their synthesis and the corresponding precursors with concentrations of 30 mmol l^{-1} were applied to the start of the activated plates (120°C, 2 h). After evaporation of the solvent, the plates were conditioned by vapour of the mobile phase for 30 min, were developed and dried in the air for 2 h. Biochemical choline esterase detection was carried out by first spraying the plates with a solution of butyrylcholine esterase (ÚSOL, Prague), prepared by dissolving 100 mg of the substance in 100 ml of phosphate buffer at pH 7·6. After 30-minute incubation at 20°C, detection was carried out by spraying with a mixture of the substrate (100 mg of acetylthiocholine iodide in 10 ml of buffer, pH 7·6) and 5,5′-dithio-bis(2-nitrobenzoic) acid (25 mg of the substance in 10 ml of buffer, pH 7·6) in a volume ratio of 1:1. After 3 min, white spots appeared

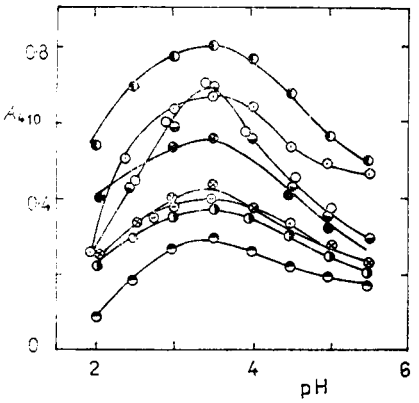


FIG. 1

The dependence of the absorbance ($\lambda =$ = 410 nm) of the ion associates of derivatives GV *XY* with bromophenol blue ($c = 0{\cdot}45$ mmol l^{-1}) on the pH. *XY*, $c_{GV\,XY}$ (μmol l^{-1}): ◓ 11, 39; ○ 12, 54; ⊖ 13, 25; ⊙ 21, 100; ◌ 22, 23; ● 23, 22; ◒ 31, 43; ◐ 32, 29; ◑ 33, 18

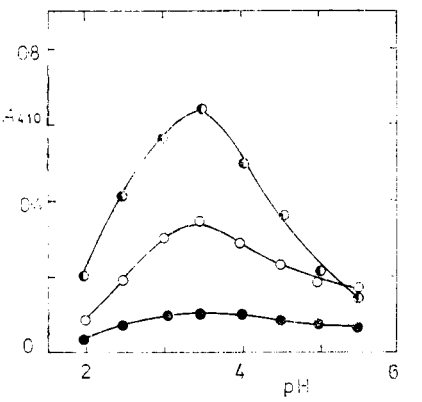


FIG. 2

The dependence of the absorbance ($\lambda =$ = 410 nm) of the ion associate of the precursor V *XO* with bromophenol blue ($c =$ = 0·45 mmol l^{-1}) on the pH. *XO*, $c_{V\,XO}$ (mmol l^{-1}): ● 10, 1·26; ○ 20, 0·32; ◐ 30, 0·06

on the yellow background. For detection with ninhydrin, the plates were heated for 10 min at 120°C. The positions of the red to blue spots were drawn on the pale pink background.

RESULTS AND DISCUSSION

The absorption curves of the yellow extract of the ion associate of substances GV 11 through 33 and precursors V 10 through 30 with bromophenol blue all exhibit an absorption maximum at a wavelength of 410 nm. It follows from the dependence of the absorbance at this wavelength on the pH (Figs 1,2) that a medium of citrate buffer solution with a pH of 3·5 is optimal for the formation and extraction of the ion associates of the studied bases and precursors of series V with bromophenol blue.

Thin-layer chromatography of the extracts of the ion associates of the technical products of the synthesis of GV substances with bromophenol blue demonstrated that the associates of all the analyzed subjected to enzyme detection yield a single spot corresponding to the effective substance[17] – dialkylaminoethyl dialkylamidofluorophosphate. Chromatographic separation of the ion associates of the GV substances, their technical products and the corresponding precursors followed by detection of the amidic and aminic compounds with ninhydrin demonstrated that the technical products of the synthesis of dialkylaminoethyl dialkylamidofluorophosphates do not contain precursors of series V. Semiquantitative TLC separation determined the ratio of the components of the ion associate, i.e. the active components or precursors of series V and the dye as 1 : 1. A methanol solution of the studied GV substance, precursor of series V or the dye were applied to the chromatographic plate in a series of samples with increasing content, so that the size of the evaluated spots of the base and bromophenol blue of the separated ion associate fell within the calibration series of standards. Knowledge of the stoichiometric ratio of the derivatives of dialkylaminoethyl dialkylamidofluorophosphate with bromophenol blue permitted use of the method of continuous variations to determine the content of active components in the technical synthesis products. The results of thin-layer chromatography confirmed that precursors of series G and the isomer of substance GV 11 – dimethylaziridinium dimethylamidofluorophosphate[16] with the studied dye do not yield an ion associate extractable into chloroform[17].

The extraction efficiency E (%) was calculated:

$$E = (\varepsilon'/\varepsilon)\,100\,, \tag{1}$$

along with the distribution ratio D, $(V_{org} = V_{aq})$,

$$D = E/(100 - E) = \varepsilon'/(\varepsilon - \varepsilon')\,, \tag{2}$$

and the conditional extraction constant K'_{ex} (ref.[18]):

$$K'_{ex} = D/\{c_L x_{L^-} - (A - A_1)/(\varepsilon' l)\}\,, \qquad (3)$$

TABLE II

Characteristics of the ion associates of dialkylaminoethyl dialkylamidofluorophosphates and dialkylaminoethanol with bromophenol blue

Base	Parameter[a]							
	k	q	ε'	ε	D	log K'_{ex}	L_D	L_Q
GV 11	3 480	0·031	3 480	17 600	0·25	3·28	0·97	1·37
12	11 250	0·043	11 230	21 520	1·09	3·99	0·42	0·77
13	18 050	0·017	18 010	22 880	3·72	4·89	0·44	0·73
21	6 580	0·029	6 570	20 140	0·48	3·64	1·06	1·43
22	11 090	0·033	11 070	20 120	1·22	5·45	0·62	0·78
23	20 270	0.0	20 320	21 300	18·61	4·54	0·74	1·42
31	11 060	0·055	11 040	21 100	1·10	4·50	0·74	1·14
32	15 480	0·010	15 450	23 770	1·86	4·75	0·63	1·02
33	19 110	0·0	19 070	19 330	89·91	6·70	0·97	1·95
V 10	720	0·013	720	9 360	0·08	1·86	1·72	3·53
20	1 000	0·029	1 000	18 180	0·06	2·13	2·01	3·27
30	8 720	0·017	8 700	21 970	0·66	3·87	0·41	1·00

[a] k (in l mol^{-1}) and q are the parameters of the linear dependence of the absorbance A at 410 nm on the concentration of the ion associate c ($A = kc + q$), ε' (in l mol^{-1} cm^{-1}) is the conditional molar absorption coefficient of the ion associate and ε (in l mol^{-1} cm^{-1}) is the molar absorption coefficient of the ion pair under the conditions of quantitative extraction at 410 nm, D is the distribution ratio, K'_{ex} is conditional extraction constant, L_D (in μg ml^{-1}) is the detection limit and L_Q (in μg ml^{-1}) is the determination limit.

TABLE III

Matrix of the extraction efficiency E (%) in of the ion associate of dialkylaminoethyl dialkylamidofluorophosphates and dialkylaminoethanols

G	V			
	0	1	2	3
1	6	20	52	79
2	8	33	55	95
3	40	52	65	99

where c_L is the initial analytical concentration of the dye, A is the absorbance of the ion associate of the base with the dye, A_1 is the absorbance of the blank solution, x_{L^-} is the mole fraction of the dye in the L^- form, ε' is the molar absorption coefficient of the ion associate, ε is the molar absorption coefficient of the ion pair of the studied substance with bromophenol blue under conditions for quantitative extraction (Table II) for $\lambda = 410$ nm and l is the cuvette pathlength.

As the number of carbons R^2 in the dialkylamide group increases for a constant number of carbon atoms R^1 in the dialkylamine group, the increasing hydrophobicity of the test derivatives leads to increasing extraction efficiency for the ion pair, as can be seen from the homologous series in Table III. A further, smaller contribution to an increase in the extraction efficiency for the ion associate can be seen in the homologous series of dialkylaminoethyl dialkylamidofluorophosphate, where alkyl R^2 on the amide nitrogen is the same and the amine alkyl R^1 is methyl, ethyl and isopropyl. An increase in the size of the alkyl in the amine group leads to an increase in the basicity of the amine nitrogen, leading to a decrease in the growth in the extraction efficiency for the ion pair. The greater effect of the alkyl of the amide group than that of the amine group on the extractability of the ion associate can be seen from the isomeric series (GV 13, 22, 31), isomeric pairs of derivatives GV 12 and 21 with extraction efficiencies of 52 and 33%, respectively, and the pair 13 and 31 with ion pair extraction efficiencies of 79 and 52%, respectively (Fig. 3).

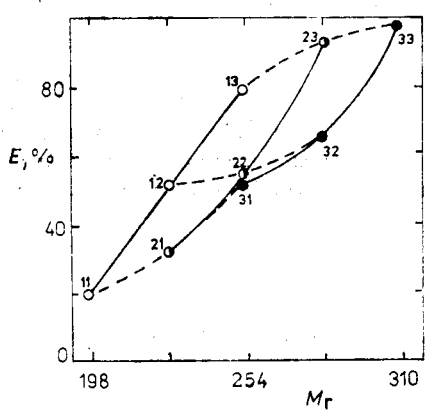


FIG. 3

Dependence of E (in %) for the ion associates of the studied derivatives GV 11 ghrough 33 with bromophenol blue on the relative molecular wieght M_r of the studied derivatives of dialkylaminoethyl dialkylamidofluorophosphates

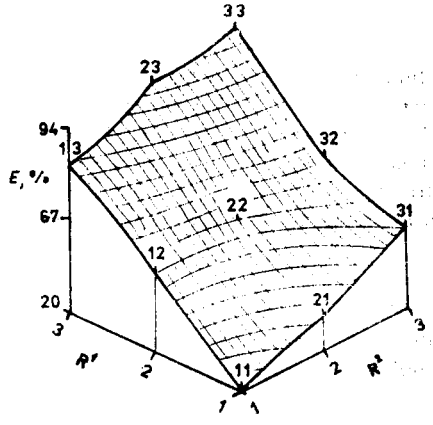


FIG. 4

Diagram of the dependence of E (in %) of the ion associates of dialkylaminoethyl dialkylamido fluorophosphates GV 11 through 33 with bromophenol blue on the number of carbons n in alkyls R^1 and R^2

Arranging of the studied derivatives of dialkylaminoethyl dialkylamidofluorophosphates on the basis of increasing extraction efficiency into eight rows is in agreement with the theoretically predicted effect of alkyls R^1 and R^2 of the amine and amide groups on the formation and extraction of the ion associates.

The results obtained are illustrated in the spatial diagram of the extraction efficiency of the ion pairs of the matrix of the nine studied derivatives of GV with bromophenol blue (Fig. 4).

It was found from the dependence $A = f(c_L)$ that a 5-fold excess of dye in the reaction mixture is optimal for the determination of the selected derivatives of GV and the precursors. A further increase in the concentration of bromophenol blue over a 10-fold excess leads primarily to an increase in the blank absorbance value.

The measured calibration curves for the studied derivatives of the GV substances and precursors of series V are linear up to a concentration of 0·1 mmol l^{-1}. All the determinations obey the Lambert–Beer law in the measured concentration range, as was found from the values of the correlation coefficient r, which is equal to 0·988 in the least favourable case. The detection and determination limits were found[19]. This method of extraction spectrophotometry can be used to determine 0·8 to 2·0 μg of the studied dialkylaminoethyl dialkylamidofluorophosphates in 1 ml of sample.

REFERENCES

1. Tammelin L. E.: Acta Chem. Scand. *11*, 859 (1957).
2. Tammelin L. E.: Ark. Kemi *12*, 287 (1958).
3. Franke S.: *Lehrbuch der Militärchemie*, Vol. 1, p. 426. Militärverlag, Berlin 1977.
4. Tammelin L. E.: Acta Chem. Scand. *11*, 1340 (1957).
5. Larsson L.: Acta Chem. Scand. *11*, 1131 (1957).
6. Heath D. F.0 *Organophosphorus Poisons*, Vol. 13. Pergamon, Oxford 1961.
7. Larsson L.: Acta Chem. Scand. *12*, 587 (1958).
8. Fredriksson T.: Arch. Int. Pharmacodyn. *113*, 101 (1957).
9. Fredriksson T.: Arch. Int. Pharmacodyn. *115*, 474 (1958).
10. Fredriksson T.: Acta Physiol. Scand. *42*, 145 (1957).
11. Fredriksson T., Tibbling G.: Biochem. Pharmacol. *2*, 286 (1959).
12. Enander I.: Acta Chem. Scand. *13*, 780 (1958).
13. Fredriksson T., Tibbling G.: Biochem. Pharmacol. *3*, 184 (1960).
14. Mouková N., Kubáň V.: Chem. Listy *73*, 1106 (1979).
15. Küster F. W., Thiel A.: *Chemical-Analytical Tables*. Academia, Prague 1988.
16. Halámek E., Tušarová I., Kobliha Z.: Collect. Czech. Chem. Commun., in press.
17. Halámek E., Tušarová I.: *Proc. Military Academy*, Ser. B.1., p. 2. VVŠ PV, Vyškov 1991.
18. Souček J., Halámek E., Kysilka R.: Collect. Czech. Chem. Commun. *53*, 1655 (1988).
19. Eckschlager K.: Chem. Listy *83*, 1009 (1989).

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