TLC Analysis of Intermediates Arising During the Preparation of Oxime HI-6 Dimethanesulfonate

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Abstract

In this study, several thin-layer chromatography (TLC) methods are described for the identification of quaternary and non-quaternary compounds (parent compounds, intermediates, by-products, and products) arisen within the synthesis of the acetylcholinesterase reactivator HI-6, at present the most promising antidote in the case of nerve agent poisonings. Using the TLC technique, particular E and Z isomers of this compound on the oxime group are separated. These TLC methods could be of high interest as quick purity control for those who are interested in development of new acetylcholinesterase reactivators and the synthesis of HI-6 in laboratories or in large-scale production.

Introduction

Organophosphorus compounds are a widespread group of substances used as pesticides, softening agents, drugs, and nerve agents. Probably the most known misuse of these compounds (as a nerve agent) seems to be the sarin Tokyo subway attack in 1995. Thanks to the quick administration of nerve agent antidotes—acetylcholinesterase (AChE; EC 3.1.1.7.) reactivator pralidoxime—only twelve people died and several thousands had mild to severe intoxication symptoms (1).

Although pralidoxime served as a very efficacious antidote in the treatment of sarin intoxications, its potency to reactivate other nerve agent intoxications is very limited. For example, it is unable to treat tabun, cyclosarin, or soman poisoning (2–5). Due to this, there are many efforts to prepare new and more promising AChE reactivators throughout the world (6–8). These days, oxime HI-6 seems to be the most promising AChE reactivator, considered as a substitute for the obsolete pralidoxime (9). The reactivation potency of HI-6 surpassed the potency of pralidoxime almost in all nerve agent intoxications. However, in the case of tabun and pesticide poisonings, there is still a lack for an optimal AChE reactivator (6,10).

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Although synthesis of the oxime HI-6 has been published and patented several times, preparation of the compound without impurities is still under investigation (11–13). To get quick monitoring of the purities of all intermediates, by-products, and products within the synthesis of HI-6, in this article, we focused our attention on the development of a convenient thin-layer chromatography (TLC) method which could be applicable in every chemical laboratory. Because dichloride salt of HI-6 will be replaced by DMS salt in few years, both salts were included in this study. Moreover, parent substances [2-hydroxyiminomethylpyridine (P2A); 4-carbamoylpyridine (INA)], intermediate [2-hydroxyiminomethyl-1-(chlormethoxymethyl) pyridinium chloride (mono P2A)], and potential by-products [1,3-*bis*(2-hydroxyiminomethylpyridinium) oxapropane dichloride (*bis* P2A); 1,3-bis(4-carbamoylpyridinium) oxapropane dichloride (*bis* INA)] were considered in this study. For a better understanding of the choice of the selected substances, the common synthetic process is outlined in Figure 1.

Experimental

Chemicals

Analytically pure isopropyl alcohol, butyl alcohol, toluene, ethyl acetate, and glacial acetic acid were purchased from Penta

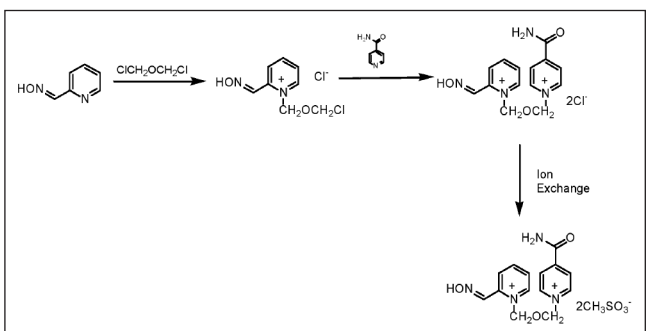

Figure 1. Scheme of the synthesis of the oxime HI-6.

(Chrudim, Czech Republic). Water was reverse osmosis pure. HI-6 and its intermediates were previously synthesized in our laboratory (12–15).

The purity of all tested compounds was tested by the determination of the melting point (Boetius block and were uncorrected); high-performance liquid chromatography (HPLC) (Column 250 × 4 mm i.d. Lichrospher 60 RP-select B [5 µm]; Merck, Darmstadt, Germany), mobile phase: 24% acetonitrile and 76% water, containing 8 mM octane-1-sulfonic acid sodium salt, 2 mM tetramethylammonium chloride, isocratic delivery at a flow-rate of 1 mL/min; UV detection at 277 nm, 25°C; and nuclear magnetic resonance (Varian Gemini 300; 300 MHz) prior to their use. According to the results of the previously mentioned methods, they were of 99% purity.

Isonicotinamide (99%) and pyridine-2-aldoxime (99%) were purchased from Sigma-Aldrich (Prague, Czech Republic). Their properties are given in Table I, and the structural formulas are given in Figure 2.

Table I. Basic Physical Properties of Tested Compounds

No.	Compound	Abbreviation	Formula	M.w.	m.p. (°C)
1	HI-6 dichloride	HI-6 2Cl	$C_{14}H_{16}Cl_2N_4O_3$	359.21	150–152
2	HI-6 dimethanesulfonate	HI-6 DMS	$C_{16}H_{22}N_4O_9S_2$	478.50	173–175
3	bisquaternary INA	bis INA	$C_{14}H_{16}Cl_2N_4O_3$	359.21	234–236
4	bisquaternary P2A	bis P2A	$C_{14}H_{16}Cl_2N_4O_3$	359.21	199–201
5	monoquaternary P2A	mono P2A	$C_8H_{10}Cl_2N_2O_2$	237.08	146–150
6	isonicotinamide	INA	$C_6H_6N_2O$	122.12	156–158
7	pyridine-2-aldoxime	P2A	$C_6H_6N_2O$	122.12	105–107

Table II. Mobile Phases Used for TLC Analysis of Tested Compounds

Mobile phase	Components	Proportions (v/v)
A	butyl alcohol–acetic acid–water	3 + 1 + 1
B	butyl alcohol–acetic acid–water	5 + 2 + 1
C	butyl alcohol–acetic acid–water–ethyl acetate	1 + 1 + 1 + 1
D	isopropyl alcohol–acetic acid–water–toluene	5 + 2 + 2 + 1

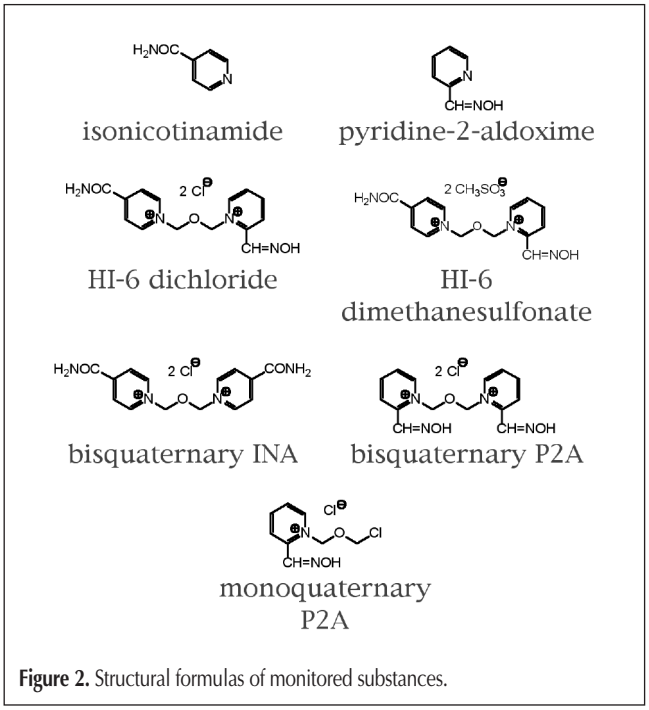

Figure 2. Structural formulas of monitored substances.

TLC measurement

TLC was performed on 75 mm × 185 mm aluminum plates coated with 0.1 mm layer of Cellulose F (Merck). According to our previous experiences (more than 30 years) in the synthesis of new AChE reactivators, we have found that cellulose seems to be the best sorbent for separation of mono and bisquaternary reactivators. Moreover, Dragendorf's reagent together with this sorbent and quaternary nitrogens show red colors to resolve the quaternary intermediate (monoquaternary salt), product (bisquaternary salt), or by-product (bisquaternary salt) from the parent substances.

All plates were tracked with a CAMAG Linomat IV automatic applicator (Camag, Berlin, Germany) and developed in twin trough chambers with stainless steel lids (Camag). Compounds were dissolved in ethanol–water (50:50 v/v). Samples (2 µL of 10mM solutions) were applied 1 cm from the bottom edge of the plates in 4 mm long spots with 5 mm spaces between them by the lowest speed of application, 15 s/µL. Development was stopped when the mobile phase passed at least 145 mm track length but maximally 5 mm from the top of the plates. Plates were developed with four acid mobile phases, listed in Table II.

HI-6 intermediates were first identified by UV light at 254 nm, visible as violet spots against a white fluorescent background with lamp model ENF–240C/F and fluorescence analysis cabinet model CM–10 (Spectronics corporation, Westbury, New York). The next step of identification was derivatization after spraying the chromatograms with Dragendorff's reagent (mixture of two solutions; Solution A: 0.5 g bismuth nitrate [$Bi[NO_3)].5H_2O$] in 20 mL of 20% acetic acid + Solution B: 5 mL of a 40% potassium iodide (KI) solution in water; mix 20 mL of solution A and 5 mL of solution B with 70 mL of water). Quaternary substances were observable within 1 min as orange spots against a yellow background.

Results

All results obtained in this study are summarized in Figure 3 and Table III. In Figure 3 is shown the TLC chromatogram of the tested compounds after the use of four different mobile phases (Figure 3A–3D). As it is clearly seen, all mobile phases used were able to separate bis-quaternary compounds (products and by-products) from non-quaternary and monoquaternary substances (parent compounds and intermediates). The monoquaternary intermediate reached the maximal resolution from the parent P2A in the mobile phase B. The TLC method was very sensitive (limits

of detection 1mM) in distinguishing HI-6 dichloride and dimethanesulfonate not only one from another (mobile phase D) but also in distinguishing their geometric isomers (E;Z) (mobile phase C), as was earlier described for obidoxime (16). In work of Spöhrer and Eyer, they found that the oxime group in obidoxime could occurr as an E or Z isomer. Due to the fact that obidoxime has two independent oxime groups, they found three different obidoxime peaks using HPLC: E,E (94.8%), E,Z (5.1%), and Z,Z (0.07%). Because there was no presence of impurities (as discussed in the Experimental section) and because of the presence of two spots, there is evidence of detection of the E and Z isomer of HI-6 and the E,E and E,Z isomer of P2A. The isomer Z,Z of P2A was probably under the detection limit. A similar result (low percentual presence and due to this no occurrence in the chromatogram) was also obtained in the previously mentioned work (16).

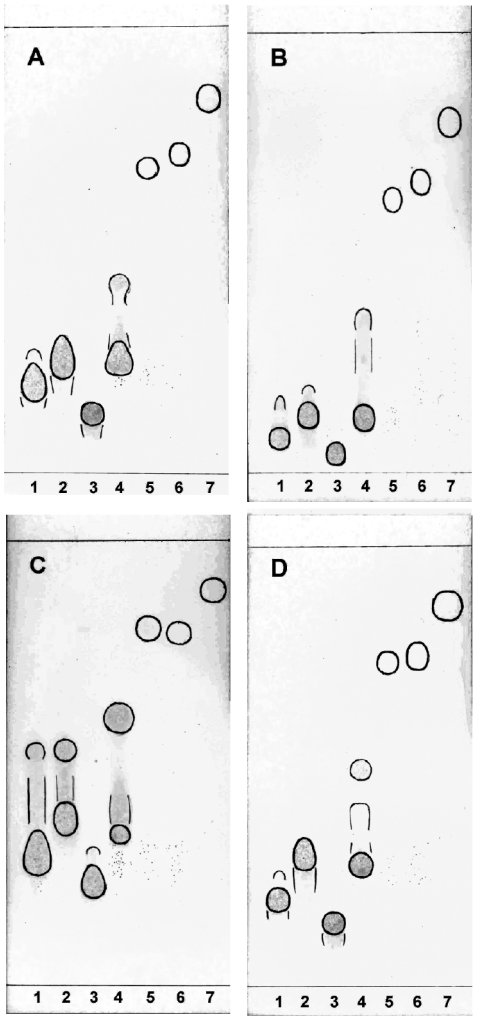

Figure 3. TLC chromatograms of tested compounds. Butyl alcohol/CH_3COOH–H_2O (3+1+1); time of development 190 min (A); Butyl alcohol–CH_3COOH–H_2O (5+2+1); time of development 130 min (B); Butyl alcohol–CH_3COOH–H_2O–ethyl acetate (1+1+1+1); time of development 90 min (C); Isopropyl alcohol–CH_3COOH–H_2O/Toluene (5+2+2+1); time of development 150 min.

Table III. R_f Values of HI-6 Intermediates in TLC on Aluminum Cellulose F Plates with Chosen Mobile Phases

Track No.	Compound	R_f A	R_f B	R_f C	R_f D
1	HI–6 2Cl	0.19	0.07	0.29	0.19
2	HI–6 DMS	0.25	0.13	0.37	0.29
3	bis INA	0.13	0.04	0.23	0.14
4	bis P2A	0.25	0.12	0.33	0.27
5	mono P2A	0.68	0.62	0.80	0.74
6	INA	0.72	0.66	0.79	0.75
7	P2A	0.84	0.79	0.88	0.86

Discussion

During the last few years, new synthetic pathways have been used for the preparation of structurally different AChE reactivators to improve standard antidotal therapy (7,8,17–19). For nerve agents, HI-6 seems to be the number one antidote (6,9). The introduction of another one—a new and promising AChE reactivator—is now unrealistic, and if there is to be any candidate it will be very expensive.

In the case of HI-6 synthesis, the preparation of the active substance is very complicated because of the high possibility of the formation of by-products (12,13). Due to this, the use of analytical methods to follow the purity of all intermediates and products is necessary. For these purposes, HPLC analysis is mainly used.

The TLC technique is a more appropriate approach because of its relatively low time-consuming sample analysis and its use of equipment which is available in every chemical laboratory (20). In this study, we have developed a method for the determination of parent compounds, intermediates, by-products, and own products arisen within the HI-6 synthesis.

The developed method could be used in the future, not only for analysis of the purity of HI-6 salts and appropriate intermediates arisen within its synthesis, but also to distinguish between different salts of HI-6 (in our case, chloride versus DMS) and its isomers, as was earlier described for obidoxime (16).

In conclusion, we have developed a TLC analysis of the purity of the oxime HI-6. The developed method could be used in the future as a quick helper for synthetic chemists who will be interested in the synthesis of HI-6 or AChE reactivators in general.

Acknowledgments

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