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SHORT COMMUNICATION

Preparation of Oxime HI-6 (Dichloride and Dimethanesulphonate)– Antidote against Nerve Agents

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ABSTRACT

Because of the threat of misuse of nerve agents as terroristic weapons by the terrorists, an immediate need is felt for the preparation of antidotes on large-scale basis. HI-6 (dichloride and dimethanesulphonate) salt are the most promising acetylcholinesterase reactivators used as causal antidotes in nerve agents intoxication. In this study, rapid and large-scale preparation of oxime HI-6, the most promising reactivator has been described.

Keywords: HI-6, acetylcholinesterase, nerve agent, quaternary salts, oxime, reactivator, antidote, organophosphate

1. INTRODUCTION

Organophosphorus compounds are available worldwide and many of these belong to the biologically active compounds[1-5]. Biological effect of several of them is based on the inhibition of the enzyme acetylcholinesterase (AChE; EC 3.1.1.7)[1,5,6-9]. The most extended organophosphorus AChE inhibitors are the pesticides. Earlier also, nerve agents were wide-spread group of these substances which were incorporated into the armament of many armies[5,10].

Although, the development and storage of these compounds are currently prohibited due to their potential misuse by terrorists. The misuse of these agents had happened in Tokyo, in 1995. Owing to the release of sarin nerve agent, 12 people died and thousands were intoxicated[11].

To overcome the threat of the nerve agent, new antidotes against nerve agents have been developed[12-16]. Among them, oxime HI-6 seems to be the number one due to its promising and relatively broad-spectrum reactivation potency[17,18]. Although the synthesis of oxime HI-6 was patented several times, the common synthesis which could be used for rapid production of pure compound on large-scale was not published yet[19-21]. Because of the commercial importance of two salts of oxime HI-6 – dichloride (formerly preferred salt of HI-6) and dimethanesulphonate (DMS; currently preferred salt of HI-6), their synthesis have been described which can be prepared in the laboratory on a large-scale.

2. SYNTHESIS

General approach how to prepare bisquaternary unsymmetrical (like HI-6) AChE reactivators was

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described many times[12-16,19-24]. It depends mainly on the reactivity of the appropriate alkylating agent used for the quaternisation. For HI-6, bis-(chloromethyl) ether (purchased from Hi-chem, Czech Republic) is used as the alkylating chain. Two possible ways for the preparation of HI-6 (Fig. 1) are: (i) from the isonicotinamide and (ii) from the 2-hydroxy-iminopyridine. In this study, a preferred way to prepare HI-6 via the monoquaternary oxime intermediate, has been described.

2.1 Analysis of Intermediates and Products

Purity of both the intermediates and the products was tested by determining the melting point (Boetius block and were uncorrected); TLC [(Kieselgel Merck; mobile phase n-*BuOH*/CH_3COOH / H_2O (5:1:2)]; detection of UV 254 (Dragendorff´s reagent); HPLC [Column 250 mm x 4 mm I.D. Lichrospher 60 RP-select B (5 mm); Merck, Darmstadt, Germany]; mobile phase - 24 per cent acetonitrile and 76 per cent water, containing octane-1-sulphonic acid sodium salt (8 mM), tetramethylammonium chloride (2 mM); isocratic delivery at a flow rate of 1 ml/min, UV detection at 277 nm; 25 °C) and NMR (Varian Gemini 300; 300 MHz)[25,26].

In Scheme 1, structures of all compounds (parent compounds, intermediates, byproducts, and final products) are shown occurring within the synthesis of *HI*-6.

2.1.1 Preparation of 2-Hydroxyiminomethyl-1-(Chlormethoxymethyl) Pyridinium Chloride

Pyridine-2-aldoxime (183.5 g, 1.5 mol) was dissolved in chloroform (1000 ml)and heated to 50 °C. Then, bis(chloromethyl)ether (172.7 g, 1.5 mol) were added. Reaction mixture was stirred for 4 h. The reaction mixture was allowed to cool to the laboratory temperature, brownish compound was separated, and then stirred with ethanol. Subsequently, yellowish compound was separated and rinsed twice by diethylether. White-grey product (146 g, yield 41 %) was collected (mp 146–150 °C; TLC: R_f = 0.5; HPLC: R_t = 8.07 min; ^1H NMR: (D_2O): was not done because of low stability of the monoquaternary salt) and without further purification subjected to other synthetic step.

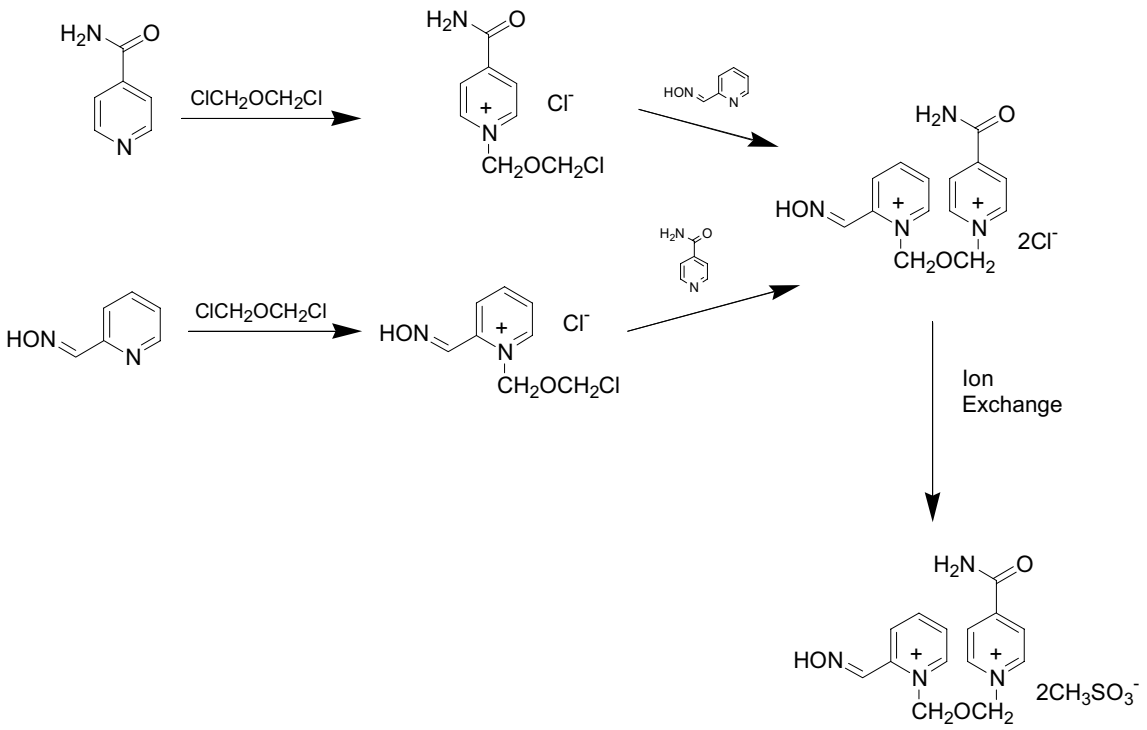
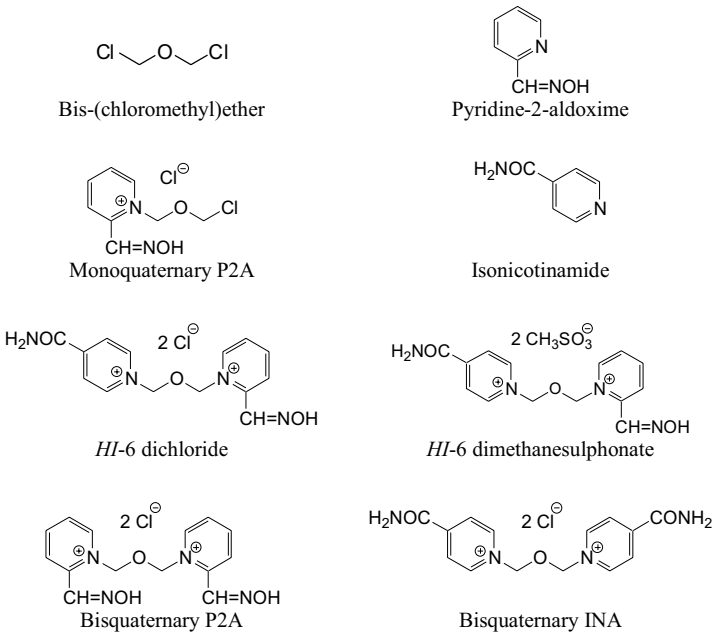


Figure 1. Scheme for the preparation of the HI-6.

2.1.2 Preparation of 1-(2-(Hydroxyimino-methyl) Pyridinium)-3-(4-Carbamoyl-pyridinium)-2-Oxapropane Dichloride (HI-6 Cl)

Solution of 2-hydroxyiminomethyl-1-chlormethoxy-methylpyridinium chloride (146.0 g, 0.616 mol) in 1200 ml of dry dimethylformamide was added a solution of (75.2 g) isonicotinamide. The reaction mixture was stirred for four hours at 50 °C under argone atmosphere. Grey product was filtered, rinsed by ethanol and diethylether. Then, the product was dissolved in isopropanole, purified with activated carbon and crystalised. 80.14 g (yield 34.5 %) of the resulted compound was collected (m.p. 138-147 °C; TLC: R_f = 0.3; HPLC: R_t = 10.30 min; ^1H NMR (D_2O): 6.35 (s, 2*H*, CH_2); 6.50 (s, 2*H*, CH_2); 8.17 (dd, 1*H*, Ph-*H*, 3J*HH* = 6.3 Hz, 3J*HH* = 7.8 Hz); 8.51 (d, 2*H*, Ph-*H*, 3J*HH* = 6.3 Hz); 8.53 (s, 1*H*, *CH=N*); 8.75 (m, 2*H*, Ph-*H*); 9.17 (d, 1*H*, Ph-*H*, 3J*HH* = 6.3 Hz); 9.24 (d, 2*H*, Ph-*H*, 3J*HH* = 6.6 Hz) ppm. *NOH* a $CONH_2$ signals were not found in the spectra).



Compound	Abbreviation	Formula	Mol Wt	Melting point (°C)
Bis-(chloromethyl)ether	BCME	$C_2H_4Cl_2O$	114.96	- *
Pyridine-2-aldoxime	P2A	$C_6H_6N_2O$	122.12	105 - 107
Monoquaternary P2A	Mono P2A	$C_8H_{10}Cl_2N_2O_2$	237.08	146 - 150
Isonicotinamide	INA	$C_6H_6N_2O$	122.12	156 - 158
HI-6 dichloride	*HI*–6 2*Cl*	$C_{14}H_{16}Cl_2N_4O_3$	359.21	150 - 152
HI-6 dimethanesulphonate	*HI*–6 DMS	$C_{16}H_{22}N_4O_9S_2$	478.50	173 - 175
Bisquaternary P2A	Bis-P2A	$C_{14}H_{16}Cl_2N_4O_3$	359.21	199 - 201
Bisquaternary INA	Bis-INA	$C_{14}H_{16}Cl_2N_4O_3$	359.21	234 - 236

* liquid at room temperature

Scheme 1: Structures and basic physical properties of parent substances, byproducts, intermediates, and final products, which could be found as byproducts of synthesis of HI-6[25,26].

2.1.3 Preparation of 1-(2-(Hydroxyimino-methyl) Pyridinium)-3-(4-Carbamoyl-pyridinium)-2-Oxapropane Dimethane-sulphonate (HI-6 DMS)

Column with, DOWEX (136 g, 2 x 8, 100/200, *Cl*-) in *OH* cycle was rinsed with methanesulfonic acid (1300 ml) solution (1 M). Subsequently, the column was rinsed with water to get neutral p*H*. *HI*-6 dichloride (27 g) in distilled water (205 ml) were tracked into the column. Yellow solution of *HI*-6 DMS was collected in twenty fractions. All fractions, except the first three, were put together and evaporated to one fifth and cooled to room temperature. Then, ethanol was added into the solution and put into the refrigerator for crystalisation. After 2 h, arisen crystals (29.50 g; yield 83 %) were collected, washed by diethylether and dried in oven (50 °C) (m.p. 165-167 °C; TLC: R_f = 0.4; HPLC: R_t = 10.30 min; ^1H NMR (D2O): 2.25 (s, 6*H*, CH_3SO_3-); 6.35 (s, 2*H*, CH_2); 6.50 (s, 2*H*, CH_2); 8.17 (dd, 1*H*, Ph-*H*, 3J*HH* = 6.3 Hz, 3J*HH* = 7.8 Hz); 8.51 (d, 2*H*, Ph-*H*, 3J*HH* = 6.3 Hz); 8.53 (s, 1*H*, *CH=N*); 8.75 (m, 2*H*, Ph-*H*); 9.17 (d, 1*H*, Ph-*H*, 3J*HH* = 6.3 Hz); 9.24 (d, 2*H*, Ph-*H*, 3J*HH* = 6.6 Hz) ppm. *NOH* a $CONH_2$ signals were not found in the spectra).

3. DISCUSSION

Development of the AChE reactivators is very important task[12-16]. For nerve agents, HI-6 seems to be the number one[17,18]. However, for cases of tabun and pesticides-intoxications, new derivatives are still designed and synthetised[1,2,27,28]. Till today, only six reactivators–pralidoxime, obidoxime, trimedoxime, methoxime (MMB-4), HI-6, and diethyxime are used as antidotes in several armies worldwide[29]. Moreover, introducing another one new and promising, is now unrealistic, and if there will be any candidate, it will be very expensive.

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