$C_8H_{12}S_2$ (172.32): C 55.76; H 7.02; S 37.22]. VPC analyses were performed on a Perkin Elmer 900 Gas Chromatograph. IR spectra were recorded on a Perkin Elmer 257 Grating Infrared Spectrophotometer, NMR spectra on a Varian A-60 spectrometer and mass spectra on an LKB 9000 mass spectrometer.

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Reactivation of Phosphorylated Cholinesterase by Some Imidazole-substituted Oximes

TOM KARLSSON, KARL-ERLAND STENSIÖ and KERSTIN WAHLBERG

Research Institute of National Defence, Department 1, S-172 04 Sundbyberg 4, Sweden

Nucleophilic agents such as oximes have been employed in restoring the activity of cholinesterase (ChE) which has been inactivated by organophosphorus compounds. Methyl-quaternized pyridinium aldoximes have been found particularly effective.[1] By a suitable modification of the substituent on the nitrogen, an increase in the reactivation rate was obtained.[2] Our intention has been to study the effect of an imidazole substituent in the pyridine aldoxime.

There is strong evidence that imidazole is part of the active site of ChE.[3] Furthermore, imidazole has a well documented catalytic capacity, *e.g.* for ester hydrolysis, both as a nucleophile itself and as a catalyst in a general acid-base catalyzed reaction.[3] Thus it would be of interest to study the effect on the reactivation process of a properly spaced imidazole group.

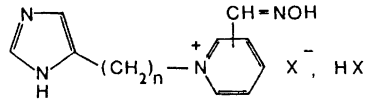
Moreover, imidazole-substituted oximes may participate in the degradation of organophosphorus compounds before they reach the active site of the enzyme.

The syntheses were performed by reacting 4(5)-chloromethylimidazole hydrochloride or 4(5)-(2-bromoethyl)imidazole hydrobromide with the appropriate pyridine aldoxime in dimethylformamide. However, it was not possible to obtain the 2-aldoxime of *N*-(imidazolylethyl)pyridinium bromide by this procedure. The difficulties with quaternization of 2-pyridine aldoximes have been pointed out previously by Poziomek *et al.*[4]

The reactivator potency against BuChE inhibited by methylisopropoxyphosphoryl fluoride (Sarin) is illustrated in Table 1. It is evident that I and II are slightly more active than 2-(hydroxyimino)-methylpyridinium methanesulphonate (P2S), a compound used as an antidote in nerve gas poisoning and as a standard in reactivation experiments. None of the compounds I – V is able to reactivate the enzyme after inhibition with dimethylamidoethoxy-

Substance	m.p.	Crude yield %	Recrystallization	Elemental analysis
I	215–217	78	EtOH-H_2O (9:1) plus acetone until turbid	Found: C 43.7; H 4.41; N 20.5. Calc. for $C_{10}H_{12}N_4OCl_2$; C 43.71; H 4.40; N 20.4.
II	212–213	85	MeOH	Found: C 43.7; H 4.44; N 20.5.
III	242–243	88	EtOH-H_2O (9:1) plus acetone until turbid	Found: C 43.4; H 4.42; N 20.4.
IV	231–232	73	95 % EtOH	Found: C 35.0; H 3.81; N 15.0. Calc. for $C_{11}H_{14}N_4OBr_2$; C 35.0; H 3.73; N 14.82.
V	232–234	68	95 % EtOH	Found: C 35.0; H 3.81; N 15.0.

Table 1. Structural formulae of some imidazole substituted pyridine oximes and their reactivating effect on Sarin inhibited BuChE. The inhibiting effect of the oximes alone on BuChE are expressed in molar I_{50} values.



Substance	n	Substitution in the pyridine ring	Reactivation: Conc. of reactivator M	Reactivation: Restored enzyme activity %	I_{50} BuChE M
I	1	2	1.25×10^{-4}	57	6.2×10^{-4}
II	1	3	1.25×10^{-4}	54	6.0×10^{-4}
III	1	4	2.5×10^{-4}	40	1.9×10^{-3}
IV	2	3	2.5×10^{-4}	25	7.7×10^{-4}
V	2	4	2.5×10^{-4}	37	3.7×10^{-3}
P2S	–	–	3.67×10^{-4}	55	8.1×10^{-4}

phosphoryl cyanide (Tabun) or methylpinacoloxyphosphoryl fluoride (Soman).

An interesting feature is that compound II with the oxime group in the 3-position is equally effective as a reactivator as the isomers (I, III) substituted in the 2- and 4 positions. This is in contrast to the behaviour of (hydroxyimino)methylpyridinium isomers, where the 3-isomer has a very small reactivation capacity.[5] The relatively good effect of II and also of IV can be attributed to the catalytic assistance of the imidazole group in the reactivation procedure and to a modification of the acid strength of the oxime group. Most of the compounds which are effective reactivators are also ChE inhibitors. This reflects the fact that a certain fit to the active site is required in order to get a reactivation. Table 1 gives the I_{50} values for the inhibition of BuChE by compounds I–V. The 4-substituted compounds III and V have only about 15–30 % of the inhibiting activity given by the other substances but show a reasonable reactivation capacity. A preliminary pharmacological investigation shows that II is not very toxic having an LD_{50} (mice, i.p.) of 184 mg/kg compared to 216 mg/kg for P2S.[6]

Experimental. Lyophilized horse serum pseudocholinesterase (BuChE) with the activity 5232 Warburg units per mg was purchased from Diosynth International NV, the Netherlands. The phosphorus compounds were synthesized in this laboratory.

Reactivation experiments. The ChE activity was measured by an electrometric method[7]

with acetylcholine (14×10^{-3} M) as substrate. ChE was completely phosphorylated by incubation with 10^{-5} M Sarin, Soman, or Tabun and excess inhibitor removed by dialysis. After dialysis a check was made that no phosphorus compound was left.[8] The reactivation experiments were performed at 25° for 90 min in a Michels buffer[7] at pH 8.14. Reactivation is given as a percentage of the ChE activity in an enzyme control containing the reactivator but no phosphorus compound.

Synthesis. 4(5)-Chloromethylimidazole hydrochloride was prepared according to Turner *et al.*[9] 4(5)-Bromoethylimidazole hydrobromide was prepared from histamine.[10] The pyridinealdoximes used have the following m.p.: 2-aldoxime 109–111° (lit.[11] 114°), 3-aldoxime 148–150° (lit.[11] 150–151°), 4-aldoxime 130–132° (lit.[11] 132°). The quaternizations were performed in dimethylformamide at 50° for about 12 h using two equivalents of aldoxime. After cooling and chloroform addition, the precipitate was filtered, washed with chloroform and recrystallized.

The reactions could be followed by TLC on silica gel with pentanol-acetic acid-acetone-water (56:6:24:14) as eluent. Compounds containing the imidazole ring were detected by spraying with a diazonium salt (Echtblausalz B). For the other compounds, Dragendorffs reagent was used.

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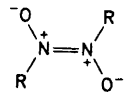
X-Ray Investigation of *N,N*-Dimethyl-*p*-nitrosoaniline, a Disordered Structure

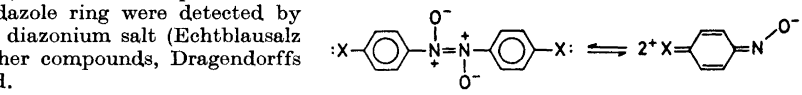
CHR. RØMMING and H. J. TALBERG

Department of Chemistry, University of Oslo Oslo 3, Norway

Recent investigations have shown the dimers of organic nitroso compounds to be azodioxy-derivatives:[1-3]



The correlation between the electron donor ability of R and the stability of these compounds has been demonstrated by Lüttke.[4-6] In *para*-substituted nitrosobenzenes a shift to the right in the equilibrium



was found when X is a strong electron donor.

In view of the powerful electron donating property of the dimethylamino group, *N,N*-dimethyl-*p*-nitrosoaniline was selected as the subject of the present investigation. A comparison of the bonds in this molecule and in those with X = H, Br, I [2,3,7] and X = OH (now being investigated in this laboratory) should be of importance for the understanding of the dimerisation process.

Owing to the disorder observed in crystals of the title compound two structure refinements were carried out with crystals grown in different ways; both gave the same results with exception of small differences in the thermal parameters. The crystals were formed (I) by slow evaporation of a diethyl ether solution and (II) by cooling a similar solution in liquid nitrogen. A third attempt was carried out by a zone melting technique; X-ray photographs proved also these crystals to be disordered. The crystal data are as follows: *N,N*-Dimethyl-*p*-nitrosoaniline, triclinic, space group $P\bar{1}$. Cell dimensions (II): $a =$