CHOLINESTERASE REACTIVATORS DERIVED FROM PYRIDINE-2-CARBALDOXIME

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Monoquaternary reactivators of cholinesterase **3a–3c** were prepared by quaternization of substituted pyridine-2-carbaldoximes with an ester (**1a**, **1b**) or carbamoyl (**2**) function in position 4. A new bisquaternary reactivator, 4-carbamoyl-2′-[(hydroxyimino)methyl]-1,1′-(but-2-ene-1,4-diyl)bispyridinium salt (**5**), was derived from the unsubstituted pyridine-2-carbaldoxime. The compounds were characterized by UV spectra and ionization constants. The substances exhibit antidote effects in intoxications with organophosphate cholinesterase inhibitors.

Key words: Reactivators; Cholinesterase; Pyridines; Pyridinium salts; Organophosphates.

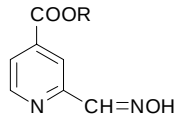
The currently used efficient cholinesterase reactivators are based on quaternized unsubstituted pyridine-2- or -4-carbaldoximes[1]. The application of substituted pyridine-2-carbaldoximes has not attracted attention as yet, although their interesting pharmacological properties can be expected. This fact may be due to a difficult preparation of substituted pyridine-2-carbaldehydes. A route to 4-substituted pyridine-2-carbaldehydes consists in the reduction of esters of pyridine-2,4-dicarboxylic acid with $LiAlH_4$ or with diisobutyl aluminium hydride using the procedure of Queguiner and Pastour[2], where only the ester function in position 2 is reduced to the aldehyde. Alternatively, corresponding 2-methylpyridines can be reacted with dimethyl sulfoxide and iodine[3]. The synthesis of a 4-substituted pyridine-2-carbaldehyde *via* the 2-pyridyllithium intermediate has been recently reported[4].

In the present work, the Queguiner–Pastour approach was applied to obtain pyridine-2-carbaldoximes with the ester function in position 4 (**1a**, **1b**), which served as the intermediates in the preparation of the carbamoyl derivative of pyridine-2-carbaldoxime **2**, which has not been described until now. The aldoximes **2** were then employed in the synthesis of monoquaternary compounds **3a–3c**.

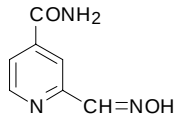
The most efficient reactivators were found in the group of aldoxime derivatives of bispyridinium salts with a $–(CH_2)_3–$ or $–CH_2–O–CH_2–$ bridge, where the aldoxime group need not to be present at both pyridinium rings. The *trans*-but-2-ene-1,4-diyl bridge between the pyridinium rings of bisquaternary reactivators has already been em-

ployed. In comparison with analogous compounds containing the $-CH_2OCH_2-$ bridge, the butenediyl bridge gives compounds which are somewhat more stable but also more toxic[5]. The new reactivator **5** with the bridge described in this paper is an analog of the efficient reactivator HI-6 (4-carbamoyl-2′-[(hydroxyimino)methyl]-1,1′-(oxydimethylene)-bispyridinium dichloride)[6].

In the experiments involving reduction of lutidinates, better results were achieved with rather aged $LiAlH_4$ preparations having lower hydride contents. The crude aldehydes obtained were oximated with hydroxylamine at pH 8 without isolation yielding corresponding aldoxime esters **1a** and **1b**.



	R
1a	CH_3
1b	C_2H_5



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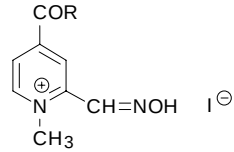
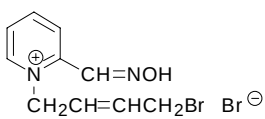


	R
3a	OCH_3
3b	OC_2H_5
3c	NH_2
3d	OH



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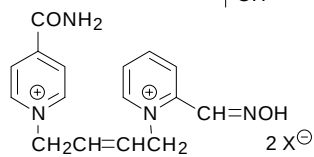

5a, X = Br
5b, X = Cl

The conversion of the esters **1a** and **1b** to the amide **2** proceeded easily by the action of concentrated aqueous ammonia on the solid esters at room temperature. Methanolic ammonia can be employed as well.

The quaternization of substituted pyridinealdoximes was more tedious than that of the unsubstituted pyridine-2-carbaldoxime.

The bisquaternary monoaldoxime **5** was prepared by a two-step procedure. In the first step, the monoquaternary compound **4** was prepared using an excess of 1,4-dibromobut-2-ene. The intermediate **4** is not very stable, and hence it was used without purification in the subsequent reaction with isonicotinamide giving dibromide **5a**, which was converted to dichloride **5b** using an ion exchanger.

Spectral properties and ionization constants of the compounds are given in Table I. As found by UV spectroscopy and confirmed by HPLC, monoquaternary substances **3**

decompose in 0.1 M NaOH to give compound **3d**, with the carboxy group presumably being in position 4. The decomposition is the fastest for methyl ester **3a**.

Compounds **3a**, **3c**, and **5a** were tested *in vivo* for their therapeutic effect by investigating their influence on the mean lethal dose LD_{50} of the relatively toxic organophosphate insecticide fosdrin (Mevinphos, 2-(methoxycarbonyl)-1-methylvinyl dimethyl phosphate) in mice. The oximes were administered intramuscularly in doses corresponding to 5% of the LD_{50} values found 1 180, 1 290, and 266 mg/kg for compounds **3a**, **3c**, and **5a**, respectively, also administered intramuscularly. The therapeutic effect is expressed by the therapeutic protection index, which is the LD_{50} ratio for the treated and untreated animals (Table II).

If monoquaternary oximes **3a** and **3c** were administered 2 min after the intramuscular intoxication, they nearly doubled the LD_{50} value of fosdrin. This relatively low efficacy

TABLE I
UV spectra and pK_a values of aldoximes **1–3** and **5a**

Compound	λ_{max} (log ε)			pK_{a1}	pK_{a2}
	MeOH	0.05 M HCl	0.05 M borax		
1a	299 (3.69)	–	–	1.92 ± 0.02	9.89 ± 0.05
1b	300 (3.65)	–	–	1.94 ± 0.04	9.90 ± 0.05
2	292 (3.65)	–	–	2.23 ± 0.02	9.91 ± 0.02
3a	–	313 (3.99)	361 (4.08)	7.60 ± 0.04	–
3b	–	315 (3.98)	355 (4.08)	7.53 ± 0.02	–
3c	–	308 (4.03)	355 (4.13)	7.57 ± 0.02	–
3d	–	309 (3.98)[a]	347 (4.09)	7.92 ± 0.05	–
5a	–	295 (4.04)	342 (4.17)	7.63 ± 0.02	–

[a] pH 6.0, λ_{max} (log ε): 303 (4.00).

TABLE II
Therapeutic protection index of aldoximes **3a**, **3c**, and **5a**

Compound	Time of administration after intoxication		
	30 s	1 min	2 min
3a	9.0	7.4	1.8
3c	11.1	8.2	1.8
5a	4.5	3.75	1.8

was due to the intoxication symptoms fully developed at the time of administration. If the time period between the intoxication and treatment was shortened to 30 s, bisquaternary oxime **5a** increased the LD_{50} value of fosdrin 4.5 times, which is considerably less than with monoquaternary oximes **3a** and **3c** bringing about a 9-fold and 11-fold increase, respectively. The increase was statistically significant ($p < 0.05$) irrespective of the time of administration and the oxime chosen. The higher efficacy of the monoquaternary oximes **3a** and **3c** towards fosdrin as compared to bisquaternary oxime **5a** is apparently due to the appreciably lower toxicity of the former oximes.

EXPERIMENTAL

Melting points were determined on a Boetius apparatus and are not corrected. IR spectra were scanned on a PU 8706 spectrophotometer, and UV spectra on an HP 8453 spectrophotometer. NMR spectra (δ, ppm; *J*, Hz) were run on a Bruker DPX 250 instrument. Ionization constants were determined spectrophotometrically. TLC of aldoximes **1** and **2** was performed on Kavalier Silufol UV 254 plates using the benzene–acetone 3 : 1 mixture and detection with UV radiation and with a 1% solution of $(NH_4)_2FeSO_4 \cdot 6\,H_2O$ (intense, very stable red to violet spots). The quaternary salts were chromatographed on Merck Cellulose F using the butanol–acetic acid–water 5 : 1 : 2 mixture, UV detection, and the Draggendorff reagent[7]. HPLC was accomplished on a Spectra Physics instrument equipped with a UV 1000 detector, SP 4000 integrator, and Tessek SGX C-18 column (150 mm long, 3.3 mm i.d.). A 70 : 30 (v/v) mixture of acetate buffer (pH 4.8) and methanol containing sodium octanesulfonate and tetramethylammonium chloride served as a mobile phase.

Methyl 2-[(Hydroxyimino)methyl]pyridine-4-carboxylate (**1a**)

Dimethyl pyridine-2,4-dicarboxylate (19.5 g, 0.1 mol) in tetrahydrofuran (600 ml), cooled under nitrogen to –60 °C, was reduced with 5 × 1.6 g of $LiAlH_4$ within 20 min. The reaction temperature was then held constant for another hour. The reaction was terminated by adding a mixture of tetrahydrofuran (100 ml), water (40 ml), and acetic acid (60 ml), the reaction mixture was evaporated to dryness in vacuum, and the residue was digested with 3 × 150 ml of ether. The extract was evaporated and the residue was mixed with a solution of hydroxylamine hydrochloride (12 g) in water (50 ml) neutralized with Na_2CO_3. The mixture was allowed to stand in a refrigerator to give 7.5 g (40%) of the crude product, m.p. 156–170 °C. After double crystallization from 50% aqueous ethanol, the melting point was 179–183 °C (ref.[3]: 184–185 °C; ref.[4]: 178 °C). For $C_8H_8N_2O_3$ (180.2) calculated: 53.35% C, 4.48% H, 15.55% N; found: 53.35% C, 4.54% H, 15.34% N. ^{1}H NMR spectrum: 11.72 s, 1 H (OH); 8.75 dd, 1 H, *J* = 0.9 and 5.0 (H-6); 8.17 bs, 2 H (H-3, CH aldoxime); 7.75 dd, 1 H, *J* = 1.6 and 5.0 (H-5); 3.93 s, 3 H (CH_3).

Ethyl 2-[(Hydroxyimino)methyl]pyridine-4-carboxylate (**1b**)

The compound was prepared analogously to **1a**; yield 45%, melting point after double crystallization from ethanol 160–162 °C (ref.[4]: 149 °C). For $C_9H_{10}N_2O_3$ (194.2) calculated: 55.67% C, 5.19% H, 14.43% N; found: 55.35% C, 5.12% H, 14.75% N. ^{1}H NMR spectrum: 11.74 s, 1 H (OH); 8.78 dd, 1 H, *J* = 1.0 and 4.7 (H-6); 8.20 dd, *J* = 1.0 and 1.6 + 8.19 s, 2 H (H-3, CH aldoxime); 7.79 dd, 1 H, *J* = 1.6 and 4.7 (H-5); 4.40 q, 2 H, *J* = 7.2 (CH_2); 1.37 t, 3 H, *J* = 7.2 (CH_3).

2-[(Hydroxyimino)methyl]pyridine-4-carboxamide (**2**)

A suspension of ester **1a** (1.12 g, 6.15 mmol) in 25% aqueous ammonia (5.6 ml) was stirred at room temperature for 24 h. The product was filtered off (0.89 g, 93%) and crystallized from 50% aqueous ethanol; m.p. 241–243 °C. For $C_7H_7N_3O_2$ (165.2) calculated: 50.91% C, 4.27% H, 25.44% N; found: 50.62% C, 4.33% H, 25.28% N. ^{1}H NMR spectrum: 11.31 s, 1 H (OH); 8.67 dd, 1 H, *J* = 0.6 and 5.0 (H-6); 8.16 s, 1 H + 8.13 dd, *J* = 0.6 and 1.6, 1 H (H-3, CH aldoxime); 7.70 dd, 1 H, *J* = 1.6 and 5.0 (H-5); 7.58 bs, 2 H (NH_2).

2-[(Hydroxyimino)methyl]-4-methoxycarbonyl-1-methylpyridinium Iodide (**3a**)

A solution of oxime **1a** (0.5 g, 3.3 mmol) and methyl iodide (1.5 g, 12 mmol) in dimethylformamide (5 ml) was heated in an ampoule at 60 °C for 12 h. Then the reaction mixture was evaporated in vacuum, and the residue was double crystallized from ethanol. Yield 0.63 (70%) of a yellow substance, m.p. 212–214 °C (dec.). For $C_9H_{11}IN_2O_3$ (322.1) calculated: 33.56% C, 3.44% H, 8.70% N, 39.40% I; found: 33.83% C, 3.24% H, 8.55% N, 39.11% I. ^{1}H NMR spectrum: 9.18 d, 1 H, *J* = 6.5 (H-6); 8.73 s, 1 H (CH aldoxime); 8.64 d, 1 H, *J* = 1.9 (H-3); 8.36 dd, 1 H, *J* = 1.9 and 6.5 (H-5); 4.48 s, 3 H (NCH_3); 4.02 s, 3 H (OCH_3).

2-[(Hydroxyimino)methyl]-4-ethoxycarbonyl-1-methylpyridinium Iodide (**3b**)

The compound was prepared analogously to **3a**; yield 70%. Crystallization from ethanol gave a yellow substance, m.p. 191.5–194 °C (dec.). For $C_{10}H_{13}IN_2O_3$ (336.1) calculated: 35.73% C, 3.90% H, 8.33% N, 37.75% I; found: 35.35% C, 4.19% H, 8.11% N, 37.43% I. ^{1}H NMR spectrum: 13.18 bs, 1 H (OH); 9.21 d, 1 H, *J* = 6.3 (H-6); 8.76 s, 1 H (CH aldoxime); 8.65 d, 1 H, *J* = 2.2 (H-3); 8.41 dd, 1 H, *J* = 2.2 and 6.3 (H-5); 4.49 s, 3 H (NCH_3); 4.48 q, 2 H, and 1.40 t, 3 H, *J* = 7.2 (CH_2CH_3).

4-Carbamoyl-2-[(hydroxyimino)methyl]-1-methylpyridinium Iodide (**3c**)

The compound was prepared analogously to **3a**; yield 65%. Crystallization from ethanol gave a yellow substance, m.p. 214–219 °C (dec.). For $C_8H_{10}IN_3O_2$ (307.1) calculated: 31.29% C, 3.28% H, 13.68% N, 41.32% I; found: 30.96% C, 3.35% H, 13.52% N, 41.08% I. ^{1}H NMR spectrum: 12.85 bs, 1 H (OH); 9.13 d, 1 H, *J* = 6.5 (H-6); 8.69 s, 1 H (CH aldoxime); 8.63 d, 1 H, *J* = 1.9 (H-3); 8.32 dd, 1 H, *J* = 1.9 and 6.5 (H-5); 8.11 bs, 2 H (NH_2); 4.41 s, 3 H (CH_3).

1-(4-Bromobut-2-enyl)-2-[(hydroxyimino)methyl]pyridinium Bromide (**4**)

A mixture of pyridine-2-carbaldoxime (12.2 g, 0.1 mol) and 1,4-dibromobut-2-ene (42.5 g, 0.2 mol) in acetone (400 ml) was allowed to stand at room temperature for 6 days. The product separated as a grey substance (11.5 g, 34%), m.p. 121–127 °C (dec.).

4-Carbamoyl-2′-[(hydroxyimino)methyl]-1,1′-(but-2-ene-1,4-diyl)bispyridinium Dibromide (**5a**)

A solution of crude monoquaternary salt **4** (11.5 g, 0.034 mol) and isonicotinamide (5 g, 0.041 mol) in dimethylformamide (100 ml) was allowed to stand for 2 days to obtain 13.44 g of the crude product, m.p. 190–199 °C (dec.). This was crystallized from ethanol (160 ml) and water (20 ml) to obtain 12.85 g (81%) of the hemihydrate, m.p. 117–123 °C. For $C_{16}H_{18}Br_2N_4O_2 \cdot 0.5\ H_2O$ (467.2) calculated: 41.14% C, 4.10% H, 11.99% N, 34.21% Br; found: 40.98% C, 4.26% H, 12.13% N, 34.01% Br. ^{1}H NMR spectrum: 13.09 s, 1 H (OH); 9.29 d, 2 H, *J* = 6.6 (H-2′, H-6′); 9.21 d, 1 H, *J* = 6.0 (H-6); 8.79 bs, 1 H + 8.31 bs, 1 H (NH_2); 8.71 s, 1 H (CH aldoxime); 8.66 t, 1 H, *J* = 8.5 (H-4); 8.51 d, 2 H, *J* =

6.6 (H-3′, H-5′); 8.45 dd, 1 H, *J* = 1.3 and 8.5 (H-3); 8.19 m, 1 H (H-5); 6.39 dt, 1 H, *J* = 5.0 and 15.7 (CH butene); 6.04 dt, 1 H, *J* = 6.6 and 15.7 (CH butene); 5.63 d, 2 H, *J* = 5.0 (CH_2); 5.47 d, 2 H, *J* = 6.6 (CH_2).

4-Carbamoyl-2′-[(hydroxyimino)methyl]-1,1′-(but-2-ene-1,4-diyl)bispyridinium Dichloride (**5b**)

Solution of bromide **5a** (4.67 g, 0.01 mol) in water (100 ml) was passed through a column of Amberlite IRA-401 (50 ml) in the chloride form. The eluate was evaporated to dryness in vacuum, and the residue was crystallized from a mixture of 40 ml of ethanol and 8 ml of water to obtain 2.5 g (66%) of the product, m.p. 220–226 °C (dec.). For $C_{16}H_{18}Cl_2N_4O_2 \cdot H_2O$ (387.3) calculated: 18.31% Cl, 4.65% H_2O; found: 18.45% Cl, 4.20% H_2O. The spectral properties of this compound were identical with those of compound **5a**.

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