PREPARATION AND PROPERTIES OF CHOLINESTERASE REACTIVATORS OF BIS-QUATERNARY MONOPYRIDINALDOXIME TYPE

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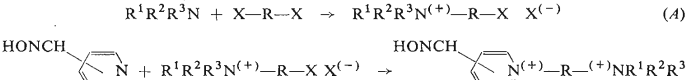
Preparation of 14 quaternary pyridinaldoximes and of 15 monoquaternary alkylating agents, isolated as intermediates is described. The pK'_a values are given of the final compounds, as well as the absorption maxima of the UV spectra and the degradation half-times in aqueous solutions of pH 7·4.

In connection with studying the reactivation of cholinesterase inhibited with organophosphates we synthesized a group of quaternarized pyridinaldoximes with another quaternary nitrogen in the N-alkyl chain. Some of these compounds with a presumed high reactivating potency were described by Nishimura and coworkers[1]. The procedure described here for the same reaction improves the yield in the first synthetic step. The higher purity of the intermediates affects favourably the yield of the final products.

$$R^1R^2R^3N + X{-}R{-}X \rightarrow R^1R^2R^3N^{(+)}{-}R{-}X \;\; X^{(-)} \qquad (A)$$

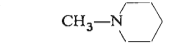
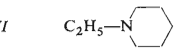
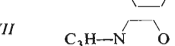
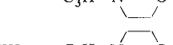
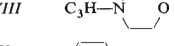


(B)

In contrast with the paper cited[1] reaction (*A*) was not conducted in ethanol but rather in acetone or benzene. In this way, with excess dihalogenide, a high yield of mostly crystalline products was obtained, there being minimum contamination by the bisquaternary compound. The monoquaternary alkylating agents obtained (Table I) were used without further purification in reaction (*B*). This reaction was carried out in ethanol in the presence of excess pyridinaldoxime. In the case of chloromethylether alkylating agents dimethylformamide was used as solvent. Crystallization of the crude products obtained by cooling the reaction mixture or by evaporating the solvent was rather laborious and mostly did not produce high yields. As crystallization solvent ethanol was used, possibly in mixture with water. The synthesized compounds are summarized in Table II. It was not possible to prepare compounds with a methylene and an ethylene bridge between the quaternary nitrogens. Quaternarization was not appreciable even in an ethanol or a dimethylformamide solution.

TABLE I

Monoquaternary Alkylating Agents $R^1R^2R^3N^{(+)}—R—X\ X^{(-)}$

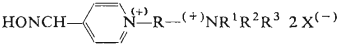
Compound	$R^1R^2R^3N$	R	X	M.p., °C (Ref.)	Yield %
XV	$(CH_3)_3N$	CH_2	Br	165—167 (155)[2]	94
XVI	$(CH_3)_3N$	$(CH_2)_2$	Br	254[a] (243[a])[2] (229)[3]	90
XVII	$(CH_3)_3N$	$(CH_2)_3$	Br	214·5 (208)[1,4] (205)[3]	95
XVIII	$(CH_3)_3N$	$(CH_2)_4$	Br	131—134 (190)[4]	80
XIX	$(CH_3)_3N$	$(CH_2)_6$	Br	107—109 (106—108)[3]	95
XX	$(CH_3)_3N$	$CH_2CH{=}CHCH_2$[b]	Br	165—167	95
XXI	$(CH_3)_3N$	CH_2OCH_2[c,d]	Cl	102—110	60
XXII	$CH_3(C_2H_5)_2N$	$(CH_2)_3$	Br	— (120—130)[3]	50
XXIII	$(CH_3)_2NCH_2CH_2OH$	$(CH_2)_3$	Br	—	75
XXIV	$(C_2H_5)_3N$	$(CH_2)_3$	Br	153—156 (129—132)[1] (146—148)[3]	62
XXV		$(CH_2)_3$	Br	202—203 (190—193)[3]	82
XXVI		$(CH_2)_3$[e]	Br	174	95
XXVII		$(CH_2)_3$	Br	145—147 (143—145)[3]	85
XXVIII		CH_2OCH_2[c,f]	Cl	—	50
XXIX		$(CH_2)_3$	Br	136 (128—129)[3]	65

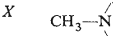
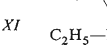
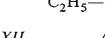
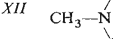
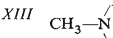
[a] Decomposition; [b] *trans*-isomer, calculated: 29·27%, found: 29·75% $Br^{(-)}$; [c] total Cl (determined after alkaline hydrolysis); [d] calculated: 40·73%, found: 38·68% Cl; [e] calculated: 25·36%, found: 25·41% $Br^{(-)}$; [f] calculated: 32·81%; found: 33·48% Cl.

The ionization constants of the hydrogen of the aldoxime group of the pyridinium-aldoximes were measured. An expected decrease of the pK_a values was found upon substitution of the middle methylene group of the trimethylene bridge with oxygen. In the UV spectrum, this substitution was reflected in a bathochromic shift of the

TABLE II

Bisquaternary Monoaldoximes



Compound	$R^1R^2R^3N$	R X	M.p., °C [a] (ref.)	Yield, % (ref.[1])	Calc./Found % X	 % N
I	$(CH_3)_3N$	$(CH_2)_3$ Br	168·5 (159—160)[1]	65 (33)	41·71 39·70	10·97 10·92
II	$(CH_3)_3N$	$(CH_2)_4$ Br	231—233[a]	31	40·24 39·97	10·58 11·35
III	$(CH_3)_3N$	$(CH_2)_6$ Br	143—149	—	—	—
IV	$(CH_3)_3N$	$CH_2CH{=}CHCH_2$ [b] Br	158—161[a]	15	40·44 39·35	—
V	$(CH_3)_3N$	CH_2OCH_2 Cl	193·5[a]	16	23·94 23·46	14·19 13·59
VI	$(CH_3)_3N$ [c]	CH_2OCH_2 Cl	173—174[a]	7	23·94 24·16	—
VII	$CH_3(C_2H_5)_2N$	$(CH_2)_3$ Br	209—211[a] (180—192)[1]	63 (15)	38·87 37·55	10·22 10·29
VIII	$(CH_3)_2NCH_2CH_2OH$	$(CH_2)_3$ Br	142—144	47	38·68 37·62	10·17 10·57
IX	$(C_2H_5)_3N$	$(CH_2)_3$ Br	238—240[a] (223[a])[1]	60 (22)	37·58 37·25	9·88 10·11
X		$(CH_2)_3$ Br	227—230[a] (217[a])[1]	57 (55)	37·72 37·50	9·93 10·08
XI		$(CH_2)_3$ Br	232—234[a] (225[a])[1]	36 (7)	36·55 35·63	9·61 9·92
XII		$(CH_2)_3$ Br	222—224[a] (213[a])[1]	48 (34)	37·59 36·81	9·88 9·81
XIII		CH_2OCH_2 Cl	196—199[a]	40	20·96 20·77	12·42 12·51
IV		$(CH_2)_3$ Br	243[a] (212)[5]	37	39·64 39·52	10·42 10·71

[a] Decomposition; [b] *trans*-isomer; [c] HON=CH in position 2.

TABLE III

pK'_a, Absorption Maxima of the UV Spectrum and Half-Times of Degradation of Reactivators Prepared

Compound	pK'_a	0·05M-HCl		0·05M-NaOH		$t_{1/2}$ h
		λ_{max}, nm	$\varepsilon . 10^{-3}$	λ_{max}, nm	$\varepsilon . 10^{-3}$	
I	8·16	280	16·70	340	24·50	88·1
II	8·29	278	16·75	337	25·00	111·8
III	8·29	275	15·10	335	19·25	—
IV	8·16	280	15·50	340	21·80	—
V	7·89	280	16·35	350	21·40	48·8
VI	7·52	300	11·25	350	16·30	0·64
VII	8·18	280	17·10	340	24·40	138·5
VIII	8·18	280	16·80	340	24·30	88·5
IX	8·17	280	16·83	340	24·50	87·8
X	8·19	280	17·50	340	24·75	91·2
XI	8·16	280	17·00	340	24·50	88·6
XII	8·17	280	16·70	340	23·90	90·0
XIII	7·86	280	16·35	350	21·40	49·2
XIV	8·17	280	17·25	340	24·50	93·0
TMB-4	—	—	—	—	—	114·9
Toxogonin	—	—	—	—	—	39·2

last absorption band of the betaine form of the molecule. Prolongation of the chain is accompanied by a rise of the pK_a value and by a hypsochromic shift in the UV spectrum of the betaine form toward the wavelength of the absorption band of monoquaternary pyridiniumaldoximes. Table III shows together with values of pK_a and data on the UV spectra also the half-times of degradation of the compounds at pH 7·4. These values document the stability of the compounds prepared as compared with the standard reactivators trimethylene-1,3-bis(4-pyridiniumaldoxime) dibromide (TMB-4) and bis(4-pyridiniumaldoximemethyl) ether dichloride (Toxogenin), the stability and mechanism of decomposition of which was taken up by Ellin and coworkers[6] and by Christenson[7]. The reactivating capacity of the synthesized compounds *in vitro* is described by Patočka[8].

EXPERIMENTAL

The melting points are not corrected. Samples for elementary analysis were dried in vacuo (0·5 Torr) over phosphours pentoxide. The purity of the compounds was further controlled by paper chromatography (Whatman No 1, 1-butanol–acetic acid–water 4 : 1 : 5) detected under UV light and with Dragendorff's reagent. The UV spectra were prepared on a VSU-1C, Zeiss (Jena) spectrophotometer. The pK_a values were measured spectrophotometrically. The stability was followed spectrophotometrically at the wavelength of the maximum of the betaine form of the molecule. 10^{-4}M solutions in 0·01M phosphate buffer (pH 7·4) were tested at 60°C. Examples of carrying out reactions (*A*) and (*B*) are shown below.

4-Methyl-4-(3-bromopropyl)morpholinium Bromide (*XXVII*)

A solution of 10·1 g 4-methylmorpholine (0·1 mol) and 101 g 1,3-dibromopropane (0·5 mol) in 250 ml acetone was refluxed for 15 h, the precipitated substance was filtered and washed with acetone. A total of 26·3 g (85%) crystalline compound was obtained.

4-Methyl-4-(3-chloro-2-oxapropyl)morpholinium Chloride (*XXVIII*)

A solution of 10·1 g 4-methylmorpholine (0·1 mol) in 50 ml benzene was mixed with a solution of 29 g bis(chloromethyl) ether (0·25 mol) in 50 ml benzene. The reaction mixture, protected from external humidity, was heated for 2 h at 60°C and then left to stand for 2 days. The filtered compound was washed with benzene and dried over phosphorus pentoxide in an evacuated desicator. A total of 12 g (50%) hygroscopic compound was obtained.

1-(4-Hydroxyiminomethylpyridinium)-3-(hydroxyethyl)dimethylamonium)propane Dibromide (*VIII*)

A solution of 15 g *XXIII* (0·05 mol) and 12·2 g 4-pyridinaldoxime (0·1 mol) in 200 ml ethanol was refluxed for 30 h. After cooling to —10°C a total of 14 g crude product was filtered. Recrystallization from ethanol yielded 10 g (47%) of a chromatographically pure compound.

1-(4-Hydroxyiminomethylpyridinium)-3-(4-methylmorpholinium)-2-oxapropane Dichloride (*XIII*)

2·5 g *XXVIII* (0·11 mol) and 4·0 g 4-pyridinaldoxime (0·033 mol) was dissolved in 25 ml dimethylformamide with heating to 50°C. After 4 days of standing at room temperature the crude product was filtered and recrystallized from 95% ethanol. A total of 1·5 g (40%) chromatographically pure product was obtained.

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