STUDIES ON PYRIDINE ALDOXIMES

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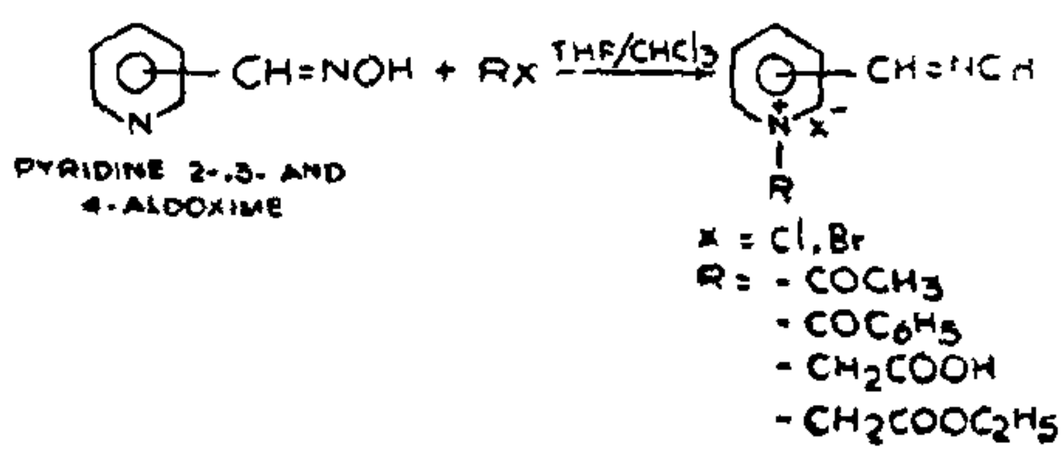
ABSTRACT

Pyridine 2,3- and 4-aldoximes have been quaternized with acetyl chloride, benzoyl chloride, bromoacetic acid and ethyl bromoacetate under carefully controlled conditions to yield the corresponding quaternary salts. The stable compounds among these have been estimated by measuring absorption at 405 mμ at pH 10·5. Efficacy of the compounds in reactivating phosphorylated cholinesterase enzymes *in vitro* has also been evaluated.

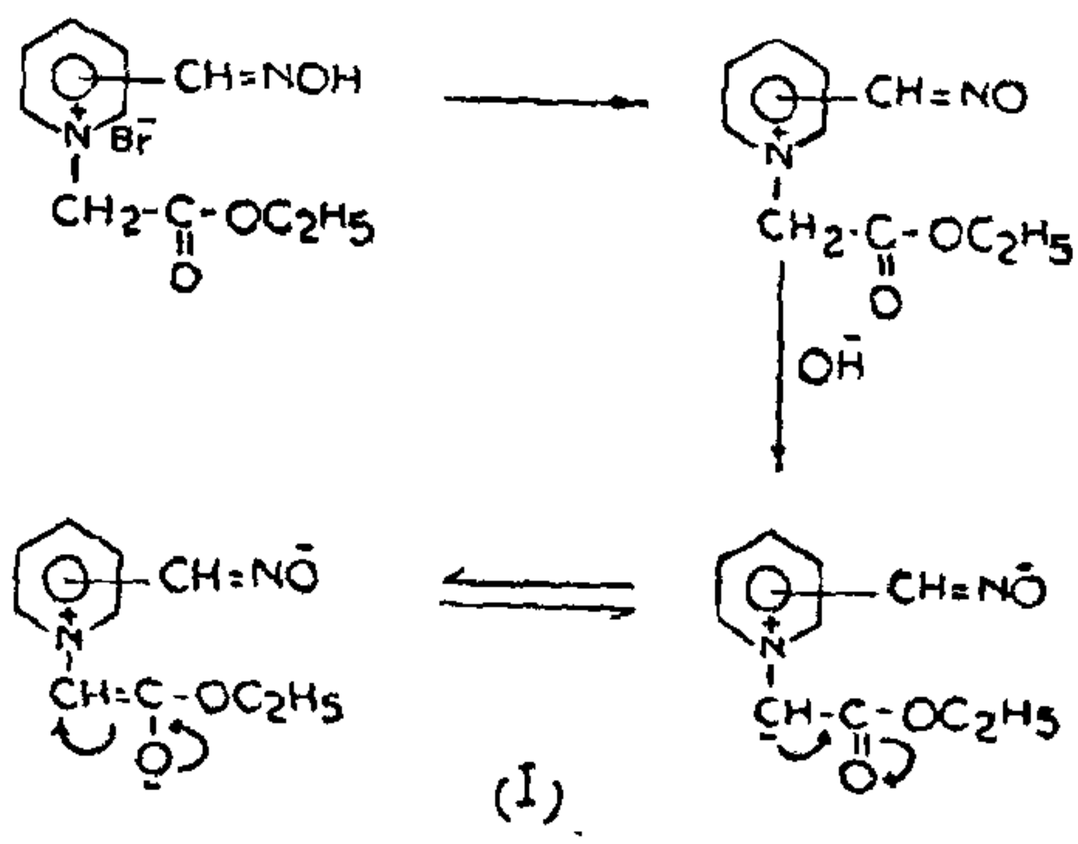
QUATERNARY pyridinium aldoximes like pyridine aldoxime methiodide (2-PAM)[1] or N,N'-trimethylene-*bis*-(4-hydroxyiminomethyl pyridinium) dibromide[2] have been used for reactivating phosphorylated cholinesterase enzyme in therapy as adjuvants to atropine with remarkable success. A practical reactivater should dissociate readily and also should have sufficient nucleophilicity in the dissociated form. A new series of quaternary salts of 2-, 3- and 4-pyridine aldoximes have been synthesised with the above end in view. Acylation of nitrogen atom of pyridine nucleus was expected to facilitate oximate anion formation and increase reactivation property[3].



Physical data on these compounds are given in Table I.

The compounds were difficult to synthesize without rigidly controlling the reaction temperatures. At higher temperature, the pyridine aldoxime hydrochloride was formed instead of the desired compounds. N-Acetyl and N-benzoyl compounds were hydrolysed very quickly with water. N-Methylene carbethoxy compounds were stable in water but hydrolysed by alkali to hydroxylamine and the corresponding quaternary pyridinium aldehyde. N-Acetyl and N-benzoyl compounds did not show any infrared absorption between 1650–1800 cm^{-1}, the normal carbonyl range. Different workers have reported divergent values for N-acyl compounds[4–6]. It appears that the)C=O group loses much of its carbonyl character on linkage to the heterocyclic nitrogen.

A simple method of estimation of the more stable of these compounds is also reported. In alkaline solution, the compounds form a yellow coloured stable ylide, which obeys the Beer-Lambert's law. Existence of such ylides as (I) given below, formed from different substituted mono- and *bis*-pyridinium aldoximes on treatment with bases has been reported recently[7].



2-, 3-, and 4-N-Methylene carbethoxy iminoformyl pyridinium bromides have been evaluated *in vitro* for their efficacy in reactivating cholinesterase enzyme phosphorylated with DDVP[8]. The 2-isomer was found to effect 13% regeneration of inhibited cholinesterase, while 3- and 4-isomers were totally inactive.

EXPERIMENTAL

Melting points given in Table I are uncorrected. IR spectra in KBr were recorded on a Perkin–Elmer model 577 double beam spectrophotometer.

Pyridine 2-, 3- and 4-aldoximes

They were prepared from the corresponding aldehydes by known methods[9].

N-Acetyl and N-benzoyl (2-, 3- *and* 4-) *iminoformyl pyridinium chlorides and N-methylene carboxylic acid-4-iminoformyl pyridinium bromide* :

Under dry conditions, pyridine aldoxime (0·01 mole) in chloroform/THF (40 ml; 25 ml) was taken in a 100 ml 2-necked round-bottomed flask fitted with a dropping funnel and a $CaCl_2$ guard tube. The flask was kept at −10 to −15° C. Freshly distilled acid

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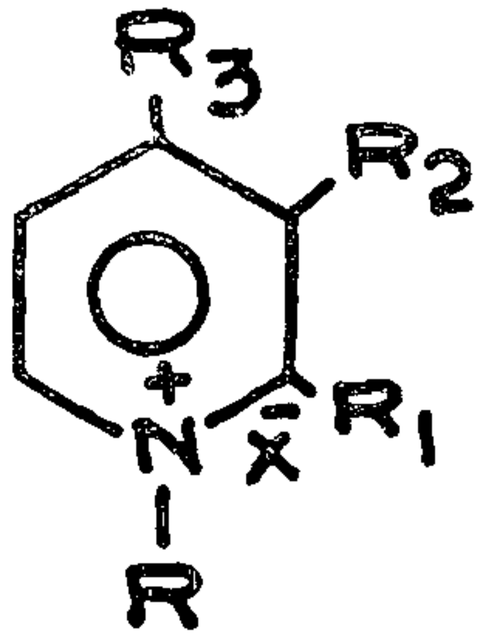
chloride (0·01 mole) in chloroform/THF (5 ml) was added dropwise to the oxime solution with continuous stirring during a period of 25–30 minutes. A pale yellow precipitate was formed during the addition. The mixture was stirred for 3 hrs. and further at room temperature for an additional one hour. The product was filtered by suction, washed with chloroform followed by ether, dried and kept in a desiccator. It was crystallised from absolute alcohol and dry ether.

N-Methylene carbethoxy (2-, 3- and 4-) *iminoformyl pyridinium bromides*

Under dry conditions, pyridine aldoxime (0·01 mole) and distilled THF (25 ml) were taken in a 50 ml round bottomed flask fitted with a reflux condensor and a $CaCl_2$ guard tube. Ethyl bromoacetate (0·01 mole) was added and the mixture was refluxed on a water-bath for 6 hrs. in the case of 4-oxime and 8 hrs. in the case of 2-oxime. In the case of 3-oxime, the reaction mixture was stirred at room temperature without

TABLE I



Physical Data of the Compounds Prepared

Sl. No.	Compounds R	R_1	R_2	R_3	X	Solvent	Temp. of reaction (°C)	Yield %	Appearance	M.P.°C (Decomp)	I. R. Max cm^{-1} (KBr)
1	2	3	4	5	6	7	8	9	10	11	12
1.	$-COCH_3$	H	H	CH=NOH	Cl	$CHCl_3$	−10 to −15	85	White powder	225–26	3150–2900 (OH) 1000 (N–O)
2.	$-COCH_3$	H	CH=NOH	H	Cl	THF	−10 to −15	80	White powder	166–68	3100–3000 (OH) 1005 (N–O)
3.	$-COCH_3$	CH=NOH	H	H	Cl	$CHCl_3$	−10 to −15	50	White powder	180–82	3200–2800 (OH) 995 (N–O)
4.	$-COC_6H_5$	H	H	CH=NOH	Cl	$CHCl_3$	−10 to −15	77	White powder	232–34	3150–2900 (OH) 1000 (N–O)
5.	$-COC_6H_5$	H	CH=NOH	H	Cl	THF	−10 to −15	77	White powder	171–73	3100–3000 (OH) 1005 (N–O)
6.	$-COC_6H_5$	CH=NOH	H	H	Cl	$CHCl_3$	−10 to −15	38	White powder	175–78	3200–2800 (OH) 995 (N–O)

TABLE I—*Contd.*

1	2	3	4	5	6	7	8	9	10	11	12
7.	$-CH_2COOH$	H	H	CH=NOH	Br	$CHCl_3$	−10 to −15	60	Colourless needles	84–86	3100–2900 (OH) 1000 (N–O)
8.	$-CH_2COOC_2H_5$	H	H	CH=NOH	Br	THF	Reflux	66	Colourless cubes	161–63	3100–2800 (OH) 1730 (C=O) 1212 (C–O–C) 1000 (N–O)
9.	$-CH_2COOC_2H_5$	H	CH=NOH	H	Br	THF	Room temp.	66	Colourless needles	163–65	3100–2900 (OH) 1000 (N–O) 1730 (C=O) 1215 (C–O–C)
10.	$-CH_2COOC_2H_5$	CH=NOH	H	H	Br	THF	Reflux	33	Colourless needles	149–50	3100–2900 (OH) 1005 (N–O) 1730 (C=O) 1210 (C–O–C)

Satisfactory elemental analysis results were obtained for all the compounds.

refluxing. The light brown solid formed was filtered, washed with (3 times; 10 ml portions) and crystallised from absolute alcohol as colourless needles.

Colorimetric estimation of N-methylene-carbethoxy 4-iminoformyl pyridinium bromide

Optical measurements were made on Bausch and Lomb spectronic-20 and readings taken at 405 mμ at pH 10·5. A standard curve was drawn and recoveries calculated.

TABLE II
(*Recovery*)

Conc. (gm/ml)	
Added	Recovered
$6·3 \times 10^{-5}$	$6·5 \times 10^{-5}$
$12·6 \times 10^{-5}$	13×10^{-5}

Cholinesterase reactivation

In vitro regenerating activity of these compounds was estimated by blocking the enzyme with an organophosphorus compound and reactivating with the oxime. Human serum (0·2 ml) was used as the source of cholinesterase and 100 μ mol acetyl choline bromide as the substrate. Reaction was done in barbital buffer at pH 8·3 ± 0·1. Acetyl choline formed was reacted with alkaline hydroxylamine to form acet hydroxamic acid which at a pH 1·2 ± 0·2 gives a red purple colour with ferric chloride. To block the enzyme 0·1 ml (1 ppm) diethyl dichlorovinyl phosphate (DDVP) was used and 0·1 ml (1 mg/ml) of the oxime was added for regeneration. Readings were taken at 540 mμ and percentage regeneration calculated. N-(Methylene carbethoxy)-2-iminoformyl pyridinium bromide (Table I, No. 10) showed maximum activity in the series with 13% regeneration while the 4- and 3-isomers of the same had virtually no activity.

1. Wilson, I. B. and Ginsburg, S., *Biochim. Biophys. Acta*, 1955, **18**, 168.
2. Poziomek, E. J., Hackley (Jr.), B. E. and Steinberg, G. M., *J. Org. Chem.*, 1958, **23**, 714.
3. Hobbiger, F., *Handbook of Experimental Pharmacology*, edited by G. B. Koelle (Springer-Verlag, Berlin), 1963, **15**, 930.
4. Paul, R. C. and Chadha, S. L., *Spectrochim. Acta*, 1966, **22**, 615.
5. Sheynkman, A. K., Portnova, S. L., Shienker, U. N. and Kost, A. N., *Dokl. Akad. Nauk. USSR*, 1964, **157**, 1416; see *Chem. Abstr.*, 1964, **61**, 14157.
6. Holecek, J., Pavlik, I. and Klikota, J., *Sb. Ved. Praci, Vysoka Skola Chem. Technol. Pardubice*, 1964, **2**, 23, see *Chem. Abstr.*, 1966, **64**, 5815.
7. Hagedorn, I. and Gundel, W. H., *Arzneimittel-Forsch.*, 1976, **26**, 753.
8. *Standard Methods of Clinical Chemistry*, edited by David Saligson, Academic Press, New York and London, 1963, **4**, 47.