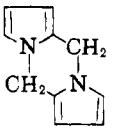
catalytically,[18] attempts were made to carry out such a conversion. Low pressure hydrogenation resulted in recovery of starting material. High pressure hydrogenation in ethyl acetate (ethanol was not satisfactory) gave an oil of b.p. 60° (1 mm.) which could not be identified satisfactorily but was neither the hoped-for V nor *N*-acetylpiperidine.[19] The relatively large quantity formed (2.52 g. from 2.0 g. of II) indicated that solvent was involved.



V

(18) A. Treibs and A. Zinsmeister, *Ber.*, **90**, 87 (1957).

(19) Piperidine and ethyl acetate at 200° are reported to yield *N*-acetylpiperidine.[20]

(20) F. B. Ahrens, *Ber.*, **27**, 2088 (1894).

Reaction of 2-pyrrolealdehyde with morpholine. Condensation of 2-pyrrolealdehyde with morpholine in the manner described above furnished 3.19 g. (97%) of tan crystals which were decolorized by recrystallization from ethyl acetate and then melted at 197–198°. The product resembled II in chemical behavior and solubility.

Anal. Calcd. for $C_{18}H_{24}N_4O_2$: C, 65.43; H, 7.32; N, 17.0. Found: C, 65.53; H, 7.46; N, 17.1.

Reaction of 2-pyrrolealdehyde with pyrrolidine. A mixture of 9.6 g. of the aldehyde and 14.2 g of pyrrolidine yielded 7.35 g. (50%) of brown product which was purified by recrystallization from ether, m.p. 93–94°. The substance decomposed rapidly on standing, and like its analogs, could not be titrated satisfactorily.

Anal. Calcd. for $C_{18}H_{24}N_4$: C, 18.9. Found: N, 18.8.

Acknowledgment. We are grateful to E. I. du Pont de Nemours and Co., Inc., for the gift of chemicals.

TALLAHASSEE, FLA.

[CONTRIBUTION FROM THE CHEMOTHERAPY BRANCH, U. S. ARMY CHEMICAL WARFARE LABORATORIES]

Pyridinium Aldoximes[1]

EDWARD J. POZIOMEK, BRENNIE E. HACKLEY, JR., AND GEORGE M. STEINBERG

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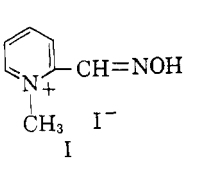
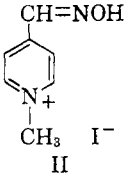
A number of 1,1′-polymethylenebis(4-formylpyridinium bromide) dioximes and *N*-substituted 2- and 4-formylpyridinium halide oximes have been prepared. The bis-quaternary dioximes are active as chemotherapeutic agents in the treatment of nerve gas and other anticholinesterase poisoning in experimental animals, when administered in conjunction with atropine. The most active, 1,1′-trimethylenebis(4-formylpyridinium bromide) dioxime, appears to have advantages over previously reported treatment agents.

The "nerve gases" such as diisopropyl phosphorofluoridate (DFP), isopropyl methylphosphonofluoridate (GB) and *O*-ethyl *N*,*N*-dimethyl phosphoramidocyanidate (GA) as well as many of the organophosphorus insecticides or their metabolites function biologically by inhibition of the enzyme acetylcholinesterase[2]; inhibition being caused by phosphorylation (or phosphonylation) of the active site of the enzyme.

Nerve gas poisoning has been treated symptomatically with drugs which are pharmacologically antagonistic to acetylcholine. Such a compound is atropine and it is presently the recommended remedy.[3] As part of a program aimed at the development of prophylactics and of therapeutics which will act to repair the biological lesion, we have been involved in a search for reagents which (*a*) react rapidly with the nerve gases under physiological conditions of *p*H and temperature and (*b*) reactivate phosphorylated (or phosphonylated) enzymes. Several groups of rapid nerve gas reactants have been reported.[4]



I



II

Recently, 2-formyl-1-methylpyridinium iodide oxime,[5] Compound I, has been reported to enhance considerably the activity of atropine in the chemotherapeusis of poisoning due to organophosphorus compounds.[6] Compound I shares the

(1) Presented at American Chemical Society, 132nd Meeting, New York, September 1957.

(2) (a) B. J. Jandorf, H. O. Michel, N. K. Schaffer, R. Egan, and W. H. Summerson, *Discussions Faraday Soc.*, **20**, 134 (1955). (b) J. E. Casida, *J. Agr. Food Chem.*, **4**, 772 (1956).

(3) W. H. Summerson, *Armed Forces Chem. J.*, **9**, 24 (1955).

(4) (a) B. E. Hackley, Jr., R. Plapinger, M. Stolberg, and T. Wagner-Jauregg, *J. Am. Chem. Soc.*, **77**, 3651 (1955). (b) G. M. Steinberg and J. Bolger, *J. Org. Chem.*, **21**, 660 (1956). (c) B. J. Jandorf, T. Wagner-Jauregg, J. O'Neill, and M. Stolberg, *J. Am. Chem. Soc.*, **74**, 1521 (1952). (d) T. Wagner-Jauregg and B. E. Hackley, Jr., *J. Am. Chem. Soc.*, **75**, 2125 (1952). (e) T. Wagner-Jauregg, B. E. Hackley, Jr., T. A. Lies, O. O. Owens, and R. Proper, *J. Am. Chem. Soc.*, **77**, 922 (1955). (f) J. Epstein, D. Rosenblatt, and M. Demek, *J. Am. Chem. Soc.*, **78**, 341 (1956).

(5) This compound has been commonly referred to in the pharmacological and biochemical literature as 2-pyridinealdoxime methiodide or 2-PAM.

(6) (a) I. B. Wilson and S. Ginsburg, *Biochim. et Biophys. Acta*, **18**, 168 (1955). (b) D. R. Davies and A. L. Green, *Discussions Faraday Soc.*, **20**, 269 (1955). (c) H. Kewitz, I. B. Wilson, and D. Nachmansohn, *Arch. Biochem. Biophys.*, **64**, 456 (1956).

property of reacting rapidly with GB *in vitro*, under physiological conditions of *p*H and temperature with many other oximes which are chemotherapeutically ineffective.[7] It does stand out among other oximes, however, in its ability to reactivate inactivated acetylcholinesterase (AChE) *in vitro* with great rapidity.[6] The reactivation process involves removal of the phosphate (or phosphonate) grouping from the enzyme.

Wilson[6a] has offered the hypothesis that the outstanding activity of Compound I results from its ability to strongly associate with the inhibited enzyme at the site of phosphorylation, and that in association complex the reactive oximino group is properly oriented for displacement of the phosphate moiety. By way of comparison, the corresponding 4-formyl-1-methylpyridinium iodide oxime,[8] Compound II, is also comparatively active as a reactivator of phosphate or phosphonate inhibited cholinesterase; however, it is considerably less effective than I. Thus, with isopropyl methylphosphonylated eel acetylcholinesterase the rate constant for reactivation at *p*H 7.4, 25°, in the presence of 7.2×10^{-3} *M* acetylcholine is $2 \times 10^{3} M^{-1}$ for I and $1.4 \times 10^{2} M^{-1}\text{min.}^{-1}$ for II.

If strong association between oxime and inhibited enzyme were an important factor in reaction, it seemed that one should be able to increase reactivation rate by combining structures which are known to strongly associate with the enzyme, such as the di- and polyquaternary compounds, with the reactive formylpyridinium halide oxime group. To this end a series of 1,1′-polymethylene bis-(4-formylpyridinium) bromide dioximes have been prepared. These compounds, Table I, are even more rapid reactivators of the inhibited eel AChE than I although they are structurally related to the less active II. Under the conditions referred to above, VI, the most active of these compounds reactivates with a rate constant of $10^{4} M^{-1}\text{min.}^{-1}$ With the exception of VII which is too toxic for chemotherapeutic use, all of the bisquaternary compounds are active, when used together with atropine, as therapeutics and to a lesser but significant extent as prophylactics against GB poisoning. The most effective of the group in the treatment of poisoned animals is compound IV, 1,1′-trimethylenebis(4-formylpyridinium bromide) dioxime,[9] which is not quite as rapid a reactivator as V; its rate constant under the conditions cited is $6 \times 10^{3} M^{-1}\text{min.}^{-1}$

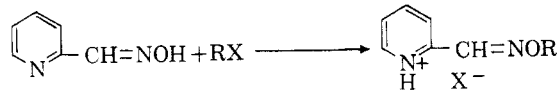
In preliminary studies in animals poisoned with GB, the combination of Compound IV and atropine appears to be more effective in both therapy and prophylaxis than the corresponding combination of I with atropine; although there is a marked species variation in their relative effectiveness. In rats challenged with a $2LD_{50}$ dose (iv) of GB, all of a group of six animals survived if the first combination was administered (iv) immediately after poisoning. The second combination saved only two of the group of six animals. On the other hand, with dogs which were given a $20LD_{50}$ dose of GB (subcutaneous) the survival ratios were the same for the two treatments (4/5) which were given intravenously when symptoms appeared; however, the recovery time was much more rapid for the surviving animals which received the first treatment, *i.e.* 2 hours *vs.* 24 hours.

The *in vitro* reactivation data and the therapy results were kindly provided by Drs. H. O. Michel and E. Bay, respectively. Detailed reports of their studies will be published elsewhere.

This paper also reports the synthesis of several monoquaternary analogs of compounds I and II, Table II. All of the compounds in this group were inferior in chemotherapeutic activity to Compound I.

Two standard procedures were employed for quaternization of the pyridine ring. In procedure A, the pyridine compound and halide were reacted at reflux temperature in absolute ethanol; in procedure B the mixture of amine, halide, and solvent was heated to 60° in a capped bottle of the carbonated beverage variety. The latter procedure was generally found to be more convenient because of its simplicity.

Quaternization of the pyridine oximes was sometimes complicated by a side reaction involving alkylation of the oximino group.[10] This side reaction



became increasingly preponderant with increased steric hindrance at the site of reaction, *i.e.* the pyridine nitrogen atom. With highly hindered oximes such as 2,6-diformylpyridine dioxime, the oxime ether was the sole product of reaction. Similarly, in an attempted preparation of 2-formyl-1-isopropylpyridinium bromide oxime using procedure A, reflux time 60 hours, the only product isolated was the *O*-alkylated derivative in 37% yield. Low yields were general with the 2-formyl derivatives, and attempts to synthesize *N,N′*-bis derivatives of 2-formylpyridinium halide oximes by quaternization of 2-pyridinealdoxime have been unsuccessful to date. The properties, yields, and analyses of the compounds prepared are summarized in Tables I and II.

EXPERIMENTAL

Oximes. 2- and 4-Pyridinecarboxaldehyde oximes were prepared by warming on the steam bath a neutralized

(7) (a) B. E. Hackley, Jr., Ph.D. dissertation, University of Delaware, 1956. (b) A. L. Green and B. Saville, *J. Chem. Soc.*, 3887 (1956).

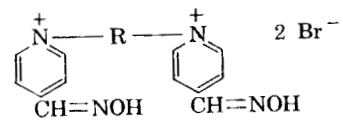
(8) Commonly referred to as 4-PAM.

(9) Commonly referred to as TMB-4.

(10) S. Ginsburg and I. B. Wilson, *J. Am. Chem. Soc.*, **79**, 481 (1957).

TABLE I

1,1′-POLYMETHYLENEBIS(4-FORMYLPYRIDINIUM HALIDE) DIOXIMES

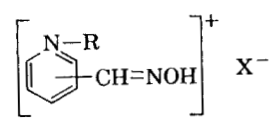


No.	Substituents, R	Conditions	Yield, %	M.P., °C.[a]	Formula	pK_a	Analysis: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	Neut. Equiv. Calcd.	Neut. Equiv. Found
III	$-(CH_2)_2-$	A, 31 hr. B, 64 hr.	35.0	300	$C_{14}H_{16}Br_2N_4O_2$	8.1	—	—	—	—	12.9	12.7	216	216
IV	$-(CH_2)_3-$	B, 48 hr.	88.2	238–241 (dec.)	$C_{15}H_{18}Br_2N_4O_2$	8.2	40.4	41.2	4.1	4.6	12.6	12.6	223	223
V	$-(CH_2)_4-$	B, 16 hr.	81.0	239–241 (dec.)	$C_{16}H_{20}Br_2N_4O_2$	8.3	41.8	41.2	4.4	4.6	—	—	230	246
VI	$-(CH_2)_5-$	B, 95 hr.	95.0	208–210 (dec.)	$C_{17}H_{22}Br_2N_4O_2$	8.4	42.3	42.3	4.6	4.9	11.6	11.4	237	236
VII	$-(CH_2)_{10}-$	B, 8 hr.	85.0	219–223 (dec.)	$C_{22}H_{32}Br_2N_4O_2$	8.5	48.6	48.3	6.0	6.0	10.3	10.7	272	271

[a] Melting points are uncorrected.

TABLE II

N-SUBSTITUTED 2- AND 4-FORMYLPYRIDINIUM HALIDE OXIMES



Oximino Formyl	R	X	Conditions	Yield, %	M.P., °C.[a]	Formula	pK_a	Analysis: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	Neut. Equiv. Calcd.	Neut. Equiv. Found
2	Ethyl	I	A, 160 hr.	34.8	176–177 (dec.)	$C_8H_{11}IN_2O$	8.1	34.5	34.3	4.0	3.9	10.1	10.3	278	265
2	Allyl	Br	A, 8 hr.	28.8	300	$C_9H_{11}BrN_2O$	8.0	44.5	44.1	4.5	4.5	11.5	12.9	243	240
4	Allyl	Br	B, 70 hr.	93.0	183–187 (dec.)	$C_9H_{11}BrN_2O$	8.4	44.5	44.3	4.6	4.6	11.5	11.7	243	234
2	2-Hydroxy ethyl	Br	A, 6 hr.	9.9	197–200 (dec.)	$C_8H_{11}BrN_2O_2$	7.8	38.9	39.1	4.5	4.6	11.3	11.4	247	245
4	2-Hydroxy ethyl	Br	B, 54 hr.	44.5	187–190 (dec.)	$C_8H_{11}BrN_2O_2$	8.3	38.9	38.8	4.5	4.5	11.3	11.4	247	241
4	Butyl	Br	A, 24 hr. B, 64 hr.	88.3	138–139 (dec.)	$C_{10}H_{15}BrN_2O$	8.4	46.4	44.9	5.8	5.5	10.8	11.0	259	232

[a] Melting points are uncorrected.

aqueous solution of the corresponding aldehyde (obtained from the Aldrich Chemical Co.) and $NH_2OH \cdot HCl$, m.p. 112.5–113.0° and 130.0–130.5°, respectively.

Quaternizations. In synthesizing the mono 2- and 4-formyl alkyl pyridinium halide oximes a 2:1 molar ratio of halide to tertiary oxime was used. In the "bis" series a 1:3 molar ratio was employed.

Procedure A. A mixture of the pyridine oxime and halide was dissolved in sufficient ethanol and refluxed for the period of time specified in Tables I and II.

Procedure B. A mixture of oxime and halide was dissolved in about 100 ml. of ethanol and heated at 60° in a 200-ml. capped pressure bottle for specified periods of time. The reaction mixtures were cooled to room temperature and the products of reaction removed by filtration. In several cases, it was necessary to add absolute ether to effect complete precipitation. The products were recrystallized from absolute ethanol.

pK_a Values. The pK_a values were determined at room temperature (25–27°), from potentiometric titration data, assuming pK_a to be the pH of half neutralization. In each case approximately 100 mg. of oxime dissolved in 5 ml. of water was titrated with 0.1N sodium hydroxide.

Analysis. Elemental analyses were performed by standard procedures. For determination of nitrogen (Dumas) the weighed samples were layered over with V_2O_5 prior to ignition. Since the oxime ether hydrohalides are isomeric with the desired quaternized oximes it was necessary to establish purity by independent determination. This was achieved readily by potentiometric titration since the oxime ether hydrohalides have pK_a values of less than 5, whereas the pK_a values of the quaternized oximes are 7.8–8.5.

Where mixtures of oxime ether hydrohalide and quaternary oximes were obtained, separation was accomplished by fractional crystallization from ethanol or by separation from neutral aqueous solution. At pH 6–7 the oxime ether (and also any unreacted pyridine aldoxime) could be extracted from aqueous solution with $CHCl_3$ leaving the quaternary compound in the aqueous layer.

Acknowledgment. The authors wish to express their gratitude to the Analytical Research Branch of the Research Directorate, U.S. Army Chemical Warfare Laboratories for the analyses here reported and to Pfc. Gerard Natarelli for many of the potentiometric titrations.

ARMY CHEMICAL CENTER, MD.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MARYLAND]

Polycyclic Compounds Containing Nitrogen. I. The Diels-Alder Reaction of 1-Nitro-1-alkenes

NATHAN L. DRAKE AND ALBERTA B. ROSS[1,2]

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As a preliminary to investigations involving substituted nitrocyclohexenes and their analogs as intermediates for the preparation of polycyclic compounds containing ring nitrogen, the reaction of various 1-nitro-1-alkenes with 2,3-dimethyl-1,3-butadiene was reexamined. The nitroalkenes studied have the general formula, $RCH{=}CHNO_2$, where R is methyl, ethyl, propyl, isopropyl, butyl, isobutyl, amyl, hexyl, and 2-ethoxyethyl. The majority of the adducts were characterized by conversion to the corresponding dibromides which were readily purified by crystallization. Two of the dibromides were not crystalline; in these instances the corresponding cyclohexenones were prepared by the Nef reaction. Reduction of the nitrocyclohexenes to the corresponding cyclohexylamines is described.

Investigations concerned with the synthesis of polycyclic compounds containing ring nitrogen by routes involving the Diels-Alder reaction demanded a reinvestigation of the use of 1-nitro-1-alkenes as dienophiles. The present paper records this work.

It has previously been shown that 1-nitro-1-alkenes participate as dienophiles in the Diels-Alder reaction.[3] In general, however, the yield of adduct has been low whenever the preparation has involved an open-chain diene; the number of such cases previously studied is very small.

2,3-Dimethylbutadiene was chosen for our work because of its ready availability and its reactivity. 2-Methoxybutadiene was also employed in a few cases.[4]

Experimental conditions employed for such reactions by previous investigators have varied from heating an ether solution of the reactants under reflux to heating the reactants in a sealed tube at 150°. We have found that no adduct was isolable from 1-nitro-1-pentene and 2,3-dimethyl-1,2-butadiene when a mixture of the two was allowed to

(1) From a thesis submitted by A. B. Ross in partial fulfillment of the requirements for the Ph.D. degree, University of Maryland, June 1957.

(2) Monsanto Chemical Company Fellow, 1956–57.

(3) H. L. Holmes, *Org. Reactions, IV*, 60 (1948); K. Klager, *J. Org. Chem.*, **20**, 650 (1955); W. E. Noland, H. I. Freeman, and M. S. Baker, *J. Am. Chem. Soc.*, **78**, 188 (1956); J. D. Roberts, C. C. Lee, and W. H. Saunders, *J. Am. Chem. Soc.*, **76**, 4501 (1954); W. C. Wildman and C. H. Hemminger, *J. Org. Chem.*, **17**, 1641 (1952); W. C. Wildman and D. R. Saunders, *J. Org. Chem.*, **19**, 381 (1954); A. Etienne, A. Spire, and E. Toromanoff, *Bull. soc. chim. France*, 750 (1952); E. E. Van Tamalen, and R. J. Thiede, *J. Am. Chem. Soc.*, **74**, 2615 (1952); D. V. Nightingale, M. Maienthal, and J. A. Gallagher, *J. Am. Chem. Soc.*, **75**, 4852 (1953); W. E. Noland and R. E. Bambury, *J. Am. Chem. Soc.*, **77**, 6386 (1955); K. Alder, H. F. Rickert, and E. Windemuth, *Ber.*, **71**, 2451 (1938); E. G. Kataev and P. S. Matveeva, *J. Gen. Chem. (U.S.S.R.)*, **23**, 405 (1953); *Chem. Abstr.*, **48**, 3272 (1954); W. E. Noland, R. E. Counsell, and M. H. Fisher, *J. Org. Chem.*, **21**, 911 (1956).

(4) See paper, II, *J. Org. Chem.*, in press.