SYNTHESIS OF TRITIUM LABELLED OXIMES: 2-PYRIDINE ALDOXIME METHIODIDE (2-PAM) AND 1-(2-HYDROXYIMINOMETHYLPYRIDINIUM)-1-(4-CARBOXYAMIDO-PYRIDINIUM)DIMETHYLETHER DICHLORIDE (HI-6), WITH HIGH SPECIFIC ACTIVITY.

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SUMMARY

Synthesis of two quaternary pyridinium oximes [^{3}H]2-PAM and [^{3}H]HI-6 (18 and 22 Ci/mmole, respectively) was performed by using unlabelled 6-bromo 2-pyridine aldehyde (I) as a common starting material. The labelling was based on a catalytic ring-tritiation of 6-bromo 2-diethylketal pyridine followed by acid hydrolysis and reaction with hydroxylamine to yield 6-[^{3}H]2-hydroxyiminomethyl pyridine (II). [^{3}H]2-PAM was obtained by reacting II with methyliodide and [^{3}H]HI-6 was prepared by alkylation of II with bischlorodimethylether followed by coupling to isonicotineamide. The radiochemical purity of [^{3}H]2-PAM and [^{3}H]HI-6 was 98% and at least 95%, respectively.

Key words: tritium, radiolabelled oximes, 2-PAM, HI-6, reactivators, acetylcholinesterase.

INTRODUCTION

The monoquaternary pyridinium oximes were developed by Ginsburg and Wilson in the early fifties (1) as reactivators of inhibited

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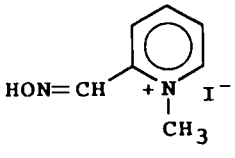
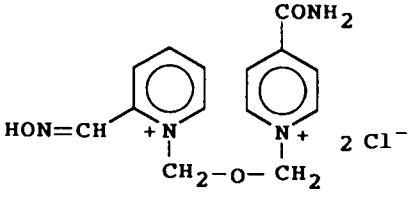
acetylcholinesterase (AChE). It was assumed that the oxime moiety attached to a positively charged methylpyridinium ring would fit into the anionic site of AChE and displace the phosphoryl group which is bound covalently to the enzyme. Indeed, pyridinium 2-aldoxime methiodide (2-PAM, scheme 1A) was the first rationally developed reactivator of AChE which also displayed antidotal activity in vivo against poisoning by organophosphorus (OP) compounds (2)

Scheme 1 : Molecular structure of 2-PAM and HI-6



1

A: 2-PAM



2

B: HI-6

The concurrent administration of 2-PAM together with atropine to various laboratory animals poisoned by certain OP compounds provides antidotal protection (3). However, certain OP compounds such as pinacolyl methyl phosphonofluoridate (soman) may produce an inhibited AChE intermediate which could not be reactivated by oximes (4). The process by which the level of reactivatable AChE is decreased is called aging and involves dealkylation of the pinacolyl group from the OP-AChE conjugate (5). Rapid aging renders the enzyme resistent to reactivation by oximes and the efficacy of the antidotal treatment following soman intoxication is therefore limited. However, it was noted that HI-6 (Scheme 1B) is the most efficient reactivator of non-aged soman-inhibited human AChE (6). Namely, before dealkylation occurs HI-6 reactivates the inhibited human AChE at the highest rate compared to other reactivators (7). Nevertheless, the antidotal efficacy of HI-6

(together with atropine) against soman poisoning may also be related to other pharmacological activities such as the inhibition of peripheral nicotinic and muscarinic receptors (8-10).

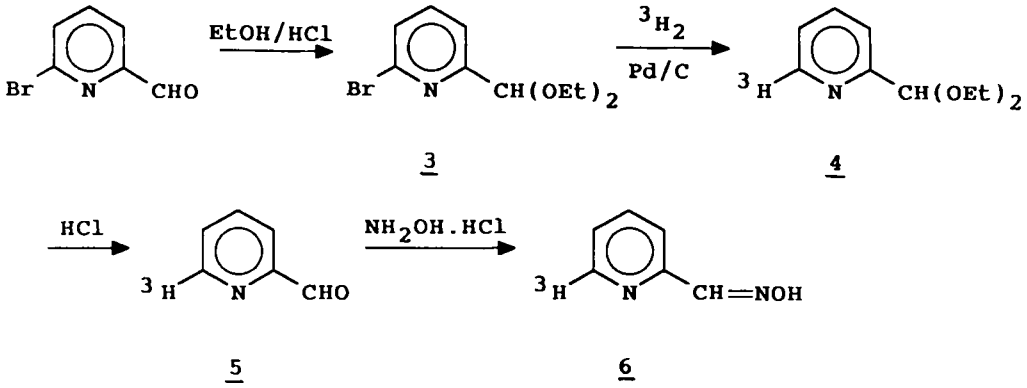
The pharmacokinetics, bioavailability and tissue distribution of quaternary oximes may be monitored by the use of radioactively labelled compounds. In addition, the presence of minute levels of quaternary oximes in the brain is controversial since it was noted that such quaternary compounds could hardly cross the blood-brain barrier (11). Radiolabelled oximes may help to resolve this problem. Carbon-14 labelled 2-PAM and HI-6 were previously prepared and used for studying the pharmacokinetics, metabolic fate and whole-body autoradiography of these drugs (12,13).

This report summarizes the synthesis of [^{3}H]2-PAM and [^{3}H]HI-6 from the unlabelled precursor 6-bromo 2-pyridine aldehyde.
We have developed a new synthetic procedure for the preparation of high specific activity (18-20 Ci/mmole) tritium labelled 2-PAM and HI-6. Such high specific activity may help to detect even low level of quaternary oximes in brain . Furthermore, tritium labelled quaternary oximes may also serve for binding of oximes to various receptors in vitro. Pharmacological data obtained with these radiolabelled oximes were published previously (14).

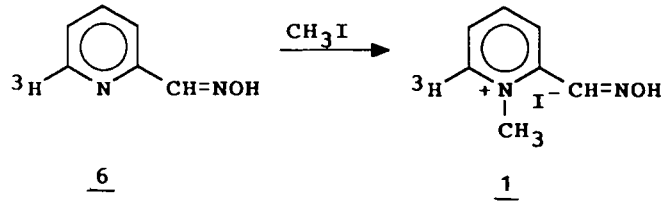
RESULTS AND DISCUSSION

The synthetic route for the preparation of [^{3}H]-2-PAM and [^{3}H]HI-6 consists of the preparation of ring tritiated 2-pyridine-aldoxime according to scheme 2 followed by either methylation with methyliodide (scheme 3) to [^{3}H]-2-PAM or alkylation with bischlorodimethylether to produce [^{3}H]-2-hydroxyiminomethyl pyridinium chloromethoxymethyl chloride which was reacted with isonicotinamide to produce [^{3}H]-HI-6 (scheme 4).

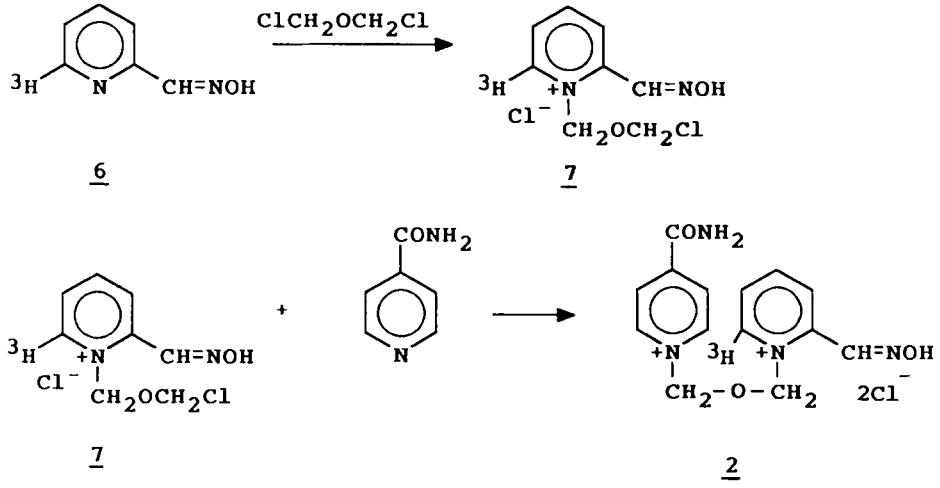
Scheme 2: Synthetic pathway of [^{3}H]-2-pyridine-aldoxime



Scheme 3: Synthetic pathway of [^{3}H]-2 PAM 1



Scheme 4: Synthetic pathway of [^{3}H]-HI-6 2



3 was efficiently reduced with tritium gas in the presence of Pd/C to [^3H]-2-pyridinemethyldiethylketal. Purification of this reaction product by coloumn chromatography on alumina yielded 4 with a radiochemical purity greater than 99%. Acid hydrolysis of 4 yielded the aldehyde 5 which was converted to the oxime 6 by reaction with hydroxylamine. Compound 6 was produced with a high specific activity of 18 Ci/mmole and a radiochemical purity > 95% . The ring tritiated 2-pyridine aldoxime 6 is a common key precursor for the synthesis of various radiolabelled quaternary pyridinium oximes. The required oxime can be produced from 6 by reacting it with the appropriate alkylating agent, as illustrated in schems 3 and 4, for the synthesis of 2-PAM and HI-6, respectively. 2-PAM was obtained by reacting 6 with methyliodide. It was obtained as a solid yellow compound. Its radiochemical purity was >98% as determined by UV absorption at 336 nm. The absorption at 336 nm of $3.35.10^{-5}$M [^3H]-2-PAM solution in 0.1N NaOH is 0.611 and since the molar absorbance of 2-PAM under the same conditions is $1.85.10^4$ $M^{-1}cm^{-1}$ the value obtained for the absorption of [^3H]-2-PAM corresponds to 98 ± 1% radiochemical purity. The specific activity was 18 Ci/mmole.

The synthesis of [^3H]-HI-6 consists of the reaction of 6 with 1,1'- bischlorodimethylether to produce 7 , which was obtained as a white solid and a subsequent reactions of 7 with isonicotinamide to yield [^3H]-HI-6. The absorption at 335 nm of 5.10^{-4} solution of [^3H]-HI-6 in 0.1 N NaOH is 0.476 and since the molar absorbance of pure HI-6 under the same conditions is $1.13.10^4 M^{-1}cm^{-1}$, the chemical purity of [^3H]-HI-6 is 84.1 ±1%. This value corresponds very well with the ^1H nmr spectrum of the labelled compound which shows the presence of ca. 15% bis - isonicotinamide (1,1' - dimethyl ether) dichloride, 8. However, according to our synthetic route compound 8 could not be radiolabelled at all. Therefore, the radiochemical purity of [^3H]-HI-6 is > 95%. The reactivation potency of [^3H]-HI-6 was measured with purified electric eel

acetylcholinesterase (AChE) inhibited by diethylphosphorochloridate (DEPC). The kinetic pattern obtained for reactivation of DEPC AChE by [^{3}H]-HI-6 is practically identical to that of pure HI-6 (not shown). Compound 8 is indeed a reasonable by-product in this reaction according to the experimental conditions (see Experimental part). In the reaction to produce 7, two fold excess of 1,1' - bischlorodimethylether was used and probably 10% - 15% of the ether remained occluded in the solid even after repeated wash. In the following step to produce 2, excess of isonicotinamide was used to assure the completion of the reaction and it is conceivable that it reacted with the residual 1,1'- bischlorodimethylether to produce 8.

Compound 8 is not radiolabelled and has no reactivation potency activity. Furtheremore, both oximes were diluted 1:100 - 1:250 by unlabelled oximes to yield a lower specific activity before injection to animals. Thus, the diluted radioactive solution of HI-6 which was used for the in vivo autoradiography (14) contains eventually only traces of the unlabelled by-product 8.

^{1}H nmr spectrum of the labelled compound 2 shows also that the position of labelling is regiospecific based on the following nmr data. In the unlabelled compound, proton 6 of the pyridinealdoxime ring appears as a doublet at 9.05 ppm, and in the the tritiated compound this signal is significantly reduced to about 10-15% of its initial intensity in the unlabelled compound (not shown).

EXPERIMENTAL

Chemical and radiochemical purity measurments were determined by the following methods:

1) Thin layer chromatography (TLC) on either silica gel (60-F-254) or alumina plates (60-F-254, neutral type E) from Merck. Visualization was carried out by UV lamp and radiation scanner LB-2722 (Berthold, FRG).

2) Quantitative measurements of the concentration of the desired compound was performed by UV spectra, and comparison of the obtained

spectra to spectra of pure unlabelled standards . These measurements were carried out on a varian 200 double beam spectrophotometer.

3) ^1H nmr spectra were obtained by using a Brucker 250 MHz .

Radioactivity measurments were made in a liquid scintillation spectrometer (Tri-Carl-460-C Packard).

The synthesis of 6-Bromo-2-formylaldehyde was performed by modification of a procedure described previously for the preparation of 5-bromo-2-formyl aldehyde. The labelling with tritium gas and the consecutive synthetic steps with tritium labelled compounds were carried out at the Nuclear Research Center - Negev, Beer-Sheva, Israel.

6 - bromo - 2 - picoline:

6 - Amino 2-picoline (50g) was dissolved in 150 ml of 48% HBr. The stirred solution was cooled to 0°C and 34.5 g of $NaNO_2$ in 62.5 ml of water were added dropwise, and the temperature was kept at 0°C by adding ice. The cold diazonium salt was added dropwise into a suspension of 40 gr of CuBr in 32 ml of HBr warmed to 80°C. When the addition was complete the reaction mixture was cooled, basified with NaOH and steam distilled. The product was purified on a silica-gel column eluted with a mixture of ether hexane, 1:1 (15g).

6 - Bromo - 2 - picoline - N - oxide.

To 7.7 gr. of 6 - bromo - 2 - picoline in 50 ml of dichloromethane were added 10.2 gr of m - chloroperbenzoic acid in 100 ml of dichloromethane .

After 24 hr the reaction mixture was filtered. A solution of saturated K_2CO_3 was added and the product was extracted. This product was purified on a silica gel column eluted with ethylacetate.

6 - Bromo - 2 - Pyridylmethanol acetate

7 gr. of 6 - Bromo - 2 - picoline - N - oxide were added slowly with stirring to 100 ml of acetic anhydride at 100-120°C.

After the exothermic reaction subsided, the dark reaction mixture was stirred and refluxed for 0.5 - 1.0 hr. Ethanol was cautiously added untill the excess of acetic anhydride was converted to ethyl acetate and acetic acid. The resulted solution was cooled and neutralized with $KHCO_3$ solution. The organic layer was extracted with CH_2Cl_2. The product was analyzed by TLC on silica gel plates hexane : CH_2Cl_2 1:1 .

6 - Bromo - 2 - pyridylmethanol.

Concentrated HCl (50 ml) was added to the 6 - bromo - 2 - pyridylmethanol acetate obtained in the previous step and refluxed for 1 hr. The solution was evaporated to dryness under reduced pressure to give the HCl salt of 6 - bromo - 2 - pyridylmethanol. It was neutralized with $KHCO_3$ solution and the product was purified by chromatography on a silica gel column eluted with ether: hexane 1:1 (2.2 gr).

6 - Bromo - 2 - formylpyridine.

To a solution of 2.2 gr. of 6 - bromo - 2 - pyridylmethanol in 20 ml of chloroform were added 5 gr of MnO_2 and the reaction mixture was stirred and refluxed for 12 hr. Following filtration the MnO_2 cake was extracted several times with boiling chloroform . The extracts were combined, dried (Na_2SO_4) and the chloroform was evaporated to yield 6-bromo 2-formylpyridine (0.8 gr).

6 - Bromo - 2 - formyldiethylacetal 3

0.8 gr of 6 - Bromo - 2 - formylpyridine were added to 25 ml of Ethanol/HCl and the solution was left for 24 hr. Then it was carefully neutralized with 10 ml of triethylamine, filtered and concentrated

under reduced pressure. The overall yield of this reaction was 0.9 gr of product. Its purity was checked by TLC on alumina plates using a mixture of 10% isopropanol in hexane for elution

Catalytic Reduction of compound 3

100 mg of 3 were dissolved in 2 ml of tetrahydrofuran (THF) and 0.25 ml of triethylamine. To this solution 25 ml of Pd/C were added and hydrogen was added at atmospheric pressure until no starting meterial was left (according to TLC analysis neutral alumina 2% isopropanol:hexane). The reaction mixture was filtered and the catalyst was washed with THF. Evaporation of solvent yielded 60 mg of 2 - formyl - pyridinediethyl acetal 4 , identical with the product obtained by reacting 2- formylpyridine with ethanol/HCl .

Hydrolysis of 4 to 2 - formylpyridine and synthesis of 2 - pyridine - aldoxime

To 60 mg of 2 - formylpyridinediethylacetal were added 1 ml of HCl 2.5N and the solution was warmed to 60 °C for 1 hr.
Water was evaporated and the product was neutralized with a solution of $KHCO_3$. To the neutralized product 1 ml of a solution of 0.5 gr NH_2OHHCl in 2 ml water and 2 ml NaOH 10% were added. The reaction mixture was warmed for 10 min. to 60°C and when cooled the product precipitated. The oxime was extracted with diethyl ether. The etheral solution was dried and evaporation of the ether left 20 mg of product. TLC was performed on silica gel ether : hexane, 1:1.

Synthesis of 2 - PAM. 1

To 26 mg of 2 - pyridine aldoxime in 1 ml of acetonitrile were added 0.5 ml of methyl iodide and the solution was warmed to

40°C for 4 hr in a sealed tube. After cooling to room temperature, 2-PAM was precipitated, the solvent was removed and the precipitate was washed several times with ether and dried under reduced pressure to yield 50 mg product.

Synthesis of 2-hydroxyiminomethyl-1-chloromethoxymethyl pyridinium chloride 7

To 20 mg of 2 - pyridine aldoxime 3 in 0.5 ml of $CHCl_3$ at 40°C were added 0.1 ml of a solution of 1 gr. of $ClCH_2OCH_2Cl$ in 4.2 ml of $CHCl_3$ (ca. 20 mg of $ClCH_2OCH_2Cl$). The reaction mixture was stirred for 5 hr at 40°C and then left overnight at room temperature. The solvent was removed and the product (white solid) was washed several times with ether and dried under reduced pressure over P_2O_5. The yield was 40 mg, mp. 138 - 143°C with decomposition. ^1H nmr (D_2O) δ ppm: 9.06(d, 1HAr); 8.7(s,1H, CH = NOH); 8.65(t, 1H, Ar); 8.5(d, 1H, Ar); 8.08(t, 1H, Ar); 6.32(s,2H,N-CH_2-O); 5.66(s, 2H, OCH_2Cl).

Synthesis of HI - 6

To a solution of 40 mg of isonicotinamide in 2 ml of acetonitrile at 50 - 60°C were added 40 mg of 7 and the reaction mixture was stirred for 12 hours at 50 - 60°C. After cooling the solvent was removed and the solid was washed with acetone and hexane. Crystalization from isopropanol/ water yielded 40 mg of product mp. 145°-147°C. nmr: $^1H(D_2O)$ ppm: 9.2 (d, 2H, Ar); 9.05 (d, 1H, Ar) ; 8.68 (s t, 3H, Ar); 8.49 (d, 1H, Ar); 8.47 (d, 2H, Ar); 8.14 (t, 1H, Ar); 6.45 (s, 2H, N-CH_2O).

Analysis C, H, N, Cl

Theoretical C=44.68% ; H = 4.78%; N = 14.89%; Cl = 18.61% experimental (=44.98%); H = 4.94 ; N = 14.82 ; Cl = 18.67%

Reduction of 3 with tritium

synthesis of [^{3}H] - 2 - pyridineformyldiethylacetal 4

6-Bromo-2- pyridylformyldiethylacetal (150 mg 0.6 mmole) was dissolved in 1 ml of dry THF and 0.5 ml of dry triethylamine, to this we added 30 mg of 10% Pd/C and 50 curies of tritium gas. The reaction mixture was stirred for 90 min. at room temperature until uptake of tritium ceased (32 curies). Labile tritium was removed under reduced pressure (30 mmHg) using methanol as solvent. 15-20 ml of CH_2Cl_2 were added, the catalyst was removed by filtration and the solvent removed at 30 mmHg to yield crude ring tritiated 2 - pyridylformyldiethylacetal ([^{3}H] - 3).

This product was purified on a short column of alumina with 2% isopropanol : hexane. After evaporation of the solvent 5 curies of 3 were obtained with radiochemical purity of >99% as determined by TLC on alumina plates 2% isopropanol : hexane and radioscanning the corresponding TLC plates.

[^{3}H] - 2 - Pyridinealdoxime

1 ml of HCl 2.5 N was added to the [^{3}H] - 3 obtained previously and the resulting solution was heated to 60°C for 1.5 hr. Then water was evaporated under reduced pressure. The residue was neutralized with 10% $KHCO_3$ and reacted with 1 ml of a solution containing 0.5 g of $NH_2OH.HCl$ in 2 ml H_2O and 2 ml 10% NaOH.

The reaction mixture was heated to 60°C for 10 min. After cooling the product precipitated and was extracted with ether. The etheral solution was dried over Na_2SO_4 and evaporated to yield 17 mg of product with total activity of 2.5 Curies. TLC analysis on silica-gel plates hexane: ether (1:1) showed the product to be identical with authentic unlabelled 2 - pyridine-aldoxime. Radioscanning of the chromatographic plate showed the product to be 95% radio-pure. The specific activity was 18 Ci/mmole.

$[^3H]$ - 2 - PAM 1

$[^3H]$ - 2 - pyridinealdoxime (17 mg) were dissolved in 1 ml of dry acetonitrile, 0.5 ml of methyliodide was added and the solution was warmed to 40°C for 4 hr. to yield $[^3H]$ - 1 which was produced as a yellow precipitate. After cooling, the mother liquor was removed and the solid was washed several times with ether until no starting material could be detected by TLC and visualization of the plates by uv or radioscanning. After drying 20 mg of product were obtained having a total activity of 1.4 Curies. The radiochemical purity was determined by uv spectra and was found 98%. The specific activity was 18 Ci/mmole.

Synthesis of $[^3H]$- 6

$[^3H]$-2- pyridinealdoxime ie the starting material which was synthesized by the same sequence of reactions as described for the synthesis of $[^3H]$-2- PAM. Reduction of (3) 156 mg (0.6 mmmole) yielded after purification by column chromatography 105 mg of 4 (15 Curies). Hydrolysis of 4 to 5 and oximation yielded 63 mg of $[^3H]$-6 (15 curies) having specific activity of 28 Ci/mmole. The radiochemical purity was >95% .

Synthesis of $[^3H]$- 7

To 36 mg of $[^3H]$-2- pyridinealdoxime in 1 ml of acetonitrile 0.4 ml of a solution of 1 gr of 1,1 dichlorodimethyl ether in 5 ml of $CHCl_3$ were added. The reaction was left at 40°C for 5 hr. and then 12 hr. at room temperature. The product was obtained as a white solid. The mother liquor was removed and the precipitate was washed several times with ether. After drying 77 mg of 7 were obtained.

Synthesis of $[^3H]$- 2

To 77 mg of $[^3H]$ - 7 in 1 ml of dry acetonitrile a solution of 77 mg of isonicotinamide in 2 ml acetonitrile was added. The reaction

mixture was warmed at 50 °C for 12 hr. After cooling the mother liquor was removed and the precipitate was washed several times with acetone and petroleum-ether.

The product was recrystallyzed from water - isopropanol. 35 mg of HI-6 (1.8 Curies) were obtained as white powder after drying. The chemical purity was 85% as determined by uv and nmr and specific activity was 22 Ci/mmole.

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