THE SYNTHESIS OF (^{3}H)-XYLAMINE,

AN IRREVERSIBLE INHIBITOR OF NOREPINEPHRINE UPTAKE

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SUMMARY

Xylamine [N-(2-chloroethyl)-N-ethyl-2-methylbenzylamine], an irreversible inhibitor of neuronal norepinephrine uptake, has been synthesized with tritium incorporated in the aromatic nucleus. Initially, 2-methylbenzyl bromide was nonselectively mono-brominated on the ring and the resulting isomeric products were condensed with 2-(ethylamino)ethanol. Unequivocal identification of the products of each reaction was provided by mass spectral analysis. A mixture of mono-bromobenzylamine isomers was isolated for catalytic reduction with ^{3}H$_2$ at a commercial facility. Carrier N-(2-hydroxyethyl)-N-ethyl-2-methylbenzylamine was added to the crude tritiated product prior to refluxing in the presence of excess thionyl chloride. (^{3}H)-Xylamine was obtained as the hydrochloride salt and repeated recrystallizations yielded a radiochemically pure product with a specific activity of 3.09 Ci/mmol. Several analytical procedures established that the radiolabel was associated with xylamine.

KEY WORDS: (^{3}H)-2-chlorethylamines, norepinephrine uptake inhibitors, bromo-
            benzylamine reduction.

INTRODUCTION

Xylamine is a selective irreversible inhibitor of norepinephrine (NE) up-take into central and peripheral noradrenergic neurons (1,2,3). The azirid-

inium ion formed by the cyclization of xylamine in aqueous solution has been shown to be responsible for the observed inhibition of uptake and long-term blockade most likely results from alkylation of a nucleophile associated with the NE carrier (4) (Fig. 1). The selective nature of this action is probably due to a high binding affinity of the carrier for the aziridinium ion. The ion is a structural analogue of bretylium, an adrenergic neuron blocking agent that is a substrate for the NE transport system. The covalent modification of the NE carrier has generated much interest in obtaining radiolabeled xylamine. Certainly, a specific, nondiffusable marker for NE uptake sites would have several important applications. Radiolabeled, irreversible ligands have been successfully employed in the quantitation, localization, and isolation of other pharmacologically important receptors (5,6,7).

This reports describes a convenient synthetic procedure for preparing a ring-brominated 2-hydroxyethylamine (3) precursor of xylamine which can be catalytically reduced with $^3H_2$ and subsequently converted to the 2-chloroethyl-amine. A feature of this synthetic method is that it can be readily adapted to yield (^3H)-xylamine with a specific activity much higher than that reported here. Currently, the specificity of binding of (^3H)-xylamine to the NE uptake carrier is being examined in this laboratory.

MATERIALS AND ANALYTICAL METHODS

2-Methylbenzylbromide, bromine, 2-(ethylamino)ethanol, and thionyl chloride were purchased from Aldrich Chemical Co., Milwaukee, WI. Silica Gel 60 was from MCB Manufacturing Chemists, Cincinnati, OH. Analytical (0.25 mm thickness) precoated silica gel and alumina thin-layer chromatography (TLC) plates were obtained from Merck AG, Darmstadt, Germany. All other chemicals were obtained through common commercial sources. Melting points were determined on a Fisher-Johns apparatus without correction. Elemental analysis of the bromobenzylamine product was performed by Galbraith Labs, Knoxville, TN.

N-(2-Hydroxyethyl)-N-ethyl-2-methylbenzylamine (xylaminol) was prepared as described by Kammerer et al. (1) and the compound was more than 97% pure when
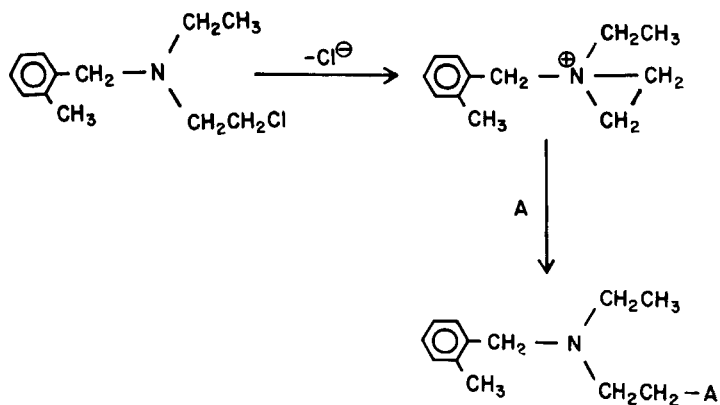
Fig. 1. Formation of the aziridinium ion and its reaction
with a nucleophile (A) that can be a small molecule
(e.g., H_2O) or a site on a tissue macromolecule
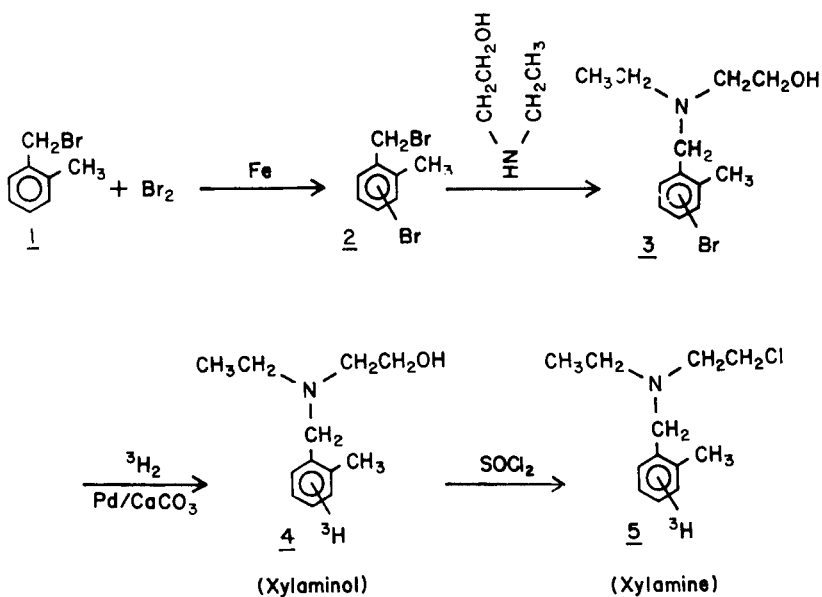(e.g., the NE transport system).

Fig. 2. Outline of the synthetic method for (^{3}H)-xylamine·HCl.

analyzed by gas chromatography.

Gas liquid chromatographic (GC) analyses of reaction components were per-
formed using a Varian 2100 gas chromatograph. A 2m x 2mm silanized glass col-
umn packed with 3% OV 17 (Supelco, Bellefonte, PA) was used for all analyses
with a carrier gas (N_2) flow rate of 30 ml/min. GC-mass spectrometry of reac-
tion products used an Hewlett-Packard 5981A GC-MS system and a column having
dimensions and a stationary phase identical to the above Varian GC column.
Ionization voltage was 20 eV with an ion source temperature of 200°C.

High-performance liquid chromatography (HPLC) of product radiochemical com-
position used an Hewlett-Packard 1080B liquid chromatograph with a 254 mm UV de-
tector. The LC column utilized was an Altex Ultrasphere ODS, 5 micron, 15 cm x
4.6 mm, and the mobile phase was water-methanol (70:30) with a flow rate of
2 ml/min.

UV absorbance measurements were made with a Cary Model 15 spectrophotom-
eter. The 272 nm absorption peak of the benzylamine [$E_{max,272nm}$ (CH_2Cl_2, xyla-
mine HCl) = 619] was used to quantitate the tritiated product. Radioactivity
was determined with a Searle Mark III liquid scintillation counter and Bray's
solution (9) was used as the scintillation fluid.

The reduction of 3 was performed by the Custom Preparations Division of
Amersham Corp. (Arlington, IL). Catalyst and labile tritium removal was done
at the commercial facility.

Radiochemical purity of the final product was evaluated by HPLC (described
above) and radiochromatograms of TLC plates scanned with a Packard Model 7201
Radiochromatogram Scanner. Dry silica gel plates were developed in tetrahydro-
furan and alumina plates were developed in toluene-acetonitrile (90:10).

Bromination of 2-Methylbenzyl Bromide

The aromatic nucleus of 2-methylbenzyl bromide (1) was brominated using
the method of Wisanski and Ansbacher for bromine substitution of o-xylene (10).
A mixture of 2.5g (0.0135 mol) of 1 and 35 mg of clean iron filings was stirred

at room temperature in 150 ml of dry carbon tetrachloride with protection from

light. To this solution, 0.75 ml (0.0135 mol) of bromine in 5 ml of dry CCl_4

was slowly added over 1 hr. The disappearance of 1 was followed by GC (180°C

column temperature) and the reaction was allowed to continue for an additional

hour after the starting material no long declined (4 hr). A single product

peak was observed and it represented approximately 90% of the starting 1.

The solution was washed successively with 100 ml of water followed by

100 ml of sodium sulfite (20%) in water and then filtered through Whatman #1

filter paper. The GCMS (180° column temperature) resolved two products with

identical mass spectra (Fig. 3) and their structures were consistent with that

of 2. Mono-bromination of the ring is indicated by the presence of ion frag-

ments at m/z 183 and m/z 185, resulting from loss of the benzylic bromine. The

three possible isotopic molecular ions are also observed (m/z 262, m/z 264 and

m/z 266), supporting the assignment of the structure indicated by 2.

Brominated Xylaminol

No attempt was made to separate unreacted 1 from 2 and the dried CCl_4 solu-

tion was directly refluxed with 2.7 ml (0.029 mol) of 2-(ethylamino)ethanol.

When 1 and 2 were no longer detectable by GC the solution was washed with water

and dried over sodium sulfate. GCMS (200°C column temperature) of the bromine

containing product resolved four isomeric compounds with identical mass spectra

(Fig. 4). The ion fragments occurring as doublets at m/z 271 and m/z 273 and at

m/z 240 and m/z 242 are diagnostic for the ring brominated xylaminol (3) pro-

duct. Two of the isomers accounted for 80% of the product and were equally

abundant. The remaining 20% of 3 was present as similar proportions of the two

lesser abundant positional isomers.

The nonbrominated congener of 3 (i.e., xylaminol) was now separated from

the desired products. The solvent was removed under reduced pressure and the

residual thick oil was taken up into 2 ml tetrahydrofuran (THF). A portion

equivalent to 0.5 g of the products was applied to a 30 cm x 2 cm silica gel
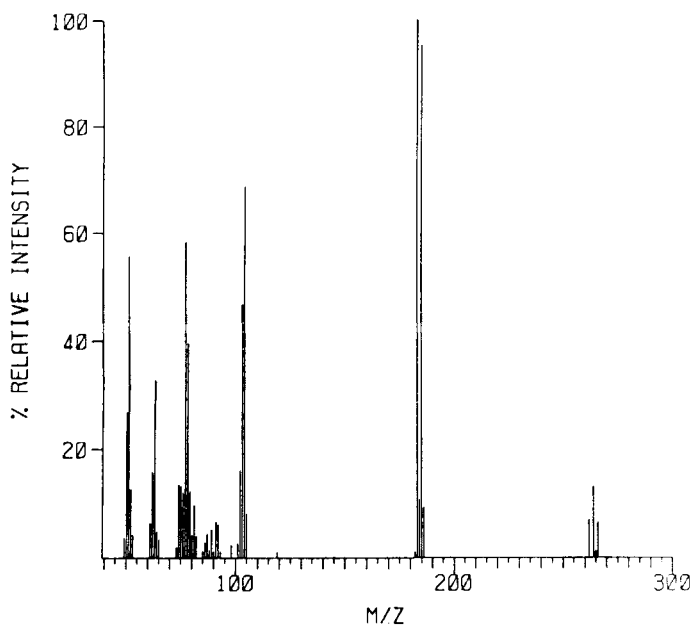
Fig. 3. Mass spectrum of one of the two resolved isomers of 2.
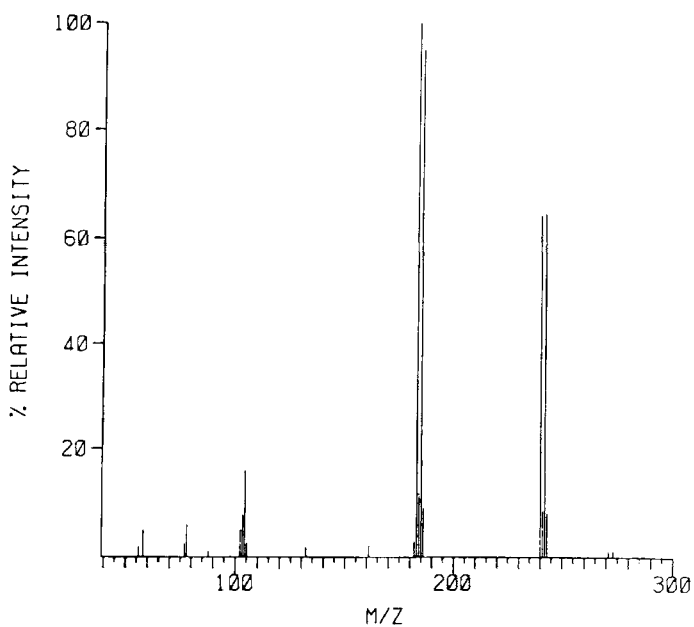

Fig. 4. Mass spectrum of one of the four resolved isomers of 3.

60 column and eluted with THF. Three ml fractions were collected and those ab-
sent of GC detectable xylaminol were combined and the solvent was removed under
vacumn (150 mg yield). The isomeric products could not be crystallized and
after extensive solvent removal the viscous oil was analyzed as the free amine.

Calculated C,H,N,Br: 52.95, 6.67, 5.15, 29.41. Found C,H,N,Br: 53.04,
6.21, 4.88, 30.26 ($C_{12}H_{18}NOBr$).

$^3H_2$ Reduction of $\underline{3}$

The reduction of $\underline{3}$ at the Amersham Corp. facility was performed under con-
ditions for minimizing hydrogenolysis of the benzylamine. Twenty-five mg (0.092
mmol) of $\underline{3}$ in 2.5 ml dioxane was exposed to 25 Ci of $^3H_2$ for 20 hr at room tem-
perature and atmospheric pressure in the presence of 25 mg of 1070 Pd/CaCO$_3$ and
50 µl of triethylamine. The catalyst and labile tritium were removed and the
acid extractable labeled material was returned to this laboratory in ethanol
(1.87 Ci total). HPLC analysis of this crude material indicated that only 60%
of the radioactivity co-chromatographed with authentic xylaminol. Efforts to
remove the contaminating tritium were not successful and it was decided to
achieve radiopurity at the expense of product specific activity. This approach
involved the addition of carrier xylaminol to provide sufficient material for
crystallization of the (^3H)-xylamine product and its purification through suc-
cessive recrystallizations.

(^3H)-Xylamine·HCl

The ethanol was removed from half of the crude tritiated product at 40°C
using a water aspirator. Thirty mg of carrier xylaminol was added to the resi-
due in 25 ml of dry dichloromethane (CH$_2$Cl$_2$). A 5-fold molar excess (0.07 mmol)
of thionyl chloride was added and the solution was refluxed (55°C) for 4 hr.
The solvent was removed using a water aspirator and the residue was taken up
into 10 ml of cold water. The water was alkalinized in the presence of 20 ml of
diethylether, and the ether layer was separated and dried over sodium sulfate.
After filtration, hydrochloric acid saturated dry ether was added dropwise to

yield a crystalline product. The crystals were filtered, washed with 50 ml of
dry ether, and dried to yield 27 mg of product (3.23 Ci/mmol). Three recrystal-
lizations from ethanol/ether produced a constant specific activity product (20
mg, 3.09 Ci/mmol). The product melting point was 147-148°C, identical to that
of authentic xylamine·HCl (1). Thin-layer chromatography on both silica gel and
alumina plates indicated that 100% of the radioactivity chromatographed with
xylamine·HCl (Fig. 5). Reverse isotope dilution analysis showed that the label
consistently extracted with xylamine·HCl without change in its specific activ-
ity. HPLC of the labeled product in the presence of detectable carrier xyla-
mine·HCl showed greater than 97% of the tritium chromatographed with the car-
rier. Also, alkaline hydrolysis of the labeled product produced a radiochemi-
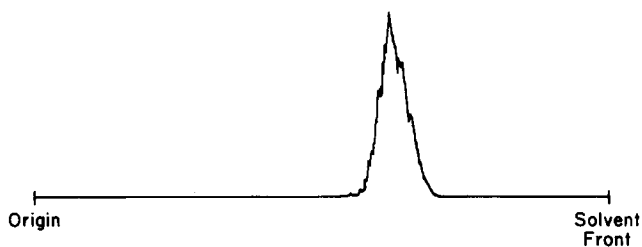cally pure product that behaved analytically as xylaminol.

Origin   Solvent
                                                                Front

Fig. 5.   Radiochromatogram of (^{3}H)-xylamine·HCl on a silica
           gel TLC plate developed in THF. The peak of radio-
           activity co-chromatographed with unlabeled xyla-
           mine·HCl (R_f = 0.65).

Stock solutions of the final recrystallized product were prepared in etha-
nol:0.005N HCl at 0.5 mM and 1.0 mM concentrations and stored at -80°C. After
6 months there was no detectable radiodicomposition or chemical decomposition of
the (^{3}H)-xylamine·HCl at either concentration.

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