Synthesis of Tritium Labeled 1-Methyl-4-[4-(7-trifluoromethyl-4-quinolyl)-aminobenzoyl]piperazine and Derivatives

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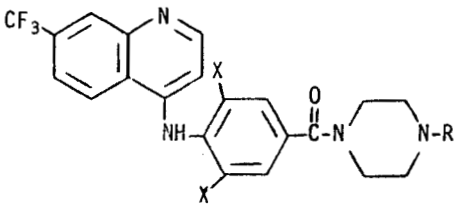
SUMMARY

Deuteration of p-aminobenzoic acid hydrochloride (PAB·HCl) was studied to provide a model for tritiating PAB·HCl. Tritium labeled PAB·HCl was used as the starting material for the synthesis of tritium labeled 1-methyl-4-[4-(7-trifluoromethyl-4-quinolyl)aminobenzoyl]piperazine (Ic) and the corresponding 1-demethyl compound (Id) and 1-oxide (II).

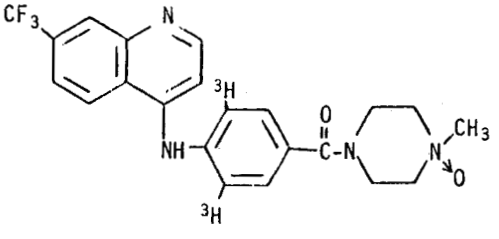
Key Words: Synthesis, Tritium, 4-Aminoquinolines,Exchange, Deuterium, p-Aminobenzoic Acid

INTRODUCTION

A number of substituted 4-aminoquinolines exhibit hypotensive activity (1-4). One such compound, 1-methyl-4-[4-(7-trifluoromethyl-4-quinolyl)aminobenzoyl] piperazine (Ia), has been found to affect the central nervous system and cardiovascular system in test animals. This report describes the synthesis of tritium



I a) X = H, R = CH_3
b) X = ^2H, R = CH_3
c) X = ^3H, R = CH_3
d) X = ^3H, R = H

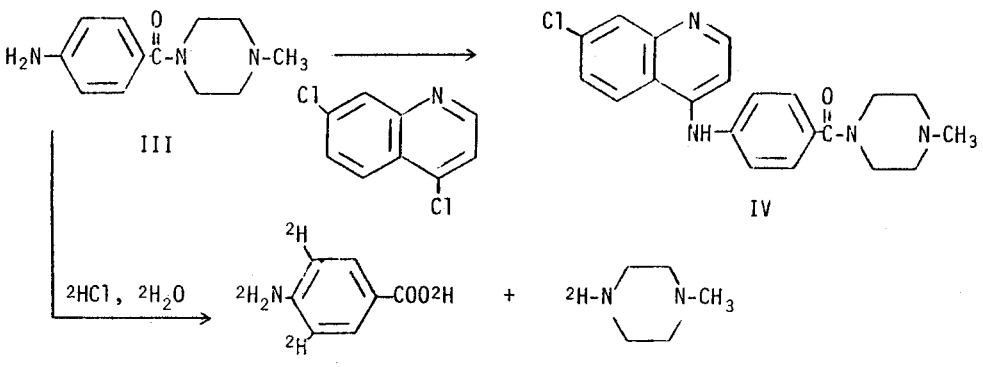


II

labeled Ic and its corresponding 1-demethyl (Id) and 1-oxide (II) derivatives for conducting absorption, biotransformation, and excretion studies with these compounds.

The *p*-aminobenzoic acid portion of the molecular structure of Ia was chosen as a suitable and convenient region to incorporate tritium labels for two reasons. First, any metabolic or chemical cleavage of the amide linkage in Ic would still leave the major fragment of the molecule, *i.e.*, 4-(7-trifluoromethyl-4-quinolyl)aminobenzoic acid (VIb), bearing the tritium labels and therefore detectable by radioactivity. Secondly, the two protiums in the phenyl ring *ortho* to the amino group could be expected to be exchangeable under acid catalysis with deuterium and tritium, thus offering two convenient sites for introducing labels at some appropriate intermediate stage in the synthetic path. Because specific labeling at known positions was desired, direct incorporation of tritium into the intact molecule of Ia was not attempted, since several positions in the quinoline ring system could also be expected to be subject to protiumtritium exchange. The preparation (Scheme 1) of 1-methyl-4-[4-(7-chloro-4-quinolyl)aminobenzoyl]piperazine (IV) from 1-methyl-4-(4-aminobenzoyl)piperazine (5) (III) has been described (6). In an attempt to synthesize Ic in an analogous manner, deuteration of III in 6N ^{2}HCl in ^2H_2O was investigated as a model for tritiating III.

Scheme 1



+ deuterated III

It was found that incorporation of deuterium into III was accompanied by the hydrolysis of III to PAB and piperazine. Therefore, an alternative synthesis for Ic, using tritium labeled PAB·HCl (Vb) as starting material, was devised. Preparation of Vb was modeled after deuterium labeling of PAB·HCl.

Deuteration of PAB·HCl in the phenyl ring at the two positions *ortho* to the amino group occurred readily in 3N ^{2}HCl in ^2H_2O at 90°C. The exchange reaction

was observed by means of nuclear magnetic resonance (nmr) spectroscopy, using tetramethylsilane as the standard for chemical shifts. As the reaction progressed, the upfield doublet of doublets in the aromatic region at 476 Hz (J = 9, 2.5 Hz), attributable to hydrogens *ortho* to the amino group, diminished in intensity as the downfield doublet of doublets at 505 Hz (J = 9, 2.5 Hz) due to the *meta* hydrogens collapsed into a singlet at 505 Hz. Constancy of the composite intensity of the downfield signals for the duration of the experiment confirmed the expectation that deuteration would not occur at the *meta* positions. The validity of the use of the composite integral of the downfield aromatic hydrogen signals, as a standard for measuring the extent of deuteration, was ascertained by adding dioxane to the reaction mixture as a second standard. The ratio of the integral of the *meta* hydrogens to that of the dioxane singlet remained constant for 24 hours under the reaction conditions. From a semi-log plot of the extent of *ortho* deuteration *vs.* time (Figure 1), the pseudo first-order deuteration

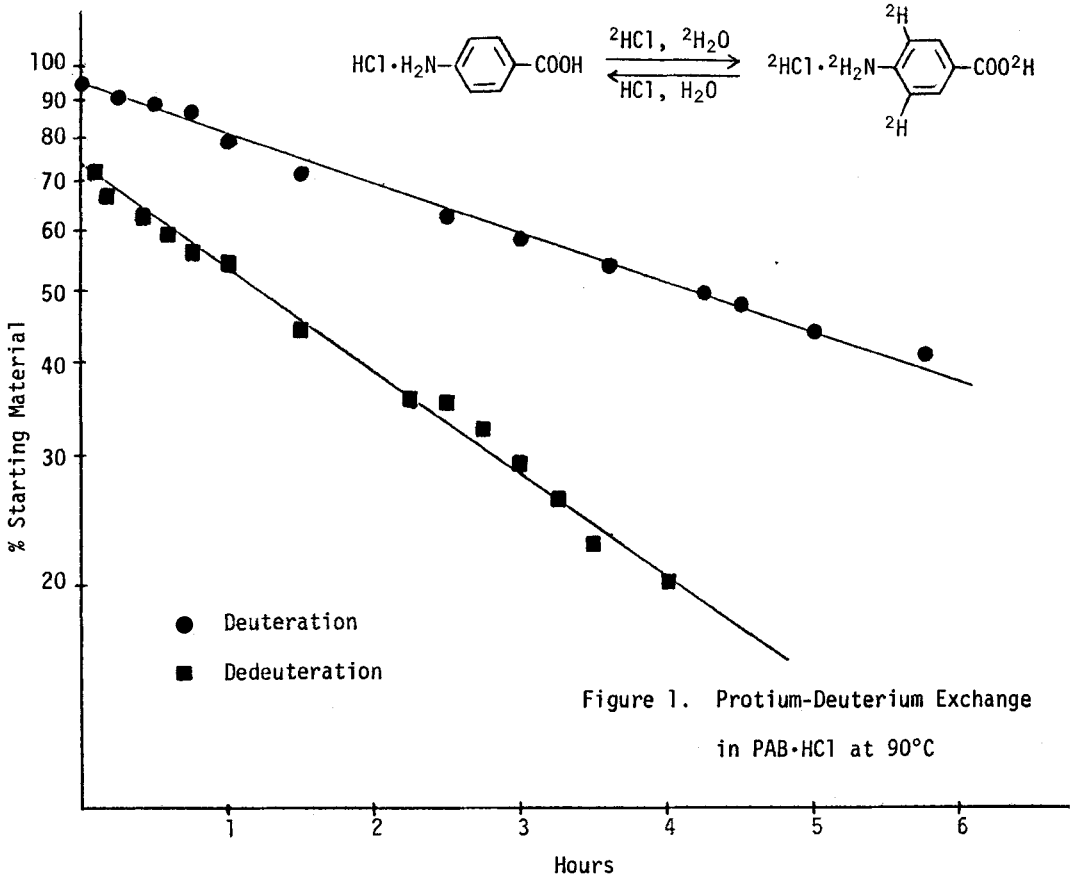
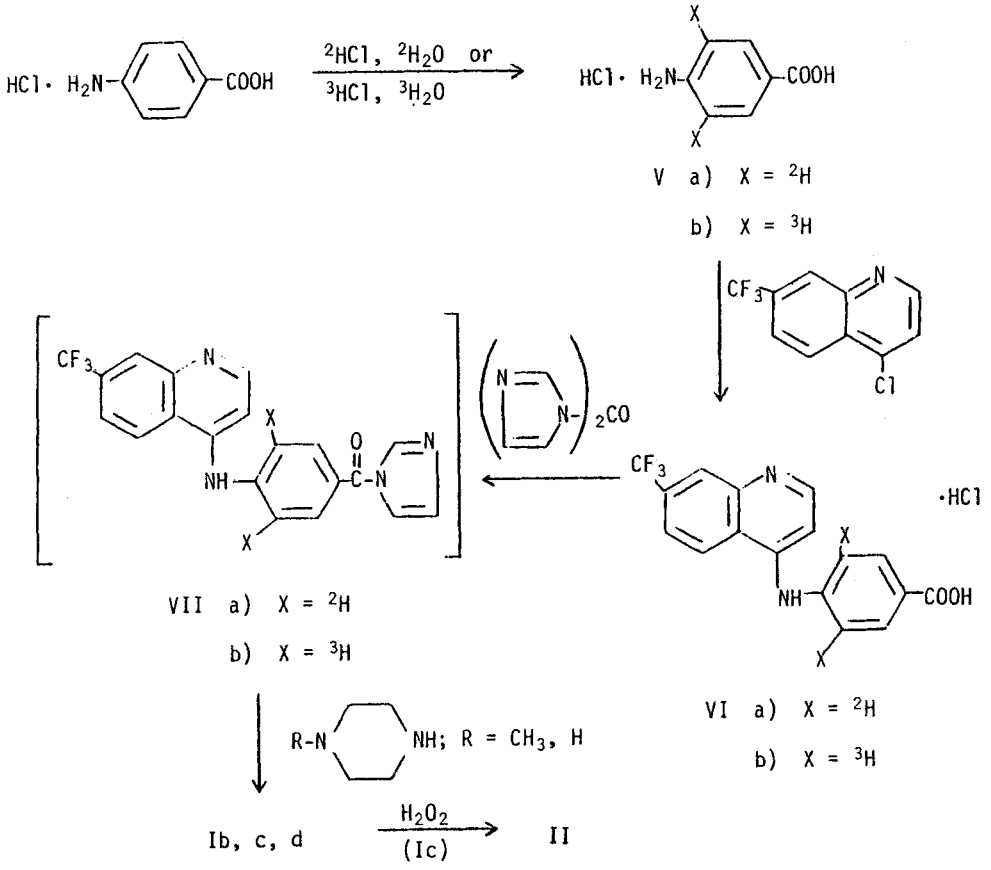


Figure 1. Protium-Deuterium Exchange in PAB·HCl at 90°C

half-time was found to be 4.4 hours. By studying the removal of the labels from deuterated PAB·HCl (Va) at 90°C in 3N HCl in H_2O, the dedeuteration half-time was similarly determined and found to be 2.2 hours.

The more facile loss of deuterium labels from PAB·HCl than their incorporation prompted an assessment of the stability of the labels in Ib. Compound Va was treated with 4-chloro-7-trifluoromethylquinoline to give the deuterated acid VIa, which was converted *via* its imidazolide VIIa to the amide Ib (Scheme 2). Comparison of the nmr spectra of Va and Ib showed that no loss of deuterium had occurred during the transformations. An aqueous solution of Va at pH 1.45 and

Scheme 2



40°C, similar to physiological conditions in the stomach, was monitored by nmr for 21 hours, and no detectable loss of deuterium was observed. In Ib, the presence of the quinolyl group, as expected, increased the chemical stability of the

labels by reducing the electron density on the aromatic amino nitrogen. All the above findings would indicate that the phenyl ring positions *ortho* to the amino group in the *p*-aminobenzoic acid portion of the Ia molecule would be satisfactory sites for tritium labeling.

Tritiation of PAB·HCl was carried out in 3N HCl in tritiated water at 90°C for 36 hours (∿8 deuteration half-time) with excellent incorporation of tritium. The conversion of tritiated PAB·HCl (Vb) to Ic *via* VIb and VIIb paralleled the synthesis of Ib from Va as shown in Scheme 2. Treatment of VIIb with piperazine, instead of 1-methylpiperazine, afforded the 1-demethyl compound Id. Oxidation of Ic with hydrogen peroxide produced the N-oxide II.

EXPERIMENTAL

Radioactivity determinations were carried out with a Packard Tri-Carb Model 2425 liquid scintillation spectrometer, using Diotol scintillation solvent and the external standard method. Nmr studies were carried out with a Varian A60A instrument. Thin layer chromatography (tlc) analyses were performed on 2.5 x 10 cm glass plates coated with 250 μm thick layer of silica gel GF (Analtech). Visualization of developed zones was by uv illumination (254 nm). Radioactive zones were detected by means of a Vanguard Model 880 Autoscanner equipped with a Model 885 Glass Plate Scanner. Uv spectra were obtained with a Cary Model 15 spectrometer. Melting points were determined in capillary tubes and were uncorrected. Microanalyses were obtained for the indicated elements, and the results were all within ±0.4% of theory.

Deuteration of PAB·HCl

A 0.526 M solution of PAB·HCl in ^2HCl-^2H_2O was prepared by warming a mixture of 137 mg of the salt and 1.5 ml of 3 N ^2HCl in ^2H_2O. A portion of the solution was placed in the nmr probe preheated to 90°C. The aromatic region of the spectrum (550-400 Hz) was scanned and integrated at intervals. The downfield doublet of doublets at 505 Hz (J = 9, 2.5 Hz) attributed to the 2- and 6-hydrogens *ortho* to the carboxyl group gradually coalesced into a singlet at 505 Hz as deuteration progressed at the 3- and 5-positions. The composite integral of

these downfield signals served as a standard. In a separate experiment where dioxane was added as a second standard, the ratio of this composite integral to that of the dioxane singlet remained constant at 90°C for 24 hrs. The extent of deuteration at a given time was calculated from the ratio of the diminishing upfield 3,5-hydrogen doublet of doublets at 476 Hz (J = 9, 2.5 Hz) to the downfield composite signals. Plotting the percentage of the remaining 3,5-hydrogens against time on semilog paper gave a straight line. From the slope of the plot a reaction half-time of 4.4 hrs was calculated (Figure 1).

Deuteration of PAB was also carried out on a preparative scale to provide material for stability studies. A mixture of 2.743 g (20 mmoles) of the free acid and 20 ml of 3 N ^2HCl-^2H_2O was heated at 90°C with stirring for 24 hrs. The mixture was evaporated at reduced pressure to dryness and the residual solids were triturated with Me_2CO. The crystals were filtered, washed with Me_2CO followed by Et_2O, and dried to give 3.292 g (91.6% yield) of 4-amino-3,5-dideuterobenzoic acid deuterochloride. Nmr analysis of this material showed it was 81.4% deuterated in the 3- and 5-positions. This material was used in all the dedeuteration and synthetic experiments which involved the deuterium label described below.

Dedeuteration of PAB-3,5-$^2H\cdot^2HCl$

A portion of a 0.5 M solution of 137 mg (0.765 mmole) of PAB-3,5-$^2H\cdot^2HCl$ in 1.5 ml of 3 N HCl in H_2O was heated at 90°C in the nmr probe. The back-exchange was observed by periodic scanning and integration of the aromatic region to note the increase in 3,5-H. The procedure and data treatment were analogous to those used in the deuteration experiment described above. The time *vs.* % 3,5-^2H (100-% 3,5-H) plot gave a dedeuteration half-time of 2.2 hrs (Figure 1).

Stability of Deuterium Labels

A. Stability of PAB-3,5-$^2H\cdot^2HCl$ at pH 1.45

The stability of the deuterium label in PAB-3,5-$^2H\cdot^2HCl$ was tested by observing the nmr spectrum of an aqueous solution of 100 mg of the material in 1 ml of H_2O. The unbuffered solution, pH 1.45, was kept at 40°C. After 21 hrs, no loss of deuterium from the 3- and 5-positions was observed.

B. Preparation of Ib

A mixture of 1.216 g (5.25 mmoles) of 4-chloro-7-trifluoromethyl-quinoline* and 868 mg (5.0 mmoles) of PAB-3,5-^{2}H·^{2}HCl in 20 ml of abs EtOH was stirred under N_2 at RT overnight. The bright yellow crystalline precipitates were filtered, washed with H_2O, EtOH and Et_2O in that order and dried to give VIa in quantitative yield. Compound VIa (1.106 g, 3.0 mmoles) was dissolved in 30 ml of hot dimethyl sulfoxide (DMSO). The solution was cooled to RT and 0.973 g (6 mmoles) of 1,1'-carbonyldiimidazole (CDI) was added. The mixture was stirred at RT for 2.5 hrs under N_2 and 1.0 ml (0.900 g, 9 mmoles) of 1-methylpiperazine was added. After the solution was stirred for another 2.5 hrs, it was poured into 60 ml of H_2O. The resulting slurry was kept in a refrigerator overnight. The solids were filtered, washed with H_2O and dried, 1.134 g, 91.2% yield. This material was chromatographed on a 3 x 70 cm column of silica gel (200 g) eluted with 6:1 v/v $CHCl_3$-CH_3OH. The eluate was collected in 15 ml fractions at the rate of 2.5 min per fraction. The residue from fractions 42-90 was dissolved in 15 ml of $CHCl_3$, filtered and the filtrate was mixed with 20 ml of PhH. The solution was boiled until the volume was reduced to 20 ml. The remaining mixture was cooled to give 929 mg (82.0% yield) of Ib, mp 216-7°C. The nmr spectrum of this material showed that no loss of aromatic deuterium had occurred during the reaction and workup procedures leading from PAB-3,5-^{2}H·^{2}HCl to Ib.

PAB-3,5-^{3}H·HCl (Vb)

A mixture of 348 mg (2 mmoles) of PAB·HCl and 1 ml of 3 N HCl containing nominally 25 Ci of tritiated H_2O was heated with stirring at 90°C for 36 hrs. The mixture was cooled and water was added to dissolve all solids. The solution was freeze-dried and the residue was twice redissolved in 10 ml of H_2O and freeze-dried to remove labile tritium** The crude material (287 mg) was added to a solution of 350 mg of non-labeled PAB·HCl in 15 ml of MeOH. The solution was treated with activated charcoal (Darco G 60), filtered and the filtrate was mixed

*Kindly supplied by Dr. C. E. Coverdale, prepared according to the procedure of Snyder, *et al.* (7).

**Tritiation of PAB HCl and removal of labile tritium was carried out by New England Nuclear Corp., Boston, Mass.

with 35 ml of Et_2O and dried to afford 500 mg of crystals, sp act 1.302 mCi/mg. Tlc (3:1 v/v $CHCl_3$-MeOH) analysis of this material showed, in addition to Vb, presence of 8.3% (by radioactivity) of the methyl ester* of Vb, evidently the result of partial esterification of the acid Vb during recrystallization from MeOH. Since Vb methyl ester would not interfere adversely with the desired reactions in the subsequent steps, the crude Vb was used without further purification.

Compound VIb

A mixture of 87 mg (0.5 mmole) of Vb and 174 mg (1.0 mmole) of non-labeled PAB·HCl was dissolved in 4.5 ml of 80% EtOH. To the solution was added 365 mg (1.575 mmole) of 4-chloro-7-trifluoromethylquinoline. After being stirred at RT for 5 min, the clear solution became a bright yellow solid mass. The mixture was kept at RT overnight and the yellow crystals were filtered, washed with ∿7 ml of EtOH followed by Et_2O and dried to give 542 mg of VIb, sp act 192.9 μCi/mg. Tlc (3:1 v/v $CHCl_3$-MeOH) analysis showed in addition to VIb the presence of 8.2% (by radioactivity) of the methyl ester** of VIb. This crude material was used without further purification in the preparation of Ic.

Compound Ic

1,1'-carbonyldiimidazole (405 mg, 2.50 mmoles) was added to a solution of 461 mg (1.25 mmole) of VIb in 14 ml of DMSO. The mixture was stirred at RT for 2.5 hrs and 0.45 ml (405 mg, 4.0 mmoles) of 1-methylpiperazine was added. The mixture was stirred for another 2.5 hrs and mixed with 30 ml of H_2O. The resulting suspension was refrigerated overnight. The solids were filtered, washed with H_2O and dried, 486 mg. Tlc (9:1, 6:1, and 3:1 v/v $CHCl_3$-CH_3OH) analyses of this crude material showed the presence of the desired Ic, the methyl ester of VIb (1.2% by radioactivity), as well as traces of other components. The crude

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* To provide tlc standard, a sample of PAB·HCl methyl ester was prepared by treating a MeOH solution of PAB methyl ester with anhydrous methanolic HCl and precipitating the salt by adding Et_2O.

**A reference sample of methyl ester of VI was prepared in 73.5% yield by treating a solution of 188 mg (1.00 mmole) of PAB HCl methyl ester in 3 ml of 80% EtOH with 244 mg (1.05 mmole) of 4-chloro-7-trifluoromethylquinoline at RT. The resulting precipitates were chromatographically homogeneous by tlc (3:1 v/v $CHCl_3$-MeOH).

material was chromatographed on a 3 x 54 cm column of 150 g of silica gel eluted with 6:1 v/v $CHCl_3$-MeOH. The eluate was collected in 15 ml fractions at the rate of 2 min per fraction. The residue from fractions 33-73 was recrystallized from PhH-hexane to give 460 mg (89% yield) of crystals. A portion of this material (185 mg) was subjected to preparative tlc (250 μm silica gel, 6:1 or 3:1 v/v $CHCl_3$-CH_3OH, 55-75 mg per 15 cm streak). Recovered materials eluted with MeOH were combined and recrystallized first from EtOAc, then from PhH-$CHCl_3$ to give 103 mg of pure Ic, mp 217-219°C; sp act 153.8 μCi/mg; radiochemically pure by tlc (6:1 v/v $CHCl_3$-MeOH); *anal* ($C_{22}H_{21}F_3N_4O$): C, H, N.

Compound Id Dihydrochloride

A mixture of 212 mg (0.57 mmole) of VIb*, 195 mg (1.20 mmole) of CDI, and 4.5 ml of DMSO was stirred at RT under N_2 for 2 hrs. The resulting yellow solution was added dropwise with stirring in 5 min to a solution of 491 mg (5.7 mmole) of piperazine in 6 ml of DMSO. The mixture was stirred at RT overnight and added with stirring to 50 ml of ice H_2O. The milky mixture was chilled in the refrigerator for 24 hrs and filtered. The crude product was chromatographed on a 3 x 28.5 cm column of 80 g of silica gel eluted with 1.7 l. of 3:1 v/v $CHCl_3$-MeOH. The eluate was collected in 10 ml fractions at 2.7 min per fraction. The residue from fractions 46-95, Id free base, was found radiochemically pure by tlc (3:1 v/v $CHCl_3$-MeOH). It was dissolved in 4 ml of abs EtOH and treated with 2 ml of 1.56 M HCl in EtOH. The mixture was added dropwise with swirling to 9 ml of Et_2O and kept at RT ~20 hrs, during which time the initially gummy solids gradually crystallized. The crystals were recrystallized from MeOH-Et_2O to give 195 mg (72.5% yield) of the dihydrochloride of Id, sp act 294 μCi/mg; λ_{max}^{EtOH} 216 nm (ε 40,450), 252 (ε 19,650), and 349 (ε 18,800); *anal* ($C_{21}H_{21}Cl_2F_3N_4O$): C, H, N.

Compound II

A solution of 8.1 mg of tritium labeled Ic, sp act 153.8 μCi/mg, 1.230 g of

*This sample of VIb, sp act 482 μCi/mg, was prepared from a purified sample of Vb according to the procedure described above. The Vb which contained 8.3% (by radioactivity) of the methyl ester was purified by treating 130 mg of the material with 2 ml of 1 N NaOH and 1 ml of H_2O at 60°C for 2 hrs, reacidifying the mixture with 2 ml of 1 N HCl, decolorizing the dark mixture with Darco G-60, filtering and freeze-drying.

non-labeled Ia and 8 ml of 30% H_2O_2 in 80 ml of MeOH was kept at RT for five days at which time tlc showed complete oxidation.* The mixture was chilled in ice bath and a suspension of 250 mg of 5% Pd-C catalyst in MeOH was added to destroy the excess H_2O_2. The mixture was stirred at RT for 24 hrs and filtered. The filtrate was concentrated and the residue crystallized after addition and evaporation of two portions of 50 ml each of abs EtOH. The crude was dissolved in $CHCl_3$ and filtered to remove traces of catalyst. The solution was concentrated to ~10 ml and mixed with 5 ml of Et_2O. The mixture was chilled and the crystals were collected, washed with Et_2O and dried, 1.136 g (88.7% yield) of II, sp act 0.858 μCi/mg. Melt solvate analysis of the material showed it contained 8.62% or one-third of a molar equivalent, of $CHCl_3$; *anal* ($C_{22}H_{21}F_3N_4O_2 \cdot {}^1/_3\ CHCl_3$): C, H, F, N. Tlc analysis (3:1 v/v $CHCl_3$-MeOH) indicated the product was 98.2% radiochemically pure and contained 1.6% of the starting material.

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