

SPECIFIC TRITIUM LABELLING OF α -(p-METHOXYPHENYL)- α' -NITRO-4 [3-(DIMETHYLAMINO)PROPOXY] STILBENE(CI-680) BY CATALYTIC DEIODINATION.

C.E. Blackburn.

Labelled Compounds, Science Product Division,
Mallinckrodt Chemical Works,
St. Louis, Missouri,
U.S.A.

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SUMMARY

In an unusual example of tritium-labelling by catalytic dehalogenation in the presence of competing groups, an iodinated precursor was selectively deiodinated with tritium gas to yield α -(4-methoxyphenyl-3- ^3H)- α' -nitro-4 [3-(dimethylamino)propoxy]stilbene(CI-680- ^3H).

The product was labelled specifically at the C-3 position of the methoxyphenyl moiety with a specific radioactivity of 27.7 Ci/mole and a net yield of 809 mCi. Characterization of CI-680- ^3H (base) established the chemical purity to be >99% and the radiochemical purity to be >97.6%. Characterization also included an evaluation of the tritium label stability in water and in aqueous acidic and basic media.

INTRODUCTION

The antiprogestational, hypocholesteremic and antifertility activity of α -(p-methoxyphenyl)- α' -nitro-4 [3-(dimethylamino)propoxy]stilbene, hereinafter referred to as CI-680, has been reported by DeWald ⁽¹⁾. The need for a high specific activity radioisotopically labelled preparation of CI-680 for use in metabolic and mechanism of action studies prompted a study of methods of labelling CI-680. A specific radioactivity of 20-40 Ci/mole was thought to be necessary to provide the detection sensitivity needed for mechanism of action studies. Tritium was an obvious choice in preference

to carbon-14 since, in any hydrogen containing compound, the replacement of one hydrogen atom with a tritium atom will result in the compound having a specific radioactivity of 29.12 Ci/mmole ⁽²⁾; the replacement of one carbon atom with a carbon-14 atom would result in the compound having a maximum specific radioactivity of only 64 mCi/mmole ⁽³⁾.

A number of methods of introducing tritium directly into the CI-680 molecular structure by exchange were tried without success. These methods included catalytic exchange of CI-680 with tritium or tritiated water in various solvents using heterogeneous platinum catalysts ⁽⁴⁾ or homogeneous platinum catalysts (K_2PtCl_4) ⁽⁵⁾. These methods failed to yield a product of high specific activity that could be purified.

Catalytic dehalogenation of a suitable halogenated precursor with tritium to yield tritium labelled CI-680 was considered as an alternative method only after the exchange procedures failed. It was originally feared that the process of dehalogenation with tritium might also saturate the double bond and/or reduce the nitro group to an amine, yielding a number of tritium labelled products from which it would be difficult to isolate pure CI-680-³H. Nevertheless, several chlorinated, brominated and iodinated derivatives and intermediates to CI-680 were synthesized for use in catalytic dehalogenation studies, including I, VI, VII and VIII (See Figure 1). Catalytic dehalogenation of precursors VI, VII and VIII with non-radioactive hydrogen gas yielded product mixtures from which CI-680 could not be isolated in satisfactory purity and yield; dehalogenation of I yielded very promising results. The body of this paper describes the catalytic dehalogenation of I with tritium gas to yield CI-680-³H in high specific radioactivity and yield.

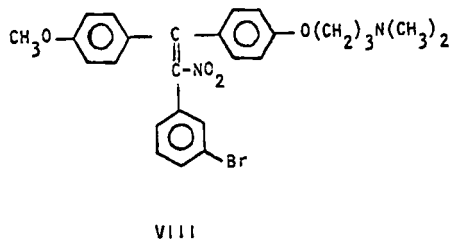
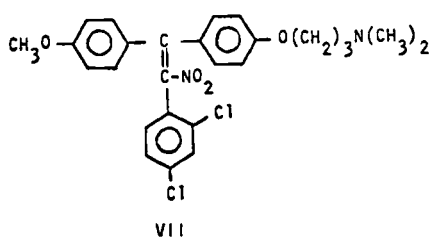
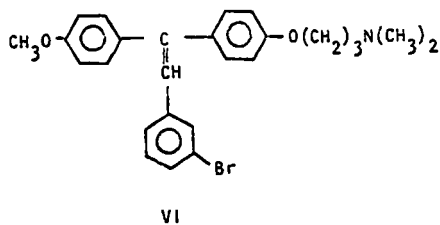
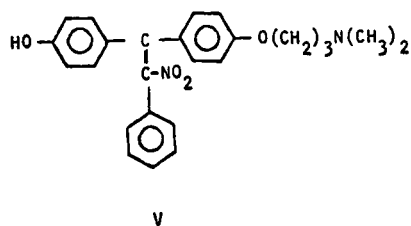
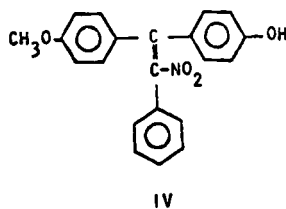
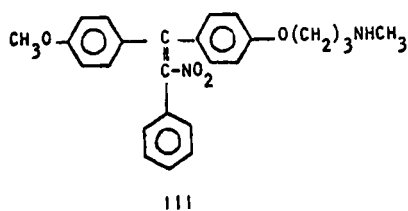
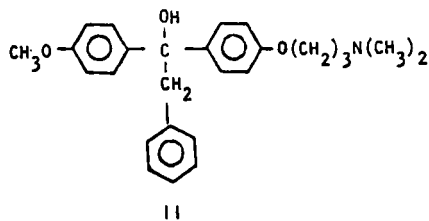
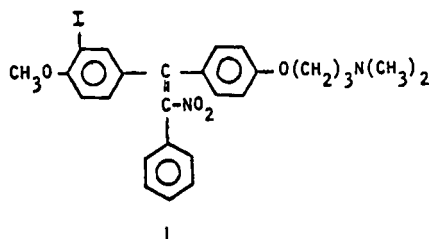


Figure 1 - Derivatives of Cl-680 (Base)

SYNTHESIS

The reaction conditions for the catalytic deiodination of 1 as shown in Figure 2 were as follows:* 56 mg (0.1 mmole) of $\alpha(3\text{-iodo-4-methoxyphenyl})\text{-}\alpha'\text{-nitro-4[3-(dimethylamino)propoxy]stilbene (1)}$ was stirred with 150 mg 5% Pd/Al₂O₃ in 2 ml dimethyl formamide in a 10 ml reaction flask on a high vacuum apparatus. The vacuum system was evacuated and then filled with 60 Curies of carrier-free tritium gas to a pressure of one atmosphere. While stirring magnetically, the deiodination reaction was allowed to proceed at 25°C for 25 minutes until the total tritium uptake was 4.0 ml. Approximately 2.5 ml tritium was adsorbed on the catalyst; the remaining 1.5 ml (0.06 mmole) tritium reacted with 1 to yield Cl-680-³H (base). The reaction was not allowed to proceed to a stoichiometric reaction of 0.1 mmole tritium as a safeguard against the possible onset of competing reactions (tritium saturation of the double bond and reduction of the nitro group to an amino group). After removing the tritium gas with a Toepler pump, the reaction mixture was vacuum distilled to remove the solvent. The resultant residue was mixed with methanol and then vacuum distilled to dryness. To remove the last traces of labile tritium, the crude product was dissolved in 10 ml 0.1 N HCl, basified with 2 ml 1 N NaOH and extracted into chloroform. The chloroform^{phase} was washed with water and vacuum distilled to dryness leaving a residue of product which was redissolved in 50 ml benzene:ethanol, 1:1. Analysis of this solution found a total of 2.11 Curies of crude tritiated product.

Chromatographic studies (Appendix A -- systems A, B, C, D, E and F) revealed that the crude product was Cl-680-³H (base) with a radiochemical purity of approximately 60%. Furthermore, the studies indicated that not all of the radiochemical impurities could be resolved from Cl-680-³H (base)

*This reaction was done at the New England Nuclear Corporation, Boston, Massachusetts, with the help and advice of Dr. Robert E. O'Brien.

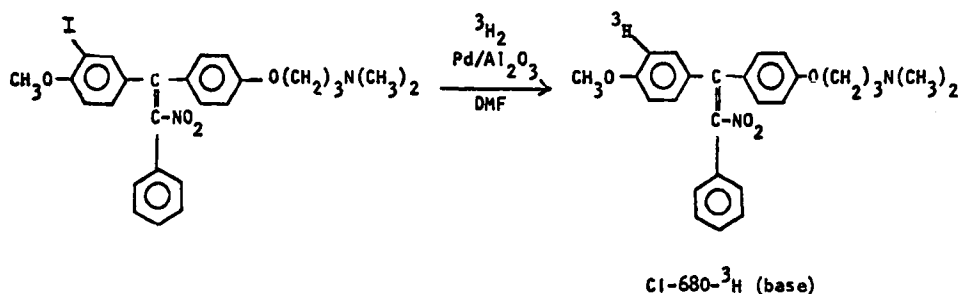


Figure 2. Synthesis of tritium-labelled CI-680 (base)

in a single TLC system. Thus, to purify the product by preparative TLC, it was necessary to use two consecutive different TLC separations. An 1812 μCi portion of crude CI-680- ^3H in the benzene:ethanol solution was vacuum distilled to dryness, redissolved in 4 ml ethyl acetate and streaked in equal amounts on each of four 20 cm x 20 cm TLC plates (1 mm) along a line 1 cm x 15 cm at the origin and chromatographed (Appendix A -- system C). The purified product was eluted with ethyl acetate to yield 755 mCi CI-680- ^3H (base) with a radiochemical purity of 91%. The ethyl acetate eluate of the product was concentrated to 3 ml and chromatographed again in a different TLC system (Appendix A -- system B) in equal amounts on each of two TLC plates (1 mm). The purified product was eluted with ethyl acetate to yield 609 mCi CI-680- ^3H (base) with a chemical purity of >99% and a radiochemical purity of $\geq 97.6\%$.

A 511.5 mCi portion of this high specific activity CI-680- ^3H (base) in ethyl acetate solution was vacuum distilled to remove ethyl acetate and redissolved in 50 ml benzene, which is a preferred solvent for protection against radiation induced decomposition during storage.⁽⁶⁾ The benzene

solution was analyzed to find (by fluorimetric analysis) 7.88 mg Cl-680-³H (base) with a total activity of 505 mCi and a specific radioactivity of 27.7 Ci/mmole. All of the characterization data for this product are summarized in Table 1.

The remaining 97.5 mCi Cl-680-³H in ethyl acetate solution was mixed with 865 mg (2.00 mmole) Cl-680 (base, nonradioactive) to lower the specific radioactivity for use in metabolic studies. Ethyl acetate was removed by vacuum distillation. The residue of Cl-680-³H (base) was redissolved in 4 ml ether and converted to the monocation salt when added slowly to 0.44 g (2.10 mmole) citric acid monohydrate in 40 ml boiling 2-butanone. The solution was concentrated to 25 ml by boiling and then diluted with ether until crystallized Cl-680-³H (citrate) began to appear. Crystallization was allowed to proceed overnight at 0°C. The yellow crystalline product was collected by filtration, washed with ether and dried to yield 1127 mg Cl-680-³H (citrate) with a specific radioactivity of 82.7 μCi/mg and a radiochemical purity of 98.0%. Characterization data for this product are summarized in Table 2.

TRITIUM LABEL STABILITY

The preparations of tritium labelled Cl-680 are intended for use in physiological mechanism studies and drug metabolism experiments where Cl-680 and its metabolic products can be detected by means of the tritium activity. To serve this function, the tritium to carbon bond must be stable under conditions existing in biological systems. As a preliminary step to the use of tritium-labelled compounds in biological systems, it has been found useful to evaluate the stability of the tritium label in water and in basic and acidic aqueous media (7, 8, 9).

The stability of the tritium activity was studied in aqueous media using Cl-680-³H (citrate) which was considered to be representative of both

preparations of $\text{Cl-680-}^3\text{H}$. $\text{Cl-680-}^3\text{H}$ (citrate) was dissolved in water, 0.1 N NaOH, 1 N NaOH, 0.1 N HCl and 1 N HCl, for 18 hours at 25°C, made basic where necessary and then extracted into chloroform. In two other experiments, $\text{Cl-680-}^3\text{H}$ (citrate) was dissolved in 4 N NaOH and 4 N HCl, heated at 100°C on a steam bath for 2 hours, made basic where necessary and then extracted into chloroform. The total tritium content of each of the aqueous and chloroform phases was determined. If the tritium activity in $\text{Cl-680-}^3\text{H}$ was readily removed under these test conditions, then it would be exchanged from $\text{Cl-680-}^3\text{H}$ for non-radioactive hydrogen in the test medium and ultimately would be detected as ^3HHO in the aqueous phase after extraction of $\text{Cl-680-}^3\text{H}$ (base) into chloroform; if the tritium label were stable, no significant exchange or loss of tritium from $\text{Cl-680-}^3\text{H}$ would occur and no ^3HHO would be detected in the aqueous phase. The experimental details of these experiments are described in Appendix B and the results are presented in Table 3.

The results of these tritium stability studies established that the tritium activity in the preparations of $\text{Cl-680-}^3\text{H}$ is stable for at least 18 hours in water and aqueous solutions of 0.1 N NaOH, 1 N NaOH, 0.1 N HCl and 1 N HCl at 25°C and in 4 N NaOH at 100°C for at least 2 hours; in these studies (Appendix B -- experiments 1-6), $\leq 3.5\%$ of the tritium activity was found in any of the aqueous phases. However, tritium activity can be removed from $\text{Cl-680-}^3\text{H}$ when it is heated at 100°C in 4 N HCl for 2 hours; in this study (Appendix B -- experiment 7), 11.3% of the tritium activity was detected in the aqueous phase. A TLC study (Appendix A -- system D) of this aqueous phase detected <10% of the total tritium spotted; of the tritium detected, 4% was detected as $\text{Cl-680-}^3\text{H}$, 51% was detected as analogue V (Figure 1), and the remaining 45% was uniformly distributed along the chromatographic lane. These results suggest that at least 90% of the tritium activity in the aqueous

phase was present in a volatile form such as ${}^3\text{H}_2\text{O}$. From these observations, it is evident that some tritium activity can be removed from CI-680- ${}^3\text{H}$ by heating in 4 N HCl, and that some O-demethylation may occur.

While it has been shown that tritium activity can be removed from CI-680- ${}^3\text{H}$ in hot 4 N HCl, it has also been established that the tritium activity in CI-680- ${}^3\text{H}$ is stable at room temperature in water and aqueous acidic and basic media. Thus, from these observations, it would appear that the tritium activity in the preparations of CI-680- ${}^3\text{H}$ should be a satisfactory tracer for the carbon skeleton of CI-680.

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Table 1. Summary of Characterization of Cl-680-³H (Base)

Parameter	Cl-680- ³ H (Base)
Color	Yellow
Chemical Form	Free base in benzene solution
Specific Activity	
mCi/mg	64.9
Ci/mmole	27.7
Chemical Purity	
- GLC	GLC mass peaks from Cl-680- ³ H (base) having identical chromatographic profiles and retention times as authentic Cl-680 (base) were detected; no iodoprecursor (I, see Figure 1) to Cl-680- ³ H (base) was detected. Chemical purity is estimated as >99%.
Radiochemical Purity	
- TLC (Appendix A - systems B, C, D, E, and F)	A major ³ H spot from Cl-680- ³ H (base) having the same R _f as authentic Cl-680 was detected in five TLC systems. Reference analogues (see Figure 1) I, II, III, and IV were resolved from Cl-680- ³ H in at least one TLC system and detected in the maximum amounts of 1.1%, 0.02%, <0.1%, and 0.3%, respectively; one other unidentified radiochemical impurity of 0.2% was detected in one TLC system; no other radiochemical impurities were detected. The radiochemical purity is estimated as >97.6%.
Tritium Label Stability	Same as Cl-680- ³ H (citrate). See Table 2.

Table 2. Summary of Characterization of Cl-680-³H (Citrate)

Parameter	Cl-680- ³ H	Authentic Cl-680
Color	Yellow	Yellow
Chemical Form	Citrate Salt	Citrate Salt
Specific Activity		
mCi/nmole	51.6 (measured)	-
μCi/mg (salt)	82.7 (measured)	-
μCi/mg (base)	119 (calculated)	-
Elemental Analysis		
Calc C 61.53%	60.95%, 60.88%	-
H 5.81%	5.91%, 5.85%	-
N 4.49%	4.43%, 4.46%	-
Ultraviolet Analysis		
in MeOH	λ 281 ε 14600 238 ε 20200	λ 281 ε 14700 238 ε 20500
Infrared Analysis	The IR of Cl-680- ³ H (citrate) is identical to the IR of authentic Cl-680.	
Chromatography (Appendix A - systems B, D, E, and F)	A major ³ H spot from Cl-680- ³ H (citrate) having the same R _f as authentic Cl-680 was detected in four TLC systems. Reference analogues (see Figure 1) I, II, III, and IV were resolved from Cl-680- ³ H (citrate) in at least one TLC system and detected in the maximum amounts of 1.5%, 0.04%, 0.05%, and 0.04%, respectively; one other unidentified impurity of 0.3% was detected in one TLC system; no other radiochemical impurities were detected. The radiochemical purity is estimated as ≥98.0%.	
Tritium Label Stability	The tritium activity in Cl-680- ³ H (citrate) is non-labile at room temperature for at least 18 hours in water and aqueous solutions of 0.1 N NaOH, 0.1 N HCl, 1 N NaOH, and 1 N HCl, and at 100°C for at least 2 hours in 4 N NaOH. As much as 11.3% of the tritium activity can be removed by heating on a steam bath in 4 N HCl for 2 hours. See Table 3.	

Table 3. Results of Tritium Label Stability Experiments on CI-680-³H

Experiment	Test Conditions ^a	% ³ H in Chloroform Phase ^b	% ³ H in Aqueous Phase ^b
1	H ₂ O, 18 hours	89.3	2.8
2	0.1 N NaOH, 18 hours	90.4	2.3
3	0.1 N HCl, 18 hours	94.2	0.3
4	1 N NaOH, 18 hours	92.8	3.4
5	1 N HCl, 18 hours	94.9	0.2
6	4 N NaOH, steam bath 2 hours	90.9	1.6
7	4 N HCl, steam bath 2 hours	82.5	11.3

^aExperiments 1-5 were conducted at room temperature (approximately 25°C). See Appendix B.

^bThe % ³H in the chloroform and aqueous phases is based on the measured tritium content of 3 ml of stock solution prior to any pH adjustments or extraction. The sensitivity of these experiments permitted the detection of $\geq 0.05\%$.

APPENDIX A: CHROMATOGRAPHIC METHODS

CI-680-³H and the authentic reference compounds (Figure 1) were chromatographed together by the ascending technique on thin layers of silica gel and aluminum oxide until the solvent front was 15 cm from the origin. Using techniques in liquid scintillation spectrometry, the radiochromatograms were scanned for tritium by measuring the dpm ³H on each of the 1 cm sections along the chromatographic lane from the origin to the solvent front. The TLC systems are listed below:

System	Solvent	Solvent Composition
A	Silica Gel	Hexane:Et ₂ NH, 5:1
B	Al ₂ O ₃	Hexane:Et ₂ NH, 5:1
C	Al ₂ O ₃	EtOAc
D	Silica Gel	EtOAc:Et ₂ NH, 30:1
E	Al ₂ O ₃	EtOAc:Et ₂ NH, 30:1
F	Silica Gel	Acetone:MeOH:NH ₄ OH, 90:30:1

APPENDIX B: TRITIUM STABILITY TESTS ON CI-680-³H (CITRATE)

A stock solution of CI-680-³H (citrate) in water was prepared as follows: 4.3 mg CI-680-³H (citrate) was mixed vigorously with 30 ml distilled water, filtered and diluted with water to exactly 50 ml. The stock solution was analyzed for tritium content per 3 ml aliquots prior to any of the following experiments. Experiment 1 - A 3 ml aliquot of the stock solution was allowed to stand at room temperature for 18 hours, then adjusted to 0.1 N NaOH with the addition of 1 ml 0.4 N NaOH and extracted with 6 ml chloroform. Experiment 2 - A 3 ml aliquot of the stock solution

was adjusted to 0.1 N NaOH with the addition of 1 ml 0.4 N NaOH, allowed to stand 18 hours at room temperature and extracted with 6 ml chloroform.

Experiment 3 - A 3 ml aliquot of the stock solution was adjusted to 0.1 N HCl with the addition of 1 ml 0.4 N HCl and allowed to stand 18 hours at room temperature. The test medium was then adjusted to 0.12 N NaOH with the addition of 1 ml 1 N NaOH and extracted with 6 ml chloroform. Experiment 4 -

A 3 ml aliquot of the stock solution was adjusted to 1 N NaOH with 1 ml 4 N NaOH, allowed to stand at room temperature for 18 hours and extracted with 6 ml chloroform. Experiment 5 - A 3 ml aliquot of the stock solution was adjusted to 1 N HCl with the addition of 1 ml 4 N HCl and allowed to stand at room temperature for 18 hours. The test medium was then adjusted to 0.08 N NaOH with the addition of 1.1 ml 4 N NaOH and extracted with 6 ml chloroform. Experiment 6 - A 3 ml aliquot of the stock solution was adjusted

to 4 N NaOH with the addition of 0.79 ml 19.3 N NaOH, heated at 100°C on a steam bath for 2 hours and extracted with 6 ml chloroform. Experiment 7 - A 3 ml aliquot of the stock solution was adjusted to 4 N HCl with the addition of 1.5 ml 12.13 N HCl and heated at 100°C on a steam bath for 2 hours. The test medium was adjusted to 0.1 N NaOH with 0.96 ml 19.3 N NaOH and extracted with 6 ml chloroform.

In all of the above experiments, the resulting aqueous and chloroform phases were separated and each was analyzed for total tritium content using techniques in liquid scintillation spectrometry.

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