PREPARATION OF A ^{125}I LABELLED [1,3H]IMIDAZOLE:

2-n-butyl-4(5)-^{125}I-iodo-5(4)-hydroxymethylene imidazole

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SUMMARY

A method for the introduction of ^{125}I in a substituted imidazole has been devised. 2-n-Butyl-4(5)-hydroxymethylene imidazole undergoes rapid and selective electrophilic substitution on the ring when treated with a halogenating agent such as, i. a., N-chlorosuccinimide. This reaction has been adapted to the preparation of 2-n-butyl-4(5)-^{125}I-iodo-5(4)-hydroxymethylene imidazole by treatment of 2-n-butyl-4(5)-hydroxymethylene imidazole by chloramine-T in the presence of sodium iodide. The radiolabelled product purified and isolated by HPLC is obtained at a high specific activity (2200Ci/ mmol) with good chemical and radiochemical yields (~70%).

Key words : imidazole, iodination, radioiodination

INTRODUCTION

The study of the biology and pharmacology of a series of substituted imidazoles required a radiolabelled ligand. The choice of ^{125}I was made based on the necessity to have a strong emitter for binding studies.

Electron rich aromatic compounds can be labelled by electrophilic radioiodination in the presence of radioiodide and iodine monochloride[1], chloramine-T[2] or "iodogen".[3] The 2-n-butyl-4(5)-hydroxymethylene imidazole reacts readily with N-chlorosuccinimide in an electrophilic substitution reaction to provide the 4(5)-chloro analogue.[5] The synthesis of the 2-n-butyl-4(5)-iodo-5(4)-hydroxymethylene imidazole was thus attempted in analogy to the synthesis of the described chloroderivative. The preparation of the radiolabelled species was then investigated.

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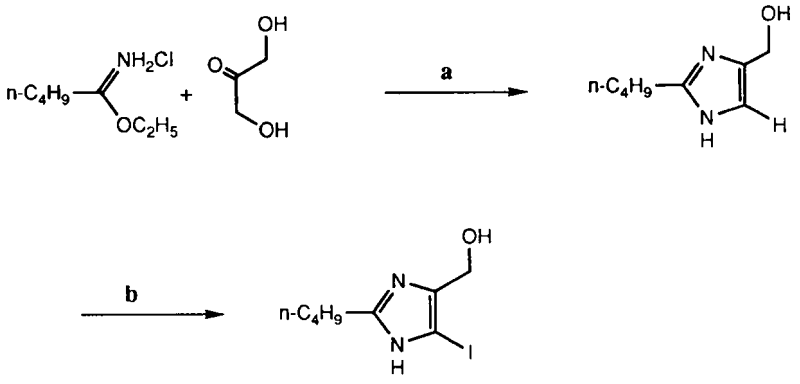
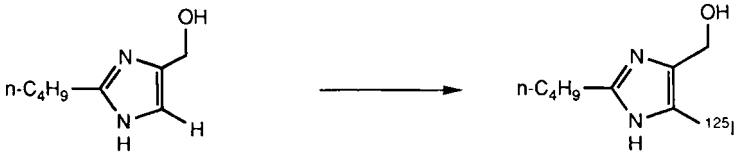
SCHEME A



a. Liquid NH3; 90°C; 16 hr. b. NCS, NaI, dioxane, 2-methoxyethanol.

SCHEME B



Conditions : chloramine-T, MeOH/H2O, 1mCi $Na^{125}I$

RESULTS

The 2-n-butyl-4(5)-hydroxymethylene imidazole can be prepared by treatment of the valerimidate hydrochloride with 1,3-dihydroxyacetone in ammonia by a procedure described earlier[4] as shown in scheme A. Halogenation of the 4(5) position on the free imidazole by treatment with one to two equivalents of of N-halosuccinimide in a polar solvent such as dioxane or 2-methoxyethanol at a temperature of 40-100°C for 1-10 hours has been described in U. S. Patent 4,355,040[5].

We have attempted several iodination procedures and have observed that 2-n-butyl-4(5)-hydroxymethylene imidazole can be selectively iodinated by treatment with N-chlorosuccinimide in the presence of sodium iodide (Scheme A). The iodo derivative can be freed from small quantities of starting material and other halogenated derivatives by crystallization or by HPLC purification (yield ~ 70%).

The synthesis of the radiolabelled imidazole derivative was performed in aqueous methanol using a large excess (10 equivalents) of the hydroxymethylimidazole over the $Na^{125}I$. An excess of chloramine-T (2.5 eq) was used to initiate the electrophilic substitution reaction (Scheme B). After 1 hour reaction time, the mixture was separated in its different constituents

by HPLC (Fig). The excess starting material eluted first followed by some of the chloro derivative. The desired iodinated product eluted last. Fractions (0.5 ml) of the eluate were taken at regular intervals and checked for radioactivity. The two fractions correponding to the peak of the 2-n-butyl-4(5)-iodo-5(4)-hydroxymethylene imidazole contained more than 75 % of the total radioactivity engaged. Thus, the radioiodinated species was pure by HPLC criteria and a total of 764 uCi at high specific activity was isolated.

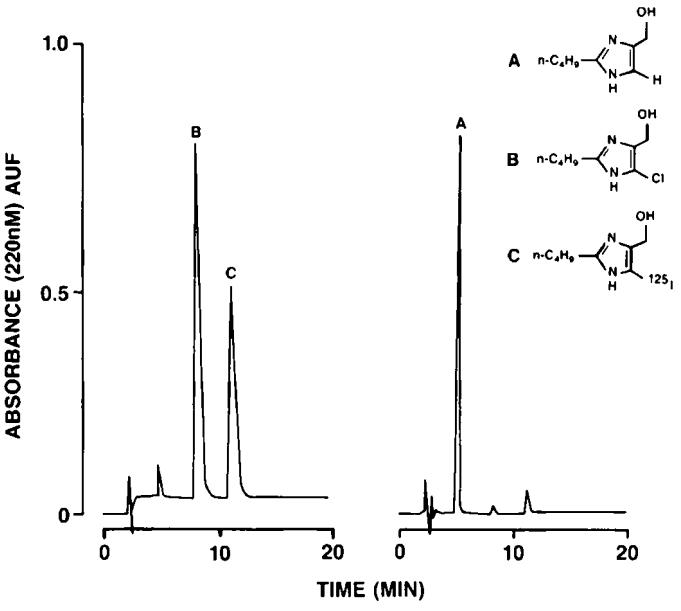


Figure legend

The left panel exhibit the chromatogram (monitored at 220 nM) obtained for a reference mixture containing the precursor 2-n-butyl-5(4)-hydroxymethylene imidazole (A), the 2-n-butyl-4(5)-chloro-5(4)-hydroxymethylene imidazole (B) and the 2-n-butyl-4(5)-iodo-5(4)-hydroxymethylene imidazole (C) upon chromatography on a C18 bonded silicagel (Vydac column 218TP54; eluant H_2O/CH_3CN:93/7/0.05% TFA; 1ml/min flow rate). The right panel show the chromatogram resulting from the injection of the radioiodination reaction mixture. The large excess of A (RT = 5.4 min) eluted first followed by a small quantity of (B; RT = 8.6 min). Eventually, the desired radioiodinated compound C was collected (RT = 11.7 min) over two fractions which contained 75% of the total radioactivity.

EXPERIMENTAL

The reagents were commercially available and of synthetic grade. $Na^{125}I$ was obtained from Amersham (IMS.30; 1.0mCi size). The purification of the final compounds was by HPLC on reverse phase C18 bonded silicagel (U-Bondapak or Vydac) with CH_3CN/H_2O containing 0.05% TFA as solvent. The eluted peaks were monitored at 254 nM or 220 nM.

The structures of the synthesized compounds were confirmed by ^1H NMR spectroscopy (Varian XR-300, 300MHz) in $CDCl_3$ or DMSO-d_6 or CD_3OD. Elemental analysis for C, H, N was obtained from Galbraigh Laboratories, Inc.

2-n-butyl-4(5)-hydroxymethylimidazole. In a steel bomb were added successively 60g of valeroyl imidate hydrochloride[6] (MW= 165; 0.36 mole), 65g of dihydroxyacetone dimer (MW=180, 0.36 mole) and 400ml (16 moles) of liquified ammonia. The bomb was sealed and heated at 90°C for 24 hr (pressure increase to about 200 psi). After cooling to 25 °C, the ammonia was vented and 1000ml acetone added to the residue and the precipitated NH_4Cl filtered out. The residual solution was quickly filtered through a pad of silicagel using one more liter of acetone to elute the product. After concentration the residual oil was dissoved in 1.2 liter ethyl acetate. A remaining oily residue was decanted and triturated with acetone. Both solutions deposited a crystalline material which was identified as the desired 2-n-butyl-4(5)-hydroxymethylimidazole. The dried pale yellow solids combined weighed 32g (58% yield). The product, which migrates as a single spot on silica gel eluted with 10% MeOH saturated with ammonia in $CHCl_3$ (Rf = 0.25), had a melting point of 88.6-88.8 °C.
^1H NMR ($CDCl_3$, δ ppm) : 6.8 (s, 1H); 4.6 (s, 2H); 2.7 (t, 2H); 1.7 (m, 2H); 1.35 (m, 2H); 0.9 (t, 3H).
FABMS (calcld for $C_8H_{14}ON_2$, found): 154.21, 155.

2-n-butyl-4(5)-iodo-5(4)-hydroxymethylene imidazole. An aliquot of 2-n-butyl-5(4)-hydroxymethylene imidazole (200mg, MW 154, 1.3 mmol) was dissolved in 2 ml of a 1:1 mixture of dioxane and 2-methoxyethanol. An excess of NaI (.68g, MW 159, 10.6mmol) and 0.178 g (MW 133.5, 1.3 mmol) in 1ml methanol were added. The reaction mixture was stirred at 25°C for 4 hours and the solvents removed under vacuum. Water was added to the residue and the solid formed collected by filtration. This solid was then purified by crystallization from boiling chloroform as a slightly yellow powder (m.p.: 102.6-104 °C).
^1H NMR ($CDCl_3$, δ ppm) : 6.8 (s, 1H); 4.6 (s, 2H); 2.7 (t, 2H); 1.7 (m, 2H); 1.35 (m, 2H); 1.9 (t, 3H).
FABMS (calcld for $C_8H_{13}IN_2O$, found): 280.11, 281.1 ($M+H^+$). Elemental analysis (calcld for $C_8H_{13}IN_2O$, found): C, 34.32, 34.49; H, 4.66, 4.58 ; N, 10.0, 9.99.

2-n-butyl-4(5)-^{125}I-iodo-5(4)-hydroxymethylene imidazole. In a vial were combined, 10 nmol of 2-n-butyl-5(4)-hydroxymethylene imidazole in 5 uL of methanol, 2.5 nmol of chloramine-T (N-chloro-4-methylbenzenesulfonamide sodium salt) in 5 uL H_2O and 1mCi of $Na^{125}I$ in 10 uL H_2O . The mixture (~ 20uL) was reacted for 1 hr at 25°C with occasional shaking and then injected on a C18 bonded silicagel (Vydac column 218TP54; eluant H_2O/CH_3CN:93/7/0.05% TFA). The fractions containing the iodo compound (monitored by UV at 220 nm) were collected and counted. Two fractions contained 764uCi of pure compound.

CONCLUSION.

A new preparation of 2-n-butyl-4(5)-iodo-5(4)-hydroxymethylene imidazole is provided. The application of this method to electrophilic radioiodination of 2-n-butyl-4(5)-hydroxymethylene imidazole provided the desired labelled material. In combination with HPLC isolation, this procedure proved to be a very viable approach suitable to isolate the radioiodinated target compound in high yield and high purity.

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