

## ELECTROCHEMICAL SYNTHESIS OF [ $^{131}\text{I}$ ]-IDO-METOPROLOL

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### SUMMARY

Using constant potential electro-chemical oxidation of iodine and radioiodide in acetonitrile, a positively-charged iodinating species, the N-iodo-acetonitrilium ion was produced. Electrophilic aromatic substitution of iodine through this species on the  $\beta_1$ -adrenergic blocking agent metoprolol resulted in the production of [ $^{131}\text{I}$ ]-Iodo-Metoprolol in an 82% yield.

Key Words: Metoprolol, Iodine-131, Electrochemical Radioiodination,  $\beta_1$ -Blockers

### INTRODUCTION

Organ localization by means of specific drug-receptor interaction is an intriguing concept in radiopharmaceutical design. The  $\beta$ -adrenergic receptor system is one which may prove successful in this regard for the design of myocardial imaging agents. The cardioselective  $\beta_1$ -adrenergic blocking drugs (acebutolol, atenolol, metoprolol, practolol) inhibit the cardiac  $\beta_1$  receptors to a much greater extent than those of the lung ( $\beta_2$ -receptors) (1).

Radio-iodobenzoyl derivatives of the cardioselective drugs, practolol and acebutolol have been found to localize to a greater degree in the myocardium than propranolol (2,3) and another radio-iodinated derivative of practolol (4) has demonstrated a promising heart/blood ratio and cardio-selectivity. Furthermore the norepinephrine storage analog meta-iodo-benzylguanidine has also been found to localize in the myocardium (5).

In this paper, we describe the electrochemical radio-iodination of the cardioselective  $\beta_1$ -adrenergic blocking agent, metoprolol, and the physical characterization of the iodinated compound.

## EXPERIMENTAL

Materials and Equipment

The acetonitrile (HPLC grade) was first dried over silica gel, then distilled from over phosphorous pentoxide, refluxed over calcium hydride and further re-distilled. The tetraethyl-ammonium perchlorate (Fisher Sci. Co., polarographic grade) was oven-dried at 120°C for 24 hours. Na<sup>131</sup>I (labeling grade) was obtained from Frosst Radiopharmaceutical Division, Canada.

Metoprolol Tartrate (Di-(±)-1-(isopropylamino)-3-[p-(2-methoxy-ethyl)phenoxy]-2-propanol L-(+)-tartrate) was generously supplied by Astra Pharmaceuticals Canada, Ltd.

Other chemicals were of analytical grade and included iodine, methylene chloride, iodine monochloride and 1,3,5-trinitrobenzene.

The potentiostat and digital coulometer were Princeton Applied Research Model 173 and Model 179, respectively. The microammeter was Phillips Model PM2503.

## METHOD

The design of the three-electrode electrochemical cell is shown in Figure 1. Electrolysis of iodine (as carrier iodine-127 and Na<sup>131</sup>I) is carried out at a constant potential of +2.000V applied to the platinum gauze anode versus the Ag/AgNO<sub>3</sub> (0.1M) reference electrode.

The supporting electrolyte-solvent system was a solution of tetraethylammonium perchlorate (TEAP) in acetonitrile using a 4:1 molar ratio of supporting electrolyte:iodine. The following relatively low specific activity radioiodination was carried out with 100.0 mg (0.394 mmols) of carrier iodine and 11.68 mCi of Na<sup>131</sup>I.

Overloading of the potentiostat during the electrolysis procedure may occur due to depletion of supporting electrolyte in the catholyte solution. This was remedied by the injection of a small volume of a concentrated solution of TEAP in acetonitrile (200.0 mg/ml) into the cathode chamber.

A pre-electrolysis run was carried out to oxidize any possible impurities in the supporting electrolyte-solvent system alone. Completion of pre-electrolysis was noted by a steady low-level current occurring after approximately 18 minutes. At this point 10 ml of the anolyte solution were removed and used to dissolve the carrier iodine. After returning this solution to the anolyte compartment, the  $\text{Na}^{131}\text{I}$  in a minimum volume of aqueous  $\text{NaOH}$  (18  $\mu\text{l}$ ) was added by syringe.

During the electrolysis procedure, the cell current, applied potential, coulombs generated and colour of the anolyte solution were

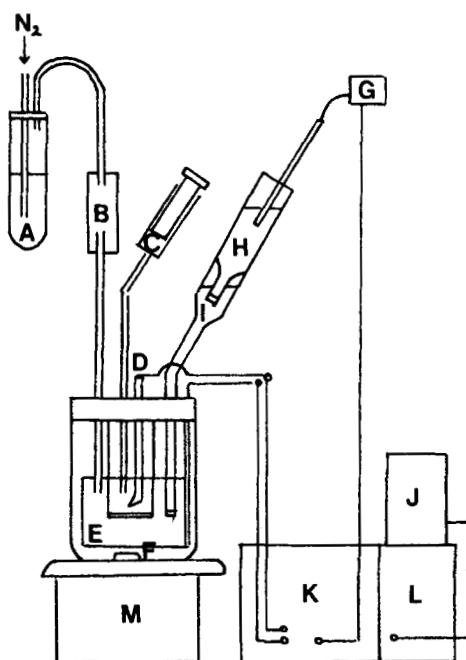


FIGURE 1

## ELECTROCHEMICAL CELL

A: acetonitrile, B:  $\text{NaOH}$  pellets, C: TEAP spiking solution, D: stainless-steel cathode, E: platinum anode, F: magnetic stirring bar, G: electrometer probe, H: reference electrode ( $\text{Ag}/\text{AgNO}_3$  0.1M in  $\text{CH}_3\text{CN}$ ), I: salt bridge, J: microammeter, K: potentiostat, L: digital coulometer, M: magnetic stirrer

recorded. Completion of oxidation of the iodine was noted by a gradual change in anolyte colour from dark brown to pale yellow with a simultaneous decrease in the current, occurring after approximately 90 minutes.

After the potentiostat was deactivated a 5.0 ml aliquot of the anolyte, equivalent to 0.073 mmol of iodine, was removed for another purpose. Then 160.27 mg of metoprolol tartrate (equivalent to 0.468 mmol of metoprolol base) was dissolved in 5.0 ml of methylene chloride and added to the anolyte compartment. The reaction mixture was allowed to stir for an additional 30 minutes before a 1.0 ml aliquot was withdrawn for HPLC analysis.\*\*

The remaining reaction mixture was removed by syringe, transferred to a 50 ml evaporation flask and the solvent evaporated at 35°C under reduced pressure. The resulting brown residue was transferred to a 50 ml teflon-capped glass tube with the aid of 10 ml of diethyl ether and 10 ml of water. Extraction of the product, [ $^{131}\text{I}$ ]-Iodo-metoprolol HCl salt, was performed as follows: the aqueous layer was acidified to pH 2.0 with 1.0 N HCl, the mixture thoroughly agitated and the ether and aqueous layers separated. The radioactivity of the ether layer measured 1.96 mCi. A 5 ml ether wash of the aqueous layer removed a further 0.049 mCi. The separated aqueous layer containing the product was then alkalized to pH 10.0 with 1.0 N NaOH and four 5 ml ether extractions of this aqueous layer were carried out. The new aqueous phase contained 1.97 mCi and the ether phase, containing the product, had an activity of 2.01 mCi. The HCl salt of the product (m.p. 142.5-144°C) was obtained by addition of a slight excess of 1.0 N HCl and evaporation under reduced pressure.

The product was dissolved in 6.0 ml of aqueous sterile 0.9% w/v sodium chloride. Qualitative radiochemical purity was determined by

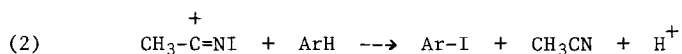
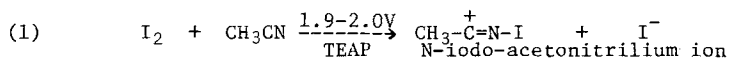
\*\*HPLC Conditions: Column-Waters  $\mu$ Bondapak-CN (3.9mm X 30cm); Mobile Phase-30% MeOH/70% 0.05 M monobasic phosphate buffer, pH 4.0; Flow Rate-1.0 ml/min; Detector-Waters Model 440, Absorbance range 0-2 at 254 nm; injection volume 5  $\mu$ l.

Assay: 1.0 ml anolyte + 0.5 ml trinitrobenzene (1.0 mg/ml in 70% MeOH/30% H<sub>2</sub>O), add sufficient mobile phase to 25.0 ml.

injection of 10  $\mu\text{l}$  of this solution into the HPLC system used previously for quantitative analysis (with omission of the internal standard) and by collection of twenty- 1.0 ml/minute fractions. Using a multichannel analyzer (Tracor Northern Model TN-1705) and a 3" X 3" NaI(Tl) well crystal, the counts/minute/1.0 ml fraction of eluate were obtained in a channel number corresponding to 364 keV.

## RESULTS AND DISCUSSION

Utilizing constant potential electrochemical oxidation of iodine in acetonitrile, Miller *et al.* (6) have demonstrated that a positively charged iodinating species, the N-iodo-acetonitrilium ion can be produced. This species will then cause the iodine to undergo electrophilic aromatic substitution with a variety of aromatic substrates (ArH) to produce the corresponding mono-iodo products:



We have adapted this technique to the radioiodination of the cardio-selective  $\beta_1$ -adrenergic blocking agent, metoprolol. This drug is strongly activated towards electrophilic substitution in the ortho position by the resonance effect of the phenoxy group. The inductive effect of the p-methoxyethyl group also activates the meta position to a lesser extent.

Quantitative HPLC analysis was performed on the 1.0 ml aliquot removed from the anolyte solution, using the peak area ratio of metoprolol/trinitrobenzene internal standard and a calibration curve ( $Y=1.115 X - 0.0975$  ;  $r=0.9993$ ) where Y is the peak area ratio and X is the corresponding metoprolol tartrate concentration. This assay showed that about 82% of the metoprolol had been iodinated with the formation of a product peak ( $t_R = 8.51$  minutes). An iodine monochloride iodination of metoprolol previously gave a product peak with the same retention time.

Figure 2 shows the radiochromatogram and HPLC chromatogram overlay produced by the collection and scintillation counting of twenty 1.0 ml/

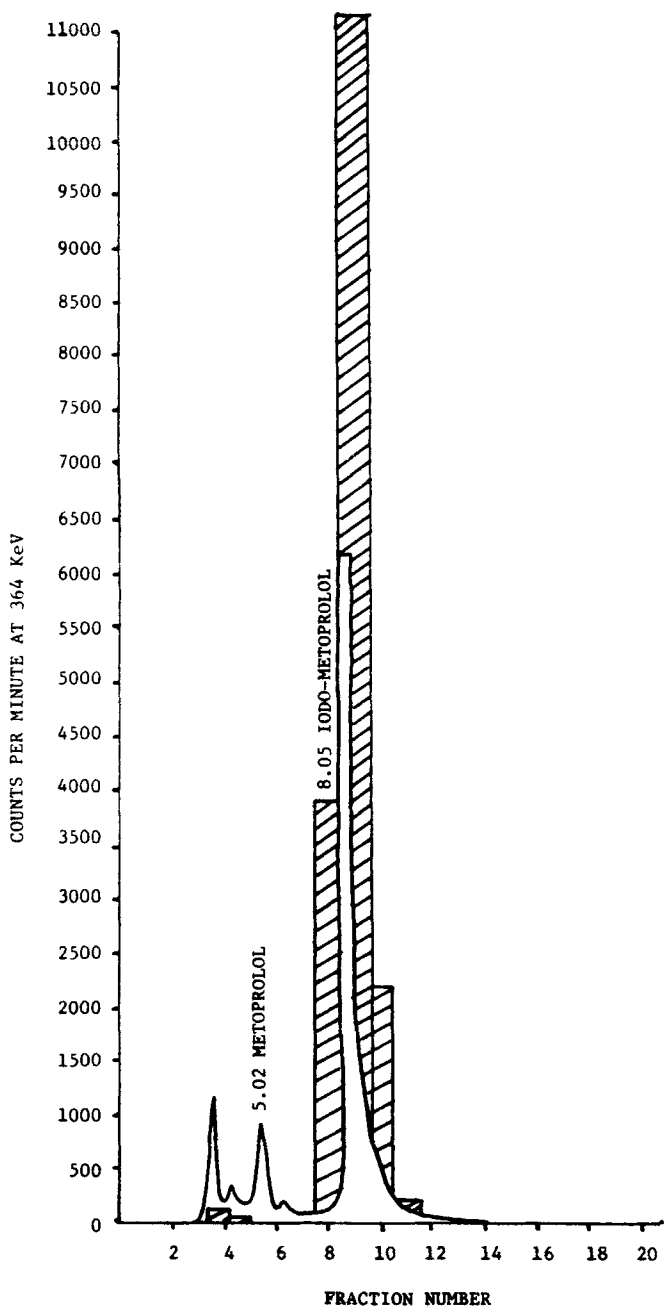




FIGURE 2

RADIOCHROMATOGRAM OF [<sup>131</sup>I]-IODO-METOPROLOL

 counts per minute at 364 KeV  
 absorbance at 254 NM

minute fractions as previously described. Essentially all of the radio-activity is contained within the product peak, i.e. fractions (7-11).

The UV spectrum of metoprolol in methanol shows  $\lambda_{\text{maxima}}$  of 230 ( $\epsilon = 8480$ ), 276 ( $\epsilon = 1398$ ) and 286 nm ( $\epsilon = 1216$ ). That of the product shows  $\lambda_{\text{maxima}}$  of 230 ( $\epsilon = 8167$ ), 284 ( $\epsilon = 2152$ ) and 290 nm ( $\epsilon = 1968$ ). A small bathochromic and hyperchromic shift of all  $\lambda_{\text{maxima}}$  values has occurred which would be consistent with aromatic substitution by a relatively weak auxochrome such as iodine.

Partial 60 MHz proton NMR spectra for metoprolol and the product are shown in Figure 3A, 3B. In the aromatic region shown, metoprolol appears as 2 doublets ( $\delta 6.85$ , 2 H,  $J = 9$  Hz/ $\delta 7.17$ , 2 H,  $J = 9$  Hz) whereas the product appears as 2 doublets ( $\delta 6.78$ , 1 H,  $J = 9$  Hz/ $\delta 7.20$ , 1 H,  $J = 9$  Hz) and 1 singlet ( $\delta 7.65$ , 1 H). While the coupling pattern of metoprolol is  $A_2^1B_2^1$ , that of the product is ABC. The ABC pattern is indicative of a tri-substituted aromatic moiety and thus mono-substitution by iodine. Although structures I and II are both consistent with this pattern it seems likely that structure II is in fact correct, given a consideration of those positions on the aromatic ring activated towards electrophilic substitution by iodine.

The IR spectrum of the product (Nujol<sup>®</sup> mull) is as follows ( $\nu_{\text{max}}^{\text{NaCl}} \text{ cm}^{-1}$ ): 3322 (O-H stretch), 1600, 1497 (C=C stretch, aromatic), 1403 (N-H bend), 1285, 1250 (asymmetric C-O stretch, aromatic ether), 1181, 1053, (C-O stretch, hydroxyl), 1119 (asymmetric C-O-C stretch, aliphatic ether), 1005 (symmetric C-O-C stretch, aromatic ether), 898, 800, 766 (aromatic C-H bend, out of plane). Comparison of the aromatic portion of the IR spectrum with that reported in the literature (7) for 1,2,4-trisubstituted benzenes, specifically 2,4-dimethyl-anisole (Figure 4A, 4B) strongly suggests substitution in the ortho position by iodine as would theoretically be expected. This is further supported by the different infrared spectral pattern for 3,4-dimethylanisole in the same region (8).

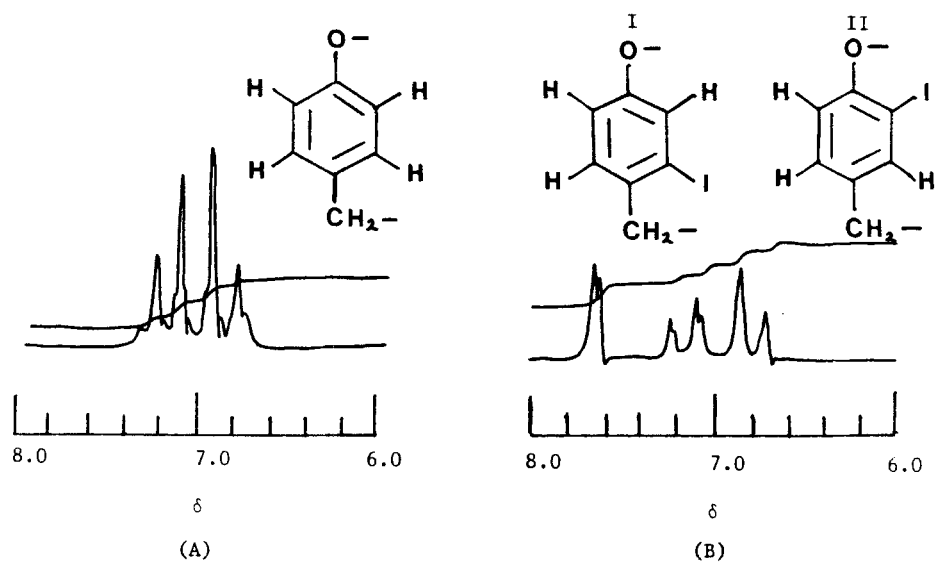


FIGURE 3

PARTIAL 60 MHz NMR SPECTRA OF METOPROLOL

(A) AND IODO-METOPROLOL (B) IN DEUTERATED CHLOROFORM

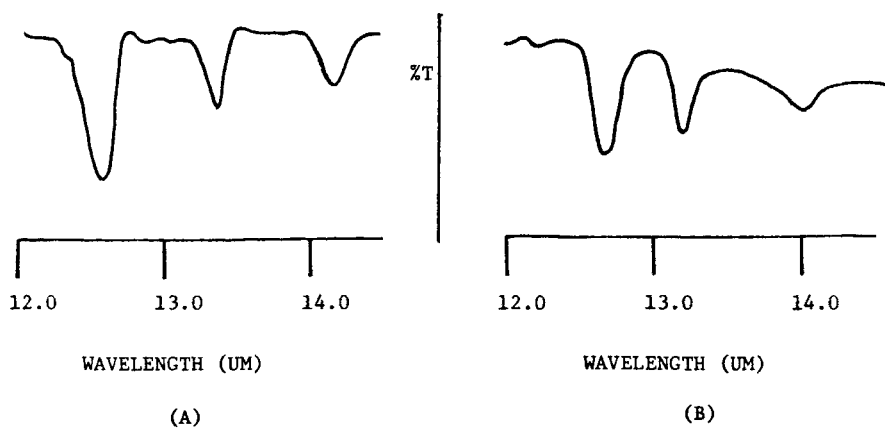


FIGURE 4

PARTIAL IR SPECTRA OF 2,4-DIMETHYL-ANISOLE (A)

AND IODO-METOPROLOL (B) AS A NUJOL MULL



Since the object of this work was the radiodination of metoprolol for characterization purposes only at this stage, with the question of specific radioactivity optimization to be addressed in the future employing a micro-electrochemical cell, precise calculation of the specific activity was not performed. It is however, approximately 26  $\mu\text{Ci}$  per mg of the hydrochloride salt of metoprolol.

All product characterization, with the exception of the radiochromatography and HPLC shown in Figure 2, was carried out on the iodinated metoprolol base or its hydrochloride salt which were shown to be free of non-iodinated metoprolol by HPLC analysis (9).

#### CONCLUSIONS

We have used constant potential electrochemical oxidation of iodine and radioiodide in acetonitrile to produce the iodinating species, N-iodo-acetonitrilium ion as reported previously by Miller *et al.* (6). Subsequent electrophilic substitution of iodine through this species on the cardio-selective  $\beta_1$ -adrenergic blocking agent, metoprolol, has resulted in the production of [ $^{131}\text{I}$ ]-iodo-metoprolol with an estimated 82% chemical yield. The product has been characterized by HPLC-radiochromatography, UV, NMR and IR as the mono-radio-iodo product with substitution in the ortho position by iodine.

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