THE PREPARATION AND PURIFICATION OF 3-(4-IODOPHENOXY)-1-ISOPROPYLAMINO-2-PROPANOL-^{125}I, A BETA ADRENERGIC ANTAGONIST

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SUMMARY

The preparation of an iodine-125 labelled beta adrenergic ligand, 3-(4-iodophenoxy)-1-isopropylamino-2-propanol, suitable for β-adrenergic receptor membrane binding studies is described. Several preparations have been carried out with radiochemical yields of 20-30%. Specific activities in the order of 150 Ci/mmol are readily obtained.

Key Words: Beta Adrenergic Antagonist, Iodine-125

INTRODUCTION

To date most studies aimed at molecular characterisation of β-adrenergic receptors have utilised tritium labelled agonists such as isoprenaline or adrenaline in binding studies to membranes containing β-adrenergic receptors. Although the bindings sites identified were of a uniform nature, their binding characteristics were not what would be expected of intact physiological β-adrenergic receptors (1,2,3). In view of these differences, we and others have been searching for suitably labelled radioactive adrenergic ligands of high specific activity, the binding characteristics of which might reflect all of the properties expected of beta receptors. Recently Mukherjee, et al. (4) and Auback, et al. (5) have reported that dihydroalprenolol-^{3}H and iodohydroxybenzyl-pindolol-^{125}I are two adrenergic ligands suitable for β-adrenergic receptor membrane binding studies. However only the synthesis and

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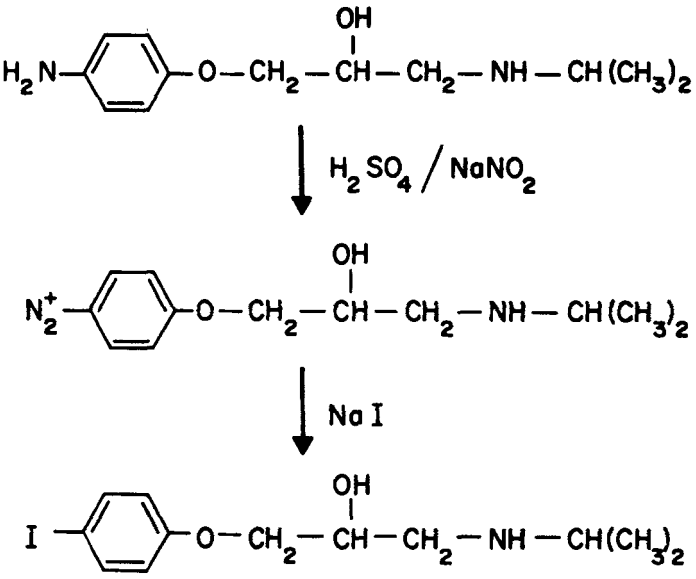
and purification of iodohydroxybenzylpindolol-^{125}I has been reported (6).

We wish to report the synthesis and purification of another beta adrenergic antagonist 3-(4-iodophenoxy)-1-isopropylamino-2-propanol-^{125}I which has also been useful in probing the molecular characteristics of beta adrenergic and its receptors (7,8). Both 3-(4-iodophenoxy)-1-isopropylamino-2-propanol and its iodine-125 derivative are readily synthetisised via the Gattermann reaction (see scheme). Diazotisation of 3-(4-aminophenoxy)-1-isopropylamino-2-propanol

Scheme



is carried out by conventional methods and excess nitrous acid is destroyed by ammonium sulphamate. Complete destruction of excess nitrous acid is imperative to stop oxidation of sodium iodide-^{125}I. Under the conditions of the Gattermann reaction (see methods) another minor basic product was detected in addition to 3-(4-iodophenoxy)-1-isopropylamino-2-propanol and identified by mass spectrometry as 1-isopropylamino-3-phenoxy-2-propanol. It is formed by ammonium sulphamate reduction of the diazonium salt. This product is readily separable from 3-(4-iodophenoxy)-1-isopropylamino-2-propanol by recrystallisation from aqueous methanol or preparative t.l.c. in solvent E (see methods).

The Radiochemical purity of 3-(4-iodophenoxy)-1-isopropylamino-2-propanol-^{125}I was in excess of 98%. The use of sodium iodide-^{125}I means that the beta adrenergic ligand may be labelled to various specific activities up to a theoretical maximum of approximately 2000 Ci/mmol.

MATERIALS AND METHODS

The following materials were obtained from the sources indicated : sodium iodide-^{125}I (Amersham-Searle, carrier free, 1 mCi in ca. 0.02 ml dilute NaOH); 3-(4-aminophenoxy)-1-isopropylamino-2-propanol (ICI, Australia); peroxide free diethyl ether (May and Baker) and all other solvents (reagent grade, distilled prior to use). Copper bronze was activated according to Vogel (9).

Radioactive counting was carried out using a Packard model 5360 autogamma scintillation spectrometer. UV and mass spectra were recorded using a Unicam SP800 spectrophotometer and an EAI Quad 2100 laboratory mass spectrometer run at 70 eV. Melting points were determined using an Electrothermal melting point apparatus and were uncorrected.

Preparative t.l.c. was carried out on 0.5 mm thick plates of silica gel (Kieselgel 60 F_{254}, Merck). Radiochemical purity determinations and purification of 3-(4-Iodophenoxy)-1-isopropylamino-2-propanol-^{125}I were carried out on 0.25 mm thick plates of the same adsorbent. Solvent systems used for chromatography were A, methanol : ammonia (100:0.5); B, butanol: acetic acid:water (85:10:5); C, benzene:dioxan:ammonia:water (62.5:35:0.5:2); D, chloroform: propanol:ammonia (75:25:0.1); E, hexane:morpholine (100:9). For the determination of radiochemical purity, the radiolabelled sample were spotted both on top of and parallel to unlabelled carrier on the t.l.c. plate. After development and visualisation under ultra violet light (245 nm), 1 cm bands were cut and their radioactivity determined.

3-(4-Iodophenoxy)-1-isopropylamino-2-propanol. 3-(4-Aminophenoxy)-1-isopropylamino-2-propanol (2.25 mmol) was dissolved in 10% sulphuric acid (10 ml) at 1-5°C. To this solution was added 10% sodium nitrite (~4.6 mmol) over 15 min until excess nitrous acid could be detected with starch-iodide indicator. After 10 min excess nitrous acid was destroyed with 10% ammonium sulphamate solution (~3 mmol, no nitrous acid could be detected by starch-iodide indicator). To

the resultant solution a 50% sodium iodide solution (2.5 mmol) and copper bronze (0.30 g) were added and the resultant solution heated in a boiling water bath for 15 min or until all the diazonium salt had decomposed.

On cooling the reaction mixture was made alkaline (pH ∿13) with 10% sodium hydroxide solution and extracted with ether (2 x 30 ml). The ethereal extract was washed with water (2 x 10 ml) and re-extracted with 1M hydrochloric acid (10 ml). The acid solution was then made basic (pH ∿ 13) with 10% sodium hydroxide solution and re-extracted with ether (2 x 20 ml). The ethereal layer was washed with water (10 ml), dried over anhydrous sodium sulphate and after evaporation in vacuo yielded a solid which was purified initially by preparative t.l.c. in solvent A. The product was eluted from the t.l.c. plate with methanol and recrystallised from aqueous methanol. Alternatively, analytical purity of the product could also be obtained by preparative t.l.c. in solvent E (3 x 15 cm.) Yield 55% mp 98-100 ^{o}C. Mass Spectrum (70 eV): m/e (relative abundance) 335 (M^{+}, 37), 334 (40), 320 (23), 290 (36), 233 (16), 220 (100), 203 (28), 191 (28), 161 (34). UV spectrum (0.1N HCl): λ_{max} (279 nm), (ε 1194). Anal. Calcd. for $C_{12}H_{18}NO_2I$: C 43.0; H 5.37, N 4.18; Found C 43.3; H 5.38; N 3.80.

3-(4-Iodophenoxy)-1-isopropylamino-2-propanol-^{125}I. 3-(4-Aminophenoxy)-1-isopropylamino-2-propanol (20 µmol) was dissolved in 10% sulphuric acid (0.25 ml) at 0-5^{o}C. To this solution was added cold 10% sodium nitrite solution (∿45 µmol) and the solution left to stand for 20 min at 1-5^{o}C. Excess nitrous acid was destroyed by 10% ammonium sulphamate solution (until no excess of nitrous acid could be detected by starch-iodide indicator). To the resultant solution sodium iodide-^{125}I (1 mC, 7 nmol) and copper bronze (0.05 g) were added and the resultant solution heated on a boiling water bath for 15 min. On cooling the resultant solution was made alkaline (pH 10-13) with 10% sodium hydroxide solution and extracted with ether (2 x 10 ml). The ethereal extract was washed with water (2 x 5 ml) and re-extracted with 1M hydrochloric acid (1 ml). The pH was readjusted to 13 with 10% sodium hydroxide solution and re-extracted with ether (10 ml). After washing the ethereal layer with water (3 ml), evaporation of the ether was carried out at room temperature in a stream of nitrogen gas.

The residue was dissolved in methanol (500 µl), applied to a preparative t.l.c. plate and chromatographed (18 cm) in solvent A. The product at Rf 0.82 was eluted from the t.l.c. plate with methanol, concentrated by evaporation at room temperature in a nitrogen stream and rechromatographed (t.l.c.) in solvent E (3 x 15 cm). 3-(4-Iodophenoxy)-1-isopropylamino-2-propanol-^{125}I at Rf 0.70 was eluted from the t.l.c. plate with methanol and the methanolic extract evaporated to dryness at room temperature in a nitrogen stream. The residue is redissolved in 0.1N HCl (0.1 ml) and stored at -20°C for up to 3 weeks.

The yield of 3-(4-iodophenoxy)-1-isopropylamino-2-propanol-^{125}I based on the total radioactivity added to the reaction mixture and that found in the final product was calculated to be 20-30% over five syntheses. Specific activity of the iodinated beta adrenergic blocker was in the order of 150 Ci/mmol. The iodine-125 product when chromatographed in solvents A,B,C & D migrated as a single radioactive peak with Rf values of 0.82, 0.37, 0.30 and 0.36 respectively. In all cases it co-chromatographed with 3-(4-iodophenoxy)-1-isopropylamino-2-propanol.

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