THE SYNTHESIS OF TRITIUM LABELED CARDIOSELECTIVE BETA-ADRENOCEPTOR ANTAGONISTS

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SUMMARY

Two cardioselective beta-adrenoceptor antagonists: dl-acetanilide-4'-[3-(isopropylamino)-2-hydroxypropoxy] ("practolol"), and dl-chloroacetanilide-4'-[3-(isopropylamino)-2-hydroxypropoxy] ("chloropractolol"), labeled with tritium at positions 3' and 5' of the aromatic ring were prepared. The starting materials for the synthesis of the aryloxypropanolamines was 2,6-di-^{3}H-4-acetamido phenol, prepared by catalytic dehalogenation of 2,6-dibromo-4-acetamido-phenol, employing tritium gas. Condensation of 2,6-di-^{3}H-4-acetamido-phenol with epichlorohydrin, followed by epoxide ring opening with isopropylamine yielded 3',5'-di-^{3}H-acetanilide-4'-[3-(isopropylamino)-2-hydroxypropoxy], (3',5'-di-^{3}H-practolol). Deacetylation of 3',5'-di-^{3}H-practolol followed by selective ar-N chloroacetylation of the resulting 1-(2',6'-di-^{3}H-4'-amino)-phenoxy-3-isopropylamino-propan-2-ol, gave 3',5'-di-^{3}H-chloropractolol with a specific activity of 750 mCi/mmol.

Keywords: Practolol, chloropractolol, ^{3}H-labeled-synthesis.

INTRODUCTION

dl-Chloroacetanilide-4'-[3-(isopropylamino)-2-hydroxypropoxy] ("chloro-practolol") is a new, selective and irreversible myocardial (beta-1) adrenoceptor antagonist[1]. It was prepared by incorporating an alkylating function into the chemical structure of a clinically useful

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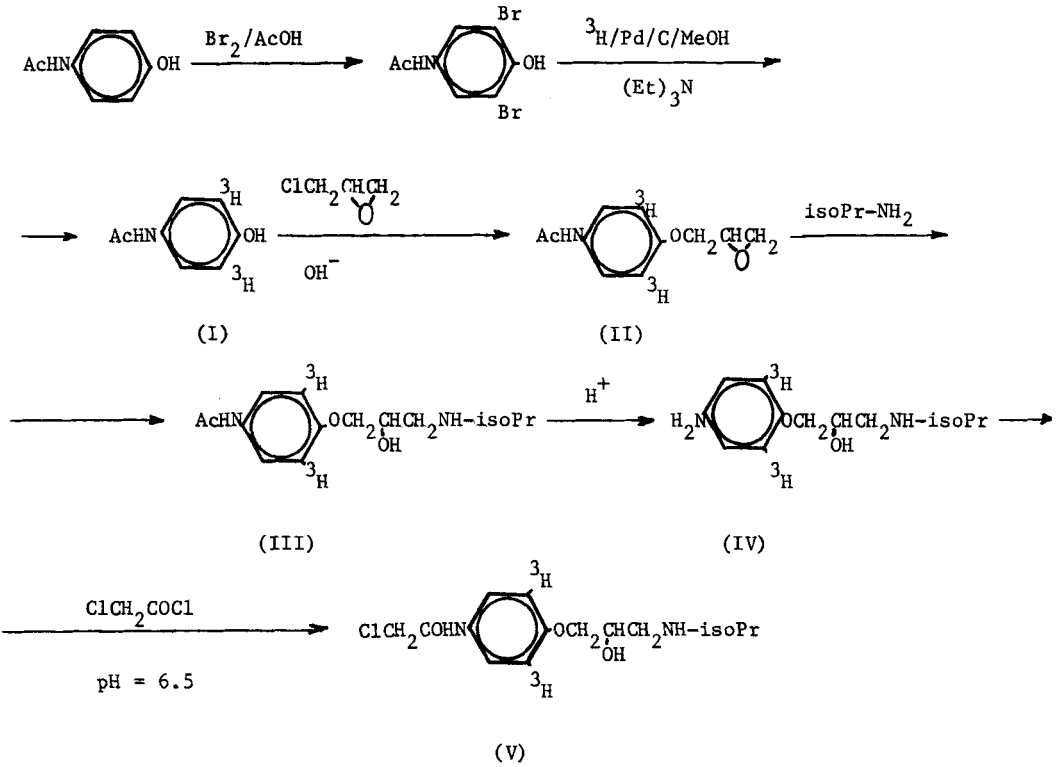
drug - "practolol", with the intention of producing a site-directed, covalently bound label of the beta-1 adrenoceptor[1]. "Practolol" (dl-acetanilide-4'-[3-(isopropylamino)-2-hydroxypropoxy]) itself, is a competitive beta-adrenoceptor antagonist, selectively blocking beta-1 (myocardial) adrenoceptors sub-type[2]. "Practolol" is used to treat patients with angina pectoris[3], and its property of selectively blocking beta-1 adrenoceptors has been claimed to reduce the incidence of bronchospasm in patients with bronchial asthma and obstructive pulmonary disease[4].

The next step towards direct labeling of the beta-1 adrenoceptor in its native state, was to prepare a highly radioactive "chloropractotol". The detailed synthetic procedures leading to 3'5'-di-^{3}H-chloropractolol (V) are reported. The synthesis of 3',5'-di-^{3}H-chloropractolol (V), shown in Scheme I, was designed to incorporate tritium in positions 3'-and 5'- of the aromatic ring, thus assuring retention of the label during subsequent biological studies. The general synthetic approach also allows for the handling of radioactive materials in small quantities in conventional apparatus. The reaction between phenols and epichlorohydrin under mild basic conditions to give the corresponding 3-phenoxy-1,2-epoxypropanes, proceeds smoothly and in good yields. Care should be taken to employ an excess of epichlorohydrine so as to avoid the formation of bis-1,3-aryloxypropan-2-ols[5]. Subsequent opening of the epoxide ring with a large excess of isopropylamine, also proceeds under mild conditions to give the desired aryloxypropanol amine in good yield. In this step again, a large excess of one of the reactants the primary amine, should be used to suppress further reaction of the secondary amino-alcohol product with another molecule of the epoxide[6]. Deacetylation of 3',5'-di-^{3}H-practolol (III) by acid hydrolysis was practically completed after two hours of gentle refluxing in EtOH-HCl. The deacetylation product-(dl)-1-(2',6'-di-^{3}H-4'-amino phenoxy)-3-isopropylamino-propan-2-ol (IV) - was chloroacetylated at a low temperature, and with efficient stirring, by an excess of chloroacetylchloride, in a phosphate buffer at a pH range of 6.0-6.5. Under these reaction conditions exclusive acetylation of the anilino nitrogen

occurred, in good yield. Tritium labeled intermediates and final product (I-V) are stable and remain chemically and radiochemically pure after cold storage (4°C) for at least several months in dilute, (2 mC/ml), aqueous solution.

In conclusion, a simple, multistep procedure for preparing aryloxypropanolamine type of beta-adrenoceptor blocking agents, tritiated in specific positions of the aromatic nucleus, is outline. The synthetic procedures are suitable for either large-scale, or ultra-high specific activity preparations.

Synthetic scheme:



EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. Infra red spectra were recorded in KBr discs on a Perkin-Elmer grating infra red spectrophotometer Model 257. UV spectra

were recorded in 0.1M phosphate buffer, pH 7.60, on a varian Techtron UV-Vis spectrophotometer, Model 635. TLC's were performed on aluminum oxide precoated plates, containing fluorescent indicator (AL F, 0.25mm, Riedel-De Haen AG, Germany). Plates were developed in the following solvent systems:

1) Chloroform - methanol (5:95 v/v).

2) Chloroform - methanol (15:85 v/v).

3) n-BuOH-AcOH-H_2O (4:2:1 v/v).

Spots were detected by UV light at 254 nm, and by exposure to I_2 vapor. Radiochemical purity was checked both by radiochromatogram scan of the TLC plates (Berthold LB 2722 Dunnschicht Scanner II), and by reverse dilution analysis. Total and specific activities of the tritiated compounds were measured by liquid scintillation (Packard Tri-Carb liquid scintillation spectrometer, Model 3375). Analytical results for C, H, N were within $\pm$ 0.2% of the theoretical values for all new compounds (II-V). UV and IR spectra of tritiated compounds were matched with those of authentic samples, and were found identical. Mixed m.p.'s of tritiated and corresponding non-tritiated compounds were unchanged.

2,6-Dibromo-4-acetamidophenol: This compound was prepared according to Heller and Soldner[7], by direct bromination of 4-acetamidophenol in glacial acetic acid. M.p. = 188° (H_2O) (lit.[7] m.p. = 188°).

2,6-Di-^{3}H-4-acetamidophenol (I):

To 50 mg (0.16 mmol) of 2,6-dibromo-4-acetamidophenol in 0.5 ml of methanol, were added 17.4 mg Pd/c (10%) and 50 µlit of triethylamine. The suspension was stirred at room temp. and 40 Ci of tritium gas were introduced into the reaction vessel. The catalytically induced tritium displacement of the two bromine atoms was completed after $^1/_2$hr., resulting in the absorption of 22 Ci of tritium. At the end of the reaction, the system was cooled (N_2), excess of tritium-removed, and the system was then allowed to return to room temp. The solvent was evaporated and the reaction mixture was washed twice with methanol to remove labile tritium. Finally, the suspension was filtered and the filtrate evaporated under reduced pressure. The residue - 2,6-di-^{3}H-4-acetamido-phenol (21.7 mg, 0.14 mmol, specific

activity: 20.7 Ci/mmol), was found to be over 95% chemically and radiochemically pure, and was therefore used without further purification. Chemical yield: 86%, Rf: 0.84 (solvent system-1).

dl-1-(4'-Acetamido-2',6'-di-^{3}H-phenoxy)-2,3-epoxy-propane (II):

The tritiated 4-acetamidophenol (I) (21.7 mg), was diluted with "cold" 4-acetamidophenol (400.3 mg), and the resulting mixture (422 mg, 2.79 mmol, spec. act.: 1.04 C/mmol) was dissolved in 10 ml NaOH (0.5 N), at room temp. The slightly turbid solution was filtered and the filtrate, added dropwise, at room temp. into a stirred, methanolic solution (15 ml methanol) of epichlorohydrin (100 mmol). After overnight stirring at room temp. and following a drop in the pH from an initial value of >11 to ca.6, the reaction mixture was evaporated in vacuo to remove the methanol and excess of epichlorohydrin. The residue was taken into ethylacetate, and the extract washed with H_2O and dried ($MgSO_4$). Evaporation of the dried extract and trituration of the resulting oily residue with ether gave 1-(4'-acetamido-2',6'-di-^{3}H-phenoxy)-2,3-epoxypropane (368 mg, 1.78 mmol), chemically and radiochemically over 98% pure. M.p. = 115-116°.
Chemical yield: 64%. Crystallization of a test sample from isopropanol, raised the m.p. of theproduct to 118°. Rf: 0.78 (solvent system-2).

dl-Acetanilide-3',5'-di-^{3}H-4'-[3-(isopropylamino)-2-hydroxypropoxy]; 3',5'-Di-^{3}H-practolol (III):

dl-1-(4'-Acetamido-2',6'-di-^{3}H-phenoxy)-2,3-epoxypropane (II) (350 mg, 1.69 mmol. >98% pure) dissolved in isopropanol (35 ml), was added slowly into a large excess of isopropylamine (ca. 400 mmol), while stirring, at room temp. The reaction mixture was left overnight and then evaporated to dryness in vacuo. The resulting 3',5'-di-^{3}H-practolol (400 mg, 1.5 mmol), was chemically and radiochemically over 98% pure. M.p. = 134.5 - 137°. Chemical yield: 89%. Crystallization of a test sample from methyl-ethyl-ketone raised the m.p. of the product to 138.5 - 140°. (lit.[6] m.p. = 142 -143°). Rf: 0.76 (solvent system-3).

dl-1-(4'-Amino-2',6'-di-^{3}H-phenoxy)-3-isopropylamino-propan-2-ol (IV):

Deacetylation of 3',5'-di-^{3}H-practolol (370 mg, 1.39 mmol, >98% pure),

was carried out in EtoH (12 ml)-HClconc. (6 ml) mixture, under gentle reflux. Within 1 hr. ca. 80% of the practolol were deacetylated and after 2 hrs. the reaction was completed, as judged both by thin-layer chromatography and by IR spectrometry (complete disappearance of the typical C = O stretching band of sec. amide at 1,660 cm^{-1}). The reaction mixture was evaporated in vacuo and the residue washed with a small volume of EtOH, filtered, and finally washed with dry ether. The resulting product 1-(4'-amino-2',6'-di-^{3}H-phenoxy)-3-isopropylamino-propan-2-ol, di-HCl, (331 mg, 1.11 mmol), was chemically and radiochemically over 99% pure. M.p. = 225-228°. Chemical yield: 80%. Crystallization of a test sample from EtOH-AcOEt, raised the m.p. of the product to 230-232°. Rf: 0.65 (solvent system-3).

dl-Chloroacetanilide-3',5'-di-^{3}H-4'-[3-(isopropylamino)-2-hydroxypropoxy]; 3',5'-Di-^{3}H-chloropractolol (V):

Selective chloroacetylation of the anilino function of 1-(4'-amino-2',6'-di-^{3}H-phenoxy)-3-isopropylamino-propan-2-ol, di-HCl (297 mg, 1.0 mmol; >99% pure), was carried out in phosphate buffer (0.1 M, pH = 6.5, 25 ml), at a temp. range of 5-10°C (ice-water cooling), by dropwise addition of chloroacetylchloride (technical grade, 1 ml), and efficient stirring. The pH of the reaction mixture which drops to ca. 1, is subsequently brought up to 6.5 by the addition of NaOH-3N.

The chloroacetylation procedure is repeated once more, and the reaction mixture is then left at pH = 6.5 , while being stirred for 1 hr at 5-10°, and an additional hour at room temp. After filtration of the reaction mixture, the cooled filtrate is alkalinized by NaOH-3N to pH of about 10-11. The alkaline solution is then left in an ice bath for ca. 15 min by which time the chloroacetylated product has completely crystallized out as colorless glistering plates. The crystalline product is filtered, washed with a small quantity of cold water, and dried at room temp. in vacuo. The dried product (224 mg, 0.75 mmol; M.p. = 118-120°; >98% chemically and radiochemically pure; chemical yield: 74.5%), is dissolved in ethanol saturated with HCl (10 ml), and the solvent is then removed by evaporation under reduced pressure. The residue, the HCl salt of 3',5'-di-^{3}H-chloropractolol is crystallized to a

constant specific activity (twice) from a mixture of ethanol, aceton, and ether. The final product - dl-3'5'-di-^{3}H-chloropractolol hydrochloride (106 mg, 0.35 mmol; chemical yield: 31.5%) is over 99% pure chemically and radiochemically. M.p. = 147-149° (lit.[1] m.p. = 147-148.5°C). Rf: 0.72 (solvent system-3). UV: λmax 247 nm (ε = 9,900). Specific activity: 750 mCi/mmol. Overall chemical yield (starting from 2,6-di-^{3}H-4-acetamidophenol): 14%.

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