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The Preparation and Purification of Labelled β-Adrenergic Blocking Agents

Part I. Synthesis of carbon-14 and tritium labelled 1-isopropylamino-3-(1-naphthyloxy)-propan-2-ol hydrochloride, (propranolol hydrochloride, Inderal*)

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SUMMARY

The preparation of ^{14}C-propranolol hydrochloride from 1-naphthol-1-^{14}C is described. Several preparations of the compound have been carried out with overall radiochemical yields of 17 %-21 %. Specific activities of 3.51 μCi/mg, 8.8 μCi/mg, and 23.2 μCi/mg have been obtained.

Propranolol has also been tritium labelled non specifically by catalytic exchange with tritiated acetic acid. After extensive purification pure material with a specific activity of 940 μCi/mg was obtained.

INTRODUCTION.

Propranolol was the first beta-adrenergic receptor antagonist to be used clinically on a wide scale for the treatment of cardiac arrythmias, angina pectoris and hypertension. ^{14}C and ^{3}H labelled propranolol were required for a study of its metabolism in man [1, 2] and for metabolic [1, 3] and distributive studies including whole body autoradiography [4] in animals. The ^{14}C material was prepared by the route indicated in the scheme. The ^{3}H labelled propranolol

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was obtained by heating pure unlabelled propranolol in 70 % ^{3}H acetic acid (containing at least 100 curies of activity) at the boil for 20 hr. This process was carried out at the Radiochemical Centre Amersham, England. The crude material produced by this method required extensive purification.

Materials.

Sulphur free toluene (May and Baker Ltd.) was used without further purification. Merck Silica GF and HF was obtained from Andermanns Ltd. The 2,5-diphenyloxazole (PPO) and 1,4-bis(4-methyl-5-phenyl oxazole) benzene (DMPOPOP) were purchased from Packard Instrument Ltd. Wembley; naphthalene (scintillation grade) was obtained from Thorn Electronics Ltd., Tolworth. The 1-naphthol 1-^{14}C was purchased from the Radiochemical Centre, Amersham.

Commercial dioxan was purified by the method of Vogel [5]. All samples were counted on a Packard Tri Carb Liquid Scintillation spectrometer Model 314; the sample containers were standard 20 ml glass screwcap vials of low potassium content (Packard Instrument Ltd., Wembley). Colloidal silica (Aerosil) was obtained from Buch, Beach, Segner Bayley Ltd. The photographic film used for autoradiography of ^{14}C isotopes was 'Ilfex' X-ray film (Ilford Ltd., Essex, England), and Kodak Royal Blue X-ray film was used to fluorograph tritium. All solvents used were either redistilled or of analytical reagent quality.

Experimental.

^{14}C Labelled Propranolol Hydrochloride.

^{14}C-1-Naphthyl glycidyl ether (1,2-epoxy-3-(1-naphthyloxy)propane).

1-Naphthol-1-^{14}C (72.7 mg) with a specific activity of 2.37 mCi/mmole was diluted with inactive freshly sublimed 1-naphthol (71.3 mg) and added to a stirred solution of sodium hydroxide (47 mg) in water (1.0 ml). The mixture was stirred for 30 min. until all the 1-naphthol had dissolved, then epichlorohydrin (0.15 ml) was added, and the reaction mixture was stirred at 27° C for 20 hr.

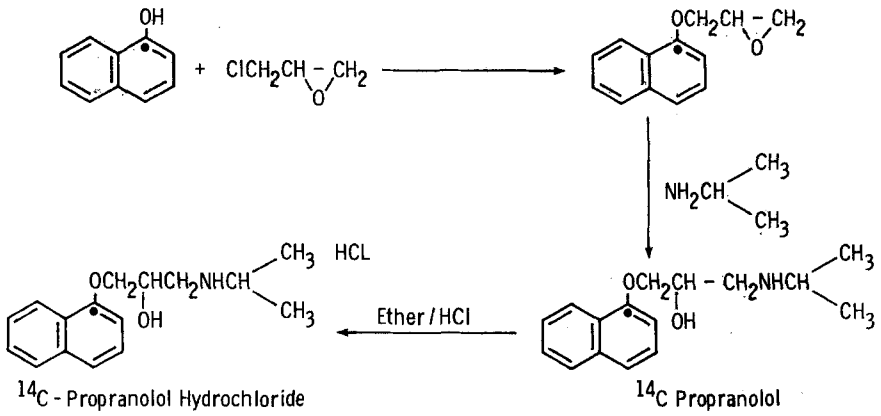
The mixture was extracted with chloroform (5 × 5.0 ml), and the total chloroform extracts were combined, washed with 2N acetic acid (2 × 5.0 ml) and with distilled water (2 × 5.0 ml), before being dried over anhydrous sodium sulphate for 16 hr. The chloroform extract, after evaporation to dryness under reduced pressure at 50° C, was transferred to the reaction tube for the next stage and then dried overnight under reduced pressure, to give a brown oil (crude yield 196 mg).

The product was examined by thin layer chromatography (T.L.C.) using silica GF developed with petroleum-ether (b.p. 60-80° C) ethyl acetate (90 : 10).

When detected under UV 2540 Å the chromatographic pattern was shown to be identical with that of cold material which had been distilled at 140° C and 0.1 mm. Comparison of the UV and autoradiographic patterns showed that all the impurities were labelled.

^{14}C-Propranolol.

A solution of ^{14}C-1-naphthyl glycidyl ether (196 mg) in isopropylamine (1.0 ml) was heated under reflux for 2 hr. The mixture was then kept at 50° C whilst excess isopropylamine was removed by evaporation in a current of dried air, the final traces being removed under reduced pressure. To the residue 2N-hydrochloric acid (1.0 ml) was added, the mixture was stirred for 20 min., and the non basic impurities were removed by extraction with diethyl ether saturated with 2N hydrochloric acid (4 × 5.0 ml). Sodium hydroxide solution 50 % w/v (0.48 ml) was added to the aqueous phase, and the solution was stirred at 0° C for 1 hr. The reaction mixture was then extracted with diethyl ether saturated with sodium hydroxide solution 50 % w/v (8 × 5.0 ml). The combined ether extracts were washed with distilled water (2 × 2.0 ml), and dried over anhydrous sodium sulphate for 16 hr. The extract was evaporated to dryness under reduced pressure at 30° C, to give crude propranolol (Yield 103 mg).



Scheme

Examination by T.L.C.

The crude base was chromatographed on silica GF developed with ethanol-ammonia (0.880)-water (80 : 4 : 5); examined under UV 2540 Å and autoradiographed for 16 hr. (Fig. 1*a*).

Purification of ^{14}C-propranolol.

A column of 1.7 cm diameter was prepared from 10 g of silica HF and eluted with ethanol-cyclohexane (60 : 40). The crude product was dissolved in the mobile phase (4.0 ml). and applied to the column; 64 × 5.0 ml fractions were collected, and 5 μl aliquots of alternate fractions were spotted on a silica GF plate and developed with ethanol-ammonia (0.880)-water (80 : 4 : 5). The plates were run for 10 cms, dried, examined under UV 2540 Å and autoradiographed for 16 hr. The appropriate fractions containing one spot material with identical R_f to that of the pure reference compound were combined and evaporated to dryness under reduced pressure from a water bath at 30° C. Traces of silica were removed by the centrifugation of hot cyclohexane extracts. The removal of the solvent under reduced pressure (bath at 50° C) gave a light brown solid (63 mg) m.p. 88-92° C.

This material was chromatographed on a silica GF plate, developed with ethanol-ammonia (0.880)-water (80 : 4 : 5), examined under UV 2540 Å and autoradiographed for 16 hr. An identical chromatographic pattern was

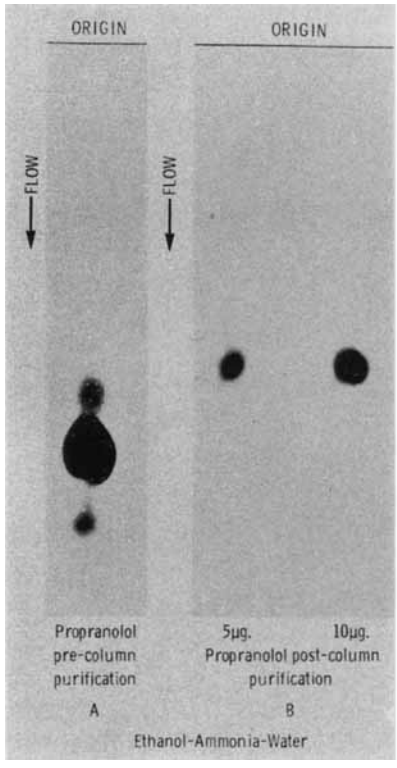


FIG. 1.

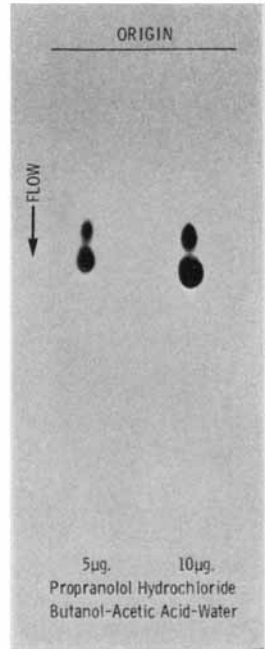


FIG. 2.

shown by both means of detection (Fig. 1*b*). The combined fractions of propranolol on chromatographic examination showed one spot material.

Conversion to ^{14}C-propranolol hydrochloride.

The propranolol was dissolved in sodium dried ether (2.5 ml), to which was added anhydrous ether saturated with hydrogen chloride gas (2.0 ml). The gum obtained hardened to a white solid, m.p. 161-3° C, on cooling. The product was recrystallised from methanol-ethyl acetate and dried at 20-24° C under reduced pressure to give white needles (59 mg), (Found; C, 64.8; H,7.2; N,4.2. $C_{16}H_{22}O_2NCl$ requires C, 64.5; H, 7.4; N,4.7) representing an overall chemical yield of 19.8 %.

The product was examined by T.L.C. in the same system as that employed for chromatography of the base and autoradiographed for 16 hr. The autoradiograph was used to "map" the plate which was then segmented, each segment being counted in dioxan-naphthalene-silica phosphor, when a minimum radiochemical purity of 99.5 % was determined. Mass spectrometry showed no detectable impurities. The specific activity was 3.51 μCi/mg (1037 μCi/mmole) which represented an overall radiochemical yield of 17.2 %.

A second preparation of this compound was carried out to produce material of a much higher specific activity (23.2 μCi/mg = 6,855 μCi/mmole). The scale of the experiment was reduced to 0.5 mmole. The crude propranolol before converting to the hydrochloride was purified by preparative T.L.C. using a silica GF plate of 0.5 mm thickness, developed with ethanol-ammonia (0.880)-water (80 : 4 : 5), 25.67 mg were obtained. An overall radiochemical yield of 19.8 % was achieved.

Thin Layer Chromatographic Behaviour.

When propranolol-^{14}C hydrochloride was chromatographed on silica GF, developed with *n*-butanol-acetic acid-water 40 : 10 : 5 and autoradiographed, double spot formation was observed (Fig. 2). This 'double spot' formation was also obtained with unlabelled material when it was run at high concentrations in butanol-acetic acid-water and viewed under UV 2540 Å (Fig. 3.1).

Fig. 3.2. If the propranolol hydrochloride solution was applied to the plate and then overspotted with concentrated hydrochloric acid before development, the lag spot was converted into lead spot.

Fig. 3.3. Conversely if propranolol hydrochloride was left in contact with the developing solvent and then applied to the plate it was found on development that only lag spot material was produced.

Fig. 3.4. Overspotting of this solution with concentrated hydrochloric acid converted all the lag spot material to lead spot.

Fig. 3.6. If some of the propranolol hydrochloride solution was spotted onto the plate and overspotted with glacial acetic acid before development, an increase in the lag spot concentration was observed.

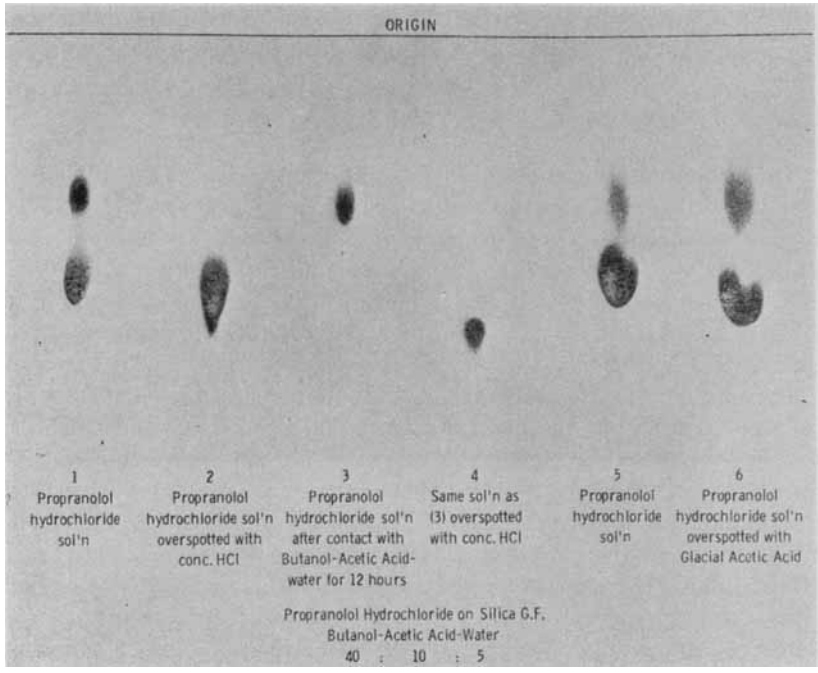


FIG. 3.

It therefore appears that propranolol hydrochloride eluted in a weakly acidic system formed an "ion pair" to give base-hydrochloride as the lead spot and base-acetate as the lag spot.

Tritium labelled propranolol.

Propranolol base (1.0 g) which had been purified by successive recrystallisation, was tritiated at the Radiochemical Centre, Amersham, England by their TRI process, and the tritiated solvent together with the labile tritium was removed. The product (0.5 g) was dissolved in ethanol and shown to contain 7.0 curies of activity.

Three T.L.C. systems were employed to examine this product.

1. Silica GF developed with Ethanol-Ammonia-Water 80 : 4 : 5
2. Silica GF developed with *n*-Butanol-Acetic Acid-Water 40 : 10 : 5
3. Silica GF developed with Ethanol 74 OP-Water 88 : 12

Examination of this solution by T.L.C. on system 1 followed by autoradiography showed that much of the radioactivity was due to N-acetyl propranolol. Some O-acetyl propranolol was also shown to be present using system 3.

Systems 1 and 2 separated propranolol and the N-acetyl propranolol but propranolol had an identical R_f to its O-acetyl derivative. They were however separated on system 3.

Thin layer electrophoresis followed by autoradiography confirmed that the major source of radioactivity in the sample was N-acetyl propranolol. This accounted for the high activity in the preparation after preliminary purification from the tritiation reaction mixture. Alkaline hydrolysis of the tritiated product was followed by resolution of the mixture by chromatographic separation on a silica column and by preparative thin layer chromatography. This purification process produced material having a specific activity of 940 μCi/mg, and with a minimum radiochemical purity of 98 %.

Thin Layer Electrophoresis was carried out on a silica thin layer with pyridine-acetic acid buffer at pH 5.4 (19 mA at 400 volts). Autoradiography followed by plate segmentation and liquid scintillation counting showed that 83 % of the radioactivity present was in the form of N-acetyl propranolol. Propranolol and O-acetyl propranolol were also shown to be present. This was confirmed by T.L.C. in system 3.

Hydrolysis.

The ethanol solution of crude ^{3}H-propranolol was evaporated to dryness under reduced pressure (bath at 30-40° C). To the residues was added an aqueous solution of 2.5 N sodium hydroxide (25 ml) and the reaction mixture was stirred at 22° C for 20 hr. The crude hydrolysate was extracted with diethyl ether (5 × 50 ml), and the combined extracts were washed with water (3 × 10 ml). The washed extract was extracted with N-hydrochloric acid (4 × 15 ml), which was basified with aqueous sodium hydroxide solution before extracting it with diethyl ether (4 × 50 ml). The combined ether extracts were dried for 16 hr. over anhydrous magnesium sulphate. An aliquot of the extract was chromatographed on silica GF plates developed with system 1 and showed material of the same R_f as propranolol together with five impurities, all of which were shown by autoradiography to be radiolabelled.

The crude extract was evaporated to dryness under reduced pressure at 30° C and applied to a silica GF column (2.5 cm diameter) prepared from silica GF (10.0 g) using ethanol-cyclohexane (60 : 40) as the mobile phase. The column was washed with the eluting solvent for 24 hr. prior to the introduction of the extract. Aliquots (5 μl) of alternate eluate fractions (2.0 ml) were chromatographed on silica GF plates developed with the same solvent and autoradiographed for 110 hr. The residue from the combined fractions gave crude propranolol (42 mg).

The product was further purified by preparative T.L.C. on a plate coated with a layer of silica HF 0.5 mm thick, and developed with system No. 1. The plate was dried and autoradiographed, and the area corresponding in R_f to propranolol was removed and extracted in a Soxhlet thimble with methanol for 16 hr. The extract on evaporation under reduced pressure at 30° C produced a residue which was triturated with boiling absolute ethyl alcohol, and the combined extracts were evaporated to dryness. The identity of the product was confirmed by T.L.C. in system 3 which separates O-acetyl propranolol

from propranolol and also by infra red spectroscopy. Yield 25.89 mg of specific activity 940 μCi/mg. The radiochemical purity was determined by chromatographing on a silica GF plate, developed in system 1, followed by autoradiography and liquid scintillation counting in a thixotropic phosphor, when the purity was shown to be a minimum of 98 %.

Fluorography.

A more rapid method for autoradiographic studies of tritium labelled compounds is the fluorographic technique described by Luthi and Wasser [6]. The tritium labelled propranolol was examined by this method. Five plates (20 × 20 cm) were prepared from Merck silica GF (15 gm) which had been ball milled for 16 hr. with anthracene (15 gm) in ethanol (96 %, 100 ml). The plates were dried at 30° C for 16 hr. before use. The R_f value of this compound on a mixture of silica and anthracene in equal proportions was not significantly different from that on silica alone. A concentration plate with a range of concentration from 2-30 μg was prepared and developed with solvent system 1.

After development the plate was covered with X-ray film (Kodak Royal Blue), placed in a hinged light proof aluminium box and immersed in dry ice at −70° C for 16 hr. The fluorograph was developed as for a normal autoradiograph and 'one spot' material at all concentrations was obtained.

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