Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXV, No. 9

THE SYNTHESIS OF BETAXOLOL LABELLED WITH TRITIUM AT HIGH SPECIFIC ACTIVITY

John ALLEN* and André TIZOT

Laboratoires d'Etudes et de Recherches SYNTHELABO (L.E.R.S.)

Department of Chemistry

Labelled Compounds and Metabolite Synthesis Group

31, avenue Paul Vaillant-Couturier

92220 BAGNEUX (FRANCE)

SUMMARY

The synthesis of betaxolol, a new β-blocking agent, labelled with tritium in high specific activity (49 Ci/mmol) is described. The tritium was incorporated into the molecule via a catalytic reductive debromination of the 2,6-dibromo-betaxolol precursor in the presence of tritium gas. This labelled compound has been used in receptor binding studies and also in studying the release of betaxolol from rat atrial slices on electrical stimulation.

Key words : Betaxolol, β-Blocking Agent, Tritium.

INTRODUCTION

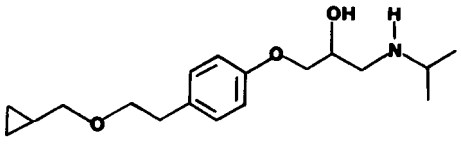
Betaxolol (KERLONE®)[1,2] (1-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-3-(isopropylamino)-2-propanol hydrochloride) (1) is a new cardioselective β_1-adrenoceptor antagonist which is currently being used in the treatment of essential hypertension[2,3,4] and in glycauma[5]. As part of our development programme of this drug the compound has been labelled with carbon-14 for pharmacokinetic, drug metabolism and protein binding studies[6,7], with deuterium

* *To whom correspondence should be adressed.*

0362–4803/88/090931–07$05.00

Received January 12, 1988

in order to determine the levels of betaxolol in biological fluids by GC-mass spectrometry[8], and with carbon-13 for bioavailability studies[7]. The syntheses of these compounds have also been discussed in a general review on the labelling of β-blockers with stable and radioactive isotopes[9]. Betaxolol labelled with tritium at high specific activity was also required, for use in receptor binding studies. This labelled compound has also proved extremely useful in studying the release of betaxolol from rat atrial slices on electrical stimulation[10].



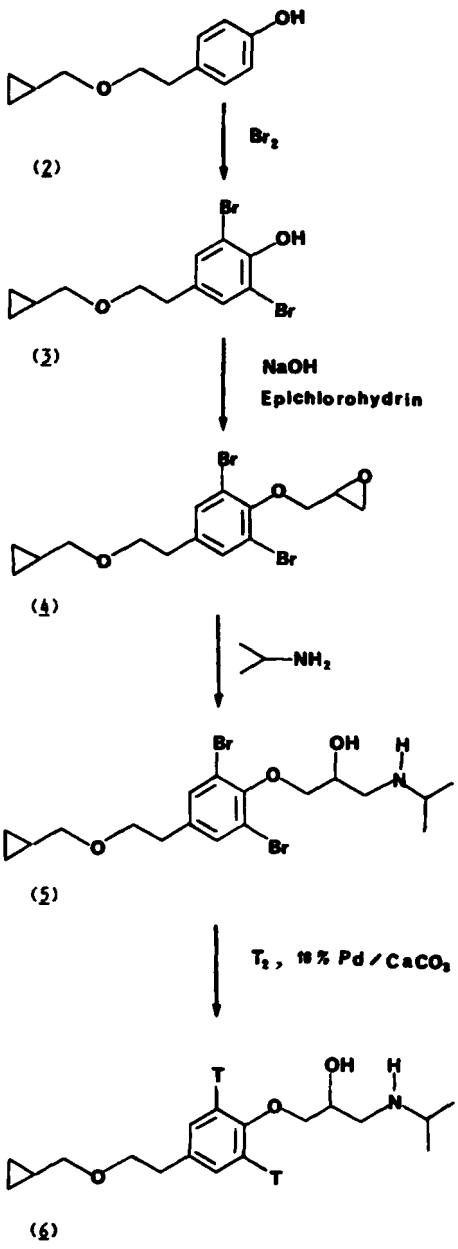
(1) HCl

DISCUSSION

A general tritium labelling procedure for β-blockers having an isopropylaminohydroxypropoxy side-chain has been described in the literature[11]. Unfortunately this method has been used to obtain compounds having relatively low specific activities. For the receptor binding and mechanism of action studies a compound labelled in high specific activity was required. An examination of the chemical structure of betaxolol led us to the opinion that in order to achieve these higher specific activities it was necessary to synthesise a ring-labelled compound. Direct bromination failed to yield any dibromobetaxolol and so this precursor was synthesized and on catalytic reductive debromination in the presence of tritium gas afforded [^{3}H]-betaxolol in high specific activity as shown in Scheme 1.

4-[2-(Cyclopropylmethoxy)ethyl]phenol, a synthetic intermediate used in the synthesis of betaxolol[1,2], was brominated to yield the 2,6-dibromophenol (3). Alkylation with an excess of epichlorohydrin under basic conditions yielded the epoxypropane (4) which when heated in a bomb at 100°C with an excess of isopropylamine gave 2,6-dibromobetaxolol (5).

SCHEME 1


OH
Br_2
(2)
Br
OH
Br
(3)
NaOH
Epichlorohydrin
Br
O
Br
(4)
NH_2
Br
OH
H
N
Br
(5)
T_2, 10% Pd / $CaCO_3$
T
OH
H
N
T
(6)

A catalytic reductive debromination of this compound in the presence of hydrogen afforded, on purification, betaxolol which was identical by tlc, nmr and mass spectrometry to an authentic sample. This experiment was repeated using deuterium to yield [$^2H_2$]-betaxolol which was confirmed by mass spectrometry.

Then catalytic reductive debromination of this precursor in the presence of tritium gas and purification of the crude product by preparative layer chromatography yielded [^3H]-betaxolol with a specific radioactivity of 49 Ci/mmol and a radiochemical purity of 98 % as determined by thin layer radiochromatography.

Since betaxolol has the general β-blocking aryloxypropanolamine chemical structure this method could be used to synthesise a wide range of β-blocking agents labelled with tritium at high specific activity.

EXPERIMENTAL

Melting points were determined using a Büchi SMP 20 (Tottoli) melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Bruker WP 80 or a WP 200 SY spectrometer using tetramethylsilane as internal reference. Chemical shifts are reported in ppm. Mass spectra were recorded on a VG Micromass 7070 instrument in the electron impact mode (70 eV). Elemental analyses were determined using a Perkin Elmer 240 analyser, linked to a Tektronix 31 calculator and are within ± 0.3 % of theoretical values. The tritiation step was carried out by Amersham International plc, UK. Radiochemical purity was determined by radiochromatography using a Berthold LB 2832 TLC Linear Analyser.

2,6-Dibromo-4-[2-(cyclopropylmethoxy)ethyl]phenol (3)

4-[2-(Cyclopropylmethoxy)ethyl]phenol (10 g, 52 mmol) was dissolved in chloroform (90 ml) and n-butylamine (11 ml, 8.1 g, 111 mmol) added. The solution was cooled to -45°C and a solution of bromine (6 ml, 18.7 g, 117 mmol) in chloroform (30 ml) was added dropwise with stirring. After stirring for 30 minutes the reaction mixture was carefully poured into water and the product

extracted with chloroform. The combined organic phases were washed with water, dried ($MgSO_4$) and evaporated to yield (3) as a yellow oil (13.6 g, 54.3 mmol) which was used directly for the next stage. ^{1}H-NMR (80 MHz, $CDCl_3$, TMS) δ 7.38 (S, 2H, ArH), 5.85 (S, 1H, OH, exchangeable with D_2O), 3.8 (t, 2H, J = 7 Hz, CH_2), 3.29 (d, 2H, J = 7 Hz, CH_2), 2.79 (t, 2H, J = 7 Hz, CH_2), 1.03 (m, 1H, cyclopropyl-H), 0.63 (m, 2H, cyclopropyl-CH_2) and 0.18 (m, 2H, cyclopropyl-CH_2).

[[2,6-Dibromo-4-[2-(cyclopropylmethoxy)ethyl]phenoxy]methyl]oxirane (4)

The 2,6-dibromophenol (3) (7.0 g, 20 mmol) was stirred in water (30 ml) for 1 hour at room-temperature in the presence of sodium hydroxide (0.98 g, 24.5 mmol). Epichlorohydrin (4 ml, 4.7 g, 51.1 mmol) was added to the reaction mixture and this stirred for 20 hours at room-temperature. The product was extracted with ether, washed with water, dried ($MgSO_4$) and evaporated to yield (4), a colourless oil (7.7 g), which was used directly for the next step.

2,6-Dibromobetaxolol (5)

The oxirane (4) (7.7 g, 19 mmol) and an excess of isopropylamine (15 ml) were introduced into a bomb and this was heated for 8 hours at 100°C and then left at room-temperature for 15 hours. The product was removed from the bomb and the excess isopropylamine evaporated. Water was added and the solution acidified with 2N hydrochloric acid. This was washed with ether, basified with ammonia and the product extracted with ether, washed with water, dried and evaporated. The product was crystallised from pentane-ether to yield 2,6-dibromobetaxolol (5.7 g, 12.3 mmol, 65 %) mp : 60-61°C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.40 (S, 2H, Ar.H), 4.08 (S, 2H, CH_2), 4.08 (m, 1H, CHOH), 3.63 (t, 2H, J = 7 Hz, CH_2), 3.32 (d, 2H, J = 7 Hz, CH_2), 2.85 (m, 5H, 2 CH_2 + N CH), 1.12 (S, 3H, CH_3), 1.02 (S, 3H, CH_3), 1.05 (m, 1H, cyclopropyl-CH), 0.55 (m, 2H, cyclopropyl-CH_2) and 0.23 (m, 2H, cyclopropyl-CH_2). IR (KBr) 3279, 2922, 2459, 1540, 1452, 1255, 1092, 993 and 740 cm^{-1}. MS, m/z 464 (M^+H), 450, 421, 273, 265, 198, 169, 72, 55 and 30. Anal. Calcd for $C_{18}H_{27}Br_2NO_3$: C, 46.47 ; H, 5.85 ; N, 3.01 ; Br, 34.35 %. Found : C, 46.28 ; H, 5.79 ; N, 2.95 ; Br, 34.22 %.

[2,6-$^{3}H_{2}$]Betaxolol (6)

The 2,6-dibromobetaxolol precursor (5) (10 mg) in ethanol (2 ml) was stirred at room-temperature for 3 1/2 hours in the presence of 10 % $Pd/CaCO_3$ (7 mg), diisopropylethylamine (100 µl) and tritium gas (5 Ci). This was filtered to remove the catalyst and evaporated. The evaporation was repeated twice with ethanol (3 ml) in order to remove the labile tritium. The crude product was purified by preparative layer chromatography on silica gel eluting with toluene : methanol : acetic acid (80:15:5). The band containing the [^{3}H]-betaxolol was located by UV and removed and the product extracted with ethanol (60 ml).This was filtered and evaporated to yield [^{3}H]-betaxolol (6) (250 mCi) with a specific activity of 49 Ci/mmol which was determined by scintillation counting and UV measurements at λ max 275 nm.

The radiochemical purity was found to be 98 % by thin-layer radiochromatography on silica gel in :

(a) toluene : methanol : acetic acid (85:15:5), and

(b) methanol.

ACKNOWLEDGEMENTS

The authors would like to thank Dr J.P. Kitcher, Amersham International plc, Cardiff Laboratories for his collaboration in the tritium work.

REFERENCES

1. Manoury P.M.J., Cavero I.A.G., Najer H. and Giudicelli D.P.R.L., Synthelabo, Paris, France, US Patent n° 4,252,984 (1981).
2. Manoury P.M.J., Binet J.L., Rousseau J., Lefèvre-Borg F.M. and Cavero I.A.G.- J. Med. Chem., 30 : 1003 (1987).
3. Morselli P.L., Kilborn J.R., Cavero I., Harrison D.C. and Langer S.Z. (eds) Betaxolol and other β1-adrenoceptor antagonists. LERS Monograph Series, vol. 1, Raven Press, New York (1983).
4. Beresford R. and Heel R.C. - Drugs, 31 : 6 (1986).
5. Berrospi A.R. and Leibowitz H.M. - Arch. Ophthalmol., 100 : 943 (1982).

6. Aubert P., Beaucourt J.-P. and Pichat L. - J. Label. Comp. Radiopharm., 9 (4) : 543 (1982).

7. Allen J. and Tizot A. - 2nd ICI Labelled Compounds Symposium, University of York, England, 1984.

8. Hermann P., Fraisse J., Allen J., Morselli P.L., and Thénot J.-P. - Biomed. Mass Spectrometry, 11 (1) : 29 (1984).

9. Allen J. - The role of isotopes in the development of β-adrenoceptor blocking agents, in Isotopes in the Physical and Biomedical Sciences, Volume 1, Labelled Compounds (Part A), Buncel E. and Jones J.R., Eds, Elsevier, Amsterdam : 425 (1987).

10. Petruzzo P., Arbilla S., Allen J. and Langer S.Z. - Naunyn-Schmiedeberg's Arch. Pharmacol., 332 : 253 (1986).

11. Arfwidsson A., Hallhagen G. and Hoffmann K.-J. - J. Label. Comp. Radiopharm., 17 (3) : 369 (1980).