A CONVENIENT METHOD FOR THE SPECIFIC TRITIUM LABELLING OF BETA-ADRENOCEPTOR ANTAGONISTS

Anders Arfwidsson, Göran Hallhagen and Kurt-Jürgen Hoffmann

Research Laboratories, AB Hässle, Fack, S-431 20 MÖLNDAL, Sweden

SUMMARY

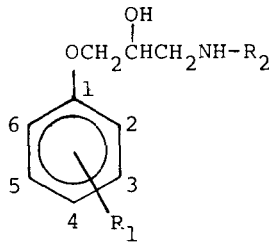
The specific tritium labelling of beta-adrenoceptor antagonists of the general structure $ArOCH_2CH(OH)CH_2NHR$ is described. The tritium label is introduced in the 2-position in the aminohydroxypropoxy side-chain starting from $ArOCH_2\overset{O}{\hat{C}}HCH_2$, an intermediate in the synthesis of the unlabelled compound. The key step is a reduction of the corresponding bromoketone $ArOCH_2COCH_2Br$ using readily available sodium borohydride-^3H as the labelling reagent. The 2-position has proven stable enough to be suitable for most biochemical applications, e.g. metabolic studies. Five examples are given, including alprenolol-^3H and metoprolol-^3H.

Key Words: Alprenolol-^3H; metoprolol-^3H; beta-adrenoceptor antagonists, specific ^3H-labelling of

INTRODUCTION

Beta-adrenoceptor antagonists with the general structure 1 (figure 1) have been synthesized and extensively studied in

0362-4803/80/0317-0369$01.00 Received May 9, 1979



1

Figure 1: Beta-adrenoceptor antagonist with the aminohydroxy-propoxy side-chain

our laboratories since the early 60's. There was soon a need for radioactively labelled compounds for pharmacokinetic and metabolic studies. Since we were dealing with a number of potential drugs with the general structure 1, and new ones could be expected in the future, we were indeed interested in a specific tritium labelling procedure to be routinely used with this category of compounds.

There are several ^{3}H- and ^{14}C-labelled preparations of beta--adrenoceptor antagonists with the general structure 1 described in the literature, as summarized in table 1. However, most of these preparations are more or less unique for each structure as the label is located in the aromatic ring, in the aromatic substituent R_1 or in the amine substituent R_2 (figure 1). Consequently these methods can only be of limited interest in the labelling of compounds with the general structure 1.

Non-specific tritium labelling, as described for propranolol (13,15), is a straightforward method, but there are limitations in the field of application since the position and therefore the stability of the label cannot be adequately controlled.

Table 1: ^3H- and ^{14}C-labelled preparations of beta-adrenoceptor antagonist, $ArOCH_2CHOHCH_2NHR$, from the literature.

Compound	Label and position	Ref.
Acebutolol	acetyl-1-^{14}C	(1)[d]
Atenolol	ring-U-^{14}C	(2)[d]
Atenolol	carbamoylmethyl-1-^{14}C	(3)[d]
Bunolol	carbonyl-^{14}C	(4)[b]
1-Bunolol	ring-7-^3H	(5)[d]
Carteolol	ring-6,8-^3H	(6)[d]
(-)Dihydroalprenolol	propyl-2,3-^3H (nominal)	(7)[b]
Nadolol	aminohydroxypropoxy-2-^{14}C	(8)[d]
Oxprenolol	ring-4,5-^3H[a]	(9)[c]
Oxprenolol	isopropyl-^{14}C	(9)[d]
Pindolol	isopropyl-^{14}C	(10)[b]
Practolol	ring-2,6-^3H[a]	(11)[b]
Practolol	acetanilide-1-^{14}C	(12)[d]
Practolol	ring-U-^{14}C	(12)[d]
Propranolol	ring-1-^{14}C[a]	(13)[b]
Propranolol	G-^3H	(13)[b]
Propranolol	isopropyl-^{14}C	(14)[d]
Propranolol	G-^3H (high spec. act.)	(15)[b]
1-Timolol	thiadiazolring-3,4-^{14}C	(16)[d]
Tolamolol	ethylamino-2-^{14}C-cresolring-G-^3H	(17)[c]

[a] Numbering of the aromatic ring is indicated in figure 1.

[b] Full experimental details.

[c] Reaction sequence is given.

[d] No experimental information at all.

In the synthesis of ring 2,6-^{3}H labelled practolol (11), the label is introduced via 2,6-dibromination of the intermediate phenol 2 (figure 2) followed by catalytic dehalogenation employing tritium gas. The isopropylaminohydroxypropoxy side-chain is then synthesized just as for the unlabelled compound. The synthesis procedure is of general interest as long as the aromatic substituent R_1, does not interfere with the bromination and dehalogenation steps.

In nadolol-^{14}C (8) the label is located in the 2-position in the aminohydroxypropoxy side-chain. This is suitable for the ^{14}C-labelling of beta-adrenoceptor antagonists with the general structure 1. However, the cost of the ^{14}C-label probably does not make this a routine operation.

Considering a specific tritium labelling procedure for the general structure 1, the 2-position, that is the carbon atom bearing the hydroxyl group in the aminohydroxypropoxy side-chain, seemed to be the most favourable alternative for two reasons. The 2-position should permit the use of readily available sodium borohydride-^{3}H as the labelled reagent. The 2-position also should be acceptable from a metabolic point of view, that is stable enough in a biological system to prevent loss of the label through exchange or degradation.

RESULTS

The method of choice to incorporate the ^{3}H-label into the 2-position in the aminohydroxypropoxy side-chain would be to oxidize the hydroxyl group in the type 1 compound to the corresponding keto function and then to resynthesize the labelled compound 1-^{3}H using sodium borohydride-^{3}H. Kurihara et al (18) have

described the oxidation of 1-phenoxy-3-diethylamino-2-propanols to the corresponding ketones with various oxidizing agents and found aluminium or potassium tert. butoxide to work in acceptable yields. However, we did not succeed in oxidizing our own structures 1 according to these instructions.

The most convenient method for the synthesis of compounds of structure 1 is outlined in figure 2. The properly substituted phenol 2 is reacted with epichlorohydrin under basic conditions, and the resulting epoxide 3 is treated with an excess of the appropriate amine, usually isopropylamine, to give the 1-amino--3-aryloxypropan-2-ol 1.

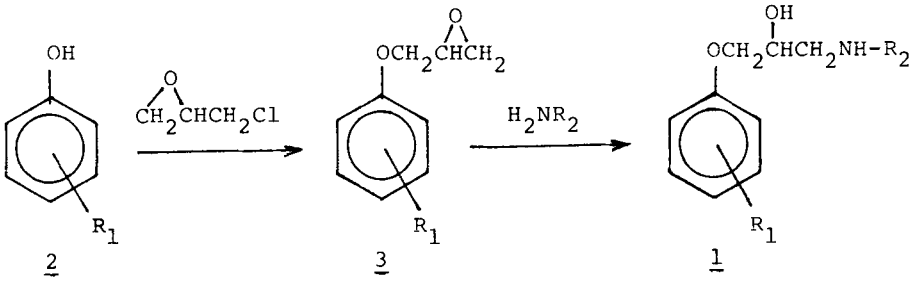
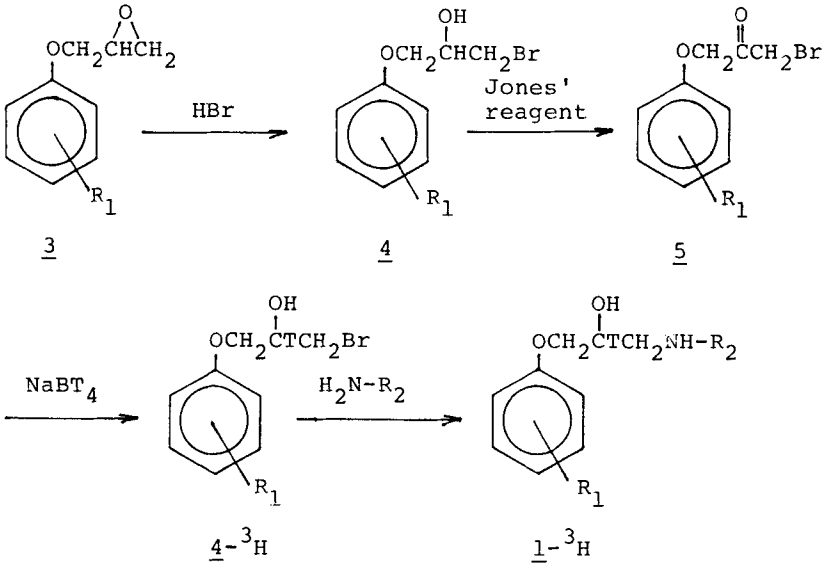


Figure 2: Synthesis of the aminohydroxypropoxy side-chain of beta-adrenoceptor antagonists 1.

Our specific tritium labelling procedure starting from the intermediate epoxide 3 is outlined in figure 3. The sequence can be divided in two parts: the two-step synthesis of the precursor, the bromoketone 5, and the two-step labelling procedure.

Starting from the intermediate epoxide 3, one mol of HBr is added and the resulting bromohydrin 4, recrystallized or as the crude oil, is oxidized with 2-3 equivalents of Jones' reagent in acetone solution. Work-up and several recrystallizations from diisopropyl



Compound 1	R_1	R_2
H 56/28[a]	2-$CH_2CH{=}CH_2$	-$CH(CH_3)_2$
H 87/07	4-$OCH_2CH_2OCH_3$	"
H 93/26[b]	4-$CH_2CH_2OCH_3$	"
H 104/08[c]	4-$CH_2CH_2NHCOOCH_3$	"
H 148/52	2-CN	-CH_2CH_2O-C$_6$H$_4$-OH

[a] alprenolol [b] metoprolol [c] pamatolol

Figure 3: The specific tritium labelling procedure for beta--adrenoceptor antagonists of structure 1.

Table 2: Bromohydrins 4 and bromoketones 5 synthesized according to figure 3.

Compound 1	Yield of 4 (%)	Mp of 4[a] (°C)	Yield of 5 (%)	Mp of 5[a] (°C)
H 56/28	93	Crude oil	30	<25
H 87/07	71	56	26	42-43
H 93/26	94	Crude oil	26	<25
H 104/08	92	Crude oil	60	109-10
H 148/52	73	74	53	105-10

[a] From diisopropyl ether.

Table 3: Tritium labelled beta-adrenoceptor antagonists 1-^3H synthesized according to figure 3.

Compound 1-^3H	Dilution factor in work up	Chemical yield 5→1-^3H (%)	Radiochemical yield (%)[a]	Radiochemical purity (%) (method of analysis)	Specific activity (GBq/mmol)	(mCi/mmol)
H 56/28-^3H (·HCl)	4.3	77	106	≥99 (HPLC)	17.6	477
H 87/07-^3H (base)	7.3	62	85	>95 (TLC)	10.2	277
H 93/26-^3H (base)	9.9	75	124	>95 (TLC)	10.0	270
H 104/08-^3H (base)	3.0	48	60	≥96 (HPLC)	36.4	983
H 148/52-^3H (·HCl)	2.6	53	56	≥98 (HPLC)	22.7	613

[a] From the nominal amount of activity in the sodium borohydride-^3H ampoule.

ether afford the pure, crystalline bromoketone 5. Some investigated bromoketones have now been stored at -20°C for several years and have been used as precursors in repeated labelled preparations. ^{1}H NMR ($CDCl_3$, TMS) of bromoketones 5 distinguish two characteristic methylene singlets at δ4.9-4.7 and 4.4-4.2, respectively ($ArOC\underline{H}_2COC\underline{H}_2Br$). Chemical yields and melting points of bromohydrins 4 and bromoketones 5 are given in table 2.

In the labelling procedure, the bromoketone 5 is reduced with sodium borohydride-^{3}H in ethanol solution. A molar ratio of at least 5:1 (bromoketone:sodium borohydride-^{3}H) is used to insure proper use of the labelled reagent, and an excess of unlabelled sodium borohydride is then added to complete the reaction. The chemical yield of crude labelled bromohydrin 4-^{3}H is quantitative and this product is treated, without further purification, with an excess of the appropriate amine to give the labelled 1-amino-3-aryloxypropan-2-ol 1-^{3}H.

Work up includes evaporation and dissolution in dilute HCl. The acidic water solution is washed with an organic solvent and made alkaline to pH 10-11. The base is extracted into an organic solvent and the organic solution is evaporated to dryness. Compound H 148/52-^{3}H was further purified by preparative TLC to separate the non-volatile primary amine. The chemical yield of the crude base is in the range of 50-80 % (over two steps) and the radiochemical purity usually ≥95 % by TLC. Specific activities are on the order of 40-200 GBq/mmol (1-5 Ci/mmol) before diluting the material. The specific activity is merely a function of the specific activity of the sodium borohydride-^{3}H used, which has been on the order of 200-700 GBq/mmol (5-20 Ci/mmol), and the actual excess of bromoketone over sodium borohydride-^{3}H in the first step of the labelling procedure.

As a rule the crude labelled base has been diluted with the unlabelled reference compound to 10-20 GBq/mmol (300-600 mCi/mmol) and transformed into the salt of a suitable acid. The salt has been stored at -20°C in crystalline form or in ethanol solution. Chemical and radiochemical purity were analysed by ^{1}H NMR, TLC and HPLC. TLC plates were scanned and HPLC fractions were liquid scintillation counted for activity. Chemical and radiochemical yields are summarized in table 3.

CONCLUSIONS

Today the metabolic pathways involved in the degradation of the aminohydroxypropoxy side-chain are well penetrated and understood (for a review see (19)). The typical degradation steps of the isopropylaminohydroxypropoxy side-chain are outlined in figure 4. For bunolol (20,21), oxprenolol (22) and propranolol (23,24), a further oxidation to the corresponding phenoxy acetic acid, $ArOCH_2COOH$, as well as direct cleavage of the ether linkage to give the corresponding phenol, ArOH, have been reported. This would mean the loss of a tritium label in the 2-position, but in general these metabolic reactions seem to be of minor importance from a quantitative point of view.

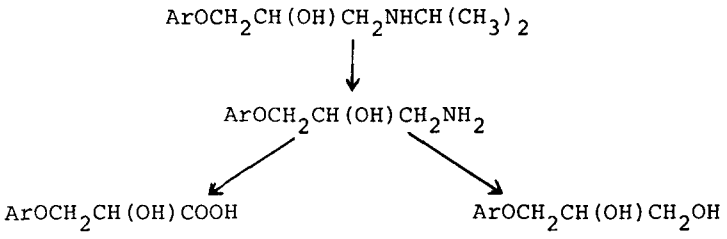


Figure 4: The metabolic degradation of the isopropylaminohydroxypropoxy side-chain.

We believe our ^{3}H-labelling procedure, as outlined in figure 3, is of general interest for the labelling of beta-adrenoceptor antagonists of the general structure 1. Once the unlabelled compound is synthesized, the intermediate epoxide 3 can be used to synthesize the corresponding ^{3}H-labelled compound, using readily available sodium borohydride-^{3}H as the source of tritium. Selectivity and mild reaction conditions in the two-step synthesis of the bromoketone 5 and in the two-step labelling procedure, together with the low cost of sodium borohydride-^{3}H, has made this sequence a routine operation in our laboratory.

EXPERIMENTAL

Sodium borohydride-^{3}H, 200-700 GBq/mmol, 5-20 Ci/mmol, was purchased from The Radiochemical Centre, Amersham. Radioactivites were measured in a Packard Tricarb Model 3375 liquid scintillation counter. TLC-plates, precoated silica gel F_{254}, Merck, were scanned on a Berthold Model II thin-layer radio-scanner. ^{1}H NMR were recorded on Varian T-60 and Varian CFT-20 instruments. Melting points were determined on a Kofler Heizbank and melting point intervals on a Mettler FP2 apparatus (1°/min).

As an example, the synthesis of metoprolol-^{3}H, H 93/26-^{3}H, (±)--1-isopropylamino-3-(4-(2-methoxyethyl)-phenoxy)-propan-(2-^{3}H)--2-ol, will be given. The synthesis of unlabelled metoprolol has been reported earlier (25).

1-Bromo-3-(4-(2-methoxyethyl)-phenoxy)-propan-2-ol, 4(R_1=4--$CH_2CH_2OCH_3$): 10.4 g (0.050 mol) of 3-(4-(2-methoxyethyl)-phenoxy)-1,2-epoxypropane, 3(R_1=4-$CH_2CH_2OCH_3$), (25) was dissolved in 25 ml of dioxane and 6.1 ml of 48 % HBr (0.055 mol) was added

dropwise at +5°C with magnetic stirring and ice-cooling. The reaction mixture was stirred for one hour at room temperature and then evaporated to dryness. The residue was dissolved in diethyl ether, washed with water, dried and evaporated to leave 13.6 g (94 %) of crude bromohydrin 4 (R_1=4-$CH_2CH_2OCH_3$) as an oil.

1-Bromo-3-(4-(2-methoxyethyl)-phenoxy)-propan-2-one, 5 (R_1=4-$CH_2CH_2OCH_3$): 13.6 g (0.047 mol) of crude bromohydrin 4 (R_1=$CH_2CH_2OCH_3$) was dissolved in 250 ml of acetone. With vigorous stirring, 11.8 ml (1.0 equivalent) of Jones' reagent (26) was added. The reagent was rapidly consumed (the solution turned from red to green in less than 5 minutes) and another portion of 11.8 ml was added. The second portion was also consumed (the solution turned from red to green in about 5 minutes), and a third portion of 11.8 ml was added. The third portion was consumed much more slowly, and the reaction mixture was stirred for 30 minutes at room temperature. The conglomerated chromium salts were filtered off and the reddish-green acetone solution was evaporated to dryness. Water was added and the bromoketone was extracted into diethyl ether, dried, treated with charcoal and evaporated. The crude bromoketone was recrystallized several times from diisopropyl ether to give 3.5 g (26%) of bromoketone 5 (R_1=4-$CH_2CH_2OCH_3$), mp <25°C. ^1H NMR ($CDCl_3$, TMS): δ 7.3 (2H,d,J=9Hz, aromatic), 6.9 (2H,d,J=9Hz, aromatic), 4.8 (2H,s, -$C\underline{H}_2COCH_2Br$), 4.2 (2H,s, -$CH_2COC\underline{H}_2Br$), 3.6 (2H,t,J=7Hz, -$CH_2C\underline{H}_2OCH_3$), 3.4 (3H,s, -$OCH_3$), 2.8 (2H,t,J=7Hz, -$C\underline{H}_2CH_2OCH_3$).

1-Bromo-3-(4-(2-methoxyethyl)-phenoxy)-propan-(2-^{3}H)-2-ol, 4-^3H (R_1=4-$CH_2CH_2OCH_3$): The contents of a sodium borohydride-^3H-ampoule (nominally 28 GBq (750 mCi), 310 GBq/mmol (8.4 Ci/mmol), 0.089 mmol) was added in one portion to an ice-cooled solution of 147 mg (0.512 mmol) of bromoketone 5 (R_1=4-$CH_2CH_2OCH_3$) in

4 ml of abs. ethanol. The resulting suspension was shaken occasionally. After 1.5 hours at 0°C and 1 hour at room temperature, the reaction mixture was again cooled to 0°C and 19 mg (0.50 mmol) of unlabelled sodium borohydride was added. The reaction mixture was left for 1 hour at 0°C and 1 hour at room temperature, and was then evaporated in a N_2-stream. The residue was partitioned between a saturated NaCl solution and diethyl ether. The water layer was further extracted twice with diethyl ether and the combined diethyl ether layers were dried and evaporated to yield 147 mg of crude bromohydrin 4-^{3}H (R_1=4-$CH_2CH_2OCH_3$), which was used without further purification in the next step.

(±)-1-Isopropylamino-3-(4-(2-methoxyethyl)-phenoxy)-propan-(2-^{3}H)-2-ol, 1-^{3}H (R_1=4-$CH_2CH_2OCH_3$, R_2=$CH(CH_3)_2$, metoprolol-^{3}H: 145 mg (0.50 mmol) of crude bromohydrin 4-^{3}H (R_1=4-$CH_2CH_2OCH_3$) was dissolved in 2 ml of 2-propanol and 5 ml of isopropylamine was added. The solution was refluxed for 4.5 hours (monitored by TLC) and then evaporated. The residue was dissolved in 4 ml of 0.5 M HCl. The HCl solution was washed three times with diethyl ether and twice with hexane, made alkaline to pH 12 using 5 N NaOH and extracted three times with diethyl ether. Evaporation of the diethyl ether extract gave 110 mg of crude metoprolol-^{3}H base. After a preliminary determination of the specific activity, the material was diluted with 984 mg of unlabelled metoprolol base and recrystallized from hexane. The chemical yield was 922 mg (75 %) of metoprolol-^{3}H base with a specific activity of 10.0 GBq/mmol (270 mCi/mmol) and a radiochemical yield of 123 % (from the nominal amount of activity in the sodium borohydride-^{3}H ampoule). The radiochemical purity was examined in three different TLC systems (A: methanol, B:ethylacetate, C: ethylacetate-methanol-water-conc. NH_4OH 50:10:5:5) and was found to be >95 %.

REFERENCES

1. Basil B., Collins R.F. and Cuthbert M.F. - Brit. J. Pharmacol. 47: 620P (1973)
2. Reeves P.R., Barnfield D.J., Longshaw S., McIntosh D.A.D. and Winrow M.J. - Xenobiotica 8: 305 (1978)
3. Reeves P.R., McAinsh J., McIntosh D.A.D. and Winrow M.J. - Xenobiotica 8: 313 (1978)
4. Merrill E.J. - J. Pharm. Sci. 60: 1589 (1971)
5. Leinweber F-J., Szpiech J.M. and DiCarlo F.J. - J. Pharm. Sci. 67: 129 (1978)
6. Kohri H., Morita S., Nakagawa K. and Nishino H. - Folia Pharmacol. Japon 72: 341 (1976)
7. Randall M.H., Altman L.J. and Lefkowitz R.J. - J. Med. Chem. 20: 1090 (1977)
8. Dreyfuss J., Shaw J.M. and Ross Jr J.J. - Xenobiotica 8: 503 (1978)
9. Riess W., Rajagopalan T.G., Imhof P., Schmid K. and Keberle H. - Postgrad. Med. J. 46(Nov.suppl): 32 (1970)
10. Kiechel J.R., Niklaus P., Schreier E. and Wagner H. - Xenobiotica 5: 741 (1975)
11. Shtacher G., Erez M., Cohen S., Cohen A. and Buchman O. - J. Labelled Compounds and Radiopharmaceuticals 13: 59 (1977)
12. Reeves P.R., Case D.E., Jepson H.T., McCormick D.J., Nicholls J.T., Felix R.H., Holt P.J.L., Zacharias F.J. and Fluke R.W. - J. Pharmacol. Exp. Ther. 205: 489 (1978)
13. Burns J. - J. Labelled Compounds 6: 45 (1970)
14. Bakke O.M., Davies D.S., Davies L. and Dollery C.T. - Life Sciences 13: 1665 (1973)
15. Buchman O., Pri-Bar I. and Hagag Y. - J. Labelled Compounds 10: 519 (1974)

16. Tocco D.J., Duncan A.E.W., Deluna F.A., Hucker H.B., Gruber V.F. and Vandenheuvel W.J.A. - Drug Metab. Disp. 3: 361 (1975)

17. Wood B.A., Stopher D.A. and Monro A.M. - Xenobiotica 5: 183 (1975)

18. Kurihara T., Takeda H. and Ito H. - Yakugaku Zasshi 89: 1528 (1969)

19. Pfeifer S. and Zimmer C. - Die Pharmazie 30: 625 (1975)

20. Leinweber F-J., Greenough R.C., Schwender C.F., Haynes L.J. and DiCarlo F.J. - J. Pharm. Sci. 60: 1516 (1971)

21. Leinweber F-J., Greenough R.C. and DiCarlo F.J. - Xenobiotica 8: 239 (1978)

22. Riess W., Huerzeler H. and Raschdorf F. - Xenobiotica 4: 365 (1974)

23. Tindell G.L., Walle T. and Gaffney T.E. - Life Sciences 11: 1029 (1972)

24. Walle T. and Gaffney T.E. - J. Pharmacol. Exp. Ther. 182: 83 (1972)

25. Arfwidsson, A., Borg K.O., Hoffmann K-J. and Skånberg I. - Xenobiotica 6: 691 (1976)

26. Chinn L.J. - Selection of Oxidants in Synthesis - Oxidation at the Carbon Atom, Marcel Dekker, Inc., New York, 1971 p. 42