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NOTE

PREPARATION OF 2-[α-(2-ETHOXYPHENOXY)BENZYL]-[5-^{14}C]MORPHOLINE METHANESULFONATE ([^{14}C]REBOXETINE) A NEW ANTIDEPRESSANT AGENT.

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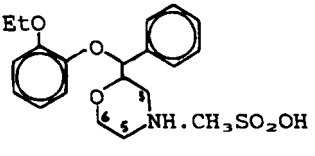
SUMMARY

The labelling with radiocarbon of the new antidepressant agent Reboxetine is described. The preparation has been carried out in a two step procedure using 2-chloro-N-(3-(2-ethoxyphenoxy)-2-hydroxy-3-phenyl)propyl-[1-^{14}C]acetamide 2 as starting material. The expected compound was prepared by cyclization of the above halogenoacylamido alcohol to the corresponding morpholone ring followed by reduction to the final [5-^{14}C]morpholine derivative 4, 98% radiochemically pure and with specific radioactivity of 988 MBq/mmol. An overall radiochemical yield of 57.5% was achieved.

Key words: Reboxetine, FCE 20124, [^{14}C]reboxetine, 2-(α-aryloxy-benzyl) morpholines, antidepressant agent.

INTRODUCTION

During a screening program for orally active new potential antidepressant agents, Reboxetine, namely 2-[α-(2-ethoxyphenoxy)benzyl]morpholine methanesulfonate (FCE 20124.methanesulfonate), was found to have an outstanding activity in pharmacological models and biochemical tests, predictive of antidepressant effectiveness [1][2].



Reboxetine

For "in vivo" and "in vitro" studies with this compound, a radiolabelled form was required. The introduction of radiolabel into the morpholine ring was

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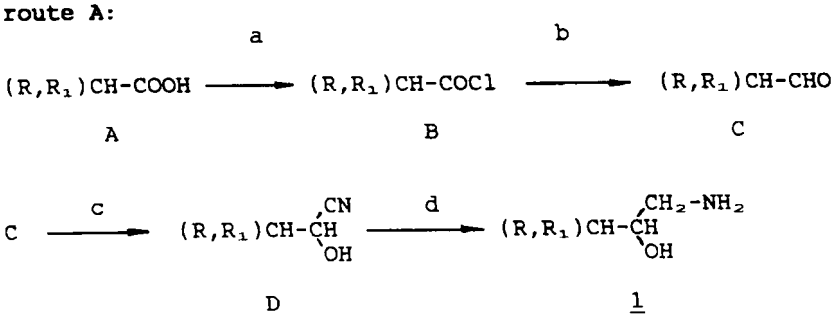
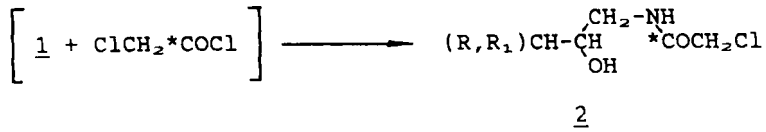
SCHEME

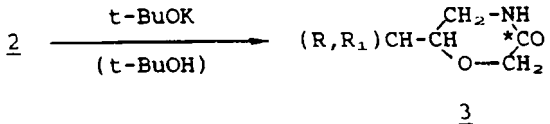
route A:

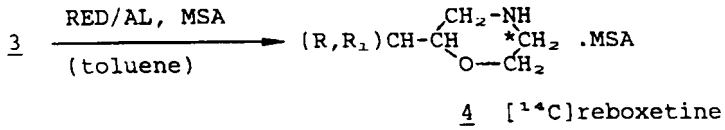


a=$SOCl_2$; b=$LiAlH(O\text{-}t\text{-}Bu)_3$; c=$NaHSO_3$,KCN; d=$LiAlH_4$

route B:







* = [^{14}C]; MSA = CH_3SO_2OH

considered suitable, not only because ready synthetic accessibility, but also there was no literature precedent for metabolic loss of label from the morpholine ring. The easy acylation of the amino-alcohol 1 with the readily available chloro-[1-^{14}C]acetic acid to give the chloro-acetamide 2, prompted us to prepare [^{14}C]reboxetine according the procedure already described in a general method by Melloni et al. [2], as shown in the scheme (route B). A preparation of [^{14}C]reboxetine, according to this route, has been described by Cocchiara et al. [3], but the instability of the labelled acyl chloride and difficult purification of [^{14}C]reboxetine gave unsatisfactory results. We describe an improved experimental protocol here.

RESULTS AND DISCUSSION

The synthetic sequence as outlined in the scheme (route A)prompted us to consider position 3 of morpholine ring as possible radiocarbon labelling site with [^{14}C]KCN. However this route was discarded due to low yields obtained for the one pot acid chloride - aldehyde - cyanohydrin sequence. The instability of the intermediate aldehyde and the small scale nature of the reactions contributed to this difficulty.
Therefore positions 5 and 6 proved to be suitable for labelling reboxetine. However the availability of chloro-[1-^{14}C]acetamido derivative 2 enabled us to obtain the morpholine ring labelled at position 5. According to the scheme (route B), cyclization of 2 with t-BuOK afforded the morpholone derivative 3, which was reduced to the corresponding morpholine compound 4 with RED-AL [Sodium bis-(2-methoxyethoxy) aluminium hydride - 70% toluene solution]. Purification of the crude 4 by preparative TLC, gave [^{14}C]reboxetine, 99% radiochemically pure with a radiochemical yield of 57% from 2.

EXPERIMENTAL

Thin layer chromatography (TLC)

TLC were generally run on Merck silica gel F 254 (0.25 mm) plates, using the following solvent systems (given by volume):

A) chloroform:methanol:ammonia(30%)	(90:10:1)
B) chloroform:methanol:formic acid(99%)	(160:30:20)
C) chloroform:ethanol:ammonia(30%)	(200:20:1.5)
D) chloroform:methanol	(175:25)

High performance liquid chromatography (HPLC)

Analysis was performed by using a Merck Lichrosorb C_8 10 um (250x 4.6 ID mm) column with a mobile phase of CH_3CN : KH_2PO_4 buffer (0.1 M) pH 5.1 (35 : 65 by volume); flow rate 1.4 ml/min; UV detection 275 nm; radiometric detection with heterogeneous cell (0.36 ml) Yttrium silicate.

Ultraviolet spectra were determined on a Beckman DU50 spectrophotometer. Measurements of radioactivity were carried out with a Packard 300C liquid scintillation counter using Rialuma (Lumac System A.G.) as liquid scintillation cocktail. Radiochemical analyses of TLC plates were performed with a Berthold 3832 automatic linear analyser.
HPLC analyses were carried out with a Perkin Elmer series 2/2 liquid chromatograph with LC 75 UV/VIS detector and Packard Trace Mod.7130 on line with 512 kRAM 3270 IBM PC as radioactivity flow monitor.
2-Chloro-N-(3-(2-ethoxyphenoxy)-2-hydroxy-3-phenyl)propyl-[1-^{14}C]acetamide 2 (98% radiochemically pure) was synthesized by Amersham International plc.

6-[α-(2-ethoxyphenoxy)benzyl]-[3-^{14}C]morpholine-3-one (3)

Compound 2 (407 MBq; 0.415 mmol) was dissolved with stirring at 40 °C in 4.4 ml t-butyl alcohol. After cooling to room temperature, potassium t-butoxide (102 mg) in 2.5 ml t-butyl alcohol was added with stirring. The mixture was stirred for about 2 hours. When the reaction was complete (checked by radio-TLC, system A; Rf 0.54), 2N HCl was added to pH at 4.5 and the mixture was evaporated to dryness under vacuum. The solid was dissolved in 22.5 ml water and the solution, made neutral with $NaHCO_3$ (powder), was transferred into a separating funnel and extracted with ethylacetate (4x12 ml). The combined organic extracts were filtered through a small plug of Na_2SO_4 and then evaporated to dryness under vacuum to give compound 3 (378 MBq), 93 % radiochemically pure (by radio-TLC, system A), which was used in the next step without further purification.

2-[α-(2-ethoxyphenoxy)benzyl]-[5-^{14}C]morpholine.methane-sulfonate ([^{14}C]reboxetine) (4)

RED/AL [sodium bis-(2-methoxyethoxy)aluminium hydride-70% toluene solution: technical grade)], 0.23 ml was added, under nitrogen, to a stirred solution of the crude compound 3 (378 MBq; about 0.393 mmol) in 2.2 ml of toluene. The stirring was continued for one hour. At the end of the reaction (checked by radio-TLC, system B; Rf 0.43), the excess of reagent was quenched with 2N sodium hydroxide (1.25 ml) and the biphasic mixture was transferred into a separating funnel. The organic layer was washed with aqueous 10% NaCl (4x10 ml), dried (by filtration through a small plug of Na_2SO_4) and evaporated to dryness under vacuum to give the crude compound 4 (351 MBq), 90% radiochemically pure (by radio-TLC, system B).
The solid was dissolved in acetone (about 1 ml), neutralized with stoichiometric amount of methanesulfonic acid (34 mg). After solvent evaporation, the solid was taken up with 10 ml water and transferred into a separating funnel. The aqueous solution was washed with toluene (3x10 ml) and with diethylether (3x10 ml). The pH was adjusted to pH 8 with 1N NaOH and the compound was extracted with toluene (3x10 ml) to give 323 MBq of crude [^{14}C]reboxetine 4 (92% by radio-TLC; system B). This product was then purified by preparative TLC (silica gel Merck F254 plate 20x20 cm; 2mm thick; eluent system D). The chromatographic band corresponding to reboxetine was removed and the product extracted from silica gel with aqueous methanol (10% water). The combined extracts were filtered through a D_4 sintered glass-filter and the compound concentration was determined spectrophotometrically. The subsequent neutralization with a stoichiometric amount of methanesulfonic acid afforded 234 MBq of [^{14}C]reboxetine 4 with a radiochemical purity >98% (by radio-TLC: systems B,C and D; rf = 0.43, 0.49 and 0.28 respectively; by radio-HPLC ; t_R = 7 minutes) and specific radioactivity of 988 MBq/mmol.
The UV spectrum (in methanol, λ_{max} = 276 nm, $E^{1\%}_{1cm}$ = 53) was concordant to that of the standard sample.
The overall radiochemical yield from compound 2 was 57.5%.

ACKNOWLEDGMENTS

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