SYNTHESIS OF [^{14}C]ALAPROCLATE

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SUMMARY

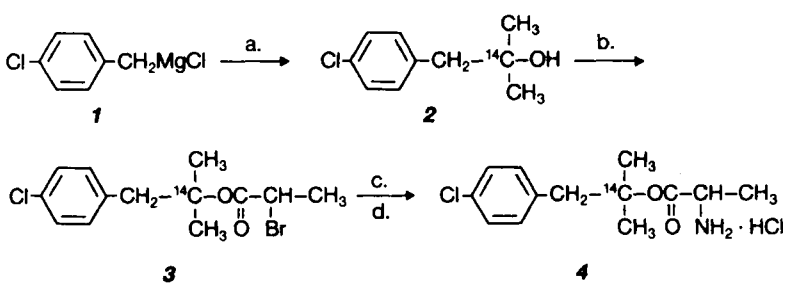
The synthesis of carbon-14 labelled alaproclate from [2-^{14}C]acetone in three steps is described. The label is introduced by the reaction of a benzylic Grignard reagent with the ketone yielding a tertiary alcohol Subsequent esterification with an α-bromocarboxylic acid chloride and amination furnished the title compound in a 13% overall radiochemical yield.

Key words: Alaproclate, antidepressant, carbon-14

INTRODUCTION

Alaproclate (4), a specific inhibitor of neuronal 5-HT re-uptake, (1), has currently undergone evaluation as an antidepressant. In the course of the studies, a carbon-14 labelled form of the drug was required for pharmacokinetic and metabolism studies. This publication describes the synthesis of [^{14}C]alaproclate utilized as a tracer in the studies of its metabolism in the mouse and the dog.

Scheme



Reagents: a) $(CH_3)_2{}^{14}CO$ b) $CH_3CHBrCOBr$ c) NH_3 d) HCl-ether

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RESULTS AND DISCUSSION

The radioactive label was incorporated at the first step of the synthesis by a Grignard reaction between 4-chlorobenzylmagnesium chloride (1) and [2-^{14}C]acetone (Scheme). This reaction, with unlabelled material, had been found to vary in the yield of the alcohol, presumably as a result of dimerization of the benzylic reagents during the *in situ* preparation of the Grignard reagent (1). Therefore, a 10-fold excess of Grignard reagent was employed in order to ensure that all of the labelled acetone could be consumed. The reaction was performed on a vacuum manifold where the [^{14}C]acetone was introduced from an ampoule by vacuum transfer. When the reaction was completed, the unlabelled alcohol (2) was added as carrier to the product mixture. After work-up, the crude product was treated with 2-bromopropionyl bromide in N,N-dimethylaniline. TLC of the reaction mixture indicated a slower reaction than expected and an additional amount of reagents was added. When TLC showed that the main part of the radioactivity co-chromatographed with the desired product (3), work-up furnished an oil which was used directly in the following amination step. The α-bromoester (3) was added to newly condensed ammonia in a culture- tube, equipped with a teflon-lined screw-cap and left at room temperature. The tube was of a thick-walled type to minimize the risk of bursting from built-up pressure when the temperature of the ammonia containing mixture was raised. After converting the produced amine to its hydrochloride, unlabelled alaproclate was added and crystallization afforded 4 in an overall radiochemical yield of 13%.

EXPERIMENTAL

[2-^{14}C]Acetone was purchased from Amersham International plc, England. Radioactivity was determined in a Packard Tri-Carb 460C liquid scintillator spectrometer using Biofluor (New England Nuclear) as the counting medium. The radiochemical purity was determined from TLC plates using a Berthold LB 283 TLC Linear Analyzer.

1-(4-Chlorophenyl)-2-methyl-2-[2-^{14}C]propanol (2)

A reaction flask, supplied with a magnetic stirring bar, was charged with an ethereal solution of 1.6M 4-chlorobenzylmagnesium chloride (1) (1 ml) and connected to an outlet of a vacuum manifold. The flask was cooled to -72°C in a dry ice-ethanol bath and a break-seal glass ampoule containing [2-^{14}C]acetone (10 mCi, 58 mCi/mmol) was attached to the vacuum line. The manifold was evacuated and by breaking the glass seal of the ampoule by means of a "magnetic hammer", the [2-^{14}C]acetone was distilled into the reaction flask. After 15 min at room temperature, the ampoule was carefully heated to complete the transfer. The

connection to the reaction flask was closed and the cooling bath removed. After stirring at room temperature for 18 hours, non-radioactive 2 (78 mg, 0.42 mmol) in ether (1 ml) and 0.5M HCl (2 ml) were added. The organic layer was separated and the aqueous solution extracted with ether (4 x 1 ml). The combined organic solutions were dried (Na_2SO_4) and the solvent evaporated to give an oil (210 mg) containing labelled alcohol (2) as identified by TLC (SiO_2/diethylether-n-hexane; 8:2 , v/v) using an authentic reference sample for comparison.

1-(4-Chlorophenyl)-2-methyl-2-[2-^{14}C]propyl-2-aminopropanoate ([^{14}C]Alaproclate) (4)

The crude product (2) from the previous step was dissolved in N,N-dimethylaniline (117 mg, 0.96 mmol) and 2-bromopropionyl bromide (181 mg, 0.84 mmol) was added. The mixture was stirred for 2 hours at room temperature and for 1.5 hours at 40°C. TLC(SiO_2/toluene) with unlabelled material showed only ~20% conversion of 2. Additional N,N-dimethylaniline (110 mg, 0.91 mmol) and 2-bromopropionyl bromide (180 mg, 0.84 mmol) were added. After 5 hours at 40°C, ~90% of the radioactivity co-chromatographed with unlabelled 3. Toluene (1 ml) and water (1.5 ml) were added. The organic phase was washed with 0.1M H_2SO_4 (4 x 1.5 ml) and aqueous $NaHCO_3$ (2 x 1.5 ml) and dried ($MgSO_4$). Evaporation of the solvent left an oil which was dissolved in ethanol (0.5 ml) and added to liquid ammonia (200 mg) in a screw-cap equipped test tube immersed in a dry ice-alcohol bath. The test tube was sealed and left at room temperature for 14 hours. The temperature was raised to 30°C and after 4 hours TLC (SiO_2/$CHCl_3$-CH_3OH-conc. NH_3; 95:5:0.025, by vol.) showed ~72% radioactive yield of 4. Ether (2 ml) and H_2O (2 ml) were added and after separation, the aqueous layer was discarded. Extraction of the organic phase with 0.5M HCl (2 ml) gave a solution which was made alkaline (pH ~11) with 2M NaOH and extracted with ether (4 x 1.5 ml). The combined ether extracts were dried (Na_2SO_4) and treated with an excess of HCl in ether. Evaporation of the solvent left a residue to which a warm solution of non-radioactive 4 (50 mg, 0.17 mmol) in acetone (700 µl) was added. Crystallization at 4°C gave off-white crystals which were recrystallized from acetone to furnish 65 mg of 4. The specific activity was 6.0 mCi/mmol and the radiochemical purity greater than 97% as shown by TLC in the system described above.

REFERENCES

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