A SYNTHESIS OF [^{14}C] PROGABIDE®- A NEW ANTICONVULSANT

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SUMMARY

Progabide®, 4-[[(4-chlorophenyl)(5-fluoro-2-hydroxyphenyl)-methylene]amino] butanamide, labelled individually with carbon-14 in the butanamide side-chain and in the imino carbon were synthesised for pharmacokinetic and metabolism studies. The former from potassium [^{14}C] cyanide and the latter from barium [^{14}C] carbonate in overall radiochemical yields of 33.8 and 42.2 % respectively.

Key words : Progabide®, Carbon-14, Synthesis.

INTRODUCTION

The important neurotransmitter 4-aminobutyric acid (GABA) has been shown to be effectively excluded from the central nervous system by the blood brain barrier when systemically administered.[1,2]

A series of GABA derivatives have been synthesised with an imine link to a lipophilic carrier in order to facilitate the passage of GABA across the blood brain barrier.[3,4]

One compound possessing anticonvulsant and GABA mimetic activity was required labelled with carbon-14 for metabolism and pharmacokinetic studies.

This compound has been designated SL 76.002, Progabide®, (I), 4-[[(4-chlorophenyl)(5-fluoro-2-hydroxyphenyl)-methylene]amino]butanamide.

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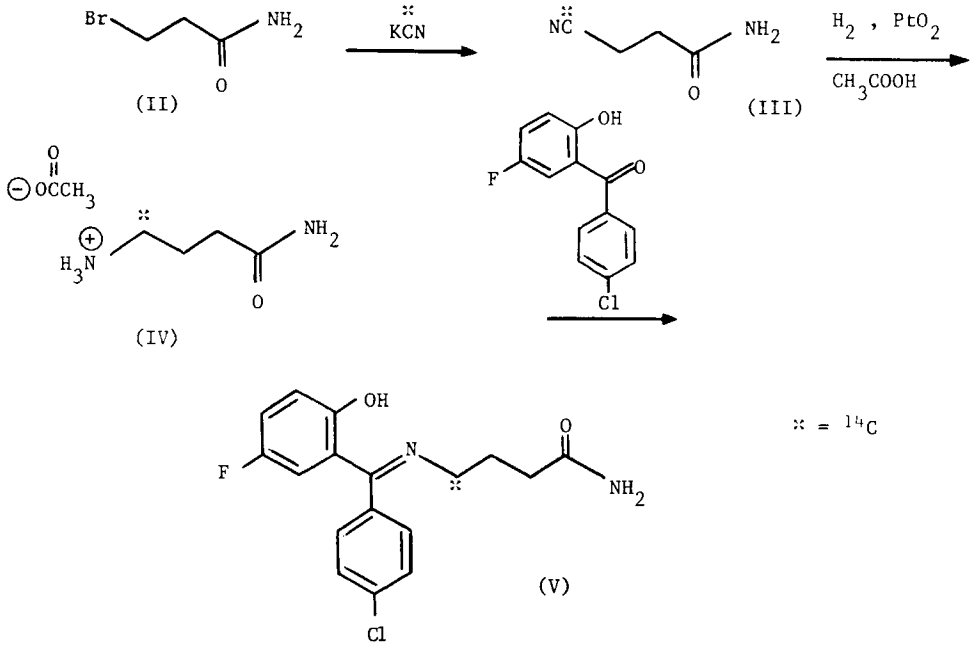
SCHEME I



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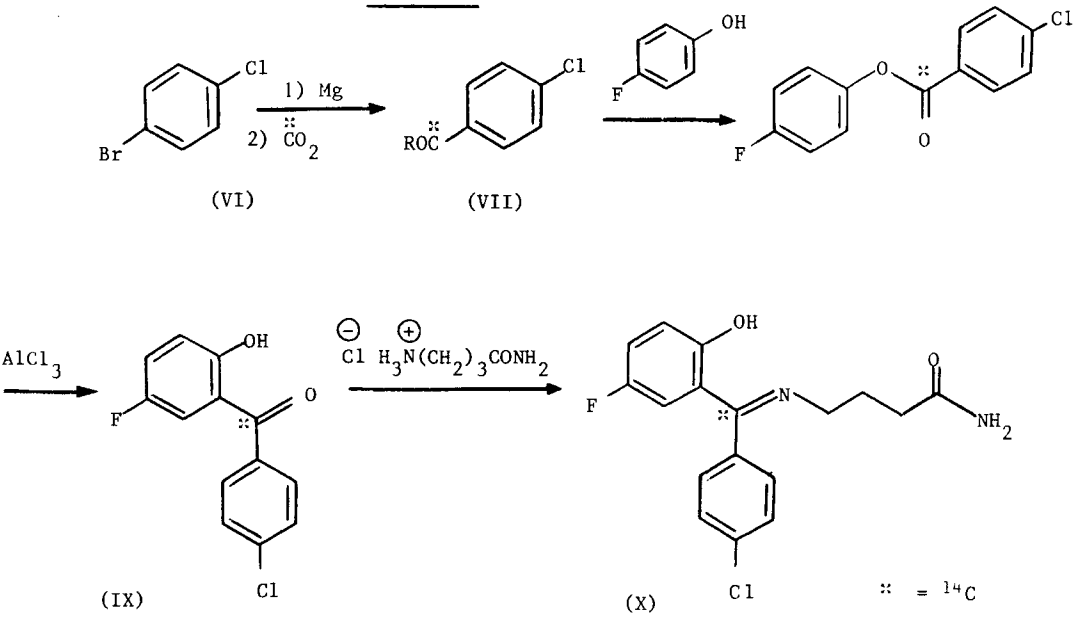

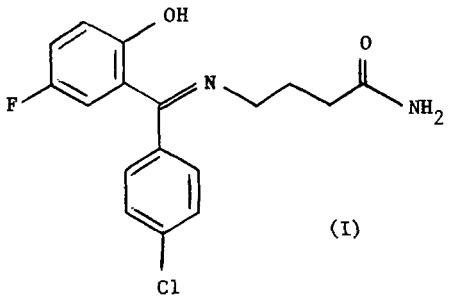

(I)

Initially, SL 76.002 (I) was labelled in the butanamide side-chain but since in an animal study a large proportion of the radioactivity was excreted as $^{14}CO_2$, presumably via an oxidative deamination of GABA to succinic acid and subsequent degradation via the Krebs cycle, it was therefore decided to have the label in the benzophenone moiety.

The compound labelled in the butanamide side-chain was however useful to study the 4-aminobutanamide and GABA distribution and metabolism pattern and especially to see if there was the desired apparition of SL 76.002, GABA, its amide and / or derived metabolites in the brain.

Two individual labelled forms were therefore required, these being the [4-^{14}C] butanamide (V) and [imino-^{14}C] (X) compounds which were synthesised as outlined in scheme I and II.

3-Bromopropanamide [5] (II) on treatment with potassium [^{14}C] cyanide gave the desired 3-[^{14}C] cyanopropanamide (III). Hydrogenation of this in acetic acid using Adams' platinum oxide catalyst afforded the 4-[4-^{14}C]aminobutanamide acetate salt (IV) which reacted with (4-chlorophenyl)-(5-fluoro-2-hydroxyphenyl)-methanone to afford [^{14}C] SL 76.002 (V) labelled in the butanamide side-chain in a 33.8 % radiochemical yield from potassium [^{14}C] cyanide.

The Grignard reagent obtained from 4-bromochlorobenzene (VI) was carbonated with [^{14}C] carbon dioxide and the 4-chloro-[carboxyl-^{14}C] benzoic acid (VII, R = OH) obtained treated with thionyl chloride to yield the corresponding acid chloride (VII, R = Cl) which reacted with 4-fluorophenol to give the ester (VIII). Fries rearrangement of this ester (VIII) gave the methanone (IX) which reacted smoothly with 4-aminobutanamide hydrochloride to yield

[^{14}C] SL 76.002 (X) labelled in the benzophenone moiety in a 42.2 % radiochemical yield from barium [^{14}C] carbonate.

EXPERIMENTAL

Barium [^{14}C] carbonate and potassium [^{14}C] cyanide were purchased from Amersham France. Samples were counted on a Searle Mark III counter using Instagel (Packard) as counting medium. The photographic film used for autoradiography was Kodak "Kodirex" X-ray film.

4-[[(4-chlorophenyl)(5-fluoro-2-hydroxyphenyl)methylene]amino][4-^{14}C]butanamide (V).

To potassium [^{14}C] cyanide (50 mCi) in water (1 ml) was added dropwise over 1 hour a solution of 3-bromopropanamide[5] (152 mg, 1 mmol) in DMF (1 ml) with heating at 90°C and stirring. After the addition the reaction was continued for a further 30 minutes and then evaporated.

The residue was dissolved in glacial acetic acid (1 ml) and filtered. The flask and filter were washed with further portions of glacial acetic acid (2 x 1 ml) which were combined with the original filtrate. Adams' platinum oxide (50 mg) was added and the product hydrogenated for 3 hours at room-temperature and pressure. This was then filtered through a 5 µ Millipore filter, rinsed with glacial acetic acid, water and then evaporated and dried under vacuum.

The product was dissolved in dry methanol (2 ml), sodium methoxide (60 mg) and (4-chlorophenyl)-(5-fluoro-2-hydroxyphenyl)methanone (250 mg) were added. The methanol was slowly evaporated under a stream of nitrogen with heating at 70°C. This procedure was repeated with dry methanol (2 ml) and then with dry ethanol (2 ml). The residue was redissolved in chloroform (10 ml), washed with water, dried (Na_2SO_4) and evaporated.

Purification of the product was carried out by hplc on silica (Merck, column lobar® A) eluting with benzene : acetone (60 : 40) to afford the desired 4-[[(4-chlorophenyl)(5-fluoro-2-hydroxyphenyl)methylene]amino][4-^{14}C]

butanamide (V)(129.9 mg, 16.9 mCi, 43.6 mCi/mmol) in a 33.8 % radiochemical yield from potassium [^{14}C] cyanide.

The radiochemical purity was found to be greater than 99 % by t.l.c. in the following systems.

1- Benzene : acetone (6 : 4)

2- Di-ethylether : methanol (95 : 5)

3- Ethyl acetate

The purity was determined using a Chromelec radioactive plate scanner.

4-Chloro- [carboxyl-^{14}C] Benzoic Acid (VII, R = OH)

4-Bromochlorobenzene (4.79 g, 25 mmol) in dry ether (50 ml) was added gradually to magnesium turnings (0.6 g, 25 mmol) in dry ether (30 ml). The reaction was initiated with a crystal of iodine, heated under gentle reflux during the addition and then subsequently for a further 90 minutes.

The Grignard reagent [6] (1.1 mmol) was then treated with [^{14}C] carbon dioxide (50 mCi) generated from barium [^{14}C] carbonate and the carbonation carried out with stirring at -20°C for one hour.

The reaction mixture was treated with sulphuric acid (5ml) and then diluted with ether and water. The aqueous layer was separated and then extracted with ether. The desired acid was isolated by extraction of the combined organic phases with 10 % sodium carbonate, acidification, extraction with ether, washing with water, drying (Na_2SO_4) and evaporation. The 4-chloro[carboxyl-^{14}C] benzoic acid (VII, R = OH) (140.3 mg, 0.89 mmol) obtained was used directly for the following stage.

4-Chloro-[carboxyl-^{14}C] Benzoic Acid, 4-Fluorophenyl Ester (VIII)

To 4-chloro-[carboxyl-^{14}C] benzoic acid (VII, R = OH) (140.3 mg) was added thionyl chloride (2 ml) and the reaction mixture heated under reflux for 1 $\frac{1}{2}$ hours. The excess thionyl chloride was evaporated, dry benzene (5 ml) added and the evaporation repeated to remove the last traces of thionyl chloride.

The acid chloride was dissolved in dry ether (5 ml) and p-fluorophenol (99.2 mg, 0.89 mmol) in dry ether (5 ml) and triethylamine (0.15 ml, 1.11 mmol) were added and the solution heated under reflux for 1 hour. The triethylamine hydrochloride formed was filtered off and washed with dry ether. The ether layer was then washed with 10 % sodium bicarbonate, water, dried (Na_2SO_4) and evaporated to afford 4-chloro-[carboxyl-^{14}C] benzoic acid, 4-fluorophenyl ester (VIII) (210 mg) in an 88 % radiochemical yield from barium [^{14}C] carbonate.

(4-Chlorophenyl)-(5-fluoro-2-hydroxyphenyl) [^{14}C] methanone (IX)

The ester (VIII) (210 mg) was heated in an oil bath at 120°C until it melted and then aluminium chloride (224 mg) was added and the temperature raised to 200°C. Heating was continued at this temperature with stirring for 5 minutes and then allowed to cool. The solid residue was slowly dissolved in a solution of 7N hydrochloric acid (19 ml) and the product extracted with ether which was subsequently washed with water, dried (Na_2SO_4) and evaporated to yield the crude methanone (IX) (184.1 mg, 38.2 mCi).

Purification of this by hplc on silica (Merck, Lichroprep Si 60) eluting with chloroform afforded (4-chlorophenyl)-(5-fluoro-2-hydroxyphenyl) [^{14}C] methanone (IX) (33.4 mCi, 161 mg, 51.8 mCi/mmol).

4-[[(4-Chlorophenyl)(5-fluoro-2-hydroxyphenyl)[^{14}C] methylene]amino] butanamide (X)

The methanone (IX) (29.5 mCi, 142.5 mg, 0.57 mmol) in dry methanol (10 ml) was treated with sodium methoxide (44 mg, 0.8 mmol) and 4-aminobutanamide hydrochloride (110 mg, 0.8 mmol) and was heated at 90-100°C under nitrogen for 3 hours. After evaporation this was redissolved in chloroform, washed with water, dried (Na_2SO_4) and evaporated.

Purification of the product was carried out by column chromatography on silica (30 g, Merck Si 60, 70-230 mesh). The unreacted methanone (IX) was recovered by elution with chloroform and then by elution with ethyl acetate; the

4-[[(4-chlorophenyl)(5-fluoro-2-hydroxyphenyl) [^{14}C] methylene] amino] butanamide (X) (21.1 mCi, 136.9 mg, 51.8 mCi/mmol) was recovered in an overall radiochemical yield of 42.2 % from barium [^{14}C] carbonate.

The radiochemical purity was found to be greater than 99 % by t.l.c. in the same systems as previously used for the [4-^{14}C] butanamide compound.

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5. Prepared from 3-bromopropionic acid via the acid chloride obtained by treatment with thionyl chloride and then subsequent treatment with ammonia.
6. Aliquots of the Grignard reagent were treated with standard hydrochloric acid and the excess acid estimated by titration with standard sodium hydroxide and the concentration of the Grignard reagent determined. This procedure was a modification of that described by Gilman H., Wilkinson P.D., Fishel W.P. and Meyers C.H.- J. Amer. Chem. Soc., 45 : 150 (1923).