A NEW SYNTHETIC ROUTE FOR PREPARING ^{14}C-LABELLED AMINOGLUTETHIMIDE

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SUMMARY

A new route for preparing aminoglutethimide labelled at C_5 (Scheme I (10)) with ^{14}C is described, which offers the advantage of introducing ^{14}C at a late stage in the synthesis.

Key words: ^{14}C-Aminoglutethimide, Glutarimides, Radiopharmaceuticals, Steroid biosynthesis, Breast cancer.

INTRODUCTION

Aminoglutethimide inhibits (a) the conversion of cholesterol into pregnenolone in the adrenal gland (1,2) and (b) the aromatisation of androgens to oestrogens in peripheral tissues (3). These endocrine effects were the main reason for withdrawing the drug as an anticonvulsant from the market in 1966 (4). However, they now form the basis for the recent re-introduction of aminoglutethimide as an alternative treatment to major endocrine ablation for postmenopausal patients with disseminated breast cancer (5). The metabolism of this drug has become therefore a subject of renewed interest. This has been studied in man by Douglas and Nicholls (6) and more recently in the rat by Egger *et al*. (7).

For metabolic mass balance studies aminoglutethimide labelled at C_5 with ^{14}C has been synthesised. Bernhard *et al*. synthesised ^{14}C-labelled glutethimide in 1956 (8) starting from benzeneacetonitrile. Our work offers the advantage that the ^{14}C label is introduced at a late stage in the synthetic route (Scheme I).

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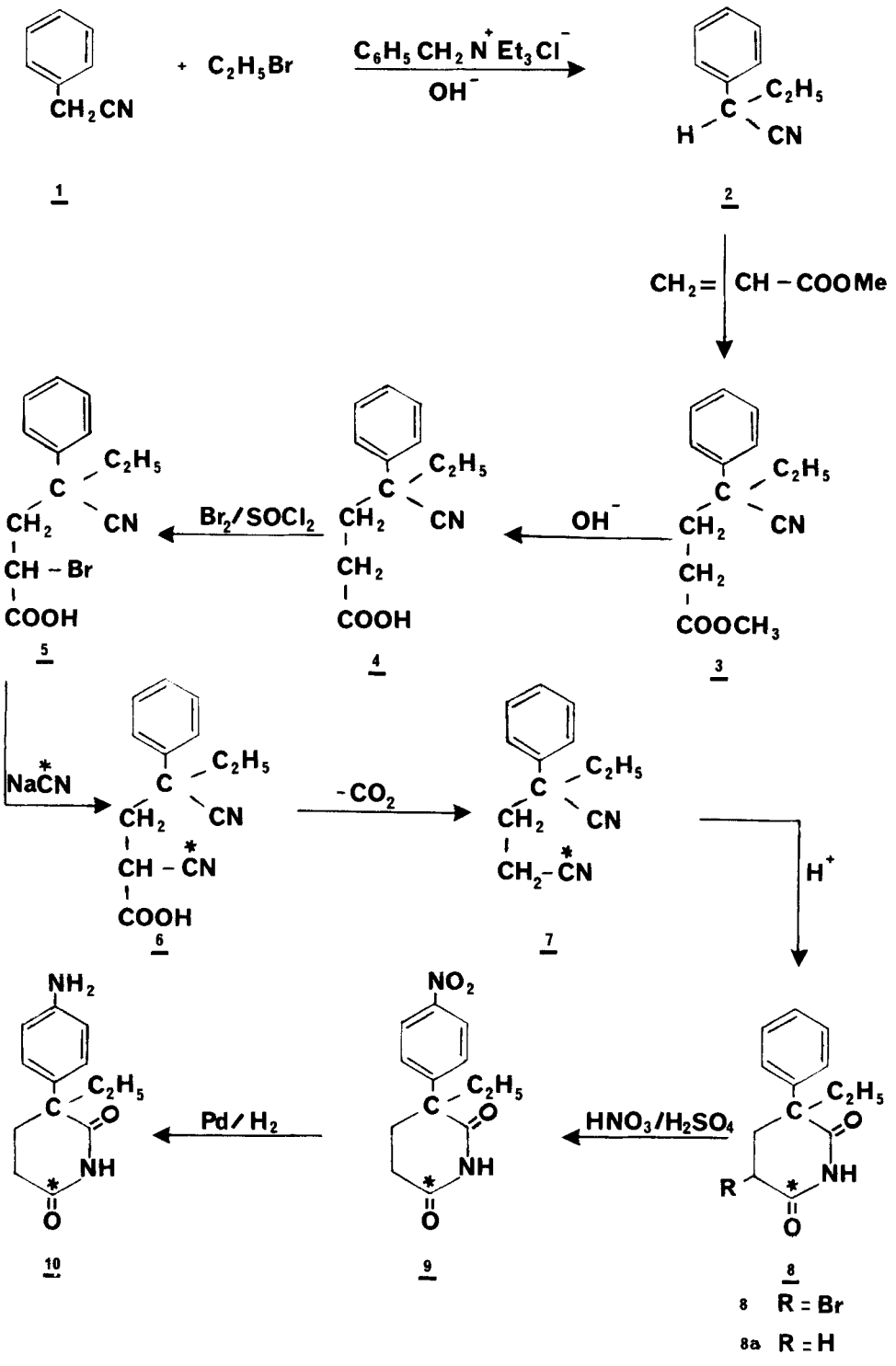
0362-4803/83/121331-08$01.00

Received March 31, 1983
Revised July 26, 1983


CH2CN
+ C2H5Br
C6H5CH2N+Et3Cl−
OH−
1
2
CH2= CH − COOMe
3
OH−
4
Br2/SOCl2
5
NaCN*
6
−CO2
7
H+
8
R = Br
8a R = H
HNO3/H2SO4
9
Pd/H2
10

SCHEME I

DISCUSSION

Benzeneacetonitrile was ethylated by applying the phase transfer method of Makosza and Jonczyk (9) - the best of a number of methods tried. Following Bernhard's method (8), the ester (3) was obtained by the action of methyl acrylate. It was subsequently hydrolysed to give the acid (4). This was brominated in thionyl chloride by Schwenk and Papa's method (10) to give the bromo acid (5), after removing the excess of thionyl chloride under reduced pressure. However, when the excess of thionyl chloride was removed by Ingold's method (11) using formic acid at 100°, the product was not the acid (5) but the bromo imide (8). This reaction offers a convenient one step process for producing the bromo imide (8). The bromo acid (5) was converted into the labelled cyano acid (6) which was then decarboxylated to give the dinitrile (7). Cyclisation of the dinitrile (7) gave glutethimide (8a) labelled at position -5, which was then converted into the required (5-^{14}C) aminoglutethimide by nitration and reduction (10).

EXPERIMENTAL

I.r. spectra were recorded on Nujol mulls using a Perkin-Elmer 357 i.r. spectrophotometer. N.m.r. spectra were scanned on a Perkin-Elmer R32 n.m.r. spectrophotometer. Microanalysis was performed by Mr. G.S. Crouch of the School of Pharmacy, London. Organic solvents were dried using molecular sieve (4A). Sodium (^{14}C) cyanide was purchased from Amersham International plc. Thin layer chromatography was performed with Merck chromatogram plastic sheets coated with silica gel containing a fluorescent indicator (Type 5735).

2-Phenylbutanenitrile (2)

Bromoethane (32.7 g, 0.3 mole) was added dropwise over 50 minutes to a stirred mixture (maintained at 30-35°) of 50% aqueous sodium hydroxide (70 ml), benzeneacetonitrile (30 g, 0.26 mole) and benzyltriethylammonium chloride (0.54 g, 0.002 mole). Stirring was continued for 2 h and then for 20 h at 55°. Subsequently the mixture was cooled to 25°, benzaldehyde (4.3 g, 0.04 mole) added and stirring continued for 1.5 h at this temperature.

Water (100 ml) and benzene (50 ml) were added and the layers were separated. The aqueous phase was extracted with benzene (100 ml) and the organic layers were combined and successively washed with water (60 ml), dilute hydrochloric acid (60 ml) and water (60 ml). The organic layer was dried (Na_2SO_4), evaporated under reduced pressure and the oily residue distilled to give (a) excess benzaldehyde (60-62°/1mm Hg) and (b) the required product as a colourless oil, b.p. 78-80°/1mm Hg (21 g, 56%); lit. (9), b.p. 102-104°/7mm Hg (78-84%). This on t.l.c. (benzene:ethyl acetate, 4:1) showed one spot (R_f 0.86) and no spot corresponding to benzeneacetonitrile.

Methyl 4-cyano-4-phenylhexanoate (3)

This was prepared in 45% yield by the method of Bernhard et al. (8), b.p. 138-142°/1.5mm Hg, lit. (8), 160-180°/12mm Hg.

4-Cyano-4-phenylhexanoic acid (4)

Methyl 4-cyano-4-phenylhexanoate (3) (10 g, 0.04 mole) was boiled under reflux with methanolic potassium hydroxide (10%, 30 ml) for 2 h. The resulting solution was cooled and acidified with dilute hydrochloric acid. The precipitate was removed by filtration, washed with water, dried and crystallised from aqueous alcohol as white crystals (8 g, 86%) m.p. 91-93°, lit. (12), m.p. 92-93°; ir 2300-3240 cm^{-1} (br COOH), 2240 cm^{-1} (CN), 1698 cm^{-1} (C=O); nmr ($CDCl_3$) δ 0.9 (t, 3H, CH_2-$\underline{CH_3}$), 2.0 (q, 2H, $\underline{CH_2}$-CH_3), 2.2-2.65 (m, CH_2-CH_2), 7.3 (s, br 5H, ArH), 8.9 (s, br 1H, COOH).

Anal. Found: C, 71.36; H, 6.96; N, 6.54. $C_{13}H_{15}O_2N$ requires: C, 71.86; H, 6.95; N, 6.45%.

2-Bromo-4-cyano-4-phenylhexanoic acid (5)

Method (A)

Thionyl chloride (12.5 ml), purified by distillation from quinoline (13), was added to 4-cyano-4-phenylhexanoic acid (9 g, 0.04 mole) in a three-necked flask fitted with a condenser protected with a calcium chloride tube. The mixture was refluxed for 2 h, and then (under gentle

reflux) bromine(6.9 g, 0.04 mole) was added dropwise over a period of 3 h. The mixture was left overnight and the excess thionyl chloride then removed under reduced pressure. The mixture was poured into ice-water to yield an oil which solidified overnight. The sticky solid was extracted with benzene and the solution washed with 10% sodium bicarbonate solution. The aqueous layer was acidified with dilute hydrochloric acid and extracted with ether; the ethereal solution was then dried (Na_2SO_4) and decolourised with charcoal. Evaporation of the ether gave a sticky residue which was stirred with light petroleum (40-60°). A yellowish solid separated which gave white crystals (6.0 g, 51%, m.p. 122-124°) from light petroleum (80-100°)/benzene; ir 2320-3240 cm^{-1} (br COOH), 2237 cm^{-1} (CN), 1713 cm^{-1} (C=O); nmr ($CDCl_3$) δ 0.93 (t, 3H, CH_2-$\underline{CH_3}$), 2.0 (q, 2H, $\underline{CH_2}$-CH_3), 2.5-3.0 (m, 2H, $\underline{CH_2}$-CHBr), 4.12 and 4.21 (2d, J=6.7, 9 Hz, 1H, $\underline{CH}$Br), 7.2-7.5 (s, br 5H, ArH), 8.7-9.6 (s, br 1H, COOH).

Anal. Found: C, 52.86; H, 4.81; N, 4.60; Br, 26.54. $C_{13}H_{14}O_2NBr$ requires: C, 52.72; H, 4.76; N, 4.73; Br, 26.9%.

Method (B)

4-Cyano-4-phenylhexanoic acid (7 g, 0.03 mole) was brominated as in the previous method using thionyl chloride (9.7 ml) and bromine (5.4 g, 0.03 mole). After removing the excess thionyl chloride under reduced pressure, formic acid (2 ml) was added dropwise (cautiously with cooling) from a dropping funnel and the mixture then heated for 20 minutes at 100° and poured into water. After standing overnight the mixture was extracted with benzene which was then washed with water, dried (Na_2SO_4) and evaporated under reduced pressure to give a black oil. This was washed with a mixture of ether/light petroleum (60-80°) when a solid separated which, on crystallisation from ethanol, gave colourless crystals (3 g, 31%) m.p. 163-165°, lit. (14), m.p. 165-167°. The product was found to be 4-bromo-2-ethyl-2-phenylglutarimide (8) previously prepared by Urech *et al.*(14) and Aboul-Enein *et al.* (15).

Ir 3100, 3200 cm^{-1} (NH), 1710, 1728 cm^{-1} (C=O imide); nmr ($CDCl_3$) δ 0.88 (t, 3H, CH_2-$\underline{CH_3}$), 2.0 (two q overlapping, 2H, $\underline{CH_2}$-CH_3), 2.4-3.0 m, 2H, $\underline{CH_2}$-CHBr), 4.4 and 4.5 (2d, J=6.7, 11.1 Hz, 1H, $\underline{CH}$Br), 7.18-7.5 (m, 5H, ArH), 8.2 (s, br 1H, NH).

Anal. Found: C, 52.62; H, 4.74; N, 4.73; Br, 26.87. Calcd for $C_{13}H_{14}O_2NBr$: C, 52.72; H, 4.76; N, 4.73; Br, 26.97%.

2-Ethyl-2-phenyl(5-^{14}C)glutarimide (8a)

(1) 2-Bromo-4-cyano-4-phenylhexanoic acid (5 g, 0.016 mole) was suspended in water (10 ml) and neutralised with sodium bicarbonate solution (10%, 50 ml) using phenolphthalein as indicator (16). The mixture was warmed to 55° and added to a solution of sodium (^{14}C) cyanide (0.11 g, 0.002 mole, specific activity 50 mCi/mmol), and "cold" sodium cyanide (0.91 g, 0.02 mole) in water (7 ml) maintained at 75°. The solution was heated at 95° for 10 h and the brown solution was then cooled, acidified with dilute hydrochloric acid (trapping the hydrogen cyanide evolved in a suspension of calcium hydroxide in ferrous sulphate solution) and extracted twice with chloroform. The extract was then washed twice with water and evaporated to dryness; fresh chloroform was added to the residue and the solution was dried (Na_2SO_4) and evaporated under reduced pressure to give a black oil. Qualitative tests showed Br to be absent, ir (film) 2240 cm^{-1} (br CN).

(2) The crude oil from the previous step (3 g, 0.01 mole) was heated in an oil bath at 150° for 10 hours with stirring. The oil was dissolved in ether (20 ml) and washed with 10% sodium carbonate (10 ml). The ether layer was dried (Na_2SO_4) and evaporated to leave a sticky oil (1,2 g, 48%). The crude oil was used for the following step.

(3) The dinitrile (1.2 g, 0.006 mole) was cyclised as reported by Aboul-Enein et al. (15). The oily product was stirred with ether-light petroleum (40-60°) to yield a solid which, on crystallisation from water-ethanol mixture, gave colourless crystals (0.6 g, 53%) m.p. 81-84°, lit. (17), 82-86°.

2-Ethyl-2-(4-nitrophenyl)(5-^{14}C)glutarimide (9)

2-Ethyl-2-phenylglutarimide (0.6 g, 0.002 mole) was nitrated as reported by Aboul-Enein et al. (15) and the product was crystallised from methanol to give white crystals (0.3 g, 43%) m.p. 135-137°, lit. (15), m.p. 135-137°. Ir (KBr) 1692, 1730 Cm^{-1} (C=O imide), 1350, 1525 Cm^{-1} (NO_2).

2-(4-Aminophenyl)-2-ethyl(5-^{14}C)glutarimide (10)

2-Ethyl-2-(4-nitrophenyl)(5-^{14}C)glutarimide (0.3 g, 0.001 mole) in ethyl acetate (30 ml) was reduced catalytically at atmospheric pressure using 10% palladium/charcoal (400 mg). After the absorption of hydrogen ceased, the catalyst was filtered off and the solvent was evaporated to yield an oily residue. The oil solidified when cooled with acetone-solid carbon dioxide mixture and gave colourless crystals from benzene/ethyl acetate (0.18 g, 69%) m.p. 149-152°, lit. (15), 148-150°. M.p. of an authentic (cold) sample (149-152°). The radioactive product was contaminated with some of the p-nitro compound as shown by t.l.c. This was removed by stirring with light petroleum (60-80°) the product then showing only one spot on t.l.c. (100µg load, chloroform:methanol 95:5). The radiochemical purity was 98.9%. The specific activity, determined against a standard (^{14}C)hexadecane sample, was 5.4mCi/mmol. For biological investigations this material was diluted fifty-fold with pure "cold" aminoglutethimide.

ACKNOWLEDGMENT

The authors thank Mr. P. Martin for technical assistance, and Dr. R. Bowman for helpful discussion.

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