SYNTHESIS OF ^{14}C-LABELED AMINOGLUTETHIMIDE

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SUMMARY

A new procedure has been developed for the synthesis of ^{14}C-labeled glutethimide with an improved yield from $K^{14}CN$. In this procedure benzyl cyanide prepared from benzyl chloride and $K^{14}CN$ was almost quantitatively monoethylated by an ion-pair extraction method using 50% excess of tetrabutylammonium hydroxide as the catalyst. Michael addition of methyl acrylate to monoethylbenzyl cyanide followed by hydrolysis with H_2SO_4 gave glutethimide which was then nitrated (HNO_3 + H_2SO_4) and catalytically reduced to aminoglutethimide. The best result was obtained when the intermediate para-nitroglutethimide was isolated in pure form by crystallization and then reduced in the presence of 10% Pd/C. When the nitration product (which was a mixture of isomers) was reduced without purification, isolation of pure p-aminoglutethimide was more difficult and the yield was much lower.

Key words: [^{14}C]Glutethimide, [^{14}C]Aminoglutethimide, Ion-pair extraction, Monoethylbenzyl cyanide

INTRODUCTION

Aminoglutethimide, 3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione (7) was originally introduced (1) as an anticonvulsant drug (Elipten™, CIBA) for treatment of epilepsy. It was subsequently withdrawn from the

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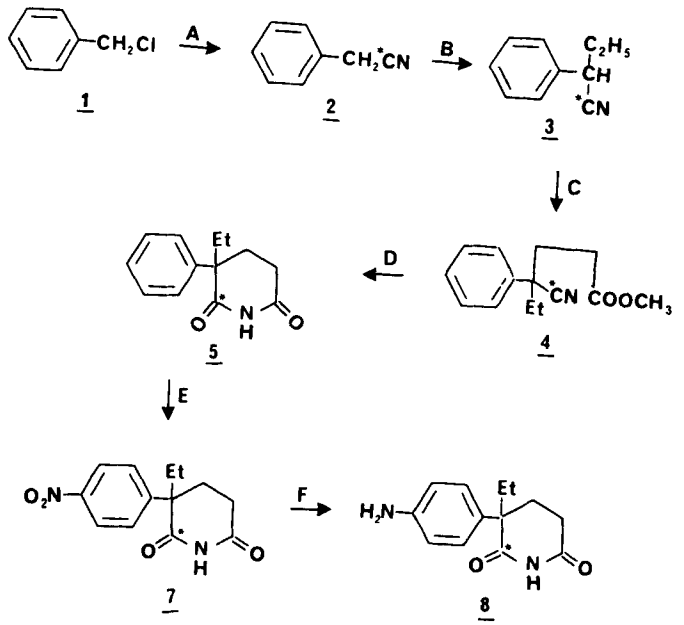
market mainly because it caused adrenal insufficiency by inhibiting adrenal steroidogenesis (2,3). However, in recent years, these inhibitory effects have been suggested to be of utility in the treatment of hormone-dependent breast carcinomas (4-6) as an alternative to adrenalectomy. It has, therefore, been reintroduced into therapy for treatment of breast cancer and Cushing's syndrome caused by adrenal hyperfunction. Metabolism studies of aminoglutethimide have, therefore, become very important. For this reason, we synthesized ^{14}C-labeled aminoglutethimide (7) on two occasions and studies with one of the preparations have already been published (7). In this paper, we describe in detail the methods of our synthesis and some of the difficulties associated with obtaining glutethimide (5) and aminoglutethimide (7) in a pure state and with a good yield.

METHODS AND RESULTS

The synthesis of aminoglutethimide which is described in a patent literature (1) involves nitration of glutethimide at the para position of the benzene ring and catalytic reduction of the resulting p-nitroglutethimide. The synthesis of ^{14}C-labeled aminoglutethimide (7) in our laboratories was accomplished by adapting this method. For this purpose, we first synthesized ^{14}C-labeled glutethimide (5) the preparation of which has been described by Bernhard et al (8). The sequence of reactions employed for the synthesis of labeled aminoglutethimide (7) from $K^{14}CN$ is given in the synthetic scheme.

In our first preparation of ^{14}C-labeled glutethimide (5), we followed the published procedure (8) and found that although the overall yield of 5 from $K^{14}CN$ was very good (53%) as reported by the authors, the product was not chromatographically pure. Purification by crystallization or column chromatography lowered the yield to 33%. Investigations by thin layer chromatography (TLC) and nuclear magnetic resonance spectroscopy (NMR) re-

SYNTHETIC SCHEME



A = $K^{14}CN$

B = n-$Bu_4N \cdot HSO_4$, NaOH, EtI or $NaNH_2$, EtBr

C = $CH_2{=}CHCOOCH_3$

D = H_2SO_4

E = $HNO_3 + H_2SO_4$

F = H_2(Pd/C)

vealed that the compounds 3 and 4 as obtained by the above method contained impurities which could not be separated by distillation without loss of some desired materials. Particularly, the separation of unreacted benzyl cyanide (2) from monoethylbenzyl cyanide (3) proved to be very difficult.

Therefore, in our later synthesis, we looked for a method which would give monoethylbenzyl cyanide in almost quantitative yield and chose the ion pair extraction procedure of Brandstrom and Junggren (9). These authors have claimed to obtain 100% monoethylation of benzyl cyanide by using two molar equivalents of ethyl iodide and 1·2 molar equivalents of tetrabutylammonium hydroxide as the catalyst. But we observed during our trial runs using unlabeled materials that the use of 1·2 molar equivalents of the catalyst gave only 75-80% monoethylation as judged by the NMR

spectrum of the product. The extent of monoethylation was measured by the disappearance of a sharp singlet at 3·7 ppm due to the methylene protons of benzyl cyanide and by the appearance in its place of a triplet due to the methine proton of monoethylbenzyl cyanide. However, when we increased the amount of the catalyst to 1·5 molar equivalents, there was practically no unreacted benzyl cyanide in the product as judged by the NMR spectrum and thin layer chromatogram of the product. We then applied this ion-pair extraction procedure for the synthesis of ^{14}C-labeled ethylbenzyl cyanide (3) which was then converted to glutethimide (5). The overall yield of chromatographically pure glutethimide (5) obtained by this method was considerably higher (51% from $K^{14}CN$) than before.

Labeled glutethimide (5) was then nitrated by following the procedure described in the patent literature (1). The preparation of pure para-nitroglutethimide (6) free from other isomeric nitro compounds with a good yield posed a problem. In the patent method (1), the nitration product was crystallized once to obtain a product of m.p. 128-36°C in 85% yield. When this material was further purified by crystallization, a product having a m.p. of 137-39°C was obtained in an unspecified yield. Later, when Aboul-Enein et al (10) prepared p-nitroglutethimide by this method, they obtained a product of m.p. 135-37°C in 53% yield. Recently, p-nitroglutethimide has been prepared by two other groups (11,12) and the reported m.p's are 140-41°C and 142-43°C. Nothing was known, however, about the purity of these materials having different melting points. In one of our preparations of the labeled p-nitroglutethimide (6), the nitration product was crystallized twice from ethyl acetate to obtain a product of m.p. 137-39°C in 50% yield. The NMR signals due to the aromatic protons of this product appeared as two A_2B_2 doublets showing thereby that it was pure p-nitroglutethimide (6). The material from the mother liquor was crystallized twice from isopropanol to give a product of m.p. 170-72°C the structure of which was confirmed as meta-nitroglutethimide by the NMR

signals of its aromatic protons. p-Nitroglutethimide (6) was then catalytically reduced and p-aminoglutethimide (7) was isolated by crystallization from ethyl acetate in 85% yield. The purity of 7 was investigated by NMR spectroscopy and TLC in a solvent system (provided by CIBA-GEIGY Analytical Research Department) which was known to separate a mixture of p-aminoglutethimide and m-aminoglutethimide. The results showed that the compound 7 was pure p-aminoglutethimide. The radiochemical purity as determined by the isotope dilution method was found to be 99%.

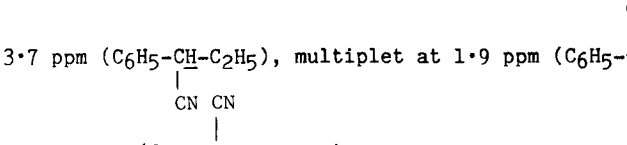
In another preparation, we decided to hydrogenate the nitration product of m.p. 128-36°C and then purify the amino compound in order to see whether the yield of aminoglutethimide could be increased thereby. The hydrogenation product was isolated by column chromatography and then crystallized twice from ethyl acetate/petroleum ether (1/1) to get a product with about 60% yield. When the purity of this product was checked by TLC it was found to be a mixture of p-amino and m-amino compounds by comparison with authentic compounds. The amount of m-aminoglutethimide in the mixture was then quantitatively determined by the isotope dilution method. A small amount of the labeled material was diluted with a large amount of pure unlabeled aminoglutethimide and then crystallized from methanol. The specific activity of the crystallized material was 15% lower than the calculated value and the specific activity of the material from the mother liquor was correspondingly higher. The labeled material was then purified by two crystallizations from ethyl acetate with considerable loss of the desired material in the mother liquor. The final product was pure by TLC and at least 99% pure by the isotope dilution method. However, the yield from glutethimide was only 25% which is much lower than 43% obtained by the first method. Because the mixture of isomeric nitroglutethimides was more readily separated by crystallization than the mixture of isomeric amino compounds it is better to purify p-nitroglutethimide before hydrogenation to p-aminoglutethimide.

EXPERIMENTAL

Melting points are uncorrected. Thin layer chromatography was performed on silica gel 60 F 254 (E. Merck) plates of 0.5 mm thickness and column chromatography with 70-230 mesh silica gel 60 (E. Merck). Organic extracts were dried with magnesium sulfate. Nuclear magnetic resonance spectra were obtained in $CDCl_3$ solution with a 90 MHz Varian EM 390 spectrometer. $K^{14}CN$ was purchased from New England Nuclear Corporation of Boston, Massachusetts, U.S.A. and it was also prepared in our Isotope Laboratory at Basle, Switzerland.

[^{14}C]Benzyl cyanide (2). This was prepared by heating an ethanolic solution of $K^{14}CN$ with benzyl chloride according to the published procedure (8). The reaction was monitored by TLC in ethyl acetate/petroleum ether (10/90). The yield was 95% on 10 mmolar scale.

2-Phenylbutyro-[1-^{14}C]nitrile (3). To a solution of 5.1 g of tetrabutylammonium hydrogen sulfate (15 mmol) in 15 ml of water was added with stirring 1.2 g of NaOH (30 mmol). A solution of 1·17 g of [^{14}C]benzyl cyanide (10 mmol) and 3·12 g of ethyl iodide (20 mmol) in 15 ml of methylene chloride was then added to the above mixture. After refluxing for 45 min, the mixture was cooled, and layers separated. The organic layer was then dried and evaporated to dryness. The residue was extracted several times by stirring with ether and filtering. The filtrate was evaporated to give 1·3 g (90% of theory) of an oil. NMR: singlet at 7·4 ppm ($C_6\underline{H}_5$), triplet at



3·7 ppm (C_6H_5-$\underline{C}\underline{H}$(CN)-$C_2H_5$), multiplet at 1·9 ppm (C_6H_5-$\underline{C}\underline{H}$(CN)-$\underline{C}\underline{H}_2$-$CH_3$) and triplet

at 1·0 ppm (C_6H_5-$\underline{C}\underline{H}$(CN)-$CH_2$-$\underline{C}\underline{H}_3$). TLC in ethyl acetate/petroleum ether (15/85): one spot.

Methyl γ-phenyl-γ[^{14}C]cyano-caproate (4). The above oil was dissolved in 5 ml of dioxane and 1·5 g of methyl acrylate was added to it followed by

0·5 ml of triton B solution. The solution was then heated at 80°C for three hours. Dioxane was removed by distillation under vacuum and the residue extracted with ether. The ether solution was then washed with 1N HCl, dried and evaporated to dryness to give 1·75 g (84% of theory) of an oil. TLC in ethyl acetate/petroleum ether (15/85) showed one major spot and two minor spots one of which was due to unreacted 3.

[^{14}C]Glutethimide (5). The above oil without any purification was dissolved in 3·5 ml of acetic acid and 1·8 ml of 85% H_2SO_4 was added to it. The mixture was then heated at 90° for 2·5 hours, cooled and added to ice-water. The organic matter was extracted with ether and the ether extract washed with 1N NaOH solution. The ether solution was then dried and evaporated to dryness to give 1·35 g of an oil which became solid on standing. TLC in 30% ethyl acetate in petroleum ether showed a major spot due to glutethimide and two minor spots one of which was more polar than the spot due to glutethimide and identical to that due to ethylphenylacetamide formed by the hydrolysis of unreacted 3. The other minor spot which was less polar than that due to glutethimide was not identified. Crude glutethimide was then crystallized from ether/petroleum ether (1/1) to give 1·15 g (70% of theory) of a crystalline solid, m.p. 84-86°C. TLC showed no impurity.

Nitration of [^{14}C]glutethimide. A solution of 1·15 g of glutethimide (5) in 3·5 ml of conc. H_2SO_4 was cooled to -5°C. A nitrating mixture of 1 ml of 71% HNO_3 and 1 ml of conc. H_2SO_4 was then added to the above solution with stirring and cooling. Stirring was continued for half an hour at 0°C and then the solution was poured onto ice-water. The organic material was extracted with methylene chloride and the extract was washed with sodium carbonate solution until neutral. The methylene chloride solution was dried and evaporated to dryness. The residue was crystallized from ethyl acetate to give 1·17 g of a solid, m.p. 128-36°C. TLC in ethyl acetate/petroleum ether (1:1) showed only one spot.

[^{14}C]p-Nitroglutethimide (6). The above solid was recrystallized from ethyl acetate to give 700 mg (50% of theory) of a solid, m.p. 137-39°C. NMR (aromatic region): doublets at 7·56 and 8·34 ppm.

[^{14}C]m-Nitroglutethimide. The material from the mother liquor was twice crystallized from isopropanol to give 175 mg of a solid, m.p. 170-72°C. NMR (aromatic region): Multiplet due to 3H at 7·85 and a multiplet due to 1H at 8·5 ppm.

[^{14}C]Aminoglutethimide. Method A. To a solution of 700 mg of pure p-nitroglutethimide (m.p. 137-39°C) in 50 ml of ethyl acetate was added 700 mg of 10% palladium-on-carbon and the mixture shaken in an atmosphere of hydrogen. The absorption of hydrogen was complete in one hour. The catalyst was removed by filtration and ethyl acetate removed by evaporation. The residue was crystallized from ethyl acetate to give 530 mg (85% of theory) of p-aminoglutethimide (7), m.p. 147-49°C. TLC: triple development in chloroform/ethyl acetate (7/3) showed only one spot. NMR (aromatic region): doublets at 6·65 and 7·1 ppm. Isotope dilution: 99% p-aminoglutethimide.

Method B. To a solution of 950 mg of [^{14}C]nitroglutethimide (m.p. 128-36°C) in 100 ml of ethyl acetate was added 800 mg of 10% palladium-on-carbon and the mixture was shaken in an atmosphere of hydrogen. The product obtained after removal of the catalyst and ethyl acetate was chromatographed on a column of silica gel. The eluate obtained with 35% ethyl acetate in chloroform was evaporated to dryness and the residue was crystallized twice from ethyl acetate/petroleum ether (1/1) to give 500 mg of a solid. TLC: triple development in ethyl acetate/chloroform (30/70) showed a major spot due to p-aminoglutethimide and a slightly faster moving minor spot due to m-aminoglutethimide.

Isotope dilution: To 1·5675 mg (22.38 μCi/mg) of the above solid was added 203.0 mg of unlabeled aminoglutethimide and the resulting mixture which had a calculated specific activity of 0.1094 μCi/mg was then crystallized from

methanol. The crystallized solid had a specific activity of 0.0935 μCi/mg, about 15% less than the calculated value. The impure product was then recrystallized twice from ethyl acetate to obtain about 210 mg (25% of theory) of a solid m.p. 148-50°C. TLC: triple development in ethyl acetate/chloroform (30/70) showed no meta-isomer. Isotope dilution: about 99% p-aminoglutethimide.

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