NOTES

SYNTHESIS OF ^{14}C-LABELLED TRANEXAMIC ACID [trans-AMINO-{^{14}C-METHYL}-CYCLOHEXANE CARBOXYLIC ACID]

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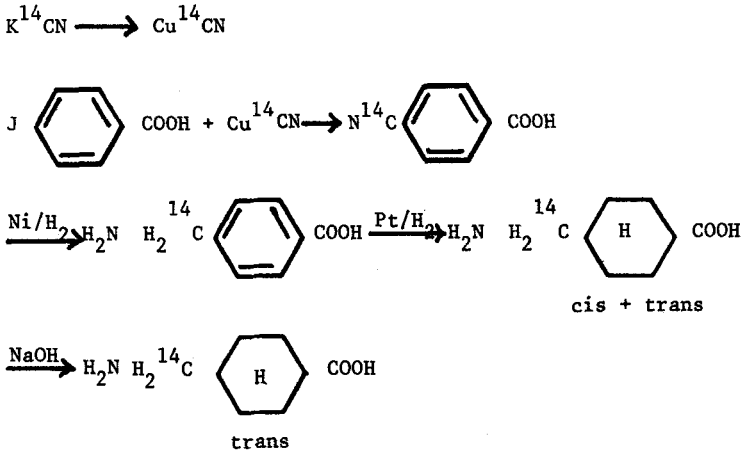
INTRODUCTION

Tranexamic acid (*trans*-4-aminomethylcyclohexane carboxylic acid) is a valuable antifibrinolytic agent which has been used clinically for several years[1]. For biomedical and pharmacological studies of this drug the synthesis of the radioactively labelled compound was required. Although tritium labelling appeared convenient, a metabolically stable ^{14}C-label was preferred due to the nature of the planned investigations, and we now report a procedure for the specific introduction of ^{14}C in the methylene group of the side chain, utilizing potassium ^{14}C-cyanide as the radioactive starting material.

DISCUSSION AND RESULTS

A convenient synthetic route leading to tranexamic acid consists in the reduction of *p*-cyanobenzoic acid[2]. Introduction of a radioactive *p*-cyano group into benzoic acid thus appeared to be an attractive step in the synthesis of ^{14}C-labelled tranexamic acid. The synthetic route is depicted below:



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The conversion of potassium cyanide to cuprous cyanide according to Vogel[3] proceeded smoothly also on a small scale. Several solvents have been suggested for the nucleophilic exchange of the *p*-halogen atom of benzoic acids[4,5,6]. In our case the best yield was obtained in dimethylsulphoxide to which a trace of pyridine had been added. *p*-Iodobenzoic acid was found to afford a slightly higher yield of nitrile than the bromo analog (cf.7).

The reduction of *p*-cyanobenzoic acid to 4-aminomethylcyclohexane carboxylic acid in one step has been reported[8]. In our hands, however, better yields and purer products were obtained if the reduction was carried out in two separate steps. The cyano group was first hydrogenated using a Raney-nickel catalyst at atmospheric pressure[8], whereafter the aromatic ring was reduced in acetic acid over platinium oxide at slightly elevated pressure and temperature[2]. This procedure afforded predominantly the *cis*-isomer (70-90% and 10-30% *trans*). Isomerization of this mixture in sodium hydroxide solution at 210° [9] afforded a product with a *trans*/*cis* ratio of approximately 9 to 1. Crystallization of the amino acid as its toluenesulphonic acid salt from acetone removed the last traces of the *cis*-form. The main fraction of *p*-aminomethylcyclohexane carboxylic acid thus obtained consisted of 98.8% of the *trans*-isomer.

The overall yield based on the radioactive potassium cyanide was about 12%, which appears satisfactory considering the numerous steps involved.

EXPERIMENTAL

Analysis

Separation of the isomers using high voltage paper electrophoresis was carried out at pH 4.5 (50 V, 3 h). The electropherograms were developed with ninhydrine. The ratio of *trans*/*cis*-isomers and residual *p*-aminomethylbenzoic acid was determined by scanning the strips in a Packard Chromatogram scanner Model 7201. Specific radioactivity was determined by liquid scintillation counting, using a Packard 3375 instrument.

^{14}C-Copper(I)cyanide

Powdered crystallized copper(I)sulphate (535 mg) was dissolved in water (1.7 ml) in a water-manteled beaker warmed to 60° (the pH should be 3.5 - 4.5). To this solution was added within 1 min sodium hydrogen sulphite (150 mg in 1 ml of water) immediately followed by potassium ^{14}C-cyanide (150 mg, 100 mCi, in 1 ml of water; The Radiochemical Centre Ltd, Amersham,England) both

solutions warmed to approximately 60°. The resulting mixture was kept at this temperature for 15 min and then filtered through a sintered glass plate. The product was washed with boiling water and by ethanol and finally dried at 110° for 24 h. The yield was 180 mg (87%, based on KCN).

p-(^{14}C-Cyano)-benzoic acid

p-Iodobenzoic acid (540 mg, 2.2 mM) was dissolved in dimethyl-sulphoxide (10 ml) and to the solution was added the foregoing cuprous-^{14}C-cyanide (180 mg, 2 mM) followed by 10 drops of pyridine. The mixture was refluxed for 4 h and then poured into a solution of iron(III)chloride (800 mg) in 2.5 N hydrochloric acid (60 ml). The mixture was kept at 60-70° for 20 min in order to decompose the nitrile-copper iodide complex[5], cooled and extracted with ether (2 x 100 ml). The extracts were dried (Na_2SO_4) and the solvent evaporated. The residue was crystallized from water, yielding 205 mg (70%) of *p*-(^{14}C-cyano)-benzoic acid, mp 212-214°, lit.[10] 213-215°.

p-Amino(^{14}C-methyl)benzoic acid

The *p*-^{14}C-cyanobenzoic acid (205 mg) was dissolved in 20% ammonia (10 ml). Raney-nickel (50 mg, obtained from Liljeholmens Stearinfabrik,Stockholm) was added and the mixture was hydrogenated at room temperature and atmospheric pressure until the consumption of hydrogen ceased (~ 2 h). The catalyst was filtered off, the solution was evaporated to dryness and the acid was crystallized from a small volume of water, yielding 140 mg (60%),mp 343°,lit.[2] 345°.

4-Amino-(^{14}C-methyl)cyclohexane carboxylic acid

The labelled 4-aminomethylbenzoic acid (140 mg) was mixed with inactive material (150 mg) and dissolved in acetic acid (10 ml). Five drops of water and 70 mg of PtO_2 were added and the mixture was hydrogenated at 70° and 4 atm for 12 h with 70 mg of PtO_2 as a catalyst. The catalyst was filtered off and the solvent removed, yielding 230 mg (56%) of amino acid acetate (predominantly *cis*-isomer).

Trans-4-amino-(^{14}C-methyl)-cyclohexanecarboxylic acid. [^{14}C-Tranexamic acid]

The above *cis-trans* mixture, sodium hydroxide (80 mg) and water (2 ml) were heated at 210° for 20 h in a sealed steel container of 7 ml volume. After cooling,water (10 ml) was added and the resulting solution was passed through a strong cation exchange column (0.5 x 10 cm, Dowex 50 x 8, NH_4^+). The eluate and washings (5 ml) were evaporated and to the residue was added a solution of *p*-toluenesulphonic acid (250 mg) in water (20 ml). The resulting mixture was evaporated *in vacuo* at 50° and the remaining semi-solid was dissolved in acetone (50 ml) whilst still warm. From the slightly turbid so-

lution the *p*-toluenesulphonic acid salt of tranexamic acid crystallized. The solid was collected and dissolved in water (25 ml). This solution was passed through an anion exchange column (1 x 12 cm; Amberlite IR-4B, OH^-). The eluate was evaporated and the residue was crystallized from water. A first yield of 63 mg (38%) of crystalline *trans*-4-amino-(^{14}C-methyl)-cyclohexane carboxylic acid was obtained. Electrophoresis showed that this product consisted of 98.8% of the *trans*-isomer. From the mother liquor, a second semi-crystalline fraction was obtained; 30 mg (18%), 94.5% *trans*.

The specific activity of the ^{14}C-tranexamic acid was 20.4 mCi/mM. The total recovery of radioactivity in the two fractions was 12.4 mCi (= 12.4% overall yield based on K^{14}CN).

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Jan Lundström[x]

Gertrud Westin-Sjödahl[xx] and N. Åke Jönsson

[x]Faculty of Pharmacy, Biomedical Center, University of Uppsala Box 570, S-751 23 Uppsala. Present address: Astra Läkemedel, S-151 85 Södertälje, Sweden

[xx]Department of Organic Chemistry, Research Department, AB Kabi,Fack,S-104 25 Stockholm, Sweden. To whom requests for reprints should be sent.

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