SYNTHESIS OF 2,9-[^{14}C]DIMETHYL-1,10-PHENANTHROLINE AND THE PRODUCTION OF ^{67}Cu.

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SUMMARY

2,9-[^{14}C]Dimethyl-1,10-phenanthroline was prepared by means of a nucleophilic methylation of 2-methyl-1,10-phenanthroline with [^{14}C]methyllithium followed by oxidation. The specific activity of the synthesized and purified 2,9-[^{14}C]dimethyl-1,10-phenanthroline was 1.78 mCi/mmol. The overall radiochemical yield was 59%.

^{67}Cu was produced by α-bombardment of ^{64}Ni.

Key Words: 2,9-[^{14}C]dimethyl-1,10-phenanthroline, ^{67}Cu, copper(I) complex, Mycoplasma gallisepticum.

INTRODUCTION

The copper(I) complex of 2,9-dimethyl-1,10-phenanthroline is known to exhibit a strong growth inhibitory effect on Mycoplasma gallisepticum, a micro-organism which causes chronic respiratory disease in poultry (1, 2).

0362-4803/81/071015-07$01.00

Received April 11, 1980
Revised May 27, 1980

Materials

Lithium

Small pieces of lithium wire were flattened with a hammer to enlarge the surface and thoroughly cleaned by rinsing in ether, methanol, tetrahydrofuran and pentane respectively.

[^{14}C]Methyl iodide

[^{14}C]Methyl iodide (10 mCi) with a specific activity of 58 mCi/mmol was obtained from the Radiochemical Centre, Amersham.

2-Methyl-1,10-phenanthroline (7)

To a stirred solution of 3.6 g of sintered 1,10-phenanthroline (20 mmol) in 60 ml of anhydrous THF and 100 ml of anhydrous ether at 0^{o}, 15 ml of a 1.6 M solution of methyllithium in ether was added dropwise under a nitrogen atmosphere. Stirring was continued for 2½ hours at a temperature of 0-10^{o}. After hydrolysis and oxidation with a concentrated solution of $KMnO_4$ in acetone the product was distilled in a sublimation apparatus at 150-190^{o}/0.5 mm, giving 3.85 g of a yellow viscous oil and subsequently crystallized twice from ether/p.e. 40-60. Exposed to air the transparent crystals turned opaque because of hydrate formation. M.p. 85-86^{o}, (8) 53^{o} for the base dried to air, 75-76^{o} for the anhydrous base (9) 78^{o}. Yield after crystallization of the hydrochloride from isopropyl alcohol/ether: 2.7 g (59%); m.p. 214-215^{o}.

Methods

The addition of reagents, reactions and isolation of products were all carried out in a fully closed apparatus of Pyrex glass (Fig. 1), freed from oxygen and moisture by flushing with helium gas and evacuation (10^{-6} mm Hg) for 18 hours. Break-seal ampoules were filled with anhydrous solvents or liquid reagents as described (10) and were connected to the reaction vessel by fusing the glass. The contents of the various ampoules could be brought into the reaction vessel

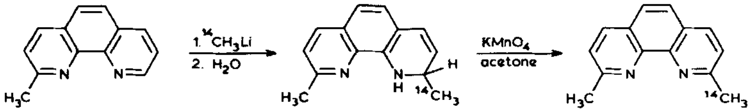
As part of our investigation concerning the mode of action of this copper(I) complex we intend to study its transport into the mycoplasma cell. Hence we decided to prepare this complex with a radioactive label in the ligand. To detect the possible dissociation of the copper complex in whole cells of Mycoplasma gallisepticum, also the metal has to be radioactive. Therefore we prepared $^{67}CuCl_2$ and 2,9-[^{14}C]dimethyl-1,10-phenanthroline.

From these compounds the copper(I) complex is formed in situ when they are dissolved in the molar ratio 1:2 in the growth medium of Mycoplasma gallisepticum (2). The synthesis of the ^{14}C-labelled ligand and the production of ^{67}Cu is reported in this paper.

EXPERIMENTAL PART AND RESULTS

The synthesis of 2,9-dimethyl-1,10-phenanthroline has been reported several times (3, 4, 5). In all cases the well-known Skraup reaction is involved in the synthetic procedure. Because of the low yield generally obtained in this reaction, the applicability of alkyllithium reagents in the preparation of a series of ortho substituted 1,10-phenanthrolines, analogous to the procedure described by Kauffmann (6), was investigated previously (7). This method proved to be very successful, giving much higher yields and being a milder and cleaner procedure in comparison with the Skraup reaction.

In this way we prepared 2,9-[^{14}C]dimethyl-1,10-phenanthroline from 2-metyl-1,10-phenanthroline and [^{14}C]methyllithium according to the following scheme:



after breaking the thin glass break-seal with an iron rod fused in a glass tube using a magnet (10).

[^{14}C]Methyllithium

A solution of [^{14}C]methyllithium was prepared basically in the same way as described by Müller and Rundel (11). Lithium (in excess) (30 mmol) was added to reaction vessel I (Fig. 1) under helium pressure, followed by sealing opening 1 and evacuation of the vessel. Subsequently 10 ml anhydrous ether, 5 mmol inactive methyl iodide and 10 mCi [^{14}C]methyl iodide, respectively, were added to the reaction vessel. After completion of the reaction, the ampoules were freed from ether and other volatile products by cooling the reaction vessel with liquid nitrogen and subsequently sealed off.

2,9-[^{14}C]Dimethyl-1,10-phenanthroline

Reaction vessel I, containing a solution of [^{14}C]methyllithium was connected with reaction vessel II. 2-Methyl-1,10-phenanthroline (5 mmol) was added to vessel II followed by sealing 5 and evacuation of the vessel. After the 2-methyl-1,10-phenanthroline was dissolved in 15 ml anhydrous benzene the [^{14}C]methyllithium solution of vessel I was added slowly. Stirring was continued for 2 hours at 0-10°C resulting in a deeply red solution. Reaction vessel I and the empty ampoule were sealed off and vessel III which contained 15 ml of water was connected to reaction vessel II and evacuated. After this the water was decanted to reaction vessel II. After hydrolysis the brightly yellow coloured organic layer was decanted partly to vessel III and the solvent was distilled back to vessel II by gentle heating of vessel III and cooling vessel II with liquid nitrogen. Repeating this procedure several times resulted in a colourless organic layer in vessel II and some yellow oil containing the product in vessel III. After cooling vessel II was sealed off. Since no volatile radioactive compounds were present anymore, vessel III was opened. The product was dissolved in ca. 3 ml of acetone and oxidized by adding small portions of a concentrated solution of $KMnO_4$ in acetone until the colour of the reaction mixture became permanently purple. After separation of the precipitated MnO_2 the solution was concentrated. TLC analysis (Merck silicagel 60 F_{254}, elution solvent ether containing 2% ethanol and 1 droplet of conc. ammonia, detection at 254 nm) revealed that this solution mainly contained 2,9-dimethyl-1,10-phenanthroline while also a small amount of 2-methyl-1,10-phenanthroline and a yellow coloured impurity were detected. These compounds were separated by columnchromatography (silicagel 0.063 - 0.200 mm; ether/p.e. 40-60 4:1; ether and ether + 3% ethanol respectively). The yield of purified 2,9-[^{14}C]dimethyl-1,10-phenanthroline was 2.95 mmol and 59% of the initial applied radioactivity, resulting in a specific activity of 1.78 mCi/mmol.

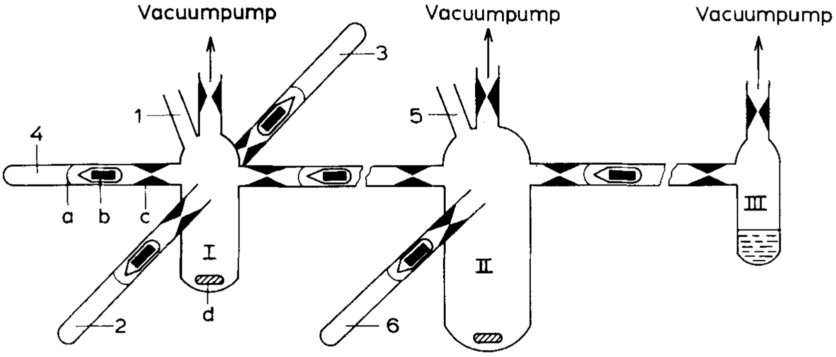


Figure I, fully closed apparatus for the synthesis of 2,9-[^{14}C]dimethyl-1,10-phenanthroline.

I. reaction vessel for synthesis of [^{14}C]methyllithium
II. reaction vessel for nucleophilic addition reaction and hydrolysis
III. vessel containing 15 ml of water

1. opening for the addition of lithium
2. ampoule with 10 ml anhydrous ether
3. ampoule with 5 mmol inactive methyl iodide
4. ampoule with 10 mCi [^{14}C]methyl iodide

5. opening for the addition of 2-methyl-1,10-phenanthroline
6. ampoule with 15 ml anhydrous benzene

a. thin glass break-seal
b. iron rod, fused in a glass tube
c. thickening of the glass for sealing off
d. magnet for stirring

The production of ^{67}Cu

^{67}Cu was produced by α-bombardment of enriched ^{64}Ni-foils (isotopic purity > 95%), using the reaction ^{64}Ni(α, p)^{67}Cu. The α-particles (E_α = 25 MeV) were accelerated with the AVF cyclotron of the Vrije Universiteit. The thickness of the Ni-foils was about 5 mg/cm^2. After irradiation (irradiation times 2-4 hours with 2 μA beam current) the target was allowed to cool down during several hours. For chemical separation the Ni-foil was dissolved in 5.5N nitric acid, carriers were added (50 μg Cu and 50 μg Zn) and after evaporation to dryness the salts were taken in 6N HCl and brought on a column of Dowex-1 anion-exchanger. Nickel was eluted with 6N HCl and copper with 2N HCl. Afterwards the also present ^{62}Zn was eluted with 0.05N HCl.

The Cu fraction containing radioactive ^{67}Cu (measured by γ spectroscopy) and traces of ^{64}Cu was evaporated to near dryness and taken in several milliliters of water. The yield of ^{67}Cu was about 30 μCi. The ^{64}Ni was recycled by electroplating the Ni from a solution of ammonium sulfate on Pt backing foil.

ACKNOWLEDGEMENT

We are indebted to Drs. F.J.M. Freijee (Department of Organic Chemistry, Vrije Universiteit, Amsterdam) for technical advice and to Dr. G. Westera and Mr. C.J. Brouwer (Radio Nucliden Centrum, Vrije Universiteit, Amsterdam) for hospitality and technical assistence.

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