SYNTHESIS OF CARBON-14-LABELLED ANTIBACTERIAL AGENT. SYNTHESIS OF 1-ETHYL-[1-^{14}C]-6-FLUORO-1,4-DIHYDRO-4-OXO-7-(1-PIPERAZINYL)-3-QUINOLINECARBOXYLIC ACID (^{14}C-AM-715)

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SUMMARY

1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (I, AM-715), a new potent antibacterial agent, was labelled with carbon-14 at the C-1 position of the N-ethyl group for metabolic studies. The synthesis was achieved according to two reaction routes. The overall radiochemical yields of ^{14}C-AM-715 based on ethyl iodide-1-^{14}C from route A and route B, were 29.4% and 9.4%, respectively.

Key Words: Antibacterial agent, 1-Ethyl-[1-^{14}C]-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, Carbon-14.

INTRODUCTION

In our investigation of antimicrobial quinolonecarboxylic acid derivatives, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (AM-715) had a potent activity against gram-positive and gram-negative bacteria.[1), 2)]

In order to study the distribution, excretion and metabolism in animals of AM-715, a radiolabelled sample was synthesized.

0362-4803/81/121765-07$01.00

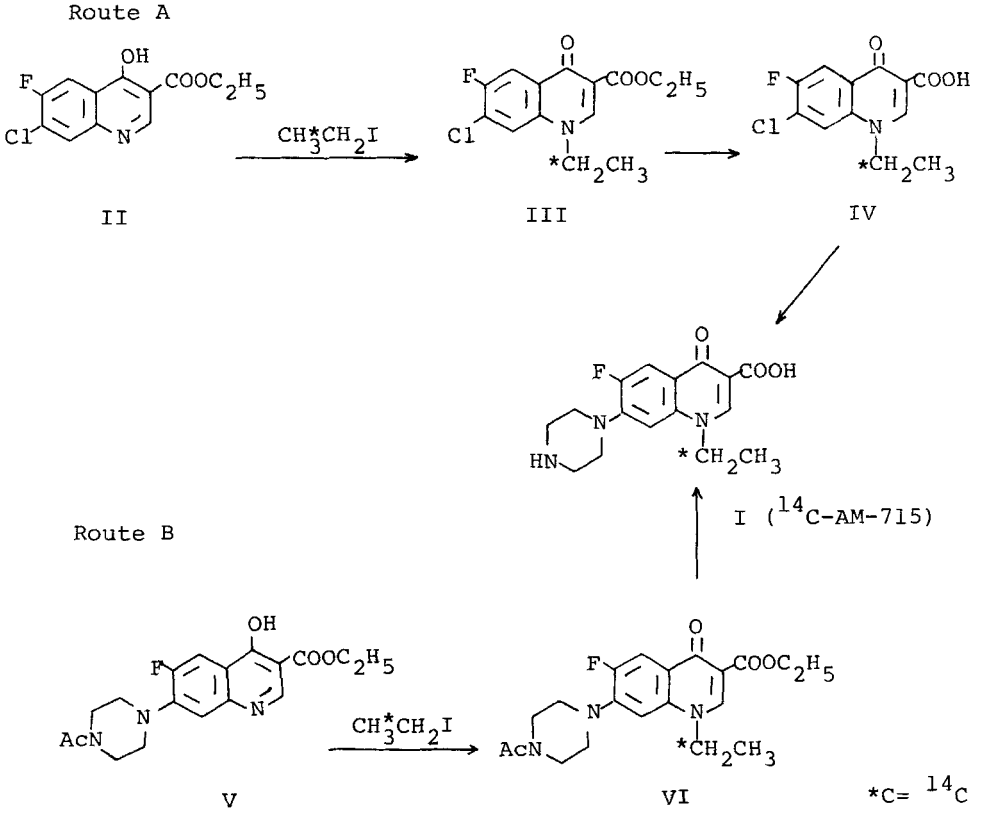
Received September 29, 1980
Revised February 1, 1981

Fujiwara et al.[3] had reported the metabolic fate of N-ethyl-1-^{14}C-oxolinic acid, and that the radioactivity was rarely removed by oxidative and metabolic degradation. It was also proved that the N-ethyl group of nalidixic acid[4], piromidic acid[5] and pipemidic acid[6] was not released in the course of metabolism. Accordingly, N-ethyl group was chosen as the labelling position of AM-715.

In this report we describe the synthesis of ^{14}C-AM-715 labelled at the C-1 position of the N-ethyl group.

DISCUSSION

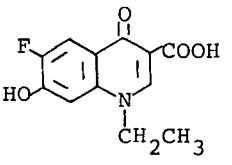
Synthetic routes of ^{14}C-AM-715 are illustrated in Scheme I.



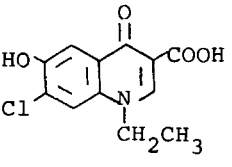
Scheme I Synthesis of ^{14}C-AM-715

Initially non-radioactive reactants were used (cold runs) to adapt the reaction sequence to microsynthetic conditions. The first step (II⟶III) of route A proceeded relatively smoothly. The yield of ethyl 7-chloro-1-ethyl-[1-^{14}C]-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylate (III) was about 68% based on ethyl iodide-1-^{14}C. (Chemical yield of III was 85.3% from II). However, in this reaction, a small amount of other products was formed, as detected by GLC and TLC. One minor product (ethyl 7-chloro-4-ethoxy-[1-^{14}C]-6-fluoro-3-quinolinecarboxylate) could be easily separated from the crude product by extraction with hot n-hexane and identified by comparision with GLC, TLC and IR spectrum of an authentic sample[2].

The ester (III) was quantitatively converted to the corresponding acid (IV) by hydrolysis with 10% aqueous sodium hydroxide for 15 min at 70°C. It was necessary to keep this hydrolysis time, otherwise there were by-products, (a) and (b), detected by TLC.



(a)



(b)

The radiochemical purity of IV was 100% by TLC. Condensation of IV with piperazine gave ^{14}C-AM-715 (I) in a 49.8% yield. The overall yield of I was 29.4% based on ethyl iodide-1-^{14}C. Its specific activity was 11.4 μCi/mg and its radiochemical purity was 100% by reverse dilution analysis and TLC.

The alternative route B involved two steps, but the yield of I was lower than that by route A. This is due to the poor reactivity

of ethy 6-fluoro-4-hydroxy-7-(4-acetyl-1-piperazinyl)-3-quinoline-carboxylate (V) with ethyl iodide. Then contaminated with the 4-O-ethyl derivative (12% from radioactive peak area of TLC) crude VI was hydrolyzed with 2 N sodium hydroxide, and afforded I in good yield. Total radiochemical yield of I from route B was 9.4% based on ethyl iodide-1-^{14}C. The specific activity was 4.62 μCi/mg and its radiochemical purity was 98% by reverse dilution analysis and TLC. This was identical with I from route A.

EXPERIMENTAL

Gas chromatograms were obtained on a Gas chromatogram GC-6A (Shimadzu). GLC analysis conditions were as follows: Column 3 mmφ X 1 m glass column, 5% OV-1 Chromosorb WAW 60-80 mesh, inj. temp. 270°C, column temp. 250°C, carrier gas He 30 ml/min, detector FID. Thin layer chromatography (TLC) was performed on Kieselgel 60 F_{254} 0.25 mm plates (Merck). TLC Radiochromatograms were scanned on Radiochromato Scanner 7201 (Packard). Radioactivity was determined on Tri-Carb Liquid Scintillation Counter 2425 (Packard).

Route A

Ethyl 7-chloro-1-ethyl-[1-^{14}C]-6-fuoro-1,4-dihydro-4-oxo-3-quinoline-carboxylate (III)

A mixture of ethyl iodide-1-^{14}C (5.0 mCi, 195 mg, purchased from the Radiochemical Centre, Amersham, England), ethyl 7-chloro-6-fluoro-4-hydroxy-3-quinolinecarboxylate[2] (II, 270 mg), 173 mg of K_2CO_3 and 7 ml of dimethylformamide (DMF) was sealed in a glass tube, and heated at 90°C in an oil bath for 22 hr with stirring. 195 mg of ethyl iodide was added to the mixture and heated for 9 hr. The reaction mixture was evaporated under reduced pressure, suspended in water and extracted with $CHCl_3$. The $CHCl_3$ layer was dried over

Na_2SO_4 and evaporated. The residue was washed by hot n-hexane. 254 mg of III was collected, whose radiochemical purity was 98% by GLC (Rt = 3.5 min) and TLC ($CHCl_3$:AcOEt = 1:1 v/v, Rf = 0.19). Ethyl 7-chloro-4-ethoxy-[1-^{14}C]-6-fluoro-3-quinolinecarboxylate was occurred in the n-hexane layer as a minor product (GLC: Rt = 0.8 min , TLC: $CHCl_3$:AcOEt = 1:1 v/v, Rf = 0.51).

7-Chloro-1-ethyl-[1-^{14}C]-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (IV)

III was hydrolyzed in 10% sodium hydroxide for 15 min at 70°C in a water bath. The reaction mixture was neutralized with conc. HCl cooling in ice water. The resulting powder was collected by filtration, washed with water and dried *in vacuo*. The product, IV, 217 mg (94.7%), was obtained. Its radiochemical purity was 100% by TLC [(i) $CHCl_3$:MeOH:28% NH_4OH = 20:12.5:5 v/v, Rf = 0.54, (ii) EtOH:AcOH:H_2O = 3:1:1 v/v, Rf = 0.61].

1-Ethyl-[1-^{14}C]-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid (^{14}C-AM-715, I)

A mixture of IV (217 mg), anhydrous piperazine (354 mg) and 5 ml of 3-methoxybutanol (bp. 158°C) was sealed in a glass tube under N_2 gas, and heated at 170°C in an oil bath for 2 hr. The reaction mixture was evaporated under reduced pressure. After washing with cold water, the residue was recrystallized with methanol , dried at 120°C for 3 hr *in vacuo*. The colorless powder of ^{14}C-AM-715, 129 mg (49.8%) was obtained with the specific activity of 11.4 μCi/mg. The final product was identical in every respect with authentic unlabelled AM-715. Its radiochemical purity was 100% by reverse dilution analysis and TLC [(i) $CHCl_3$:MeOH:28% NH_4OH = 20:10:3

v/v, Rf = 0.20, (ii) Me_2CO:MeOH:28% NH_4OH = 2:1:1 v/v, Rf = 0.48, (iii) EtOH:AcOH:H_2O = 3:1:1 v/v, Rf = 0.15]. Non-labelled I was similary prepared from non-labelled ethyl iodide, recrystallized from methanol. m.p. 222-223°C Anal. for $C_{16}H_{18}N_3O_3F$ Calcd. C: 60.18, H: 5.68, N: 13.16, Found. C: 60.34, H: 5.68, N: 12.85.

Route B

Ethyl 1-ethyl-[1-^{14}C]-6-fluoro-1,4-dihydro-4-oxo-7-(4-acetyl-1-piperazinyl)-3-quinolinecarboxylate (VII)

A mixture of ethyl iodide-1-^{14}C (5.0 mCi, 195 mg, purchased from the Radiochemical Centre, Amersham, England), ethyl 6-fluoro-4-hydroxy-7-(4-acetyl-1-piperazinyl)-3-quinolinecarboxylate[2] (VI, 176 mg), 104 mg of K_2CO_3 and 6 ml of DMF was sealed in the glass tube, and heated at 90°C in an oil bath for 17 hr with stirring. The reaction mixture was evaporated under reduced pressure. Water was added to the residue, and extracted with $CHCl_3$. The $CHCl_3$ layer was dried over Na_2SO_4, evaporated to dryness. The crude product contained 95.8 mg of VII, whose radiochemical purity was 88% as revealed by TLC ($CHCl_3$:AcOEt = 1:1 v/v, Rf = 0.40, 88%, Rf = 0.59, 12%). The product (VII) was used for the following hydrolysis without any purification.

A mixture of VII (95.8 mg), 10 ml of 2 N sodium hydroxide and 2 ml of ethanol was refluxed for 3 hr. The mixture was neutrallized with conc. HCl during in ice water. The precipitated solid was filtered, washed with cold water and then recrystallized from methanol. The crystalline residue was dried at 120°C for 3 hr *in vacuo*, giving ^{14}C-AM-715 whose specific activity was 4.62 μCi/mg. Its radiochemical purity was 98% by reverse dilution analysis and TLC [(i) $CHCl_3$:MeOH:28% NH_4OH = 20:10:3 v/v, Rf = 0.18, (ii) Me_2CO:

MeOH:28% NH_4OH = 2:1:1 v/v, Rf = 0.48, (iii) EtOH:AcOH:H_2O = 3:1:1 v/v, Rf = 0.15].

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