SYNTHESIS OF CARBON-^{14}LABELLED ANTIMICROBIAL AGENTS. I. SYNTHESIS OF 1,4-DIHYDRO-1-METHOXY-6,7-METHYLENEDIOXY-4-OXO-QUINOLINE-3-CARBOXYLIC-3-^{14}C ACID (AB-206-3-^{14}C).

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SUMMARY

1,4-Dihydro-1-methoxy-6,7-methylenedioxy-4-oxoquinoline-3-carboxylic acid (I) (AB-206), a new synthetic antimicrobial agent, was labelled with carbon-14 at C-3 position of the quinolinone ring for metabolic studies. The synthesis was achieved according to the reaction schemes shown in Fig. 1 and 4; which involved a newly devised key-reaction, the condensation of diethyl malonate-2-^{14}C with ethyl orthoformate and 3,4-methylenedioxyaniline in the presence of anhydrous zinc chloride as a catalyst to ethyl α-carbethoxy-β-(3,4-methylenedioxyanilino) acrylate-2-^{14}C (III). The overall radiochemical yield of AB-206-3-^{14}C (I) was 9.6% from methanol-^{14}C.

INTRODUCTION

In our investigation of antimicrobial agents, 1,4-dihydro-1-methoxy-6,7-methylenedioxy-4-oxoquinoline-3-carboxylic acid (AB-206, I)[1] has been found to be active especially against gram-negative microorganisms. The preliminary metabolic studies[2] of the agent with the use of AB-206-^{14}C labelled at N-methoxy group revealed that the agent readily underwent O-demethylation in rats, so that it became necessary to prepare ring-

labelled AB-206 in order to follow up the metabolic fate of the quinolinone residue.

Crew[3] reported the metabolism of oxolinic acid, 1,4-dihydro-1-ethyl-6,7-methylenedioxy-4-oxoquinoline-3-carboxylic acid, in which they employed oxolinic acid-^{14}C labelled at C-3 position. To our knowldge, however, no report concerning the synthesis of the labelled compound has appeared so far. Recently, Volford[4] reported a synthesis of 3-carbethoxy-4-hydroxy-7-methyl-1,8-naphthyridine-^{14}C. In this case, the labelling position was C-4 and/or carbonyl since the starting material was α,α-picolylaminomethylenemalonic ester-(carbonyl-^{14}C).

In this report we describe the synthesis of AB-206-^{14}C labelled at C-3 position of the quinolinone-ring.

DISCUSSION

The synthesis of diethyl malonate-2-^{14}C was achieved through the well-known reaction scheme[5-9] illustrated in Fig. 1. The labelled run utilized 6.5 mmoles of methanol-^{14}C with a total activity of 75 mCi. The yield of diethyl malonate-2-^{14}C was 35% based on methanol-^{14}C.

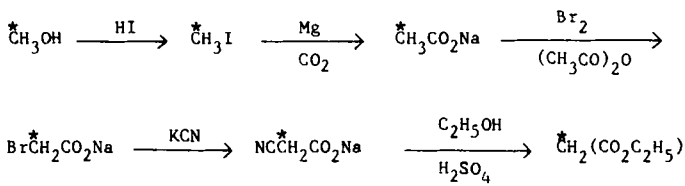


Fig. 1. Scheme for the synthesis of diethyl malonate-2-^{14}C.

General methods utilized so far for the synthesis of unlabelled 3-carbethoxy-4-hydroxyquinolines with the use of diethyl malonate as a raw material comprise that[10] which is illustrated in Fig. 2.

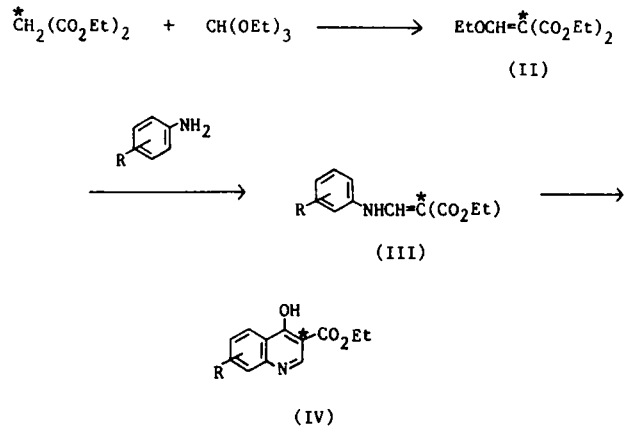
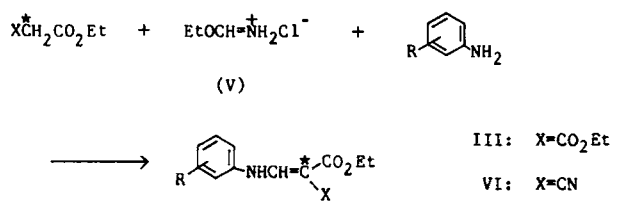
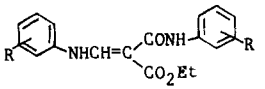


Fig. 2. Scheme for the synthesis of 3-carbethoxy-4-hydroxy-quinolines through ethoxymethylenemalonic ester.

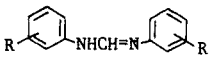
However, principal disadvantages of this method through ethoxymethylene-malonic ester (EMME) (II) are the mediocre yield and the technical complexity in the small-scale preparetion of EMME itself. In fact, by our hands, EMME-2-^{14}C was obtained in the yield of only 50% at the hightest from diethyl malonate-2-^{14}C. An investigation of alternate routes, therefore, was considered desirable.

The observation of Egli[11], that ethyl cyanoacetate when treated with ethoxyformimine (V) undergoes condensation with anilines to β-anilinoacrylates (VI) suggests the possibility that diethyl malonate might react like cyanoacetate to give β-anilinoacrylates (III).





(VII)

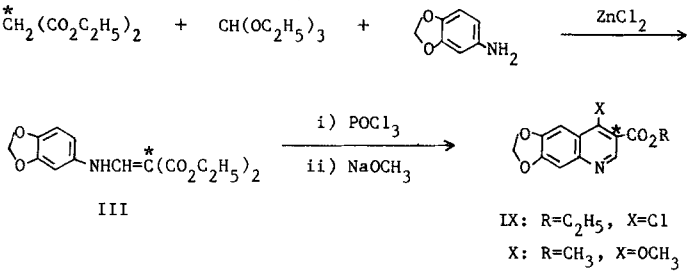


(VIII)

Fig. 3. Scheme for the synthesis of β-anilinoacrylates by Egli's method.

Accordingly, we tried this method under a variety of reaction conditions, but we only obtained a low yield of III as a mixture with by-products (VII and VIII).

In the course of these experiments, while investigating the use of various acids as catalysts, it was observed that ethyl orthoformate, instead of formimine (V), and diethyl malonate could condense with anilines in the presence of anhydrous zinc chloride as a catalyst to form III (Fig. 4). After considerable investigation, a condition was established under which good yields of β-anilinoacrylates could be obtained without any significant amount of by-products**. Thus heating a mixture of diethyl malonate-2-^{14}C, ethyl orthoformate, 3,4-methylenedioxyaniline and zinc chloride at 120 - 130° for 4 hr, followed by column-chromatography gave β-anilinoacrylate-2-^{14}C (III) in 65% yield and diethyl malonate-2-^{14}C in 15% recovery.

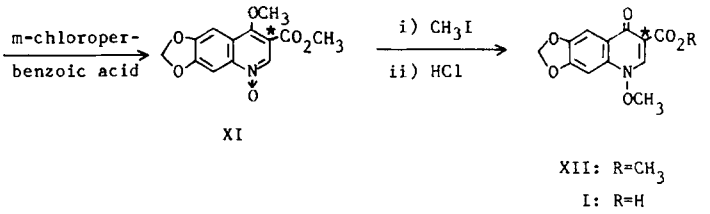


III

IX: R=C_2H_5, X=Cl

X: R=CH_3, X=OCH_3

** Synder and Jones[12] reported the same type of reactions in which no catalyst was employed, but they only obtained α-carbethoxy-β-anilinoacrylaniline (VII).



XI

XII: R=CH_3

I: R=H

Fig. 4. Scheme for the synthesis of AB-206-3-^{14}C from diethyl malonate-2-^{14}C.

Conversion of β-anilinoaceylate-2-^{14}C (III) to AB-206-3-^{14}C (I) was accomplished according to the reaction scheme demonstrated in Fig. 4, which was established by Nakagome[13] for the large-scale preparation of unlabelled compounds. Cyclyzation of β-anilinoacrylate-2-^{14}C (III) with phosphoryl chloride gave 3-carbethoxy-4-chloroquinoline-3-^{14}C (IX) in 82% yield, which was then treated with sodium methoxide to afford 3-carbomethoxy-4-methoxy-quinoline-3-^{14}C (X) in 63%. N-Oxidation of X with m-chloroperbenzoic acid to N-oxyquinoline-3-^{14}C (XI) (87% yield), followed by treatment of the latter with methyl iodide yielded N-methoxyquinoline-3-^{14}C (XII). Finally, hydrolysis of the ester (XII) to AB-206-3-^{14}C (I) was readily achieved in 1 N HCl in a quantitative yield. The overall yield of AB-206-3-^{14}C was 9.6% from methanol-^{14}C.

EXPERIMENTAL

Sodium acetate-2-^{14}C -- Sodium acetate-2-^{14}C was synthesized in 57% (43.2 mCi) yield from methanol-^{14}C (75.0 mCi, 6.5 mmol) by a standard method[5]; which comprised iodination of methanol-^{14}C in 57% hydriodic acid, followed by Grignard reaction of the resulted methyl iodide-^{14}C with magnesium and carbon dioxide.

Sodium bromoacetate-2-^{14}C -- Sodium acetate-2-^{14}C (43.2 mCi, 3.7 mmol) was transformed into sodium bromoacetate-2-^{14}C by the method of Kögl, Emmelot and den Beer[6]. The yield of sodium bromoacetate-2-^{14}C was 90% based on sodium acetate-2-^{14}C.

Sodium cyanoacetate-2-^{14}C -- Sodium cyanoacetate-2-^{14}C was prepared from sodium bromoacetate-2-^{14}C (38.9 mCi, 6.7 mmol) according to the method of Gal and Shulgin[7]. The product was used for the following reaction without any purification.

Diethyl malonate-2-^{14}C -- This substance was prepared directly from sodium cyanoacetate-2-^{14}C according to the method devised by Christie[8] and applied for the first time to the radioactive preparation by Gianetto[9]. Concentrated sulfuric acid (4 ml) was added dropwise at 0° to a mixture of sodium cyanoacetate-2-^{14}C (ca. 6.7 mmol), anhydrous benzene (1.5 ml) and absolute ethanol (9 ml). The mixture was heated to reflux for 4.5 hr. After cooling, ice-water (ca. 30 ml) was added to the mixture, which was extracted with ether. The ethereal extract was washed with 5% sodium carbonate solution and water, dried, and evaporated to give crude diethyl malonate-2-^{14}C (27.0 mCi, 70% from sodium bromoacetate-2-^{14}C), which, by radio-gaschromatogram analysis, was revealed to contain 2.6% (0.7 mCi) of ethyl cyanoacetate-2-^{14}C. This mixture was used without purification for the following reactions.

Ethyl ethoxymethylenemalonate-2-^{14}C -- A mixture of diethyl malonate-2-^{14}C (5.0 g, 31 mmol, 0.38 mCi), ethyl orthoformate (5.2 g, 36 mmol), acetic anhydride (6.6 g, 48 mmol) and anhydrous zinc chloride (2 mg) was prepared in a 30 ml flask equipped with a thermometer, a gas inlet tube and a 14 cm column. The column was attached to a still head and condenser. The mixture was agitated for 5 min. by a stream of dry air and then heated as follows:

102 - 115° for 2.5 hr, 115 - 127° for 7 hr (at this point, 1.1 g of ethyl orthoformate and 1.3 g of acetic anhydride were added), 127 - 145° for 2 hr, and 145 - 155° for 2 hr. After cooling, the mixture was distilled under reduced pressure to give ethyl ethoxymethylenemalonate-2-^{14}C, bp. 106 - 110° / 0.25 Torr, in 49% yield (3.1 g, 0.18 mCi).

Condensation reaction of diethyl malonate with ethoxyformimine hydrochloride (V) and 3,4-methylenedioxyaniline. -- A mixture of diethyl malonate (1.05 g, 8.20 mmol), ethoxyformimine hydrochloride (V) (0.90 g, 8.26 mmol), 3,4-methylenedioxyaniline (1.13 g, 8.20 mmol) and triethylamine (1.20 g, 12 mmol) was heated at 110 - 120° for 6 hr. The mixture was extracted with ether, and the extract was washed with water, dried and evaporated to give a crystalline residue. To the residue n-hexane was added, and the mixture was stirred for 5 min. and filtered. From the filtrate, diethyl malonate (0.31 g, 30%) was recovered. The crystalline residue was chromatographed on silica gel in chloroform to afford ethyl α-carbethoxy-β-(3,4-methylenedioxyanilino)acrylate (III) (0.45 g, 18%, mp. 100 - 102°), α-carbethoxy-β-(3,4-methylenedioxyanilino)-acrylanilide (VII) (0.21 g, 7%, mp. 167 - 169°), bis-(3,4-methylenedioxyphenyl)-formamidine (VIII) (0.26 g, 11%, mp. 165 - 167°) and 3,4-methylenedioxyaniline (0.21 g, 19%). These products were identical with authentic samples in the infra-red spectra.

Ethyl α-carbethoxy-β-(3,4-methylenedioxyanilino)acrylate-2-^{14}C -- A mixture of diethyl malonate-2-^{14}C (1.06 g, 10.0 mmol, 20.1 mCi), ethyl orthoformate (1.78 g, 12.0 mmol), 3,4-methylenedioxyaniline (1.64 g, 12 mmol) and anhydrous zinc chloride (50 mg) was heated at 122 - 125° for 7.5 hr. The mixture was dissolved in chloroform. The chloroform solution was washed with water, dried and evaporated to give a residue. Column chromatography of the residue in chloroform on silica gel yielded diethyl malonate-2-^{14}C (0.24 g, 3.0 mCi) as

the first eluate, and ethyl α-carbethoxy-β-(3,4-methylenedioxyanilino)acrylate-2-^{14}C (III) (1.99 g, 13.0 mCi) as the second eluate; the latter showed the identical R_f-value (0.46) on silica gel-TLC, developed with chloroform/methanol = 20/1, and IR-spectrum identical with the authentic unlabelled sample.

3-Carbethoxy-4-chloro-6,7-methylenedioxyquinoline-3-^{14}C (IX) -- A mixture of α-carbethoxy-β-(3,4-methylenedioxyanilino)acrylate-2-^{14}C (III) (1.99 g, 6.48 mmol, 13.0 mCi) and phosphoryl chloride (1.53 g, 10.0 mmol) in anhydrous toluene (15 ml) was heated under reflux for 8 hr. The mixture was concentrated to dryness under reduced pressure. The residue taken up in chloroform was washed with 10% sodium carbonate solution and water, dried and evaporated. The crude product was subjected to column chromatography on silica gel. Elution with chloroform gave 3-carbethoxy-4-chloro-6,7-methylenedioxyquinoline-3-^{14}C (IX) (1.49 g, 10.7 mCi), mp. 109 - 110°, identical with the authentic unlabelled compound in their IR-spectra.

3-Carbomethoxy-4-methoxy-6,7-methylenedioxyquinoline-3-^{14}C (X) -- To a solution of 3-carbethoxy-4-chloro-6,7-methylenedioxyquinoline-3-^{14}C (IX) (1.49 g, 5.31 mmol, 10.7 mCi) in absolute methanol (25 ml) was added dropwise a solution of sodium methoxide (6.5 mmol) in absolute methanol (6 ml) at room temperature. The mixture was then heated to 80 - 85° for 3 hr. The mixture was concentrated under reduced pressure and taken up in chloroform. The chloroform solution was washed with water and dried. Concentration of the solution, followed by column chromatography on silica gel in chloroform yielded 3-carbomethoxy-4-methoxy-6,7-methylenedioxyquinoline-3-^{14}C (X) (874 mg, 6.72 mCi), mp. 148 - 150°, identical with the authentic sample in their IR-spectra.

3-Carbomethoxy-4-methoxy-6,7-methylenedioxy-1-oxoquinoline-3-^{14}C (XI) -- To a solution of 3-carbomethoxy-4-methoxy-6,7-methylenedioxyquinoline-3-^{14}C (X)

(874 mg, 3.35 mmol, 6.72 mCi) in chloroform (25 ml) was added m-chloroperbenzoic acid (865 mg, 5.0 mmol) at room temperature. The mixture was stirred at room temperature for 24 hr. After dilution with chloroform (50 ml), the solution was washed with 10% sodium carbonate solution and water. The dried solution was evaporated to dryness to give a crystalline residue, which was triturated with ethyl acetate (10 ml). Filtration of the mixture gave 3-carbomethoxy-4-methoxy-6,7-methylenedioxy-1-oxoquinoline-3-^{14}C (XI) (806 mg, 5.85 mCi), mp. 200 - 203°, identical in every respect with the authentic sample.

1,4-Dihydro-1-methoxy-6,7-methylenedioxy-4-oxoquinoline-3-carboxylic-3-^{14}C acid (I) (AB-206-3-^{14}C) -- A solution of 3-carbomethoxy-4-methoxy-6,7-methylenedioxy-1-oxoquinoline-3-^{14}C (XI) (806 mg, 2.91 mmol, 5.85 mCi) and methyl iodide (5 ml) in chloroform (10 ml) was heated to reflux for 5 hr. Evaporation of the reaction mixture afforded a crystalline residue, 3-carbomethoxy-1,4-dihydro-1-methoxy-6,7-methylenedioxy-4-oxoquinoline-3-^{14}C (XII), which was then heated with 1 N hydrochloric acid at 90 - 95° for 3 hr. After cooling, the crystalline precipitate was collected by filtration and washed with water and then methanol; giving 1,4-dihydro-1-methoxy-6,7-methylenedioxy-4-oxoquinoline-3-carboxylic acid-3-^{14}C (I) (714 mg, 5.50 mCi, 9.6% based on methanol-^{14}C). The final product had the specific activity of 2.01 mCi/mmol and mp. 264 - 266° (decomp.), and was identical in every respect with the authentic unlabelled compound.

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