PREPARATION OF 1-[^{14}C] HYDROXYMETHYL-6-ETHOXYCARBONYL-5,7-DIMETHYL-4-PHTHALAZONE (EG-626)

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SUMMARY

1-[^{14}C] Hydroxymethyl-6-ethoxycarbonyl-5,7-dimethyl-4-phthalazone ([^{14}C] EG-626, 12) was synthesised from 6-ethoxycarbonyl-5,7-dimethyl-4-methoxyphthalazine-2-N-oxide (8) and potassium [^{14}C] cyanide by the Reissert-Henze reaction.

The overall radiochemical yield from potassium [^{14}C] cyanide was 21.0 %.

Key word: EG-626, ^{14}C labelling

INTRODUCTION

EG-626 exhibits a platelet aggregation inhibition, phosphodiesterase inhibition, PGI_2 potantiating action and smooth muscle relaxing action.[1,2,3]

The present paper deals with the synthesis of EG-626 labelled with carbon-14 at the 1-hydroxymethyl group for the investigation of its metabolism and disposition.

RESULT AND DISCUSSION

6-Ethoxycarbonyl-5,7-dimethyl-4-methoxyphthalazine-2-N-oxide (8) was prepared from 1,2,4-triethoxycarbonyl-3,5-dimethylbenzene (1) by seven steps as shown in scheme I.

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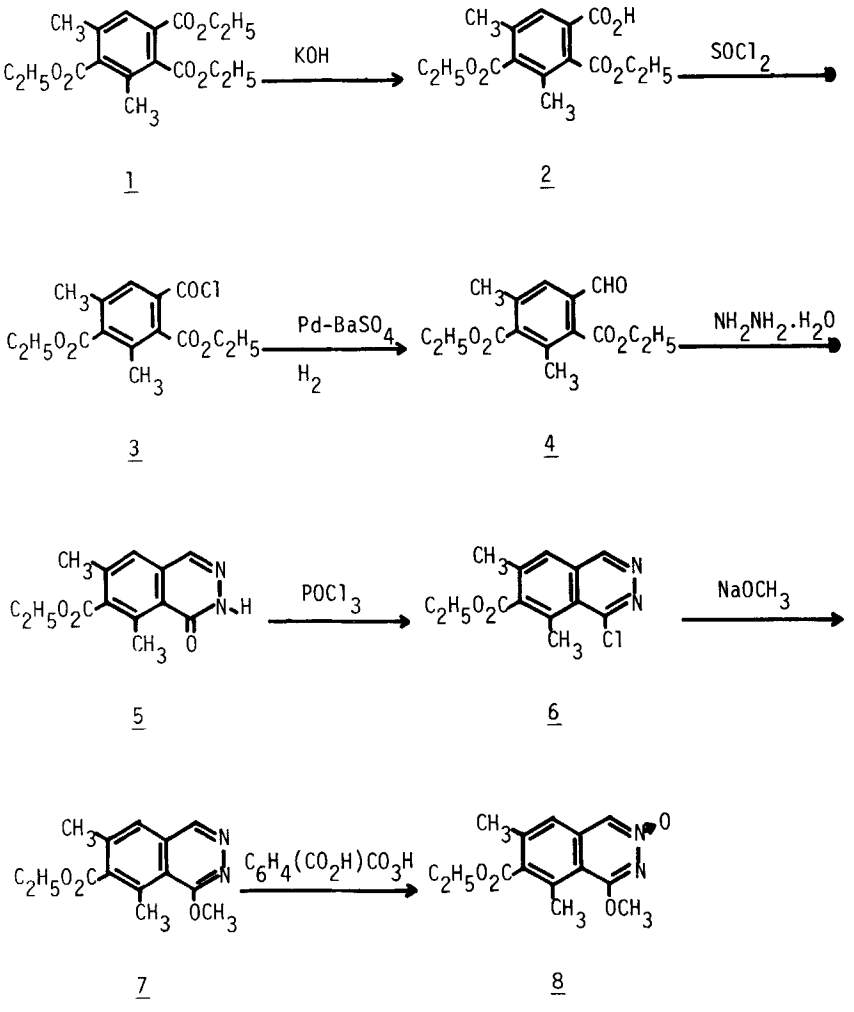


1 2

3 4

5 6

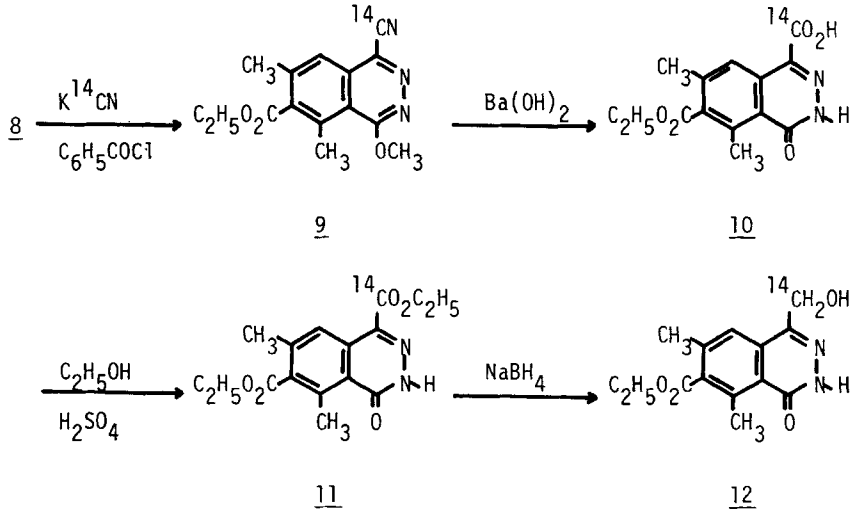
7 8

SCHEME I

The overall yield of the synthesis was 25.6 % and the yield of each reaction was more than 80 % except for the reaction 2 to 4 (see the EXPERIMENTAL).

Scheme II shows the synthetic route for introducing a carbon-14 atom into the 1-hydroxymethyl position of EG-626 using potassium [^{14}C] cyanide as a starting material of carbon-14 source.

Thus, a carbon-14 atom was introduced into the hydroxymethyl group of the



SCHEME II

phthalazine ring by the Reissert-Henze reaction using a solution of 8 and potassium [^{14}C] cyanide in 50 % dioxane in the presence of benzoyl chloride.[4] The yield of this reaction was poor owing to the formation of two by-products. Pure 1-cyanophthalazine (9) was obtained from the reaction mixture by extraction with chloroform and thin layer chromatography (t.l.c). The radiochemical yield of this reaction was found to be 26.3 %.

The resulting 1-cyanophthalazine 9 was refluxed with barium hydroxide in 50 % ethanol solution for 6 hr. The cyano and the methoxy groups in 9 were hydrolyzed simultaneously to give 1-carboxy-6-ethoxycarbonyl-5,7-dimethyl-4-phthalazone (10). The radiochemical yield of this reaction was 78.0 %.

1-Carboxy-6-ethoxycarbonyl-5,7-dimethyl-4-phthalazone (10) was esterified with ethanol and sulfuric acid in the usual manner and the resulting ethyl ester (11) was reduced with sodium borohydride to EG-626.[5] The radiochemical yield of these two steps were excellent; essentially quantative.

The pure [^{14}C] EG-626 was obtained by recrystallization from 50 % ethanol

solution. When 205.46 mg (0.744 mmol) of 6-ethoxycarbonyl-5,7-dimethyl-4-methoxyphthalazine-2-N-oxide (8) and 44.3 mg (0.682 mmol) of potassium [^{14}C] cyanide (10 mCi) were used as starting materials, 57.8 mg (0.209 mmol) of EG-626 (12) (1.94 mCi) was obtained. The specific activity of [^{14}C] EG-626 was 33.64 μCi/mg and the overall radiochemical yield was 21.0 % from potassium [^{14}C] cyanide.

EXPERIMENTAL

2,4-Diethoxycarbonyl-3,5-dimethylbenzoic acid (2)[6]

A mixture of 1,2,4-triethoxycarbonyl-3,5-dimethylbenzene (1) (3.22 g, 10 mmol) and a solution of potassium hydroxide (1.12 g, 20 mmol) in methanol (20 ml) was refluxed for 1 hr. After cooling, the reaction mixture was evaporated to dryness under reduced pressure and the residue was dissolved with benzene (50 ml). The benzene solution was washed with 5 % hydrochloric acid (10 ml) and water (10 ml), and dried over anhydrous sodium sulfate.

Removal of the solvent gave a crude powder which was recrystallized from benzene-n-hexane solution to afford white prisms of 2 (2.71 g, 92.2 %); m.p 118.3°C, NMR δ($CDCl_3$) 9.4 [1H, singlet, -COO<u>H</u>] 7.75 [1H, singlet, aromatic-<u>H</u>] 4.4 [4H, quartet, J=7.0 Hz, -O<u>CH</u>$_2$CH$_3$] 2.3 [3H, singlet, aromatic-<u>CH</u>$_3$] 2.2 [3H, singlet, aromatic-<u>CH</u>$_3$] 1.4 [6H, triplet, J=7.0 Hz, -OCH$_2$<u>CH</u>$_3$].

2,4-Diethoxycarbonyl-3,5-dimethylbenzaldehyde (4)

A solution of 2 (2.94 g, 10 mmol) and thionyl chloride (3 ml) in benzene (30 ml) in the presence of 5 drops of dimethylformamide was heated at 50-60°C and stirred for 2 hr. The solvent and an excess thionyl chloride were evaporated under reduced pressure and then the residue was dissolved with fresh benzene (10 ml). Removal of the solvent gave an oily residue of 2,4-diethoxycarbonyl-3,5-dimethylbenzoylchloride (3) which was used the next reaction without further purification.

To a solution of 3 in xylene (30 ml), 5 % Pd-$BaSO_4$ (1 g) and quinoline-

sulfur poison solution[7] (0.1 ml) were added, and then the solution was stirred at 110°C under H_2 stream for 12 hr. After cooling, the reaction mixture was washed 5 % hydrochloric acid (10 ml) and water (10 ml) and dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave a crude oil which was chromatographed on silica gel with benzene-ethyl acetate (9/1) to afford 4 as a colorless oil (1.63 g, 58.6 %); NMR δ($CDCl_3$) 9.95 [1H, singlet, -CHO] 7.5 [1H, singlet, aromatic-H] 4.4 [4H, quartet, J=7.0 Hz, $-OCH_2CH_3$] 2.35 [3H, singlet, aromatic-CH_3] 2.3 [3H, singlet, aromatic-CH_3] 1.45 [6H, triplet, J=7.0 Hz, $-OCH_2CH_3$].

6-Ethoxycarbonyl-5,7-dimethyl-4-phthalazone (5)

A solution of 4 (2.78 g, 10 mmol) and hydrazine hydrate (5 ml) in ethanol (60 ml) was refluxed for 3 hr. After cooling, the solution was evaporated under reduced pressure and the residue was dissolved with benzene (50 ml).

The benzene solution was washed with 5 % hydrochloric acid (10 ml) and 5 % sodium bicarbonate solution (10 ml) and dried over anhydrous sodium sulfate.

Removal of the solvent under reduced pressure gave a crude powder which was recrystallized with benzene-ethyl acetate solution to afford white prisms of 5 (1.77 g, 72.2 %); m.p 177.2°C, NMR δ($CDCl_3$) 10.8 [1H, singlet, -NH] 8.0 [1H, singlet, aromatic-H (1 position)] 7.3 [1H, singlet, aromatic-H (8 position)] 4.45 [2H, quartet, J=7.0 Hz, $-OCH_2CH_3$] 2.9 [3H, singlet, aromatic-CH_3 (5 position)] 2.45 [3H, singlet, aromatic-CH_3 (7 position)] 1.44 [3H, triplet, J=7.0 Hz, $-OCH_2CH_3$].

6-Ethoxycarbonyl-5,7-dimethyl-4-methoxyphthalazine (7)

A mixture of 5 (2.66 g, 10 mmol) and phosphorus oxychloride (5 ml) was heated at 100°C and stirred for 20 min. The reaction mixture was poured into ice-water (10 ml) with stirring and then the water solution was made alkaline (pH > 10) with conc. ammonia solution. The solution was extracted with chloroform (50 ml) and then the chloroform solution was dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave 6-ethoxycarbonyl-5,7-dimethyl-4-chlorophthalazine (6) as a crude yellow residue.

A mixture of crude 6 and sodium methylate solution (20 ml, 1 mmol/ml methanol) was refluxed for 2 hr. After cooling, the solvent was evaporated under reduced pressure, and then the residue was dissolved with benzene (50 ml). The benzene solution was washed with water (10 ml) and dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave a crude white powder which was recrystallized with isopropyl ether-ethyl acetate to afford white prisms of 7 (1.74 g, 67.0 %); m.p 132.0°C, NMR δ($CDCl_3$) 9.0 [1H, singlet, aromatic-H (1 position)] 7.5 [1H, singlet, aromatic-H (8 position)] 4.45 [2H, quartet, J=7.0 Hz, $-OCH_2CH_3$] 4.2 [3H, singlet, $-OCH_3$] 2.8 [3H, singlet, aromatic-CH_3 (5 position)] 2.45 [3H, singlet, aromatic-CH_3 (7 position)] 1.4 [3H, triplet, J=7.0 Hz, $-OCH_2CH_3$].

6-Ethoxycarbonyl-5,7-dimethyl-4-methoxyphthalazine-2-N-oxide (8)

A mixture of 7 (2.60 g, 10 mmol) and 5 % phthalic monoperacid ether solution[8] (50 ml) was stirred at room temperature for 24 hr. The reaction mixture was washed with 5 % sodium hydroxide solution (10 ml) and dried over anhydrous sodium sulfate. Removal of solvent under reduced pressure gave a yellow powder which was recrystallized with chloroform-isopropyl ether to afford yellow needles of 8 (2.70 g, 97.8 %); m.p 185.8°C (decomp.), NMR δ ($CDCl_3$) 8.15 [1H, singlet, aromatic-H (1 position)] 7.25 [1H, singlet, aromatic-H (8 position)] 4.45 [2H, quartet, J=7.0 Hz, $-OCH_2CH_3$] 4.2 [3H, singlet, $-OCH_3$] 2.7 [3H, singlet, aromatic-CH_3 (5 position)] 2.4 [3H, singlet, aromatic-CH_3 (7 position)] 1.4 [3H, triplet, J=7.0 Hz, $-OCH_2CH_3$].

1-[^{14}C] Cyano-6-ethoxycarbonyl-5,7-dimethyl-4-methoxyphthalazine (9)

To a solution of 8 (205.46 mg, 0.744 mmol), potassium cyanide (30.4 mg, 0.468 mmol) and potassium [^{14}C] cyanide (13.9 mg, 0.214 mmol, 10 mCi) in 50 % dioxane (4 ml), a solution of benzoyl chloride (95.87 mg, 0.682 mmol) in dioxane (1 ml) was added dropwise under continuous stirring for 5 min.

The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was extracted with chloroform (50 ml). The chloroform solution was washed with 5 % sodium carbonate solution (10 ml) and dried over anhydrous

sodium sulfate. Removal of the solvent under reduced pressure gave an oily residue which was purified by t.l.c on silica gel with benzene-ethyl acetate (9/1). Extraction from the part corresponding to Rf value 0.45 on t.l.c plate with ethyl acetate gave 9 as a white powder (2.63 mCi, 26.3 %).

1-[^{14}C] Hydroxycarbonyl-6-ethoxycarbonyl-5,7-dimethyl-4-phthalazone (10)

A solution of 9 (2.63 mCi) and barium hydroxide (2 g) in 50 % ethanol was refluxed for 6 hr with stirring. After cooling, the solution was concentrated and the residue was adjusted to pH 1-2 with conc. hydrochloric acid.

The acidic solution was extracted with ethyl acetate (30 ml) and the ethyl acetate solution was dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave 10 as s white powder (2.05 mCi, 78.0 % [20.5 % from 8]).

1-[^{14}C] Hydroxycarbonyl-6-ethoxycarbonyl-5,7-dimethyl-4-phthalazone (11)

A solution of 10 (2.05 mCi) and conc. sulfuric acid (0.75 ml) in absolute ethanol (30 ml) was refluxed for 4 hr. After cooling, the solution was evaporated under reduced pressure and the residue was dissolved with water (10 ml) and ethyl acetate (30 ml). The ethyl acetate extract was dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave 11 as a white powder (2.05 mCi, ca 100 % [20.5 % from 8]).

1-[^{14}C] Hydroxymethyl-6-ethoxycarbonyl-5,7-dimethyl-4-phthalazone (12), EG-626

A solution of 11 (2.05 mCi) and sodium borohydride (1 g) in ethanol (20 ml) was cooled below 0°C and a solution of calcium chloride (2 g) in ethanol (10 ml) was added dropwise to the solution for 2 hr. After addition, the reaction mixture was stirred for more 4 hr at room temperature and allowed to stand at room temperature overnight. The reaction mixture was concentrated to dryness under reduced pressure and the residue was dissolved with water (10 ml). The solution was adjusted to pH 4.5-5.0 with conc. acetic acid and heated at 85-95°C for 15 min. After cooling, the solution was readjusted to pH 7.0 with 5N sodium hydroxide and the solution was extracted with ethyl acetate (30 ml). The ethyl acetate solution was dried over anhydrous sodium sulfate.

Removal of the solvent under reduced pressure gave a crude powder of 12 (2.01 mCi, 97.9 % [21.0 % from 8]) which was recrystallized with 50 % ethanol to afford pure [^{14}C] EG-626 (57.8 mg, 1.94 mCi, 33.64 μCi/mg).

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