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Synthesis of [^{14}C]-Labelled Pelrinone

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SUMMARY

[^{14}C]Pelrinone (1,4-dihydro-2-methyl-4-oxo-6[(3-pyridinylmethyl)-amino]-5-pyrimidine-[^{14}C]-carbonitrile hydrochloride; AY-28,768 hydrochloride), a potent inotropic agent, was prepared by reacting chloroacetic acid with [^{14}C]sodium cyanide to give the labelled cyanoacetic acid. The acid was esterified, converted to the bis-S-methyl compound, condensed with acetamidine hydrochloride, coupled with 3-aminomethylpyridine, and treated with methanolic hydrochloric acid. Two batches of [^{14}C]pelrinone were produced, giving a combined overall yield of 15.6% from [^{14}C]sodium cyanide (sp. act. 66.2 ± 1.5 and 64.2 ± 1.9 μCi/mg; 98.8 and 98.0% radiochemical purity, respectively).

Key words: Inotropic agent, Pelrinone, AY-28,768 hydrochloride, ^{14}C

INTRODUCTION

Pelrinone (I) is a potent inotropic agent that has been selected for clinical development (1, 2). In order to study the metabolic disposition of pelrinone in laboratory animals and man, a synthesis of the [^{14}C] labelled compound was undertaken as shown below:

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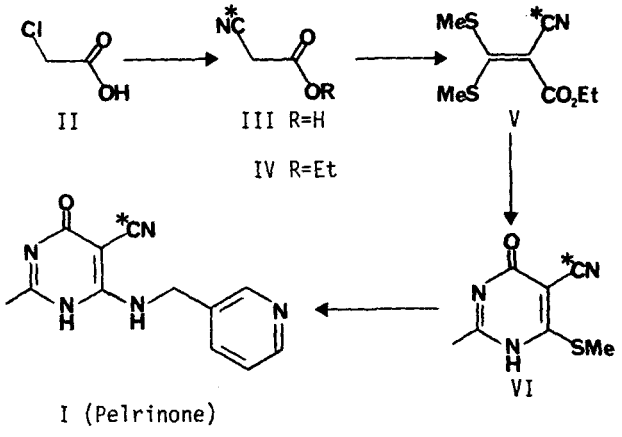

* Position of [^{14}C] label

DISCUSSION

[^{14}C]Cyanoacetic acid (III), prepared from chloroacetic acid (II) and [^{14}C]sodium cyanide, was esterified with ethanol in the presence of sulfuric acid (3). The [^{14}C]labelled ethyl cyanoacetate (IV) was reacted with carbon disulfide (4) and the resulting product alkylated with dimethyl sulfate to give the bis-S-methyl compound V. Condensation of IV with acetamidine hydrochloride gave the pyrimidone VI. [^{14}C]Pelrinone was obtained when VI was reacted with 3-aminomethyl pyridine. The free base was converted to its hydrochloride salt and crystallized from methanol-ether. The [^{14}C]pelrinone was obtained in an overall radiochemical yield of 15.6% based on [^{14}C]sodium cyanide: 0.0916 g, sp. act. 66.2 ± 1.5 μCi/mg and 0.0275 g, sp. act. 64.2 ± 1.9 μCi/mg. The radiochemical purity was determined to be 98.8 and 98.0%, respectively, by TLC autoradiography in three solvent systems.

EXPERIMENTAL

The synthesis of [^{14}C]pelrinone was carried out incorporating [^{14}C]sodium cyanide (50 mCi, sp. act. 58.0 mCi/mmole) purchased from New England Nuclear, Boston, Mass. The intermediates in the synthesis were characterized in trial experiments using unlabelled material. The

reactions in the labelled synthesis were monitored by TLC using unlabelled reference compounds.

Ethyl [2-^{14}C]Cyanoacetate (IV)

Ethyl [2-^{14}C]cyanoacetate was prepared from chloroacetic acid (0.235 g, 2.5 mmole) by reaction with [^{14}C] sodium cyanide (0.044 g, 0.86 mmole) and unlabelled sodium cyanide (0.086 g, 1.79 mmole) as described by Mandel and Brown (3). The crude ethyl [2-^{14}C]cyanoacetate was isolated as a yellow oil (0.191 g, 47% based on cyanide) and was used without further purification.

[2-^{14}C]Cyano-3,3-bis(methylthio)-2-propenoic acid ethyl ester (V)

The crude ethyl cyanoacetate (0.191 g, 1.66 mmole) and carbon disulfide (0.10 ml, 0.126 g, 1.66 mmole) dissolved in dioxane (0.85 ml) were added to a suspension of pulverized potassium hydroxide (0.186 mg, 3.31 mmole) in dioxane (1.7 ml) while maintaining the temperature between 15-20°. After the addition was complete, the reaction mixture was warmed to room temperature and stirred for an additional 20 minutes. The bright yellow suspension was diluted with ether (4.2 ml), centrifuged, and decanted. The potassium salt was washed with a single portion of dioxane : ether = 1 : 1 (10 ml), centrifuged, decanted, and dried under a stream of nitrogen.

Dimethyl sulfate (0.34 ml, 0.458 g, 3.53 mmole) was added to the crude bis-potassium sulfide (0.441 g, 1.65 mmole) suspended in absolute ethanol (10 ml) at 0°. The progress of the reaction was followed by TLC (chloroform). When the reaction was complete, the ethanol was removed at 40° under reduced pressure. The residue was partitioned between chloroform (40 ml) and water (20 ml), the layers separated, and the chloroform extracts dried ($MgSO_4$). Removal of the chloroform at 30° under reduced pressure yielded the crude bis-S-methyl compound (V) (0.261 g).

1,4-Dihydro-2-methyl-6-(methylthio)-4-oxo-5-pyrimidine-[^{14}C]carbonitrile (VI)

Sodium hydride (50% oil dispersion, 0.117 g) was placed in a Craig tube, washed with hexane, and suspended in dry dimethylformamide (0.60 ml). Acetamidine hydrochloride (.0137 g, 1.44 mmole) was dissolved in dry dimethylformamide (1.2 ml) and added dropwise to the mixture, which was then stirred at room temperature for 1 hr. A solution of the [^{14}C]labelled bis-S-methyl compound (0.261 g, 1.20 mmole) in dry dimethylformamide (1.8 ml) was added. The reaction was stirred at room temperature for 4 hr, diluted with water (1.5 ml), acidified with concentrated hydrochloric acid, and cooled in an ice bath. The precipitated product (0.187 g) was collected by centrifugation, dried under vacuum, and used without further purification.

1,4-Dihydro-2-methyl-4-oxo-6-[(3-pyridinylmethyl)amino-5-pyrimidine-[^{14}C]carbonitrile hydrochloride (pelrinone) (I)

A solution of the [^{14}C]cyano-pyrimidone (0.187 g) and 3-aminomethyl pyridine (0.41 ml) in dimethoxyethane (5.0 ml) was heated at reflux for 18 hr in a 30 ml centrifuge tube. The mixture was cooled to room temperature, diluted with methanol (0.6 ml), and centrifuged. The precipitated product was collected, washed with ether, and dried under vacuum. The pelrinone free base (0.151 g) was suspended in methanol (1.7 ml), and methanolic hydrochloric acid (2N, 1.7 ml) was added. The mixture was stirred at room temperature for 2 hr, diluted with ether (8.6 ml), and centrifuged. The collected [^{14}C]pelrinone hydrochloride was dried under vacuum at 60° : 0.165 g.

The crude [^{14}C]pelrinone hydrochloride was crystallized from methanol/water (0.0916 g, sp. act. 66.2 ± 1.5 μCi/mg). A second crop of crystals was obtained from the mother liquors by recrystallizing from methanol/water (0.0275 g, sp. act. 64.2 ± 1.9 μCi/mg).

The radiochemical purity of [^{14}C]pelrinone hydrochloride was determined by TLC autoradiography in three solvent systems : (a) ethyl acetate : methanol : ammonium hydroxide = 20 : 5 : 1; (b) chloroform : methanol : acetic acid = 95 : 9.5 : 0.5; (c) chloroform : methanol : ammonium hydroxide = 16 : 4 : 0.2 (for solvent systems (a) and (b) the TLC plates were pre-wetted with solvent systems before the pelrinone was applied). The radioactive zones were located by exposing the TLC plates to X-Omat XAR Medical X-ray film. The silica gel (1 cm sections) was scraped into counting vials, digested with water (0.2 ml) and 50% hydrofluoric acid (0.2 ml), and counted in Aquasol scintillation cocktail (15.0 ml).

The TLC, IR, NMR, and MS properties of the [^{14}C]pelrinone were identical to those of an authentic sample of pelrinone: i.r. (nujol) 3240, 3150, 2800, 2210, 1655, 1625 1590, 1495 cm^{-1}; n.m.r. (dmso-d_6) 12.2 (1H, broad, NH), 9.5 (1H, broad, NH), 8.45 (2H, m, aromatic), 7.5 (2H, m, aromatic), 4.6 (2H, d, CH_2N), 2.25 (3H, s, CH_3); m.s. m/e 241 (M^+).

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