SYNTHESIS OF ^{14}C-LABELED ANTITUMOR AGENTS. I. SYNTHESIS OF THE RING- AND SIDE-CHAIN-^{14}C-LABELED DL-4,4'-PROPYLENEDI-2,6-PIPERAZINEDIONES

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SUMMARY

The ring- and side-chain-^{14}C-labeled DL-4,4'-propylenedi-2,6-piperazinediones (I) were synthesized from chloroacetic acid-1-^{14}C in two steps and from DL-analine-1-^{14}C in six steps, respectively. The radiochemical yield for the ring labeling was 24.5%; for the side-chain labeling it was 13.7%. In the side-chain labeling, the synthesis involved a new method for preparing DL-proprylenediamine from DL-alaninamide.

Stability and purification of the 2,6-piperazinedione are described.

Key Words: Carbon-14, DL-4,4'-Propylenedi-2,6-piperazinedione, Antitumor Agent, DL-Propylenediamine-1-^{14}C

INTRODUCTION

Ethylenediamine tetraacetic acid (EDTA) is a powerful chelating agent but has no significant antitumor activity [1], whereas DL-4,4'-propylenedi-2,6-piperazinedione* (I) and its analog 4,4'-ethylenedi-2,6-piperazinedione, less polar and possibly latent forms of EDTA, are antitumor agents [2].

Various interesting biological and biochemical properties of I have been reported in the past few years. These include antitumor activity [1-6], control of malignant metastases [7], inhibition of DNA, RNA, and protein synthesis in

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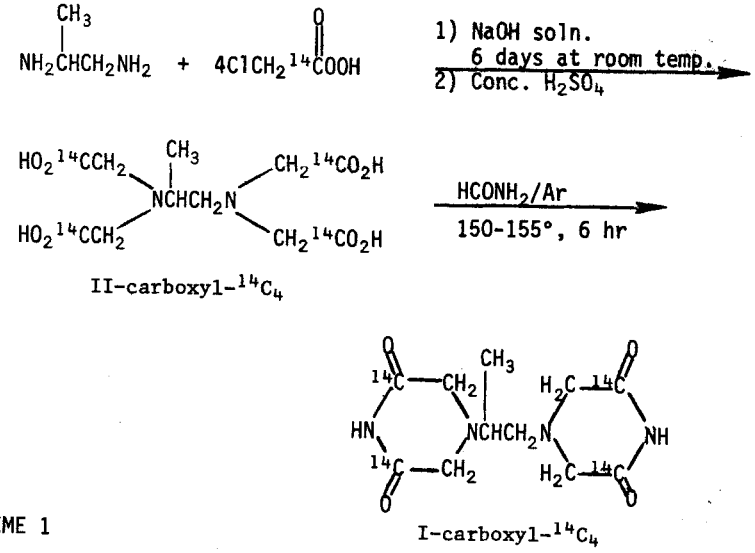
* Its synonym is DL-4,4'-(1-methyl-1,2-ethanediyl)-bis-2,6-piperazinedione or DL-1,2-bis-(3,5-dioxopiperazin-1-yl)propane. Also, this compound is designated as ICRF-159 or NSC-129,943.

mouse-embryo fibroblasts [8], and the cell-cycle specificity [9]. Therefore, metabolic, pharmacological, and other biochemical aspects of this compound are of current interest. Synthesis of the ring- and side-chain-^{14}C-labeled I certainly will facilitate these studies.

CHEMISTRY

In preparing the ^{14}C-ring-labeled I-carboxyl-$^{14}C_4$, the method for synthesis of ethylenediamine tetraacetic acid-I-$^{14}C_4$ by Murray and Ronzio [10] was adapted and tried without success. Perhaps the complete carboxymethylation of propylenediamine with formaldehyde and sodium cyanide is much more difficult than that of ethylenediaminine because of steric effect.

Alternatively, Dwyer and Garvan's method [11] was used to prepare the key intermediate DL-propylenediamine tetraacetic acid-I-$^{14}C_4$(II-carboxyl-$^{14}C_4$) (Scheme 1). The reaction requires an equivalent ratio of chloroacetic acid-I-^{14}C to DL-propylenediamine of 1.5:1. In the cold experiments, ratios such as 1.25:1 and 1.1:1 did not give as high a yield, calculated on the basis of chloroacetic acid. When bromoacetic acid was substituted for chloroacetic acid, the condensation reaction failed, probably because the rate of hydrolysis of bromoacetic acid is much higher than the rate of its condensation with DL-propylenediamine.



SCHEME 1

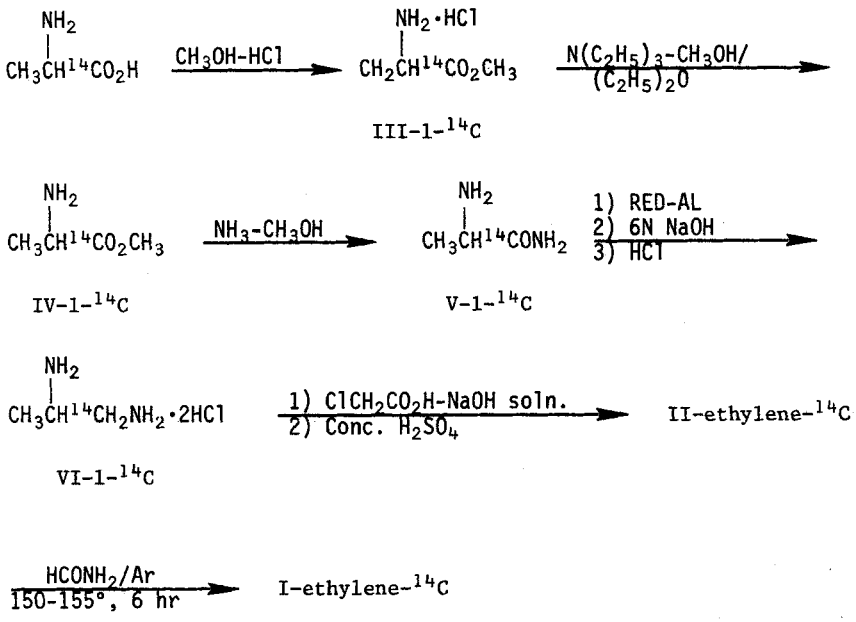
Imidation of II-carboxyl-$^{14}C_4$ was accomplished by heating it with formamide at 150 to 155° for 6 hours in an argon atmosphere.* Recently, it was reported that I also can be prepared in a good yield by reacting II with ethyl carbamate and/or propyl carbamate [3] or from tetraamide of II [5,6]. In this two-step synthesis, ring-^{14}C-labeled I was obtained in 24.5% radiochemical yield on the basis of the excess chloroacetic acid-1-^{14}C and in 41.6% chemical yield on the basis of DL-propylenediamine.

^{14}C-Labeled DL-propylenediamine dihydrochloride (VI) is the key intermediate for synthesis of side-chain-^{14}C-labeled I. Preparation of VI from acetaldehyde (with or without sodium bisulfite), ammonium chloride, and sodium cyanide via reduction of α-aminopropionitrile resulted in a poor yield [12,13]. However, VI was successfully synthesized from DL-alanine.

As shown in Scheme 2, DL-alanine-1-^{14}C was esterified to give the hydrochloride salt of its methyl ester (III-1-^{14}C) [14]; hydrogen chloride was removed by triethylamine [15]; aminolysis of the ester-^{14}C (IV-1-^{14}C) yielded DL-alaninamide-1-^{14}C (V-1-^{14}C) [15,16]; and reduction of V-1-^{14}C with RED-AL--sodium bis-(2-methoxyethoxy)-aluminum hydride [17]--followed by acidification with hydrogen chloride, afforded VI-1-^{14}C. For convenience, the reduction product was isolated as VI-1-^{14}C, a dihydrochloride. The dihydrochloride instead of the free base was used directly for condensation with excess chloroacetic acid using a slightly modified procedure. Subsequent imidation of the II-ethylene-^{14}C yielded the side-chain-^{14}C-labeled I. The radiochemical yield of the entire synthesis was 13.7% from DL-alanine-1-^{14}C.

Reduction of DL-alaninamide (V) by diborane in tetrahydrofuran was explored, and VI was obtained in 23% yield. However, 44 to 55% yield of VI was achieved when RED-AL was used as a reducing agent. Since V can be prepared from DL-alanine in three steps with a high yield (∿95%), DL-alanine-1-^{14}C is a convenient inter-

* In 1972, Dr. A. M. Creighton suggested that we conduct this reaction under a nitrogen atmosphere. In the cold experiments, the reaction in the argon atmosphere gave a slightly better yield (66%) than that in the suggested condition (58-61%). However, 70% yield of this imidation reaction was claimed in Dr. Creighton's patents by heating II and formamide at 110 to 120° and at reduced pressure under nitrogen for 1 hour and at 155 to 160° for 4 hours [5,6]. We became aware of the patents after we had completed the synthesis of the ring- and side-chain-^{14}C-labeled I.



SCHEME 2

mediate for synthesis of I-ethylene-^{14}C. This approach provides a new method for synthesizing D- or L-propylenediamine and their respective derivatives, D- or L-I or D- or L-II, without resorting to resolution of either optical isomer [18].

After 9 months of storage in a freezer under dry conditions, 5 to 10% of the ring- or side-chain-^{14}C-labeled compound had decomposed. The products resulting from the decomposition cannot be identified because the radioautogram of the thin-layer chromatogram of the piperazinedione-^{14}C in System A indicated that the spot due to the piperazinedione-^{14}C tailed all the way to the origin.

Purification can be accomplished simply by washing the compound with absolute methanol at room temperature. The recovery resulting from purification is about 65 to 75%.

EXPERIMENTAL

Melting points were determined by using Mel-Temp (Laboratory Devices, Cambridge, Mass.) and were not corrected. Radioactivity was measured by an LF-250 Liquid Scintillation System (Beckman Instrument, Inc., Fullerton, Calif.). p-Dioxane or an aqueous solution of the radioactive compound (0.1-0.5 ml) in

10 ml of Scintisol-Complete (Isolab, Inc., Akron, Ohio) was used for counting.

The purity of the product was checked by radioautography of the thin-layer chromatogram: MN-polygram, Silica G, UV/254, Brinkmann; System A, prewashed and developed by n-amyl alcohol:pyridine:water (4:4:2, by volume); System B, developed by n-butyl alcohol:acetic acid:water (450:50:125, by volume) visualized with the aid of I_2 vapor. Unless indicated as an estimation, the purity of the ^{14}C compound was determined by actual counting of the collected silica gel or cut-up plastic foil from the radioactive spots of the chromatogram.

DL-Propylenediamine(tetraacetic acid-1-$^{14}C_4$), (II-carboxyl-$^{14}C_4$) [11]

Essentially, the procedure for preparing II-ethylene-$^{14}C_4$ (described below) was followed to yield II-carboxyl-^{14}C, except that DL-propylenediamine, the free amine, was used, and no extra equivalent of sodium hydroxide solution was required to neutralize the dihydrochloride, as it is in preparing II-ethylene-$^{14}C_4$. From 2.05 g of chloroacetic acid-1-^{14}C (21.7 mmole, 53 mCi at 2.4 mCi/mmole; mp 60 to 62°; Dohm Products, Ltd., No. Hollywood, Calif.), 1.42 g (4.63 mmole) of II-carboxyl-1-$^{14}C_4$ (mp 225-230°; literature [11] mp 236°) was obtained in 85% crude yield from $ClCH_2$-$^{14}CO_2H$* and was used directly in the next step.

DL-4,4'-Propylenedi-(2,6-piperazinedione-2,6-$^{14}C_2$), (I-carboxyl-$^{14}C_4$)

By following the procedure for synthesis of I-ethylene-^{14}C, described below, 401 mg (1.49 mmole, 13 mCi at 8.73 mCi/mmole or 32.5 μCi/mg) of I-carboxyl-$^{14}C_4$ was obtained from the crude II-carboxyl-1-$^{14}C_4$. The piperazinedione-$^{14}C_4$ was obtained in 24.6% radiochemical yield (mp 230 to 232°, in a sealed evacuated tube identical to that of nonlabeled authentic sample; 99% pure by radioautography; R_f 0.63 in System A).

Methyl ester of DL-alanine-1-^{14}C hydrochloride (III-1-^{14}C) [14]

A stirred suspension of DL-alanine-1-^{14}C (924 mg, 10.4 mmole, 100 mCi; New England Nuclear, Boston, Mass.) and absolute methanol (9 ml) was bubbled with

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* It was found in the next step that the partially purified acid-$^{14}C_4$ contained a small quantity of inorganic salt. However, this salt did not affect the imidation reaction. A longer period of stirring with water may help to dissolve most of the salt.

hydrogen chloride until a clear solution resulted. Then hydrogen chloride delivery was continued until a saturated solution at ice-bath temperature was obtained. The flask was stoppered, and the mixture was allowed to stir at room temperature for 20 hours. Methanol and excess hydrogen chloride were carefully removed at reduced pressure below 40°. A residue of 1.6 g (quantitative) of the ester-1-^{14}C hydrochloride was obtained.

Methyl ester of DL-analine-1-^{14}C (IV-1-^{14}C) [15]

To a stirred solution of the hydrochloride (1.6 g) in 2.9 ml of absolute methanol was slowly added 1.5 ml (1.09 g, 10.9 mmole) of triethylamine (99%, Aldrich Chemical Co., Inc., Milwaukee, Wis.), followed by 60 ml of anhydrous ether. The mixture was stirred at room temperature for 10 min and then cooled at 4° for 1.5 hour. The triethylamine hydrochloride was removed by filtration and washed with anhydrous ether (5 X 10 ml). The solvents from the combined filtrate were distilled off through a Claisen distilling head below 70°. The residue dissolved in absolute methanol (15 ml) was used for the next transformation.

DL-Alanineamide-1-^{14}C (V-1-^{14}C) [15,16]

In an apparatus protected from moisture by a Drierite drying tube, the stirred and cooled methanol solution of the alanine-1-^{14}C ester was saturated with gaseous ammonia. The reaction solution in the stoppered flask was stirred at room temperature for 3 days. The solution was carefully evaporated to dryness *in vacuo* below 40°, and the residual water was removed with benzene by azeotropic distillation at reduced pressure. The residue was then dried in the presence of phosphorus pentoxide and sodium hydroxide pellets in two separate beakers at 0.3 torr for 2 hour. The crude amine-^{14}C (863 mg, 9.8 mmole; 94% crude yield based on alanine-1-^{14}C) was used for the next reduction step.

DL-Propylenediamine-1-^{14}C dihydrochloride (VI-1-^{14}C)

To a stirred mixture of the crude amide-^{14}C in 58 ml of dry benzene under the protection from the moisture was added dropwise 17.5 ml of RED-AL, sodium bis-(2-methoxyethoxy)aluminum hydride [17] (62.2 mmole: Aldrich Chemical Co.,

Inc.), followed by 5 ml of the benzene. The reaction mixture was heated in an oil bath at 75 to 90° for 20 hour. Sodium hydroxide solution (26 ml, 6N) was added, followed by 17 ml of water, and the mixture was allowed to continue stirring at room temperature for 0.5 hour. The benzene layer was separated, dried over anhydrous potassium carbonate for 1 day, and filtered. The cooled benzene filtrate was acidified with hydrogen chloride gas. The diamine-^{14}C dihydrochloride mixture was carefully evaporated to dryness *in vacuo* below 40°. The residue with benzene (15 ml) was evaporated again. This process was repeated to yield a dry residue (461 mg).* The aqueous layer of the mixture was extracted continuously with 115 ml of chloroform for 2 days. The chloroform layer was separated, dried over potassium carbonate, filtered, and combined with the residue from the benzene fraction. The combined mixture was acidified with hydrogen chloride and carefully evaporated to a volume of approximately 50 ml. The insoluble product (635 mg, 4.32 mmole; 44% yield from the amide-^{14}C) was collected by filtration. The analysis using System B indicated that the product was homogenous and identical to DL-propylenediamine dihydrochloride.

DL-Propylenediamine-1-^{14}C tetraacetic acid (II-ethylene-^{14}C) [11]

To a stirred and cooled solution of chloroacetic acid (2.46 g, 26.0 mmole) in water (1 ml) was added dropwise 4.86 ml of 10.7N sodium hydroxide solution (52 meq) through a buret. Then 635 mg (4.32 mmole) of the diamine-^{14}C dihydrochloride (VI-1-^{14}C) was added. Finally, 0.8 ml of 10.7N sodium hydroxide solution (8.6 meq) was added to neutralize the hydrochloride. The ice-cold mixture was stirred in a water bath to allow the solids to dissolve without significantly elevating the temperature of the mixture; then it was stirred at room temperature for 6 days. The cold reaction mixture was acidified with concentrated sulfuric acid (723 µl, 26.0 meq) using a syringe microburet and then stored at 4° overnight. The cold mixture, which contained some colorless crystals, was swirled for a few minutes and then was kept at 4° for 48 hours. The white precipitates were collected, and the filtrate was acidified with 180 µl (2.1

* The drying by evaporation of the residue with benzene is necessary only if one needs to know the weight of the residue from the benzene layer.

mmole) of concentrated hydrochloric acid and kept at 4° overnight. The second crop was collected similarly. The combined crude product (1.69 g) was stirred with 4.3 ml of water for 15 min. The insoluble tetraacid-^{14}C was collected by filtration, washed with 95% ethanol and then anhydrous ether, and dried. The purified product (1.09 g, 3,56 mmole; 82% yield from the diamine-^{14}C salt) was used for the final step of the synthesis.

DL-4,4'-Propylene-1-^{14}C-di-2,6-piperazinedione (I-ethylene-^{14}C)

Under an argon atmosphere, a stirred mixture of the tetraacid-^{14}C (1.08 g, 3.56 mmole) and the redistilled formamide (5.6 ml) was heated at 150 to 155° (oil bath) for 6 hours. The reaction mixture was cooled, and the excess solvent was distilled off below 100° at 0.45 torr to yield a nearly dry residue. The residue was triturated with 5.4 ml of absolute methanol. The mixture was kept at 4° overnight and then stirred at room temperature for 5 min. The solid (602 mg, 2.24 mmole) was collected by filtration and partially dissolved in 24 ml of boiling p-dioxane. The insoluble impurities were removed while the mixture was warm. The filtrate was diluted with absolute methanol (6 ml), and the resulting solution was kept at 4° for 48 hours. The precipitate (398 mg) was collected, and this filtrate was evaporated to dryness *in vacuo* below 40°. The residue was almost completely dissolved in 4 ml of methanol:p-dioxane solution (20:80, v/v) by heating (hot water bath). The mixture then was kept at 4° overnight. A second crop was obtained after filtering and washing with 0.3 ml absolute methanol and 3 ml anhydrous ether. The combined solid product (488 mg) was dried (Drierite) at 50 to 60° for 2 hours. The melting point of the product (227-230°) in a sealed evacuated capillary tube was slightly lower than that of the authentic sample (230 to 232°). The light-brown product was purified by stirring with 15 ml of absolute methanol at room temperature for 2 hours. The purified product was collected by filtration and washed with 10 ml of absolute methanol and then 10 ml of anhydrous ether. Gentle suction was applied to afford the dry piperazinedione-^{14}C (394 mg, 1.47 mmole; mp 229 to 230°) in 13.7% radiochemical yield from DL-alanine-1-^{14}C. The desired ^{14}C-compound (13.7 mCi at 9.34 mCi/mmole or 34.9 μCi/mg) was 98% pure by radioautography using the tlc System A (R_f 0.56, which is identical to that of the nonlabeled authentic sample).

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