PREPARATION OF CARBON-14 LABELED 3,4-DIHYDRO-5-METHYL-1(2H)-ISOQUINOLINONE

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SUMMARY

Carbon-14 labeled 3,5-dihydro-5-methyl-1(2H)-isoquinolinone, a potent inhibitor of poly(ADP-ribose) polymerase (ADPRP) was prepared from 1-bromo-2-methylbenzene in 7.8 % overall yield. The C-14 label was introduced from $Ba^{14}CO_3$ via metal-halogen exchange and carboxylation reactions. Subsequent transformations yielded a propenecarbonyl azide 8 which was rearranged by Curtius reaction to the isocyanate, and a cyclization produced 5-methyl-1(2H)-[4-^{14}C]isoquinolinone (9). From the compound 9 by Pd/C catalyzed hydrogenation 3,4-dihydro-5-methyl-1(2H)-[4-^{14}C]isoquinolinone (10) was made.

KEY WORDS: poly(ADP-ribose) polymerase, radiotherapy, radiation induced lethality, 1(2H)-isoquinolinone.

Introduction

Poly(ADP-ribose) polymerase (ADPRP) is a chromatin-bound enzyme found in the nucleus of nearly all eukaryotic cells. The known physiological functions of ADPRP include the catalysis of poly(ADP-ribose) synthesis from NAD^+ and involvement in the repair of induced DNA damage (1). In spite of the numerous studies, the mediation of such cellular repair by ADPRP is as yet poorly understood (2). However, compounds such as 3-aminobenzamide that are known to inhibit poly(ADP-ribose)polymerase activities have been described to potentiate radiation induced lethality of mammalian cells (3), the cytotoxicity of chemotherapeutic agents (4) and to impair DNA excision repair (5). Some other compounds have shown enhanced radiation-induced cell killing that correlates with their potency as inhibitors of ADPRP (6). Therefore the specific inhibition of poly(ADP-ribose) polymerase activities could have useful application in chemo- and radio-therapies.

Cellular repair of radiation-induced DNA damage is currently an important limitation in the effectiveness of radiotherapy (7). An approach to maximizing the lethality of tumor cells in target tissues may be by the adjunct administration of ADPRP inhibitors as potentiating agents of induced DNA

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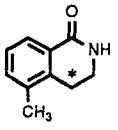
damage. In our laboratories we have directed efforts at finding such inhibitors of poly(ADP-ribose) polymerase (ADPRP). We also hope that such compounds would be useful tools as probes of the mechanism of this enzyme action, and thus further our insight into tumor biology.



(10) PD 128763

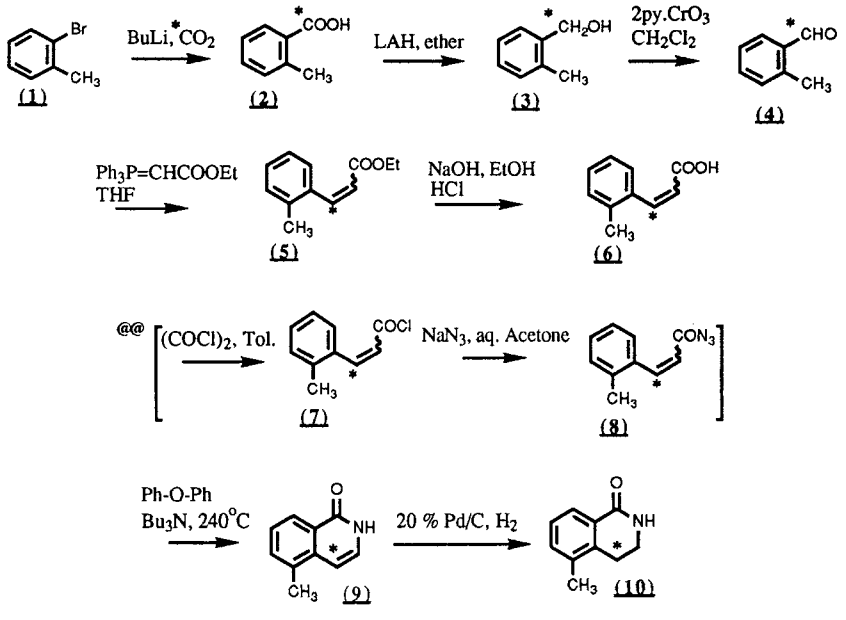
3,4-Dihydro-5-methyl-1(2H)-[4-^{14}C]isoquinolinone (10) PD128763 is a very potent inhibitor of ADPRP that was discovered in our laboratories (8), and it possesses many desirable properties (9) that warrant continuation of its evaluation as a possible clinical lead compound. Using $Ba^{14}CO_3$, we prepared a carbon-14 labeled analog of 3,4-dihydro-5-methyl-1(2H)-isoquinolinone. The sequence included a Curtius rearrangement reaction, and the cyclization of an isocyanate derived therefrom to an isoquinolinone. From this isoquinolinone by catalytic hydrogenation, we prepared the target compound 10. Details of the synthesis are presented.

RESULTS AND DISCUSSION

The conversion of 2,3-dihydroxy-4-methyl-1H-inden-1-one with trichloroacetic acid and sodium azide to 3,4-dihydro-5-methyl-1(2H)-isoquinolinone was a pivotal step in the original synthesis of our target compound. In that preparation (8), 2,3-dihydroxy-4-methyl-1H-inden-1-one was itself accessible from o-tolualdehyde by sequential transformation into trans-2-methylcinnamic acid, and methyl-benzenepropanoic acid. Cyclization thereof yielded 2,3-dihydroxy-4-methyl-1H-inden-1-one. We chose a modified synthesis of 3,4-dihydro-5-methyl-1(2H)-[4-^{14}C]isoquinolinone (10) as illustrated in **scheme 1.** By this approach we were able to prepare the target labeled version from $Ba^{14}CO_3$. We employed metal-halogen exchange and carboxylation (10) reactions to expeditiously make the desired 2-methyl-[carboxy-^{14}C]benzoic acid (2). Once obtained, compound 2 was converted by reduction with LAH to 2-methylbenzene-[^{14}C]methanol (3), and then oxidized with Collins reagent to 2-methyl-[carbony-^{14}C]benzaldehyde (4). The aldehyde 4 was homologated by reaction with (carboethoxymethylene)-triphenylphosphorane under standard conditions to afford the predominantly (calc. 93 %) trans-cinnamic acid ester, ethyl 3-(2-methylphenyl)-2-[3^{14}C]propenoic ester (5). The compound 5 was subjected to base hydrolysis, and upon acidification provided 3-(2-methylphenyl)-2-[3-^{14}C]propenoic acid (6). Without purification the compound 6 was transformed to the corresponding propenoyl chloride 7 , and then to 2-[3-^{14}C]propenecarbonyl azide 8, according to the method of Poulter

et al (11). From 3-(2-methylphenyl)-2-propenoic acid (6), we also prepared by catalytic hydrogenation the saturated derivative, 3-(2-methylphenyl)-propanoic acid. By a protocol similar to that used to make the compound 8, 3-(2-methylphenyl)-propanoic acid was converted to 3-(2-methylphenyl)-propanecarbonyl azide. Although the acid chlorides and acyl azides were isolated in both series of compounds, they were neither purified nor characterised, and were used as such in the subsequent steps.

Scheme I



@@ not characterized

We planned to execute in one pot either a Curtius rearrangement reaction on 3-(2-methylphenyl)-2-[3-^{14}C]propenecarbonyl azide (8) or its 2-[3-^{14}C]propanecarbonyl azide derivative, and cyclize the resulting isocyanate product to afford 5-methyl-1(2H)-[4-^{14}C]isoquinolinone (9) or 3,4-dihydro-5-methyl-1(2H)-[4-^{14}C]isoquinolinone (10) respectively. However, the very limited examples of such transformation in literature necessitated the investigation of this step. We were unable to cyclize the isocyanate from 3-(2-methylphenyl)-propanecarbonyl azide to obtain the unlabeled title compound 10. But the compound 3-(2-methylphenyl)-2-[3-^{14}C]propenecarbonyl azide 8 under the same reaction conditions, heating in phenyl ether-tributyl amine (7:3, v/v) at 240 °C, was converted to 5-methyl-1(2H)-[4 -^{14}C]isoquinolinone (9). Evidently, the presence of the olefin group was crucial to effecting cyclization in the putative isocyanate from the compound 8. In part therefore, this reactivity may be explainable in terms of hindered rotation about the olefin group. After isolation by filtration and

recrystallization, the compound 9 was converted by Pd/C catalyzed hydrogenation to the titled compound 10. With the exception of compounds 7 and 8, all intermediate compounds were characterized by tlc comparison with authentic samples obtained from commercial sources. Proton nmr analyses gave results that were identical with those obtained for the reference compounds, and they were consistent with assigned structures. The labeled compound (10) was identical in all appropriate physical and spectroscopic measurements with 'cold' authentic material.

In conclusion, we have synthesized 3,4-dihydro-5-methyl-1(2H)-[4-^{14}C]isoquinolinone (10) starting from 1-bromo-2-methylbenzene. We used $Ba^{14}CO_3$ as a source of a carboxy-^{14}C which was subsequently transformed to an acyl azide, rearranged to the isocyanate and cyclized to give 5-methyl-1(2H)-[4-^{14}C]isoquinolinone (9). A catalytic hydrogenation afforded the target compound 10.

Experimental

General Methods.

All reactions were carried out under inert atmosphere. ^{1}H-NMR spectra were recorded with Varian (EM 390) 90 MHz spectrometer, a Gemini 200 MHz or a Varian XL 300 MHz spectrometer. Radiochemical purity of every labeled compound was determined by tlc radiochromatogram with Bioscan 200 imaging scanner. Carbon-14 labeled $BaCO_3$ was purchased from American Radiolabeled Chemicals, Inc. St. Louis, MO, and diluted to the required specific activity prior to use in synthesis. Radiochemical counting was performed on a Packard 574 liquid scintillation counter using Beckman Readi-Solv MP cocktail. HPLC analyses of final products were performed on a Waters Associates 600E system with on line Applied Biosystems 1000S diode array detector and either a β-RAM radioactivity detector or Radiomatic series A-200 radioactivity flow detector. Column chromatography was carried out on a Merck Kieselgel 60 (230μ).

2-Methyl-[carboxy-^{14}C]benzoic acid (2)

A carbon dioxide generator was assembled from a 100 mL round bottom flask and a 50 mL pressure equalizing dropping funnel. The flask, and funnel were respectively charged with $Ba^{14}CO_3$ (1.37 g, 6.95 mmol, specific activity 10.07 mCi/mmol), conc. H_2SO_4 (30 mL, 2160 mmol), and via a $CaCl_2$ tube, was connected to a vacuum manifold. After the system was degassed, conc. H_2SO_4 was added dropwise to the stirred $Ba^{14}CO_3$, generating $^{14}CO_2$ which was collected and stored in a 'cold finger' cooled in a liquid nitrogen bath. Next, n-BuLi (1.6 M solution in hexane) (4.40 mL, 7.04 mmol) was added dropwise to a solution of 1-bromo-2-methylbenzene (1. 0 mL, 8.60 mmol) in dry THF (10.0 mL) maintained at -78° C under argon atmosphere. After stirring for 45 min, the cold-bath was replaced with a liquid nitrogen bath, and the reaction was connected to the vacuum manifold.

The reaction was degassed, and the above pre-generated carbon dioxide was sublimed to collect in the reaction vessel. The liquid nitrogen bath was replaced with a dry ice-acetone bath, and the reaction was stirred while it slowly warmed up to room temperature over 3 hours. The solvent was removed, and the residue was re-suspended in ether (50 mL) and water (20 mL). The organic phase was extracted with a further 6N. NaOH (20 mL), the combined aqueous portion was acidified with conc. HCl, and extracted with ether (3X100 mL). After it was dried on $MgSO_4$, the solvent was removed to give 750mg (80 %). NMR 300 MHz ($CDCl_3$) δ 2.69 (s, 3H, *ortho*-C<u>H</u>3); 7.28 (overlapped d&tr, 2H, J = 7.06 Hz, *para and meta* protons); 7.45 (tr, 1H,, J = 7.5 Hz, *meta* proton); 8.10 (d, 1H, J = 1.6 and 7.5 Hz, *ortho* proton); 12.28 (brs, exch. D_2O, -COO<u>H</u>).

<u>2-Methylbenzene-[^{14}C]methanol (3)</u>

To a solution of 2-methyl-[carboxy-^{14}C]benzoic acid (750 mg, 5.51 mmol) in dry ether (100 mL) at 0 °C was added in one portion lithium aluminum hydride (LAH) (500 mg). The cold bath was removed and the mixture was stirred at room temperature overnight. The temperature was again lowered to 0°C with an ice cold bath and excess LAH was destroyed by the dropwise addition of EtOAc. It was diluted with methylene chloride (200 mL) and saturated Na_2SO_4 solution was added dropwise till the aluminate coagulated, and dried with anhydrous Na_2SO_4. It was filtered and solvent was removed to give an oil (592 mg, 88 %). Tlc on silica gel developed with hexane:ethylacetate (75:25, v/v) showed product to be identical (Rf 0.21) with commercially (Aldrich) obtained 2-methylbenzyl alcohol. NMR 300 MHz ($CDCl_3$) δ 1.83 (s, -O<u>H</u>, exch. D_2O); 2.37 (s, 3H, Ar-C<u>H</u>3); 4.75 (s, 2H, benzylic -C<u>H</u>2), 7.22 and 7.45 (m, 4<u>H</u>, aromatic protons).

<u>2-Methyl-[carbony-^{14}C]benzaldehyde (4)</u>

Collins reagent (CrO_3.2py) (10.0 g, 8 equiv.) was added to a stirred solution of 2-methylbenzene-[^{14}C]methanol (592 mg, 4.85 mmol) in methylene chloride (30 mL). After 15 min it was filtered through a bed of Celite, and the filterate was applied to a short column of florisil and eluted with methylene chloride. Solvent was removed to give 580 mg (99 %). Tlc on silica gel developed with hexane: ethyl acetate (75:25 v/v) showed product to be identical (Rf 0.43) with o-tolualdehyde purchased from Aldrich Chemical Company. NMR 300 MHz ($CDCl_3$) δ 2.67 (s, 3H, -C<u>H</u>3); 7.25, (d, J = 7.2 Hz, 1<u>H</u> *meta*); 7.35, (tr, J = 7.4 Hz, 1<u>H</u>, *para*); 7.47, (dtr, J =1.5, 7.5 Hz, 1<u>H</u> *meta*); 7.84, (1<u>H</u> , dd, J = 1.5, 7.5 Hz *ortho*) and 10.26 (s 1H, aldehyde).

<u>Ethyl-3-(2-Methylphenyl)-2-[3-^{14}C]propenoic ester (5)</u>

A mixture of 2-methyl-[carbony-^{14}C]benzaldehyde (580 mg, 4.8 mmol) and (carboethoxymethylene)-triphenylphosphorane (7.045 g, 20.2 mmol) in dry THF (40 mL) was refluxed

under nitrogen atmosphere, and the reaction was monitored by tlc. After the aldehyde had been consumed (calc. 2 hr) the solvent was removed, and the residue was taken up in ether:hexane 1:3 (400 mL) and filtered. The filterate was concentrated, applied to a column of silica gel and eluted with 5 % EtOAc in hexane to give ester (808 mg, 88 %). NMR 300 MHz ($CDCl_3$) δ 1.33 (tr, 3H, -C<u>H</u>$_3$ J = 7.20 Hz); 2.43 (s, 3H, Ar-C<u>H</u>$_3$); 4.36 (q, 2H, -OC<u>H</u>$_2$ J = 7.20 Hz); 6.35 (d, 1H, =C<u>H</u>COOEt , J = 15.8 Hz); 7.23 (m, 3H, *meta and para* protons); 7.54 (d, 1H, *ortho* proton); 8.0 (d, 1H, Ar-C<u>H</u>=CHCOOEt, J = 15.8 Hz). The following peaks calculated to be 6.8 % in proportion and presumably due to the *cis* -isomer, δ 1.15 (tr); 2.30 (s); 4.10, (q), and 6.05 (d, J = 12.10 Hz) were observed in the spectrum.

<u>5-Methyl-1(2H)-[4-^{14}C]isoquinolinone (9)</u>

Ethyl-3-(2-methylphenyl)-2-[3-^{14}C]propenoic ester (808 mg 4.20 mmol) and NaOH (1.0 g 25 mmol) in ethanol (50 mL) were refluxed for 1.5 hr, and the solvent was removed. Ethyl acetate (60 mL) and 1.0 N HCl (25 mL) were added and the organic phase was separated. The aqueous portion was further extracted with ethyl acetate (3X40 mL), and the combined organic extract was washed with brine and dried. The solvent was removed to give 3-(2-methylphenyl)-2-[3-^{14}C]propenoic acid (<u>6</u>) as a white solid. NMR 300 MHz ($CDCl_3$ + DMSO) δ 2.39 (s, 3H, Ar-C<u>H</u>$_3$); 6.31 (d, 1H, J = 15.89 Hz, =C<u>H</u>COOH); 7.22 (m, 3H, *two meta and a para*, protons); 7.51 (d, 1H, J = 7.5 Hz, *ortho* proton); 7.92 (d, 1H, J = 15.89 Hz, Ar-C<u>H</u>=CHCOOH); and 10.35 (brs, exch. D_2O, -COO<u>H</u>).

The above 3-(2-methylphenyl)-2-[3-^{14}C]propenoic acid (<u>6</u>) (assuming 100 % yield) was suspended in dry toluene, and 10 equiv. of oxalyl chloride followed by a drop of dry DMF were added. It was stirred for 1 hr. after which evolution of gas stopped. The solvent was removed, and the liquid product, 3-(2-methylphenyl)-2-[3-^{14}C]propenoyl chloride (<u>7</u>), was dried on a vacuum pump for 2 hr. It was dissolved in dry acetone and added dropwise to a vigorously stirred solution of NaN_3 (1.10 g, 17.0 mmol) in water (4.0 mL) maintained at 0-4°C. After addition was completed, it was stirred for additional 20 min and diluted with hexane (300 mL). The organic phase was separated, the aqueous phase was extracted with hexane 2 X 40 mL, the combined organic extract was dried, and the solvent was removed to give 3-(2-methylphenyl)-2-[3-^{14}C]propenecarbonyl azide (<u>8</u>) as an oil which crystallized on standing. To this solid was added phenyl ether-tributylamine (7:3) (100 mL) and then transferred to an oil bath kept at 240 °C and the reaction was monitored. After 2 hr the reaction was complete, it was cooled to room temperature, poured onto pet. ether (800 mL) and set aside overnight during which 5-methyl-1(2H)-[4-^{14}C]isoquinolinone (<u>9</u>) separated as a solid. The solid product was collected by filteration, crystallized from methanol to give 120 mg (18 % based on <u>5</u>) and checked by NMR 300 MHz ($CDCl_3$) δ 2.56 (s, 3H, C_5-C<u>H</u>$_3$); 6.71 (d, 1H, J = 7.4 Hz, C_3 =C<u>H</u>-); 7.26 (d, 1H, J =

7.4 Hz, C_4 -CH=); 7.38 (tr, 1H, J = 7.9 Hz, C_7 -CH=); 7.52 (d, 1H, J = 7.5 Hz, C_6 =CH-); 8.31 (d,1H , J = 7.9 Hz, C_8 =CH-); and 12.03 (brs exc. D_2O, 1H , -NH).

3,4-Dihydro-5-methyl-1(2H)-[4-^{14}C]isoquinolinone (10)

A solution of 9 (120 mg), in acetic acid : methanol (80:20 30 mL) and 20 % Pd/C (200 mg) was hydrogenated at 60 psi overnight and filtered. The solvent was removed at reduced presuure to give crude 3,4-dihydro-5-methyl-1(2H)-[4-^{14}]isoquinolinone. It was purified by medium pressure reverse phase (C_{18}-silica gel) chromatography (water : methanol (55 : 45)), followed by crystallization from methylene chloride:hexane to give 81.0 mg (67 %, specific activity 67.8 μCi/mg), TLC silica gel 60 F-254, (EtOAc : cyclohexane; 21:9), Rf 0.12, 100 %. HPLC T_r 7.24 min, PCP 100 %, CP 99.63 % on Alltech Econosil C_{18}, 10 μ, 4.6 mm ID X 250 mm, 0.025 M ammonium phosphate (pH = 4.0 with H_3PO_4) : CH_3CN (70 :30), flow rate 1.5 mL/ min, uv 215 nm. NMR 300 MHz ($CDCl_3$) δ 2.32 (s, 3H , C_5-CH_3); 2.92 (tr, 2H , J = 6.5 Hz, C_3-CH_2); 3.58 (m, 2H , J = 2.50, 6.7 Hz, C_4-CH_2); 7.07 (brs exc. D_2O,1H , NH); 7.25 (tr, 1H, J = 7.5 Hz, C_7-CH); 7.31 (d, 1H, J = 7.5 Hz, C_6-CH); 7.95 (d, 1H, J = 7.5 Hz, C_8-CH).

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