CONVENIENT METHODS FOR THE PREPARATION OF [5-^{14}C]-4-IPOMEANOL AND [^{3}H(G)]-4-IPOMEANOL OF HIGH SPECIFIC RADIOACTIVITY

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SUMMARY

Procedures are described for the preparation of radiolabeled 4-ipomeanol [1-(3-furyl)-4-hydroxypentanone], a highly selective pulmonary alkylating agent, of sufficiently high specific radioactivity for biochemical and autoradiographic studies. The ^{14}C-labeled material is prepared from the reaction of ethyl 3-furoylacetate with [3-^{14}C]-propylene oxide; the tritiated product is obtained by catalytic tritiation using T_2O or tritium gas as the source of the radiolabel.

Key Words: [5-^{14}C]-4-Ipomeanol, [^{3}H(G)]-4-Ipomeanol

INTRODUCTION

4-Ipomeanol [1-(3-furyl)-4-hydroxypentanone] is a highly selective pulmonary alkylating agent which currently is receiving attention as a potential antitumor agent and as a valuable experimental tool in pulmonary toxicology (1-6). The parent compound is not reactive with biological macromolecules, but it is metabolized in the lungs to a highly reactive product that binds covalently to nucleophilic tissue constituents.

Although the capacity of tissues to metabolically activate 4-ipomeanol in vivo and in vitro can be assayed by biochemical methods (6), these procedures do not allow the unequivocal assignment of this activity to individual cells within mixed cell populations such as found in the whole lung, or in partially purified whole-cell preparations, or in pulmonary tumors. Thus, an autoradiographic technique has been developed for evaluating the cellular and intracellular sites of metabolic activation of 4-ipomeanol in systems containing heterogeneous

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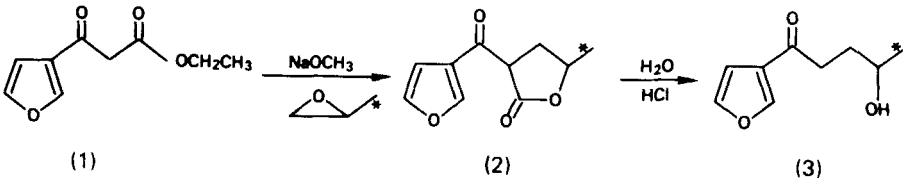
lung cell populations (1). This autoradiographic approach requires 4-ipomeanol of high specific radioactivity.

Previous methods (2,3) for the preparation of ^{14}C-radiolabeled 4-ipomeanol were rather tedious, required extensive purification procedures, or yielded product of relatively low specific radioactivity (adequate for biochemical studies but but not for autoradiography). Moreover, a preparation method for ^{3}H-labeled 4-ipomeanol also was needed, since such material is necessary for high-resolution autoradiography. Therefore, we describe here some convenient procedures for the preparation of [5-^{14}C]-4-ipomeanol and [$^{3}H(G)$]-4-ipomeanol of sufficiently high specific activity for a wide variety of biological studies, including in vitro and in vivo autoradiographic investigations.

EXPERIMENTAL

[5-^{14}C]-4-Ipomeanol (3). The synthesis of 3 is outlined in Figure 1. The method is an adaptation of one which previously has been used for the large-scale preparation of unlabeled 4-ipomeanol (4,5). The starting material, ethyl 3-furoylacetate (1), was prepared from ethyl 3-furoate as described previously (4,5).



(1) (2) (3)

Figure 1. Synthesis of [5-^{14}C]-4-ipomeanol (3). Asterisk shows location of radiolabeled atom.

Sodium methoxide solution was prepared freshly by dissolving 223 mg (0.01 mol) of metallic sodium in 10 ml of anhydrous methanol; 0.95 ml of the solution (containing 0.95 mmol $^{-}OCH_3$) was transferred to a 3 ml conical-bottom Reacti-Vial fitted with a magnetic cone stirrer and Mininert valve closure (Pierce Chemical Co., Rockford, IL). All reactants were introduced into the vessel through the valve closure using Hamilton microliter syringes.

After adding 167 µl (1.0 mmol) of 1, the reaction mixture was stirred for 15 min. Next, 70 µl (1.0 mmol) of [3-^{14}C]-propylene oxide (specific activity 2.0 mCi/

mmol) (American Radiochemical Corp., Sanford, FL) was added and the mixture stirred for 60 hr at room temperature. The lactone product (2) was decarboxylated without purification; 120 µl of 10 N HCl was added to the reaction vessel and the mixture was stirred for 15 hr at 50-55° over a water bath. The solution then was evaporated under a stream of N_2 to yield a brown residue ($\sim$0.2 ml). A mixture of 200 mg of NaCl and 2 ml H_2O were added and vigorously shaken with the residue. The mixture was extracted three times with 6 ml portions of ethyl ether. The extracts were combined, dried over anhydrous Mg_2SO_4, filtered, and evaporated under N_2. The residue (about 150 mg) was dissolved in 200 µl of ethyl ether and purified by thin-layer chromatography as described below.

[^{3}H(G)]-4-Ipomeanol. A mixture of 100 mg of 4-ipomeanol (prepared as described previously (4,5), 30 mg of 5% Rh/Al_2O_3 (Macalaster Bicknell, Millville, NJ), 0.5 ml of absolute ethanol, and 0.1 ml of glacial acetic acid was added to a 15 ml combustion tube. The tritium source was either T_2O or tritium gas obtained from ICN Pharmaceuticals Inc., Irvine, CA. The T_2O (25 Ci; specific activity 59 Ci/mmole) was added directly to the reaction mixture after flushing the tube with argon; for addition of tritium gas (25 Ci), the tube was evacuated on a vacuum line, followed by introduction of the tritium. The tube then was sealed and shaken at room temperature for 15 hrs. The catalyst was separated by centrifugation and the solution evaporated in vacuo. Labile tritium was back-exchanged by repeatedly (10-15 times) dissolving the sample in 10 ml portions of methanol and evaporating the solvent under a stream of N_2. The resulting crude product (about 95 mg) was dissolved in 200 µl of ethyl ether and purified by thin-layer chromatography as described below.

Purification of [5-^{14}C]-4-ipomeanol and [^{3}H(G)]-4-ipomeanol. Thin-layer chromatographic plates (silica gel GF in 1000 µ and 250µ thicknesses) were obtained from Analtech, Inc., Newark, DE. The 250 µ plates were deactivated by dipping them in methanol and allowing them to dry in room air 2 hr prior to use. The 4-ipomeanol bands were viewed on the eluted plates by short wave U.V. and were identified by comparative chromatography with authentic samples of unlabeled 4-ipomeanol. The chemical purity of the products was verified by high-pressure liquid

chromatography (Waters Model ALC 202 liquid chromatograph fitted with a 30 x 0.39 cm Bondapak column eluted with 30% (v/v) methanol/water at a rate of 2 ml/min (4-ipomeanol retention time = 10 min). The radiochemical homogeneity of the products was checked by counting 2 ml fractions from the high-pressure liquid chromatograph (ACS counting scintillant, Amersham Corp; Beckman Model LS-2122P Scintillation Counter) or by TLC using a Packard Model 385 TLC radiochromatogram scanner.

The crude [5-^{14}C]-4-ipomeanol was chromatographed on a 1000μ TLC plate using 40% (v/v) ethyl acetate/hexane as the eluant. The product band (R_f = 0.6) was scraped from the plate and extracted three times with 10 ml portions of ethyl ether. The extracts were combined, evaporated, and rechromatographed on a deactivated 250μ plate eluted with 10% (v/v) methanol/benzene. The product band (R_f = 0.4) was removed and extracted three times with 10 ml portions of ethyl ether. The solvent was removed using a stream of N_2. The product (average of 38 mg [0.23 mmol] (± 10 mg); 24% final yield based on six replications) was a viscous, colorless liquid and was chemically and radiochemically homogeneous, and had a specific activity of 2.0 mCi/mmol.

The crude [^{3}H(G)]-4-ipomeanol was chromatographed first on four deactivated 250μ TLC plates using 10% (v/v) methanol/benzene as the eluent. The product band (R_f = 0.4) was removed and extracted with ethyl ether. The sample was re-chromatographed twice more by the same procedure using one 250μ plate each time. The product obtained from the exchange procedure using either tritium gas or T_2O had a specific activity of approximately 1.7 Ci/mmol, although the yield generally was higher with the gaseous exchange method. The amount of labeled 4-ipomeanol typically recovered in several replications of these procedures was 2-5% of the amount of unlabeled starting material. The tritiated 4-ipomeanol was chemically and radiochemically homogeneous. Moreover, analysis of a sample of tritiated product stored in methanol solution at -20° for 6 months showed less than 5% decomposition.

DISCUSSION

Procedures are described herein for the preparation of radiolabeled 4-ipomeanol of high purity and high specific radioactivity. The product is suitable for use in biochemical and autoradiographic assays employing the compound. Prod-

uct with the highest specific radioactivity can be prepared using the tritiation procedures, and this material is most useful in autoradiographic investigations which require the highest possible cellular and intracellular resolution. Biochemical and autoradiographic data obtained with the ^{14}C-labeled material versus the tritiated 4-ipomeanol otherwise appear quite similar. For example, the same pattern of pulmonary cell-specific alkylation, as assessed by autoradiography, was obtained when animals were administered [5-^{14}C]-4-ipomeanol or [^{3}H(G)]-4-ipomeanol (1).

Previously-described methods for the preparation of ^{14}C-labeled 4-ipomeanol have several disadvantages, compared to the present method. A procedure (2) for making [4-^{14}C]- or [3,5-^{14}C]-4-ipomeanol, using [2-^{14}C]- or [1,3-^{14}C]-acetone, respectively, as the source of radiolabel, involved a 4-step reaction sequence, and yielded a complex mixture of isomeric 1,4-dioxygenated-1-(3-furyl)-pentanes, including 4-ipomeanol. Thus, with this method, purification of the desired product was rather difficult, the yield was relatively low, and the final product was of relatively low specific radioactivity (15 μCi/mmole). Another procedure (3), which used [^{14}C]-methyl iodide as the source of radiolabel, yielded [5-^{14}C]-4-ipomeanol of higher (140 μCi/mmole) specific radioactivity than the previous method; however, the preparation of precursor materials required for this procedure was rather tedious. Clearly therefore, the presently-described methods offer the simplest and most convenient means of preparation of radiolabeled 4-ipomeanol suitable for a variety of biological investigations.

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