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SYNTHESIS OF [METHOXY-^{14}C]EUGENOL

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SUMMARY

A short, efficient synthesis for [methoxy-^{14}C]eugenol from unlabelled eugenol is described. The free phenolic group is protected as a methanesulfonate ester prior to removal of the methoxyl group. Reaction of the resulting phenolic mesylate with [^{14}C]methyl iodide under basic conditions followed by treatment with warm sodium hydroxide gave the title compound in good overall yield.

KEY WORDS: Eugenol, Synthesis, Carbon-14

INTRODUCTION

Eugenol (4-allyl-2-methoxyphenol) **1** is a major constituent of cloves and clove oil. Eugenol has been used extensively as a local anesthetic in dentistry. The toxicological and pharmacological properties of eugenol have been recently reviewed (1). Eugenol is generally considered to be nontoxic when ingested. A recent study has shown, however, that when rats and hamsters are given low doses by intratracheal instillation, eugenol can be acutely toxic (2). This finding is particularly significant in view of the fact that the smoking of clove cigarettes, which can deliver up to 16 mg of eugenol per cigarette to the lung, has become increasingly popular among young people (3).

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There have been a number of cases of adverse health effects associated with the smoking of clove cigarettes which have been reported to the Center for Disease Control in Atlanta, Georgia, USA (4,5). In our investigation of potential health effects associated with the smoking of clove cigarettes, radiolabelled eugenol was needed to evaluate the transfer and fate of eugenol during the smoking of clove cigarettes as well as to determine its deposition in various organs of rodents exposed to clove cigarette smoke. We report, herein, a convenient and efficient synthesis of [methoxy-^{14}C]eugenol which is applicable to the preparation of specific ^{14}C-labelled methyl ethers of other polyphenols.

RESULTS AND DISCUSSION

This synthesis of [methoxy-^{14}C]eugenol (Figure 1) employs unlabelled eugenol as the starting material. The hydroxyl group was protected as the methanesulfonate (mesyl) ester by reaction with methanesulfonyl chloride in pyridine. This protective group was chosen because it is stable under acidic conditions, which are required for cleavage of the methoxyl group (6). Boron tribromide in methylene chloride selectively removed the methoxyl group at -78°C to give 5-allyl-2-mesyloxyphenol **3** in 84% overall yield from eugenol. This intermediate **3** could be prepared in large quantity and stored for later conversion to [methoxy-^{14}C]eugenol as required. Both reactions proceed in high yield and the intermediates can be isolated in high purity without the need for chromatographic separations. For the actual labelling of intermediate **3**, the compound was heated in acetone solution with [^{14}C]methyl iodide over anhydrous potassium carbonate. This reaction was performed in a reusable heavy-walled pressure tube closed with a threaded Teflon stopper (Ace Glass, Inc., Vineland, NJ) to

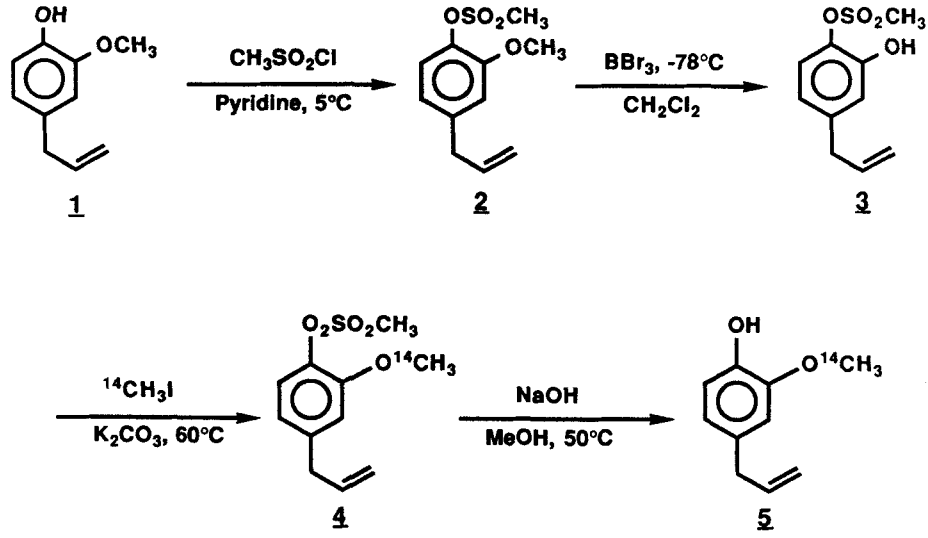

Figure 1. Synthesis of [methoxy-^{14}C]eugenol.

minimize loss of the volatile methyl iodide on the small chemical scale employed. The reaction with [^{14}C]methyl iodide proceeded in high yield to give the labelled anisole derivative which was converted quantitatively to [methoxy-^{14}C]eugenol upon warming in methanolic sodium hydroxide solution (7). The yield of [methoxy-^{14}C]eugenol from intermediate 3 was 81%. Capillary gas chromatographic analysis of the product showed less than 2% contamination by isoeugenol. Isoeugenol can be formed by migration of the double bond in the allyl group of eugenol. This preparation represents an improvement both in yield and in simplicity of work-up over the previously reported synthesis of [methoxy-^{14}C]eugenol (8) which relied on Claisen-rearrangement of [methoxy-^{14}C]-2-allyloxyanisole, yielding the target compound as a mixture with [methoxy-^{14}C]isoeugenol which was then separated by preparative gas chromatography.

EXPERIMENTAL

Infrared spectra were recorded on a Perkin Elmer Model 267 spectrophotometer. Nuclear magnetic resonance spectra

were obtained on a JEOL FX-90Q spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard. Mass spectra were recorded on a Hewlett Packard Model 5988A instrument. Gas chromatography was performed on a Hewlett Packard capillary gas chromatograph Model 5710A. A Radiomatic Flo-One radioactive flow detector equipped with a 2.5 ml detection cell was employed for high performance liquid chromatographic analysis of the labelled products. Flow-Scint II (Radiomatic, Inc., Tampa, FL) was used as the scintillation cocktail. Eugenol was purchased from Aldrich Chemical Co., Milwaukee, Wisc. and was distilled prior to use. [^{14}C]Methyl iodide was purchased from Amersham, Arlington Heights, Ill. with a specific activity of 56 mCi/mmol.

Eugenol mesylate 2.

A solution of eugenol (820 mg, 5 mmol) in dry pyridine (5 ml) was stirred under nitrogen at 5°C as methanesulfonyl chloride (855 mg, 7.5 mmol) was added slowly dropwise *via* syringe. The reaction was complete after 1 hour. The reaction mixture was poured into water and extracted with ether (3 x 40 ml). The ether extracts were washed with 1N HCl (10 ml), water (25 ml), and brine and dried (Na_2SO_4). After evaporation of the solvent under reduced pressure (<30°C) the product **2** was obtained as a colorless oil which crystallized on standing; mp 28-30°C; (1.1g,90%); IR (neat) 3070,3020,2980,2950,2920,2850,1595,1500,1455,1415,1350,1270, 1170,1150,1105,1025,965,905,850,820,780,747,685 cm^{-1}; NMR δ7.22(d,1,H_6,$J_{5,6}$=9.0 Hz), 6.88(s,1,H_3), 6.78(d,1,H_5), 5.95 (m,1,CH=C), 5.10(m,2,H_2C=C), 3.89(s,3,OCH_3), 3.39(d,2,$ArCH_2$-C=,J=6.4 Hz), 3.17(s,3,CH_3SO_3-); mass spectrum, m/e(relative intensity) 242(29%,M^+), 163(100) 135(15), 103(40), 91(46), 79(50).

5-Allyl-2-mesyloxyphenol 3.

A solution of **2** (100 mg, 0.4 mmol) in CH_2Cl_2 (10 ml)

was cooled to -78°C under nitrogen as boron tribromide (0.5 ml, 1.0M in CH_2Cl_2) was added via syringe. The reaction was allowed to warm to room temperature over a 30 min period and was stirred for an additional 30 min. The reaction mixture was poured into 5% $NaHCO_3$ (25 ml) and extracted into ether (2 x 25 ml). The ether layer was washed with water (30 ml) and brine and dried (Na_2SO_4). After filtering, the solution was evaporated to a colorless oil under reduced pressure below 30°C. This compound crystallized on standing at 0°C to give colorless crystals ; mp 70-72°C. The yield of **3** was 85 mg (93%). This compound gave the following spectral data; IR (nujol) 3475, 3030, 1590, 1490, 1350, 1260, 1155, 1085, 960, 920, 840, 800, 775, 750, 720 cm^{-1}; NMR δ7.16(d,1,H_6,$J_{5,6}$=9 Hz) 6.75(dd,1,H_5), 5.91(m,1,CH=C), 5.08 (m,2,H_2C=C), 3.31 (d,2,$ArCH_2$-C=C,J=6.4 Hz), 3.21 (s,3, CH_3SO_3-); mass spectrum, m/e(relative intensity) 228 (49%,M^+), 149(80), 121(53), 91(94), 79(100).

5-Allyl-2-mesyloxy-[methoxy-14C]anisole **4**.

A mixture of **3** (4.0 mg, 18 μmol), K_2CO_3 (10 mg), [^{14}C]methyl iodide (1.0 mCi, 18 μmol) and acetone (4mL) was heated in a sealed tube for 2 hr at 60°C. After cooling, the mixture was filtered, and the filter was rinsed with several portions of acetone until an aliquot gave no counts above background by liquid scintillation counting. The filtrate was evaporated yielding 0.85 mCi (85 % yield) of **4** which was used in the next reaction without further purification .

[Methoxy-^{14}C]Eugenol **5**.

A solution of **4** (0.85 mCi,15 μmol) was dissolved in methanol (25 mL) and 1M NaOH was added (5 mL). The solution was warmed at 50°C for 5 hr, cooled to room temperature, and the methanol was removed under reduced pressure below 40°C. The basic solution was extracted with ether (3 x 20 ml)and

the extracts (which contained a small amount of unreacted 4) were recycled. The aqueous solution was neutralized with 1 N HCl and extracted with ether (3 x 25 ml) (checking for recovery by liquid scintillation counting). The extracts were combined, dried (Na_2SO_4), and evaporated under reduced pressure below 30°C. The product 5 was purified by normal phase HPLC (EM Hibar II LiChrosorb Si-60 10 μm column, 4.6 mm x 25 cm, i.d.) eluting with 5% THF/hexane (2 ml/min) and monitoring at 254 nm. The yield of 5 was 2.2 mg and 0.85 mCi (95% yield, based upon the amount of radioactivity consumed). The specific activity of [methoxy-^{14}C]eugenol as prepared above was 63.4 mCi/mmol (based upon the weight of 5). Capillary gas chromatography (12m cross-linked methylsilicone column, 100-200°C, 2°/min, flow rate 1 ml/min, purge 4 ml/min, split 10 ml/30 sec) of the product revealed it to be >98% chemically pure with less than 2% isoeugenol detected. The retention time of eugenol under these conditions was 7.57 min, while isoeugenol eluted at 10.72 min. Radiochemical purity was determined to be >96% by reverse-phase HPLC using a LiChrosorb 10 μm RP-18 column, 4.6 mm x 25 cm (EM Reagents, Gibbstown, N.J.) and a radioactive flow detector. The retention time of eugenol using an isocratic solvent system of 50% MeOH/H_2O and a flow rate of 2 ml/min was 8.5 min.

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