SYNTHESIS OF CARBON-14 LABELED CAPSAICIN

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SUMMARY

The synthesis and purification of carbon-14 labeled capsaicin are described.

Key Words: Carbon-14, Synthesis, Capsaicin, Purification, Low Pressure Liquid Chromatography

INTRODUCTION

The fruits (red peppers) of various species of the genus Capsicum contain an intensely sharp and pungent substance, capsaicin, which is important as a food and cosmetic additive. Since capsaicin has significant neuronal effects (1) and has been shown to stimulate and potentiate prostaglandin formation (2), capsaicin-^{14}C, (E)-N-(4-hydroxy-3-methoxybenzyl-α-^{14}C)-8-methyl-6-nonenamide [7], was prepared in order to perform metabolism/binding studies.

SYNTHESIS

The synthesis of capsaicin-^{14}C [7] is shown in the following scheme. Treatment of 1-benzyloxy-4-bromo-2-methoxybenzene [1] with butyllithium followed by carbon-^{14}C dioxide provided the labeled benzoic acid [2] in 90% overall radiochemical yield. Reduction of the acid [2] with diborane gave the benzyl alcohol [3] in 82% yield; oxidation to the aldehyde [4] using DDQ was accomplished in 81% yield. Preparation of the corresponding oxime [5] in 92% yield was followed by hydrogenolysis to give the key intermediate 4-hydroxy-3-methoxybenzylamine-α-^{14}C hydrochloride [6] in 64% yield. Capsaicin-^{14}C [7] was prepared from [6] by coupling with (E)-8-methyl-6-nonenoyl chloride using the procedure from the

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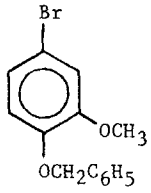
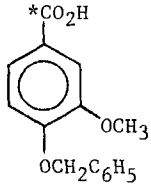


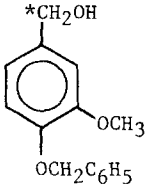
1

1) BuLi
2) *CO2



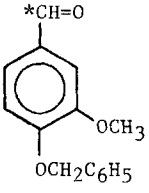
2

B2H6
THF



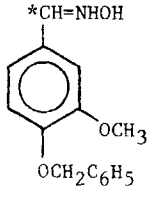
3

DDQ



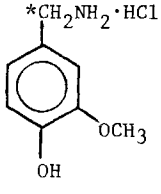
4

NH2OH
pyridine



5

H2,Pt
HCl



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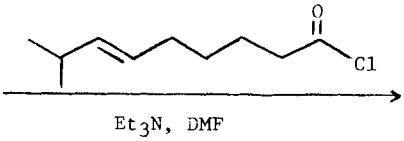

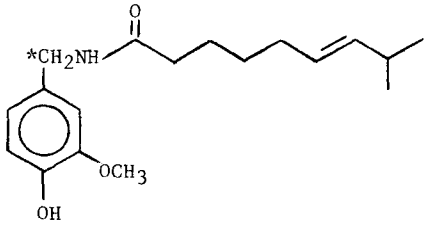

7

unlabeled synthesis (3). After purification by low pressure LC, the capsaicin-^{14}C [7] was obtained in 15% yield and was shown to have chemical and radiochemical purity of ~ 97%.

EXPERIMENTAL

The 1-benzyloxy-4-bromo-2-methoxybenzene [1] was prepared by treatment of 2-methoxyphenol (Aldrich) with bromine in acetonitrile followed by formation of the benzyl ether by treatment with benzyl chloride in triethylamine. The (E)-8-methyl-6-nonenoyl chloride was prepared using the procedure of Crombie et al. (3). Barium carbonate-^{14}C was obtained from Amersham. TLCs were run on E. M. Science Associates' 60 F-254 silica gel plates (5 x 20 cm). TLC radiochromatogram scans were run on a Packard A7230 two-dimensional radiochromatogram scanner. Low pressure LC purification of capsaicin-^{14}C was done on Lichroprep Si 60 silica gel using 1:1 Skelly B/ethyl acetate. Chemical and radiochemical purity of capsaicin-^{14}C were determined by reverse-phase (60% methanol, 40% water) HPLC using a Waters HPLC with Waters 440 detector and Spherisorb 5 μ column; retention time was 14 min at 1 mL/min at λ = 254 nm.

4-Benzyloxy-3-methoxybenzoic-carboxy-^{14}C acid [2]. A solution of 1-benzyloxy-4-bromo-2-methoxybenzene [1] (1.481 g, 5.05 mmol) in 10 mL of anhydrous THF was cooled to -80° and then treated with 1.6 M n-butyllithium in hexane (4.6 mmol). After being stirred at -80° for 10 min, the mixture was cooled with liquid nitrogen bath and evacuated in a vacuum manifold. The carbon-^{14}C dioxide, which had been generated from 335 mg of barium carbonate-^{14}C (91 mCi, 54 mCi/mmol) with 20 mL of concentrated sulfuric acid, was introduced by vacuum transfer. The liquid nitrogen bath was then replaced by a Dry Ice-acetone bath and the mixture was stirred at -76° for 20 min before argon was introduced. Stirring was continued for 2 hr. The reaction mixture was allowed to warm to -60° and quenched by addition of 10 mL of 5% sodium hydroxide solution. The aqueous layer was separated and the organic phase extracted again with 10 mL of 5% sodium hydroxide. The combined aqueous phase was washed with ether and acidified at 0°

to pH 1 with dilute hydrochloride acid. The precipitate which formed was filtered by suction, washed with water and dried in vacuo to furnish 390 mg (90% yield) of 4-benzyloxy-3-methoxybenzoic-carboxy-^{14}C acid [2] as white needles (81.6 mCi, 54 mCi/mmol) which was identified by TLC with unlabeled material. The radiochemical purity was greater than 98%, as determined by radiochromatographic scan. TLC: 10:1 ethyl acetate/methanol, R_f = 0.61.

4-Benzyloxy-3-methoxybenzyl-α-^{14}C alcohol [3]. Ten milliliters of 1 M borane-THF solution (10 mmol) was added dropwise to 4-benzyloxy-3-methoxybenzoic-carboxy-^{14}C acid [2] (390 mg, 81.6 mCi, 1.51 mmol) dissolved in 30 mL of THF. The mixture was stirred for 2 hr. After excess diborane was cautiously destroyed by addition of 20 mL of water, the THF was evaporated under reduced pressure. The aqueous phase was extracted with ether (4 x 25 mL) and the combined ether extract was washed with water (3X), dried ($MgSO_4$) and evaporated. The oily residue was found to have a total radioactivity of 67 mCi (82% yield) and was identified as the desired benzyl alcohol [3] by TLC with unlabeled material. The radiochemical purity was greater than 98% and the product was used without further purification in the next step. TLC: ethyl acetate, R_f = 0.45.

4-Benzyloxy-3-methoxybenzaldehyde-formyl-^{14}C [4]. A solution of 2,3-dichloro-5,6-dicyanobenzoquinone (303 mg, 1.38 mmol) in 5 mL of dioxane was added in several portions to a solution of the benzyl alcohol [3] (304 mg, 67 mCi, 1.24 mmol) in 10 mL of dioxane. A precipitate formed immediately. The mixture was allowed to stir at ambient temperature overnight. Two more small portions of DDQ were added when the presence of a small amount of unreacted benzyl alcohol was detected. After stirring for one more day, the mixture was filtered and the filtrate evaporated under reduced pressure. The residue was triturated with methylene chloride, filtered, and the filtrate was chromatographed over silica gel (50 g). The desired aldehyde, identified by TLC with unlabeled material, was eluted with methylene chloride to yield 255 mg (85% yield) of 4-benzyloxy-3-methoxybenzaldehyde-formyl-^{14}C [4] (57 mCi, 54 mCi/mmol). The radiochemical purity was greater than 98%. TLC: ethyl acetate, R_f = 0.60.

4-Benzyloxy-3-methoxybenzaldehyde-formyl-^{14}C oxime [5]. A mixture of 4-benzyloxy-3-methoxybenzaldehyde-formyl-^{14}C [4] (255 mg, 57 mCi, 1.06 mmol) and hydroxylamine hydrochloride (200 mg, 2.9 mmol) in 30 mL of ethanol and 1 mL of pyridine was stirred at ambient temperature for 1 day. The reaction mixture was diluted with 20 mL of water. After the ethanol was evaporated under reduced pressure, the aqueous phase was extracted several times with ether (total 100 mL). The ether extract was washed with dilute hydrochloric acid (2X) and water (3X), and dried ($MgSO_4$). Evaporation of the ether solution afforded 251 mg (92% yield) of 4-benzyloxy-3-methoxybenzaldehyde-formyl-^{14}C oxime [5] (52.7 mCi, 54 mCi/mmol), which was identified by TLC with unlabeled material. The radiochemical purity was greater than 98%. TLC: ethyl acetate, R_f = 0.62.

4-Hydroxy-3-methoxybenzylamine-α-^{14}C hydrochloride [6]. A solution of 4-benzyloxy-3-methoxybenzaldehyde-formyl-^{14}C oxime [5] (251 mg, 52.7 mCi, 0.97 mmol) in 25 mL of ethanol and 1 mL of concentrated hydrochloric acid was hydrogenated in the presence of platinum catalyst under atmospheric pressure for 12 hr. After removal of the catalyst, the solution was evaporated to dryness under reduced pressure. The residue was triturated with 10 mL of anhydrous ethanol and evaporated again to dryness. The gray colored solid residue was further dried *in vacuo* at room temperature to yield 33.6 mCi (64% yield) of 4-hydroxy-3-methoxybenzylamine-α-^{14}C hydrochloride [6]. This material, which was identified by TLC with unlabeled material, was used without further purification in the next step. TLC: 10:1 methanol/Et_2NH, R_f = 0.55.

Capsaicin-^{14}C [7]. The crude 4-hydroxy-3-methoxybenzylamine-α-^{14}C hydrochloride [6] (33.6 mCi, 0.62 mmol) from the previous step was dissolved in 9 mL of anhydrous DMF. This solution was treated with 1.5 mL of triethylamine, which was immediately followed by a solution of (E)-8-methyl-6-nonenoyl chloride in 3 mL of anhydrous DMF. The reaction mixture was stirred at ambient temperature for 20 hr. After filtering, the solvents were removed by vacuum distillation at

30°C. The residue was taken up in water (20 mL) and extracted with ether (3 x 40 mL). The combined ether solution was extracted with 1% sodium hydroxide (4 x 10 mL). The aqueous sodium hydroxide solution was acidified with dilute hydrochloric acid and extracted with ether (4 x 25 mL). The combined ether extract was washed with water (3X), dried ($MgSO_4$) and evaporated to give 18.5 mCi of crude capsaicin-^{14}C, which was further purified by low pressure liquid chromatography. The purified product thus obtained could be readily induced to crystallize, and was triturated with hexane and filtered to provide 29 mg (15% yield) of capsaicin-^{14}C [7] as beige crystals (5.1 mCi, 53.6 mCi/mmol). The chemical purity was found to be 96.8% by reverse-phase HPLC, and the radiochemical purity was determined to be 97.1% by plotting radioactivity of the HPLC eluant (1 fraction/min) versus fractions. UV λ_{max} 279 nm (ε 3000, methanol).

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