SYNTHESIS OF [^{14}C]-LABELLED TOLRESTAT(N-[[5-(TRIFLUOROMETHYL)-6-METHOXY-1-NAPHTHALENYL]-[^{14}C]THIOXOMETHYL]-N-METHYLGLYCINE; AY-27,773)

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SUMMARY

[^{14}C]-Tolrestat(N-[[5-(trifluoromethyl)-6-methoxy-1-naphthalenyl]-[^{14}C]-thioxomethyl]-N-methylglycine; [14C]AY-27,773), a new aldose reductase inhibitor, was prepared by incorporating [14C]carbon dioxide. The intermediate, 6-methoxy-5-trifluoromethyl-[1-14C]-naphthoic acid, prepared by carbonation of the corresponding lithiated derivative, was condensed with sarcosine methyl ester hydrochloride, converted to the thioamide and hydrolyzed. [14C]Tolrestat, having a specific activity of 52.7 μCi/mg, was obtained in 26% overall yield from [14C]barium carbonate and had a radiochemical purity of 97%.

Key words: Aldose reductase inhibitor, tolrestat, AY-27,773, ^{14}C, carbonation.

INTRODUCTION

Tolrestat (N-[[5-(trifluoromethyl)-6-methoxy-1-naphthalenyl]-[^{14}C]-thioxomethyl]-N-methylglycine; AY-27,773) (1) is a potent aldose reductase inhibitor presently under development (1). In order to study the metabolic disposition of tolrestat in laboratory animals and man, a synthesis of the [^{14}C]labelled compound was undertaken as outlined below:

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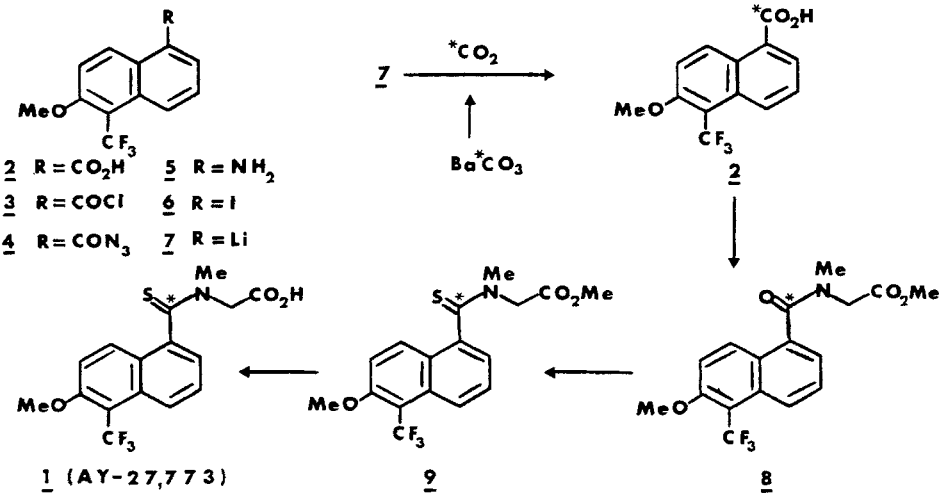

* Position of ^{14}C label.

DISCUSSION

The key intermediate in the synthesis of [^{14}C]tolrestat was 6-methoxy-5-trifluoromethyl-[1-^{14}C]-naphthoic acid (2) (2). The preparation of this compound required the prior synthesis of an appropriately substituted halogen derivative which could be lithiated by reaction with n-butyllithium and then carbonated with [^{14}C]carbon dioxide.

The bromodecarboxylation of 2 via the sodium (3) or mercuric salt using a modified Hunsdiecker reaction (4) proved unsuccessful. However, the 1-iodo-6-methoxy-5-trifluoromethyl-naphthalene (6) was successfully prepared using the following reaction sequence. The acyl azide 4, obtained from 2 via the acid chloride 3, underwent a Curtius

rearrangement to give the isocyanate which on hydrolysis gave the substituted amine 5 (5). The reaction of the diazonium salt, prepared from 5, with potassium iodide gave the required iodide 6 in an overall yield of 30% from 2 (6). The lithiated derivative 7, prepared by the reaction of the iodide 6 with n-butyllithium, was carbonated at -60° with [^{14}C]carbon dioxide to give 6-methoxy-5-trifluoromethyl-[1-^{14}C]-naphthoic acid (2) in an 88% radiochemical yield.

The acid 2 was reacted with sarcosine methyl ester hydrochloride to give the amide 8. When 8 was dissolved in dry pryidine and refluxed in the presence of phosphorous pentasulfide the thioamide 9 was obtained. Hydrolysis of the ester group in 9 gave [^{14}C]tolrestat in an overall crude yield 64% from [^{14}C]barium carbonate. The crude [^{14}C]tolrestat was crystallized from chloroform-hexane and then recrystallized twice from methanol-water. The purified [^{14}C]tolrestat (0.166 g, 8.75 mCi, specific activity 52.7 μCi/mg) was obtained in an overall radiochemical yield of 26% based on [^{14}C]barium carbonate. The radiochemical purity of the labelled compound was determined to be 97% by TLC-autoradiography in three solvent systems.

EXPERIMENTAL

The synthesis of [^{14}C]tolrestat was carried out starting with [^{14}C]barium carbonate (25 mCi, 51.5 mCi/mmole, and 8.8 mCi, 55.9 mCi/mmole) purchased from New England Nuclear, Boston, MA. The intermediates in the synthesis were characterized in trial experiments using unlabelled material. The reactions in the labelled synthesis were monitored by TLC using unlabelled reference compounds.

1-Iodo-6-methyl-5-trifluoromethyl-naphthalene (6)

The acid 2 (2.70 g, 10 mmole) was refluxed in thionyl chloride (50 ml) for 2 hr. The reaction mixture was repeatedly dissolved in toluene

and evaporated to dryness to remove the excess thionyl chloride. The crude acid chloride 3 (2.96 g) was dissolved in acetone (25 ml) and cooled in an ice bath. Sodium azide (0.677 g, 10.4 mmole) dissolved in water (2 ml), was added and the reaction was stirred at 0° for 0.5 hr. The mixture was diluted with water (50 ml) and the precipitated acyl azide was collected by filtration, washed with water, pressed dry and dissolved in toluene (100 ml).

The toluene solution was dried over magnesium sulfate, evaporated to a small volume ($\sim$ 25 ml) and refluxed for 0.5 hr. The reaction was removed from the oil bath and a 50% aqueous potassium hydroxide solution (50 ml) was added. The mixture was stirred for 15 min, heated at 100° for 15 min, cooled to room temperature, diluted with water (50 ml) and extracted with toluene (3 x 50 ml). The toluene extracts were washed with water and combined. Concentrated hydrochloric acid (20 ml) was added and the amine hydrochloride was collected by filtration. The solid was dissolved in 1 N sodium hydroxide (100 ml) and extracted with ethyl acetate (3 x 50 ml). The ethyl acetate extracts were washed with water, combined, dried over magnesium sulfate and evaporated to dryness.

The crude amine (1.59 g, 6.59 mmole) was suspended in water (15 ml), cooled to 0° and 2 N sulfuric acid (10.1 ml) was added. The reaction was stirred at 0° while a solution of sodium nitrite (0.48 g, 6.96 mmole) in water (25 ml) was added dropwise. After 45 min urea (0.083 g, 1.38 mmole) was added to the cold mixture. Potassium iodide (2.032 g, 12.2 mmole) was dissolved in water (20 ml) and 2 N sulfuric acid (9.05 ml) and cooled to 0°. The diazonium salt mixture was slowly added and the reaction was allowed to warm up to and remain at room temperature for 1 hr. The mixture was briefly warmed in a boiling water bath for $\sim$ 10 min, cooled and extracted with ether (3 x 50 ml). The ether extracts were washed successively with a sodium bisulphite

solution (2 x 50 ml), 0.1 N sodium hydroxide (50 ml) and saline (50 ml), combined, dried over magnesium sulfate and evaporated to dryness. The residue was chromatographed on a silica gel column using hexane as the eluting solvent. The fractions containing the desired product were combined and evaporated to dryness. The iodide was recrystallized from hexane: 1.09 g, 30% from 2;m.p. 89-91°; n.m.r. ($CDCl_3$) δ 4.0 (s, 3H, OCH_3), 7.7 (m, 5H, aromatic); m.s. m/e 352 (M^+); 225 $(M-I)^+$.

5-Trifluoromethyl-6-methoxy-[1-^{14}C]-naphthoic acid (2)

The iodide 6 (1.26 g, 3.5 mmole) in dry ether (50 ml) was cooled to -60° under nitrogen and n-butyllithium (2.4 M in hexane) (1.06 ml 2.55 mmole) was added. The reaction flask was attached to a vacuum manifold and the lithiated derivative was carbonated at -60° with [^{14}C]carbon dioxide generated from [^{14}C]barium carbonate (0.095 g, 25 mCi, 0.48 mmole, and 0.031 g, 8.8 mCi, 0.16 mmole) and unlabelled barium carbonate (0.208 g, 1.06 mmole). After the last of the carbon dioxide had been transfered to the reaction flask, the mixture was stirred at -60° for 5 min to complete the reaction.

Nitrogen was introduced into the system and the reaction mixture was quenched by the addition of 2 N hydrochloric acid (50 ml). The acidic solution was extracted with ether (3 x 50 ml) and the combined ether layers were extracted with a 5% sodium bicarbonate solution (4 x 50 ml). The bicarbonate extracts were combined, cooled in ice water, acidified and extracted with ether (3 x 50 ml). The ether extracts were combined, dried over magnesium sulfate and evaporated to dryness. The product was a white solid (0.406 g, 88%) and was used without further purification.

N-[[5-(Trifluoromethyl)-6-methoxy-1-naphthalenyl]-[^{14}C]carbonyl]-N-methyl-
glycine methyl ester (8)

The crude acid 2 (0.406 g, 1.5 mmole), dicyclohexylcarbodimide (0.372 g, 1.8 mmole) and 1-hydroxy-1H-benzotriazole (0.305 g, 2.26

mmole) were stirred at room temperature in dry dimethylformamide (2.7 ml) for 1 hr. Sarcosine methyl ester hydrochloride (0.426 g, 3.05 mmole) and N-ethylmorpholine (0.38 ml) in dry dimethylformamide (1.0 ml) were added. The reaction mixture was stirred at room temperature for 4 hr, filtered and evaporated to dryness. The residue was dissolved in ethyl acetate (50 ml) and washed successively with 1 N hydrochloric acid (50 ml), a saturated sodium bicarbonate solution (50 ml) and brine (50 ml). The ethyl acetate extracts were combined dried over magnesium sulfate and evaporated to dryness. The residue (0.586 g) was used in the next reaction without further purification.

N-[[5-(Trifluromethyl)-6-methoxy-1-naphthalenyl]-[^{14}C]thioxomethyl]-N-methylglycine methyl ester (9)

The crude ester (0.586 g) was dissolved in dry pryidine (10 ml) and phosphorous pentasulfide (0.621 g, 2.8 mmole) was added. The reaction was heated to reflux for 4 hr, cooled to room temperature, poured into warm water (100 ml) and extracted with ethyl acetate (3 x 75 ml). The ethyl acetate extracts were washed successively with 3 N hydrochloric acid (2 x 75 ml), a saturated sodium bicarbonate solution (75 ml) and brine (75 ml), combined, dried over magnesium sulfate and evaporated to dryness. The crude residue (0.511 g) was purified by chromatography on a silica gel column using ethyl acetate: hexane = 1:1 as solvent. The fractions containing the desired thioamide were combined and evaporated to dryness (0.423 g).

N-[[5-(Trifluoromethyl)-6-methoxy-1-naphthalenyl]-[^{14}C]thioxomethyl]-N-methylglycine (AY-27,773) (1)

The thioamide (0.423 g, 1.14 mmole) was dissolved in 2-methoxy ethanol (6 ml) and 2 N sodium hydroxide (1.14 ml). The reaction mixture was stirred at room temperature for 2.5 hr, diluted with water (40 ml) and extracted with ethyl acetate (20 ml). The aqueous solution was acidified with 1 N hydrochloric acid and extracted with ethyl acetate

(4 x 50 ml). The ethyl acetate extracts were washed with brine (2 x 30 ml), combined, dried over magnesium sulfate and evaporated to dryness. The residue (0.392 g) was crystallized from chloroform-hexane. The crystals (0.197 g) were collected and recrystallized twice from methanol-water to give [^{14}C]tolrestat: 0.166 g, sp. act. 52.71 ± 0.86 μCi/mg.

The radiochemical purity of [^{14}C]tolrestat was determined by TLC-autoradiography in three solvent systems: (a) ethyl acetate: toluene: acetate acid = 5:4:1; (b) chloroform: ethyl acetate:acetic acid = 10:10:.5; (c) chloroform: methanol: acetic acid = 90:9.5:0.5. The radioactive zones were located by exposing the TLC plates to X-Omat R Medical X-ray film. The silica gel (1 cm sections) was scraped into counting vials, digested with water (0.2 ml) and 50% hydrofluoric acid (0.2 ml) and counted in Aquasol scintillation cocktail (15 ml).

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