SYNTHESIS OF ^{131}I-LABELLED β-IODO-D-ALANINE AND RELATED COMPOUNDS

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SUMMARY

Methyl N-carbobenzoxy-β-^{131}I-D-alaninate (2) was synthesized by the melt method. Partial hydrolysis of compound 2 gave methyl β-^{131}I-D-alaninate (3). Hydrolysis of 2 with 2 N HCl gave β-^{131}I-D-alanine. The ^{123}I and ^{125}I labelled compounds were synthesized by the same method.

Key Words: Methyl N-carbobenzoxy-β-^{131}I-D-alaninate, Methyl β-^{131}I-D-alaninate, β-^{131}I-D-alanine, Melt Method, ^{125}I, ^{123}I.

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INTRODUCTION

β-chloro-D-alanine and β-fluoro-D-alanine have been shown to be potent antibacterial agents and are non-toxic to mammalian cells (1-5). The mechanism of antimicrobial action of these two haloamino acids was shown to involve irreversible inhibition of alanine racemase--an enzyme which catalyzes the formation of D-alanine, an essential constituent of bacterial cell walls. Alanine racemase was first isolated by Wood from a variety of bacteria (6). The content of alanine racemase is species variable; however, all of the bacteria tested exhibited racemase activity. It was also demonstrated that mammalian tissues, such as rabbit liver, kidney, brain, heart and muscle did not reveal any alanine racemase activity. The synthesis of these compounds labelled with a positron emitting nuclide was thus of interest for their possible use in abscess localization.

Since the reported syntheses of β-fluoro-D-alanine (2,3) are not suitable for labelling purposes, a new amino acid analog, β-^{131}I-D-alanine, was synthesized.

^{131}I-labelled β-iodo-D-alanine was prepared from ^{131}I-labelled methyl N-carbobenzoxy-β-iodo-D-alaninate (2) (7). Compound 2 was synthesized by the isotopic exchange between the radioactive iodide and methyl N-carbobenzoxy-β-iodo-D-alaninate (1) in a molten state (75°C) for 30 min. Treatment of compound 2 with hydrogen bromide in glacial acetic acid afforded methyl β-^{131}I-D-alaninate hydrobromide (3). Subsequent hydrolysis of compound 3 in 2 N HCl gave β-^{131}I-D-alanine (4). The ^{123}I and ^{125}I labelled compounds were synthesized by the same method. The biological activity and biodistribution of these compounds will be published elsewhere (7).

EXPERIMENTAL

Melting points were determined on a Fisher-Jones melting point apparatus and were uncorrected. NMR spectra were measured on a JEOL MH-100 spectrometer and TMS used as an internal standard. $Na^{125}I$ and $Na^{131}I$ in 0.1 N NaOH was obtained from New England Nuclear, Boston, MA. ^{123}I-iodide was produced via the $^{124}Te(p,2n)^{123}I$ reaction at the BNL 60" cyclotron (8). Elemental analyses were performed by Schwarzkofp Microanalytical Laboratories, Woodside, NY.

Methyl N-Carbobenzoxy-β-iodo-D-alaninate (1)

A mixture of 18-crown-6-ether (0.11 g) and KI (1.92 g, 11.58 mmol) in 50 mL of CH_3CN was stirred at room temperature for 30 min after which methyl N-carbobenzoxy-O-tosyl-D-serinate (1.90 g, 4.67 mmol) was added. The mixture was refluxed for 2 hr, the precipitate was filtered by suction and washed with 20 mL of CH_3CN. The combined filtrate was evaporated to dryness and the residue was extracted with ethyl ether. The ethereal solution was evaporated and the product was recrystallized from ethyl ether-petroleum ether to give 1.61 g (95%) of compound 1, m.p. 65-65.5°C; TLC (petroleum ether;cellulose) showed a single spot at R_f = 0.95. NMR ($CDCl_3$) δ3.60 (d, J=4Hz,2H), 3.80 (s,3H), 4.60 (t, J=4Hz,1H), 5.14 (s,2H), 5.68 (d,1H), 7.40 (s,5H). Anal. ($C_{12}H_{14}INO_4$).

In the absence of 18-crown-6-ether, the reaction gave a similar yield.

Methyl N-Carbobenzoxy-β-^{131}I-D-alaninate (2)

A solution of methyl N-carbobenzoxy-β-iodo-D-alaninate (1) (10.30 mg) and ^{131}I-NaI (3.73 mCi) in 2 mL of ethanol was placed in a 5 mL V-shaped flask and was heated briefly under a gentle stream of nitrogen to dryness.

The residue was allowed to melt (75°C) for 30 min and then cooled to room temperature. The mixture was dissolved in 5 mL of ethyl ether and passed through a cellulose column (0.5 x 12 cm). The column was eluted with petroleum ether and the solvent was evaporated to give 8.75 mg (3.03 mCi) of 2 (85% chemical and 81.2% radiochemical yield). Radiochemical purity was determined to be >98% by thin layer chromatography on cellulose, R_f = 0.95 (petroleum ether).

Methyl β-^{131}I-D-alaninate Hydrobromide (3)

A solution of methyl N-carbobenzoxy-β-^{131}I-D-alaninate (2) (465 μCi) in 2 mL of HOAc-HBr was kept at room temperature overnight and evaporated to dryness. The residue was extracted with ether (5 mL), dissolved in a small amount of MeOH and then passed through a cellulose column (0.5 x 12 cm). The column was eluted with MeOH to give 247 μCi (53.1% radiochemical yield) of product 3.

β-^{131}I-D-alanine (4)

A solution of methyl N-carbobenzoxy-β-^{131}I-D-alaninate (2) (192 μCi) in 3 mL of 2 N HCl was refluxed for 2 hr. The solution was evaporated to dryness and the residue was extracted with ether, dissolved in MeOH and passed through a cellulose column. The column was washed with ether and then eluted with MeOH to give 120 μCi (62.5% radiochemical yield) of product 4. Radiochemical purity was determined to be >99% by thin layer chromatography on cellulose. HOAc-n-BuOH - H_2O (15:60:25), R_f = 0.52.

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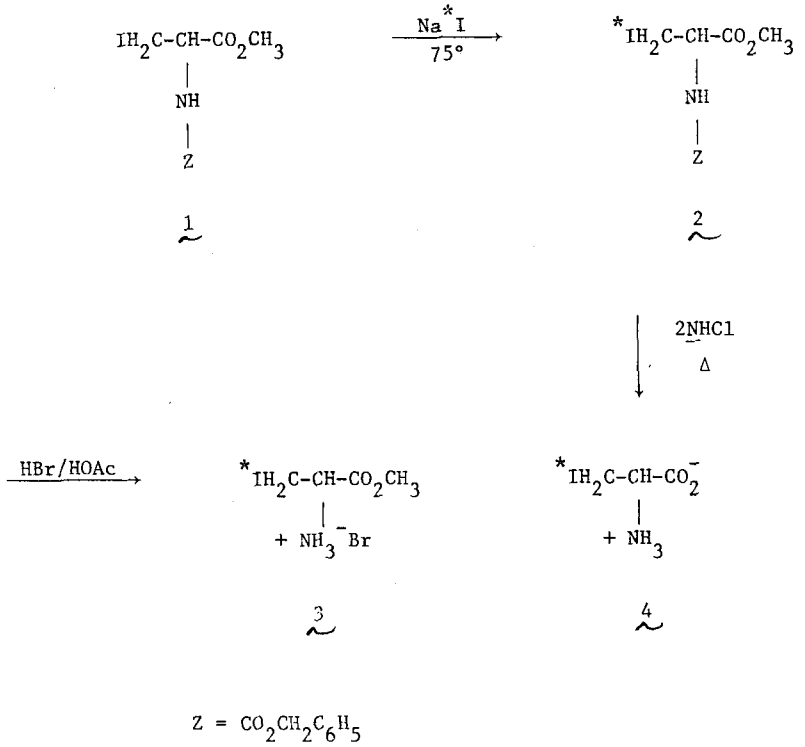

Scheme

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