SYNTHESIS OF 3-CYANO-4-METHYL-5(^{14}C)-METHYL-2-(5-^{14}C)PYRROLYLOXAMIC ACID

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SUMMARY

A synthesis of 3-cyano-4-methyl-5 (^{14}C)-methyl-2-(5-^{14}C)pyrrolyloxamic acid is described. The compound, having specific activity of 66.7 μCi/mmole, was obtained in 19.68% overall yield from uniformly labelled ^{14}C-L-alanine.

Key Words: 3-Cyano-4-methyl-5(^{14}C)-methyl-2-(5-^{14}C)pyrrolyloxamic acid, heterocyclic oxamic acid, antiallergy activity.

INTRODUCTION

The title compound is one of a group of heterocyclic oxamates (1) which were screened for biological activity and found to possess antiallergy activity (2). The active form was shown to be the free acid (3). Investigations of the metabolic fate and mechanism by which this compound prevents mediator release from the mast cell required the synthesis of the ^{14}C-labeled compound, which has not been previously reported.

DISCUSSION

Ethyl (3-cyano-4,5-dimethyl-2-pyrrolyl)oxamate 4 was first prepared as one of a series of heterocyclic oxamates synthesized as possible indole isostere precursors. In the synthesis of the title compound no intermediates were isolated, and only the final product was recrystallized. A convenient synthesis of the oxamate, as reported by Blanton, et al. (1), begins with the preparation of the amino-ketone 2 by the method of Wiley and Borum (4) and the amine-substituted pyrrole 3 by the method of Gewald (5). The radiolabel was

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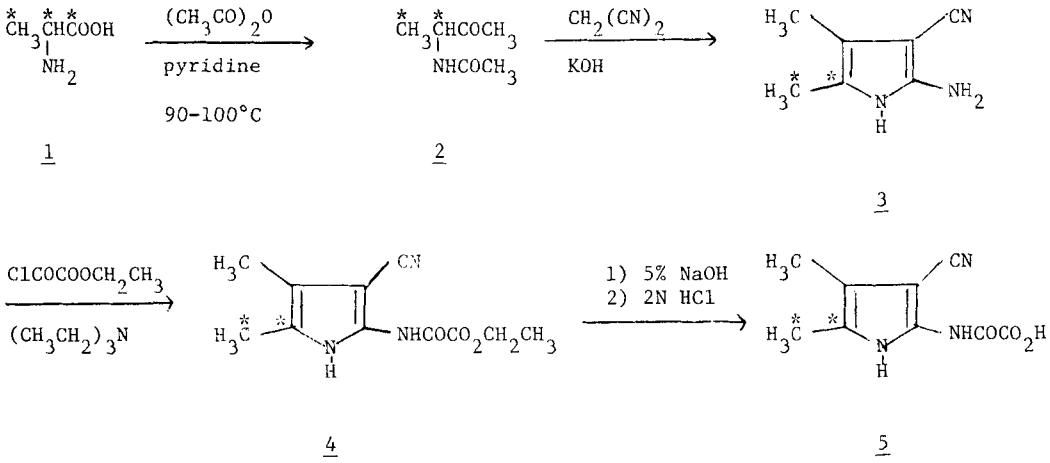
incorporated into the 5-position of the pyrrole nucleus and the carbon of the C-5 methyl group by the use of 500 μCi of uniformly labelled, (UL), ^{14}C-L-alanine, 1, in the preparation of the Dakin-West product 2. When 2 was treated with malononitrile in the presence of a stoichiometric amount of potassium hydroxide, the amine-substituted pyrrole was obtained. A small quantity of unlabelled, recrystallized 3 was added to this pyrrole derivative as additional carrier. Ethyl oxalyl chloride was then reacted with the pyrrole derivative to form 4. A small amount of unlabelled recrystallized 4 was added as additional carrier. The ester 4 was then hydrolyzed in 5% sodium hydroxide and the acid 5 was obtained upon acidification with 2N hydrochloric acid.

The acid 5 (specific activity 66.7 μCi/mmole) was obtained in 19.7% overall yield from UL-^{14}C-L-alanine with radiochemical purity of 98% as determined by radiochromatographic scanning of TLC plates developed in 3 TLC solvent systems.



1 2 3 4 5

EXPERIMENTAL

Pilot experiments were carried out with unlabelled compounds and suitable infrared and nuclear magnetic resonance spectra were obtained for each inter-

mediate as well as the target compound. Radioactivity measurements were carried out using a Beckman LS 100 C Liquid Scintillation System.

UL-^{14}C-L-alanine(Lot #2092; specific activity 135 mCi/mmole, 98% radiochemical purity, solvent 95% ethyl alcohol) was purchased from California Bionuclear Corporation. All other chemicals were of reagent quality.

2-Amino-3-cyano-4-methyl-5(^{14}C)-methyl)pyrrole 3.

The ethanol from 500 μCi of UL-^{14}C-L-Alanine was removed under reduced pressure. The white residue was dried, and then 351.0 mg (3.9 mmol) of unlabelled alanine was added. Pyridine (1.59 ml; 19.8 mmol) and acetic anhydride (2.24 ml; 23.5 mmol) were added and the reaction mixture was stirred for 8 hr. at 90-100°C. The excess pyridine, acetic anhydride and acetic acid were removed in vacuo. The brown oil 2 was dissolved in 1 ml of methanol and treated with 264.0 mg of malononitrile (4 mmol). The solution was then placed in an ice bath and 2.975 ml of 1.312N KOH (4 mmol) was added dropwise with stirring. The solution turned red and a solid formed within 30 minutes.

The reaction mixture was allowed to stir for 1 hr. at room temperature and for an additional hour at 50-55°C. The hot solution was poured into 10 ml of ice water and the purple solid isolated by vacuum filtration. The product was dried in a vacuum oven to obtain 149.3 mg 3 (26.96% yield). The unlabeled pilot compound was recrystallized from water-methanol to yield tan needle-like crystals: m.p. 164-166°C (lit. 166°); IR (KBr): 3220 (-NH_2) and 2180 (-CN) cm^{-1}; NMR (DMSO-d_6): δ 1.85 (s, 3H, CH_3), 1.95 (s, 3H, CH_3), 5.3 (s, 2H, NH_2), 10.0 (s, 1H, NH).

Ethyl (3-cyano-4-methyl-5(^{14}C)-methyl-2-(5-^{14}C)pyrrolyl)oxamate 4.

An additional 65.5 mg (0.48 mmol) of recrystallized unlabelled 3 were added to the 149.3 mg of labelled 3. Then 6.22 ml of ethyl acetate were added. The solution was then treated with 155.2 mg of triethylamine (1.53 mmol) followed

by 209.5 mg of ethyl oxalyl chloride (1.53 mmol) at approximately 0°C. A yellow precipitate formed immediately. The solution was stirred for 2 hr. in an ice bath and for 1 hr. at room temperature. The triethylamine hydrochloride was removed by vacuum filtration and the filtrate allowed to stand overnight at room temperature. The filtrate was refiltered to remove a small amount of precipitate which formed upon standing, and the excess ethyl acetate was removed in vacuo. The pilot compound was recrystallized from 95% ethanol to yield a bright yellow crystalline material melting at 132-133°C (Lit. 132-133°); IR (KBr): 2225 (CN), 1760 (COC_2H_5) and 1680 (-NHCO-) cm^{-1}; NMR (DMSO-d_6): δ 1.3 (t, 3H, ethyl CH_3), 1.95 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 4.3 (q, 2H, ethyl CH_2), 10.1 (s, 1H, NH), 10.4 (s, 1H, -NHCO-).

3-Cyano-4-methyl-5(^{14}C)-methyl-(5-^{14}C)-pyrrolyloxamic acid 5.

To the yellow-colored, labelled solid 4, 50 mg (0.23 mmol) of recrystallized unlabelled 4 were added and 2.0 ml of 5% NaOH were added. The solution was then made acidic to congo red paper by the dropwise addition of 2N HCl. The resulting yellow precipitate was isolated by vacuum filtration and oven dried. The yellow powder (218.7 mg) obtained following recrystallization from methanol melted at 234-236°C (Lit. 237°C; specific activity 66.7 μCi/mmole; 98% radiochemical purity). IR (KBr): 3500 (OH), 2205 (CN), 1680 (-NH CO-); NMR (DMSO-d_6): δ 1.95 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 7-8 (broad, 1H, -COOH), 10 (s, 1H, NH), 10.4 (s, 1H, -NHCO-).

The radiochemical purity of the final product was determined on 0.25 mm silica gel GF 254 plates (Analtech, Inc., Newark, Delaware) in three different solvent systems: (A) chloroform: ethyl acetate: formic acid, 35:55:10, (R_f:0.25) (B) 1-propanol: concentrated ammonia, 70:30, (R_f:0.54); (C) pyridine: H_2O, 65:35, (R_f:0.72).

The radioactive zones on the plate were detected by the use of a Perkin Model 7201 Radiochromatogram Scanner. The percent radiochemical purity was then calculated from the determination of total peak areas for the final

compound and any labelled impurities. Radiochemical purity in each of the 3 TLC systems was > 98%.

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