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SYNTHESIS AND CHARACTERIZATION OF 2-HYDROXYMETHYL-1-METHYL-4-NITRO-5-IMIDAZOLCARBONITRILE-4,5-^{14}C

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SUMMARY

Preparation of the title compound, a key intermediate required to assist in the investigation and development of a new class of substances for controlling coccidiosis, is described. This labeled substance was synthesized in 10 steps, with a 17% overall radiochemical yield, from oxalic-1,2-^{14}C acid.

Key Words: 2-Hydroxymethyl-1-methyl-4-nitro-5-imidazolcarbonitrile-4,5-^{14}C, N,N'-dimethyloxamide-1,2-^{14}C, 5-chloro-1-methyl-imidazole-4,5-^{14}C, 2-acetoxymethyl-5-chloro-1-methyl-4-nitroimidazole-4,5-^{14}C, coccidiostats, ring-labeled imidazoles.

INTRODUCTION

During the course of investigations in these laboratories for new substances with coccidiostat activity, 2-hydroxymethyl-1-methyl-4-nitro-5-imidazolcarbonitrile (XI) and related compounds obtained by derivatization of the hydroxyl function of this compound were found to possess considerable biological activity (1). To assist in the establishment of a residue pattern for this series of substituted imidazoles, a substantial quantity of ^{14}C-ring labeled (XI) was required as a key intermediate. This paper presents the procedure developed to prepare (IV) with carbon-14 labeling at carbons C-4 and C-5 of the imidazole nucleus, and the subsequent chemistry that led to the labeled title compound (XI). The reaction sequence for the preparation of (XI) from oxalic-1,2-^{14}C acid (I) is shown in the following scheme:

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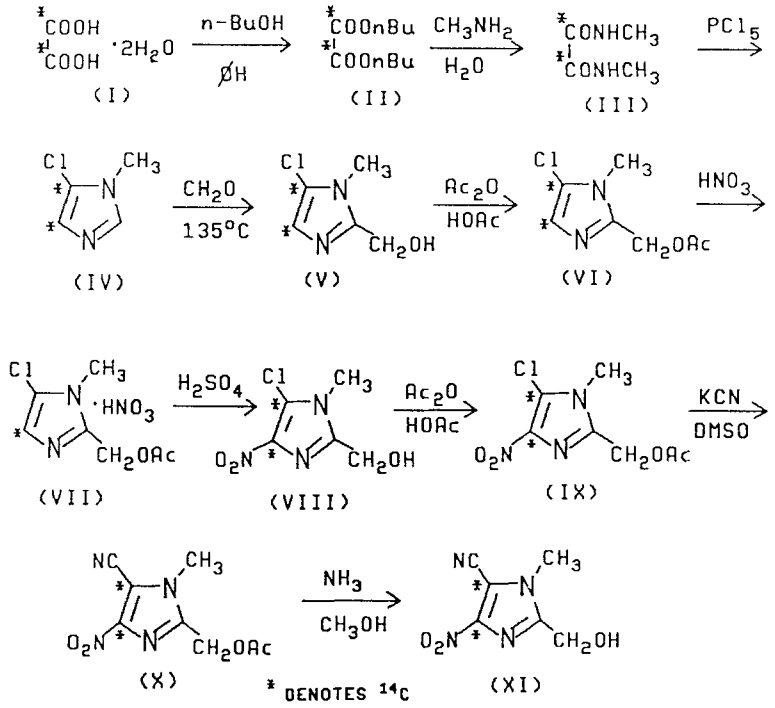

(I) (II) (III) (IV) (V) (VI) (VII) (VIII) (IX) (X) (XI)

DISCUSSION

Oxalic-1,2-^{14}C acid dihydrate (I) was converted to N,N'-dimethyloxamide-1,2-^{14}C (III) (2,3) by, first, azeotropic esterification to form di-n-butyl oxalate-1,2-^{14}C (II), followed by amidation of this intermediate with methylamine in water. Conversion to the imidazole (IV) was accomplished in 49.2% yield by treating the oxamide (III) with phosphorus pentachloride at 110°C following a procedure described by Blicke and Godt (3). The resulting 5-chloro-1-methylimidazole-4,5-^{14}C (IV) was hydroxymethylated with aqueous formaldehyde at 135°C to give 5-chloro-2-hydroxymethyl-1-methyl-imidazole-4,5-^{14}C (V) (4). This carbinol (V) was protected from oxidation during the following nitration by acetylation, using acetic anhydride in acetic acid, to provide 2-acetoxymethyl-5-chloro-1-methyl-imidazole-4,5-^{14}C (VI) in an overall yield (from IV) of 72%. Nitration of this intermediate was accomplished following a procedure based on that described by Sarasin and Wegman

(5). Thus careful neutralization of (VI) in the cold with dilute nitric acid, followed by removal of water in vacuo yielded (VII), the dry nitrate salt of (VI), which upon addition of concentrated sulfuric acid and warming was readily nitrated, but also de-acetylated to give 5-chloro-2-hydroxymethyl-1-methyl-4-nitroimidazole-4,5-^{14}C (VIII). In order to insure a substrate suitable for halogen/cyanide exchange the unisolated crude (VIII) was re-acetylated using acetic anhydride to yield 2-acetoxymethyl-5-chloro-1-methyl-4-nitroimidazole-4,5-^{14}C (IX) in an 81.9% yield (from VI). Replacement of chlorine or another suitable leaving group by cyano in similarly substituted imidazoles is a well established approach to 4-nitroimidazole-5-carbonitriles (1,4,6). For the corresponding displacement in our series potassium cyanide in dry dimethyl sulfoxide served to furnish 92% of 2-acetoxymethyl-1-methyl-4-nitro-5-imidazolcarbonitrile-4,5-^{14}C (X). Conversion of (X) to the targeted title compound was finally accomplished by deacetylation using anhydrous ammonia in cold methanol. A single slurry of the crude (XI) isolate provided a 60% yield of 2-hydroxymethyl-1-methyl-4-nitro-5-imidazolcarbonitrile-4,5-^{14}C (XI), with a radiochemical purity of >99% by TLC analysis. The overall yield to (XI) from oxalic-1,2-^{14}C acid (dihydrate) was 17%.

EXPERIMENTAL

Analytical TLC was carried out on 5x20 cm glass plates precoated with silica gel 60 F-254 (E. Merck, Darmstadt, Germany). Radioactive zones were located with a Berthold Model LB2722 scanner. Radioactivity was measured with a Packard Tri-Carb^{R} Model 3320 liquid scintillation spectrometer, using 0.4% OMNIFLUOR^{R} in toluene-ethanol (7:3) as scintillator medium. Purity and specific activity of the starting material, oxalic-1,2-^{14}C acid dihydrate, were taken as given by the supplier (ICN). ^{1}H NMR spectra were determined with a Varian EM-360, 60 MHz spectrometer. Mass spectra

were obtained with an LKB Model 9000 spectrometer, operated in the electron-impact mode (70 ev).

N,N'-Dimethyloxamide-1,2-^{14}C (III) -- To a mixture of 262 mg (2.08 mmol, @ 12 mCi/mmol) of oxalic-1,2-^{14}C acid dihydrate and 1310 mg (10.4 mmol) of carrier acid in a one-necked, 100 mL flask was added 9.3 mL of n-butanol and 7.5 mL of benzene. This mixture was heated to reflux, and water was removed by azeotropic distillation. After 20 hours the reaction was judged to be complete, and the solvents were removed by distillation at atmospheric pressure. The residual material, consisting mostly of di-n-butyl oxalate-1,2-^{14}C along with some n-butanol, was cooled to -25°C and 10 mL of 40% aqueous methylamine was added in one portion. An exothermic reaction ensued and the product, N,N'-dimethyloxamide-1,2-^{14}C (III), precipitated as an insoluble, colorless solid. The resulting thick slurry was stirred at 0-5°C for 1/2 hour, then the excess methylamine and water were removed at 5°C under reduced pressure. The solid product (III) was dried to constant weight (1420 mg) representing a 98% yield from the oxalic-1,2-^{14}C acid.

5-Chloro-1-methylimidazole-4,5-^{14}C (IV) -- Following the procedure described by Blicke and Godt (3) N,N'-dimethyloxamide-1,2-^{14}C (III) (1420 mg, 12.2 mmol @ 2.0 mCi/mmol) and 5500 mg (26.4 mmol) of phosphorus pentachloride reacted to give 700 mg of product (IV), a liquid, b.p. 67-70°C/2 mm. (b.p.200°C/760 mm (5))

2-Acetoxymethyl-5-chloro-1-methylimidazole-4,5-^{14}C (VI) -- A mixture of 5-chloro-1-methylimidazole-4,5-^{14}C (700 mg, 6.0 mmol @ 2.0 mCi/mmol) and 3.8 mL of 40% aqueous formaldehyde was placed in a heavy walled glass tube. The tube was sealed and then immersed in an oil bath preheated to 135°C and kept at that temperature for ten hours. After cooling and opening, the contents of the tube were removed and concentrated in vacuo to a waxy solid product, crude 5-chloro-2-hydroxymethyl-1-methylimidazole-4,5-^{14}C (V). This

material was dissolved in 25 mL of acetic anhydride:acetic acid (1:1) and the resulting solution was refluxed for six hours. After standing at 25°C overnight the excess solvents were removed by vacuum distillation to leave a straw colored oil. Distillation of this residue at 0.25 mm gave 864 mg of an almost colorless oil, which upon standing solidified to a waxy solid, 2-acetoxymethyl-5-chloro-1-methylimidazole-4,5-^{14}C (VI). Analysis by TLC (developed with $CHCl_3/CH_3OH$ (9:1)) with radioscan indicated a radiochemical purity of 98%, and this intermediate was carried on without further purification into the nitration procedure.

2-Acetoxymethyl-5-chloro-1-methyl-4-nitroimidazole-4,5-^{14}C (IX) -- To a solution of 864 mg (4.61 mmol, 2.0 mCi/mmol) of 2-acetoxymethyl-5-chloro-1-methylimidazole-4,5-^{14}C (VI) in 3.5 mL of water at 0-5°C was added 3.4 mL (4.52 mmol) of 1.3 N nitric acid. After stirring at 0-5°C for 1/4 hour the water was removed by vacuum distillation and further drying under vacuum to constant weight to leave 1040.9 mg (90.2%) of 2-acetoxymethyl-5-chloro-1-methylimidazole-4,5-^{14}C nitrate (VII). To this dried salt, in a 100 mL flask immersed in an ice bath, was added 5.0 mL of ice cold concentrated sulfuric acid. The ice bath was removed and the viscous solution was stirred and warmed to 25°C over a 1/4 hour period, kept at that temperature for one hour, and then heated to 100°C and aged at that temperature for thirty minutes. After cooling to 0-5°C the mixture was quenched with 15 mL of ice cold water. Sodium bicarbonate was added portionwise until the resulting slurry was just alkaline, and the product (VIII) was then extracted into 5 x 10 mL of ethyl acetate. This extract was dried over magnesium sulfate and concentrated to a residual oil. TLC analysis of this crude product suggested that nitration of the imidazole ring had been accompanied by deacetylation of the side chain. The crude 5-chloro-2-hydroxymethyl-1-methyl-4-nitroimidazole-4,5-^{14}C (VIII) was dissolved in 15 mL of acetic anhy-

dride:acetic acid (1:1). After the addition of a few mg of p-toluenesulfonic acid the solution was heated at reflux for one hour. Solvent was removed by vacuum distillation to leave a residue which, after solution in ethyl acetate and washing with water, gave a waxy solid weighing 878.5 mg (91%) upon again removing the solvent. Single spot TLC now showed that this material was conpletely acetylated 2-acetoxymethyl-5-chloro-1-methyl-4-nitroimidazole-4,5-^{14}C (IX) as expected. Radioscan of a thin layer plate developed in chloroform:methanol (9:1) indicated a radiochemical purity of 99%.

2-Acetoxymethyl-1-methyl-4-nitroimidazolcarbonitrile-4,5-^{14}C (X) -- To 878.5 mg (3.77 mmol @ 2.0 mCi/mmol) of (IX) in a 25 mL flask was added 2.8 mL of dry dimethyl sulfoxide. After stirring at 25°C for 1/4 hour, during which time the (IX) was completely dissolved, 370 mg (5.69 mmol) of freshly pulverized potassium cyanide was added in one portion. The colorless solution at once became black-brown. The flask was then immersed in an oil bath at 80°C and held at that temperature for thirty minutes. The dimethyl sulfoxide was removed in vacuo at the lowest reasonable temperature. The black tarry residue which remained was extracted with two 25 mL portions of chloroform, and the combined extracts were filtered to remove some insoluble material. This filtrate was in turn extracted with 3x5 mL of water and then concentrated to a volume of 2-3 mL. Chromatography on silica gel (E. Merck AG, Darmstadt, 5-25 μ) eluting with methanol: chloroform (1:9) gave 774.9 mg (92%) of 2-acetoxymethyl-1-methyl-4-nitroimidazolcarbonitrile-4,5-^{14}C (X). The radiochemical purity of this isolate was shown to be >99% by TLC (silica, chloroform:methanol: 9:1). That the structure of this substance was as expected was confirmed by NMR and mass spectral analysis; ^{1}H NMR ($CDCl_3$): 5.19 (s, 2H, 2-$\underline{CH_2}$-O-CO-CH_3), 3.95 (s, 3H, 1-CH_3), 2.13 (s, 3H, 2-CH_2-O-CO-$\underline{CH_3}$). The electron impact mass spectrum (EIMS) showed the following signals: low intensity

molecular ion at m/z 224; M-42 (CH_2CO), m/z 182; M-43 (CH_3CO), m/z 181; M-59 (CH_3COO), m/z 165; M-89 (CH_3CO & NO_2), m/z 135.

2-Hydroxymethyl-1-methyl-4-nitroimidazolcarbonitrile-4,5-^{14}C (XI)--A solution of 774.9 mg (3.47 mmol @ 2.0 mCi/mmol) of (X) in 5 mL of methanol was prepared by dissolving the imidazole at 25°C, then after cooling to -15°C a vigorous stream of gaseous ammonia was introduced above the surface while stirring briskly. After five minutes the ammonia addition was stopped, with stirring at -15°C continued for exactly thirty five minutes. (A longer reaction time had been shown to result in the formation of byproducts.) The solvent and residual ammonia were removed under vacuum to leave a solid residue. This was then slurried with 7.5 mL of ether, filtered and dried to yield 374.9 mg (59.5%) of product (XI). Radiochemical purity was shown to be >99% by TLC in two systems, (silica, chloroform:methanol 9:1; and chloroform:acetonitrile: acetic acid 8:1:1) with R_f identical to that of reference material. Both the NMR and mass spectra were in good agreement with the expected structure, 2-hydroxymethyl-1-methyl-4-nitro-5-imidazolcarbonitrile-4,5-^{14}C (XI). ^1H NMR ($CDCl_3$): 4.84 (s, 2H, 2-CH_2OH), 3.97 (s, 3H, 1-CH_3). The electron impact mass spectrum (EIMS) showed the following signals: intense molecular ion at m/z 182; M-17 (OH), m/z 165; M-46 (NO_2), m/z 136.

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