PREPARATION OF 3,5-DIMETHYLPYRAZOLE-3,5-^{14}C AND ITS HYPOGLYCEMIC METABOLITE, 5-METHYLPYRAZOLE-3-CARBOXYLIC ACID-5-^{14}C

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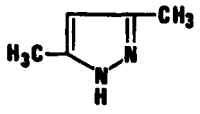
SUMMARY

3,5-Dimethylpyrazole-3,5-^{14}C and its hypoglycemic metabolite, 5-methylpyrazole-3-carboxylic acid-5-^{14}C, were each prepared by separate two-step reaction sequences from acetone-2-^{14}C in 38% and 46% yields, respectively.

Key Words: Carbon-14, Hypoglycemic agent, Pyrazole

INTRODUCTION

3,5-Dimethylpyrazole (I) is a potent hypoglycemic agent in the glucose-primed, fasted rat (1). Metabolism studies with I resulted in isolation of



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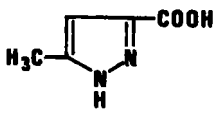

II

5-methylpyrazole-3-carboxylic acid (II) as a urinary metabolite of I in the rat (2). This metabolite was found to have an even greater hypoglycemic potency than I and indeed accounts for the hypoglycemic activity of I when it is orally administered to the rat. A radioactive form of I was required

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for additional studies of the metabolism of I in the rat (3). A radioactive form of II also was required in order to perform metabolism studies (4) necessary for development of this active metabolite as a hypoglycemic agent. Carbon-14, rather than tritium, was chosen as the label since all of the hydrogen atoms of I and II are potentially susceptible to metabolic loss.

EXPERIMENTAL

Radioactivity Measurements

All counting was performed with a Packard Tricarb, model 314EX2A liquid scintillation spectrometer at -8° under conditions suitable for measuring carbon-14. Appropriate aliquots of samples were dissolved in 15 ml of scintillation solvent {toluene-dioxane-methanol (350:350:210 by volume) containing 73 g of naphthalene, 4.6 g of 2,5-diphenyloxazole, and 0.08 g of 1,4-bis [2-(5-phenyloxazolyl)] benzene per liter}. The absolute counting efficiency for each sample was determined by recounting following addition of an internal standard of carbon-14 labeled toluene and results were then converted to mCi.

Paper and Thin-Layer Chromatography

Paper chromatograms were developed by the descending method using 57-cm lengths of Whatman No. 1 paper in the following systems: (a) 1-butanol-piperidine-H_2O (81:2:17 by volume) and (b) 1-butanol-acetic acid-H_2O (4:1:1 by volume). Rf values for II are 0.37 and 0.76 in systems (a) and (b), respectively. Thin layer chromatography was carried out in the ethyl acetate-water-formic acid (95:5:1 by volume) system on 0.25-mm films of silica gel GF. The Rf value of I in this thin-layer chromatography system is 0.57. The UV absorption of standards and products was detected by viewing the paper and thin-layer chromatograms under short-wavelength UV light. Zones of radioactivity were located by scanning the paper strips with a Vanguard, Model 880 automatic chromatogram scanner. Radioactive zones on the thin-layer plates were located by transferring sequential 0.5-cm segments of the silica-gel film into individual vials and counting using scintillation solvent containing 3% H_2O.

Synthesis

2,4-Pentanedione-2,4-^{14}C (III) - A mixture of 0.29 g (5 mM) of acetone-2-^{14}C* and 1.28 g (12.5 mM) of acetic anhydride was cooled to -10° with an ice-salt bath. Gaseous BF_3 was passed through the reaction mixture at a flow rate of 2-3 bubbles per second for 0.75 hours. During this period the mixture turned to a viscous, semi-solid, yellow mass. A solution of 1.2 g of sodium acetate in 4 ml of H_2O was added and, after a 1-hour period at 0°, the reaction mixture was steam distilled to obtain 20 ml of distillate. A hot, filtered solution of 0.6 g of cupric acetate monohydrate in 7.5 ml of H_2O was added to the distillate. The resulting copper complex of III was allowed to crystallize in the refrigerator overnight. The copper complex was filtered, washed with cold water and dried at 50° *in vacuo* to yield 0.559 g (85%) of the copper complex of I having a specific activity of 0.127 mCi per mM. The copper complex was dissolved in 2 ml of 20% H_2SO_4. The liberated 2,4-pentanedione-2,4-^{14}C (III) was extracted with three 2-ml portions of ethyl ether, and the ether was removed with a gentle stream of nitrogen from the flask used for the following step.

3,5-Dimethylpyrazole-3,5-^{14}C (I) - A mixture of the 2,4-pentanedione-2,4-^{14}C (III), 0.548 g (4.2 mM) of hydrazine sulfate and 3.2 ml of 10% sodium hydroxide was stirred at 0-5° for one hour. The reaction mixture was diluted with 2 ml of H_2O and extracted with 3 portions of ethyl ether. The residue from the ether extract was recrystallized from Skellysolve B to obtain 0.182 g (45%) of 3,5-dimethylpyrazole-3,5-^{14}C (I) having a specific activity of 0.133 mCi per mM; m.p. 107-107.5°. The I.R. spectrum [(Nujol mull) 3200, 3130, 3030 (NH,CH); 2780, 2720, 2600 (NH); 1665, 1595, 1550, 1485 (C=N, C=C); 1305, 1125, 1010, 850, 780, 735 cm^{-1}] of the product corresponded to that of standard I. Thin-layer chromatography of the product revealed a

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* One mCi (nominal) of acetone-2-^{14}C, purchased from New England Nuclear Corp., was diluted to 0.29 g with nonradioactive acetone.

single UV-absorbing and radioactive zone corresponding to that of standard I. *Anal.* Calcd. for $C_5H_8N_2$: C, 62.5; H, 8.4; N, 29.1. Found: C, 62.2; H, 8.4; N, 29.7.

2,4-Dioxopentanoic Acid-4-^{14}C. Ethyl Ester (IV), Sodium Salt - Sodium ethoxide was prepared from 0.3 g (13 mM) of freshly cut sodium and 6 ml of ethanol in a flask equipped with a stirring bar, a dropping funnel having a drying tube and a reflux condenser having a nitrogen inlet. After the sodium had been completely consumed, a mixture of 0.6 g (10.3 mM) acetone-2-^{14}C[†] and 1.5 g (10.3 mM) diethyloxalate in 7.5 ml of benzene was added to the sodium ethoxide solution at room temperature with stirring. The product, which separated as a thick mass, was allowed to stand at room temperature overnight. The reaction mixture was then diluted with 10 ml of benzene-ethanol (1:1 by volume), filtered and washed with an additional 10 ml of benzene:ethanol. The product was dried *in vacuo* at room temperature to yield 1.4 g (76%) of the sodium salt of 2,4-dioxopentanoic acid-4-^{14}C ethyl ester (IV). This material was used in the next step without futher purification.

5-Methylpyrazole-3-carboxylic acid-5-^{14}C (II) - A solution of 0.63 g (12.2 mM) of sodium acetate in 5.5 ml was saturated with nitrogen in a flask equipped as described in the previous step. Hydrazine sulfate (1.0 g, 7.7 mM) was added to the flask and the reaction mixture was heated at 50° and stirred while a solution of 1.3 g (7.7 mM) of the sodium salt of 2,4-dioxopentanoic acid-4-^{14}C ethyl ester (IV) in 8 ml of H_2O was added over a 0.25 hr- period. The reaction mixture was stirred and heated at 50° for an additional 4 hours. It was then cooled to room temperature, 0.62 g of powdered NaOH was added and the reaction mixture was stirred at room temperature overnight. Sufficient 6 N H_2SO_4 was added to acidify the reaction mixture and the product was allowed to crystallize in the refrigerator overnight. This material was crystallized twice from water

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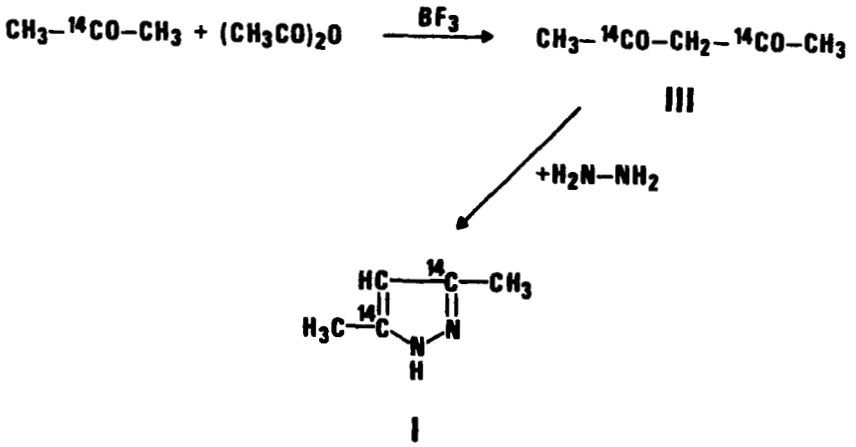
[†] Two mCi. (nominal) of acetone-2-^{14}C, purchased from New England Nuclear Corp., was diluted to 0.6 g with nonradioactive acetone.

to yield 0.589 g (61%) of 5-methylpyrazole-3-carboxylic acid-5-^{14}C (II) having a specific activity of 0.133 mCi per mM. The IR[(Nujol mull) 3240 (NH); 3140, 3100 (CH); 2680 (OH); 1715 (C=O); 1595, 1565, 1510 (C=N, C=C); 1430, 1270, 1200, 1120, 1015, 770 cm^{-1}] and UV [(EtOH) 218 mμ (ε9150)] spectra of the product corresponded to those of standard II. *Anal.* Calcd. for $C_5H_6N_2O_2$: C, 47.6; H, 4.8; N, 22.2. Found: C, 47.8; H, 4.9; N, 22.5.

RESULTS AND DISCUSSION

3,5-Dimethylpyrazole-3,5-^{14}C (I) was prepared from acetone-2-^{14}C by the two-step synthetic sequence shown in Scheme 1. The first step,

Scheme 1



I

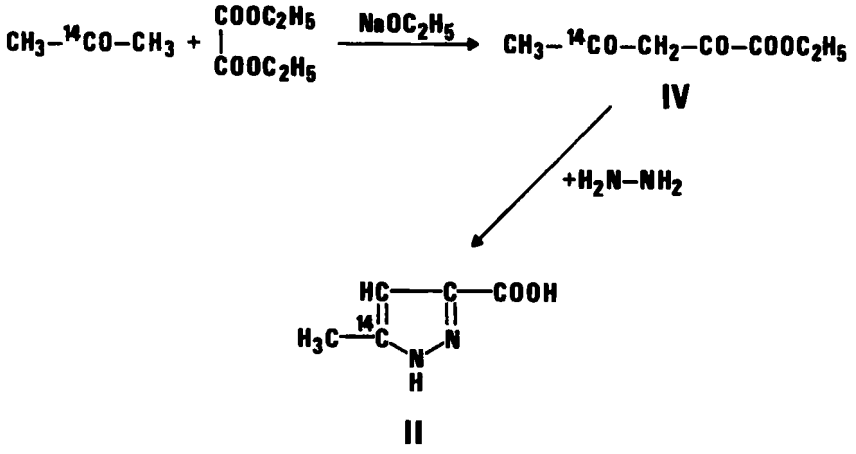
leading to 2,4-pentanedione (III), is well suited to small-scale work since the product, a volatile liquid, is isolated in solid form as a copper complex. Initial attempts to prepare III using commercially available BF_3 etherate were not successful. Trial runs using gaseous BF_3 by the method of Denoon (5), however, were successful resulting in 75-85% yields (by weight) of the copper-complex of III on a mM scale. The copper complex of III also was obtained in 85% weight yield using radioactive acetone. However, the

apparent radiochemical yield was only 53%. Possible explanations for this lower radiochemical yield are (a) loss of acetone-2-^{14}C during its transfer and dilution with nonradioactive acetone and (b) self condensation of acetic anhydride to produce nonradioactive 2,4-pentanedione, thus diluting the desired radioactive 2,4-pentanedione, as described by Doorenbos, *et al.* (6). This self condensation can be minimized by inverse addition of the acetone and acetic anhydride to a complex of the BF_3 (6).

The second step, leading to 3,5-dimethylpyrazole-3,5-^{14}C (I), was carried out in 45% weight and radiochemical yields (based on the copper complex of III) as described by Wiley and Hexner (7). The yield was lower than anticipated, possibly due to loss of III during generation from its copper complex. The product, 3,5-dimethylpyrazole-3,5-^{14}C (I), was obtained in 38% overall weight yield from acetone-2-^{14}C. It was shown to be both chemically and radiochemically pure.

5-Methylpyrazole-3-carboxylic acid-5-^{14}C (II) was prepared by the two step synthetic sequence shown in Scheme 2. The first step,

Scheme 2



leading to 2,4-dioxopentanoic acid ethyl ester (IV), also is well suited to small scale work since it can be isolated in solid form as its sodium salt as described by Marvel and Dreger (8). This step, however, proved to be trouble-

some due to the difficulty of filtering the sodium salt of IV. As a result of numerous trial runs, it was found that using 30% excess sodium ethoxide and diluting the reaction mixture with ethanol:benzene immediately prior to filtration resulted in an easily filterable product of apparently high purity. The sodium salt of IV was obtained in 74% weight yield using radioactive acetone. The apparent radiochemical yield was only 65%. In this case the lower radiochemical yield likely resulted from loss of acetone-2-^{14}C during its transfer and dilution with nonradioactive acetone.

The second step, leading to 5-methylpyrazole-3-carboxylic acid-5-^{14}C (II), was carried out in 61% weight and radiochemical yields (based on IV) by a modification of the method of Knorr and Macdonald (9). This modification$^{\Delta}$, involving inverse addition of IV to hydrazine sulfate, resulted in an improved yield and a purer product. The product, 5-methylpyrazole-3-carboxylic acid-5-^{14}C (II), was obtained in 46% overall weight yield from acetone-2-^{14}C. It was shown to be both chemically and radiochemically pure.

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