

SYNTHESIS OF N,N'-[METHYL-<sup>14</sup>C]-DIMETHYLHYDRAZINE  
OF HIGH SPECIFIC ACTIVITY

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## SUMMARY

N,N'-[Methyl-<sup>14</sup>C]-dimethylhydrazine dihydrochloride (2) having a specific activity of 112.5 mCi/mmol was prepared in two steps from ethyl hydrazinedicarboxylate (3) in dry THF and <sup>14</sup>C-methyl iodide with a specific activity of approximately 58 mCi/mmol. Methylation of 3 following treatment with KOEt and subsequent hydrolysis of the intermediate ethyl N,N'-[methyl-<sup>14</sup>C]-dimethylhydrazinedicarboxylate (4) in conc. HCl afforded 2 in 64% yield following purification by thin layer chromatography.

Key Words: Carbon-14; N,N'-[methyl-<sup>14</sup>C]-dimethylhydrazine; Methyl iodide; Ethyl hydrazinedicarboxylate.

## INTRODUCTION

A number of alkyl hydrazines are known to be carcinogenic in small animals.<sup>1</sup> Their extensive use as high energy propellant fuels and their presence in the environment<sup>2</sup> provided the impetus for this research effort. In order to investigate their interaction with human cells, particularly in connection with their transformation potential in tissue culture,<sup>3</sup> radio-labeled methylhydrazines were required to assess metabolic pathways and interaction with critical macromolecules.

Recently we reported<sup>4</sup> a new synthesis of N,N-[methyl-<sup>14</sup>C]-dimethylhydrazine hydrochloride (UDMH·HCl,<sub>1</sub>) of high specific activity and our preliminary DNA binding studies using cultured human fibroblast cells.<sup>5</sup> For comparative binding and transformation studies the radiolabeled regioisomer (2, DMH·2HCl) was desired. Methodology utilized for the preparation of [<sup>14</sup>C]-DMH·2HCl is described and provides a specific activity of 112.5 mCi/mmol. The reaction sequence is shorter (two steps) and affords product of considerably higher specific activity than the route reported by Horisberger

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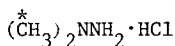
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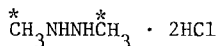
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and Matsumoto.<sup>6</sup>



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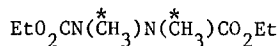
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#### RESULTS AND DISCUSSION

Whereas ethyl hydrazinedicarboxylate<sup>7</sup> (3) may be bismethylated by reaction with dimethyl sulfate in 10% NaOH solution<sup>8</sup>, the unavailability of high specific activity dimethyl sulfate precluded its usefulness in our studies. Rather, 3 (21.3 mg; 0.166 mmol) was dissolved in 5 ml of absolute ethanol and freshly cut potassium metal (15 mg; 0.334 mg atom) was added. The solvent was removed on a rotavaporator and the residue dried under reduced pressure and dissolved in THF freshly distilled from sodium. <sup>14</sup>C-Methyl iodide (2.07 equivalents; 20 mCi; approximately 58 mCi/mmol) was transferred to the reaction mixture in a vacuum manifold. Following stirring overnight at room temperature, filtration and solvent removal, a light yellow oil (99.7%) of ethyl N,N'-[methyl-<sup>14</sup>C]-dimethylhydrazinedicarboxylate (4) was obtained having identical spectral and physical properties with unlabeled compound prepared under analogous conditions. No monomethylated compound was present in the mixture as determined by NMR analysis. Interestingly, N,N'-dibenzoylhydrazine, under these conditions, afforded only monomethylated product and these results are in agreement with Horisberger and Matsumoto.<sup>6</sup> Refluxing 4 in excess concentrated HCl solution for 72 hrs followed by solvent removal under reduced pressure (rotavaporator) yielded 2 which was purified using Avicel cellulose thin layer chromatographic plates (14.6 mg; 64% from 3; 112.5 mCi/mmol) and shown to be chromatographically identical to authentic unlabeled DMH·2HCl.



3



4

## EXPERIMENTAL SECTION

Nuclear magnetic resonance data were obtained in  $\text{CDCl}_3$  using a Bruker HX-90 spectrometer. Radioactive disintegrations were measured on a Beckman LS-355 liquid scintillation counter using Amersham PCS as a counting cocktail. Thin layer chromatography was carried out on glass plates precoated with Avicel cellulose (250 $\mu$  thickness) purchased from Analtech. Plates were developed in 2-propanol:conc. HCl: water (150: 35:15) and were visualized with Folin Ciocalteu reagent obtained from Fisher Scientific.

<sup>14</sup>C-Methyl iodide (58 mCi/mmol) was purchased from Amersham prepared to our specifications by aqueous base extraction and  $\text{P}_2\text{O}_5$  drying and used without further purification. Transfer of <sup>14</sup>C-methyl iodide was carried out under reduced pressure in a Pope glass vacuum manifold. All glassware were predried under reduced pressure.

The specific activity of 2 was determined by drying and weighing an aliquot in a desiccated balance. The weighed material was dissolved in one ml of  $\text{H}_2\text{O}$  and 10 ml of PCS (Amersham) was added. Counting efficiency was determined using Oxi-Test standards from the Radiomatic Instrument and Chemical Company. The [<sup>14</sup>C]-DMH $\cdot$ 2HCl was found to be stable over 5 months when stored as a solid at  $-70^\circ\text{C}$ . Appreciable chemical decomposition was observed following 1 month at  $-20^\circ\text{C}$ . Ethyl hydrazinedicarboxylate (3) was synthesized according to the method of Rabjohn.<sup>7</sup>

Ethyl N,N'-[Methyl-<sup>14</sup>C]-dimethylhydrazinedicarboxylate (4): Under anhydrous conditions, absolute ethanol (5 ml) was added to 29.3 mg (0.166 mmol) of ethyl hydrazinedicarboxylate (3) in a 10 ml pear shaped flask. To this suspension was added 15 mg (0.384 mg atom) of freshly cut potassium metal; on shaking a clear solution was obtained. Excess ethanol was removed under reduced pressure (rotavaporator) at room temperature. The residue was dried under reduced pressure (0.1 mm;  $65^\circ\text{C}$ ; 4 hr) and suspended in 5 ml of THF freshly distilled from sodium. The suspension was frozen in liq.  $\text{N}_2$  and [<sup>14</sup>C]- $\text{CH}_3\text{I}$  (0.344 mmol; 20 mCi; 58 mCi/mmol) was transferred

to the reaction flask under reduced pressure. The reaction flask was maintained under liq.  $N_2$  for 1 hr, allowed to warm to room temperature and stirred overnight (~18 hr.). The reaction mixture was filtered (scintered glass funnel) and the residue washed thoroughly with dry THF. The filtrate and washings were combined. Following solvent removal at room temperature and under reduced pressure, a residual light yellow oil was obtained. This oil was further dried under reduced pressure (rotavaporator,  $40^\circ C$ ; 15 min.) affording 34.5 mg (99.7% by weight) of radiolabeled 4,  $^1H$ -NMR ( $CDCl_3$ )  $\delta$  1.24 (t, 6H,  $J=7.5$  Hz, 2  $-CH_2-CH_3$ ), 3.09 (s, 6H,  $-NCH_3NCH_3-$ ), 4.16 (q, 4H,  $J=7.5$  Hz, 2  $-O-CH_2-CH_3$ ).

$N,N'$ -[Methyl- $^{14}C$ ]-dimethylhydrazine Dihydrochloride (2): Ethyl  $N,N'$ -[methyl- $^{14}C$ ]-dimethylhydrazinedicarboxylate (4) (34.5 mg; 0.166 mmol) was dissolved in 4 ml of conc. HCl solution and refluxed with stirring for 72 hr (oil bath temp.  $150^\circ C$ ). The reaction mixture was cooled (room temperature), the condenser rinsed with conc. HCl and the combined HCl solutions concentrated under reduced pressure ( $40^\circ C$ ). The residue was dried utilizing several additions of methanol followed by distillation under reduced pressure (rotavaporator) affording 20.6 mg of white residue (MeOH soluble). The residue was dissolved in a minimum volume of methanol and applied to one 20 x 20 cm Avicel cellulose plate and developed in 2-propanol:conc. HCl: water (150: 35:15). The radioactive band corresponding to  $DMH \cdot 2HCl$  was scraped and eluted with 30 ml of 50% HCl (v/v). Solvent removal of the eluate under reduced pressure and azeotropic drying with methanol afforded 14.6 mg (64% from 3) of  $\overset{*}{C}H_3NHNH\overset{*}{C}H_3 \cdot 2HCl \cdot 0.5H_2O$  (112.5 mCi/mmol). Water of hydration was independently determined by elemental analysis of unlabeled material prepared under identical conditions.

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