A Simple, One-Step Synthesis of 1-^{14}C-Acetylhydrazine

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Summary

A synthesis of 1-^{14}C-acetylhydrazine by acetylation of hydrazine using 1-^{14}C-ethyl acetate and a catalytic amount of activated alumina is described.

Key Words: 1-^{14}C-acetylhydrazine, 1-^{14}C-ethyl acetate, activated alumina, Isoniazid, toxic metabolite.

Introduction

Acetylhydrazine, a toxic metabolite of Isoniazid, has been implicated in the serious hepatitis that occasionally follows the use of the drug in the treatment of tuberculosis (1). *In vitro* studies with rat and human liver microsomes have indicated that acetylhydrazine is oxidized by a microsomal cytochrome P-450 enzyme to produce a reactive acylating agent that acylates the hepatic macromolecules (2). Also, it has been postulated that such acylation of hepatic macromolecules by acetylhydrazine may produce, directly or indirectly, antigenic substances that result in an allergic reaction directed at the liver (3). In our work on the toxicity of Isoniazid, we required a pure sample of radiolabelled (^{14}C) acetylhydrazine to study the metabolic events initiating Isoniazid-induced liver injury, and herein, we wish to report a simple, one-step synthesis of 1-^{14}C-acetylhydrazine.

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Discussion

A search of the literature indicated that acetylhydrazine has been prepared either by acetylation of a suitably protected hydrazine derivative, followed by subsequent removal of the protecting group (4); or by controlled monoacetylation of hydrazine itself (5,6,7,).

Acetylation of a protected hydrazine (e.g., tertiary butyl carbazate) required acetic anhydride as the acylating agent and gave acetic acid as a by-product (cf. equation 1) thus leading to a loss of half the radioactivity if this procedure is adapted for the radiochemical synthesis. Substantial loss of radioactivity coupled with an extra reaction required to remove the protecting group makes this method not viable for the synthesis of 1-^{14}C-acetylhydrazine.

$$(CH_3CO)_2O + H_2NNHCO_2C(CH_3)_3 \rightarrow CH_3CONHNHCO_2C(CH_3)_3 + CH_3COOH \quad (1)$$

Several procedures have been reported for the preparation of acetylhydrazine by controlled acetylation of hydrazine. The most common method of preparation seems to be the reaction of hydrazine hydrate with an ester (usually methyl or ethyl) of acetic acid under reflux (5). This reaction requires at least 18 hours for completion; however, it has been reported that the use of anhydrous hydrazine at ambient temperature reduces the reaction time to less than 3 hours (6). This transacetylation reaction involving hydrazine hydrate has been adapted for the synthesis of labelled acetylhydrazine (4) and the acetylhydrazine so formed was isolated as the fumarate salt and purified by recrystallization. This procedure in our hands gave unsatisfactory results; the isolated product was contaminated with substantial amounts of hydrazine fumarate as evidenced by nmr and tlc.

Another procedure reported for the controlled acetylation of hydrazine involves the condensation of acetic acid with hydrazine hydrate at low temper

atures under azeotropic conditions using activated alumina as a catalyst (7). This reaction, usually completed in 2 to 12 hours giving 80-99% yield of acetylhydrazine, requires a Dean-Stark set-up which could be cumbersome for microscale reactions.

Inspired by the remarkable catalytic activity of alumina towards acylhydrazine formation and the ready availability of 1-^{14}C-ethyl acetate, we undertook a study of the reaction of hydrazine hydrate and ethyl acetate in the presence of alumina (cf. equation 2) under a variety of conditions. Absolute ethanol was used as a solvent and the reaction was carried out both at room temperature and under reflux with varying amounts of hydrazine hydrate and ethyl acetate. The progress of the reaction was monitored by gc analysis (8) of the p-chlorobenzaldehyde acetylhydrazone and p-chlorobenzaldehyde azine formed with p-chlorobenzaldehyde. We found that the reaction at room temperature with slight excess (10%) of ethyl acetate gave more than 98% yield of acetylhydrazine (by gc analysis) after 7 days. Also, it was observed that the reaction carried out under reflux proceeded rapidly in the initial stages to a maximum of 65-75% completion within 2 days (cf. Table I) but that prolonged heating led to the decomposition of the acetylhydrazine formed. On the other hand, the reaction at room temperature progressed steadily to 98% completion over 7 days with less than 1% diacetylhydrazine

$$CH_3CO_2C_2H_5 + H_2NNH_2H_2O \xrightarrow[\text{Abs.}C_2H_5OH]{Al_2O_3} CH_3CONHNH_2 + C_2H_5OH \qquad (2)$$

formed during this period. Accordingly, the reaction of hydrazine hydrate with a slight excess (10%) of ethyl acetate and activated alumina at room temperature was adapted for the synthesis of 1-^{14}C-acetylhydrazine.

Labelled acetylhydrazine was obtained in 85% isolated yield with more than 98% purity assessed by a tlc-liquid scintillation counting method. This simple, one-

step synthesis also has an additional advantage in that it involves minimal handling of radioactive compounds and requires only a simple set-up (a sealed Reacti-vial and a stirrer) and an equally simple work-up (filtration and evaporation under reduced pressure) to obtain the product in better than 98% purity.

Experimental

IR spectra were recorded with a Beckman 620 MX computing infrared spectrophotometer. NMR spectra were obtained with a Bruker WP-80 spectrometer, using tetramethylsilane as an internal standard. Mass spectra were recorded with a Hewlett Packard 5985A GC-MS data system. 1-^{14}C-ethyl acetate (4.9 mCi/mM) was purchased from Pathfinder Labs. Inc., St. Louis, MO 63141. Activated alumina (Baker Reagent Grade) was heated at 120° for 2 hours and cooled in a desiccator.

Reaction of Ethyl Acetate with Hydrazine Hydrate-Temperature Study

Ethyl acetate (1.76g, 20 mmoles) and hydrazine hydrate (1.0 g, 20mmoles) in absolute ethanol (6 ml) were stirred at room temperature with activated alumina (0.5 g). The progress of the reaction was monitored by gc analysis of the p-chlorobenzaldehyde acetylhydrazine and p-chlorobenzaldehyde azine.

In a separate study, the above experiment was repeated under reflux. The results are summarized in the Table below.

Table I: Percentage Yield of Acetylhydrazine with Time.

Temperature	3 hr	24 hr	48 hr
Room temp	19	70	80
Reflux(80°)	43	64	68

A better yield of acetylhydrazine was obtained by reaction at room temperature.

Reaction of Ethyl Acetate with Hydrazine Hydrate-Concentration Study

Ethyl acetate (176 mg, 2.0 mmoles) and hydrazine hydrate (100 mg, 2.0 mmoles) in absolute ethanol (400 µl) were stirred with activated alumina (100 mg) at room temperature for several days. The reaction was monitored by gc as described before.

In another study, the above experiment was repeated with 10% excess ethyl acetate (184 mg, 2.2 mmoles).

Gc analyses of the reaction mixtures from two runs indicated that after 7 days at room temperature, 92 and 96% of acetylhydrazine were formed respectively. The reaction mixture was filtered through a sintered glass funnel to remove alumina and the residue washed with more absolute ethanol. The combined filtrate and washings were evaporated to dryness under reduced pressure. The white solid obtained was dissolved in chloroform and the resulting solution evaporated to dryness (to remove traces of occluded solvent). Isolated yield of the product is 125 mg (85%) and 140 mg (95%) respectively.

m.p. 62-4° (lit. 67° (9)); IR (DMSO) C=0 1675 cm^{-1}; NMR ($CDCl_3$) δ1.97 (s,3H), 4.17 (br.s, 2H), and 7.71 (br.s, 1H); MS (solid probe) m/z 74, 43 and 42 with base peak at 43 (m/z 116 indicated trace amount of diacetylhydrazine present).

Preparation of 1-^{14}C-Acetylhydrazine

1-^{14}C-Ethyl acetate (2 mCi; 4.9 mCi/mM) was diluted with ethyl acetate to give 2.1 mmoles with a specific activity of 0.95 mCi/mM. This was stirred at room temperature with hydrazine hydrate (100 mg, 2.0 mmoles), activated alumina (100 mg) and absolute ethanol (400 µl) in a sealed Reacti-vial. After 7 days, the

reaction mixture was worked-up as described before. The yield of the product was 125 mg (85%). Specific activity was 0.85 mCi/mmole*.

Estimation of the Purity of 1-^{14}C-Acetylhydrazine

A small amount of the product was dissolved in methanol (3 ml) and the methanolic solution (50 µl) was incubated for 1 hr at 37° with p-chlorobenzaldehyde (50 µl, 150 mg/5 ml methanol). Thin layer chromatography of the p-chlorobenzaldehyde 1-^{14}C-acetylhydrazone on silica gel using ethyl acetate: acetic acid (70:30) as eluent, followed by liquid scintillation counting of the dried silica gel plate in 0.5 cm segments, indicated that the product was more than 98% pure.

* This specific activity is about 10% lower than that of the diluted starting ^{14}C-ethyl acetate and the reason for the discrepancy is not known. The specific activity of the undiluted ^{14}C-ethyl acetate (4.9 mCi/mM) was from the supplier and was not confirmed.

References

1. Summary of the report of the Tuberculosis Advisory Committee and Special Consultants to the Director, Centre for Disease Control, Morbidity and Mortality 23 : 97 (1974)

2. Nelson S.D., Mitchell J.R., Timbrell J.A., Snodgrass W.S. and Corcoran G.B. Science 193 : 901 (1976)

3. Mitchell J.R., Thorgeirsson U.P., Black M., Timbrell J.A., Snodgrass W.S., Potter W.Z., Jollow D.J. and Keiser H.R. Clin. Pharmacol. Ther. 18 : 70 (1975)

4. Nelson S.D., Hinson J.A. and Mitchell J.R. Biochem. Biophys. Res. Comm. 69 : 900 (1976)

5. Allen C.F.H. and Bell H. Org. Syn. 24 : 12 (1944)

6. Rush W.A. M.S. Thesis, Air Force Institute of Technology, Ohio, U.S.A. (June 1960)

7. Rabini T. and Vita G. J. Org. Chem. 30 : 2480 (1965)

8. Timbrell J.A., Wright J.M. and Smith C.M. J. Chromatogr. 138 : 165 (1977)

9. Smith P.A.S. Open Chain Nitrogen Compounds, Vol. 2, pp 176, W.A. Benjamin Inc., N.Y. 1966.