Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **XLI**, 377-385 (1998)

Synthesis of ^{14}C-labeled Octahydro-1,3,5,7-Tetranitro-1,3,5,7-Tetrazocine (HMX)

Chi-Yu Huang, Robert A. Mah, and Shane S. Que Hee*

Department of Environmental Health Sciences, School of Public Health, University of California, Los Angeles. Los Angeles, CA 90095-1772, U.S.A.

ABSTRACT

The ^{14}C-uniformly labeled (UL) explosive, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) was synthesized in 40% yield by nitrolysis of ^{14}C-labeled hexamethylenetetramine (hexamine) in the presence of boron trifluoride diethyl etherate as catalyst. The labeled hexamine was synthesized in 77% yield from ^{14}C-labeled formaldehyde and ammonium hydroxide. The specific activity of ^{14}C-labeled HMX was 0.24 mCi/mmol, a total of 58 μCi was prepared. The radiochemical purity of the labeled substance was 95% by HPLC-Liquid scintillation counting and 98% by HPLC-UV at 232 nm.

INTRODUCTION

The nitramine explosive, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) is one of the most important explosives used in military high-yield munitions due to its high density, detonation rate, and energy yield per unit volume. HMX and its cyclohexane analog, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) play central roles in the field of explosives and

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KEY WORDS: ^{14}C-labeled octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; HMX; ^{14}C-labeled hexamethylenetetramine; hexamine.

CCC 0362–4803/98/050377–09$17.50

Received 7 August 1997
Revised 9 December 1997
Accepted 10 December 1997

propellants (1). HMX residues are commonly found in wastewaters generated by the military explosive industry. HMX is a health concern because it adversely affects the central nervous system (2) and is classified as a class D carcinogen by the US Environmental Protection Agency. Therefore the fate of HMX after its release to the environment and during remediation and disposal is important.

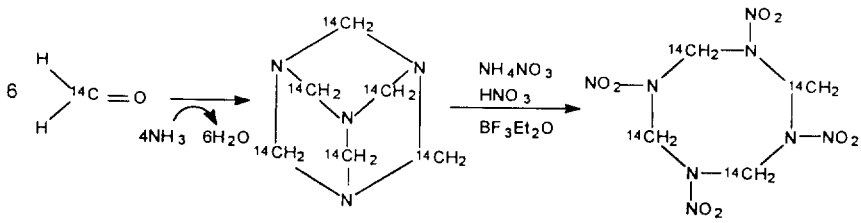
Though much data exist on biological degradation of RDX there is little published information on the biodegradation of HMX. A pathway for the anaerobic biodegradation of RDX has been proposed (3). RDX is biodegraded to mono-, di-, and tri-nitroso products arising from sequential reductions of the nitro groups ($-NO_2$) to nitroso groups (-NO) on supplementing carbon sources. These intermediate nitroso derivatives are then transformed to formaldehyde and methanol with trace amounts of hydrazine, 1,1-dimethylhydrazine, and 1,2-dimethylhydrazine. Though HMX is an RDX analog, none of the final products from RDX biodegradation have been reported for HMX biodegradation.

A species of anaerobic thermophilic bacterium capable of degrading HMX has been isolated in this laboratory from an uncontaminated lake sediment (4). To aid in the investigation of the fate and disposition of HMX during the biodegradation process, a labeled compound is necessary.

The batch process currently practised for the industrial manufacture of HMX is a variation of the original 2-step Bachmann process that gives lower yield (5). This process requires large amounts of reagents and is not practical for microscale radiosynthesis. Due to the high cost of radiolabeled material, a procedure which is suitable for small scale synthesis and is able to produce a high yield and high radioactivity of HMX is needed. Sufficient material must be provided to conduct the biodegradation studies as well as for purity confirmation. In the present work, ^{14}C-labeled HMX has been synthesized by a modified 2-stage Bachmann reaction as shown in Scheme I (6, 7). Hexamethylenetetramine (hexamine) was first prepared from ^{14}C-labeled formaldehyde and ammonium hydroxide (8). HMX was then synthesized by direct nitrolysis of hexamine with a mixture of 15:14 (w/w) 90% nitric

acid and ammonium nitrate in the presence of boron trifluoride diethyl etherate catalyst. This report details the practical microsynthesis of ^{14}C-uniformly labeled (UL) HMX, and the characterization of its radiochemical purity.

SCHEME I



Formaldehyde	Hexamine	HMX
CH_2O	$C_6H_{12}N_4$	$C_4H_8N_8O_8$

MATERIALS AND EXPERIMENTAL

^{14}C-labeled formaldehyde in water (1:99, v/v) solution was purchased from Du Pont NEN Research Products (Boston, Massachusetts). Ammonium hydroxide (29% w/w), glacial acetic acetic, and nitric acid (fuming, 90%) were purchased from Fisher Scientific (Tustin, CA). Hexamethylenetetramine (hexamine, 99.2%), ammonium nitrate, boron trifluoride diethyl etherate, and acetic anhydride were ordered from Sigma (St. Louis, MO). GC-mass spectra were obtained using a HP 5890A gas chromatograph/5988A mass spectrometer (Hewlett-Packard, Wilington, DE) using a 30 m x 0.32 mm I.D. DB-1701 chemically bonded fused-silica capillary column (J&W Scientific, Folsom, CA) and helium carrier gas at 3.0 $\pm$ 0.1 mL/min. The column temperature was kept at 150°C, and the injection port at 200°C. The mass spectrometer was operated in the full scan mode for the 70 e.V. electron impact source at 250°C. A Varian 5020 liquid chromatograph (Varian, Sugar Land, TX) equipped with a 250 mm x 4.6 mm platinum C-18 reverse-phase column (Alltech, Deerfield, IL) using 60%/40% water/methanol (v/v) at 1.0 $\pm$ 0.1ml/min allowed the effluent to be collected by a Gilson FC-80K microfractionator. The collected radioactivity in each fraction was assayed by a Beckman LS 1800 liquid scintillation spectrometer to assess the radiochemical purity.

Synthesis of unlabeled hexamethylenetetramine (hexamine)

Ammonium hydroxide (29% ammonia, 0.95 mL, total 14.70 mmol) was added dropwise with magnetic stirring to 1.280 ml of 37% formalin solution (total 17.06 mmol) in a 25 ml round bottom flask over 15 minutes at 15-20°C in a water bath. Water then was removed using a rotary evaporator under reduced pressure at 90°C until a precipitate began to form. An additional 0.95 ml of 29% ammonium hydroxide was added and the above procedure repeated. A volume of 0.74 ml of 98% ethanol was added and the mixture refluxed until the solid product was completely dissolved. The liquid was then totally evaporated by rotary evaporation. The solid product was just dissolved in the minimum amount of warm 98% ethanol. Hexamine was then precipitated by adding the same volume of diethyl ether with shaking, and by leaving the vessel in a salt-ice bath overnight. The cold slurry was then filtered, the filter washed with diethyl ether, and the filtered solid dried in a desiccator under vacuum until constant weight. More ethyl ether was added to the filtrate and the above procedures repeated to precipitate more hexamine. The yield was 220 mg (55%). Further crystallization of the filtrate yielded impure product, even though the total yield greatly increased to 83%. The sublimation point of the product was 292 ± 3°C [literature: 285 - 295°C (9)]. The ultraviolet molar absorptivity at wavelength 192 nm was 2,552.0 ± 5.9 L $mole^{-1}cm^{-1}$ and the infrared absorption spectrum of the product was identical to that of commercial hexamine (Sigma, 99.2% purity). The mass spectrum of hexamine thus prepared (m/z: **140**, 112, 96, 85, 71, 56, 42) was also identical to that of authentic hexamine.

Synthesis of ^{14}C-hexamethylenetetramine

^{14}C-Hexamethylenetetramine was prepared by the above procedure. A mixture of 53 µl of ^{14}C-formaldehyde solution (total activity 1 mCi, specific activity 53 mCi/mmol) and 1.28 ml of unlabeled 37% formalin solution was used (overall, 17.08 mmol) and 1.0 ml of ammonium hydroxide was employed to compensate for the higher content of formaldehyde. The yield of product was 305.2 mg or 76.5% of theory, or 765 µCi. This ^{14}C-labeled hexamine was not analyzed but used directly in the synthesis of ^{14}C-labeled HMX.

Synthesis of Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)

A nitric acid-ammonium nitrate solution was prepared by dissolving ammonium nitrate in 90% fuming nitric acid in the weight ratio of 15 to 14. This nitrolysis mixture was kept above 20°C to prevent precipitation of ammonium nitrate from solution. To a stirred mixture in a 50 ml round bottom flask containing 1.33 ml of glacial acetic acid, 0.02 ml of acetic anhydride and 0.03 ml boron trifluoride diethyl etherate maintained at 35-38° C by a water bath, the following solutions were added simultaneously over a 15-min period via two Pasteur pipets and a Claisen adapter: (a) 0.18 g hexamine dissolved in 0.283 ml of acetic acid (b) 0.212 ml of nitric acid-ammonium nitrate solution (15/14, w/w), and (c) 0.53 ml of acetic anhydride. At the end of each 3-min period, 0.085 ml of acetic anhydride was added to the reaction mixture. In the final addition 0.19 ml was added to constitute a total of 0.53 ml. The temperature of the water bath was increased to 44 $\pm$ 1°C and so the reaction mixture was then allowed to stand for 15 min. To the reaction mixture was then added (a) 0.32 ml of 15/14 (w/w) nitric acid - ammonium nitrate solution, prepared as above, and (b) 0.32 ml of acetic anhydride, both via Pasteur pipet simultaneously over 15 min followed by 0.48 ml acetic anhydride. The mixture was again allowed to stand for 60 min at 44 $\pm$ 1°C, then diluted with 7.45 ml of hot water (75 - 85°C) and refluxed for 30 min. The reaction mixture was cooled to 20°C by the addition of crushed ice of ASTM Type I water. The resulting white precipitate was filtered under vacuum, washed with 3 portions of ice-cold ASTM I water and dried. The crude yield was 202 mg (35.4%). Recrystallization of the crude product from hot acetone (55°C) gave 96.2 mg of HMX (16.9% yield) which had a melting point of 265 $\pm$ 0.5°C. The m.p. of authentic HMX standard was 264 - 265°C. The retention times of synthesized HMX and HMX standard on HPLC were identical, 4.9 min (Figure 1). Attempts to isolate additional pure HMX from the filtrate were unsuccessful.

^{14}C-Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)

180 mg (1.28 mmol) of ^{14}C-hexamine (451 μCi) yielded 228.4 mg (40.1%) of crude ^{14}C-HMX by the above procedure. Two recrystallizations of the crude product gave 73.5 mg

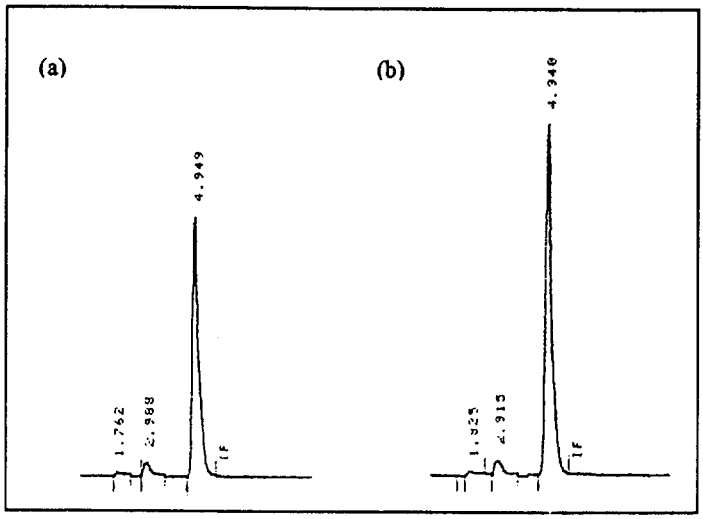


Fig. 1 HPLC chromatograms of the HMX standard (a), and synthesized HMX (b). HMX was prepared as a 10 mg/L solution in acetonitrile. Mobile phase, 60/40 water/methanol. Flow rate, 1 ml/min. Injection volume, 20 µl. UV detector, 232 nm. Peaks before HMX were also observed in an acetonitrile control injection..

of ^{14}C-HMX (12.9% relative to hexamine or 9.87% relative to starting formaldehyde). The specific activity of the labeled HMX was 0.79 µCi/mg (0.24 mCi/mmol), and the total activity was 58 µCi.

Radiochemical purity of ^{14}C-HMX

^{14}C-HMX was dissolved in 0.5 ml of acetonitrile. 20 µL of 100x diluted solution in acetonitrile was then injected into the HPLC using a Rheodyne manual injector (Rheodyne Inc., Cotati, CA). The column was a 250 mm x 4.6 mm platinum C-18 reverse-phase column and the mobile phase was 60%/40% water/methanol (v/v) with flow rate 1.0 ± 0.1 ml/min. The effluent was collected by a fractionator at 0.5 ml per fraction, and each mixed with 10 ml of Ecolite™ scintillation cocktail (ICN Biomedical Inc., Ohio). The radioactivity of each fraction was then counted on a Beckman LS 1800 liquid scintillation spectrometer. The radiochemical purity of synthesized ^{14}C-HMX was 95% (Figure 2) and the radioactivity of the collected HMX was accounted for by two peaks. The major peak and the impurity peak were collected separately twice, and analyzed by MS-MS. The major peak showed identical m/z to the HMX standard: 113, 297, 409.

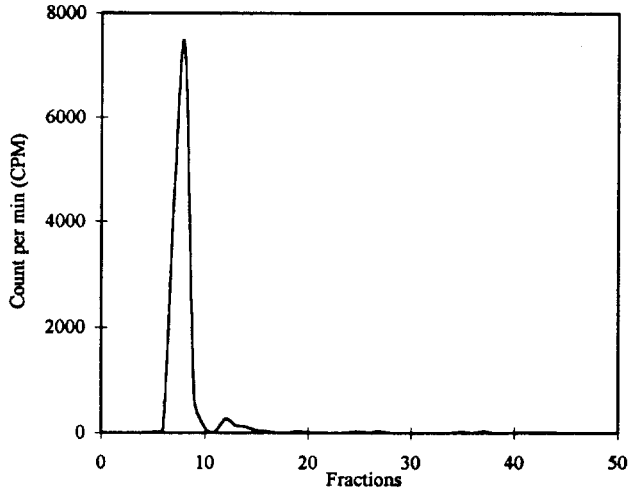


Fig. 2 Radiochemical purity of synthesized ^{14}C-UL labeled HMX. Mobile phase: 40% methanol/water; flow rate, 1 ml/min. Injection volume: 20 ul. Liquid scintillation was done on each 0.5 ml effluent fraction (10 fractions = 5.7 min). The maximum activity occurred at 4.940 min.

DISCUSSION

Minimization of HMX by-products during the synthetic process is necessary since by-products can be mistaken for degradation products. A labeled starting material is essential to isolate the products of biodegradation. The product produced here has 95% radiopurity. The use of a cold/hot label synthetic method provided sufficient material for handling. The spectroscopic and melting point purity were confirmed with cold compound previous to the labeled compound synthesis.

Several methods for the preparation of HMX on an industrial as well as laboratory scale are described in the literature (10, 11). Though hexamine is used as a precursor for manufacturing HMX, the synthesis of ^{14}C-labeled HMX with these procedures has not been reported. The present procedure was adapted from a large scale process (7) that gave the highest published yield of HMX. Boron trifluoride was used as a catalytic agent to enhance the conversion of hexamine into HMX. Higher amounts of ammonium hydroxide (15.35 mmol) than stoichiometric (11.40 mmol) were used to compensate for the high water content

in ^{14}C-labeled formaldehyde to produce ^{14}C-labeled hexamine. The reaction was conducted at low temperature (20°C) to prevent loss of formaldehyde from volatilization. Once produced, 14C-labeled hexamine was nitrated under mild conditions. In order to prevent the temperature of the reaction mixture from rising too much during the nitration, the temperature of the water bath was kept at a low temperature (35-38°C) initially, instead of 45°C in the original literature. The water bath was then warmed up to 44 ± 1°C after the first stage of HMX synthesis. This process prevented the formation of the analog, RDX, as an impurity, since it is formed beyond 55°C. Though having a lower yield of crude product, the present procedure, after two recrystallizations of the product with acetone, gave a higher purity (> 95%) of HMX than the original procedure (80-83%). This small scale procedure produced sufficient ^{14}C-labeled HMX with high radiochemical purity and activity, suitable to explore the fate of HMX during its biodegradation.

ACKNOWLEDGMENTS

Robert Silverman of the UCLA Department of Pharmacy is thanked for his assistance with infrared and magnetic resonance spectroscopy. Kym Faull in the UCLA Department of Chemistry and Biochemistry is thanked for performing the MS-MS analyses. Professor Michael Stenstrom is thanked for providing a pure HMX standard and the labeled formaldehyde. Shih-Wei Tsai is thanked for supervising the gas chromatography/mass spectrometry analyses.

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