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Preparation of deuterium-labeled biotransformation products of 2,4,6-trinitrotoluene

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Methods for the preparation of deuterium-labeled analogs to six prominent biotransformation products of the explosive 2,4,6-trinitrotoluene were developed. These are useful as reference standards for stable isotope dilution techniques and for solid state ^{2}H NMR spectroscopic studies. Although syntheses for most of the target compounds in protiated form had been reported in the past, most of those were found to be poorly suited for the preparation of the deuterated materials. Selective reduction of [$^2H_5$]trinitrotoluene furnished [$^2H_5$]-4,6-dinitro-2-hydroxylaminotoluene, [$^2H_5$]-2,6-dinitro-4-hydroxylaminotoluene, [$^2H_5$]-2-amino-4,6-dinitrotoluene, and [$^2H_5$]-4-amino-2,6-dinitrotoluene. The syntheses of [$^2H_{10}$]-2,2′-azo-4,4′,6,6′-tetranitrotoluene and [$^2H_{10}$]-4,4′-azo-2,2′,6,6′-tetranitrotoluene were accomplished by selective oxidation of [$^2H_5$]-2-amino-4,6-dinitrotoluene and [$^2H_5$]-4-amino-2,6-dinitrotoluene, respectively.

Keywords: trinitrotoluene; explosives; biotransformation; degradation

Introduction

Contamination of soils with 2,4,6-trinitrotoluene (TNT) results in the formation of transformation products including hydroxylamino-, amino-, azo-, and azoxydinitrotoluenes, which constitute a significant environmental hazard and are confirmed or suspected TNT degradation products.[1–3] Samples of the deuterated analogs shown in Figure 1 were required as reference materials for isotope dilution mass spectrometry (MS), as well as for the elucidation of soil binding characteristics by solid state ^{2}H NMR spectroscopy.[4] The preparation of the targeted products by reduction with baker's yeast in analogy to a procedure reported for ^{14}C-labeled TNT metabolites[5] was considered but found to be less suitable for the preparation of deuterated products than targeted synthesis due to its tendency to form complex mixtures.

Methods for the preparation of 4-hydroxylamino-2,6-dinitrotoluene **5** and 4-amino-2,6-dinitrotoluene **6** described in the literature[6–8] could be adapted for the synthesis of the deuterated analogs after some modification. In contrast, an adaptation of the synthesis of 2-amino-4,6-dinitrotoluene **3** from 2-methyl-3,5-dinitrobenzoic acid by Hoffmann degradation[2] was considered impractical, because of the extensive synthetic effort that would be required to obtain the precursor acid in labeled form. The selective ortho reduction of TNT has been reported,[7,9] but the published procedures were not found to be satisfactory for the small-scale preparation of high purity reference material. Likewise, the only published preparation of 2-hydroxylamino-4,6-dinitrotoluene **2**[10] was regarded as unsuitable for the deuterated analog, because of the complexity of the reported procedure and its low yield. Consequently, novel methods for the synthesis of **2**, **3**, **5**, and **6** by selective ortho reduction of [$^2H_5$]-TNT **1** were developed. The synthesis of the nonlabeled analog of **7** in low yield by partial electrolytic reduction of **1** has been reported[8] and a laborious procedure for its purification described.[2] In contrast, no methods for the preparation of **4** or its nonlabeled analog could be found in the literature.

Experimental

General

All [$^2H_5$]-TNT required for the synthesis of transformation products were prepared from commercial (Sigma-Aldrich) [$^2H_8$]-toluene, IP >99%, by stepwise nitration following a published procedure for the preparation of nonlabeled TNT.[11] MS analyses were performed on an Agilent Technologies ESI TOF 6210 mass spectrometer using a capillary voltage of 4200 V and nitrogen drying gas at 325° at 8 L/min. The fragmentor voltage was set to 150 V. The sample was delivered by the flow through method by an Agilent 1200 HPLC using a mobile phase of acetonitrile and water (90:10) with 0.1% formic acid at a flow rate of 0.4 mL/min. Nuclear magnetic resonance spectra were recorded on a Jeol Eclipse 300 MHz spectrometer. Sigma-Aldrich brand basic and acidic alumina, Brockmann, 150 mesh were used for column chromatography. All other chemicals employed in this study were purchased from Sigma-Aldrich and used as received. Melting points were taken on a Mel-Temp II. Protiated analogs of **3**, **5**, **6**, and **7** used as reference materials were prepared according to published procedures.[12] Products were characterized by ^{1}H NMR spectroscopy (to verify deuteration at >95%), ^{13}C NMR spectroscopy, MS, and comparison (thin layer chromatography, mp) to authentic protiated analogs.

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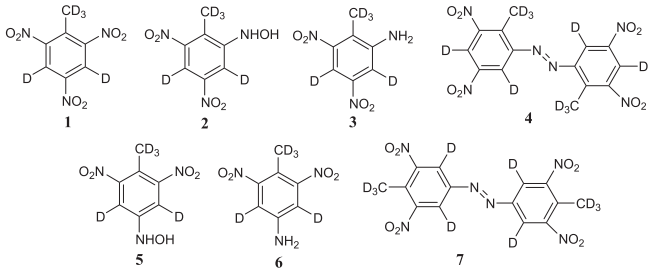

Figure 1. Deuterated 2,4,6-trinitrotoluene biotransformation products referenced in this study.

[$^2H_5$]-4,6-Dinitro-2-hydroxylaminotoluene ***2***

A total of 10 g (43 mmol) of **1** was dissolved in 15 mL dioxane. This solution was added to 11.0 g (72 mmol) of stannous chloride dihydrate suspended in 200 mL absolute ethanol. The mixture warmed spontaneously and was set aside for 5 h. All ethanol was then removed by rotary evaporation. The mixture was added to 200 mL water and the product extracted with 2 × 100 mL dichloromethane. The organic phase was dried over sodium sulfate, and solvents were removed by rotary evaporation. Crude **2** was taken up in 80 mL absolute ethanol, leaving an oily residue behind. The solvent was removed, and the remaining, partially solidified material was treated with 50 mL dichloromethane–hexane 1:1 v/v. The resulting solid was collected on a Buchner funnel, washed with approximately 5 mL dichloromethane, and recrystallized from toluene and then from acetic acid–water 1:10 v/v. Pale yellow needles, yield: 1.9 g (20%); mp 126–128°. MS (EMI) *m/z*: 219.0773 [M + H].

[$^2H_5$]-2-Amino-4,6-dinitrotoluene ***3***

A total of 10 g (44 mmol) of **1** was dissolved in 150 mL dioxane. The mixture was placed in an ice bath and saturated with hydrogen chloride gas. To the continuously stirred and chilled mixture, 30.0 g stannous chloride dihydrate (196 mmol) was added in portions of approximately 3 g. The reaction mixture was stirred at ambient temperature for exactly 30 min and then added to 500 g crushed ice and 500 mL water. The hydrochloride of **3** hydrolyzes under these conditions, and a mixture of **3** and unreacted **1** was collected by filtration. For further purification, crude **3** was dissolved in 250 mL dichloromethane, precipitated as the hydrochloride salt by saturation with hydrogen chloride gas, and collected by filtration. Unreacted **1** remained in solution and was collected by rotary evaporation for further use (approximately 1.5 g). The collected hydrochloride was suspended in 50 mL water, and sodium bicarbonate was added until pH 7 was reached. For further purification, **3** was recrystallized from benzene. Yellow needles, yield: 1.8 g (24% based on consumed **1**); mp 173–174°; literature, protiated analog: 155°, 173–174°.[13,2] MS (EMI) *m/z*: 203.0826 [M + H].

[$^2H_5$]-2,6-Dinitro-4-hydroxylaminotoluene ***5*** *and [$^2H_5$]-4-amino-2,6-dinitrotoluene* ***6***

A total of 10 g (44 mmol) of **1**, dissolved in 20 mL dioxane, was amended with 0.2 mL concentrated aqueous ammonia and the mixture saturated with gaseous hydrogen sulfide. After a 1- to 5-min initiation period, the mixture warmed spontaneously, and sulfur began to precipitate. No attempts were made to moderate the reaction, which was considered complete when hydrogen sulfide was no longer absorbed (10–15 min). Sulfur was removed by filtration and dioxane by rotary evaporation. The remaining material was slurred with 10 mL dichloromethane, and solids were collected by filtration (total yield, 10.5 g). A total of 1 g batches of this mixture was chromatographed on basic alumina. Elution of **6** with dichloromethane–acetonitrile 10:1 v/v was followed by elution of **5** with acetonitrile–ethanol (1:1). The combined fractions of **5** were taken to dryness and recrystallized from approximately 50 mL hot toluene. Orange crystals, 2.6 g (30%), mp 171–172°; literature, protiated analog: 172°.[13] (EMI) *m/z*: 219.0769 [M + H]. Compound **6** was purified by crystallization from ethanol–water 1:1 v/v, followed by crystallization from toluene. Yellow needles, 2.7 g (28%); mp 145–146°; literature, protiated analog: 135–136°, 141°, 144–146°.[2,6,14] MS (EMI) *m/z*: 203.0826 [M + H].

[$^2H_{10}$]-2,2′-Azo-4,4′,6,6′-tetranitrotoluene ***4*** *and isomer* ***7***

The respective amines **3** or **6** (1.01 g, 5 mmol) were dissolved in 50 mL dichloromethane, stirred, and amended with an equimolar amount of iodobenzene diacetate. The reaction mixtures gradually turned orange and were set aside for 6 h, and volatiles were then allowed to evaporate under a hood in an open beaker. The remaining, partially crystallized products were purified by flash chromatography (acidic alumina, dichloromethane) and subsequently recrystallized from approximately 5 mL hot toluene. Compound **4**, orange crystals, 0.24 g (24%); mp 244–246°. (EMI) *m/z*: 401.1256 [M + H]. Compound **7**, orange crystals, 0.81 g (81%); mp 267–268°; literature: 248–250°, 266–268°.[2,8] MS (EMI) *m/z*: 401.1260 [M + H].

Results and discussion

It was found that the preparation of [$^2H_5$]-TNT **1** from commercial [$^2H_8$]-toluene by stepwise nitration following a published procedure[11] could be carried out in protic media. By comparison with a single batch of [$^2H_5$]-TNT prepared in perdeuterated media, it was found that no loss of isotopic purity occurred, greatly reducing the cost of the nitration procedure. Compounds **2** and **3** are accessible by selective ortho reduction of **1** with stannous chloride in empirically optimized molar ratios. Highly acidic reaction conditions were chosen for this step to assure the immediate formation of the hydrochloride salts of the

Table 1. ^{13}C NMR chemical shifts of deuterated 2,4,6-trinitrotoluene transformation products in [$^2H_6$]-DMSO

Compound	^{13}C chemical shifts, ppm
[$^2H_5$]-4,4,6-Trinitrotoluene **1**	15.0[a], 122.2[b], 134.2, 145.7, 151.6
[$^2H_5$]-2-Hydroxylamino-4,6-dinitrotoluene **2**	12.4[a], 107.9[b], 122.6, 146.6, 150.5, 151.5, 152.8
[$^2H_5$]-2-Amino-4,6-dinitrotoluene **3**	12.9[a], 105.1[b], 110.1[b], 121.0, 146.2, 150.3, 151.4
[$^2H_{10}$]-2,2′-Azo-4,4′,6,6′-tetranitrotoluene **4**[c]	13.5[a], 114.8[b], 121.7, 139.2, 146.8, 151.6, 151.9
[$^2H_5$]-4-Hydroxylamino-2,6-dinitrotoluene **5**	13.4[a], 111.0[b], 114.7, 151.5, 151.8
[$^2H_5$]-4-Amino-2,6-dinitrotoluene **6**	13.6[a], 110.7, 111.5[b], 148.6, 152.1
[$^2H_{10}$]-4,4′-Azo-2,2′,6,6′-tetranitrotoluene **7**[c]	14.5[a], 121.7[b], 130.1, 150.0, 152.3

[a]Peak appears as septet.
[b]Peak appears as triplet.
[c]Sample heated to 100° during acquisition to increase solubility.

mono-reduction products, which resisted further reduction. It is noteworthy that numerous attempts to modify the procedure used for the preparation of **3** to improve its yield resulted in drastically reduced, rather than increased, yields. Oxidation of **3** to **4** and **6** to **7** represented a synthetic challenge, as **3** and **6** are readily oxidized to the corresponding azoxy compounds if conditions are not chosen carefully. Trials employing lead tetraacetate, *m*-chloroperbenzoic acid, and peroxide-based oxidizers under varying conditions did not produce the desired results. In contrast, treatment of **3** and **6** with hypervalent iodobenzene diacetate resulted in the relatively clean formation of **4** and **7**. Conflicting information exists about the reduction of TNT with hydrogen sulfide gas.[15] We invariably obtained mixtures of both **5** and **6**, which were separated chromatographically. Attempts to complete the reduction of **5** to **6** by repeated addition of small quantities (1 mL) of ammonia and subsequent saturation with hydrogen sulfide or by addition of ammonium sulfide solution as suggested by Michels and Gottschalk[10] (no details given in reference) resulted in the formation of both [$^{2}H_{5}$]-2,4-diamino-6-nitrotoluene and highly colored reduction byproducts of unknown nature, which were difficult to remove.

The presence of deuterium labels gave rise to characteristic ^{13}C NMR spectra, which showed the expected characteristic triplets for aryl carbons bearing deuterium, as well as septets for trideuteromethyl groups (Table 1). The chemical identities for **1–7** were further confirmed by comparison with authentic protiated analogs. Furthermore, protiated analogs of **2** and **4**, previously prepared by similar procedures[12], were characterized by X-ray crystallography.

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Conflict of Interest

The authors did not report any conflict of interest.

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