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Research Article

Microbiotic synthesis of ^{14}C-ringlabelled aminodinitrotoluenes (ADNT) and diaminonitrotoluenes (DANT)

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Summary

The four ^{14}C-ringlabelled TNT-metabolites 2-aminodinitrotoluene (2-ADNT), 4-aminodinitrotoluene (4-ADNT), 2,4-diaminonitrotoluene (2,4-DANT) and 2,6-diaminonitrotoluene (2,6-DANT) were synthesized in one step from TNT by reduction with baker's yeast (*Saccharomyces cervisiae*). Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: baker's yeast; TNT; microbiotic ^{14}C-synthesis; aminodinitrotoluenes; diaminonitrotoluenes

Introduction

Extensive production of the explosive 2,4,6-trinitrotoluene (TNT) over the last century, particularly during World War II, has caused severe contamination of former TNT-manufacturing and –handling sites.[1] The toxic, cancerogenic and mutagenic properties of TNT as well as the chemical persistency have turned it into a major environmental hazard and the effective degradation of TNT is still a challenge for environmental research.[2]

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We are investigating the degradation of TNT in aqueous environments by chemical and biological processes.[3,4] In order to determine the mineralization of TNT and its metabolites we were in need of the reduced, ^{14}C-ring-labelled TNT-transformation products 2-aminodinitrotoluene (2-ADNT), 4-aminodinitrotoluene (4-ADNT), 2,4-diaminonitrotoluene (2,4-DANT) and 2,6-diaminonitrotoluene (2,6-DANT).

These compounds occur as natural TNT metabolites and their reactivity results in a higher toxicity as compared to TNT itself.[2] On the other hand, the compounds are more susceptible to degradation reactions, and there is a growing interest in their role as possible intermediates in the conversion of TNT into commercially interesting derivatives.[5,6]

So far, 4-ADNT and 2,4-DANT were prepared by way of reduction by hydrogen sulfide in the presence of catalytic amounts of ammonia as reducing agent. Using dioxane as solvent this has led to a high regioselectivity for *para*-reduction (99:1).[7] Until recently, 2,6-DANT could only be obtained via a six-step synthesis starting from 2,6-dichlorotoluene.[8] A new procedure now allows us to selectively reduce TNT at the *ortho*-nitro group by iron powder in acetic acid. Thus 2-ADNT and 2,6-DANT are now available by direct reduction of TNT.[6]

Considering our demand for all four ADNT- and DANT-isomers, a separate synthesis for each compound would have been inefficient. Hence we looked for an alternative method to obtain all four compounds in one step.

Many microorganisms are known to reduce TNT, which has already been used to prepare ^{14}C-labelled aminodinitrotoluenes on a microscale level.[9] In addition baker's yeast was successfully employed to convert dinitronbenzenes into aminonitro-benzenes on a preparative scale.[10] Baker's yeast is advantageous for such reactions as it is cheap, readily available, easy to handle and quite versatile. Furthermore it is not toxic and as a whole cell system no cofactors have to be added.[11]

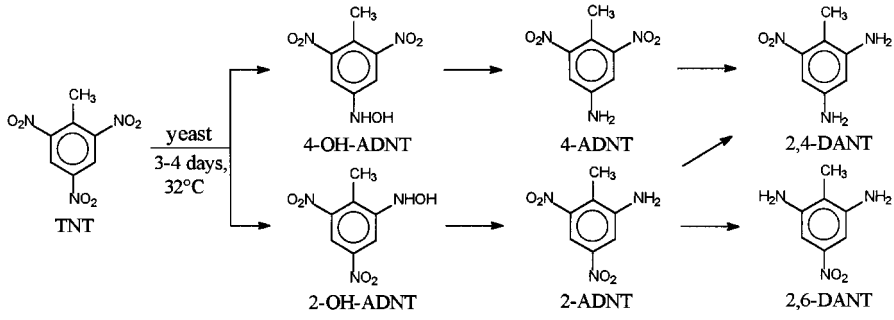
All these facts encouraged us to investigate the reaction of TNT with baker's yeast, which eventually yielded a procedure for the preparation of all four desired ring-labelled aminoaryl compounds in a single step staring from ^{14}C-TNT and yeast.

Results and discussion

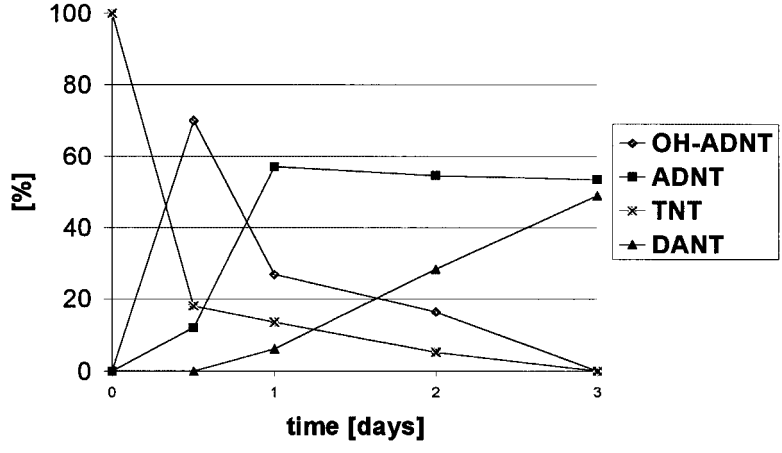
The reduction of nitroaromatic to arylamine compounds is a six-electron reduction sequence which includes two-electron steps via a

nitroso and a hydroxylamino intermediate. HPLC analysis of the reaction products showed that baker's yeast can convert TNT into the diaminonitrotoluenes within 3–4 days. Since the nitroso intermediates are unstable, the first observable intermediates are hydroxyl-aminotoluenes (OH-ADNTs) which are easily reduced and under oxidative conditions are transferred into azoxy-dimers.

OH-ADNTs can be observed after a few hours, but these intermediates are completely reduced to ADNTs after 3 days. Finally the diaminonitrotoluenes build up without any formation of detectable intermediates (see Schemes 1 and 2).



Scheme 1. Reduction pathways from TNT to DANTs



Scheme 2. Progress of TNT reduction over a period of 3 days

In order to arrive at the ADNTs and DANTs we monitored the reaction to when all four compounds are present in approximately equal amounts, while almost no TNT and OH-ADNT are remaining. If a single batch of yeast did not produce enough DANT, the reaction was extended by simply adding more yeast.

Separation of all four products was achieved by preparative TLC, using a toluene: methanol mixture to separate amino from diamino compounds followed by a second chromatography step with ethylacetate:hexane to isolate each of the regioisomers. Detection of the products was facilitated by their colour. With a growing level of reduction, the intensity of the colour increases: hydroxylaminodinitro compounds are pale yellow, aminodinitrotoluenes are yellow and diaminotoluenes are orange. All analytical data are listed in Table 3 in the experimental section. In the 'hot' run of the reaction the separation was also documented by autoradiography.

The most crucial point regarding control and reproducibility of the reaction turned out to be the regioselectivity of the reduction. Use of different batches of baker's yeast yielded different ratios of *ortho* and *para* products (see Table 1).

Though in a few experiments (R21, R20) 2-ADNT exceeded 4-ADNT, usually *para*-reduction was favoured over *ortho*. Similar results with an *ortho:para* ratio of 1:3 were found for an electrochemical reduction of TNT by a Russian group.[12] The excess of 2,4-DANT over 2,6-DANT is expected, as further reduction of 4-ADNT exclusively yields 2,4-DANT, while reduction of 2-ADNT leads to both regioisomers (Scheme 1). Based on the data in Table 1, batch 37 was most appropriate for our needs and chosen for the radioactive synthesis.

Employing recrystallized ^{14}C-TNT synthesized earlier in our laboratory[13] we obtained the desired radioactively labelled four amino-derivatives with yields as given in Table 2.

Table 1. Yields (%) from selected batches

Batch no.	37	32	30	22	21	20	16	13
4-ADNT	17.3	37.6	26.4	42.8	16.1	13.2	20.3	16.3
2-ADNT	10.2	1.5	8.1	2.9	20.3	23.4	9.2	10.2
2,4-DANT	28.7	28.7	21.6	6.2	10.8	15.6	30.0	19.2
2,6-DANT	4.8	9.6	13.2	1.5	15.6	9.6	11.0	12.0
Total	61	77	69	53	63	62	70	57

Table 2. Results of the radiolabelled synthesis

Compound	Yield (%)	Radioactivity (MBq)	Radioactivity (mCi)
4-ADNT	25	148	4.0
2-ADNT	6	40	1.1
2,4-DANT	19	114	3.1
2,6-DANT	2	11	0.2
Total	52	512	8.4

Chemical and radiochemical purities of the separated compounds were $>95\%$ as determined by HPLC.

Experimental

HPLC analysis was performed with a water–methanol gradient from 55:45 to 100% methanol on a Nucleosil RP18 column and Merck-Hitachi equipment (655A pump, L-5000 controller, L-3000 diode array detector). Before injection each sample was neutralized and extracted with CH_2Cl_2.

NMR measurements were performed in $CDCl_3$ on a Bruker 200 MHz spectrometer.

Synthesis of ^{14}C-2-ADNT, ^{14}C-4-ADNT, ^{14}C-2-4-DANT and ^{14}C-2, 6-DANT

Commercially available baker's yeast (6 g, RuF Lebensmittelwerk, batch JAN 019303) was suspended in tap water (40 ml) and stirred carefully at 32°C. To start the reaction [ring-^{14}C]-TNT (0.5 mmol, 114 mg)[13] in hot ethanol (1.6 ml) was quickly added. The course of the reaction was controlled by HPLC. To obtain an equal ratio of ADNT to DANT another 1.5 g of yeast was added on day four. The reaction was stopped after 5 days. Extraction with dichloromethane (250 ml) yielded 119 mg of raw material. The four products were applied to two preparative TLC plates (Macherey-Nagel, SIL G-200) and separated by consecutive use of a toluene:methanol 9:1 and a hexane:ethylacetate 1:1 solvent mixture, respectively (for R_f-values see Table 3). Products were detected via their color and by autoradiography. For the latter a photographic film was applied to the TLC plates for 1 min and

Table 3. Analytical data

Compound	Colour	R_t (HPLC)	R_f	*NMR*: chemical shifts			
		(min)	(TLC)	Ar-H	Ar-H	CH_3	NH_2
TNT	—(UV)	23.3	0.90	8.85	8.85	2.69	
2-OH-ADNT	Pale yellow	21.1	0.54				
4-OH-ADNT	Pale yellow	21.1	0.54				
2-ADNT	Yellow	26.3	0.60	8.03	7.72	2.36	4.21
4-ADNT	Yellow	25.5	0.66	7.28	7.28	2.44	4.22
2,4-DANT	Orange	5.7	0.32	6.56	6.23	2.16	3.79
2,6-DANT	Orange	5.1	0.39	6.96	6.96	2.00	5.17

developed as usual. The products were eluted from silica with ethylacetate and the purity of each product was determined by HPLC.

Conclusion

The reduction of TNT with baker's yeast is a new and effective synthetic approach towards the aminodinitro- and diaminonitrotoluenes. All four compounds can be obtained in satisfactory yields. Together with ^{14}C-TNT[13] the ^{14}C-labelled TNT metabolites will now be employed in combined biological and photochemical degradation experiments, a procedure that has been shown to represent a very effective degradation procedure for nitroaromatic compounds.[14,15] The aim of this investigation is to study the synergistic effects of such a combined technique and to quantify the transformation products with particular emphasis on the mineralization of starting material.

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