CONVERSION OF ANILINE INTO PYROCATECHOL BY A *NOCARDIA sp.*; INCORPORATION OF OXYGEN-18

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Received 11 December 1974

1. Introduction

A great number of the numerous pesticides which enter the environment are aniline derivatives. The biochemical transformation of anilines in soil [1–4], in the liver microsome system [5–7], and by pure cultures of microorganisms [8], has been studied, but the pathway of aniline degradation by soil microorganisms is still unknown.

Therefore, the conversion of aniline into pyrocatechol by a mutant strain of a *Nocardia sp.* was studied in an atmosphere of $^{18}0_2$. The results suggest that both atoms of oxygen in the pyrocatechol were derived from molecular oxygen.

2. Materials and methods

The mutant AM 144 was isolated after treatment of the *Nocardia* wildtype strain A 1 [9] with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) according to Adelberg et al. [10].

Strain AM 144 was grown to late logarithmic phase in 100 ml glucose (1%)-mineral salts medium [9] in a 500 ml flask at 30°C on a rotary shaker. After addition of 50 µl aniline, the flask was evaporated and filled up with nitrogen three times. In the labelling experiment the flask was evaporated again, 100 ml of oxygen-18 gas (99.9% enriched, Miles) were added, and the flask was filled up with nitrogen. The culture was incubated at 30°C with shaking, and after 24 hr the cellfree culture fluid was acidified to pH 1 and extracted three times with 50 ml of diethylether. The ether extract was dried over anhydrous Na_2So_4 and evaporated to dryness in vacuo. The oxygen-18 content of the residue was determined by mass spectrometry with the spectrometer CH 7 from Varian MAT (Bremen, Germany).

Thin-layer chromatography was carried out using silica gel with fluorescent indicator (Merck GF_{254}).

Solvent systems: Benzene/methanol (3:1, v/v) and 1-butanol/acetic acid/water (4:1:1, v/v). The catechols were identified on the chromatograms by spraying with a 2% (w/v) $FeCl_3$ solution. Ultraviolet spectra were recorded on a DMR 21 photometer from Carl Zeiss (Oberkochen, Germany).

3. Results

A *Nocardia sp.* (A 1) isolated from soil, is able to grow on carboxanilide fungicides as sole sources of carbon and energy.

Following treatment with MNNG [9], mutants were

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*Dedicated to Professor Dr Eugen Müller on the occasion of his 70th birthday.

Table 1

	Unlabelled					Labelled							
m/e	92	93	109	110	111	92	94	110	111	112	113	114	115
R.I.	12	2	4	100	7	2	13	16	1	2	4	100	7

R.I. = relative intensity

Mass spectrum of unlabelled (control experiment) and labelled pyrocatechol, respectively, derived from the oxidation of aniline by cells of AM 144. The isotopic composition of the oxygen portion of the atmosphere at the end of the labelling experiment was approximately 18% ^{16}O-^{16}O, 0%^{16}O-^{18}O, and 82% ^{18}O-^{18}O, as measured by mass spectrometry.

isolated which are blocked in the degradation pathway of carboxanilide compounds [11]. When grown in glucose minimal medium supplemented with aniline, 9 mutants accumulated a compound, which decomposes forming brown oxidation-products. This compound was isolated as described in materials and methods. It could be identified as pyrocatechol by means of UV-spectrometry and by thin-layer chromatography. One of these mutants (AM 144) was used in the following experiments.

Incubated in a nitrogen atmosphere mutant AM 144 does not produce detectable amounts of pyrocatechol. When the nitrogen gas is replaced by air accumulation of pyrocatechol is observed. This result suggests that the presence of oxygen is essential for the transformation of aniline to pyrocatechol in this bacterium.

In order to measure the number of oxygen atoms incorporated into pyrocatechol an $^{18}O_2$-labelling experiment was performed. The mass spectrum of the isolated accumulate shows a parent peak at m/e 114 which corresponds to double-labelled pyrocatechol (table 1). The smal peak at m/e 110 is in accord with the formation of pyrocatechol from the low percentage of $^{16}O_2$ in the medium.

Only a neglegible amount of part-labelled pyrocatechol is formed. We assume that 2 atoms of oxygen are incorporated at the same time into the aromatic nucleus.

4. Discussion

Accumulation studies in an $^{18}O_2$-atmosphere revealed that both hydroxyl groups of pyrocatechol are equally ^{18}O-labelled. This finding eliminates an epoxide-intermediate, formed by mono-oxygenation of aniline, since the hydrolysis of the 1,2-epoxide should lead to pyrocatechol with but one hydroxyl group containing ^{18}O. We assume that a cyclic peroxide, formed after dioxygenation is the more likely intermediate in aniline oxidation. The conversion of the peroxide to pyrocatechol may result in both hydroxyl groups being equally ^{18}O-labelled.

This conclusion is in agreement with the observation that mono-oxygenation is the mechanism of biological oxidation of nonhydroxylated aromatic compounds in mammalian liver [12] whereas dioxygenation is often found in bacteria [13].

Cyclic peroxide intermediates have been postulated in the oxidation of anthranilic acid [14], benzoic acid [15] and 2-fluorbenzoic acid to pyrocatechol [16], and in the oxidation of other aromatic compounds by micro-organisms [13].

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie.

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