

Metabolism of Dibenzofuran and Dibenzodioxin as Model for 2, 3, 7, 8-Tetrachlorodibenzodioxin Degradation

V. Strubel, H.-J. Knackmuss, and K. H. Engesser

Institut für Mikrobiologie, Universität Stuttgart, D-W 7000 Stuttgart 1, FRG

Dibenzofuran (DBF) has been employed in some recent studies as a model compound for studying the microbial degradation of cyclic biaryl ethers (2,3,5). Public attention has focussed on this class of compounds since it comprises some of the most pernicious and at same time persistent molecules, such as TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin). For dibenzofuran a novel angular dioxygenation mechanism is described and a degradation sequence via a biphenyl related pathway outlined (2).

RESULTS

Dibenzofuran degrading bacteria were enriched from various environmental sources. One of these isolate, *Brevibacterium* strain DPO 1361 showed limited growth on dibenzofuran (OD_{546nm} 4) as sole source of carbon and energy, when the substrate (10 mM) was added in a suspended form. The use of a two-liquid-phase system substantially improved the growth rate and increased the optical density (546nm) up to 10. This two-liquid-phase system consisted of phosphate buffer and an organic phase of 2,2,4,4,6,8,8-heptamethylnonane (HMN) (10%, [vol/vol]), in which DBF (10 mM referred to the whole culture volume) was completely dissolved. Turnover of substrate was monitored by following its concentration in the organic phase using HPLC (see Fig. 1).

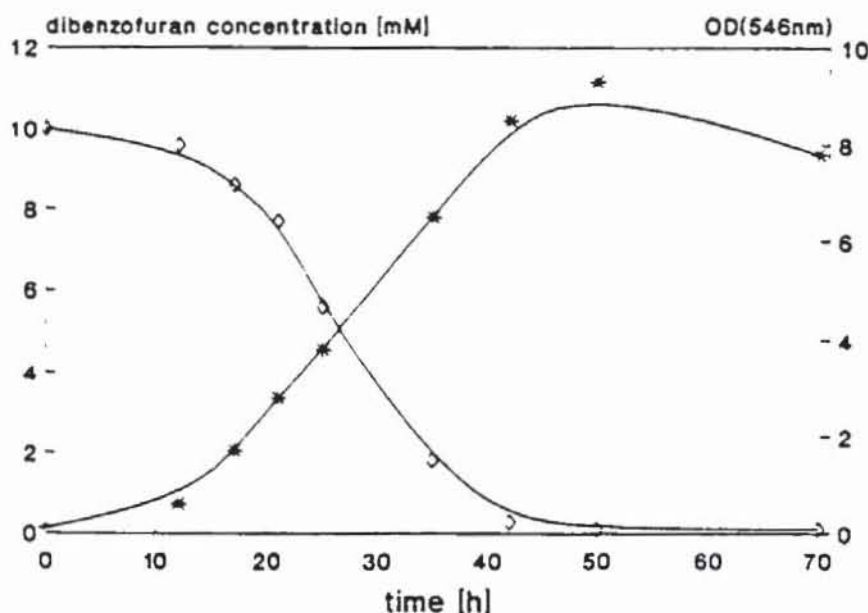


Fig.1: Growth of strain DPO 1361 in a two liquid-phase system with dibenzofuran as sole source of carbon and energy. (Explanation see text.)

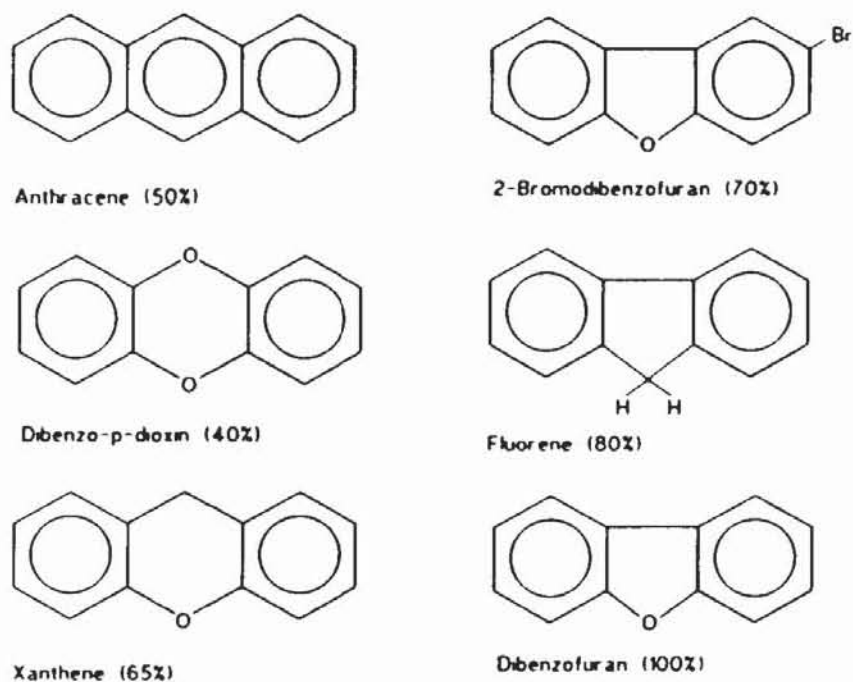


Fig. 2: Turnover of cyclic biarylether and related compounds by strain DPO 220 (Explanation see text). The per centage of substrate degraded after 60 minutes is given in brackets (DBF=100%).

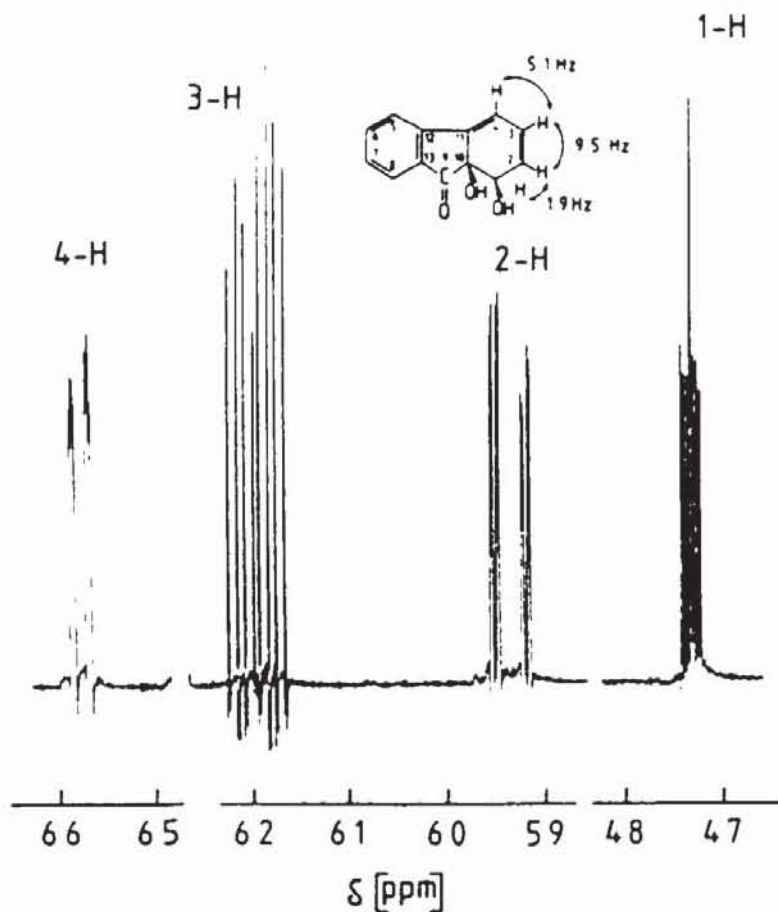
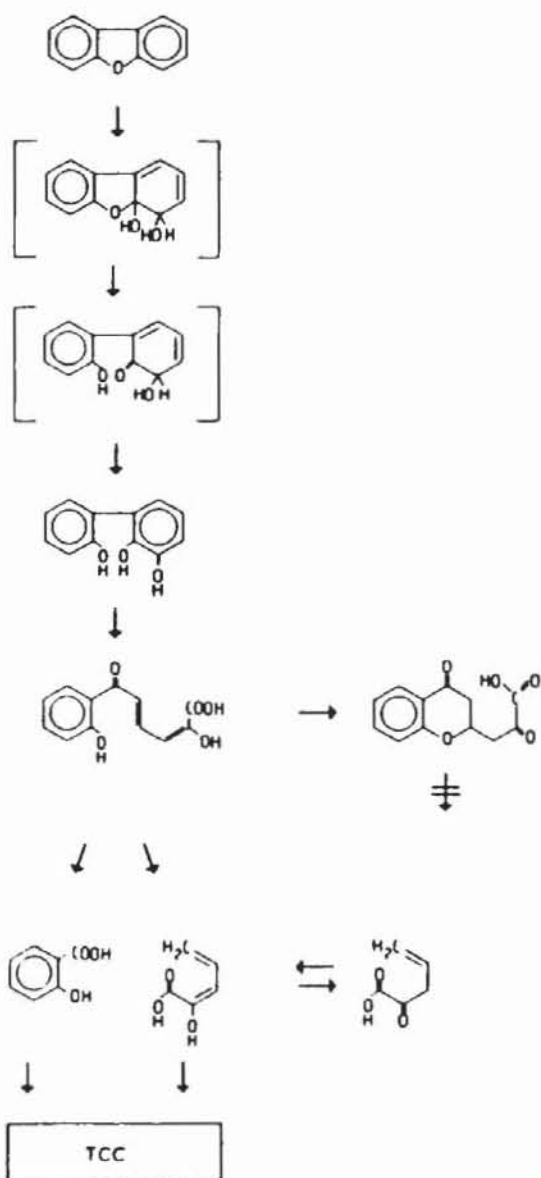


Fig. 3:
Legend see next side

Fig. 3: Partial 300 MHz $^1\text{H-NMR}$ spectrum of 1,10-dihydro 1,10-dihydroxyfluoren-9-one (20 mg in 1 ml CDCl_3 , 330K, TMS as internal standard, FID manipulated by Gaussian multiplication for improved resolution)

The DBF degrading bacteria transformed a broad range of anellated aromatics and heteroaromatics. For co-metabolism experiments cells of the strain DPO 220 (DBF-grown) were resuspended in phosphate buffer to an optical density (546nm) 5 and substrate (1 mM) added from a DMSO stock solution (100 mM). Two flasks were used respectively for each substrate. After an incubation period of 0 and 60 min the substrate was totally solubilized by adding dioxane and the concentrations were determined using HPLC (see Fig. 2)(5).

In resting cells experiments, fluorene was used as substrate analogue of DBF in order to show the mode of initial attack. After cooxidation of fluorene by cells of strain DPO 1361 (DBF-grown), one metabolite was isolated and unambiguously characterized by high-resolution MS, $^1\text{H-NMR}$ and $^{13}\text{C-H-NMR}$ as 1,10-dihydro-1,10-dihydroxyfluoren-9-one (see figure 3). This indicated the presence of a novel dioxygenase, which attacks aromatic ethers in angular position (2).



Applying the concept of angular dioxygenation likewise to DBF, the extremely stable aryl ether bond is transformed into a labile hemiacetal structure. Spontaneous cleavage of the hemiacetal and subsequent rearomatization affords 3-(2-hydroxyphenyl)catechol (HPC). HPC was transformed by a partially purified metapyrocatechase to an unstable yellow ring cleavage product, 2-hydroxy-6-(2-hydroxyphenyl)-6-oxo-2,4-hexadienoic acid (2'-Hydroxy-HOPDA). This compound showed spontaneous cyclization by straightforward intramolecular Michael addition to a dead end metabolite, which was unequivocally characterized as 3-(chroman-4-on-2-yl)pyruvate. When HPC was incubated together with partially purified metapyrocatechase and HOPDA-hydrolase enzymes, it was converted to salicylate and 2-oxo-4-pentenoate (see Fig. 4).

Fig. 4: Pathway proposed for the degradation of dibenzofuran by strain DPO 1361.

DISCUSSION

The poor water solubility of biarylethers appears one of the factors which limits the biodegradation of these substances. By the use of a two-liquid-phase system the growth rates were improved and strain DPO 1361 reached twice of the optical density, compared to a culture fed conventionally by adding the substrate in a suspended form.

Bacterial strains, enriched on DBF as sole carbon source, showed high cometabolic potential towards anellated aromatics and heteroaromatics, which is correlated by the accumulation of metabolites. The ability of these strains can be used for production of optical active diendiols, for example 1,2-dihydro-1,2-dihydroxynaphthalene from naphthalene.

We demonstrated that fluorene and DBF are attacked by strain DPO 1361 via an angular dioxygenation (2). A pathway for DBF degradation is presented, which indicates an analogy to the pathway established for diphenyl (1,4). Further investigations will have to show whether the same angular dioxygenation mechanism is involved also in the degradation of dibenzodioxin.

ACKNOWLEDGMENT

We thank P. Fischer and W. Rozdzinski for MS and NMR measurements respectively. We gratefully acknowledge H.G. Rast (Bayer AG) for supplying us with the strain DPO 1361. This work was in part supported by the BMFT, Federal Ministry for Research and Technology [grant 0329309 C]

REFERENCES

- 1) Catelani, D. and Colombi, A. (1974). Structure and physicochemical properties of 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid, the meta-cleavage product from 2,3-dihydroxybiphenyl by *Pseudomonas putida*. *Biochem. J.* **143**: 431-434.
- 2) Engesser, K.H., Strubel, V., Christoglou, K., Fischer, P. and Rast, H.G. (1989). Dioxygenolytic cleavage of aryl ether bonds: 1,10-dihydro-1,10-dihydroxyfluorene-9-one, a novel arene dihydrodiol as evidence for angular dioxygenation of dibenzofuran. *FEMS. Microbiol. Lett.* **65**: 205-210.
- 3) Fortnagel, P., Hauke, H., Wittich, R.-M., Krohn, S., Meyer, H., Sinnwell, V., Wilkes, H., and Francke, W. (1990). Metabolism of dibenzofuran by *Pseudomonas* sp. strain HH69 and the mixed culture HH27. *Appl. Environ. Microbiol.* **56**: 1148-1156.
- 4) Furukawa, K., Simon J.R., and Chakrabarty A.M. (1983). Common induction and regulation of biphenyl, xylene/toluene, and salicylate catabolism in *Pseudomonas paucimobilis*. *J. Bacteriol.* **154**: 1356-1362.
- 5) Strubel, V., Rast, H.G., Fietz, W., Knackmuss, H.-J. and Engesser, K.H. (1988). Enrichment of dibenzofuran utilizing bacteria with cometabolic potential towards dibenzodioxin and other anellated aromatics. *FEMS. Microbiol. Lett.* **58**: 233-238.