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NOTES

Transformation of Dibenzo-*p*-Dioxin by *Pseudomonas* sp. Strain HH69

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Dibenzo-*p*-dioxin was oxidatively cleaved by the dibenzofuran-degrading bacterium *Pseudomonas* sp. strain HH69 to produce minor amounts of 1-hydroxydibenzo-*p*-dioxin and catechol, while a 2-phenoxy derivative of muconic acid was formed as the major product. Upon acidic methylation, the latter yielded the dimethylester of *cis,trans*-2-(2-hydroxyphenoxy)-muconic acid.

The bacterial cooxidation of dibenzo-*p*-dioxin (DD) and its monochlorinated derivatives to *cis*-1,2-dihydro-1,2-dihydroxydibenzo-*p*-dioxin and the diol as dehydrogenation products has been described previously (3,4) along with the extremely slow degradation and/or cooxidation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory model ecosystems (5, 7).

We are studying the biodegradation of diphenylethers and related compounds, and we present here the results of investigations of the initial ring-cleaving reactions of DD by a *Pseudomonas* strain.

(This paper is based in part on a doctoral study by Hauke Harms in the Faculty of Biology, the University of Hamburg.)

Synthesis of DD, growth conditions, and preparation and extraction of the medium, as well as analytical procedures, were as previously described (1, 2). The cooxidation experiments with DD as the target compound were performed with the dibenzofuran (DF)-degrading bacterium *Pseudomonas* sp. strain HH69 and the mutant HH69-II, which no longer can grow with DF as a carbon source. The mutant strain is able to convert DF to 2,2',3-trihydroxybiphenyl (2). Both strains were grown with peptone as the carbon source throughout the following experiments.

For metabolite production, solid DD (1 g/liter; solubility in water at 28°C, about 1.8 mg/liter) was added to washed-cell suspensions of strain HH69 or HH69-II. The formation of a single metabolite was monitored by high-performance liquid chromatography. Additionally, trace amounts of 1-hydroxydibenzo-*p*-dioxin and catechol could be easily identified by coupled gas chromatography-mass spectroscopy, while the major metabolite was methylated under acidic conditions after isolation and characterized by ^1H nuclear magnetic resonance spectroscopy (Fig. 1). The solvent was $CDCl_3$, and tetramethylsilane was the internal standard; the spectrum was recorded at 400.13 MHz. δ (ppm): 4-H = 8.08, 5'-H = 7.09, 6'-H = 7.03, 3'-H = 6.93, 4'-H = 6.86, 3-H = 6.25, 5-H = 6.11, 6a-CH_3/1a-CH_3 = 3.92/3.74. Coupling constants [Hz]: $J_{3,4}$ = 11.8, $J_{3,5}$ = 0.8, $J_{4,5}$ = 15.4, $J_{3',4'}$ = 8.0, $J_{3',5'}$ = 1.6, $J_{4',5'}$ = 7.0, $J_{4',6'}$ = 1.6, and $J_{5',6'}$ = 8.0. The chemical shift for 3-H in the substituted muconic acid dimethylester (found δ = 6.25; calculated δ = 6.43 for a *cis* configuration, δ = 4.80 for a *trans* configuration [6]) indicated that the first double bond keeps the same configuration as in *cis,cis*-muconic acid, while the coupling constant of $J_{4,5}$ = 15.4 Hz (*trans*) clearly showed a rearrangement of the second double bond.

In accordance with the initial attack on the DF system (2), a derivative of 2-phenoxymuconic acid is generated by dioxygenation at positions 1 and 1a of the DD system, furnishing the dihydrodiol, which represents a hemiacetal. This unstable compound may rearomatize upon dehydration to yield 1-hydroxydibenzo-*p*-dioxin, or it may be cleaved in the course of rearomatization to form 2,2',3-trihydroxybiphenyl ether (Fig. 2).

In contrast to the postulated *meta*-cleavage of 2,2',3-trihydroxybiphenyl derived from DF (2), the corresponding trihydroxybiaryl ether undergoes an unexpected *ortho* cleavage to form the above-mentioned derivative of muconic acid. The same muconic acid derivative was also obtained from DD by DF or from peptone-grown mutant HH69-II cells, which exhibit the blocked *meta*-cleaving enzyme activity (2).

Our results strongly point to the participation of an additionally induced *ortho*-cleaving activity. This assumption was confirmed by the observation that the simultaneous conversion of stoichiometric amounts of DD and DF by peptone-grown resting cells of strain HH69 led to the excretion of the muconic acid derivative formed from DD and of 2,2',3-trihydroxybiphenyl formed from DF as the dominant products. The intense yellow color of the medium, which was detected during turnover of the trihydroxylated biphenyl produced from DF by strain HH69 (2), did not occur. A probable poisoning (obviously competitive type of inhibition) of the *meta*-cleavage activity was also suspected from

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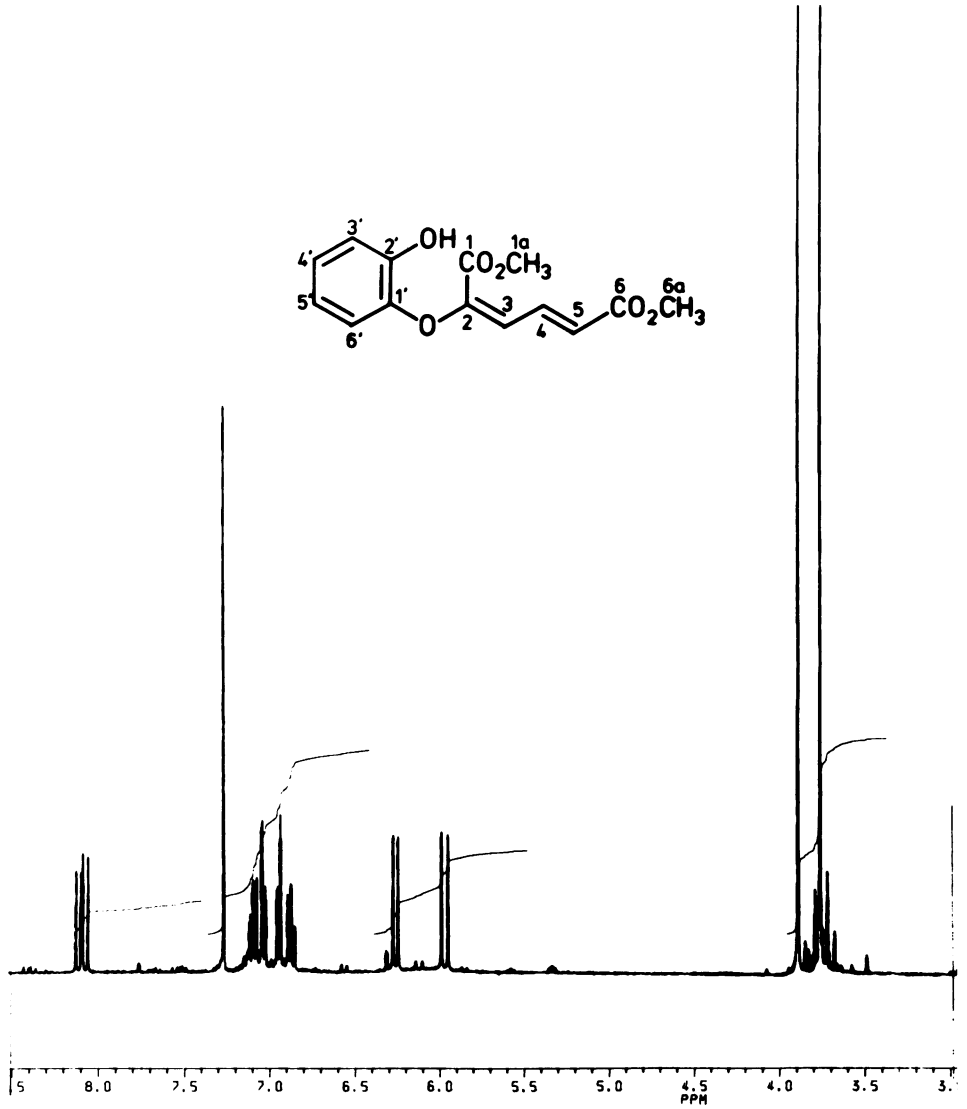

FIG. 1. ^1H nuclear magnetic resonance spectrum of *cis,trans*-2-(2-hydroxyphenoxy)-muconic acid dimethyl ester.

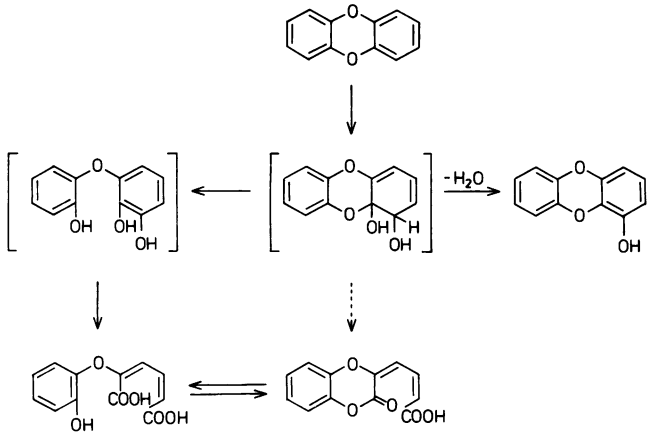

FIG. 2. Proposed pathway for the oxidation of DD by *Pseudomonas* sp. strain HH69.

the observation that strain HH69 is not able to grow with DF (1 g/liter) as the carbon source in the presence of small amounts of DD (10 mg/liter).

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