Chloroaromatics, a major class of industrial pollutants, may be oxidatively metabolized to chlorocatechols by soil and water microorganisms that have evolved catabolic activities toward these xenobiotics. We show here that 4-chlorocatechol can be further transformed by enzymes of the ubiquitous 3-oxoadipate pathway. However, whereas chloromuconate cycloisomerases catalyze the dechlorination of 3-chloro-cis-cis-muconate to form cis-dienelactone, muconoyl cycloisomerases catalyze a novel reaction, i.e. the dechlorination and concomitant decarboxylation to form 4-methylenebut-2-en-4-olide (protoanemonin), an ordinarily plant-derived antibiotic that is toxic to microorganisms.

Industrially produced halogenated aromatic compounds constitute a major class of environmental pollutants. Whereas some compounds are recalcitrant to degradation and accumulate in the environment, others disappear rapidly. Microorganisms exhibit an exceptional range of metabolic versatility, evolutionary potential and opportunism, which enables them to colonize a variety of habitats too hostile for higher organisms and to metabolize, and thereby grow, at the expense of a wide spectrum of noxious compounds, including some that are highly toxic.

The aerobic degradation of a wide range of aromatic compounds involves their successive activation and modification such that they are channelled toward one of a few key dihydroxylated intermediates such as catechol, gentisate, or protocatechuate, which are the substrates for cleavage of the aromatic ring. In the case of the haloaromatics, although halide elimination during an early step in the catabolic sequence has been documented in a few instances (1-4), the major degradative route involves conversion to corresponding halocatechols, intradiol (ortho) cleavage of the aromatic ring, and halide elimination during a subsequent reaction (Ref. 5; Fig. 1). This so-called “modified” ortho-cleavage pathway is thought to essentially parallel the classical 3-oxoadipate pathway, and enzymes of the classical 3-oxoadipate pathway have been assumed to carry out reactions identical to those of their counterparts in the modified ortho-pathway, albeit with much lower activities (6-8). In both pathways, catechols are orthodehalogenated (by (chloro-) catechol 1,2-dioxygenases, to form the corresponding cis,cis-muconates, which are then cycloisomerized by muconoyl cycloisomerase to muconolactone and muconate cycloisomerase (6-8). In analogy to muconolactone (4-carboxymethylbut-2-en-4-olide) as product of cis,cis-muconate cycloisomerization, halomuconoyl cycloisomerases were believed to be intermediates of cycloisomerization of halomuconoyl cis,cis-muconates, and spontaneous dehalogenation of those intermediates was postulated to give rise to 4-carboxymethylenebut-2-en-4-olides (dienelactones; Ref. 8). Those are subsequently hydrolyzed to maleylacetate by dienelactone hydrolase (8), followed by the enzymatic reduction of maleylacetate to 3-oxoadipate, the first common intermediate of the classical and the modified ortho-cleavage pathways (Ref. 9; Fig. 1B).

Based on the models of Ngai and Kallen (10) and Chari et al. (11) of syn addition to a cis double bond of muconate proceeding via a carbanionic intermediate, Schlömann et al. (12) suggested that the cycloisomerization reaction of 3-chloro-cis-cis-muconate is completed by the elimination of chloride rather than the protonation of the carbanionic intermediate, excluding 4-chloromuconolactone as an intermediate. An analogous elimination of chloride from the carbanion is not possible during cycloisomerization of 2-chloro-cis-cis-muconate. Recently, it has been shown that 2- and 5-chloromuconolactone were formed by muconoyl cycloisomerase (13), proving that this class of enzymes is not able to cause dechlorination during conversion of 2-chloro-cis,cis-muconate and that dechlorination by chloromuconoyl cycloisomerases is actually an enzyme-catalyzed process. We report here that both muconate and chloromuconate cycloisomerases cause dehalogenation during conversion of 3-chloro-cis,cis-muconate but that different reactions were catalyzed.

MATERIALS AND METHODS

Strains, Media, and Growth—Most of the bacterial strains used in this study have been described previously: Pseudomonas sp. B13 (14), Pseudomonas putida KT 2442 (15), Sphingomonas sp. RW1 (16), Pseudomonas sp. LB400 (17), P. putida P111 (18), Sphingomonas paucimobilis Q1 (19), Alcaligenes eutrophus HB850 (20), Rhodococcus globus P6 (21), Escherichia coli DH5α (Life Technologies, Inc.). P. putida RW10 was isolated based on its capability to mineralize 4- and 5-chlorosalicylate. Cells were grown in M9 medium supplemented with the indicated carbon source (usually 5 mM) and incubated at 30°C on a rotary shaker at 140 rpm in baffled Erlemeyer flasks. Preparation of Cell Extracts—Cells were harvested by centrifugation at the end of the logarithmic growth phase, resuspended in the appropriate buffer (50 mM Tris, pH 7.5, supplemented with 2 mM MnSO4 for analysis or purification of muconate cycloisomerase) and passed once through a French pressure cell (Amino) operating at 18,000 p.s.i. The

From Xenobiotic to Antibiotic, Formation of Protoanemonin from 4-Chlorocatechol by Enzymes of the 3-Oxoadipate Pathway*

(Received for publication, September 5, 1995, and in revised form, October 2, 1995)

Rafael Blasco†, Rolf-Michael Wittich, Megharaj Mallavarapu§, Kenneth N. Timmis¶, and Dietmar H. Pieper†

From the Division of Microbiology, National Research Center for Biotechnology, D-38124 Braunschweig, Federal Republic of Germany

*This work was supported by the Federal Ministry of Research and Technology (Award 0319-4334A). A preliminary report of this work has been presented in Ref. 44. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
†Supported by the Spanish Ministerio de Educación y Ciencia and the European Environmental Research Organization. Present address: Departamento de Bioquímica y Biología Molecular, Universidad de Córdoba, Avenida S. Alberto Magno S/N, 14071 Córdoba, Spain.
§Present address: Department of Microbiology, University of Otago, POB 56, Dunedin, New Zealand.
¶To whom correspondence should be addressed: Div. of Microbiology, GBF National Research Center for Biotechnology, Mascheroder Weg 1, D-38124 Braunschweig, Germany. Tel.: 49-531-6181-467; Fax: 49-531-6181-411; E-mail: pieper@gbf-braunschweig.de.
||Supported by the Fonds der Chemischen Industrie.
Cell debris was removed by centrifugation at 100,000 × g for 45 min. The clear supernatant is referred to as the cell extract.

Enzymatic Assays—Catechol 1,2-dioxygenase (EC 1.13.11.1) was measured by following the formation of cis,cis-muconate at 260 nm by a modification of the method of Dorn and Knackmuss (6). The cuvettes initially contained 33 mM Tris, pH 8.0, 2 mM EDTA, and an appropriate amount of enzyme. After a 5-min incubation, the reaction was started by the addition of catechol or 4-chlorocatechol to a final concentration of 0.2 mM.

Muconate cycloisomerase (EC 5.5.1.1) was measured following the consumption of cis,cis-muconate at 260 nm by a modification of the method of Sistrom and Stanier (22). The cuvettes initially contained 50
For high resolution gas chromatography-mass spectroscopy, the sample was analyzed by using a Carlo Erba Biomega series gas chromatograph equipped with a 30-m DB1 column. The gas chromatogram was linked to a Kratos MS 50 mass spectrometer. Helium was used as the carrier gas, and a potential of 70 eV was used for electron ionization. Dynamic high resolution MS of the molecular ion and the major fragment ions was performed using perfluorokerosene as internal reference.

High resolution 1H NMR spectra were recorded on a CXP 300 spectrometer (Bruker) using deuterated dimethylsilane as internal standard and deuterated methanol as solvent.

Chemicals—d5-cis-Muconate was prepared in vitro from 0.2 mM catechol using partially purified catechol 1,2-dioxygenase from Pseudomonas sp. B13. This solution was used to follow the muconate cycloisomerase activity during the purification procedure.

The syntheses of cis-dienelactone ((E)-4-carboxymethylenebut-2-en-4-olide), trans-dienelactone ((Z)-4-carboxymethylenebut-2-en-4-olide) and 3-chlorocatechol have been described previously (25, 26).

Chlorocatechol was purchased from Aldrich. All other chemicals were of analytical grade and obtained from Fluka AG, Merck AG, and Aldrich Chemie GmbH.

RESULTS

Transformation of 4-Chlorocatechol by Enzymes of the 3-Oxoadipate Pathway of Pseudomonas sp. B13—According to the currently accepted scheme for the metabolism of chlorinated catechols, 4-chlorocatechol will be converted by catechol 1,2-dioxygenase type I and muconate cycloisomerase to cis-dienelactone with reasonable activities (6–8). Theoretically, due to low activity of dienelactone hydrolyase (8) maleylacetate will be the final metabolite when cell extracts of benzoate-grown B13 are incubated with 4-chlorocatechol. However, we observed the formation of a new absorption maximum at 260 nm when the conversion of 4-chlorocatechol (100 μM) by such extracts was followed spectrophotometrically. Those spectrophotometric changes neither fit with the formation of maleylacetate (λmax = 243 nm; Ref. 27), nor of cis- or trans-dienelactone (λmax = 275 nm). Analysis of the reaction mixture by HPLC revealed the formation of a single metabolite that did not co-chromatograph with any of those compounds described to be formed during chlorocatechol metabolism via an ortho-cleavage pathway (6–9, 13, 28).

In order to analyze whether this new metabolite was formed from 4-chlorocatechol in a single catalytic step or in a coupled reaction and whether enzymes of the 3-oxoadipate pathway were responsible for its formation, both the catechol 1,2-dioxygenase type I and the muconate cycloisomerase from Pseudomonas sp. B13 were purified to homogeneity. Specific activity of catechol 1,2-dioxygenase type I and the muconate cycloisomerase from Pseudomonas sp. B13 were purified to homogeneity. Specific activity (29 units/mg of protein) was reported to be stable. The reaction was carried out at 25 °C under continuous agitation, and the progress of the reaction was monitored by tracing the increase in absorbance at 260 nm. After 2 h, the absorbance at 260 nm remained constant, and HPLC analysis confirmed the complete consumption of 4-chlorocatechol. The pH of the reaction mixture was adjusted to 6.5 by the addition of 60 ml of 500 mM MES-NaOH buffer, pH 6.5, and the reaction was initiated by addition of MnSO4 (2 mM) and purified muconate cycloisomerase (7 units). After 2.5 h protoanemonin was the major metabolite detected by HPLC (>98%) and was extracted twice with 250 ml of diethyl ether. About 4 mg of a pale yellow oil were obtained after careful evaporation of the ether.

Spectrophotometric analysis of 4-chlorocatechol (100 μM) turnover by purified muconate cycloisomerase of B13 revealed
an increase in absorption at 260 nm as predicted for 3-chloro-cis,cis-muconate formation (7). Turnover of 4-chlorocatechol by catechol 1,2-dioxygenase type II gave the same spectroscopic changes. In both cases, increase in absorption of 1.2 ± 0.05 is indicative for quantitative 3-chloro-cis,cis-muconate formation. In both cases, the quantitative consumption of 4-chlorocatechol by catechol 1,2-dioxygenase types I and II carry out the same reaction with 4-chlorocatechol as substrate, transforming 3-chloro-cis,cis-muconate (retention volume, 9.5 ml) as the product.

When a 3-chloro-cis,cis-muconate containing reaction mixture was incubated with muconate cycloisomerase (2 mM Mn²⁺ was added to stabilize muconate cycloisomerase), practically no spectroscopic changes were observed. Monitoring of the enzyme-catalyzed turnover by HPLC, however, clearly showed the disappearance of 3-chloro-cis,cis-muconate and the concomitant production of the new compound, which was shown to be formed from 4-chlorocatechol by cell extract of benzoate-grown B13 cells. Because this compound was found to be unstable at basic pH (Table I), muconate cycloisomerase-catalyzed preparative transformation of 3-chloro-cis,cis-muconate was carried out under slightly acidic conditions.

Structural Identification of 4-Methylenbut-2-en-4-olide as Cycloisomerization Product of 3-Chloro-cis-cis-Muconate—The product formed from 3-chloro-cis,cis-muconate by purified Pseudomonas sp. B13 muconate cycloisomerase was isolated on a micropreparative scale as described under “Materials and Methods.” The 1H NMR spectrum (Table II) shows four olefinic protons, centered at three different carbon atoms. A geminal coupling of 2.8 Hz between H-3 and H-4, a vicinal coupling of 5.6 Hz between H-1 and H-2, as well as long range couplings between H-1 and H-4 (1.9 Hz) and H-1 and H-3 (0.7 Hz) were observed. High resolution gas chromatography-mass spectrometry verified the composition C₅H₄O₂: m/e 96.0222 (M⁺, 100%, base peak); m/e 68.0264 (M⁺ – CO, 46%); m/e 54.0115 (M⁺ – C₃H₂O, 41%); m/e 42.0103 (M⁺ – C₂H₄O, 69%). Those results confirm the identification as 4-methyl-2-en-2-4-oxide (protoanemonin).

UV-visible spectroscopic data for protoanemonin isolated from Anemonopsis pulsatilla have been reported by Shaw (30) (λmax = 260 nm; ε260 nm = 14,000 M⁻¹ cm⁻¹). Enzyme-catalyzed transformation of 4-chlorocatechol into protoanemonin resulted in an increase of absorbance slightly higher than expected from those data, indicative for quantitative formation of this product (λmax = 259.5 nm; ε = 15,100 M⁻¹ cm⁻¹).

Formulation of Protoanemonin by Different Muconate Cycloisomerases—In order to analyze whether or not the formation of protoanemonin from 3-chloro-cis,cis-muconate is a unique characteristic of the muconate cycloisomerase from Pseudomonas sp. B13, the corresponding enzyme from salicylate-grown cells from P. putida RW10 was purified. 3-Chloro-cis,cis-muconate was prepared in vitro from 4-chlorocatechol by partially purified (only by anion-exchange chromatography) catechol 1,2-dioxygenase from Pseudomonas sp. B13. Following enzymatic conversion, proteins were eliminated by passage of the reaction mixture through a 10-kDa cut-off pore filter. Bioconversion of 3-chloro-cis,cis-muconate was carried out in a final volume of 1 ml with 20 milliliters of P. putida RW10 muconate cycloisomerase under the conditions described for the preparative production of protoanemonin with Pseudomonas sp. B13 enzymes. After 30 min, the reaction was completed by stoichiometric production of protoanemonin as judged by HPLC analysis.

Formation of protoanemonin (always greater than 70% of the theoretical yield) was also observed when cell extracts from cultures of different bacteria producing enzymes of the 3-oxoadipate pathway, i.e. benzoate-grown Pseudomonas sp. B13, P. putida KT2442 and Sphingomonas sp., RW1, and salicylate-grown P. putida RW10 were incubated with 10.2 mM 4-chlorocatechol. This is indicative of a general reaction mechanism for muconate cycloisomerizing enzymes. The specific activity for protoanemonin production by cell extracts was always >50 units/g protein, and further metabolism was negligible (<1 unit/g of protein), indicating that protoanemonin is probably nonmetabolizable, dead-end product of the classical 3-oxoadipate pathway.

In contrast to the situation with bacteria induced for type I ortho-pathway enzymes (3-oxoadipate pathway) enzymes only, no formation of protoanemonin from 4-chlorocatechol was detected when an extract of a 3-chlorobenzoate-grown culture of strain B13, i.e. an extract containing type II-modified ortho-pathway enzymes, was used. In this case, maleylacetate resulting from the concerted action of chloromuconate cycloisomerase and diene lactone hydrolase, was the only product observed. It should be noted at this juncture that 3-chlorobenzoate-grown B13 cells are induced for both the modified and the classical pathways and that the cell extract contained both muconate cycloisomerase and chloromuconate cycloisomerase. These two enzymes were partially purified from the extract by anion-exchange chromatography; individually they catalyzed the expected reactions, i.e. protoanemonin formation by muconate cycloisomerase and cis-diene lactone formation by chloromuconate cycloisomerase, but combined fractions quantitatively transformed 3-chloro-cis,cis-muconate into cis-diene lactone, indicative for the reported high activity of chloromuconate cycloisomerase with this substrate (8).

TABLE I
Stability of protoanemonin at different pH values

| Time (h) | pH 6.5 | pH 8 | pH 10 | pH 12 |
|---------|--------|------|-------|-------|
| 0       | 100    | 100  | 100   | 100   |
| 1       | 100    | 98   | 14    | 0     |
| 2       | 100    | 83   | 0     | 0     |
| 4       | 95     | 75   | 0     | 0     |

TABLE II
1H NMR data of protoanemonin

| Nucleus | Chemical shift | Coupling assignment | Coupling constant |
|---------|----------------|---------------------|-------------------|
| H¹      | 6.38           | 3J(H¹,H²)           | 5.6               |
| H²      | 7.72           | 3J(H²,H¹)           | 5.6               |
| H³      | 5.08           | 3J(H³,H⁴)           | 2.8               |
| H⁴      | 5.28           | 3J(H⁴,H¹)           | 2.8               |
|         | 5J(H¹,H³)     |                     | 0.7               |
In Vivo Production and Antibiotic Effect of Protoanemonin—When benzoate-grown cells of Pseudomonas sp. B13 or P. putida RW10 (A₆₀₀ nm = 0.8) were incubated with 1 mM 4-chlorocatechol, they completely consumed the substrate in less than 1 h. The optical density remained constant throughout the incubation period of 6 h, however, there was a drastic decrease (one order of magnitude) in the number of colony-forming units in the course of the experiment. This effect was not observed when catechol was added. The toxicity of 4-chlorocatechol toward Pseudomonas sp. B13 cells has already been reported (26). In order to analyze whether this toxic effect is due to 4-chlorocatechol itself or to protoanemonin formed during the incubation, washed cells of Pseudomonas sp. B13 grown on acetate, benzoate, or 3-chlorobenzoate were incubated with 4-chlorocatechol. A significant decrease in colony-forming units was observed only for benzoate-grown cells (Fig. 2). Whereas benzoate- and 3-chlorobenzoate-grown cells showed a rapid turnover of 4-chlorocatechol in less than 15 min, acetate-grown cells consumed the compound within 4 h. 3-Chlorobenzoate-as well as acetate-grown cells metabolized 4-chlorocatechol without any detectable accumulation of intermediates. Accumulation of protoanemonin was, however, observed in the supernatant of benzoate-grown cells. The amount of protoanemonin produced accumulated up to 30% of that expected for the stoichiometric transformation of 4-chlorocatechol. We therefore conclude that 4-chlorocatechol itself has no major toxic effect but that cell death is due to protoanemonin.

In order to study the effect of protoanemonin on various xenobiotic-degrading bacteria, we synthesized this compound from 4-chlorocatechol in a coupled reaction (catechol 1,2-dioxygenase type I and muconate cycloisomerase) catalyzed by cell-free extracts of Pseudomonas sp. B13 bacteria grown on benzoate. Pure preparations of protoanemonin were obtained after extraction with diethyl ether with a yield higher than 90%. In all cases, protoanemonin inhibited the growth of microorganisms, concentrations between 15 and 150 ppm being bacteriostatic (Table III).

### DISCUSSION

Mechanistic and Evolutionary Implications of Protoanemonin Formation—Since several studies have shown that muconate cycloisomerase and chloromuconate cycloisomerase are evolutionarily closely related (31–34), a common reaction mechanism for both types of enzymes has been assumed (12). The stereocchemistry of the reaction catalyzed by the muconate cycloisomerase of P. putida with muconate as substrate has been elucidated (Fig. 3A) and a carbamion has been postulated as the mechanistic intermediate (10). The currently accepted mechanism for the enzymatic reaction involves the formation of a metal-stabilized enol/enolate intermediate followed by a vinyllogous E2 reaction (1,4-elimination) (35, 36). Although this reaction model explains the dechlorination of 4-chloromuconolactone catalyzed by chloromuconate cycloisomerase (Fig. 3B), because of the double bond linking the carboxylate anion, the proposed intermediate does not explain the formation of protoanemonin in the reaction catalyzed by muconate cycloisomerase. Other workers have postulated that 4-chloromuconolactone is formed by protonation of the corresponding carbamion as intermediate and have assumed that this compound is chemically unstable, such that cis-dienelactone is generated spontaneously by anti-elimination of hydrogen chloride or chloride from the carbanion (6) in a nonenzymatic collateral reaction (Fig. 3C, part 2). However, if this were the case, the product of the reaction with 3-chloromuconate should be always cis-dienelactone, independent of the type of enzyme catalyzing the reaction. This reasoning, together with our new findings, has led us to propose 4-chloromuconolactone as the intermediate from which decarboxylation and dechlorination occur concomitantly in a single reaction catalyzed by muconate cycloisomerase (Fig. 3C, part 1). For the reaction catalyzed by chloromuconate cycloisomerase, an active site base must be postulated, which catalyzes fast elimination of the solvent-derived C-5 proton and the halide, thus generating cis-dienelactone (Fig. 3C, part 2).

Despite their high degree of homology, it is evident that muconate cycloisomerase and chloromuconate cycloisomerase are not merely isoenzymes with distinct substrate specificities. Different mechanisms were observed not only with 3- but also with 2-chloro-cis,cis-muconate (13), indicating different active site structures. It is interesting to speculate that evolution of the chloromuconate cycloisomerase active site structure may have been selected by the need to prevent formation of the antibiotic protoanemonin. The three-dimensional structure of both muconate cycloisomerase from P. putida (37) and of chloromuconate cycloisomerase from Alcaligenes eutrophus JMP 134 (38) have now been elucidated, and conformational differences in the active site as well as differences in the polarity and size of the channel leading to the active site were reported. Site-directed mutagenesis will give insights into amino acids responsible for variation in substrate specificity and transformation mechanisms.

The Ecological Implication of Protoanemonin—Protoanemonin has been known since 1945 as an active constituent of plants of the Ranunculaceae family, which is antibiologically active against a wide spectrum of microorganisms (39). We
Report here on its bacterial formation from 4-chlorocatechol, a central intermediate of the catabolism of chloroaromatic compounds, by enzymes of the widespread 3-oxoadipate pathway. Protoanemonin formation was observed in vitro and in vivo, resulting in cell death. As being released from the cells, it may subsequently affect the entire ecosystem in which it is produced. Formation of protoanemonin has to be taken into consideration when microorganisms harboring incomplete catabolic pathways are confronted with chloroaromatics. Accordingly, the inhibitory effects of chlorobenzoates on chlorobiphenyl metabolism and degradation by co-inoculation of chlorobenzoate degraders has been observed. Havel and Reineke (40) attributed the inhibition of mineralization of polychlorinated biphenyls by laboratory-selected microorganisms in soil microcosms to an unknown toxic metabolite produced from 4-chlorobenzoate (the end-product of 4-chlorobiphenyl metabolism by the biphenyl degraders used in this study) by the native microflora present in their soil slurries. The positive effect on such survival achieved by co-inoculation with chlorobenzoate degraders (41, 42) is consistent with this hypothesis. Microcosm experiments performed in our laboratories have shown protoanemonin formation by the natural microflora to be responsible for the poor survival of polychlorinated biphenyls metabolizing bacteria in environmental settings (44). We assume that the microbial formation of protoanemonin will occur not only in the presence of chlorinated aromatic pollutants but also during the catabolism of fluoroaromatics. The cycloisomerization product of 3-fluoro-cis,cis-muconate was reported to be transformed by enolactone hydrolase of P. putida strain A 3.12 predominantly into a new compound that was tentatively identified by its UV-spectroscopic properties as protoanemonin (43). If this result is subsequently confirmed, it seems that protoanemonin can be formed by two completely different enzyme reactions, namely those carried out by muconate cycloisomerase and by enolactone hydrolase, according to the nature of the halosubstituent of cis,cis-muconate.

Acknowledgments—We thank Manfred Nimtz and Victor Wray for performing gas chromatography-mass spectrometry and NMR analysis, Michael Schömann for fruitful discussions, and Dave McKay for critical reading of the manuscript.

REFERENCES
1. Apajalahti, J. H. A., and Saikin(1987) J. Bacteriol. 169, 675–681
2. Schotten, J. D., Chang, K.-H., Babitt, P. C., Charosh, H., Sylvestre, M., and Dunaway-Mariano, D. (1992) Science 253, 182–185
3. Sander, P., Wittich, R.-M., Fortnagel, P., Wilkes, H., and Francke, W. (1991) Appl. Environ. Microbiol. 57, 1430–1440
4. Fettner, S., Müller, D., and Lingens, F. (1992) J. Bacteriol. 174, 279–290
5. Reineke, W., and Knackmuss, H.-J. (1988) Ann. Rev. Microbiol. 42, 263–287
6. Dorn, E., and Knackmuss, H.-J. (1978) Biochem. J. 174, 73–84
7. Dorn, E., and Knackmuss, H.-J. (1978) Biochem. J. 174, 85–94
8. Schmidt, E., and Knackmuss, H.-J. (1980) Biochem. J. 192, 339–347
9. Reineke, W. (1984) in Microbial Degradation of Organic Compounds (Gibson, D. T., ed. pp. 319–360, Marcel Dekker, New York
10. Ngai, K.-L., and Kallin, R. G. (1983) Biochemistry 22, 5231–5236
11. Chari, R. V. J., Whitman, C. P., Kozarich, J. W., Ngai, K.-L., and Ornston, L. N. (1987) J. Am. Chem. Soc. 109, 5514–5519
12. Schömann, M., Fischer, P., Schmidt, E., and Knackmuss, H.-J. (1990) J. Bacteriol. 172, 5129–5139
13. Vollmer, M. D., Fischer, P., Knackmuss, H.-J., and Schömann, M. (1994) J. Bacteriol. 176, 4366–4375
14. Dorn, E., Hellwig, M., Reineke, W., and Knackmuss, H.-J. (1974) Arch. Microbiol. 99, 61–70
15. Franklin, F. C. H., Bagdasarian, M., Bagdasarian, M. M., and Timmis, K. N. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 7458–7462
16. Wittlich, R.-M., Wilkes, H., Sinnerfell, V., Francke, W., and Fortnagel, P. (1992) Appl. Environ. Microbiol. 58, 1003–1010
17. Bopp, L. H. (1986) J. Indust. Microbiol. 3, 23–29
18. Hernandez, B. S., Higon, K., Kondrat, R., and Focht, D. D. (1991) Appl. Environ. Microbiol. 57, 3361–3366
19. Taïra, K., Hayase, N., Arimura, N., Yamashita, S., Miyazaki, T., and Furukawa, K. (1988) Biochemistry 27, 3990–3996
20. Bedard, D. L., Wagner, R. E., Bresnan, M., Habel, M., and Brown, J. F. (1987) Appl. Environ. Microbiol. 53, 1094–1102
21. Astorius, C. A., and Timmis, K. N. (1993) J. Bacteriol. 175, 4631–4640
22. Sistrom, W. R., and Stainer, R. Y. (1954) J. Biol. Chem. 210, 821–836
23. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
24. Laemmli, U. K. (1970) Nature 227, 680–685
25. Reineke, W., and Knackmuss, H.-J. (1984) Appl. Environ. Microbiol. 47, 395–402
26. Schreiber, A., Hellwig, M., Dorn, E., Reineke, W., and Knackmuss, H.-J. (1980) Appl. Environ. Microbiol. 39, 58–67
27. Evans, W. C., Smith, B. S. W., Mass, P., and Ferry, H. N. (1971) Biochem. J. 122, 509–517
28. Schneid, E., Remberg, G., and Knackmuss, H.-J. (1980) Biochem. J. 192, 331–337
29. Piek, W. A., and Kozarich, J. W. (1990) J. Org. Chem. 55, 3029–3035
30. Shaw, E. (1946) J. Am. Chem. Soc. 68, 2510–2513
31. Aldrich, T. L., Frantz, B., Gill, J. F., Kilbane, J. J., and Chakrabarty, A. M. (1987) Gene (Amst.) 52, 189–195
32. Frantz, B., and Chakrabarty, A. M. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 4460–4464
33. Ghosal, D., and You, I. S. (1988) Mol. & Gen. Genet. 211, 13–120
34. Ghosal, D., and Ghosal, P. G. (1992) J. Am. Chem. Soc. 114, 5928–5934
36. Mazur, P., Pieken, W. A., Budihas, S. R., Williams, S. E., Wong, S., and Kozarich, J. W. (1994) Biochemistry 33, 1961–1970
37. Goldman, A., Ollis, D. L., and Steitz, T. A. (1987) J. Mol. Biol. 194, 143–153
38. Hoier, H., Schlömann, M., Hammer, A., Glusjer, J. P., Carrell, H. L., Goldman, A., Stezowski, J. J., and Heinemann, U. (1994) Acta Crystallogr. Sect. D 50, 75–84
39. Seegal, B. C., and Holden, M. (1945) Science 101, 413–415
40. Havel, J., and Reineke, W. (1992) Appl. Microbiol. Biotechnol. 38, 129–134
41. Hickey, W. J., Searles, D. B., and Focht, D. D. (1993) Appl. Environ. Microbiol. 59, 1194–1200
42. Sondossi, M., Sylvestre, M., and Ahmad, D. (1992) Appl. Environ. Microbiol. 58, 485–495
43. Schlömann, M. (1988) Die verschiedenen Typen der Dienlacton-Hydrolase und ihre Rolle beim bakteriellen Abbau von 4-Fluorobenzol. Ph.D. thesis, University of Stuttgart, Germany
44. Pieper, D. H., Blasco, R., Wittich, R.-M., Megharaj, M., Fock, U., Prucha, M., Seege, M., Hofer, B., and Timmis, K. N. (1995) The Year of Louis Pasteur International Symposium: Microbes, Environment, Biotechnology, Tahiti, French Polynesia, May 8–12, 1995 (Aubert, J. P., and Martin, D. M. V., eds) pp. 85–89, Institut Louis Malardé, Papeete, Tahiti
From Xenobiotic to Antibiotic, Formation of Protoanemonin from 4-Chlorocatechol by Enzymes of the 3-Oxoadipate Pathway
Rafael Blasco, Rolf-Michael Wittich, Megharaj Mallavarapu, Kenneth N. Timmis and Dietmar H. Pieper

J. Biol. Chem. 1995, 270:29229-29235.
doi: 10.1074/jbc.270.49.29229

Access the most updated version of this article at http://www.jbc.org/content/270/49/29229

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 41 references, 23 of which can be accessed free at http://www.jbc.org/content/270/49/29229.full.html#ref-list-1