3-(2-Hydroxyphenyl)catechol as Substrate for Proximal meta Ring Cleavage in Dibenzofuran Degradation by Brevibacterium sp. Strain DPO 1361

VOLKER STRUBEL,1 KARL-HEINRICH ENGESSER,1* PETER FISCHER,2* AND HANS-JOACHIM KNACKMUSS1

Institut für Mikrobiologie1 and Institut für Organische Chemie,2 Universität Stuttgart, D-7000 Stuttgart 1, Federal Republic of Germany

Received 5 July 1990/Accepted 2 January 1991

Brevibacterium sp. strain DPO 1361 oxygenates dibenzofuran in the unusual angular position. The 3-(2-hydroxyphenyl)catechol thus generated is subject to meta ring cleavage in the proximal position, yielding 2-hydroxy-6-(2-hydroxyphenyl)-6-oxo-2,4-hexadienoic acid, which is hydrolyzed to 2-oxo-4-pentenoate and salicylate by 2-hydroxy-6-0xo-6-phenyl-2,4-hexadienoic acid hydrase. The proximal mode of ring cleavage is definitely established by isolation and unequivocal structural characterization of a cyclization product of 2-hydroxy-6-(2-hydroxyphenyl)-6-oxo-2,4-hexadienoic acid, i.e., 3-(chroman-4-on-2-yl)pyruvate.

Dibenzofuran (DBF) has been used in some recent studies as a model compound for investigating the microbial degradation of cyclic biaryl ethers (5-7, 19). Public attention has focused on this class of compounds, since it comprises some of the most pernicious and persistent molecules, such as TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin). For DBF, the most simple cyclic biaryl ether, a novel degradation mechanism involving angular dioxygenation has been described (5-7), with 3-(2-hydroxyphenyl)catechol (HPC) as a central intermediate. Definite proof for this mechanism is presented in this paper, and the total degradation of DBF is described.

**MATERIALS AND METHODS**

Organisms. Strain DPO 1361 was characterized preliminarily by the Deutsche Sammlung für Mikroorganismen, Braunschweig, Federal Republic of Germany (FRG) as a Brevibacterium species. Pseudomonas pseudoalcaligenes KF744 was kindly supplied by K. Furukawa (Tsukuba, Japan), who described it as a constitutive mutant of a biphenyl-degrading organism lacking metapyrocatechase (8). Strain BN6, which was characterized as metabolizing sulfonated naphthalenes to substituted salicylates (13), was used to accumulate definite ring cleavage products of phenylcatechols (12).

Growth conditions. Strain DPO 1361 was cultivated as described previously (19) with DBF as the sole source of carbon; vitamin B12 was added to the growth medium to a final concentration of 10 ppm. Strain KF744 bpcH was cultivated on a complex medium (19). Strain BN6 was grown in 1 liter of mineral medium containing 10 mM glucose. 2-Naphthalenesulfonic acid (0.5 mM) was added during the early exponential growth phase to obtain cells induced to catalyze biphenyl derivatives. Cells were harvested in the late exponential growth phase (optical density at 546 nm, 0.8).

Enzyme assays. One unit of enzyme activity was defined as the amount of enzyme converting 1 μmol of substrate per min. Methods for preparing cell extracts and for measuring protein content were described previously (15, 16).

* Corresponding authors.

**Metapyrocatechase activity (EC 1.13.11.2; catechol:oxygen 2,3-oxidoreductase)** was determined in phosphate buffer (50 mM, pH 7.5). For the individual preparations, the following extinction coefficients of the ring cleavage products were used: 3-phenylcatechol (PC) (λ_max, 434 nm), 22 cm² μmol⁻¹; catechol (λ_max, 375 nm), 36 cm² μmol⁻¹; 3-methylcatechol (λ_max, 382 nm), 32 cm² μmol⁻¹; 4-methylcatechol (λ_max, 388 nm), 17 cm² μmol⁻¹; and 3-isopropylcatechol (λ_max, 389 nm), 13 cm² μmol⁻¹ (3). For monitoring enzyme activity during the purification procedures, the enzyme was reactivated by incubating the eluted fractions with a mixture of (NH₄)₂Fe(SO₄)₂ (2 mM) and L-ascorbic acid (5 mM) for 30 min.

2-Hydroxy-6-oxo-6-phenyl-2,4-hexadienoic acid (HOPDA)-hydrolyzing enzyme activity (EC category, 3.1.13.11.2) was measured in phosphate buffer (50 mM, pH 7.4) by a modification of the method of Omori et al. (14). HOPDA was produced from PC by resting cells of strain BN6 in phosphate buffer, and its concentration was determined photometrically (12). The culture broth was centrifuged, and the supernatant was diluted fivefold to a final HOPDA concentration of 0.08 mM. The decrease in the HOPDA concentration in crude extracts and partially purified enzyme fractions of DBF-grown cells of strain DPO 1361 was measured photometrically at 434 nm. The reaction rates were calculated on the basis of an extinction coefficient of 22 cm² μmol⁻¹.

**Enzyme purification.** Proteins were purified at the ambient temperature on a fast protein liquid chromatography system consisting of an LCC 500 controller, a 555 pump, a UV-1 monitor, an REC-482 recorder, and an FRAG autosampler (all from Pharmacia, Uppsala, Sweden). Crude extracts of DBF-grown cells of strain DPO 1361 were filtrated and applied to a Mono-Q column (HR 5/5; Pharmacia). Samples were eluted with 80 ml of a linear gradient of NaCl (0 to 2 M) in Tris HCl (50 mM, pH 7.5; flow rate, 0.7 ml/min). Fractions (1 ml) were collected, and the respective enzyme activities were determined after reactivation.

**HPLC.** High-pressure liquid chromatography (HPLC) analyses were carried out with an HPLC system from Merck, Darmstadt, FRG, an RP-8 Lichrosorb column (125 by 4.6 mm [internal diameter]; Bischoff, Leonberg, FRG),...
and water-methanol and water-acetonitrile as the mobile phases, both adjusted with H₃PO₄ to a final pH of 2.1.

**Spectroscopy.** ¹H Fourier transform nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz (CD₃CN, with tetramethylsilane as an internal standard; 32 K transforms; NMR spectrometer CXP 300 with data system Aspect 2000 [Bruker, Karlsruhe, FRG]). The mass spectrum of 2-oxo-4-pentenoate was recorded on a API 3 mass spectrometer (Sciex, Toronto, Ontario, Canada) by atmospheric pressure ion-spray ionization. The sample was diluted in methanol-water (50:50 [vol/vol]) with 100 ppm of ammonium acetate and injected at a flow rate of 5 µl/min. The electron impact (EI) high-resolution mass spectrum of 3-(chroman-4-0x0-2-yl)pyruvate was determined on a MAT 711 mass spectrometer (20 eV; source temperature, 360 K [Varian MAT, Bremen, FRG]). The mass spectrum of the bishydrazone was determined on a Finnigan 4023/Incos 2300 quadrupole mass spectrometer with chemical ionization (CH₄; direct probe inlet; ballistic heating [Finnigan, San Jose, Calif.]).

**Chemicals.** Chemicals were of the highest purity commercially available (Merck; EGA-Chemie, Steinheim, FRG; and Serva, Heidelberg, FRG). PC was obtained from Wako Chemicals (Neuss, FRG) and 4-phenylcatechol was obtained from Promochem (Wesel, FRG). 3-Chlorocatechol was obtained by chlorination of catechol by the method of Willstätter and Müller (20).

**RESULTS**

*Brevibacterium* sp. strain DPO 1361 was shown to degrade DBF via initial angular dioxygenation (5). In this reaction, which is quite unexpected from a chemical point of view, the chemically very stable aryl ether bond is transformed into a hemiacetal structure. Spontaneous cleavage of the hemiacetal and subsequent rearomatization produces HPC, which has been proposed to be a central intermediate in this novel DBF degradation pathway (5).

**HPC as the first product of DBF dioxygenation.** When DBF-grown cells of strain DPO 1361 were incubated with a mixture of DBF and 3-chlorocatechol (3 mM and 1 mM, respectively), an established inhibitor of metapyrocatechase (4, 11), one main metabolite was shown to accumulate by HPLC (retention volume, 3.41 min; methanol-water-H₃PO₄, 50:49:9.0:1 [vol/vol/vol]). The UV spectrum, measured in situ (methanol-water-H₃PO₄, 50:49:9.0:1 [vol/vol/vol]), of this metabolite displayed a characteristic maximum at 284 nm. The metabolite was characterized as HPC (see below). In this experiment, 3-chlorocatechol was metabolized very slowly. Only after its total conversion was HPC, which accumulated as an intermediate, metabolized further, with the concomitant formation of a bright yellow color which was due to 2-hydroxy-6-(2-hydroxyphenyl)-6-oxo-2,4-hexadienoic acid (2'-OH-HOPDA) (Fig. 1, compound 5).

Strain KF744 bphC was used to prepare HPC by an independent microbial pathway. Resting cells of this mutant strain, which is blocked in the biphenyl pathway, were confirmed to accumulate PC from biphenyl. This product was identified with authentic material by HPLC (retention volume, 3.81 min; methanol-water-H₃PO₄, 50:49:9.0:1 [vol/vol/vol]; λₘₚₓ, 272 nm). When strain KF744 bphC cells were incubated under identical conditions with 2-hydroxybiphenyl, a single product was observed to accumulate. This product had UV-visible spectrum and HPLC behavior identical to those of the HPC metabolite obtained from the transformation of DBF by strain DPO 1361 after inhibition of ring cleavage enzymes with 3-chlorocatechol. When the two metabolites were applied, as trimethylsilyl derivatives, to coupled gas chromatography-mass spectroscopy analysis, the same parent peak was obtained at m/z 419, confirming the trihydroxybiphenyl structure of the DBF metabolite (Fig. 1, compound 4).

**Stoichiometry of HPC formation.** Resting cells of strain DPO 1361 grown on DBF (optical density at 546 nm, 5) were incubated with a mixture of DBF (3 mM) and 3-chlorocatechol (1 mM) as described above. This time, transformation was stopped after 30 min by centrifugation. Water-soluble metabolites were analyzed directly from the culture medium by HPLC (methanol-water-H₃PO₄, 50:49:9.0:1 [vol/vol/vol]). Suspended unsoluble substrate was dissolved by adding 4
volumes of dioxane, and the resulting solution was analyzed by HPLC (acetonitrile-water-H₃PO₄, 60:39:9:0.1 [vol/vol/vol]). In this manner, the respective DBF, HPC, and salicylate concentrations after 30 min were determined as 1.1, 1.3, and 0.2 mM, respectively. Thus, 70% of the DBF transformed was accumulated as HPC, unequivocally establishing that this metabolite is the key intermediate in DBF degradation.

Catabolism of HPC with partially purified enzymes. Crude extracts from DBF-grown cells of strain DPO 1361 transformed HPC into salicylate and one additional metabolite, which was shown by HPLC to be identical to the metabolite found in the supernatants of DBF-grown cells of strain DPO 1361 and strain DPO 220 (empirical formula, C₁₂H₁₀O₃) (19). For a detailed analysis of the HPC-metabolizing enzyme system, a crude extract from DBF-grown cells of strain DPO 1361 was fractionated on Mono-Q–Sepharose with a NaCl gradient (Fig. 2). With catechol and PC as substrates, metapyrocatechase activity could be detected photometrically in three fractions. The first metapyrocatechase enzyme (type I) was eluted at 0.27 M NaCl and, after reactivation with Fe²⁺ and ascorbic acid, showed high activity (4.5 U/ml) for catechol. The second metapyrocatechase enzyme (type II) was eluted at 0.3 M NaCl and showed comparable activity for PC and catechol (0.41 and 0.40 U/ml, respectively). Low activity (0.1 U/ml for both PC and catechol) was present in the third fraction (0.34 M NaCl) and was attributed to contamination with the type II enzyme. Activities for a series of catechol derivatives are shown in Table 1.

Only the type II metapyrocatechase (0.3 M NaCl) was able to substantially metabolize HPC (as monitored by HPLC; Table 1).

![FIG. 2. Mono-Q ion-exchange chromatography of a crude extract of strain DPO 1361. A crude extract of strain DPO 1361 (protein content, 20 mg/ml) was applied to a Mono-Q column (HR 5/5) and eluted with 80 ml of a linear gradient of NaCl (0 to 2 mM) in Tris HCl (50 mM, pH 7.5; flow rate, 0.7 ml/min). The protein content of the eluent was detected photometrically at 280 nm. The rate of enzyme activity in the eluted fractions (0.7 ml) was determined after reactivation with (NH₄)₂Fe(SO₄)₂ and L-ascorbic acid as follows: +, rate of HOPDA hydrolysis (units per milliliter); □, rate of catechol cleavage (units per milliliter); ×, rate of PC cleavage (units per milliliter).](image-url)

TABLE 1. Enzyme activities of partially purified metapyrocatechases in DBF-grown strain DPO 1361

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Activity of metapyrocatechase of type:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>U/ml</td>
</tr>
<tr>
<td>PC</td>
<td>4.5</td>
</tr>
<tr>
<td>4-Phenylcatechol</td>
<td>0.00</td>
</tr>
<tr>
<td>Catechol</td>
<td>0.11</td>
</tr>
<tr>
<td>3-Methylcatechol</td>
<td>0.31</td>
</tr>
<tr>
<td>4-Methylcatechol</td>
<td>0.1</td>
</tr>
<tr>
<td>3-Isopropylcatechol</td>
<td>0.13</td>
</tr>
</tbody>
</table>

* Metapyrocatechase activities were measured as described in Materials and Methods. The type I enzyme was collected in fraction 45 of the fast protein liquid chromatography purification run, and the type II enzyme was collected in fraction 48 (see the text). The reaction mixture contained 20 ml of substrate (0.4 mM) in 960 ml of phosphate buffer (50 mM, pH 7.5). The reaction was started with 20 ml of the respective protein fraction. The protein contents of fractions 45 and 48 were 1.86 and 0.84 mg/ml, respectively. The increase in the absorbances of the products was monitored at the respective wavelength of maximal absorption.

* Reported as a percentage. The specific activity is given in parentheses (units per milligram).
VOL. 6 h. The enzyme could not for product characterization, see below). The type I metapyrocatechase showed no activity at all with HPC. Both enzymes had an optimum at pH 7.5 in potassium phosphate buffer system. The type I enzymes had an optimum at pH 7.5 in potassium phosphate buffer system. The type II enzymes had an optimum at pH 7.5 in potassium phosphate buffer system. The type II enzymes had an optimum at pH 7.5 in potassium phosphate buffer system.

acetone (10 and 20% [vol/vol]); ethanol (10 and 20% [vol/vol]); mercaptoethanol (5 mM); glutathione (4 mM); (NH₄)₂Fe (SO₄)₃ (2 mM); or ascorbic acid (5 mM).

In contrast, only one metapyrocatechase could be detected when a crude extract from salicylate-grown cells of strain OBF-grown cells. Metabolite M was therefore incorporated into the DBF C₁₂ skeleton in the course of the metabolism. On the basis of this evidence and the NMR spectral evidence presented below, the chromanone structure was proposed for this metabolite (Fig. 3). This compound was formed from the primary meta ring cleavage product, 2'-OH-HOPDA, by straightforward intramolecular Michael addition of the phenolic OH group to the α,β-unsaturated ketone side chain.

The 300-MHz ¹H NMR spectrum of metabolite M displayed a typical salicylate-type pattern in the aromatic region. The aliphatic region showed, besides a broad OH signal, just one intercorrelated -CH²H-OH₂CH₂CH₂H² spin system (Fig. 3). The highly negative values for the respective geminal couplings between H² and H² and between H² and H² showed that the two methylene groups were in a fixed bisected orientation in relation to a neighboring π-bond (Table 2).

Definite proof of this structure came from the mass spectrum [chemical ionization with methane as reagent gas, CI (CH₄)] of bis(2,4-dinitrophenylhydrazone) prepared from the metabolite by a standard procedure (38). The ¹H NMR spectrum of the bishydrazone showed two sets of AMX subspectra for two 2,4-dinitrophenylamino moieties in slightly different chemical environments in addition to the more or less unchanged spectrum of the metabolite backbone. The first fragment ion in the chemical ionization mass spectrum appeared at m/z 368 (8%) and originated from the N-protonated bishydrazone of metabolite M via a McLafferty-type rearrangement which eliminated 2,4-dinitrophenylhydrazine (m/z 199; adduct ions at m/z 227, 239) was observed. Both primary fragment ions could be rationalized only on the basis of the chromanone structure (Fig. 3) proposed for the metabolite in question.

This structure in turn proves that HPC is cleaved in meta fashion. Accordingly, we propose that 2'-OH-HOPDA is the primary ring cleavage product.

**Table 2. ¹H NMR data for 3-(chroman-4-on-2-γ)pyruvate (metabolite M)⁴**

<table>
<thead>
<tr>
<th>Proton assignment</th>
<th>δ (ppm)</th>
<th>⁴J (Hz)</th>
<th>⁵J (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H²</td>
<td>7.816</td>
<td>7.8 (5, 6)⁵</td>
<td></td>
</tr>
<tr>
<td>H⁶</td>
<td>7.062</td>
<td>7.3 (6, 7)⁵</td>
<td></td>
</tr>
<tr>
<td>H⁷</td>
<td>7.526</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H⁸</td>
<td>6.942</td>
<td>8.5 (7, 8)⁵</td>
<td></td>
</tr>
<tr>
<td>H⁹</td>
<td>5.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H²⁶</td>
<td>3.479</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H²⁶′</td>
<td>3.223</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H²⁶''</td>
<td>2.724</td>
<td>-16.9 (C, D)</td>
<td>3.7 (C, D)⁵</td>
</tr>
<tr>
<td>H²⁶''′</td>
<td>2.818</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

⁴ Nominal frequency, 300.13 MHz; <0.01 M in d₄-acetone; 298 K; internal standard, tetramethylsilane; digital resolution, ±0.025 Hz per point.

⁶ meta couplings: ⁴J (5, 7), 1.6 Hz; ⁵J (6, 8), 0.8 Hz.

The molecular composition of this metabolite was established to be C₃H₂O₃ from the parent peak of an EI high-resolution mass spectrum (m/z 234.0527; theoretical, 234.0528). Two moles of oxygen and 1 mol of hydrogen were therefore incorporated into the DBF C₁₂ skeleton in the course of the metabolism. On the basis of this evidence and the NMR spectral evidence presented below, the chromanone structure was proposed for this metabolite (Fig. 3). This compound was formed from the primary meta ring cleavage product, 2'-OH-HOPDA, by straightforward intramolecular Michael addition of the phenolic OH group to the α,β-unsaturated ketone side chain.

The 300-MHz ¹H NMR spectrum of metabolite M displayed a typical salicylate-type pattern in the aromatic region. The aliphatic region showed, besides a broad OH signal, just one intercorrelated -CH²H-OH₂CH₂CH₂H² spin system (Fig. 3). The highly negative values for the respective geminal couplings between H² and H² and between H² and H² showed that the two methylene groups were in a fixed bisected orientation in relation to a neighboring π-bond (Table 2).

Definite proof of this structure came from the mass spectrum [chemical ionization with methane as reagent gas, CI (CH₄)] of bis(2,4-dinitrophenylhydrazone) prepared from the metabolite by a standard procedure (38). The ¹H NMR spectrum of the bishydrazone showed two sets of AMX subspectra for two 2,4-dinitrophenylamino moieties in slightly different chemical environments in addition to the more or less unchanged spectrum of the metabolite backbone. The first fragment ion in the chemical ionization mass spectrum appeared at m/z 368 (8%) and originated from the N-protonated bishydrazone of metabolite M via a McLafferty-type rearrangement which eliminated 2,4-dinitrophenylhydrazine (m/z 199; adduct ions at m/z 227, 239) was observed. Both primary fragment ions could be rationalized only on the basis of the chromanone structure (Fig. 3) proposed for the metabolite in question.

This structure in turn proves that HPC is cleaved in meta fashion. Accordingly, we propose that 2'-OH-HOPDA is the primary ring cleavage product.

**Hydrolysis of the HPC ring cleavage product.** No further conversion of metabolite M was observed with either crude extracts or resting cells (DBF grown). In analogy to the pathway established for diphenyl, it seemed reasonable to
assume that the primary product of ring cleavage, 2'-OH-HOPDA, was hydrolyzed in the same manner as HOPDA (14). Crude extracts from DBF-grown cells of strain DPO 1361 indeed exhibited hydrolysis activity for 2'-OH-HOPDA. The hydrolyase was detected photometrically by monitoring the mass spectrum of the product showed the expected molecular ion peak at m/z 113.

**DISCUSSION**

We have demonstrated previously that fluorene is attacked by strain DPO 1361 via dioxygenation in the angular position (5). Since the same mechanism is operative in DBF degradation, as shown in the present paper, this newly discovered angular dioxygenation seems to be of crucial importance for the degradation of cyclic biaryl ether structures. The same mechanism was proposed recently for a gram-negative strain as well (6, 7).

DBF-grown cells of strain DPO 1361 accordingly accumulated HPC when incubated with DBF in the presence of 3-chlorocatechol, a well-established metapyrocatechase inhibitor (4, 11). Gas chromatography-mass spectrometry analysis of the 2-hydroxybibenyl metabolite product of mutant strain KF744 bphC confirmed the proposed structure. The dienediol generated by this angular dioxygenation (Fig. 1) has a chemically labile hemiacetal structure and is rearranged with cleavage of the ary ether bond and subsequent reamination to HPC. The first step of this reaction sequence may be spontaneous or enhanced enzymatically.

When crude extracts of DBF-grown cells of strain DPO 1361 were incubated with HPC or PC, a yellow color was observed, once more indicating meta ring cleavage of these substrates. Two different metapyrocatechases were discovered in the course of protein purification. One of them (type I) exhibited high activity for PC but was essentially unable to transform either HPC or catechol. The type II metapyrocatechase showed low activity for PC and catechol but was able to transform HPC. This enzyme (type II) is the relevant enzyme for the DBF-degrading pathway. The function of the type I metapyrocatechase is not yet understood. Its presence in DBF-grown cells may be rationalized in terms of an evolutionary relationship of DBF and biphenyl pathways (5). Whereas the yellow color of the PC ring cleavage product (HOPDA) remained stable for days (1), the HPC ring cleavage product was transformed to the colorless metabolite M within seconds. Its generation can be rationalized in terms of spontaneous intramolecular Michael addition of 2'-OH-HOPDA (Fig. 2). Metabolite M showed no substantial biological turnover but was chemically unstable (19). As described above, it was unequivocally characterized by mass spectroscopy and 1H NMR as 3-(chroman-4-on-2-yl)pyruvate.

2'-OH-HOPDA was shown to be hydrolyzed in crude extracts or by a partially purified enzyme of strain DPO 1361 (DBF grown) in the same manner as that described for HOPDA (1, 14). Both of the products expected for 2'-OH-HOPDA hydrolysis via this pathway, i.e., salicylate and 2-oxo-4-pentenooate, could actually be identified under these conditions.

Further investigations will be required to show whether the same angular dioxygenation mechanism is involved in the degradation of dibenzodioxin as well. First results indicate that DBF-grown cells of Brevibacterium sp. strain DPO 1361 indeed attack dibenzodioxin in the angular position, as has also been suggested for a *Pseudomonas* species (10).

**ACKNOWLEDGMENTS**

We thank P. Metzger and G. Jung for HPLC-coupled mass spectroscopy, we gratefully acknowledge the preparation of 2-oxo-4-pentenooate by A. Schmid. We are indebted to H. G. Rast (Bayer AG) for many helpful discussions. We are grateful to W. Rozdzinski for performing mass spectroscopy and to J. Reboll for running the NMR measurements.
REFERENCES


3-(2-Hydroxyphenyl)Catechol as Substrate for Proximal \textit{meta} Ring Cleavage in Dibenzofuran Degradation by \textit{Brevibacterium} sp. Strain DPO 1361

VOLKER STRUBE, KARL-HEINRICH ENGESSER, PETER FISCHER, AND HANS-JOACHIM KNACKMUSS

Institut für Mikrobiologie and Institut für Organische Chemie, Universität Stuttgart, D-7000 Stuttgart 1, Federal Republic of Germany

Volume 173, no. 6, p. 1932, column 2, line 7: "3-methylcatechol" should read "4-methylcatechol"; "4-methyl-" should read "3-methyl-".