Synthesis of Bacterial Metabolites from Haloaromatic Degradation. 1. Fe(III)-Catalyzed Peracetic Acid Oxidation of Halocatechols, a Facile Entry to *cis,cis*-2-Halo-2,4-hexadienedioic Acids and 3-Halo-5-oxo-2(5*H*)-furanylideneacetic Acids

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Introduction

Haloaromatic compounds, which play an important role as industrial products, present considerable environmental problems because of their toxicity, bioconcentration, and persistence in the biosphere. The microbial degradation of these substances has thus received increased attention.

Many chloroaromatics have been shown to be degraded by bacteria via chloro-substituted catechols as key intermediates. The catechols are cleaved in a 1,2-dioxygenation reaction, yielding the corresponding *cis,cis*-2,4-hexadienedioic acids.[1] Elimination of Cl^- occurs during or directly after cycloisomerization of the *cis,cis*-chloro-2,4-hexadienedioic acids. Further degradation of the resulting 5-oxo-2(5*H*)-furanylideneacetic acids proceeds through 4-oxo-2-hexenedioic acids.[2]

Until now, the interesting intermediate halogenated *cis,cis*-2,4-hexadienedioic acids as well as 5-oxo-2(5*H*)-furanylideneacetic acids were only available with expenditure of time through bioconversion reactions with whole cells or purified enzyme preparations in low yield.[3-6] Thus, here we report the chemical synthesis of **2a–d** and **4c–d**.

Results and Discussion

Probably the best method for stereospecific synthesis of *cis,cis*-2,4-hexadienedioic acids is the oxidative ring-cleavage of a phenolic system. 3,5-Di-*tert*-butylcatechol as well as different methylcatechols has been converted to *cis,cis*-2,4-hexadienedioic acids under various conditions.[7-14] We have applied oxidation with peracetic acid or with lead tetraacetate, both proceeding via *o*-quinones as intermediates.

Oxidation of aromatic compounds with peracetic acid was first developed for phenol by Boeseken and Engelberts[15] and applied to catechol by Wacek and Fiedler.[16] The catalytic influence of various metal ions as well as the reaction mechanism was extensively studied by Pandell.[17,18] Application of the method for the preparation of *cis,cis*-3-halo-2,4-hexadienedioic acids yielded only mixtures of *cis,trans*-isomers and lactonization products.[19,20] One possible explanation is the instability of *cis,cis*-3-halo-2,4-hexadienedioic acids, which can be attributed to the steric and stereoelectronic repulsion between the 3-halo substituent and the C-6-carboxyl group.[21] In *cis,cis*-2-halo-2,4-hexadienedioic acids this interaction is absent; therefore, they should be more stable in weakly acidic media.

Oxidation of the 3-halocatechols was carried out at room temperature by very slow addition of a saturated acetic acid solution of the corresponding catechol to a stirred solution of peracetic acid containing catalytic amounts of Fe(III). After an additional reaction time of 24–36 h at room temperature, the precipitated hexadienedioic acids can be isolated as white solids in moderate yield and high purity (Scheme 1). The configuration of **2a–d** was proven by ^{1}H NMR. A typical vicinal $^3J_{4,5}$ value of 11.6 Hz indicates *cis* geometry of the 4-double bond. In case of **2b,c** the chemical shifts of H-3, H-4, and H-5 corresponded with those reported for the identical *cis,cis* isomers isolated from haloaromatic degradation.[1] The configuration of the 2-double bond of **2d** was shown to be *cis* by biological activity toward muconate cycloisomerase, since only the *cis,cis* isomer shows enzymatic turnover.[22]

The 3,4-dihalo-2,4-hexadienedioic acids, expected from turnover of the corresponding 4,5-dihalocatechols should, like their 3-halo analogues, also be unstable in acidic medium. In fact, using the above method with a longer reaction time (48 h) for **3c,d**, we could only isolate (*Z*)-3-halo-5-oxo-2(5*H*)-furanylideneacetic acids, products of a spontaneous lactonization of the 3,4-dihalo-2,4-hexadienedioic acids and elimination of hydrogen halide.

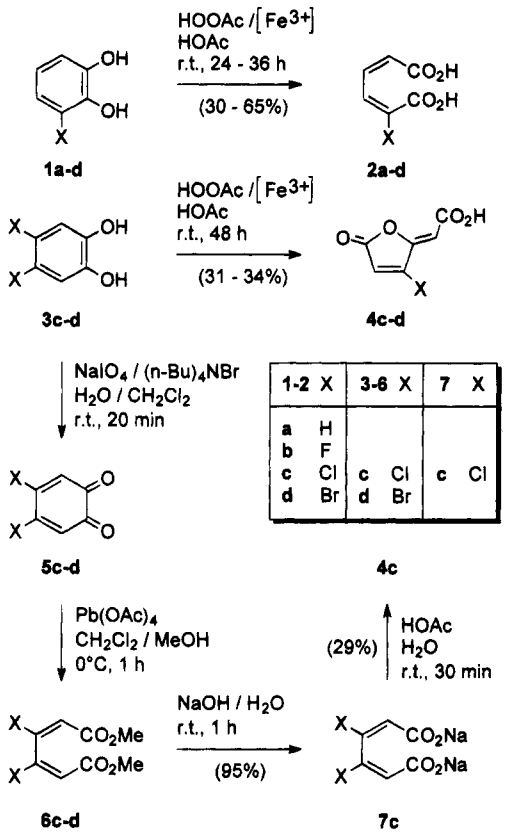
The *Z* configuration of **4c,d** was established by comparison of ^{1}H NMR data with those from (*E*)- and (*Z*)-3-fluoro-5-oxo-2(5*H*)-furanylideneacetic acid.[23] Typical chemical shifts of the exocyclic C-6 proton (H-6) were measured to be 5.8 ppm for the *Z* isomer and 6.2 ppm for the *E* isomer.

For the synthesis of 3,4-dihalo-2,4-hexadienedioic acids, we applied the oxidation of 3,4-dihalo *o*-quinones **5c,d** with lead tetraacetate,[20] from which the stable dimethyl esters were obtained. In a first step **3c,d** were oxidized with $NaIO_4$ at room temperature under phase-transfer catalysis to the corresponding labile *o*-quinones **5c,d**. The *in situ* generated quinones became directly turned over in a second oxidation step at 0 °C with lead tetraacetate in methanol to yield the dimethyl *cis,cis*-3,4-dihalo-2,4-hexadienedioic acids **6c,d**. The following alkaline hy-

(1) Schmidt, E.; Remberg, G.; Knackmuss, H.-J. *Biochem. J.* **1980**, *192*, 331.
(2) Schmidt, E.; Knackmuss, H.-J. *Biochem. J.* **1980**, *192*, 339.
(3) Fernley, H. N.; Evans, W. C. *Biochem. J.* **1959**, *73*, 22 P.
(4) Schmidt, E. *Appl. Microbiol. Biotechnol.* **1988**, *27*, 347.
(5) Schmidt, E.; Knackmuss, H.-J. *Appl. Microbiol. Biotechnol.* **1984**, *20*, 351.
(6) You, I. S.; Bartha, R. *J. Agric. Food Chem.* **1982**, *30*, 274.
(7) Adler, K.; Magnusson, R. *Acta Chem. Scand.* **1959**, *13*, 505.
(8) Farrand, J. C.; Johnson, D. C. *J. Org. Chem.* **1971**, *36*, 3606.
(9) Matsuura, T.; Matsushima, H.; Kato, S.; Saito, I. *Tetrahedron* **1972**, *28*, 5119.
(10) Tsuji, J.; Takayanagi, H. *J. Am. Chem. Soc.* **1974**, *96*, 7349.
(11) Saito, I.; Takami, M.; Matsuura, T. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2865.
(12) Moro-oka, Y.; Foote, C. S. *J. Am. Chem. Soc.* **1976**, *98*, 1510.
(13) Wiessler, M. *Tetrahedron Lett.* **1977**, 233.
(14) Speier, G.; Tyeklar, Z. *Chem. Ber.* **1979**, *112*, 389.
(15) Boeseken, J.; Engelberts, R. *Proc. Acad. Sci. Amsterdam* **1931**, *34*, 1292.
(16) Wacek, A.; Fiedler, R. *Monatsh. Chem.* **1949**, *80*, 170.
(17) Pandell, A. J. *J. Org. Chem.* **1976**, *25*, 3992.
(18) Pandell, A. J. *J. Org. Chem.* **1983**, *48*, 3908.
(19) Evans, W. C.; Smith, B. S. W.; Moss, P.; Fernley, H. N. *Biochem. J.* **1971**, *122*, 509.
(20) Pieken, W. A.; Kozarich, J. W. *J. Org. Chem.* **1989**, *54*, 510.
(21) Pieken, W. A.; Kozarich, J. W. *J. Org. Chem.* **1990**, *55*, 3029.
(22) Sistrom, W. R.; Stanier, R. Y. *J. Biol. Chem.* **1954**, *210*, 821.
(23) Manuscript in preparation.

0022-3263/94/1959-4001$04.50/0

Scheme 1



drolyses with aqueous sodium hydroxide only succeed with the chloro derivative **6c** to give the disodium salt **7c**. Weak acidification of an aqueous solution of **7c** with acetic acid yielded **4c**.

For the dimethyl esters **6c,d**, *cis,cis* configuration could be proved only indirectly. Since the ^1H and ^{13}C NMR spectra of **6c** and **6d** show only two or four signals, respectively, both compounds have to be symmetrical. Together with the inclination of **7c** for lactonization, making one *cis*-fixed double bond necessary, a *cis,cis* configuration can be assumed.

Experimental Section

All melting points were determined with a Büchi melting point apparatus and are uncorrected. ^1H NMR, ^{13}C NMR, and ^{19}F NMR spectra were recorded on a Bruker AC 250 spectrometer at 250, 63, and 235 MHz, respectively. IR spectra were recorded on a Perkin-Elmer 1420 spectrophotometer. Mass spectra were obtained with a Varian MAT 311 instrument using ionization potentials of 70–80 eV. Elemental analyses were performed in the Analytical Laboratory of this university. Catechol (**1a**), 3-fluorocatechol (**1b**), and 2,3-dimethoxybenzonitrile were purchased commercially. The dihalocatechols **3c,d** were prepared from **1a** by halogenation with sulfuryl chloride[24] or bromine,[25] respectively. 3-Chlorocatechol (**1c**) and 3-bromocatechol (**1d**) were prepared according to the literature[26] from 2,3-dimethoxybenzonitrile.

Peracetic Acid Oxidation of Catechols: General Procedure. A solution of 32% peracetic acid (36.0 g, 150 mmol), glacial acetic acid (10 g), and ferric ammonium citrate (20 mg) was placed in a flask (100 cm^3 volume). To the magnetically stirred mixture was added a solution of catechol (5.5 g, 50 mmol) in glacial acetic acid (15 g) by a syringe pump during 8 h at room temperature. After complete addition, the reaction mixture was stirred for an additional 24 h at room temperature.

(24) Willstätter, R.; Müller, H. E. *Chem. Ber.* **1911**, *44*, 2182.
(25) Kohn, M. *J. Am. Chem. Soc.* **1951**, *73*, 480.
(26) Mason, H. S. *J. Am. Chem. Soc.* **1947**, *69*, 2241.

Table 1. Peracetic Acid Oxidation of 1a and the Halocatechols 1b–d and 3c–d

starting material	time (h)	product	yield (%)	mp (°C) lit. mp (°C)	molecular formula
1a	24	**2a**	65	180–181 dec 180 dec[17]	$C_6H_6O_4$ (142.1)
1b	24	**2b**	52	185–187 dec	$C_6H_5FO_4$ (160.1)
1c	24	**2c**	40	159–160 dec 160[3]	$C_6H_5ClO_4$ (176.6)
1d	36	**2d**	30	149–150 dec	$C_6H_5BrO_4$ (221.0)
3c	48	**4c**	31	220–222 dec	$C_6H_3ClO_4$ (174.5)
3d	48	**4d**	34	220–222 dec	$C_6H_3BrO_4$ (219.0)

The resulting suspension was then concentrated in vacuo, without heating, to a final volume of approximately 40 mL. After the solution was cooled to 0–4 °C, the product was collected by suction filtration and washed with ice-cold water until the filtrate was colorless. Drying in vacuum over KOH yielded the respective *cis,cis*-2,4-hexadienedioic acid or 5-oxo-2(*5H*)-furanylideneacetic acid (Table 1).

***cis,cis*-2,4-Hexadienedioic acid (2a):** ^1H NMR (acetone-d_6) δ 6.05 (dd, 2 H, J = 8.1 Hz, J = 2.4 Hz), 7.91 (dd, 2 H, J = 8.1 Hz, J = 2.4 Hz); IR (KBr) 3200–2500, 1680, 1590, 1250, 1200; MS m/z 143 (M^+ + H), 142 (M^+). Anal. Calcd for $C_6H_6O_4$: C, 50.71; H, 4.26. Found: C, 50.63; H, 4.20.

***cis,cis*-2-Fluoro-2,4-hexadienedioic acid (2b):** ^1H NMR (acetone-d_6) δ 6.04 (m, 1 H), 7.67 (dd, 1 H, J = 11.6 Hz, J = 11.7 Hz), 7.86 (m, 1 H); ^{19}F NMR (acetone-d_6) δ −106.90; IR (KBr) 3200–2500, 1695, 1595, 1240; MS m/z 161 (M^+ + H), 160 (M^+). Anal. Calcd for $C_6H_5FO_4$: C, 45.01; H, 3.15. Found: C, 44.96; H, 3.17.

***cis,cis*-2-Chloro-2,4-hexadienedioic acid (2c):** ^1H NMR (acetone-d_6) δ 6.05 (dd, 1 H, J = 11.6 Hz, J = 1.3 Hz), 7.60 (dd, 1 H, J = 11.6 Hz, J = 11.7 Hz), 8.27 (dd, 1 H, J = 11.7 Hz, J = 1.3 Hz); IR (KBr) 3200–2500, 1695, 1565, 1255, 1230; MS m/z 178 (M^+, ^{37}Cl), 176 (M^+, ^{35}Cl). Anal. Calcd for $C_6H_5ClO_4$: C, 40.81; H, 2.85. Found: C, 40.73; H, 2.90.

***cis,cis*-2-Bromo-2,4-hexadienedioic acid (2d):** ^1H NMR (acetone-d_6) δ 6.04 (dd, 1 H, J = 11.5 Hz, J = 0.9 Hz), 7.47 (m, 1 H), 8.48 (dd, 1 H, J = 11.6 Hz, J = 0.8 Hz); IR (KBr) 3200–2500, 1695, 1255, 1230; MS m/z 222 (M^+, ^{81}Br), 220 (M^+, ^{79}Br). Anal. Calcd for $C_6H_5BrO_4$: C, 32.61; H, 2.28. Found: C, 32.88; H, 2.31.

(*Z*)-3-Chloro-5-oxo-2(*5H*)-furanylideneacetic acid (4c): ^1H NMR (acetone-d_6) δ 5.80 (s, 1 H), 6.89 (s, 1 H); ^{13}C NMR (acetone-d_6) δ 100.56, 121.40, 150.33, 155.62, 164.27, 166.48; IR (KBr) 3200–2500, 1790, 1705, 1250; MS m/z 176 (M^+, ^{37}Cl), 174 (M^+, ^{35}Cl). Anal. Calcd for $C_6H_3ClO_4$: C, 41.29; H, 1.73. Found: C, 41.18; H, 1.85.

(*Z*)-3-Bromo-5-oxo-2(*5H*)-furanylideneacetic acid (4d): ^1H NMR (acetone-d_6) δ 5.78 (d, 1 H, J = 0.7 Hz), 7.06 (d, 1 H, J = 0.7 Hz); ^{13}C NMR (acetone-d_6) δ 102.04, 125.85, 139.61, 156.81, 164.39, 167.27; IR (KBr) 3200–2500, 1800, 1710, 1250; MS m/z 220 (M^+, ^{81}Br), 218 (M^+, ^{79}Br). Anal. Calcd for $C_6H_3BrO_4$: C, 32.91; H, 1.38. Found: C, 32.82; H, 1.43.

Synthesis of 4,5-Dihalo-1,2-benzoquinones: General Procedure. To a magnetically stirred solution of the respective 4,5-dihalocatechol (30 mmol) and tetra-*n*-butylammonium bromide (50 mg) in CH_2Cl_2 (80 mL) was added a solution of $NaIO_4$ (7.5 g, 35 mmol) in water (80 mL). After vigorous stirring of the solution at room temperature for 20 min, the dark red organic layer was separated and dried with $MgSO_4$. Due to the lability of the *o*-quinone, the solution was immediately employed for further oxidation.

Synthesis of Dimethyl *cis,cis*-3,4-Dihalo-2,4-hexadienedioic Acids: General Procedure. To the solution of the respective 4,5-dihalo-1,2-benzoquinone (crude product from the step before) in CH_2Cl_2, was added dry methanol (80 mL). The stirred mixture was cooled to 0 °C and solid lead tetraacetate (16.2 g, 37 mmol) was added. After the solution was stirred at 0 °C in the dark for 1 h, the solvent was evaporated in vacuo and the oily residue was repeatedly extracted with hexane/ethylacetate 50:50 (4 × 75 mL). The combined extracts were

carefully (CO_2 evolution) stirred with an excess of saturated aqueous solution of sodium bicarbonate. After being washed with water, the organic layer was dried with $MgSO_4$. Evaporation of the solvent and condensing under reduced pressure (70–90 °C/0.03 mbar) yielded the respective pure dimethyl *cis,cis*-3,4-dihalo-2,4-hexadienedioic acid.

Dimethyl *cis,cis*-3,4-dichloro-2,4-hexadienedioic acid (6c) was prepared from **5c** as shown above: 78% yield; oil; ^1H NMR ($CDCl_3$) δ 3.70 (s, 6 H), 6.23 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 51.84, 121.70, 142.38, 162.71; IR (film) 1720, 1215, 1190, 1165; MS m/z 240 (M^+, ^{35}Cl, ^{37}Cl), 238 (M^+, ^{35}Cl). Anal. Calcd for $C_8H_8Cl_2O_4$: C, 40.19; H, 3.37. Found: C, 40.08; H, 3.26.

Dimethyl *cis,cis*-3,4-dibromo-2,4-hexadienedioic acid (6d) was prepared from **5d** as shown above: 52% yield; mp 46–48 °C; ^1H NMR ($CDCl_3$) δ 3.72 (s, 6 H), 6.36 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 51.87, 124.38, 134.20, 162.83; IR (film) 1730, 1220, 1200, 1170; MS m/z 330 (M^+, ^{81}Br), 328 (M^+, ^{81}Br, ^{79}Br), 326 (M^+, ^{79}Br). Anal. Calcd for $C_8H_8Br_2O_4$: C, 29.30; H, 2.46. Found: C, 29.30; H, 2.52.

Synthesis of Disodium *cis,cis*-3,4-Dichloro-2,4-hexadienedioate (7c). A mixture of the dimethyl ester **6c** (2.4 g, 10 mmol) was stirred with NaOH (0.8 g, 20 mmol) in water (5 mL) for 1 h. Extraction of the mixture with CH_2Cl_2 and evaporation of the aqueous phase at 3 mbar yielded **7c** (2.4 g, 94%) as a light yellow-brown powder. Attempted purification of the disodium salt by recrystallization from various solvent systems failed. ^1H NMR (D_2O): δ 5.29 (s, 2 H).

Lactonization of 7c. A solution of the disodium salt **7c** (1020 mg, 4 mmol) in water (3 mL) was mixed with acetic acid (1 mL). After standing at room temperature for 30 min, the mixture was cooled to 0 °C. After an additional 15 min, the precipitate was collected by suction filtration. It was washed with ice-cold water (2 mL) and dried in vacuum. Subsequent sublimation (120 °C/0.03 mbar) yielded **4c** (200 mg, 29%) as a colorless powder, mp 219–221 °C dec. Anal. Calcd for $C_6H_3ClO_4$: C, 41.29; H, 1.73. Found: C, 41.22; H, 1.81.

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