Synthesis of Oxygenated Derivatives of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin†

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Four monomethoxy, five dimethoxy and four dihydroxy derivatives of polychlorodibenzo-*p*-dioxin have been synthesized, and their physical and spectral properties (NMR and MS) have been reported. The general method for the synthesis of specific mono- and dimethoxy derivatives in reasonable yields has been found to be the condensation of 4,5-dichlorocatechol with appropriately substituted *o*-chloronitrobenzenes. The monomethoxy derivatives thus prepared were demethylated by boron tribromide–dimethyl sulfide. Several mono- and dihydroxypolychlorodibenzo-*p*-dioxins have been identified as metabolites of the highly toxic environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). However, most of these metabolites have not been fully characterized due to the lack of appropriate standards. The availability of these synthetic oxygenated derivatives of TCDD can be used to establish the structure and the potential toxicity associated with these metabolites.

Polychlorinated dibenzo-*p*-dioxins (PCDDs) pose a potential risk to human health and to the environment due to their inherent toxicity, ubiquitous environmental contamination, and persistence (Gray et al., 1976; Crummet and Stehl, 1973). PCDDs elicit a broad spectrum of congener-specific and tissue-specific biological and toxicological response, with the induction of hepatic cytochrome P-450 1A1 and 1A2 representing one of the most sensitive responses associated with exposure to these chemicals (Kedderis et al., 1991; Tritscher et al., 1992). Induction of cytochrome P-450 1A1 and 1A2 has been implicated in the cancer risk from other endogenous and exogenous compounds that are metabolized to active intermediates by these isozymes (Guingerich and Shimada, 1991; Nebert, 1989).

Chlorine substituents at the 2-, 3-, 7-, and 8-positions are generally considered to be necessary for dioxin-like activity, with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), representing the most potent and extensively studied congener of its class. TCDD has been identified as a trace impurity in the widely used herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and as a byproduct during incineration of solid municipal and hospital waste (Kitchin and Woods, 1978; Lewis et al., 1986; Fiedler et al., 1990; Travis and Hattemer-Frey, 1991). Binding of TCDD and related compounds to the cytosolic aryl hydrocarbon (ah) receptor is generally considered the initial event necessary for the expression of the dioxin-like activity of these compounds. Therefore, congener-specific affinity of PCDDs for the ah receptor and congener-specific pharmacokinetics are two factors that contribute to the relative *in vivo* potency of a given PCDD in a given species. The pharmacokinetics of PCDDs is congener-, dose-, and species-specific, with urinary and biliary excretion being dependent on the metabolism of these compounds (Neal et al., 1984; Olson et al., 1983). There is now evidence that a wide range of mammalian and aquatic species are capable of biotransforming TCDD to polar metabolites (Ramsey et al., 1982; Poiger et al., 1982; Poiger and Buser, 1984; Olson et al., 1980; Olson, 1986; Gasiewicz et al., 1983; Kleeman et al., 1988). Although metabolites of TCDD have not been directly identified in humans, recent data regarding feces samples from humans in a self-dosing experiment suggest that humans can metabolize TCDD (Wendling et al., 1990).

The metabolism of TCDD is generally believed to be a detoxification process (Weber et al., 1982; Mason and Safe, 1986). However, indirect evidence has suggested that TCDD metabolites may inhibit uroporphyrinogen decarboxylate activity and lead to TCDD-induced porphyria (De Verneuil et al., 1983; Wainstok de Calmanovici et al., 1986). *In vitro* studies have also reported that TCDD metabolites have high affinity for transthyretin, a transport protein for thyroid hormones and vitamin A; however, there is no evidence at present to indicate that metabolites of TCDD play a significant role in these processes *in vivo* (Lans et al., 1991). Therefore, it is essential that the metabolites of TCDD be identified and evaluated for their toxicity to understand the role of metabolism of TCDD in the expression of its toxicity. Metabolite identification will also help to identify biotransformation pathways that regulate the excretion and toxicity of TCDD.

Only a few metabolites of TCDD have been tentatively identified by mass spectrometry techniques, which in a number of cases did not confirm the substitution pattern of the metabolites (Poiger et al., 1982; Poiger and Buser, 1984; Neal et al., 1984; Sawahata et al., 1982). The isolation of these metabolites in large quantities for unequivocal structural chacterization is impractical since TCDD is very toxic and only nano- to microgram doses can be administered. Therefore, we have undertaken the synthesis of several oxygenated derivatives of TCDD (phenols and their methyl ethers) of high purity with a view to use them as GC or HPLC standards for characterizing TCDD metabolites and also for evaluating the toxicity of some of the phenolic compounds. In a previous study, Sawahata et al. (1982) reported the synthesis of 1-methoxy-2,3,7,8-tetrachloro-, 2-methoxy-1,3,7,8-tetrachloro-, and 3-methoxy-2,7,8-trichlorodibenzo-*p*-dioxin and characterized their identity only by their mass spectra. Synthesis and spectral characterization of the latter two compounds and their hydroxy analogues in microgram quantities also have been reported by Mason and Safe (1986).

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Several methods are currently available for the synthesis of PCDDs (Gray et al., 1976). The most attractive method for the synthesis of PCDDs was developed by Pohland and Yang (1972) and later modified by Gray et al. (1976) to facilitate the synthesis of relatively less chlorinated PCDDs. This procedure requires the condensation of appropriately substituted catechol with *o*-chloropolychloronitrobenzenes in the presence of a base. The condensation reaction generally proceeds via Smile rearrangement and consequently produces a mixture of products from which the isolation of the pure compound in practical quantity has proved to be extremely difficult. However, this problem can be circumvented by keeping one of the reactants symmetrically subsituted. In that event, the product of Smile rearrangement would be the same as that of direct condensation. Since our main interest has been the synthesis of oxygenated derivatives of TCDD in which only one benzo ring has been modified, we hoped that the procedure developed by Gray et al. (1976) for the synthesis of PCDDs would also be more appropriate for the synthesis of required oxygenated compounds.

EXPERIMENTAL PROCEDURES

Caution: Although the toxicity of the compounds synthesized in the present work is not known, extreme precaution is warranted, especially during the final step of the synthesis of oxygenated analogues of 2,3,7,8-TCDD because of their resemblance with 2,3,7,8-TCDD. Work was performed in a limited-access laboratory equipped with fume hoods with at least 100 linear ft/min draw of air. The synthesis of the final compounds was performed in semimicro scale (<200 mg), and the possible contamination within the fume hoods was contained through lining hoods with plastic-backed absorbent paper.

4,5-Dichlorocatechol (3) (Gray et al., 1976), 2,5-dichloro-4-nitroanisole (**2a**) (Goi and Konishi, 1954), 6-nitro-2,3,5-trichloroanisole (**2b**) (Huffer, 1921), 4-nitro-2,3,6-trichloroanisole (**2c**) (Huffer, 1921), 2-chloro-4,5-dimethoxynitrobenzene (**2e**) (Quelet and Ezz, 1959), 2,6-dichloro-*p*-hydroquinone (**7**) (Akita, 1962), 3,6-dichlorocatechol (**9**) (Knuutinen and Tarhanan, 1981), and 2,3-dichloro-4,6-dimethoxynitrobenzene (**2i**) (Castelfranchi and Perrotti, 1957) used in the present work were synthesized as described in the literature. ^{1}H NMR spectra with tetramethylsilane as an internal standard were recorded at 270 MHz in the Department of Biochemistry, and mass spectra were obtained on a Kratos MS80RFA spectrometer in the Department of Biophysics, State University of New York, Buffalo, NY. Silica gel TLC plates (0.25-mm thickness) were purchased from EM Science. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Melting points are uncorrected.

2-Methoxy-3,7,8-trichlorodibenzo-*p*-dioxin (1a). A mixture of 1.78 g (10 mmol) of 4,5-dichlorocatechol (**3**), 1.10 g (5 mmol) of 2,5-dichloro-4-nitroanisole (**2a**), and 3.0 g of freshly ignited potassium carbonate in 50 mL of dry dimethyl sulfoxide was stirred at 130 °C for 24 h. The reaction mixture was cooled, poured onto 100 mL of water, and extracted with ethyl acetate (3 × 100 mL). The organic phase was washed with water (1 × 100 mL), dried (Na_2SO_4), and distilled under reduced pressure to yield a crude product. Preparative TLC of the crude product yielded a solid which was recrystallized from chloroform/methanol to produce 0.14 g (8.8%) of **1a** as light yellow crystals: mp 213–214 °C; TLC R_f 0.35 (5% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3 H), 6.50 (s, 1 H), 6.85 (m, 1 H), 6.88 (s, 2 H); MS, *m/z* (relative intensity) 316 (M^+, 100), 318 (M + 2, 98), 320 (M + 4, 34), 301 (M – CH_3, 30), 273 (M – CH_3CO, 36). Anal. Calcd for $C_{13}H_7Cl_3O_3$: C, 49.1; H, 2.2. Found: C, 49.3; H, 2.4.

2-Hydroxy-3,7,8-trichlorodibenzo-*p*-dioxin (1j). A solution of boron tribromide–dimethyl sulfide complex (0.5 mL, 1 M in CH_2Cl_2) in 5 mL of dichloroethane was added dropwise to a solution of 60 mg of **1a** in 5 mL of dichloroethane, and the solution was refluxed for 72 h. After this time, the mixture was concentrated *in vacuo*, and the residue was partitioned between 50 mL of ether and 25 mL of 10% sodium bicarbonate. The ether layer was dried over magnesium sulfate and evaporated to produce a crystalline solid. The crystalline solid was further purified by preparative TLC on a silica gel plate using 30% ethyl acetate in hexane as a developing solvent to give 21 mg (35%) of **1j** as a colorless crystalline solid: mp 253–254 °C; TLC R_f 0.49 (30% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 5.35 (s, 1 H, exchangeable with CD_3OD), 6.57 (s, 1 H), 6.85 (s, 1 H), 6.94 (s, 1 H), 6.96 (s, 1 H). Accurate mass measurement for $C_{12}H_5Cl_3O_3$, calcd: 301.9304. Found: 301.9303.

1-Methoxy-2,3,7,8-tetrachlorodibenzo-*p*-dioxin (1b). A solution of 1.78 g (10 mmol) of 4,5-dichlorocatechol (**3**) and 1.27 g (5 mmol) of 6-nitro-2,3,5-trichloroanisole (**2b**) in 50 mL of dry dimethyl sulfoxide containing 2.8 g of freshly dried potassium carbonate was heated at 110 °C for 24 h. The usual workup and column chromatography of the crude product on silica gel using 2% ethyl acetate/hexane produced 0.11 g (6.4%) of **1b** as a colorless crystalline solid: mp 66–68 °C; TLC R_f 0.55 (5% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 3.97 (s, 3 H), 6.81 (s, 1 H), 6.99 (s, 1 H), 7.09 (s, 1 H); MS, *m/z* (relative intensity) 350 (M^+, 77), 352 (M + 2, 100), 354 (M + 4, 50), 356 (M + 6, 10), 307 (M – CH_3CO, 22). Accurate mass measurement for $C_{13}H_6Cl_4O_3$, calcd: 349.9141. Found: 349.9063.

1-Hydroxy-2,3,7,8-tetrachlorodibenzo-*p*-dioxin (1k). **1b** (35 mg) was demethylated with boron tribromide–dimethyl sulfide complex (0.5 mL, 1 M in CH_2Cl_2) in 5 mL of dichloroethane as described above for **1j** to yield 9 mg (27%) of **1b** as a colorless solid: mp 268 °C; TLC R_f 0.47 (30% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 6.65 (s, 1 H), 7.0 (s, 1 H), 7.1 (s, 1 H). Accurate mass measurement for $C_{12}H_4Cl_4O_3$, calcd: 335.8914. Found: 335.8850.

2-Methoxy-1,3,7,8-tetrachlorodibenzo-*p*-dioxin (1c). Condensation of 1.78 g (10 mmol) of 4,5-dichlorocatechol (**3**) with 1.27 g (5 mmol) of 4-nitro-2,3,6-trichloroanisole (**2c**) was carried out in dry dimethyl sulfoxide (50 mL) in the presence of anhydrous potassium carbonate as described in the preparation of **1b**. Preparative TLC (silica gel) using 2% ethyl acetate/hexane gave 0.12 g (8.5%) of **1c**: mp 167–168 °C; TLC R_f 0.59 (5% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3 H), 6.85 (s, 1 H), 6.96 (s, 1 H), 7.09 (s, 1 H); MS, *m/z* (relative intensity) 350 (M^+, 100), 352 (M + 2, 97), 354 (M + 4, 51), 356 (M^+ + 6, 4), 335 (M – CH_3, 46), 307 (M – CH_3CO, 19). Anal. Calcd for $C_{13}H_6Cl_4O_3$: C, 44.3; H, 1.7. Found: C, 43.9; H, 2.1.

2-Hydroxy-1,3,7,8-tetrachlorodibenzo-*p*-dioxin (1l). Demethylation of **1c** (70 mg) with boron tribromide–dimethyl sulfide complex (0.5 mL, 1 M in CH_2Cl_2) in 5 mL of dry dichloroethane produced 14 mg (24%) of **1l** as a colorless crystalline solid: mp 121–122 °C; TLC R_f 0.57 (30% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 6.85 (s, 1 H), 6.96 (s, 1 H), 7.10 (s, 1 H). Accurate mass measurement for $C_{12}H_4Cl_4O_3$, calcd: 335.8914. Found: 335.8905.

2-Nitro-3,4,5-trichloroanisole (2d). A solution of 3,4,5-trichloroanisole (**4**) (0.42 g, 2 mmol) in 32 mL of nitromethane and 6 mL of trifluoroacetic anhydride was cooled to 0 °C, and then 2.0 g of ammonium nitrate was added. The reaction mixture was stirred at 0 °C for 5 min and then immediately diluted with 20 mL of water. Nitromethane was distilled off *in vacuo*, and the solid separated was filtered, dried, and recrystallized from methanol to produce 0.4 g (78.4%) of **2d** as a yellow crystalline solid: mp 116–118 °C; ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 3 H), 7.12 (s, 1 H). Anal. Calcd for $C_7H_4Cl_3NO_3$: C, 32.8; H, 1.4; N, 5.46. Found: C, 33.0; H, 1.6; N, 5.31.

4-Methoxy-1,2,7,8-tetrachlorodibenzo-*p*-dioxin (1d). A mixture of 0.3 g (1.2 mmol) of **2d**, 0.42 g (2.4 mmol) of 4,5-dichlorocatechol (**3**), and 1.4 g of freshly dried potassium carbonate in 15 mL of anhydrous dimethyl sulfoxide was heated at 110 °C for 16 h. The mixture was cooled, poured onto 50 mL of water, and then extracted with ethyl acetate (3 × 20 mL). The combined organic phase was washed with water (1 × 20 mL), dried (Na_2SO_4), and distilled under reduced pressure to yield a yellow crude compound. Preparative TLC using 2% ethyl acetate/hexane gave 0.075 g (18%) of **1d** as a colorless crystalline solid: mp 188–190 °C; TLC R_f 0.25 (5% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3 H), 6.71 (s, 1 H), 7.09 (s, 1 H), 7.10 (s, 1 H); MS, *m/z* (relative intensity) 350 (M^+, 74), 352 (M + 2, 100), 354 (M + 4, 44), 356 (M^+ + 6, 5), 307 (M – CH_3CO, 33). Accurate mass measurement for $C_{13}H_6Cl_4O_3$, calcd: 349.9141. Found: 349.9071.

4-Hydroxy-1,2,7,8-tetrachlorodibenzo-*p*-dioxin (1m). A solution of boron tribromide–dimethyl sulfide complex (0.5 mL, 1 M in CH_2Cl_2) in 5 mL of dichloroethane was added dropwise to a solution of 17 mg of **1d** in 5 mL of dichloroethane. Following refluxing for 72 h, the mixture was concentrated to dryness. The residues was partitioned between 30 mL of ether and 10 mL of 10% sodium carbonate, and the ether layer was separated, dried ($MgSO_4$), and evaporated to yield a crude product. This product was chromatographed over dry column chromatography grade silica gel using ethyl acetate as eluting solvent to produce 3 mg (18%) of **1m** as pale yellow crystals: mp 154–156 °C; TLC R_f 0.40 (30% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 6.79 (s, 1 H), 7.04 (s, 1 H), 7.15 (s, 1 H). Accurate mass measurement for $C_{12}H_4Cl_4O_3$, calcd: 335.8914. Found: 335.8901.

2,3-Dichloro-7,8-dimethoxydibenzo-*p*-dioxin (1e). This compound was prepared by the reaction of 4,5-dichlorocatechol (**3**, 1.42 g, 8 mmol) and 2-chloro-4,5-dimethoxynitrobenzene (**2e**, 0.86 g, 4 mmol). The crude product was isolated as described above for **1d** and chromatographed over preparative TLC using 10% ethyl acetate/hexane as developing solvent to give 0.16 g (13%) of the desired compound **1e** as colorless crystals: mp 177–178 °C; TLC R_f 0.10 (5% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 3.82 (s, 6 H), 6.45 (s, 2 H), 6.91 (s, 2 H); MS, *m/z* (relative intensity) 312 (M^+, 86), 314 (M + 2, 100), 297 (M – CH_3, 35), 269 (M – $COCH_3$, 16). Anal. Calcd for $C_{14}H_{10}Cl_2O_4$: C, 53.6; H, 3.2. Found: 53.6; H, 3.4.

3,5-Dichloro-1,2-dimethoxybenzene (6). To an ice-cooled solution of 3.5 g (20 mmol) of 3,5-dichlorocatechol (**5**) in 8% sodium hydroxide (30 mL) was added dropwise 3.5 mL (40 mmol) of dimethyl sulfate in 10 min with vigorous stirring. The reaction mixture was allowed to warm to room temperature and stirred for an additional 4 h at room temperature followed by 8 h under reflux. The mixture was cooled and extracted with ether (2 × 50 mL), and the combined ether extracts were washed with 10% sodium hydroxide (1 × 20 mL) and water (2 × 20 mL). The ether layer was dried (Na_2SO_4) and evaporated *in vacuo* to yield 2.0 g (50%) of the desired compound **6** as an oil: ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3 H), 3.87 (s, 3 H), 6.80 (d, 1 H, J = 3 Hz), 9.95 (d, 1 H, J = 3 Hz). This product was used as such in the next synthesis.

2,6-Dichloro-3,4-dimethoxynitrobenzene (2f). Concentrated nitric acid (2 mL) was added dropwise with stirring to a solution of **6** in 30 mL of acetic anhydride at room temperature. After 4 h of stirring at room temperature, the mixture was poured on ice (200 g). Solid separated out was filtered, dried, and then recrystallized from methanol to produce 2.0 g (80%) of yellow crystals: mp 65–66 °C; ^{1}H NMR ($CDCl_3$) δ 3.90 (s, 3 H), 3.96 (s, 3 H), 6.92 (s, 1 H). Anal. Calcd for $C_8H_7Cl_2NO_4$: C, 38.1; H, 2.7; N, 5.5. Found: C, 38.0; H, 2.7; N, 5.4.

2,3-Dimethoxy-1,7,8-trichlorodibenzo-*p*-dioxin (1f). A solution of 1.42 g (8 mmol) of 4,5-dichlorocatechol (**3**), 1.0 g (4 mmol) of **2f**, and 2 g of anhydrous potassium carbonate in 40 mL of dry dimethyl sulfoxide was stirred at 130 °C for 36 h. The mixture was worked up as described before for **1d** and purified by preparative TLC using 5% ethyl acetate/hexane as developing solvent to produce a crystalline solid. Recrystallization of the solid from chloroform/methanol yielded 0.11 g (7.9%) of **1f** as colorless crystals: mp 193–194 °C; TLC R_f 0.26 (5% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 3.84 (s, 3 H), 3.87 (s, 3 H), 6.59 (s, 1 H), 7.09 (s, 2 H); MS, *m/z* (relative intensity) 346 (M^+, 98), 348 (M + 2, 100), 350 (M + 4, 42), 331 (M – CH_3, 56), 316 (M – $2CH_3$, 41). Anal. Calcd for $C_{14}H_{10}Cl_2O_4$: C, 53.7; H, 3.2. Found: C, 53.6; H, 3.4.

2,6-Dichloro-1,4-dimethoxybenzene (8). 2,6-Dichloro-*p*-hydroquinone **7** (1.8 g, 10 mmol) was methylated with dimethyl sulfate (3 mL, 33 mmol) as described above for the preparation of **6** to yield 1.2 g (60%) of **8**: mp 35–36 °C; ^{1}H NMR ($CDCl_3$) δ 3.78 (s, 3 H), 3.86 (s, 3 H), 6.85 (s, 2 H). Anal. Calcd for $C_8H_8Cl_2O_2$: C, 46.4; H, 3.9. Found: C, 46.1; H, 4.0.

2,4-Dichloro-3,6-dimethoxynitrobenzene (2g). Nitration of **8** (1.0 g, 5 mmol) with concentrated nitric acid (1 mL) in acetic anhydride (20 mL) as described above for **2f** was performed except that the reaction mixture was stirred for 2 h at room temperature. Recrystallization of the product from methanol gave 1.1 g (87%) of pure **2g** as a yellow crystalline solid: mp 74–75 °C; ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 6 H), 7.00 (s, 1 H). Anal. Calcd for $C_8H_7Cl_2NO_4$: C, 38.0; H, 2.8; N, 5.5. Found: C, 38.1; H, 2.8; N, 5.4.

1,4-Dimethoxy-2,7,8-trichlorodibenzo-*p*-dioxin (1g). A mixture of 1.42 g (8 mmol) of 4,5-dichlorocatechol (**3**), 1.0 g (4 mmol) of **2g**, and 2.0 g of freshly dried potassium carbonate in 50 mL of dry dimethyl sulfoxide was stirred at 130 °C for 72 h. The crude product isolated as described above for **1d** was purified by column chromatography over silica gel using 5% ethyl acetate/hexane as eluting solvent and then recrystallized from chloroform/methanol to yield 0.12 g (8.6%) of **1g** as colorless crystals: mp 157–158 °C; TLC R_f 0.19 (5% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 6 H), 6.41 (s, 1 H), 6.94 (s, 1 H), 7.07 (s, 1 H); MS, *m/z* (relative intensity) 346 (M^+, 100), 348 (M + 2, 83), 350 (M + 4, 37), 331 (M – CH_3, 28), 288 (331 – $COCH_3$, 16), 268 (M – $COCH_3$ – Cl, 57). Anal. Calcd for $C_{14}H_9Cl_3O_4$: C, 48.4; H, 2.6. Found: C, 47.9; H, 2.7.

3,6-Dichloro-1,2-dimethoxybenzene (10). Methylation of 1.7 g (10 mmol) of 3,6-dichlorocatechol (**9**) with 1.8 mL (20 mmol) of dimethyl sulfate in 15 mL of 8% sodium hydroxide following the procedure described above for the synthesis of **6** resulted in 1.0 g (50%) of **10** as a colorless viscous oil: ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 6 H), 7.08 (s, 2 H). This oily product was used as such in the next step.

2,5-Dichloro-3,4-dimethoxynitrobenzene (2h). The title compound **2h** was obtained in 68% yield by the nitration of **10** (1.0 g, 5 mmol) with concentrated nitric acid at room temperature using the analogous procedure described for the synthesis of **2f**. The pure nitro compound **2h** showed mp 40–41 °C; ^{1}H NMR ($CDCl_3$) δ 3.97 (s, 3 H), 4.02 (s, 3 H), 7.77 (s, 1 H). Anal. Calcd for $C_8H_7Cl_2NO_4$: C, 38.1; H, 2.8. Found: C, 37.9; H, 2.8.

1,2-Dimethoxy-3,7,8-trichlorodibenzo-*p*-dioxin (1h). A mixture of 0.5 g (2 mmol) of **2h**, 4,5-dichlorocatechol (**3**) (0.7 g, 4 mmol), and 4 g of freshly ignited potassium carbonate in 20 mL of dry dimethyl sulfoxide was stirred at 120 °C for 16 h. The reaction mixture was worked up as described for the synthesis of **1d** to produce a colorless crystalline solid which was recrystallized from chloroform/methanol to yield 0.12 g (18%) of **1h**: mp 142–143 °C; TLC R_f 0.35 (5% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3 H), 3.95 (s, 3 H), 6.66 (s, 1 H), 6.95 (s, 1 H), 7.07 (s, 1 H); MS, *m/z* (relative intensity) 346 (M^+, 90), 348 (M + 2, 100), 350 (M + 4, 27), 331 (M – CH_3, 35), 288 (331 – $COCH_3$, 14), 268 (M – $COCH_3$ – Cl, 57). Anal. Calcd for $C_{14}H_9Cl_3O_4$: C, 48.4; H, 2.6. Found: C, 48.9; H, 2.8.

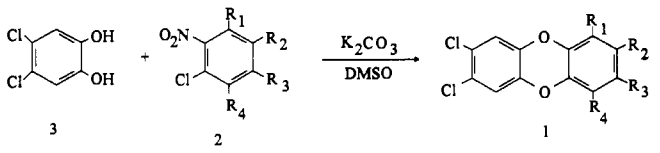
2,4-Dimethoxy-1,7,8-trichlorodibenzo-*p*-dioxin (1i). 4,5-Dichlorocatechol (**3**) (0.89, 5 mmol), 2,3-dichloro-4,6-dimethoxynitrobenzene (**2i**) (1.27 g, 5 mmol), and anhydrous potassium carbonate (2.5 g) were heated with stirring at 130 °C for 60 h. Workup, chromatographic purification, and recrystallization of the reaction product yielded 0.13 g (7.5%) of **1i** as colorless crystals: mp 54–55 °C; TLC R_f 0.07 (5% ethyl acetate in hexane); ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 6 H), 5.61 (s, 1 H), 6.90 (s, 1 H), 7.00 (s, 1 H); MS, *m/z* (relative intensity) 346 (M^+, 100), 348 (M + 2, 95), 350 (M + 4, 25), 331 (M – CH_3, 20), 303 (M – $COCH_3$, 23), 288 (303 – CH_3, 28), 268 (303 – Cl, 2.5). Accurate mass measurement for $C_{14}H_9Cl_3O_4$, calcd: 345.9567. Found: 345.9580.

RESULTS AND DISCUSSION

The oxygenated PCDDs (**1a–m**) were synthesized by condensation of 4,5-dichlorocatechol (**3**) with appropriately substituted *o*-chloronitrobenzene (see Scheme I).

The *o*-chloronitrobenzenes **2a–c**, **2e**, and **2i** were obtained according to the literature procedures (see Experimental Procedures). The remaining *o*-chloronitrobenzenes **2d** and **2f–h** have not been synthesized previously and are reported for the first time in the present paper. Thus, the nitration of commercially available 3,4,5-trichloroanisole (**4**) with ammonium nitrate in a mixture of nitromethane and trifluoroacetic anhydride at 0 °C for 5 min produced 75–80% yield of the desired **2d**. When this reaction was performed at room temperature or for longer time at 0 °C, a significant amount of dinitrated product was also present in the reaction mixture. *o*-Chloronitrobenzenes **2f–h** were obtained in 68–87% yield from the corresponding dimethyl ether derivatives **6**, **8**, and **10** of known 3,5-dichlorocatechol (**5**), 2,6-dichloro-*p*-hydro-

Scheme I



a	$R_1 = R_4 = H$	$R_2 = Cl$	$R_3 = OCH_3$
b	$R_4 = H$	$R_2 = R_3 = Cl$	$R_1 = OCH_3$
c	$R_1 = H$	$R_2 = R_4 = Cl$	$R_3 = OCH_3$
d	$R_2 = H$	$R_3 = R_4 = Cl$	$R_1 = OCH_3$
e	$R_1 = R_4 = H$		$R_2 = R_3 = OCH_3$
f	$R_4 = H$	$R_1 = Cl$	$R_2 = R_3 = OCH_3$
g	$R_2 = H$	$R_3 = Cl$	$R_1 = R_4 = OCH_3$
h	$R_1 = H$	$R_2 = Cl$	$R_3 = R_4 = OCH_3$
i	$R_2 = H$	$R_4 = Cl$	$R_1 = R_3 = OCH_3$
j	$R_1 = R_4 = H$	$R_2 = Cl$	$R_3 = OH$
k	$R_4 = H$	$R_2 = R_3 = Cl$	$R_1 = OH$
l	$R_1 = H$	$R_2 = R_4 = Cl$	$R_3 = OH$
m	$R_2 = H$	$R_3 = R_4 = Cl$	$R_1 = OH$

quinone (7), and 3,6-dichlorocatechol (9). In all three cases nitration was achieved by concentrated nitric acid in acetic anhydride at room temperature. The position of the nitro group in **2f** was assigned by comparing the ^1H NMR spectra of **2f** and its precursor **6**. **6** showed two aromatic proton signals at δ 6.80 (H-6) and 9.95 (H-4). However, the corresponding nitro derivative **2f** showed only one aromatic proton signal at δ 6.92. This observation in conjunction with the fact that the nitro group, owing to its electronegative character, causes a deshielding effect on the aromatic protons supports the structure given to **2f**. Alternatively, if nitration would have occurred at H-6 of **6**, the aromatic proton signal of **2f** should have a chemical shift equal to or higher than δ 9.95.

One hydroxylated trichlorodibenzo-*p*-dioxin, **1j**, and its methyl ether, **1a**, were synthesized. **1j** has been tentatively characterized as a metabolite as well as an aereal oxidation product of TCDD and is presumably formed by direct hydroxylation of the chloro substituent of TCDD (Poiger et al., 1982). Condensation of 4,5-dichlorocatechol (**3**) with 2,4-dichloro-5-nitroanisole (**2a**) in dimethyl sulfoxide at 130 °C in the presence of anhydrous potassium carbonate produced 3-methoxy-2,7,8-trichlorodibenzo-*p*-dioxin (**1a**). Demethylation of **1a** with boron tribromide–dimethyl sulfide complex resulted in 3-hydroxy-2,7,8-trichlorodibenzo-*p*-dioxin (**1j**). Using similar reaction conditions, three monohydroxylated tetrachlorodibenzo-*p*-dioxins (**1k–m**) and their methoxy analogues (**1b–d**) were also obtained in reasonable yields. Two of these hydroxylated tetrachlorodibenzo-*p*-dioxins (**1k** and **1l**) are expected to be formed from TCDD by rearrangement of the metabolically produced 1,2-oxide of TCDD. Both of these compounds have also been characterized as metabolites of TCDD in previous studies (Poiger et al., 1982; Sawahata et al., 1982). The compound **1m** has not been identified as a metabolite of TCDD; however, its formation is speculated. One of the possible pathways that may be involved in the formation of **1m** from TCDD is given in Scheme II. The arene oxide intermediate proposed in this pathway could also be involved in the metabolism of TCDD to the ring-cleaved product 4,5-dichlorocatechol (**3**) (Poiger et al., 1982).

Scheme II

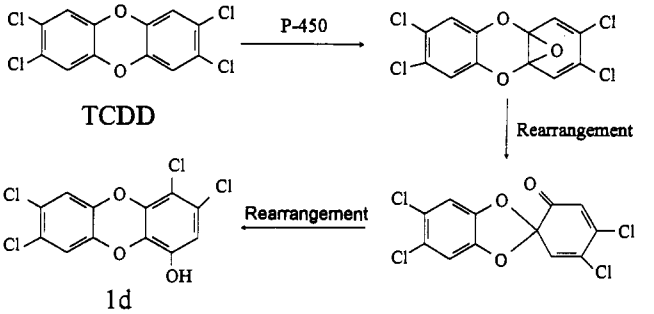


Table I. Summary of ^1H NMR Spectra of the Dibenzo-*p*-dioxin Derivatives Synthesized

	chemical shift, δ						
compd	H-1	H-2	H-3	H-4	H-5	H-8	OCH_3
1a	6.50			6.85	6.88	6.88	3.85
1b				6.81	6.99	7.09	3.97
1c				6.85	6.96	7.09	3.88
1d			6.71		7.09	7.10	3.88
1e	6.45			6.45	6.91	6.91	3.82
1f				6.59	7.09	7.09	3.84, 3.87
1g			6.41		6.94	7.07	3.83
1h				6.66	6.95	7.07	3.85, 3.95
1i			5.61		6.90	7.00	3.89
1j	6.57			6.85	6.94	6.96	
1k				6.65	7.00	7.10	
1l				6.85	6.96	7.10	
1m			6.79		7.04	7.15	

In addition to monomethoxylated dibenzo-*p*-dioxins, several dimethoxylated dichloro- and dimethoxylated trichlorodibenzo-*p*-dioxins have also been synthesized for identifying potential metabolites of TCDD. In the previous study Poiger et al. (1982) and Poiger and Buser (1984) identified at least two metabolites which upon methylation were characterized as dimethoxytrichlorodibenzo-*p*-dioxins by GC/MS technique. However, the exact position of the substituents remained unknown, presumably due to lack of synthetic standards. Dihdyroxychlorinated dibenzo-*p*-dioxins are likely to be produced by the further oxidative metabolism of the monohydroxylated metabolites of TCDD. Synthesis of dimethoxylated dibenzo-*p*-dioxin (**1e–i**) was achieved by the condensation of 4,5-dichlorocatechol (**3**) with the corresponding *o*-chloronitrobenzenes **2e–i** in the presence of potassium carbonate in dimethyl sulfoxide. Compared to monomethoxy-*o*-chloronitrobenzene, dimethoxy-*o*-chloronitrobenzenes required a slightly higher temperature (120–130 °C) for reasonable condensation to occur. In each case the desired product was separated and purified from other polar polymeric byproducts by silica gel column chromatography or preparative TLC to produce pure crystalline compounds in 7–18% yield.

Unlike other monomethoxy or dimethoxy-*o*-chloronitrobenzenes, **2f** was expected to produce two isomeric dibenzo-*p*-dioxins, namely 2,3-dimethoxy-1,7,8-trichlorodibenzo-*p*-dioxin (**1f**) or 1,2-dimethoxy-4,7,8-trichlorodibenzo-*p*-dioxin depending upon which chlorine (Cl-6 or Cl-2) participated in the condensation reaction with 4,5-dichlorocatechol (**3**). However, the mass spectrum of **1f** with molecular ion at m/z 346 and three chlorine atoms showed strong $M^+ - 15$ ($M - CH_3$) and $M^+ - 30$ ($M - 2CH_3$) ions, suggesting that both CH_3O groups of **1f** are at lateral positions (Poiger et al., 1982; Tulp and Hutzinger, 1978), which should then identify compound **1f** as 2,3-dimethoxy-1,7,8-trichlorodibenzo-*p*-dioxin. For the other isomer, 1,2-dimethoxy-4,7,8-trichlorodibenzo-*p*-dioxin, only one MeO group is in the lateral position.

Table II. Summary of Mass Spectra of Methoxylated Derivatives of Dibenzo-*p*-dioxins

	relative intensity				
compd	M^+	$M^+ - CH_3$	$M^+ - 2CH_3$	$M^+ - CH_3CO$	$M^+ - CH_3CO - Cl$
1a	100	30[a]		36	
1b	77	6		22	
1c	100	46[a]		19	
1d	74			33	
1e	86	35[a]		16	
1f	98	56[a]	40		
1g	100	28[a]			57
1h	90	35[a]			57
1i	100	20[a]		23	2.5

[a] These compounds can produce *p*-quinone-type radical cation.

^1H NMR chemical shift assignments of various proton signals of methoxy- and hydroxy-substituted chlorodibenzo-*p*-dioxins are given in Table I and are consistent with their structures. Aromatic proton signals in all of these compounds expectedly came as singlets. For all of the compounds, the aromatic protons H-5 and H-9 were the most deshielded protons, with the chemical shifts ranging from δ 6.88 to δ 7.15. H-5 or H-9 protons appeared relatively downfield (δ 6.95–7.15) if a substituent (Cl, OCH_3, or OH) was present at C-4 or C-1, respectively. A similar observation was made previously with 2,3,4,7,8-pentachlorodibenzo-*p*-dioxin, for which the H-5 protons signal appeared downfield compared to the H-9 proton signal (Gray et al., 1976). As expected, the aromatic protons ortho to methoxy or hydroxy substituents appeared as the most shielded protons in these compounds. No unique or consistent pattern was observed for methoxy proton signals which could reflect the position of the methoxy substituent in the aromatic ring. In an earlier study with the analogous derivatives of dibenzofuran, the proton signal of the methoxy group appeared downfield if the methoxy group was present at C-4 (peri to furan oxygen) rather than at the C-1-, C-2-, or C-3-position (Burka and Oberstreet, 1989).

All of the dibenzo-*p*-dioxin derivatives synthesized gave a strong M^+ ion peak (see Table II). The appearance of the $M^+ - CH_3$ fragment as a strong peak for all of the compounds except **1b** and **1d** was consistent (Tulp and Hutzinger, 1978) with the presence of a methoxy group in a lateral position (C-2 and/or C-3) in these molecules. Compounds **1b** and **1d**, for which $M^+ - CH_3$ was either not present or present with weak relative intensity, do not possess a methoxy substituent in the lateral position. The presence of a strong $M^+ - CH_3$ fragment in the mass spectrum of **1g** in which none of the methoxy substituents was in the lateral position can be explained by the resonance stabilization of oxonium ion ($M^+ - CH_3$) with its *p*-methoxy substituent. The presence of a strong fragment $M^+ - COCH_3$ or $M^+ - COCH_3 - Cl$ in dibenzo-*p*-dioxin derivatives was not indicative of any unique substituent pattern.

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