Separation and Isomer Identification of PCDDs by Reversed-Phase Liquid Chromatography Using Electron-Donor and Electron-Acceptor Bonded Silicas.

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Summary

Electron-donor and acceptor bonded stationary phases, 2-(1-pyrenyl)ethylsilyl (PYE), 2-(nitrophenyl)ethylsilyl (NPE), and 3-(p-nitrophenoxy)propylsilyl (NPO) bonded silicas, permitted the separation and isomer identification of all the isomer pairs coproduced in the synthesis of PCDD standard, including all the DCDDs and TrCDDs as well as 1,2,4,6-/1,2,4,9-TCDDs, 1,2,4,7-/1,2,4,8-TCDDs, 1,2,4,6,7-/1,2,4,8,9-PnCDDs, 1,2,4,6,8-/1,2,4,7,9-PnCDDs, 1,2,3,6,7,9-/1,2,3,6,8,9-HxCDDs, and 1,2,4,6,7,9-/1,2,4,6,8,9-HxCDDs that have not been characterized individually.

1. Introduction

The isolation of individual congeners of polychlorodibenzo-p-dioxins (PCDDs) is indispensable to provide pure analytical reference standard compounds for determinations of equivalency in teratogenicity, toxicity, carcinogenicity, and other human health effects[1]. Although the spectroscopic characterization of mixtures has been attempted for isomers that are hard to separate, complications arose when quantitative ratios approached unity for the mixtures of isomers with the highest degrees of similarity[2]. Contradicting and ambiguous structure assignments have been reported for some isomers even after the comprehensive GC study including the use of a liquid crystal stationary phase[3].

When PCDD reference compounds are prepared, each one of an isomer pair, coproduced by the Smiles rearrangement in the synthesis[4], must be purified. We report here the application of electron-donor and acceptor stationary phases for the separation of all the synthesis isomer pairs of PCDDs by reversed-phase liquid chromatography (RPLC)[5]. The stationary phases also permitted isomer identification for all the congeners based on the elution orders on PYE phase, preferentially retaining the PCDDs with the more symmetrical chlorine substitution, and on nitroaromatic bonded phases, showing the preference for the PCDDs with the more proximal chlorine substituents.

2. Method

Equipment and measurement A Beckman bioseparation system with System Gold workstation, Model 126 pump, and Model 166 detection module, and a Shimadzu system with LC-6A pump, SIL-6A injector, SPD-6A UV detector, and C-R3A data processor were used. Column temperature was maintained at 30° C.

Columns. The NPE stationary phase possesses a mixed functionality of about 70% p- and 30% o-nitro-substituted phenylethyl groups. The C_{18}, NPO, and PYE stationary

phases were octadecylsilyl, 3-(p-nitrophenoxy)propylsilyl, and 2-(1-pyrenyl)ethylsilyl groups bonded onto silica particles with surface areas of 330 m^2/g. The columns are available commercially as Cosmosil $5C_{18}$, 5PYE, 5NPE, and 5NPO from Nacalai-Tesque.

3. Results and discussion

Characterization of stationary phases

The two types of aromatic stationary phases, PYE phase and nitroaromatic phase, displayed opposite retention preferences for aromatic compounds with electron-withdrawing substituents. Fig. 1 shows the chromatograms obtained for dinitronaphthalenes with the C_{18}, PYE, NPE, and NPO phases. The more dipolar 1,8-dinitronaphthalene was retained longer than 1,5-dinitronaphthalene on NPE and NPO with the latter resulting in the greater separation fator. The opposite retention order was observed on PYE phase. Similarly, nitroaromatic phases showed preferential retention for ortho-disubstituted benzenes, whereas the more symmetrical para-disubstituted benzenes were favored by PYE phase.

As shown in Fig. 2, PYE displayed longer retention for chlorobenzene molecules with chlorine atoms spaced as far as possible. In contrast, NPE and NPO showed preferential retention for chlorobenzenes with chlorine atoms positioned as close as possible. PYE preferentially retained isomers with the more symmetrical substitution or charge separation (which results in the more electron deficient aromatic ring leading to the more favorable charge-transfer interaction), whereas the nitroaromatic bonded phases preferentially retained those isomers with the more crowded chlorine substitution (which also have the greater dipolar character leading to the more favorable dipole-dipole interaction). The retention on C_{18} phase is dominated by hydrophobic interaction. Using these characteristic retention me-

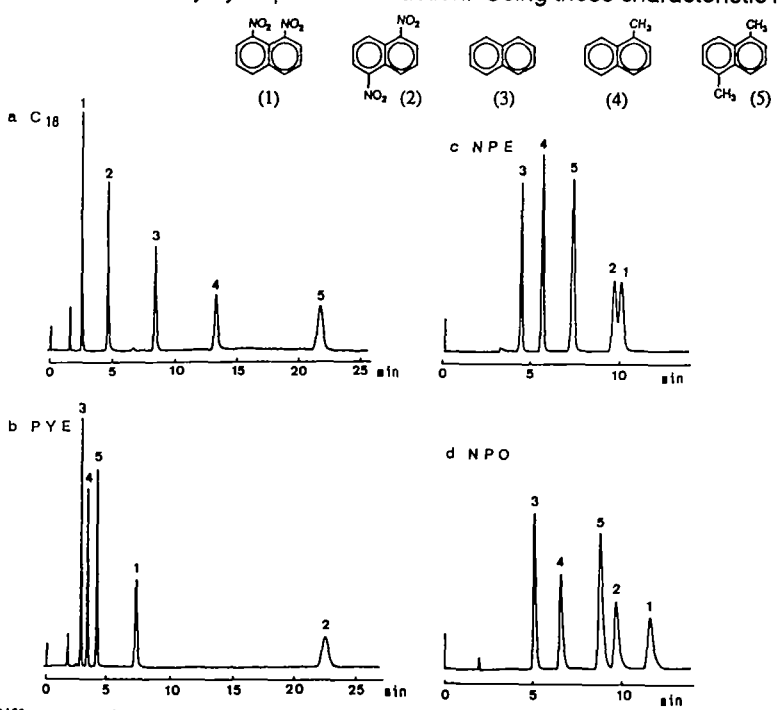


Fig. 1. Differences in selectivity of stationary phases for naphthalene derivatives: Peaks, (1) 1,8-dinitro, (2) 1,5-dinitro, (3) naphthalene, (4) 1-methyl, (5) 1,5-dimethyl. Mobile phase, methanol-water, 90:10 v/v for PYE and 70:30 for C_{18}, NPE, and NPO.

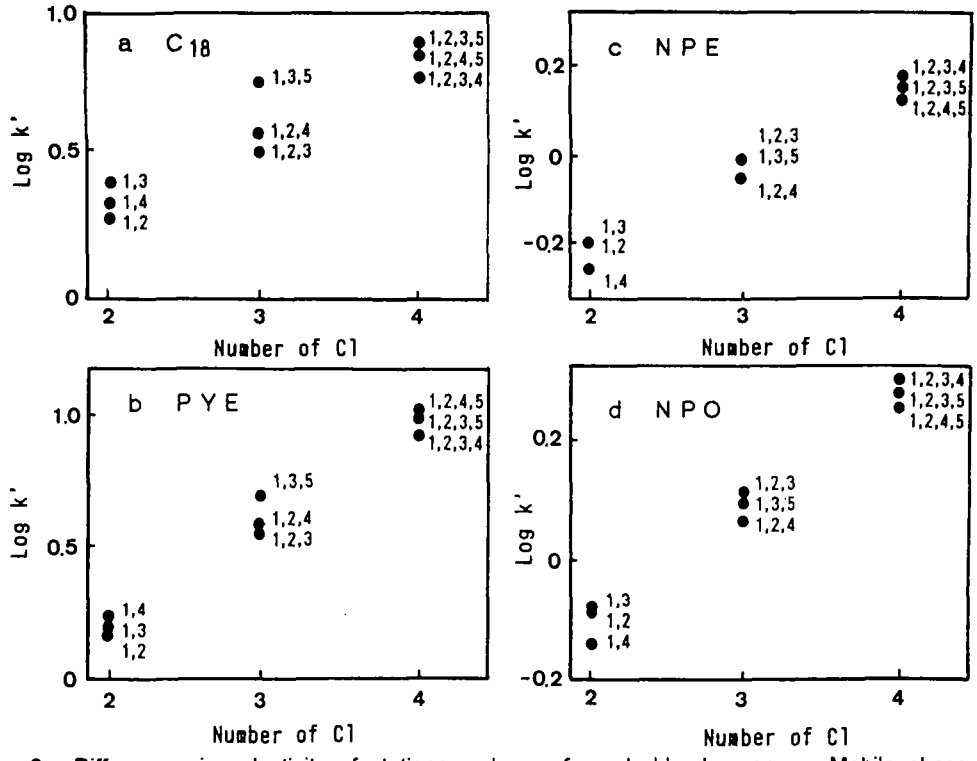


Fig. 2. Differences in selectivity of stationary phases for polychlorobenzenes. Mobile phase, methanol-water (80:20 v/v).

chanisms, it appeared possible to chromatographically identify the positional structure of substituents in isomer pairs of PCDDs.

Separation of PCDD isomers

In the preparation of PCDD standard from chlorocatechols and chloronitrobenzenes, mixtures of isomers with opposite configuration of chlorine substitution are produced by Smiles rearrangement[4]. There are 22 pairs (44 isomers) of PCDDs to be separated from the preparation mixtures. Unambiguous peak assignment following chromatographic separation has not been reported for DCDDs and TrCDDs and also for the six pairs of PCDDs with four to six chlorine substituents even by HRGC as mentioned above.

Separation of several isomer pairs of PCDDs from the preparation mixtures have been reported by using C_{18} phase[6]. The chromatographic behavior of these PCDDs was examined first to show the difference in selectivity of the electron-donor and acceptor phases. In fact, this provided basic understanding of the correlation among structure, retention, and peak size, that is to be used for the assignment of separated isomer peaks never characterized individually.

Among the seven pairs of TCDDs coproduced, four pairs were separated relatively easily. 1,2,3,6-/1,2,3,9-TCDDs gave such an example, as shown in Fig. 3. The first smaller peak on the C_{18} phase is reported to be 1,2,3,9-TCDD and the second larger one to be 1,2,3,6-TCDD[6]. This is understandable, because the more symmetrical 1,2,3,6-TCDD isomer should be produced more (to give the larger peak) based on the higher thermody-

namic stability than 1,2,3,9-TCDD that has the greater steric congestion between chlorines, and because 1,2,3,6-TCDD should be retained longer on C_{18} phase due to the greater hydrophobic surface area than 1,2, 3,9-TCDD with the greater steric congestion.

The same retention order for 1,2,3,6-/1,2,3,9-TCDDs on PYE phase as on C_{18} is readily understandable based on the greater charge-transfer interactions for 1,2,3,6-TCDD with the more symmetrical chlorine substitution. The elution order on NPE phase, however, was just opposite. This is understandable based on the greater dipole-dipole interactions with the 1,2,3,9-TCDD isomer supposed to have a greater molecular dipole than 1,2,3,6-TCDD.

Similar observations were made with the following PCDD isomer pairs with four to six chlorine substituents, 1,2,6,7-/1,2,8,9-TCDDs, 1,2,6,8-/1,2,7,9-TCDDs, 1,3,6,8-/1,3,7,9-TCDDs, 1,2,3,6,7-/1,2,3,8,9-PnCDDs, 1,2,3,6,8-/1,2,3,7,9-PnCDDs, 1,2,3,6,7,8-/1,2,3,7,8,9-HxCDDs, for which separations by C_{18} phase have been obtained. The correlation among the structure, peak size, and retention order on the four stationary phases is shown in Table I. The results can be generalized as follows. The more symmetrically substituted isomer is produced more by the reaction involving Smiles rearrangement based on the greater ther-

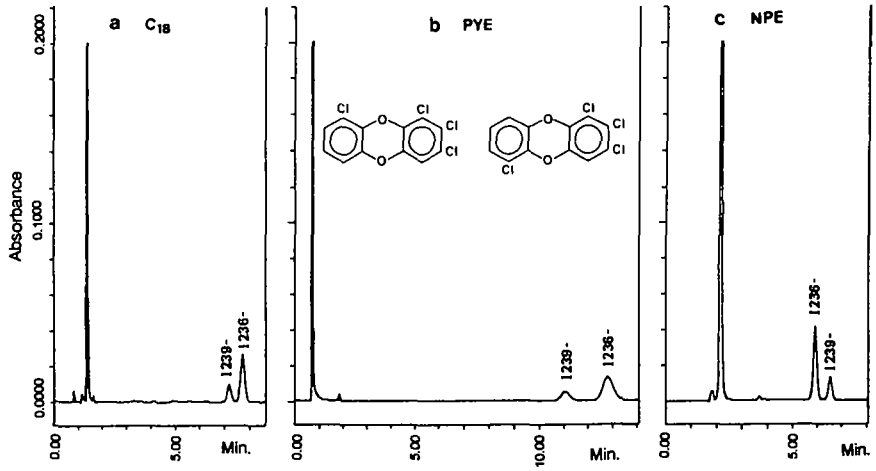


Fig. 3. Separation of 1,2,3,6-/1,2,3,9-TCDDs on C_{18}, PYE, and NPE phases. Mobile phase: methanol-water = 90/10 with C_{18}, 100/0 with PYE, and 90/10 with NPE.

Table I. Retention characteristics and peak identification scheme for PCDDs.

Stationary phase	TCDD preference (Longer retention)	*Mechanistic understanding*
C_{18}	Cl atoms more spaced (Major peak)	Greater hydrophobicity
PYE	Cl atoms more spaced (Major peak)	Greater charge-transfer interaction
NPE and NPO	Cl atoms in proximity (Minor peak)	Greater dipole-dipole interaction

An isomer with less steric congestion between chlorine atoms is preferentially produced during preparation due to the greater thermodynamic stability.

modynamic stability, or the less steric congestion between chlorine atoms. The more symmetrically substituted isomer is retained longer on C_{18} phase based on the greater hydrophobic property, and is also retained longer on PYE based on the greater charge-transfer interactions. On the other hand, the more dipolar isomer with the more congested chlorine substitution is produced less due to the lower thermodynamic stability, and retained longer on NPE and NPO phase based on the greater dipole-dipole interactions.

Structure identity assignment

We tried to separate all the isomer pairs from the preparation mixtures, and applied the generalized scheme for the identification of each separated peak. Fig. 4 shows the separation of 1,2,4,6-/1,2,4,9-TCDDs on C_{18}, PYE, and NPE phases together with 1,6-/1,9-DCDDs and 1,2,6-/1,2,9-TrCDDs. These pairs of isomers have never been characterized individually. Easy separation was observed for the DCDDs and TrCDDs on C_{18} and PYE with the smaller peak eluted first. It is logical to assume that these DCDDs and TrCDDs should follow the same tendency as 1,2,3,6-/1,2,3,9-TCDDs shown in Fig. 3, because these DCDDs and TrCDDs possess structures with one or two chlorine atoms removed from the structures of 1,2,3,6-/1,2,3,9-TCDDs. The tentative peak assignments shown in Fig. 4 agrees with the scheme in Table I. The separation of 1,2,4,6-/1,2,4,9-TCDDs, however, was not possible with C_{18} or PYE phase.

The elution orders for 1,6-/1,9-DCDDs and 1,2,6-/1,2,9-TrCDDs were reversed on NPE phase with the larger peak eluted first in agreement with the scheme in Table I. The nitroaromatic stationary phase was able to separate the 1,2,4,6-/1,2,4,9-TCDDs easily. Here it is logical to assume that the 1,2,4,6-/1,2,4,9-TCDD pair should follow the same tendency as 1,2,6-/1,2,9-TrCDDs, because the addition of one chlorine atom at the 4-position of the 1,2,6-/1,2,9-TrCDDs should not invert the relative dipolar character of the entire molecule between the isomers. Therefore the first larger peak on NPE phase was tentatively assigned

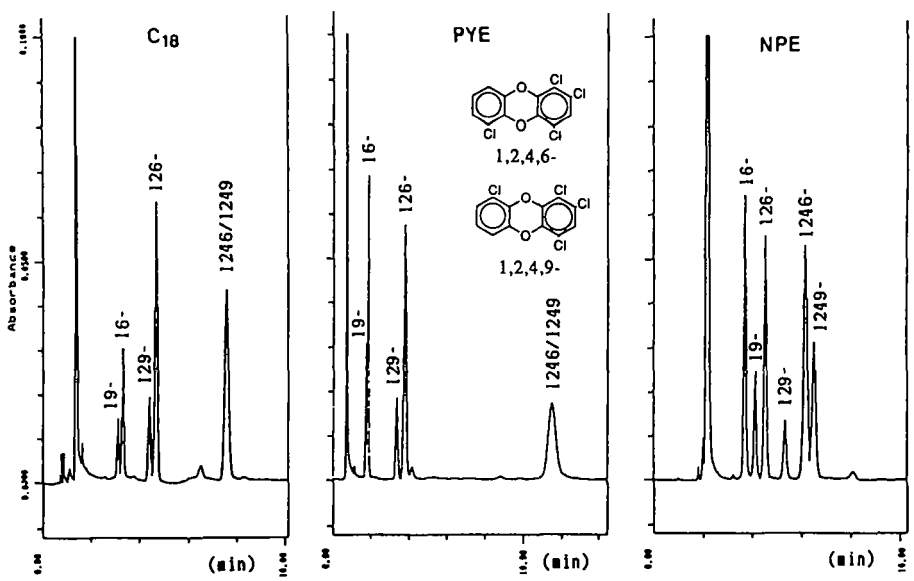


Fig. 4. Separation of 1,6-/1,9-DCDDs, 1,2,6-/1,2,9-TrCDDs, and 1,2,4,6-/1,2,4,9-TCDDs. Conditions as in Fig. 3.

as 1,2,4,6-TCDD and the second smaller peak to be 1,2,4,9-TCDD. The assignments agree with the scheme in Table I and the calculated dipole moments for these TCDDs[4b].

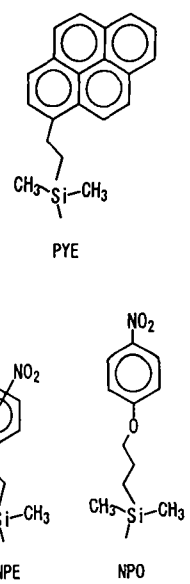
Fig. 5 shows similar results with 1,7-/1,8-DCDDs, 1,2,7-/1,2,8-TrCDDs, and 1,2,4,7-/1,2,4,8-TCDDs. The nitroaromatic stationary phases were able to separate the DCDD isomers and TrCDD isomers easily. The elution order reversal was observed for 1,2,4,7-/1,2,4,8-TCDDs between PYE and the nitrophenyl phases. The tentative peak assignments shown in Fig. 5 based on the scheme in Table I agree with the calculated dipole moments for the TCDDs[4].

In a similar manner, we were able to separate all the isomers in the 22 preparation mixtures of PCDDs. The results are summarized in Table II. In all cases where the isomer separation was successful with C_{18} or PYE phase, nitroaromatic phase showed the opposite elution order for the isomer pair. In all cases, NPO phase gave the greater separation factor than NPE phase, as shown in Table II and in Fig. 5. Now it is possible to separate and

Table II. Separation factors for PCDDs coproduced in preparation.

Dioxin mixture	Separation factor C_{18} [a]	PYE [b]	NPE [c]	NPO [c]
12/14	1.00	1.00	0.90	0.87
16/19	1.09	1.10	0.81	0.76
17/18	1.00	1.00	0.87	0.85
27/28	1.00	1.00	0.95	0.93
126/129	1.08	1.14	0.79	0.67
127/128	1.04	1.00	0.85	0.81
136/139	1.10	1.12	0.93	0.89
137/138	1.00	1.00	0.93	0.92
1236/1239	1.09	1.17	0.86	0.79
1237/1238	1.00	1.04	0.90	0.88
1246/1249	1.00	1.00	0.92	0.87
1247/1248	1.00	1.05	0.96	0.93
1267/1289	1.05	1.20	0.68	0.57
1268/1279	1.10	1.13	0.88	0.83
1368/1379	1.11	1.21	1.00	0.96
12367/12389	1.07	1.13	0.83	0.75
12368/12379	1.10	1.08	0.95	0.89
12467/12489	1.00	1.00	0.92	0.87
12468/12479	1.00	1.00	0.96	0.93
123678/123789	1.08	1.09	0.90	0.82
123679/123689	1.00	1.00	0.94	0.91
124679/124689	1.00	1.04	1.00	0.97
No. of separated pairs	11	13	20	22

a) Mobile phase: 90% methanol. b) Mobile phase: methanol for DCDD-TCDD, 50% dichloromethane-50% ethanol for PnCDD-HxCDD. c) Mobile phase: 80% methanol for DCDD-TCDD, 90% methanol for PnCDD-HxCDD.



Scheme 1. Structures of stationary phase.

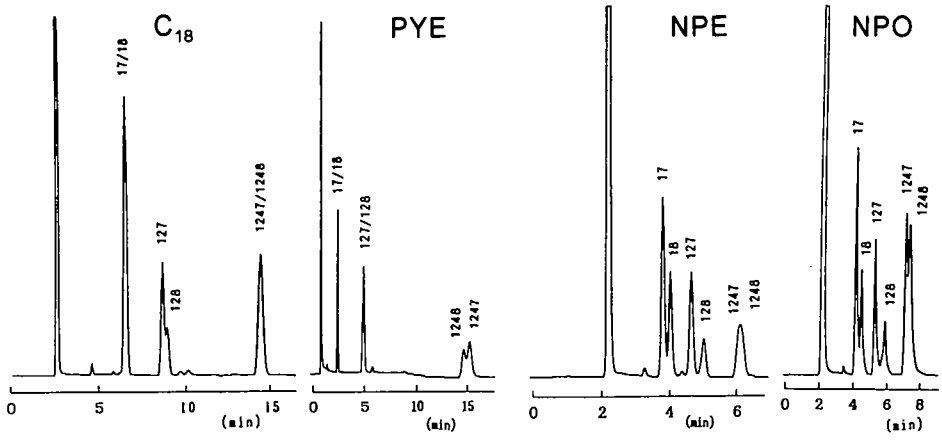


Fig. 5. Separation of 1,7-/1,8-DCDDs, 1,2,7-/1,2,8-TrCDDs, and 1,2,4,7-/1,2,4,8-TCDDs. Conditions as in Fig. 3.

assign structures of all the isomer pairs of PCDDs in preparation mixtures, by using electron-donor and acceptor phases for RPLC that show opposite retention characteristics depending on the position of chlorine substituents.

4. Conclusion

The NPO phase, which distinguishes PCDD isomers primarily by dipole-dipole interaction, can separate all of the isomer pairs coproduced in preparation. The nitroaromatic phases showed opposite retention orders for PCDDs that were separated by C_{18} or PYE phase. These stationary phases permitted structure assignment for the peaks of those congeners omitted in previous studies. The chromatographic peak assignments, which are based on the retention order, are in agreement with predictions of calculated dipole moments[4b].

5. References

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