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Note

Clean-up and separation of chlorobiphenyl isomers after synthesis by Cadogan coupling using preparative high-performance liquid chromatography

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The analysis of environmental samples for polychlorinated biphenyl (PCB) content is a common procedure. There are 209 possible PCB congeners with many exhibiting widely differing toxicities[1]. Levels of PCB contamination are generally expressed as a "total PCB" figure, usually obtained by a gas chromatographic (GC) pattern-matching technique or by a perchlorination method. Doubts have been expressed as to the accuracy and precision of these approaches to the quantification of PCBs[2,3]. Furthermore, these methods provide little or no information with regard to the identities of the individual congeners present in the sample, thus rendering the analysis of little value for toxicological evaluation. Consequently, the trend is now towards congener-specific analyses for PCBs using capillary GC methods[2,4]. Such methods require the use of individual congeners either as surrogate standards[5,6] or, preferably, as individual standards for each chlorobiphenyl analyte[2]. Unfortunately, less than half of the 209 congeners are available commercially and so workers frequently have to resort to in-house synthesis of their own standards[7,8]. Syntheses are relatively straightforward and for most chlorobiphenyls are achieved by the coupling of a chloroaniline-generated radical to a chlorobenzene in an excess of the chlorobenzene[7,9]. Unfortunately, the yields of such syntheses are low and produce a number of by-products. Additionally the use of a chlorobenzene with non-equivalent hydrogens gives rise to a mixture of chlorobiphenyl isomers. The clean-up of the chlorobiphenyls produced by this type of coupling (Cadogan coupling[10]) is laborious and the separation of chlorobiphenyl isomers can be extremely difficult, often being achieved by using repeated preparative thin-layer chromatography (TLC)[7]. Described below is a clean-up procedure followed by a preparative high-performance liquid chromatographic (HPLC) separation method for selected pairs of chlorobiphenyl isomers produced by Cadogan coupling.

EXPERTIMENTAL AND RESULTS

The coupling reactions were chosen such that no more than two chlorobiphenyl isomers would be produced. The Cadogan coupling was performed in an excess of the selected chlorobenzene and after the reaction had taken place the first step in the clean-up procedure was the removal of this excess. This was done by

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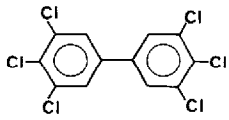
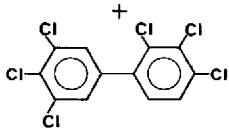
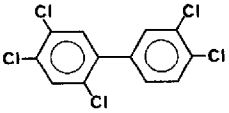
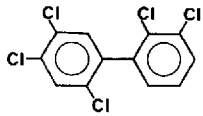
distillation under vacuum. The residue, which was a deep red-brown colour, was dissolved in a minimum of hexane and was transferred to the top of a 300 mm × 50 mm I.D. column of silica (Kieselgel 60, 70–230 mesh ASTM, Merck; wet-packed in hexane) and eluted with hexane. The chlorobiphenyls and any remaining chlorobenzene were virtually unretained by the silica and were the first components of the residue to elute from the column. Most of the by-products of the reaction appeared to be coloured compounds ranging from a deep red-brown to a light yellow colour. These were separated by the silica–hexane system with the darker pigments being retained at the top of the column and the lighter ones eluting close to the chlorobiphenyls. It was found necessary to collect a little of the light-yellow band with the chlorobiphenyl fraction in order to obtain maximum recovery of the chlorobiphenyls. The chlorobiphenyl fraction was then evaporated to dryness using a rotary evaporator.

TABLE I

PCB ISOMER PAIRS STUDIED AND MOBILE PHASE USED FOR THEIR SEPARATION

Identity	*Congener number (after Ballschmiter and Zell[11])*	*Synthesis starting materials*	*Mobile phase for separation*
 3,3′,4,4′,5,5′-Hexachlorobiphenyl	169	3,4,5-Trichloroaniline + 1,2,3-trichlorobenzene	THF–water 75:25 (v/v)
+			
 2,3,3′,4,4′,5′-Hexachlorobiphenyl	157		
 2,3′,4,4′,5-Pentachlorobiphenyl	118	2,4,5-Trichloroaniline + 1,2-dichlorobenzene	THF–water 65:35 (v/v)
+			
 2,2′,3′,4,5-Pentachlorobiphenyl	97		

The next step was the preparative-scale isolation of the individual chlorobiphenyl isomers by HPLC. This was achieved using a 250 mm × 21.2 mm I.D. Zorbax ODS preparative column (5–6 μm particle size) with tetrahydrofuran (THF)–water as mobile phase. As may be expected, the relative proportions of THF and water in the mobile phase required for baseline separation of a given pair of chlorobiphenyl isomers depends on the two isomers concerned. Table I shows the isomers that we synthesised in pairs and the composition of the mobile phase that was required for their separation.

The chlorobiphenyls were dissolved in a minimum of mobile phase (the water content of the mobile phase limits the solubility of the chlorobiphenyls). The instrument used for the separation was a DuPont 830 preparative HPLC unit fitted with a Rheodyne injection valve and a 2.0-ml loop. The mobile phase was pumped through the system *ca.* 12 ml min^{-1}. The column and injection systems were maintained at 55°C in order to assist solubility and maintain adequate peak shape. Detection was achieved by a DuPont UV detector at 254 nm, 2.56 a.u.f.s., fitted with a preparative-scale flow cell. Repeated injections of 2.0 ml of the crude chlorobiphenyl solutions were made and the individual chlorobiphenyl isomer fractions collected. This separation system also functioned as a secondary clean-up for the removal of any remaining chlorobenzene or pigments. The chlorobiphenyls should be the largest peaks in the chromatogram unless a large amount of chlorobenzene is present in which case the chlorobenzene can be distinguished because the chlorobiphenyls are retained much more strongly than the chlorobenzene. The elution order of the two chlorobiphenyls may be determined from theoretical considerations (see below) but must, of course, be confirmed by other means such as melting points (where these are known), and NMR. Fig. 1 shows a typical chromatogram for the isomer pair 118/97. Note the residual chlorobenzene and other impurities.

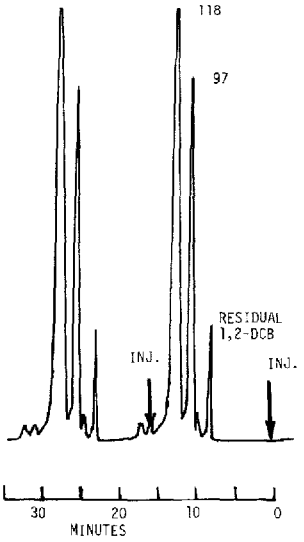


Fig. 1. Replicate chromatograms for the separation of PCBs 118 and 97 by RP-HPLC using Zorbax ODS column (5 to 6 μm) 250 mm × 21.2 mm. Mobile phase, THF–water (65:35, v/v) at 12 ml min^{-1}. 2 ml injection. UV detection at 254 nm, 2.56 a.u.f.s. System maintained at 55°C.

The isolated isomer fractions were evaporated to dryness in a rotary evaporator (starting at a low temperature to remove the THF, then increasing the temperature to remove the water). The individual isomers were then recrystallised from methanol and dried. Identities and purities were confirmed using capillary GC, ^{1}H and ^{13}C NMR and mass spectrometry. Purities as determined by capillary GC are shown in Table II.

TABLE II

PURITY OF PCB ISOMERS BY CAPILLARY GC AFTER CLEAN-UP SEPARATION AND RECRYSTALLISATION

PCB	*Purity* (%)*
97	99.0
118	>99.5
157	99.4
169	98.0

* Assuming equal response factors for all peaks in the chromatogram.

DISCUSSION

Chlorinated biphenyls are very non-polar substances and are not retained by silica. Reversed-phase systems provide more retention and therefore better conditions for attempting a separation of chlorobiphenyl isomers. The isomers produced by the Cadogan synthesis are inevitably very similar, containing the same number of chlorines and the same substitution pattern in one of the rings (the ring provided by the chloroaniline, see Fig. 2). This leaves relatively few differences to exploit in order to achieve a separation. However, although the chlorines in the ring provided by the chlorobenzene will have the same pattern with respect to each other in all the chlorobiphenyl products, the pattern of substitution of the chlorines with respect to the ring bridge will be different (see Fig. 2). This has two main consequences; firstly, increasing the degree of *ortho* substitution will affect the dihedral angle between the rings and restrict the rotation about the ring bridge[12]. Secondly, the difference in the

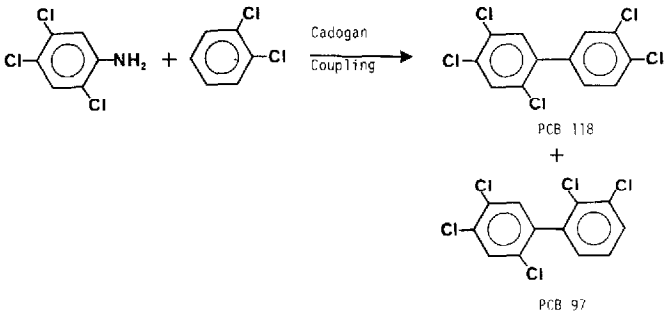


Fig. 2. Synthesis of PCBs 118 and 97 by Cadogan coupling.

chlorine pattern in one of the rings gives rise to differences in dipole moment between the isomers. Attempting to exploit differences in dihedral angle alone would be difficult since differences may be slight and would require a high degree of selectivity in the surface structure of the stationary phase and considerable restriction of rotation about the biphenyl bridge. However, increasing *ortho* substitution also increases water solubility[12] and exploiting this, together with differences in dipole moment is likely to be more successful.

For several reasons, THF was considered to be a useful solvent for attempting this type of separation. Firstly, chlorobiphenyls are very soluble in THF enabling high loadings per injection to be achieved. Secondly, THF is able to induce selectivity on the basis of differences in dipole moment (X_n = 0.42, ref. 13). Thirdly, THF, being a cyclic molecule with oxygen in the ring, suggests that any differences in the chlorine substitution pattern as well as the dihedral angles of the chlorobiphenyls may change the way the THF interacts with the chlorobiphenyls in terms of orientation about the biphenyl nucleus and the way the THF and chlorobiphenyl molecules stack in the solvation process. Consequently, we considered that there would be some scope for achieving selectivity between chlorobiphenyl isomers using THF as a mobile phase component. Using 100% THF as mobile phase with an ODS column resulted in virtually no retention of chlorobiphenyls. However, adding water to the mobile phase at a level of between 25 and 35% (v/v) dramatically increased the retention of the chlorobiphenyls and enabled the THF to exercise its selectivity. Fine tuning of the relative proportions of THF and water in order to achieve baseline separation has to be done largely by experiment. With regard to the elution order of a given pair of isomers, we found it possible to predict which isomer is likely to have the greater dipole moment simply by inspecting the structures. This gives a useful indication of the elution order since one would expect the isomer with the greater dipole moment to favour the mobile phase more than the isomer with the lesser dipole moment. Consequently, one would expect the isomer with the greater dipole moment to elute first, and this was found to be the case for the isomer pairs studied. It should be stressed, however, that this is only a guide and other factors are involved in the retention mechanism; consequently, elution order must be confirmed by other means.

Total elution time for the chlorobiphenyls is typically about 15 min with some impurities eluting just after the chlorobiphenyls. Injections may be overlapped to save time.

The isolated isomers were generally quite pure at this stage, but since some minor impurities may co-elute with the individual chlorobiphenyls, recrystallisation from methanol was carried out.

It can be seen from Fig. 1 that some tailing is evident in the chlorobiphenyl peaks and if the isomers elute close together, the earlier eluting isomer may contaminate the later. In view of this it may be necessary to collect the later eluting isomer as a late cut of the peak. One could, of course, increase the water content of the mobile phase in order to increase retention and therefore separation, but this has the disadvantages of prolonging the elution time and, more importantly, reducing the solubility of the chlorobiphenyls in the mobile phase, Thus making it necessary to introduce the chlorobiphenyl products over more injections. The problem of contamination of one isomer with the other must be resolved by the chromatography stage

since eliminating such contaminants by recrystallisation could prove to be very difficult.

CONCLUSION

The use of a preparative ODS column with a THF–water mobile phase has been shown to exhibit useful selectivity for the separation of chlorobiphenyl isomers after synthesis and preliminary clean-up. This HPLC approach is deemed to be more convenient and less time-consuming than repeated preparative TLC.

It should be borne in mind that certain chlorobiphenyl congeners are very toxic both by ingestion and skin contact. Appropriate precautions should be taken to prevent contamination of workers, and equipment should be decontaminated after use.

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