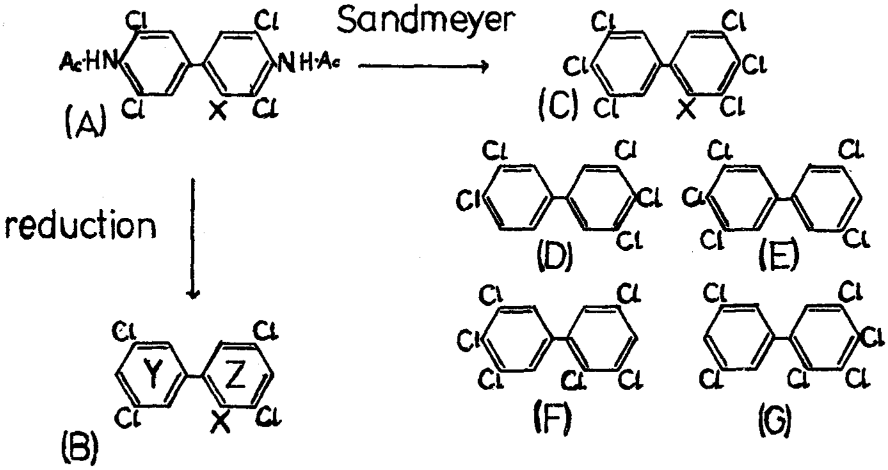
On the Diversity of Products Obtained During Synthesis of Polychlorobiphenyls by the Van Roosmalen Procedure

by David C. Ayres and Gary R. W. Denton*
Departments of Chemistry and Zoology
Westfield College
Hampstead, London

This method (VAN ROOSMALEN 1934) was selected because, after a common initial chlorination of N,N-diacetylbenzidine, the product (Scheme; A, X=H) can either be reduced to 3,3',5,5'-tetrachlorobiphenyl or converted into the highly chlorinated Sandmeyer product (C, X=H). These compounds were required for _in vivo_ toxicity testing with the American hard-shelled clam _M. mercenaria_ and the importance of pure substrates for this work has already been stressed in a previous communication (AYRES and DENTON 1972).

SCHEME



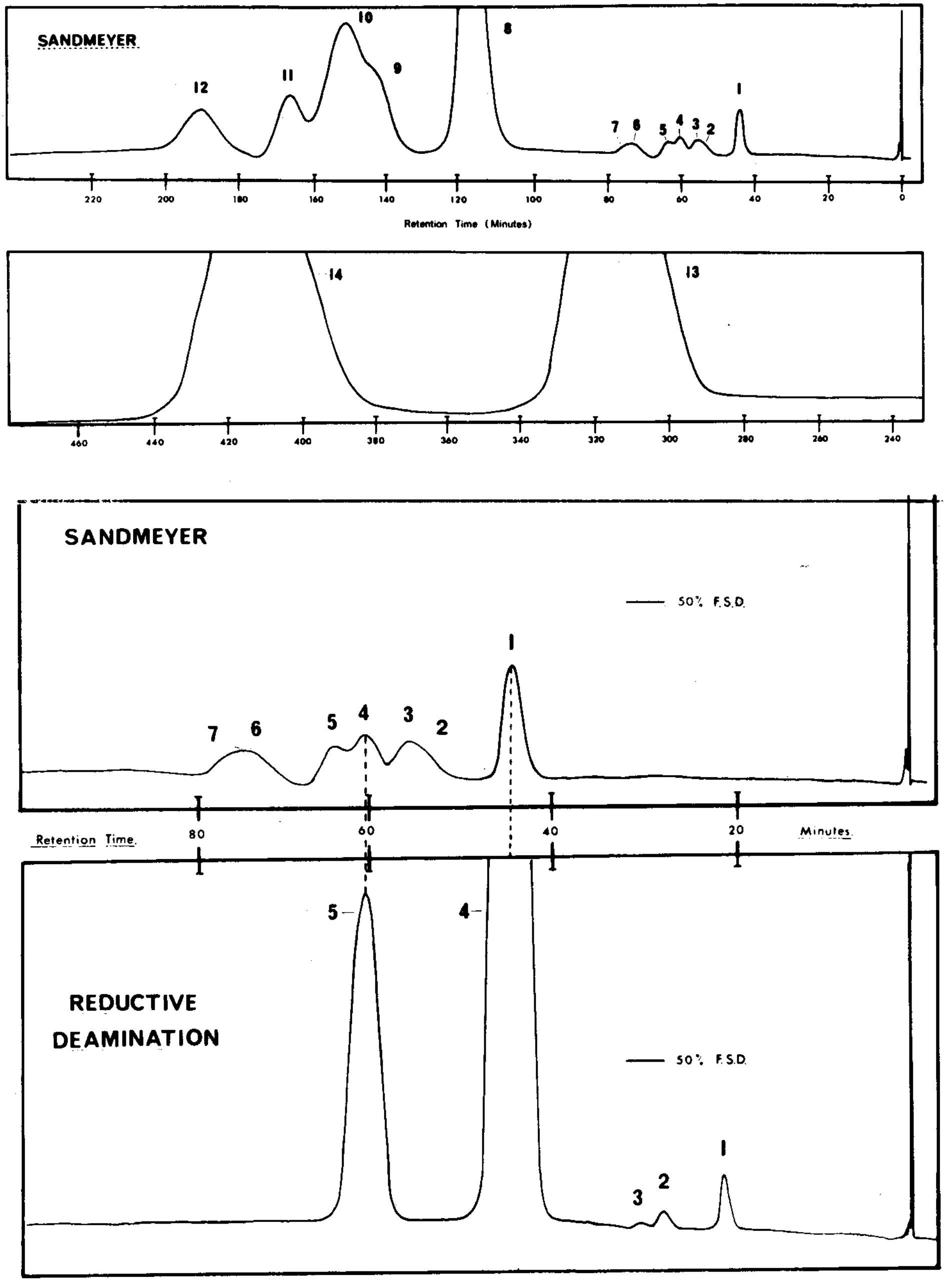
Polychlorobiphenyls formed from N,N-diacetylchlorobenzidines

---

* present address: Marine Biology Department, James Cook University Queensland 4811, Australia.

Bulletin of Environmental Contamination & Toxicology,
Vol. 14, No. 3 © 1975 by Springer-Verlag New York Inc.

Figure 1



GLC TRACINGS

Upper pair: at high sensitivity to show low retention peaks.

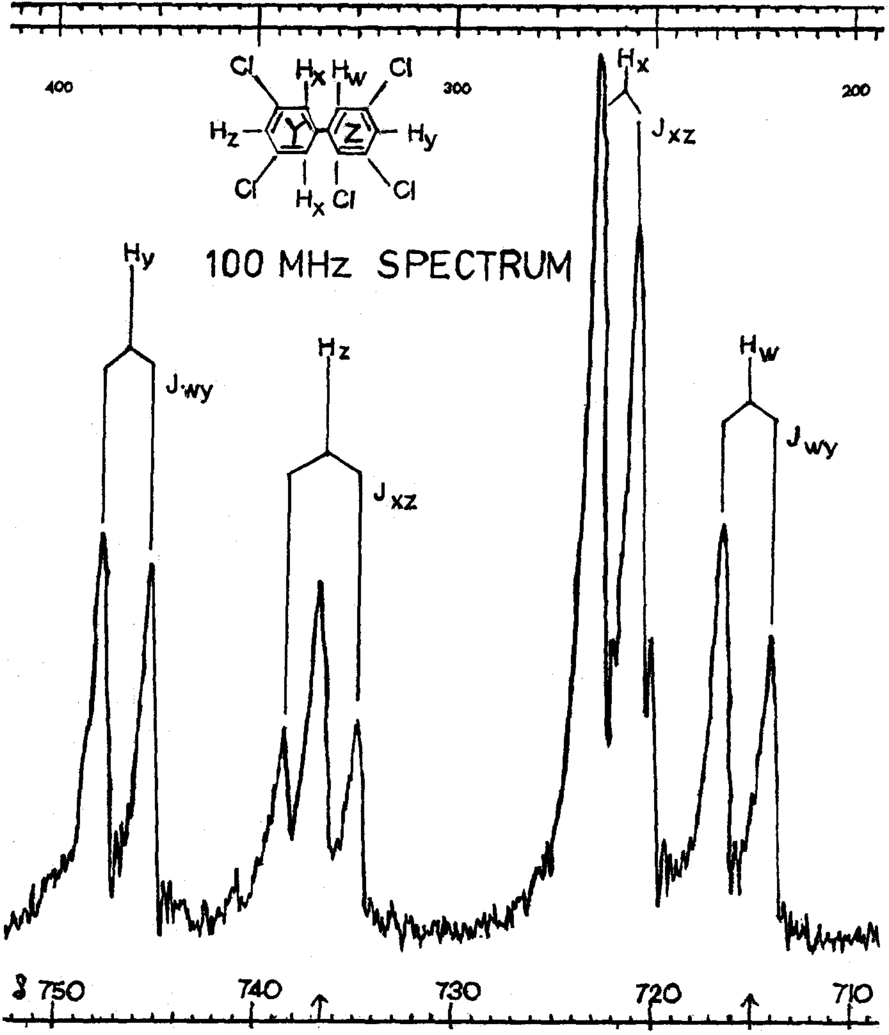
Lower pair: comparison of these peaks with those of the major deamination products.

Peak 10 resolves as a doublet when a capillary column is used.

The initial preparation of N,N-diacetylbenzidine (STRAKOSCH 1872) served the dual purpose of purifying the precursor and of increasing its solubility during chlorination in hot acetic acid. Full details of the analytical gas chromatography of the products will be reported elsewhere (DENTON to be published), but the detection of two major and three minor peaks in the reduction product and of eight significant and seven minor peaks from the Sandmeyer reaction (Figure 1) is evidence of a number of competing pathways. Although the Van Roosmalen procedure has been used recently by other workers (MELVAS 1971; HUTZINGER et al 1971; SAFE and HUTZINGER 1972) this critical feature has not been referred to.

The major components (peaks 4 and 5) from reduction were readily separated by preparative g.c. and the peak of lower retention was identified as 3,3,5,5'-tetrachlorobiphenyl having the reported (HUTZINGER et al 1971) physical constants. This compound has a 100 MHz n.m.r. spectrum in which the six protons are unresolved at a chemical shift of δ 7.34. The other main component had a more complex n.m.r. spectrum (Figure 2) which was assigned as that of 2,3,3',5,5'-pentachlorobiphenyl (B, X=Cl), so characterising the excessively chlorinated precursor (A, X=Cl). In view of Welti and Sissons analysis (WELTI and SISSONS 1972) of the spectra of a group of polychlorobiphenyls the chemical shift of the triplet at δ 7.37 (Figure 2) may be assigned to H_z. This is expected to be close to the C4-H signal observed (coincidentally with C2-H) in the 3,3',5,5'-tetrachlorobiphenyl as the additional 2-chloro substituent will not affect this remote proton significantly. A similar shift (δ 7.47) is assigned to the doublet (1H) arising from H_y. Protons in the 2-positions of (B, X=Cl) will be shielded relative to those of the tetrachloro compound (B, X=H) because the rotational mean position of rings Y and Z will diverge from coplanarity; hence the doublet (2H) at δ 7.22 is assigned to H_x and the doublet (1H) at δ 7.15 to H_w. These assignments are substantiated by the observation that the meta-couplings experienced by the protons differ, that in the trichlorinated ring Z (J_{wy} = 2.5H_z) being greater than for the disubstituted ring Y (J_{xz} = 1.75H_z).

Figure 2



In a re-investigation of the chlorination step Mr. R. GOPALAN (personal communication) showed by deamination and g.l.c. that peaks 4 and 5 (Figure 1) were detectable after a reaction time of only five min. Moreover their intensity was greater than those arising from products of lower chlorine number having retentions similar to peaks 1-3. Limitation of the number of Sandmeyer products by varying the reaction time was not therefore attempted and the two most abundant components (peaks 13 and 14) were separated by prepative g.c. That of lower retention proved to be the known (HUTZINGER et al 1971) 3,3',4,4',5,5'-hexachlorobiphenyl having four equivalent protons in its n.m.r. spectrum at δ 7.45. Peak 14 was shown by mass spectrometry (Experimental section) to arise from a heptachlorobiphenyl. The absence of coupling in the n.m.r. spectrum shows that the three protons are divided between rings Y and Z and the two singlet peaks have shifts close to those recorded for C-2 protons in B (X=Cl). This is characteristic of 2,3,3',4,4',5,5'-heptachlorobiphenyl, the logical product of dichlorination of the excessively substituted compound (A, X=Cl) already characterised as a major component by analysis of the reduction mixture.

A third significant component (peak 8) was separated and was shown by its mass number to be a pentachlorobiphenyl with a different retention time to that formed by reduction of A (X=Cl). This is not to discount the possibility that reduction had occurred during the Sandmeyer reaction as there are many known (COWDREY and DAVIES 1952) instances of reduction competing with the chlorination on monofunctional diazonium salts; a number of authors (RICHARDT et al 1970) have reviewed mechanistic aspects. The unknown could have been formed by the normal Sandmeyer reaction of incompletely chlorinated material, N,N-diacetyl-3,3',5-trichlorobenzidine or, more probably, from the major chlorination product (A, X=H) by chlorine insertion at one centre and reduction at the other. The first of these possibioities (D) was excluded by a comparison of the 220 MHz n.m.r. spectrum with those of 3,4-dichloro- and 3,4,3',4'-tetrachlorobiphenyl, when only the models showed typical <u>ortho</u> coupling patterns. The test spectrum contained only singlet peaks at δ 7.51 (2H) and 7.36 (3H) which closely ressemble the shifts of 3,3',4,4',5,5'-hexachlorobiphenyl (δ 7.45) and of 3,3',5,5'-tetrachlorobiphenyl (δ 7.34). This correspondence established the unsymmetrical structure (E) for the unknown having signals typical of one ring of each symmetrical model. The shifts shown by 3,3',4,5,5'-pentachlorobiphenyl (E) will differ slightly from the reference compounds as they have one chlorine atom more, or less, at the remote C-4 position.

It was important to determine whether any of the remaining products of the Sandmeyer reaction were chlorophenols which can arise by the hydroxylation of an intermediate carbonium ion (NAKATANI 1970). Small amounts of these compounds could invalidate toxicity tests of the polychlorobiphenyls themselves: in fact, no alkali-soluble phenolic compounds were detected in our products. A detailed GLC analysis (DENTON) has confirmed that the peaks 1 and 4 of the Sandmeyer products arise from 3,3',5,5'-tetrachloro- and 2,3,3',5,5'-pentachlorobiphenyl respectively. They are seen here to coincide with peaks 4 and 5 from the major products of reduction. This reaction competes effectively with chlorine insertion because replacement of the hindered diazonium function by hydrogen in the 4-position is sterically favoured; 2,4,6-trichlorobenzene diazonium halides yield mainly 2,4,6-trichlorobenzene under similar conditions (HODGSON and MAHADEVAN 1947). It was observed that chlorination of N,N-diacetylbenzidine rapidly affords a range of products differing in chlorine number and/or orientation (GOPALAN). Further variations arising from replacement of the acetylamino groups by hydrogen and/or chlorine point to a range of possible structures for unidentified polychlorobiphenyls. For example, further analysis (DENTON) has shown that **A** (X=Cl) gives rise to 2,3,3',4',5,5'-hexachlorobiphenyl (**F**, peak 10, Figure 1) and also to 2,3,3',4,5,5'-hexachlorobiphenyl (**G**, peak 11).

EXPERIMENTAL

M.p.'s were taken on a hot stage apparatus. 60 MHz n.m.r. spectra of polychlorobiphenyls were recorded for deuteriochloroform solutions with a Varian A 60A spectrometer and 100 MHz spectra were taken on a Varian HA 100 instrument by the Science Research Council unit at Imperial College. 220 MHz n.m.r. spectra were obtained by the S.R.C. unit at Harwell, whose A.E.I. MS 903 instrument was used to record the mass spectra.

Analytical Gas Chromatography was carried out with a Pye 104 instrument fitted with dual electron capture detectors maintained at 220° and purged at 120 ml/min. The glass chromatography column (6.5' x 0.125" i.d.) was packed with Apiezon 'L' (1.3%) + Epicote (0.2%) on acid washed D.M.C.S. Chromosorb W (80-100 mesh Perkin Elmer Corp.) and maintained at 180° with a nitrogen flow rate of 40 ml/min. Column loadings were necessarily high to ensure detection of low retention minor components (Figure 1).

Response factors for the chlorobiphenyls mentioned later in this section were obtained with a 3' x 0.125" i.d. glass column packed with 6% QF1 and 4% MS 200 on GC-2 (120-140 mesh) at 190°. The detector at 220° was purged at 100 ml/min. The tabulated values were used to calculate the relative yields of these compounds in mixtures separated, or partially separated, by column chromatography.

TABLE

Substituted biphenyl	Retention time	Response Factor (relative to p.p.-DDE=1.00)
3,3',5,5'-tetrachloro	0.74	0.48
2,3,3',5,5'-pentachloro	1.00	0.61
3,3',4',5,5'-pentachloro	1.63	0.64
3,3',4,4',5,5'-hexachloro	3.56	0.72
2,3,3',4,4',5,5'-heptachloro	4.43	1.12

The last three compounds (peaks 8, 13 and 14) accounted for 92.5% of the Sandmeyer product mixture.

Preparative Gas Chromatography was used to obtain samples for n.m.r. and mass spectra determination; here the Pye 104 was fitted with a flame ionisation detector and fraction collector. The column (6' x 0.24" i.d.) was packed with 10% QF1 on Chromosorb W (80-100 mesh) and maintained at 190°.

Reactions of Benzidine with Acetic Acid and Chlorine. Benzidine (25 g) afforded the crude diacetyl derivative (STRAKOSCH 1872) (35 g) after 2 hr reflux in glacial acetic acid (250 ml). A solution of recrystallised material in fresh acid (750 ml) was refluxed for 4 hr with continuous passage of chlorine gas, insoluble material, m.p. 340° (VAN ROOSMALEN 1934, 340°), was divided into two equal portions for the diazonium salt reactions without purification as originally described.

Deamination. The crude acetylaminochlorobiphenyl (10 g) was hydrolysed in sulphuric acid (40 g), tetrazotised using 10% sodium nitrite solution (50 ml) after the addition of water (60 ml), and the tetrazonium salt solution was refluxed with ethanol (500 ml) for 1.5 hr. The solid which separated on cooling was shown to be a mixture of at least five compounds by gas chromatography (Figure 1). The two main fractions (peak 4; 82%) and (5; 18%) were largely separated by chromatography on alumina (Spence type H, activated at 500° for 5 hr) using hexane as eluent; the minor fractions were not quantified. After further purification by preparative gas chromatography fraction 4 was identified as 3,5,3',5'-tetrachlorobiphenyl, m.p. 165° (HUTZINGER et al 1971; 164°) (Found: M^+ 290, calculated for $C_{12}H_6{}^{35}Cl_4$ M=290). Fraction 5 (B, X=Cl in scheme) was identified as 2,3,3',5,5'-pentachlorobiphenyl, m.p. 105°. (Found: M^+ 323.8870, $C_{12}H_5{}^{35}Cl_5$ requires M=323.8834) with the n.m.r. spectrum previously described (Figure 2).

Sandmeyer Reaction. A second portion of the acetylaminochlorobiphenyl (10 g) was hydrolysed and tetrazotised as above. With the tetrazonium salt solution maintained at 0° copper(I) chloride (4 g) dissolved in concentrated hydrochloric acid (20 ml) was added dropwise and the precipitated material was extracted into benzene. The mixture of polychlorobiphenyls obtained on evaporation of benzene could not be completely separated by any one chromatographic technique, although gas chromatography revealed (Figure 1) three major components (peaks 8, 13 and 14), five intermediate and at least six minor components. A two-stage application of the alumina column chromatography described above led to the separation of three pure fractions eluted in the order - compound 14 (18%), compound 8 (5.5%), and compound 13 (69%) - together with a combined fraction containing 8, 13 and other unidentified compounds (combined yield 7.5%). PLC on Kiesel gel and elution with hexane gave a pure slow running fraction of 13, but fractions of higher R_f were not pure.

Fraction 8 was identified as *3,3',4,5,5'-pentachlorobiphenyl*, m.p. 153-4°, by its mass spectrum (Found: M^+ 324 with ^{35}Cl and ^{37}Cl combination ions in the calculated relative abundances, $C_{12}H_5{}^{35}Cl_5$ requires M=324) and by n.m.r. spectra. Samples obtained by preparative gas chromatography showed four signals in the 100 MHz spectrum at δ 7.14 (not $CHCl_3$), 7.36, 7.51 and 7.63. Subsequent recrystallisation (ethanol) gave a pure sample which was examined at 220 MHz and which showed only the two singlet peaks characteristic of this pentachloro compound which were discussed above.

Fraction 13 - was the anticipated product 3,3',4,4',5,5'-hexachlorobiphenyl, m.p. 203° (HUTZINGER et al 1971, 202°). (Found: M^+ 358. Calc. for $C_{12}H_4{}^{35}Cl_6$ M=358).

Fraction 14 - was identified as *2,3,3',4,4',5,5'-heptachlorobiphenyl*, m.p. 130°, (Found: M^+ 391.8053, $C_{12}H_3{}^{35}Cl_7$ requires M=391.8055) further typified by two singlet peaks in the 100 MHz spectrum at δ 7.29 (H) and δ 7.33 (2H).

Time Dependence of the N,N-Diacetylbenzidine Chlorination (by R. Gopalan). The chlorination in refluxing acetic acid was carried out as above and samples were withdrawn every 5 min. Each sample was chilled in ice-water and then tetrazotised as rapidly as possible. Reduction in ethanol, benzene extraction and GLC showed that five compounds having t_r less than 3,3',5,5'-tetrachlorobiphenyl (Peak 4, Figure 1) had been formed in the first 5 min., whilst significant amounts of the major end products were already present.

After 45 min. at least four products of low t_r (cf. peaks 1-3) remained and their combined response exceeded that of the pentachloro compound (B, X=Cl, Peak 5).

REFERENCES

AYRES, D.C. and G.R.W. DENTON: Nature 240, 161 (1972).
COWDREY, W.A. and D.S. DAVIES: Quart. Rev. 6, 370 (1952).
DENTON, G.R.W.: to be published.
HODGSON, H.H. and A.P. MAHADEVAN: J. Chem. Soc. 1947, 173 (1947).
HUTZINGER, O., S. SAFE and V. ZITKO: Bull. Environ. Contam. Toxicol. 6, 209 (1971).
MELVAS, B.: Nord. Hyg. Tidsskr. 52, 404 (1971).
NAKATANI, Y.: Tetrahedron Letters 1970, 4455 (1970).
RICHARDT, C. E. MERZ, B. FREUDENBERG, H.-J. OPGENORTH, C.C. TAN and R. WERNER: Chem. Soc. Spec. Pub. 24, 51 (1970).
SAFE S. and O. HUTZINGER: J. Chem. Soc. Perkin I 1972, 686 (1972).
STRAKOSCH, J.: Chem. Ber. 5, 236 (1872).
VAN ROOSMALEN: Rec. Trav. chim. 53, 359 (1934).
WELTI, D. and D. SISSONS: Org. Mag. Resonance 4, 309 (1972).