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DECOMPOSITION OF CHLORINATED AROMATICS: MICROBIOLOGICAL DECOMPOSITION OF POLYCHLORINATED BIPHENYLS (PCB): Part III. CHLORINATED BENZOIC ACIDS AS PCB METABOLITES

[Abbau von chlorierten Aromaten: Mikrobiologischer Abbau der polychlorierten Biphenyle (PCB). III. Chlorierte Benzoesäuren als Metabolite der PCB]

by

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The decomposition [1] of polychlorinated biphenyls (1)[1] by monoxygenases progresses across the intermediate stage of arenoxides [2](2) by spontaneous isomerization to phenylphenols [3](3), or by enzymatic hydration across phenylcyclohexadiene-transdiols (4) to o-dihydroxy-biphenyls (7), whose ring openings lead one to expect a complex system of chlorophenylcarbonic acids (8) [4,5,6](Fig. 1). By a renewed reaction, the phenylphenols can also lead to dihydroxy-biphenyls [3].

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[1] [Underlined numbers in parentheses refer to numbered groupings in Fig. 1. Numbers in brackets indicate references. -- Translator]

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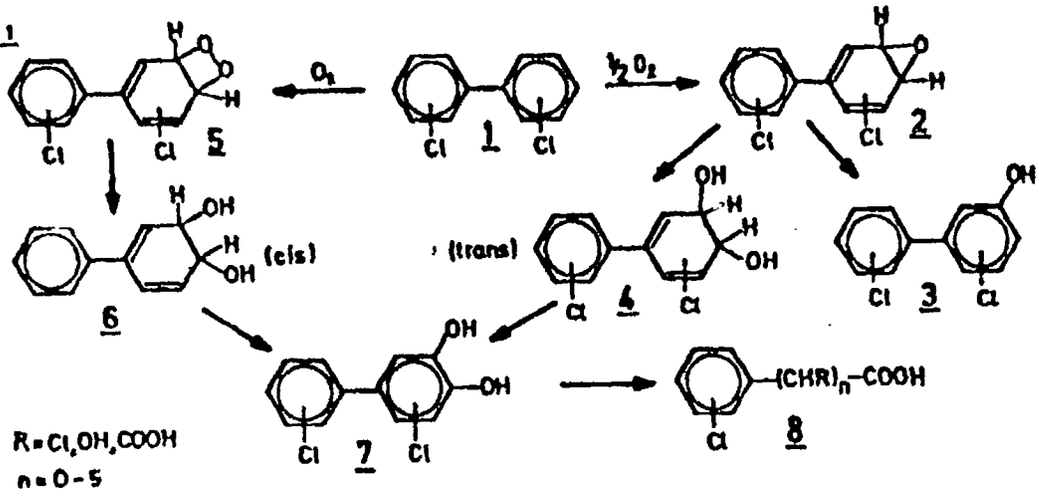
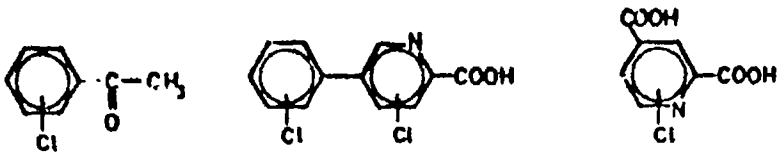
Fig. 1



Alternatively, if a reaction with dioxygenases to 1,2-dioxetanes (5) follows as the primary intermediate step in PCB decomposition, then phenylhexadiene-cis-diols (6) are to be expected (Fig. 1) [7]. These aromatize easily to o-dihydroxy-biphenyls (7), with chlorophenyl-carbonic acids (8) again to be expected as the result.

If one applies the general scheme suggested by Dagley et al. [4] for microbial decomposition of aromatics, as a working hypothesis for polychlorinated biphenyls, then in addition to chlorobenzoic acids (9) and chlorophenylacetic acids (10), chloroacetophenones (11) should develop as phenyl-replacing metabolites.

Furthermore, chlorophenyl-chloropicolinic acids (12) and chlorolutidinic acids (13) are to be expected as artifacts [5], depending on the presence of ammonium ions in the nutrient solution after meta-splitting



(11) (12) (13)

of the o-dihydroxyphenyls (phenyl pyrocatechins) and pyrocatechins by a non-enzymatic closing of the rings of the muconic acid semialdehydes.

The aromatic carbonic acids will be structurally determined by: 1) the type of o-dihydroxy-biphenyls formed, and 2) the type of ring opening of the o-dihydroxy-biphenyls.

The basic equivalence of the 2,3-position in biphenyl with the 5,6-position is eliminated by axially asymmetric chlorine substitution and then leads to different resulting products, depending on the formation of the o-dihydroxy-biphenyl. The same applies for the formation of 3,4-biphenylols, which are to be distinguished from the 4,5-biphenylols.

Various ring openings are possible for a given dihydroxy-biphenyl. The possibility of an ortho-splitting of the pyrocatechines is increased by two different kinds of meta-splitting during decomposition of chlorinated o-dihydroxy-biphenyls. For the case of 2,3,3',4,5-pentachlorobiphenyl, assuming the formation of 2',3'-dihydroxy-biphenyl, the result of ortho-splitting and of the two types of meta-splitting is presented here (Fig. 2, following page).

Without taking into account the intermediary stages (e.g. isomerization, lactone formation), the accumulation products to be discussed as metabolites in the present instance are tetrachloride derivatives of benzoic acid, of phenylacetic acid, of phenyl-pyroracemic acid and of 2-phenylmalonic acid,

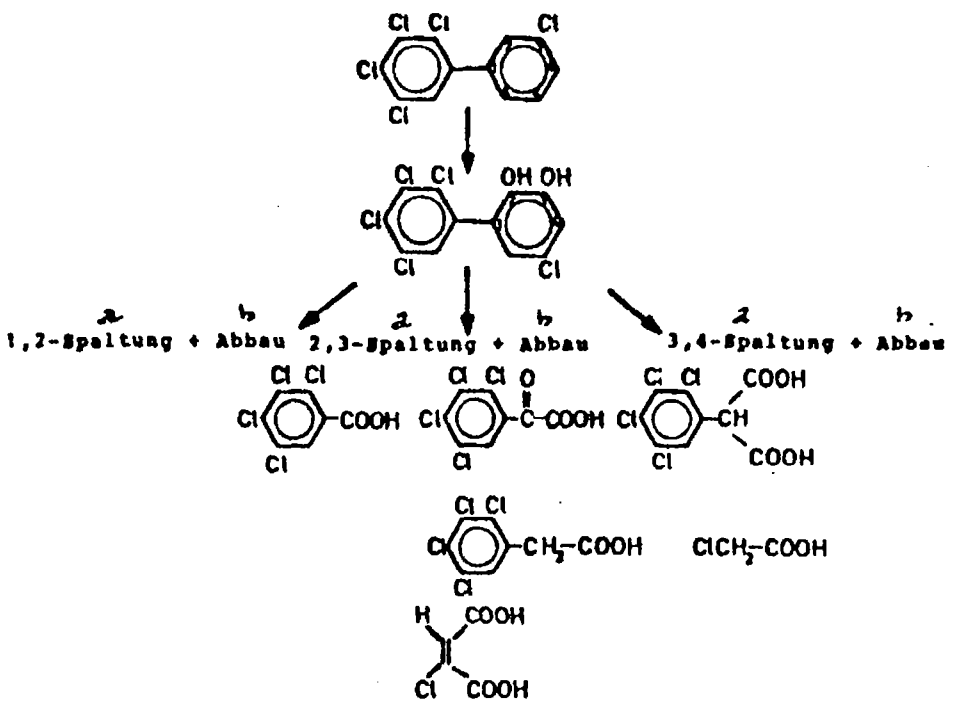


Fig. 2. Carbonic acids as possible metabolites of 2,3,3',4,5-pentachlorobiphenyl after formation of 2,3,4,5,5'-pentachloro-2',3'-dihydroxy-biphenyl.

Key: a. X-splitting b. Decomposition

together with chloromaleic acid and chloroacetic acid.

Materials and Apparatus

The PCB isomers (Analabs, NEN Chemicals, Dreieichenhain; Bayer Co., Leverkusen), the chlorobenzoic acids and chlorocinnamic acids (Fluka, Neu-Ulm and Aldrich Europe, Düsseldorf), as well as the solvents, for reaction (Merck, Darmstadt), were used as obtained. To concentrate the solutions, superpurified nitrogen was used. The gas-chromatographic experiments were performed with a Carlo Erba Model 2300 gas chromatograph, with macrosplitter, Ni-63 electron capture detector and temperature

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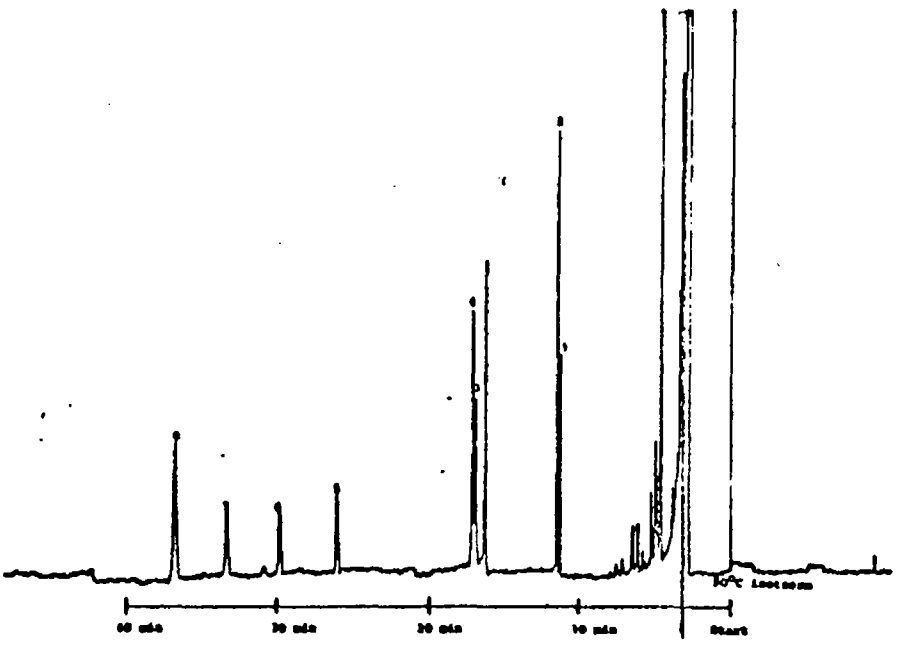

Fig. 3. Methylesters of monochloro- and dichlorobenzoic acids (90°C, Carbowax 20 M, 50 m, glass)

control. The class capillaries (SE 30, 27 m, i.d. 0.25 mm, TZ[1] C_{12}-C_{13} = 42; Carbowax 20 M. 50 m, i.d. 0.20 mm, TZ[1] C_{12}-C_{13} = 40) were manufactured according to modified data from the literature. Electronic retention time determination and surface evaluation was performed with a Spectra Physics Autolab System I integrator.

A Carlo Erba Model 2300 gas chromatograph with splitter connected to a magnetic field mass spectrometer, vacuum generators, micromass 16 F (pump delivery 1300 ℓ/sec.) with a UV recorder was available as a gas chromatograph-mass spectrometer combination with a glass capillary column

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[1] [Expansion unknown -- Translator.]

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Table I. Retention indices of methyl esters of chlorobenzoic acids, referred to trichloroacetic acid esters of the n-alcohols ($C_n > 5$); RI of trichloroacetic acid-n-hexylester = 1400.

	Fig. 3	SE 30 (120°C)	Carbowax 20M (90°C)
2-Chlorbenzoesäure[a]	(3)	1289,5	1495,2
3-Chlorbenzoesäure[a]	(2)	1284,4	1420,3
4-Chlorbenzoesäure[a]	(1)	1280,3	1416,3
2,4-Dichlorbenzoesäure[b]	(6)	1424,3	1600,0
2,5-Dichlorbenzoesäure[b]	(7)	1426,6	1618,2
2,6-Dichlorbenzoesäure[b]	(8)	1394,4	1634,2
3,4-Dichlorbenzoesäure[b]	(5)	1445,6	1577,7
3,5-Dichlorbenzoesäure[b]	(4)	1411,7	1500,0
2,3,4,5-Tetrachlorbenzoesäure[c]		1652,1[x]	---

[x]Stationary liquid OV 101, Temperature 100°C

Key: a. Chlorobenzoic acid
b. Dichlorobenzoic acid
c. Tetrachlorobenzoic acid

and a direct connection over a Pt-Ir capillary. The EI[1] source was run with 25 V.

The chlorobenzoic acids were identified gas chromatographically, with glass capillary columns and an EC detector, as their methyl esters (Fig. 3 and Table I), by relating their retention indices to authentic substances and by mass fragmentography with the aid of the gas chromatograph - mass spectrometer combination.

Reference substances for the calculation of retention indices with the use of the EC detector were the trichloroacetic acid esters of normal

[1] [Expansion unknown -- Translator.]

C_n alcohol ($n \geq 6$), which were synthesized according to standard by esterization with trichloroacetyl chloride. These homologs can be used for retention index determination with either a flame ionization detector or an electron capture detector. For the trichloroacetic acid-n-hexyl ester one gets, with reference to the n-alkanes, a retention index value of 1345.6 at 120°C on SE 30; and a value of 1494.6 at 90°C on Carbowax 20 M. To simplify evaluation, a retention index of 1400 was assigned to trichloroacetic acid-hexyl ester.

Culture Conditions

A mixed culture was used as microorganisms, obtained by water extraction of a garden mold via an intermediate enrichment by growth on benzene as a C-source, with the use of a standard phosphate salt solution (pH 6.80) with additional trace elements. This mixed culture contained mainly gram-negative bacilli with polar cilia, gram-positive cocci and paramecia.

Decomposition experiments with PCB isomers were performed with benzene as an additional C-source. The benzene could enter the culture medium by diffusion from a paraffin bottom stratum [8](2 g benzene/20 g paraffin histoplast, melting point 56°, Serva Heidelberg, per 2 ℓ culture medium).

On the lower half of the wall of a 2-liter steep-sided bottle, 1-5 mg PCB isomers were applied as a film from 20 ml acetone. After only 4 days, the culture medium was found to be saturated with the PCB isomers. The cultures were incubated for 2, 4 and 6 weeks at 28° - 32°C. After suspension of the culture on aromatic acids as described, 200 ml samples were processed [9].

Results

The PCB isomers listed in Table 2 were examined for their decomposability to chlorobenzoic acid. The chlorobenzoic acids obtained as metabolites are listed.

TABLE II.

PCB Isomers	Benzoic acids as PCB metabolites
2-Chlor-biphenyl [a]	---
4-Chlor-biphenyl [a]	4-Chlorbenzoesäure [e]
2,2'-Dichlor-biphenyl [b]	---
2,4'-Dichlor-biphenyl [b]	4-Chlorbenzoesäure [e]
2,2',5-Trichlor-biphenyl [c]	2,5-Dichlorbenzoesäure [f]
3,4,4'-Trichlor-biphenyl [c]	4-Chlorbenzoesäure [e], 3,4-Dichlorbenzoesäure [f]
2,4,4'-Trichlor-biphenyl [c]	4-Chlorbenzoesäure [e], 2,4-Dichlorbenzoesäure [f]
2,3,3',4,5-Pentachlor-biphenyl [d]	2,3,4,5-Tetrachlorbenzoesäure [g]
2,3,4,4',5-Pentachlor-biphenyl [d]	2,3,4,5-Tetrachlorbenzoesäure [g]
Clophen A 30® (Bayer)	4-Chlorbenzoesäure [e], 2,4-Dichlorbenzoesäure [f]

Key: a. Chlorobiphenyl
b. Dichlorobiphenyl
c. Trichlorobiphenyl
d. Pentachlorobiphenyl
e. Chlorobenzoic acid
f. Dichlorobenzoic acid
g. Tetrachlorobenzoic acid

During decomposition of 2,4,4'-trichlorobiphenyl, 4-chlorobenzoic acid and 2,4-dichlorobenzoic acid were concentrated in the ratio of 5:2.

The results confirm the findings of other authors on the decomposition of 4-chlorobiphenyl to 4-chlorobenzoic acid [10,11]. The concentration of chlorobenzoic acid during decomposition of PCB isomers agrees with the finding that 4-chloro-, 2,4-dichloro-, 3,4-dichloro-, 2,5-dichloro-, and 2,3,4,5-tetrachlorobenzoic acids are not noticeably decomposed by ground bacteria in the course of 60 days [12].

For 2-chlorobenzoic acid, with ground bacteria, both a comparatively fast metabolism [12] and complete stability [13] have been observed.

Our own experiments on the microbial transformation of 2-chloro- and 4-chlorobenzoic acids, with quantitative determination of the isomeric benzoic acids by means of gas chromatography, yielded almost the same decomposition rate for both acids.

Likewise, decomposition experiments with 2,4-dichloro- and 2,5-dichlorocinnamic acid, under the conditions of the PCB experiments, yielded an accumulation of 2,4-dichloro- and 2,5- dichlorobenzoic acid, respectively, which confirms the results of the decomposition of 2,2',5-trichloro- and 2,4,4'-trichlorobiphenyls, respectively.

Clophen A 30^{R} (Bayer) (42% Cl) is 80% composed of PCB isomers with chlorine in 2-, 4-, 2,2'-, 2,4'-, 2,2',3-, 2,2',5-, 2,3,4'-, 2,4,4'-, 2,4',5-, 2,2',3,5'-, 2,2',4,5'- and 2,2',5,5'- position. According to the above findings, 2-chloro-, 4-chloro-, 2,3-dichloro-, 2,4-dichloro- and 2,5-dichlorobenzoic acid should thus preferably develop. But in the decomposition of Clophen A 30^{R} (Bayer), only 4-chloro- and 2,4-dichlorobenzoic acid have been detected in the culture medium to date. The absence of both 2-chlorobenzoic acid and 2,5-dichlorobenzoic acid is conspicuous.

In the capillary chromatogram from acid fraction [9], however, there are further acid methyl esters with higher boiling points, and their identification is in progress.

Since the slow microbial breakdown of polychlorinated biphenyls (PCB) is demonstrably followed by an even slower breakdown of certain chlorobenzoic acids, one may easily conclude that as a result of the well-known

global presence of PCB, chlorinated benzoic acids are likewise present globally as environmental chemicals.

Acknowledgments

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