# DDT Metabolites and Analogs: Ring Fission by Hydrogenomonas

Abstract. *A* Hydrogenomonas *cleaved one of the rings of* p,p'*-dichlorodiphenylmethane, a product of DDT metabolism, to yield* p*-chlorophenylacetate and further metabolized the latter compound. Products of microbial degradation of other diphenylmethanes were also identified. Substituents on the methylene-carbon and* para*-chloro substitution are critical factors governing resistance of DDT and related compounds to aerobic metabolism and decomposition by the bacterium.*

Despite the enormous concern with the persistence of DDT and its degradation products in nature, surprisingly little is known about the products of biological decomposition in natural ecosystems, wherein microorganisms are likely to be the chief agents of biodegradation. The concern has been almost entirely with the loss of but a single one of the 14 carbon atoms, as exemplified by studies showing the bacterial conversion of DDT to *p,p'*-dichlorobenzophenone (*1*), but the identities of the subsequent decomposition products and consequently their possible ecological or toxicological hazards remain undefined. We report here the microbiologically effected ring cleavage of metabolites known to be generated from DDT, as well as of structural analogs, and demonstrate the influence of chemical substituents on biodegradation of these kinds of environmental pollutants.

A *Hydrogenomonas* sp. was isolated from sewage by enrichment culture techniques employing diphenylmethane as sole carbon source. In the metabolism of diphenylmethane, one aromatic ring was cleaved to give rise to phenylacetic acid. This product was isolated by thin-layer chromatography and identified by its melting point, infrared spectrum, and by mass spectral analysis, all of which give results identical to those obtained with the authentic compound. Manometric data revealed that the ring of phenylacetic acid was cleaved.

The ability of this species of *Hydrogenomonas* either to grow in media containing DDT, diphenylmethane, and related compounds as sole carbon sources or to cometabolize these chemicals aerobically was investigated. Cometabolism refers to the ability of a microorganism to metabolize a substrate that it cannot use as a source of energy or of one of the elements contained in the molecule (*2*). The observations are summarized in Fig. 1. Diphenylmethane, benzhydrol, and *p*-chlorobenzhydrol were utilized for growth, whereas suspensions of washed cells neither grew on nor cometabolized DDT or *p,p'*-dichlorobenzophenone. *p,p'*-Dichlorodiphenylmethane, *p,p'*-dichlorobenzhydrol, *p*-chlorobenzophenone, 1,1-diphenyl-2,2,2-trichloroethane, and benzophenone were cometabolized by washed cells with the accumulation of yellow-colored products that had absorption maxima of 395, 403, 423, 394, and 330 nm, respectively, in neutral to alkaline solutions; the colors were absent in acid. These characteristics are indicative of unsaturated keto-enol acids, which are produced when catechols are cleaved adjacent to the hydroxyls (*3*). Phenols, catechols, and chloride were not detected in any of the liquids after incubation of these substrates with the cell suspensions. Phenols and catechols were determined by the procedures described by Harborne (*4*) and Evans (*5*), respectively.

The observations suggest that two features contribute to making DDT and related substances refractory to aerobic attack (see Fig. 1). First is the *para*-chlorine substitution. This is evident in the inability of *Hydrogenomonas* sp. to grow on *p,p'*-dichlorodiphenylmethane and *p,p'*-dichlorobenzhydrol, although it is able to grow on the three corresponding monochloro- and unsubstituted compounds tested, and in the inability to cometabolize the *p,p'*-dichlorobenzophenone and DDT, whereas cometabolism of the three corresponding monochloro- and unsubstituted substances investigated did occur. Second is the kind of substitution on the carbon atom linking the two benzene rings, the data suggesting that a carbonyl-carbon or a trichloromethyl group impart resistance to the molecule.

A product having a melting point, infrared spectrum, and mass spectrum identical to *p*-chlorophenylacetic acid was isolated from a washed cell suspension incubated with *p,p'*-dichlorodiphenylmethane, a known metabolite generated from DDT (*1*). This finding

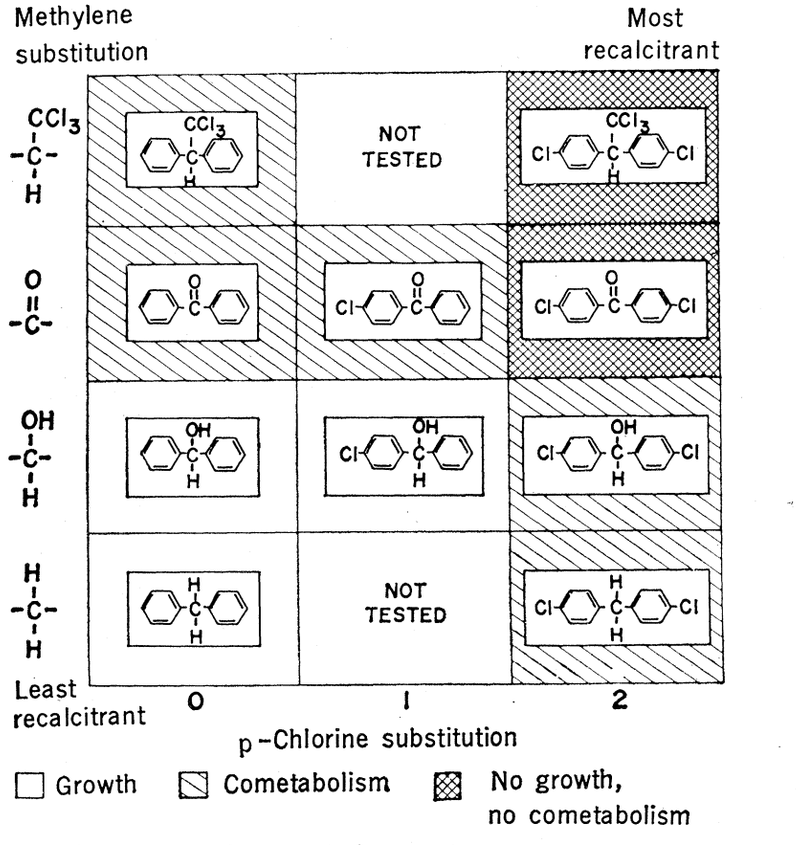


Fig. 1. Growth on or aerobic cometabolism of diphenylmethanes and related compounds by *Hydrogenomonas* sp.

shows that one of the two rings was cleaved. Moreover, further degradation of this metabolite occurred; thus, when washed cell suspensions were incubated with *p*-chlorophenylacetic acid, a yellow-colored product accumulated. This product had an absorption maximum of 379 nm in neutral to basic solutions which disappeared upon acidification. Nevertheless, no chloride was released microbiologically.

Furthermore, when a suspension of the cells was incubated with 1,1-diphenyl-2,2,2-trichloroethane, a product appeared that was identified by its melting point, infrared spectrum, and by mass spectral analyses as 2-phenyl-3,3,3-trichloropropionic acid. The infrared spectrum showed bands at 1700 and 690 $cm^{-1}$ characteristic of an aromatic acid. Mass spectrometry showed (i) a mass of 252; (ii) the loss of HCl, carboxyl, and chlorine from the parent ion; (iii) a base peak at mass-to-charge ratio (*m/e*) 102, representing a loss of three chlorines and a carboxyl; and (iv) a peak at *m/e* 77 for the phenyl ion. One of the two rings in this substrate too, therefore, was opened.

It has been reported that DDT may be converted by *Culex trasalis* larvae to *p*-chlorobenzoic acid (*6*). Although no such intermediate has been observed to be excreted by the bacterium, it has not been excluded either. Nevertheless, the evidence for a role for *p*-chlorobenzoic acid in DDT degradation must be considered as equivocal in view of the lack of a rigorous identification of the compound.

The present findings that diphenylmethane, *p,p'*-dichlorodiphenylmethane, and 1,1-diphenyl-2,2,2-trichloroethane are attacked, that at least one and sometimes both of the benzene rings are cleaved, and that there is extensive microbial modification of the chemicals focus attention on the ultimate fate of DDT in soil and water, environments that have received enormous quantities of this insecticide. Microorganisms under anaerobic conditions are capable of converting the parent pesticidal molecule to compounds of the kind metabolized aerobically by *Hydrogenomonas* sp. (*1*), so that a biological model now exists for tracing the pathway of DDT decomposition in nature.

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**References and Notes**

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## Peptides with Juvenile Hormone Activity

Abstract. *Peptide derivatives of juvenile hormone analogs which show substantial hormonal activity for certain insects were prepared. The most active compound,* L-*isoleucyl*-L-*alanyl*-p-*aminobenzoic acid ethyl ester, was up to twice as active as juvabione. Like juvabione, the peptide analog showed selective action on pyrrhocorid bugs.*

The juvenile hormone (JH) analogs are mainly aliphatic or monocyclic sesquiterpenes or aliphatic monoterpenes attached to para-substituted aromatic rings. A few examples of active compounds without isoprenoid structure include dodecylmethylether (*1*) and insecticide synergists of the sesoxane type (*2*). We were interested in polypeptide chains that bear a general resemblance to polyisoprenoids and have prepared and assayed several peptides which mimic selected terpenic and terpenoid models.

The first compound investigated was the methyl ester of 3,7,11-trimethyl-2-dodecenic acid (compound **1**), which is active on both hemipteran larvae and pupae of the beetle *Tenebrio* (Table 1). Its partly peptidic counterpart (*2*) was prepared from amino acids L-valine and L-alanine attached to a short-chained, unsaturated, dicarboxylic acid ester. One end of the molecule contains the necessary $-CH_2{\cdot}C(CH_3){\cdot}CH{\cdot}COOR$ structure, which in many straight chain terpenic analogs is necessary for JH activity (*3*). In addition, compound **2** contains a carbomethoxy group on the

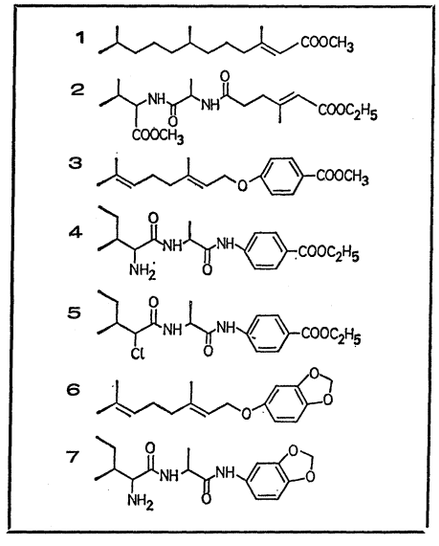


Table 1. Juvenile hormone activity. The values indicate the amount of the substances (micrograms per specimen) which caused formation of half-larval (hemipterans) or half-pupal (*Tenebrio*) adultoids. For topical applications we used standard 1-$\mu$l drops of acetone solution and for injections 1 $\mu$l of olive oil; freshly molted last instar larvae or freshly molted pupae (0 to 20 hours) were used for the assays.

| Compound | Pyrrhocoridae | | Pentatomidae | Tenebrionidae | |
|---|---|---|---|---|---|
| | *Pyrrhocoris apterus* Topical (larvae) | *Dysdercus cingulatus* Topical (larvae) | *Graphosoma italicum* Topical (larvae) | *Tenebrio molitor* Injections (pupae) | *Tenebrio molitor* Topical (pupae) |
| **1** | 5 | 10 | 100 | 1 | 10 |
| **2** | >1000 | >1000 | >1000 | >1000 | >1000 |
| **3** | 0.5 | 0.5 | 0.8 | 10 | 100 |
| **4** | 0.5 | 0.5 | >1000 | >1000 | >1000 |
| **5** | 0.5 | 0.2 | >1000 | >1000 | >1000 |
| **6** | 5 | 5 | 1 | | 0.3 |
| **7** | 100 | 100 | >1000 | 500 | |
| ± Juvabione | 1 | 0.5 | >1000 | >1000 | >1000 |
| Methyl farnesoate | 50 | 50 | 50 | 10 | |