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DDT Metabolism: Oxidation of the Metabolite 2,2-bis(p-Chlorophenyl)ethanol by Alcohol Dehydrogenase

Abstract. *A metabolite of DDT, 2,2-bis(*p*-chlorophenyl)ethanol, is a substrate of crystalline liver alcohol dehydrogenase. The oxidation of the substrate was detected spectrophotometrically. The* p*-nitrophenylhydrazone derivative of the product, 2,2-bis(*p*-chlorophenyl)acetaldehyde, was identified by comparing its mass spectrum and thin-layer chromatographic behavior with that of an authentic sample.*

The metabolism of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] in mammals gives rise to 2,2-bis(*p*-chlorophenyl)ethanol (DDOH) and 2,2-bis(*p*-chlorophenyl) acetic acid (DDA) (*1*). An intermediate aldehyde has been proposed but has not yet been found to occur in vivo. We have synthesized the proposed intermediate, 2,2-bis(*p*-chlorophenyl)acetaldehyde (DDCHO) (*2*).

Because the aldehyde has never been found in vivo and because preliminary studies indicate that the synthetic compound is highly unstable and reactive, the aldehyde was examined as a possible product of oxidation of DDOH by crystalline liver alcohol dehydrogenase (E.C.1.1.1.1).

The oxidation of DDOH was detected in a double-beam spectrophotometer by following the reduction of nicotinamide-adenine dinucleotide (NAD) at 340 nm in the presence of crystalline horse liver alcohol dehydrogenase (*3*). Because DDOH is insoluble in aqueous media, the compound was dissolved in 50 percent glycerolformal before its addition to the buffered incubation media. The resulting cloudy suspension prevented accurate spectrophotometric determination of reaction rates, but definite increases in absorbance were observed. No reaction was detected in the absence of enzyme or NAD. The reverse reaction, the reduction of DDCHO, was similarly observed with the substitution of reduced NAD for the oxidized form and by following the decrease in absorbance at 340 nm. Glycerolformal in the absence of DDOH also catalyzes the reduction of NAD, but the reverse reaction was not observed for glycerolformal.

Direct chemical evidence for the enzymatic oxidation of DDOH to DDCHO was obtained by formation of the *p*-nitrophenylhydrazone derivative. An incubation mixture was prepared containing 0.016*M* sodium pyrophosphate, *p*H 8.8, 0.008*M* NAD, and 2 mg of DDOH in a final volume of 6 ml. Crystalline liver alcohol dehydrogenase (2 mg) was added, and the mixture was incubated at 37°C for 30 minutes; then 0.01*M* *p*-nitrophenylhydrazine (0.5 ml) was added, and the mixture was shaken. A chloroform extract of the mixture was prepared and evaporated to a minimum volume. Portions of the extract were chromatographed on silica-gel plates with a mixture of benzene and petroleum ether (75:25) or benzene and ethyl acetate (95:5). The extract yielded a spot on the chromatograms whose R_F values, 0.28 and 0.12 for the respective solvent systems, corresponded to those of the authentic *p*-nitrophenylhydrazone derivative of DDCHO.

The identity of the derivative obtained from the enzymatic incubation was established by low-resolution, electron-impact mass spectrometry at 20 ev. High resolution measurements (*4*) confirmed the elemental composition of the ion fragments observed in the low resolution spectrum. An authentic sample of the *p*-nitrophenylhydrazone of DDCHO shows a prominent molecular ion at *m/e* (mass to charge) 399 and the base peak at 261. Other prominent ions of interest were *m/e* 249, 235, 226, 199, 200, 125, 122, and 111. The mass spectrum of the chloroform-extractable derivative was the same as that of the authentic sample of the DDCHO derivative. These findings strongly support the probability that DDCHO is a metabolite of DDT.

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Phenolic Aldehydes: Generation from Fossil Woods and Carbonaceous Sediments by Oxidative Degradation

Abstract. *Aromatic aldehydes derived from fossil woods and carbonaceous sediments were identified by gas-liquid chromatography; their geochemical significance is discussed.*

To help characterize organic matter in sediments and to test the possible significance of phenolic aldehydes as geochemical indices, we have analyzed oxidation products for *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde, three products of mild oxidation of lignin in woody tissues. The samples were ground with a mortar and pestle, Wiley mill (contemporary woods), or Angstrom disk mill (indurated sediments), and sieved to 60 mesh. Oxidation was carried out in a stainless steel tube in an oil bath at 183°C with about 15 ml of 8 percent aqueous NaOH and 1 ml of base-washed, redistilled nitrobenzene per gram of sample, for about 2 hours (*1, 2*). The reaction mixture was filtered, and the filtrate was washed with methylene chloride to remove nonpolar byproducts. The aqueous phase was acidified with 6*N* HCl, and the phenolic aldehydes were extracted with methylene chloride. The extract was concentrated