5. J. E. Heath and P. A. Adams, *Nature* **205**, 309 (1965).
6. P. A. Adams and J. E. Heath, *ibid.* **201**, 20 (1964).
7. J. E. Heath and P. A. Adams, *J. Exp. Biol.* **47**, 21 (1967).
8. Supported by NSF grant GB 5139 X to Dr. G. A. Bartholomew. I thank Dr. Bartholomew for assistance and encouragement, Dr. F. Engelmann for helpful comments and criticisms, and Dr. R. Thurston (Univ. of Kentucky) for generous help in establishing and maintaining the moth colony.

12 December 1969; revised 20 February 1970 ■

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.

JOSEPH E. SUGGS, ROBERT E. HAWK
AUGUST CURLEY
ELIZABETH L. BOOZER
Atlanta Toxicology Branch, Division of Pesticides, Bureau of Science, Food and Drug Administration, Atlanta, Georgia 30333
JAMES D. MCKINNEY
National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

References and Notes

1. J. E. Peterson and W. H. Robinson, *Toxicol. Appl. Pharmacol.* **6**, 321 (1964).
2. J. D. McKinney, E. L. Boozer, H. P. Hopkins, J. E. Suggs, *Experientia* **25**, 897 (1969).
3. B. L. Vallee and F. L. Hoch, *Proc. Nat. Acad. Sci. U.S.* **41**, 327 (1955).
4. We thank Prof. Klaus Biemann and Dr. Charles Hignite, Mass Spectral Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts for high resolution measurements. Supported, in part by NIH research grant RR-00317 from Division of Research Facilities and Resources.

16 February 1970 ■

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

under vacuum, and an aliquot was dried under a nitrogen stream. The residue was silylated with Tri-Sil Z reagent (Pierce Chemical Co.) and examined by gas-liquid chromatography. Reference solutions were freshly prepared by silylation of authentic compounds.

A gas chromatograph (F&M 402) equipped with a hydrogen flame-ionization detector and containing a glass column (6 feet by ¼ inch) packed with 5 percent Apiezon L on Gas Chrom Q [60/80 mesh (Applied Science)] was used. With isothermal operation at 161°C and a helium flow rate of about 60 ml per minute, satisfactory resolution of the silylated phenolic aldehydes and their acid analogues was achieved (Table 1 and Fig. 1).

Lignin, a major component of all woody tissue in vascular plants, is a complex polymer with minor variations in structure among different plant taxa (*3–5*). One variation is in the degree to which the propylphenol units of the polymer are methoxylated. In general, the greater the evolutionary specialization of a vascular plant, the more extensively methoxylated is its lignin. Angiosperm lignin, for example, contains both syringyl and vanillyl groups in abundance, whereas gymnosperm lignin is characterized primarily by the latter.

Lignin is readily oxidized by alkaline nitrobenzene. Some aromatic residues are released as the phenolic aldehydes *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde (*3, 6*).

The relative proportions of aldehydes reflect the composition of the lignin in the original wood. Generally, conifers yield primarily vanillin, dicotyledons yield both vanillin and syringaldehyde, and woody monocotyledons yield *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde. Other aldehydes and acids are also formed but in lower yields.

Primitive vascular plants contain relatively little lignin. The kinds and amounts of aldehydes obtained through oxidative treatment suggest that this lignin may be of simpler structure than that of higher plants.

Vascular tissues of plant remains do not deteriorate en masse in the course of sedimentation and degradation but in a sequence of stages, the cellulosic fraction of the cell walls being depleted rapidly relative to the lignin (*7*). Under anaerobic conditions, lignin is the most decay-resistant constituent of the woody plant cell wall (*8*). It has been observed histologically intact in fossil woods of considerable age (*7–9*).

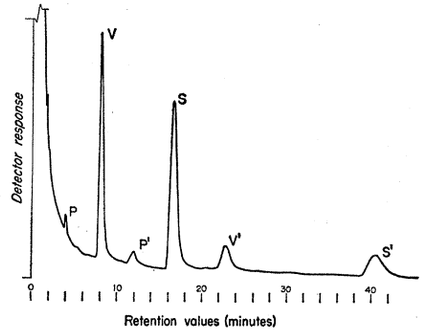

Fig. 1. Gas-liquid chromatogram on Apiezon L of trimethylsilyl ethers of *p*-hydroxybenzaldehyde (*P*), vanillin (*V*), *p*-hydroxybenzoic acid (*P'*), syringaldehyde (*S*), vanillic acid (*V'*), and syringic acid (*S'*) from *Gordonia* wood of early Tertiary age.

However, lignin appears to undergo some alteration even in relatively early stages of plant decay, for example, an initial reduction in methoxyl content (*10, 11*). It appears that demethoxylation proceeds differentially, preferentially converting syringyl groups into vanillyl groups; these also undergo methoxyl loss, but only at a much slower rate.

The yield of vanillin from wood of an extinct Miocene conifer (*Cedrus penhallowii*) is close to that produced from a related contemporary wood (*Cedrus deodara*). The ratio of vanillin to *p*-hydroxybenzaldehyde is nearly the same for both. Vanillin yields from Oligocene coniferous cone parts and seeds were comparable with those from modern species (*4*). Evidently, vanillyl residues are retained well in the lignin if the sedimentary conditions are favorable.

Ratios of vanillin to *p*-hydroxybenzaldehyde calculated for an early Tertiary (Oligocene?) dicotyledonous wood (*Gordonia*) and its modern counterpart are also nearly alike (Table 1). Here, however, the identity may have been maintained by methoxyl loss, primarily from syringyl groups. Syringaldehyde production from the fossil wood is quite low. The syringaldehyde : vanillin ratio in the contemporary wood is around 3.8; in the fossil wood, only 1.1. Nevertheless, the state of preservation of the lignin in this sample is quite remarkable.

Our oldest fossil wood, a Cretaceous conifer, gave the largest yield of *p*-hydroxybenzaldehyde of any of our samples. Vanillin, ordinarily the predominant aldehyde from coniferous woods, was produced in comparatively small yield. Again, this may indicate demethoxylation of guaiacyl residues during the geologic history of the specimen.

Aromatic aldehydes related to lignin have also been obtained from recent marine sediments (*12*), soils (*2, 13*), and peats (*2, 13, 14*).

Phenolic aldehydes, principally *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde, have been generated from offshore California sediments by acid hydrolysis (*12*). Our work with two recent sediments from the Black Sea and the Wilkinson Basin (Gulf of Maine) confirms these findings.

It is assumed that the phenolic aldehydes are derived from terrestrial plant debris. These three aldehydes are characteristic of lignin. Marine plants are devoid of lignin (*15*), except possibly for traces in certain shallow-water,

Table 1. Yield (percent) of phenolic aldehydes from woods and carbonaceous sediments upon oxidation with alkaline nitrobenzene. Abbreviations: Phb, *p*-hydroxybenzaldehyde; Van, vanillin; Syr, syringaldehyde; fm., formation; tr, approximately 0.01 percent or less; n.d., not detected.

Sample	Age	Phb	Van	Syr
Contemporary woods				
Araucaria bidwillii		0.2	63.1	<0.1
Cedrus deodara		2.1	49.4	<0.1
Betula lenta		0.3	7.9	35.4
Gordonia lasianthus		.9	21.9	84.1
Fossil woods				
Cedrus wood, Sierra Nevada mtns., Calif.	Miocene	1.5	41.2	n.d.
Gordonia wood, Brandon lignite, Vt.	Lower Tertiary	1.2	30.3	33.9
Wood (coniferous), Kreischerville lignite, N.Y.	Cretaceous	8.7	5.4	0.6
Carbonaceous sediments				
Sediment, Gatun Lake, 09°10′N, 79°50′W	Recent	0.3	2.5	2.0
Sediment, Black Sea, 43°03′N, 33°02′E	Recent	.07	0.18	tr.
Sediment, Gulf of Maine, 42°22′N, 69°29′W	Recent	.05	.14	tr.
Peat, Montclair bog, Mass.	Quaternary	.5	6.1	2.6
Shale, Green River fm., Utah	Eocene	.02	0.13	n.d.
Clay, Magothy fm., N.J.	Upper Cret.	.03	.18	n.d.
Black shale, Burro Canyon fm., Colo.	Lower Cret.	.02	.06	n.d.
Lignitic siltstone, Ischigualasto fm., Argentina	Upper Triassic	.02	.11	n.d.
Coal ball (concretion), Des Moines coal, Iowa	Carboniferous	.02	.06	n.d.
Coal, Michigamme fm., Mich.	1.8×10^9 years	n.d.	(.01)?	n.d.
Anthraxolite, Gunflint Iron fm., Canada	2.0×10^9 years	n.d.	n.d.	n.d.

truly marine angiosperms such as *Zostera* or *Thalassia*. Aldehyde production from bacteria is too limited to be of consequence in sediments rich in organic matter.

A freshwater sediment from an inlet near the northeastern shore of Barro Colorado Island, Gatun Lake, Panama, was also examined. This sediment is rich in partially degraded organic material derived from a low-lying tropical rain forest inundated during the formation of Gatun Lake, in the development of the Panama Canal. Oxidation with alkaline nitrobenzene yielded chiefly vanillin and syringaldehyde—a result consistent with the source material, mainly residue of deciduous forest trees.

Postglacial peat from Montclair bog, North Quincy, Mass., was examined. The contribution of coniferous wood to this temperate, freshwater deposit is reflected in the relatively high yield of vanillin. The correspondence between the aldehydes generated from peat specimens and the parent plant was clearly demonstrated in earlier studies with peat and soil (*2*, *13*).

On oxidative degradation of nonmarine ancient carbonaceous sediments, small yields of *p*-hydroxybenzaldehyde and vanillin were obtained from Mesozoic and younger specimens. Syringaldehyde was not detected. Lignin remnants may be the progenitors of the aldehydes from these older sediments as well. In sediments, where conditions are anaerobic and anoxic, lignin persists. Lignin is rapidly degraded only under aerobic conditions, or when oxygen can be transferred or dehydrogenation can take place (*11*).

Although older sediments are markedly different in many respects, their aldehyde yields are quite similar, and of the same order of magnitude as those of recent marine sediments.

A sample of Chattanooga shale (Upper Devonian) from northwest Georgia yielded *p*-hydroxybenzaldehyde and vanillin in amounts like those from Mesozoic sediments. This is not surprising, since the Chattanooga shale is rich in humic material (*16*). The specimen examined may have been rich in woody matter, having been collected near the probable shoreline of the Chattanooga Sea during its period of greatest extent.

Investigation of several Precambrian samples did not afford equivocal results. No aldehydes were detected from a Middle Precambrian anthraxolite, an anomalous, anthracite-like carbonaceous substance containing about 95 percent carbon and less than 0.1 percent extractives. Michigamme coal, also of Middle Precambrian age, however, gave approximately 0.01 mg of vanillin per gram of sample. Confirmation by other means is needed because the vanillin yield from our controls approaches this amount.

Paleobotanical evidence suggests that the capacity of plants to synthesize lignin did not evolve until late Silurian and early Devonian time. However, analysis of crude oil 1 billion years old from Precambrian Nonesuch Shale (Upper Michigan) gave 0.07 mg of *p*-hydroxybenzaldehyde and 0.14 mg of vanillin per gram of sample. This might be due to contamination, however, since the precise history of the specimen is not known.

Thus, it seems that phenolic aldehydes can be generated from plant materials of greatly differing ages. The phenolic aldehydes can be separated and identified; their ratios appear to be related to the chemical structure of the lignin in the initial plant. The phenolic aldehydes from the sediments analyzed in this study may be a geochemical index of the contribution of lignin to the organic sediments.

RICHARD F. LEO
Department of Biology, Harvard University, Cambridge, Massachusetts
ELSO S. BARGHOORN
Department of Biology and *Botanical Museum, Harvard University*

References and Notes

1. J. G. Bicho, E. Zavarin, D. L. Brink, *Tappi* **49**, 218 (1966).
2. R. I. Morrison, *J. Soil Sci.* **9**, 130 (1958).
3. F. E. Brauns and D. A. Brauns, *The Chemistry of Lignin* (Academic Press, New York, 1960).
4. S. M. Manskaya and T. V. Drozdova, *Geochemistry of Organic Substances* (Pergamon, New York, 1968).
5. A. C. Neish and K. Freudenberg, *Constitution and Biosynthesis of Lignin* (Springer-Verlag, New York, 1968); I. A. Pearl, *The Chemistry of Lignin* (Dekker, New York, 1967); G. H. N. Towers and R. D. Gibbs, *Nature* **172**, 25 (1953); S. M. Siegel, in *Comprehensive Biochemistry*, M. Florkin and E. Stotz, Eds. (Elsevier, New York, 1968), chap. 1, pp. 1–51.
6. K. Freudenberg, in *The Formation of Wood in Forest Trees*, M. H. Zimmermann, Ed. (Academic Press, New York, 1964), pp. 203–218.
7. E. S. Barghoorn, "Degradation of Plant Materials and Its Relation to the Origin of Coal," *Conference on the Origin and Constitution of Coal*, 2nd conf., Crystal Cliffs (Nova Scotia Research Foundation, Halifax, 1952), pp. 181–203.
8. W. W. Varossieau and I. A. Breger, "Chemical Studies on Ancient Buried Wood and the Origin of Humus," *Congr. Int. Stratigr. Geol. Carbonifere C.R., 3rd, Heerlen* (1951), pp. 637–646.
9. E. S. Barghoorn, in *The Boylston Street Fishweir II*, F. Johnson, Ed. (R. S. Peabody Foundation for Archaeology, Andover, Mass., 1949), pp. 49–83; *J. Sediment. Petrol.* **22**, 34 (1952); P. R. Morey and E. D. Morey, *Palaeontogr. Abt. B. Palaeophytol.* **125**, 73 19(→ *Science* **164**, 836 (1969); J. Sen, *Bot. Rev.* **22**, 343 (1956).
10. W. Flaig, *Geochim. Cosmochim. Acta* **28**, 1523 (1964).
11. ———, in *Coal and Coal-Bearing Strata*, D. G. Murchison and T. S. Westoll, Eds. (Elsevier, New York, 1968), pp. 197–232.
12. E. T. Degens, J. H. Reuter, K. N. F. Snow, *Geochim. Cosmochim. Acta* **28**, 45 (1964).
13. R. I. Morrison, *J. Soil Sci.* **14**, 201 (1963).
14. V. C. Farmer and R. I. Morrison, *Geochim. Cosmochim. Acta* **28**, 1537 (1964); S. M. Manskaya and L. A. Kodina, *Geochemistry* **4**, 389 (1963).
15. J. M. Harkin, in *Oxidative Coupling of Phenols*, W. Taylor and A. Battersby, Eds. (Dekker, New York, 1967), pp. 243–321.
16. I. A. Breger and A. Brown, *Science* **137**, 221 (1962).
17. We thank D. H. Dolphin, J. A. Doyle, M. G. Ettlinger, B. Maynard, E. D. Morey, P. R. Morey, and T. Swain for helpful suggestions. The Gulf of Maine sediment was received from M. Blumer and the Black Sea sediment from E. T. Degens and D. Ross, all of Woods Hole Oceanographic Institution. J. A. Doyle and B. Maynard, both of Harvard University, provided the Cretaceous clay and Chattanooga shale samples. Supported in part by NASA grant NGR 22-007-069 and NSF grant GA-650.

14 November 1969; revised 9 February 1970 ■

Mutagenicity of Trimethylphosphate in Mice

Abstract. *Subtoxic concentrations of trimethylphosphate, administered orally or parenterally to male mice, produced mutagenic effects, dependent on dosage, in the dominant lethal assay.*

Trimethylphosphate (TMP) is used as a gasoline additive, at a concentration of approximately 0.25 g per gallon, for controlling surface ignition and spark plug fouling (*1*). It is also used as a methylating agent (*2*), a chemical intermediate in production of polymethyl polyphosphates (*3*), a flame retardant solvent for paints and polymers, and a catalyst in preparation of polymers and resins (*1*). Recently the related triethyl phosphate has been proposed as a food additive for stabilizing egg whites (*4*).

Trimethylphosphate is weakly toxic to rodents when administered orally, parenterally, or cutaneously (*5*); long-term administration of high doses in rats induces weight loss and paralysis. The compound is rapidly degraded to dimethyl phosphate, and S-methylcysteine has been identified as a urinary metabolite, an indication that it functions as an alkylating agent in vivo