Microbial Degradation of 2,3,5-Triiodobenzoic Acid in Soil

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Abstract □ The metabolic fate of the plant growth regulator 2,3,5-triiodobenzoic acid was determined in soil using ^{14}C-carboxyl-labeled 2,3,5-triiodobenzoic acid. 1-^{14}C-2,3,5-Triiodobenzoic acid was degraded to $^{14}CO_2$ by the microflora in soil; no radioactive CO_2 was evolved from sterilized soil. Following incubation of 1-^{14}C-2,3,5-triiodobenzoic acid in soil, unchanged 2,3,5-triiodobenzoic acid and three metabolites (2,5-diiodobenzoic acid, 3,5-diiodobenzoic acid, and one unknown) were recovered. Peak concentrations of 2,5-diiodobenzoic acid and 3,5-diiodobenzoic acid were 15–20 and 14–15%, respectively, at 8 weeks of incubation and decreased thereafter.

Keyphrases □ 2,3,5-Triiodobenzoic acid—microbial degradation in soil, identification of metabolites □ 2,5- and 3,5-Diiodobenzoic acids—formation from microbial degradation of 2,3,5-triiodobenzoic acid □ Microbial degradation in soil—2,3,5-triiodobenzoic acid □ Plant growth regulators—microbial degradation of 2,3,5-triiodobenzoic acid

2,3,5-Triiodobenzoic acid is used as a plant growth regulator on soybeans. It has been the subject of numerous metabolic fate studies in a variety of animals and plants. This note summarizes studies on the degradation of 2,3,5-triiodobenzoic acid by the microflora of soil.

EXPERIMENTAL

Apparatus—Four 50-ml. conical flasks were connected in series with glass tubes and rubber stoppers so that attachment to a vacuum source would create a slow rate of aeration through the flasks. The contents of the flasks, beginning at the source of air, were as follows: Flask 1 contained 200 ml. of 0.5 *N* NaOH to remove CO_2 from the air; Flask 2, a light-protected conical flask, contained 100 g. of soil and 20 ml. of an aqueous solution of 1-^{14}C-2,3,5-triiodobenzoic acid as the sodium salt; and Flasks 3 and 4 each contained 100 ml. of 0.1 *N* NaOH to trap any $^{14}CO_2$ liberated from the soil. Periodically, portions of the sodium hydroxide solutions were removed and counted in a liquid scintillation spectrometer[1].

Reagents—The carboxyl-labeled 1-^{14}C-2,3,5-triiodobenzoic acid obtained[2] had a specific activity of 10.5 μc./mg. (5248 μc./mole). The compound was shown to be radiochemically pure on the basis of paper chromatography in three solvent systems (1).

Thixotropic gel (5 g./100 ml. Bray's solution) was used for counting ^{14}C in soil suspension.

Peotone soil, a silty clay loam indigenous to the Great Lakes region, was used for this experiment. As determined by plate counts, the soil contained 4.6×10^5 microorganisms/g. soil sample, not differentiated between bacteria, yeasts, and actinomycetes.

Procedures—Twenty 10-g. air-dried Peotone soil samples were put into tared 60-ml. glass-stoppered bottles. Two milliliters of 1-^{14}C-2,3,5-triiodobenzoic acid in the form of the sodium salt (1.884×10^6 d.p.m.) was applied to half the samples, and each of the remaining samples was treated with 4 ml. of 1-^{14}C-2,3,5-triiodobenzoic acid (3.768×10^6 d.p.m.) to effect 20 and 40% moisture content of the soils, respectively. The soil samples were kept at room temperature and protected from light.

At 2–8-week intervals, a soil sample from each group was acidified with 5 ml. of 1 *N* HCl and extracted twice with 40 ml. of acetone. The pooled acetone phases were concentrated by evaporation and reconstituted in 10 ml. acetone. Portions of the extract were counted in the liquid scintillation spectrometer. The acetone extract of soil was chromatographed on thin-layer plates of silica gel G in solvent systems of petroleum ether–propionic acid (10:1) and benzene–methanol–propionic acid (10:2:1). Radioactive zones on the chromatograms were quantitated with a radiochromatogram scanner. The soil residue, after acetone extraction, was counted in thixotropic gel with the spectrometer by the method of Page *et al.* (2).

The possible liberation of $^{14}CO_2$ from the soil after 1-^{14}C-2,3,5-triiodobenzoic acid application was investigated with the apparatus described earlier. One hundred grams of Peotone soil was placed in a 500-ml. light-protected conical flask. Twenty milliliters of a solution, containing 1-^{14}C-2,3,5-triiodobenzoic acid in the form of the sodium salt (1.88×10^7 d.p.m.), was added to the soil. The liberated $^{14}CO_2$ was trapped in two dilute solutions of sodium hydroxide. Periodically, 0.5 ml. of the sodium hydroxide solution was counted in 15 ml. of Bray's solution (3) in the liquid scintillation spectrometer. Fresh dilute sodium hydroxide solution was placed in the traps after each determination of radioactivity. A sterile soil sample was similarly treated as a control.

RESULTS AND DISCUSSION

The amount of $^{14}CO_2$ evolved from soil treated with 1-^{14}C-2,3,5-triiodobenzoic acid is illustrated in Fig. 1. During a period of 51 weeks, more than 80% of the radioactivity added to soil was accounted for as $^{14}CO_2$. Approximately 8% of the radioactivity remained in the soil at the end of this period. The other 12% apparently was also in the form of $^{14}CO_2$ and was lost over the 51-week test period due to periodic sampling of the sodium hydroxide solutions. Note that the sterile soil sample (control) released little or no $^{14}CO_2$ over a period of 14 weeks, while the nonsterile soil sample evolved 43% of its radioactivity as $^{14}CO_2$ in the same time period.

Acetone extraction and chromatography of soil samples treated with 1-^{14}C-2,3,5-triiodobenzoic acid for intervals of 2–28 weeks revealed three or four radioactive zones (Table I). The zones were identified, on the basis of chromatography with reference compounds, as unchanged 2,3,5-triiodobenzoic acid, 2,5-diiodobenzoic

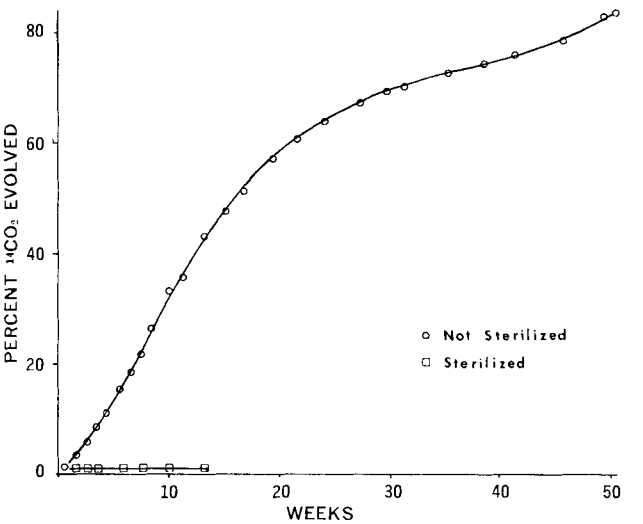

Figure 1—*Cumulative release of $^{14}CO_2$ resulting from application of 1-^{14}C-2,3,5-triiodobenzoic acid to Peotone soil.*

[1] Packard Tri-Carb.
[2] From Dr. J. E. Christian and associates, Bionucleonics Department, Purdue University, Lafayette, Ind.

Table I—Percent Radioactivity of 2,3,5-Triiodobenzoic Acid and Metabolites following Incubation in Soil

Weeks	Nonextracted	Extracted			Total
		Unknown	2,3,5-Triiodobenzoic Acid	2,5-Diiodobenzoic Acid	3,5-Diiodobenzoic Acid	
			20% Moisture			
2	2.40	0	76.19	9.93	5.01	95.53
5	3.90	0	42.90	13.90	14.47	75.17
8	4.60	0	21.76	15.05	14.33	55.74
11	6.03	0	13.01	10.33	10.10	39.47
14	6.90	13.71	12.54	4.96	5.51	43.62
22	N.D.	0.70	4.59	2.01	3.45	10.75
28	N.D.	2.42	6.68	2.42	5.18	16.70
			40% Moisture			
2	2.30	4.03	63.76	13.43	8.42	91.94
5	3.40	1.21	33.37	20.16	12.60	70.74
8	5.80	0.30	7.39	20.21	15.55	49.25
11	7.94	0.79	5.25	2.54	5.07	21.59
14	8.11	4.20	2.96	0.62	0.92	16.81
22	N.D.	1.09	2.26	1.07	1.44	5.86
28	N.D.	2.13	3.59	1.49	1.82	9.03

acid, and 3,5-diiodobenzoic acid. An unknown metabolite was also found on the chromatograms. These data demonstrate that 2,3,5-triiodobenzoic acid was decarboxylated in soil by the microorganisms present. Deiodination of 2,3,5-triiodobenzoic acid to 2,5-diiodobenzoic acid and/or 3,5-diiodobenzoic acid also occurred, reaching a peak at approximately 8 weeks. 2,3,5-Triiodobenzoic acid was metabolized more rapidly at higher soil moisture content (40%) than at 20% moisture.

Spitznagle *et al.* (4), in their studies of 2,3,5-triiodobenzoic acid metabolites in soybeans, reported that the radioactivity decreased rapidly in 1-^{14}C-2,3,5-triiodobenzoic acid-treated soybeans. Residues of 1-^{14}C-2,3,5-triiodobenzoic acid, 1-^{14}C-2,5-diiodobenzoic acid, and 1-^{14}C-3,5-diiodobenzoic acid were found in the various plant parts and in the harvested seeds. Some ^{14}C activity as a hexane-soluble material was also found in the seeds. However, studies on the expiration of $^{14}CO_2$ were not carried out.

Decarboxylation of 2,3,5-triiodobenzoic acid appears to be a major metabolic route by soil microorganisms. Metabolic fate studies on 2,3,5-triiodobenzoic acid in a variety of laboratory and domestic animals provided no evidence of decarboxylation in the metabolism of 2,3,5-triiodobenzoic acid. Rowles *et al.* (5), using 1-^{14}C-2,3,5-triiodobenzoic acid, reported 95.3% of the dose in the excreta of chickens at the end of 4 days. These authors identified 2,3,5-triiodobenzoic acid, 2,3-diiodobenzoic acid, 2,5-diiodobenzoic acid, and 3,5-diiodobenzoic acid in the excreta of chickens. Barker *et al.* (1), using 1-^{14}C-2,3,5-triiodobenzoic acid, found 72–75% of the radioactivity in rat urine and 24–28% in feces over the 4 days after dosing. Gutenmann *et al.* (6) recovered 67% of a dose of 2,3,5-triiodobenzoic acid in bovine urine in 9 days after 2,3,5-triiodobenzoic acid feeding. No expiration of CO_2 was reported in these studies.

Alexander and Aleem (7) studied the effect of molecular structure on the persistence and microbial decomposition of several phenoxyalkyl carboxylic acid herbicides and chlorophenols. They found that the aromatic nucleus of halogenated compounds remained intact for long periods when a halogen was in the *meta*-position. DeRose and Newman (8) reported that 2,4,5-trichlorophenoxyacetic acid persisted for 147 days under greenhouse conditions or 93 days in the field. 2,4-Dichlorophenoxyacetic acid and 2-methyl-4-chlorophenoxyacetic acid disappeared more rapidly, apparently due to the lack of a substituent in the *meta*-position.

In this study of 2,3,5-triiodobenzoic acid metabolism in soil with 20 and 40% moisture content, 42 and 17%, respectively, of the original radioactivity were recovered from the soil at the end of 14 weeks, of which 12.5 and 3% were due to unchanged 2,3,5-triiodobenzoic acid. This disappearance of radioactivity showed that 2,3,5-triiodobenzoic acid was metabolized at a moderate rate. In contrast, many halogenated herbicides such as monuron and insecticides such as DDT or chlordane persist for 1.5–3 and 6–12 years, respectively, in soil (9).

REFERENCES

(1) W. M. Barker, P. L. Moy, and A. G. Ebert, *J. Agr. Food Chem.*, **19**, 916(1971).

(2) A. L. Page, J. R. Sims, and F. T. Bingham, *Soil Sci. Soc. Proc.*, **28**, 359(1964).

(3) G. A. Bray, *Anal. Biochem.*, **1**, 279(1960).

(4) L. A. Spitznagle, J. E. Christian, and A. J. Ohlrogge, *J. Pharm. Sci.*, **58**, 1234(1969).

(5) S. G. Rowles, G. S. Born, J. E. Christian, and W. V. Kessler, *ibid.*, **59**, 257(1970).

(6) W. H. Gutenmann, C. A. Bache, and D. J. Lisk, *J. Agr. Food Chem.*, **15**, 600(1967).

(7) M. Alexander and M. I. H. Aleem, *ibid.*, **9**, 44(1961).

(8) H. R. DeRose and A. S. Newman, *Soil Sci. Soc. Proc.*, **12**, 222(1947).

(9) M. Alexander, *ibid.*, **29**, 1(1965).

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