Malonic Acid Conjugation by Soil Microorganisms of a Pesticide-Derived Aniline Moiety

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The microbial and chemical fate of substituted anilines resulting from pesticide degradation in soil has been of recent concern. They are oxidatively condensed to azobenzenes and related products (1) and acylated to form acylanilides (2). We report another, novel conjugative pathway for the aniline formed from the microbial degradation of the acaricide chlordimeform [N'-(4'-chloro-o-tolyl)-N,N-dimethylformamidine] in which the aniline moiety of this compound is converted to its malonanilic acid derivative.

Cultures of mixed populations of soil microorganisms were prepared by suspending 0.5 g of soil (Louisiana Commerce Silt Loam and Indiana Clay Loam, 4 replicates each) in 50 ml of synthetic medium containing 25 µg/ml of chlordimeform (tolyl-methyl-^{14}C: purified by TLC just prior to use). The cultures were incubated three weeks in the dark at 27° on a gyratory shaker. The parent compound and its degradation products were extracted with three portions of 2:1 acetonitrile-water totaling 140 ml. Chloroform (80 ml) was added to the extracts to form aqueous and neutral non-aqueous fractions. The aqueous fraction was acidified and reextracted with chloroform to give an acidic non-aqueous fraction. Both chloroform fractions were concentrated and analyzed by TLC (System A: Whatman SG-81 silica gel-loaded paper developed with 50:1 - benzene: diethylamine. System B: Whatman SG-81 paper developed with 90:10:4 - benzene: dioxane: acetic acid). Radioactive spots were located by radioautography. These spots were cut out, suspended in scintillation fluid and the amounts of radioactivity determined with a scintillation counter.

As expected (3), extensive hydrolysis to yield 4'-chloro-o-formotoluidide occurred in control mixtures of sterile soil with a 50% recovery of the parent compound being realized. The parent compound and this degradation product were identified on the basis of their chromatographic patterns. In the soil cultures 70% degradation was found. A major, previously unreported metabolite which was not present in the controls was found in each inoculated culture. This new metabolite accounted for as much as 46% of the total added radioactivity and averaged 22%.

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It was present predominantly in the acidic, non-aqueous fractions, and its Rf values were 0.00 and 0.43 for Systems A and B, respectively. The compound was isolated and purified as follows: the remaining acidic, non-aqueous fractions were chromatographed on several Brinkman Pre-coated TLC Plates, Silica Gel F-254, 0.25 mm thickness with 90:25:4-benzene: dioxane: acetic acid (System C). The unidentified metabolite was found at Rf 0.14. The radioactive area at 0.14 was removed from the plates and extracted with ethyl acetate. One-half of the combined extracts was evaporated in a quartz capillary tube for introduction by means of the solid inlet system into a Perkin-Elmer Model 270 Mass Spectrometer. The remaining portion was evaporated and methylated with diazomethane. This derivative was chromatographed (System C) and yielded a single radioactive spot at Rf 0.70. This spot was similarly extracted and the mass spectral analysis was made as above.

The mass spectrum of the acidic metabolite obtained immediately after insertion of the solid inlet probe contained a chlorine isotope cluster at m/e 227/229, a base peak cluster at m/e 141/143 and other peaks at m/e 183/185. The apparent molecular ion at m/e 227/229 rapidly disappeared and the spectrum became that of 4'-chloro-o-aceto-toluidide (M^+ 183/185, base peak m/e 141/143). This behavior suggested a malonanilic acid which readily could undergo decarboxylation to the acetotoluidide. This structural assignment was corroborated by the mass spectrum of the methylated derivative which had a molecular ion at m/e 241/243 (26% rel int.) and the base peak at 241/243, a spectrum expected for the methyl ester of the originally observed malonanilic acid.

Confirmation of structure was obtained by synthesizing (4) authentic 4'-chloro-2'-methyl-malonanilic acid, mp 156-158° (exact mass calcd. for $C_{10}H_{10}NO_3{}^{35}Cl$, 227.03492; found (5), 227.03421) from 4-chloro-o-toluidine, and the methyl ester, mp 97-98° (exact mass calcd. for $C_{11}H_{12}NO_3{}^{35}Cl$, 241.05057; found, 241.04873). The Rf values and mass spectra of these compounds were identical to those of the metabolite and its methyl ester.

Conjugation of the aniline moieties of pesticides with malonic acid may thus be a significant transformation in the soil. Such conjugations, presumably involving malonyl-coenzyme A, previously have been observed only in plants as a mechanism

for D-amino acid detoxification (6). They may, however, be important pathways for detoxifying aromatic amines in various organisms and could account for a portion of previously unidentified polar metabolites of these compounds.

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