Conversion of 4-(2,4-DB) to 2,4-Dichlorophenoxycrotonic Acid (2,4-DC) and Production of 2,4-D from 2,4-DC in Soil

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Crotonic acid is hypothesized as the first intermediate in the beta-oxidation of butyric to acetic acid. In the work reported, 2,4-dichlorophenoxycrotonic acid has been shown to yield 2,4-D in soil. Also, gas chromatographic analysis showed production of a peak in 4-(2,4-DB)-treated soil identical in retention time and shape to 2,4-dichlorophenoxycrotonic acid. These observations offer further support for the existence of an operative beta-oxidation reaction in soil.

2,4-Dichlorophenoxyacetic acid (2,4-D) has been identified (*1–3*) as a metabolite of 4-(2,4-dichlorophenoxybutyric) acid [4-(2,4-DB)] in timothy grass, birdsfoot trefoil, mixed forage, sterile pea plants, and soil. This conversion is believed to involve simple beta-oxidation of the butyric acid side-chain with resultant formation of an acetic acid chain. The first step in beta-oxidation of butyric acid is believed to involve formation of a double bond (by enzymatic dehydrogenation) between the alpha and beta carbon atoms (*4*). The resulting unsaturated acid would be crotonic acid. If 2,4-dichlorophenoxycrotonic acid (2,4-DC) could be shown to yield 2,4-D in a biological system, the hypothesis of a beta-oxidation mechanism would be further substantiated. In the work reported, 2,4-DC was added to soil and was shown to yield 2,4-D. Also, when 4-(2,4-DB) was added to the same soil, gas chromatographic analysis showed the production of a peak having a retention time and shape identical to 2,4-DC.

Procedure

Ten-gram samples of sieved Canfield silt loam soil (pH 5.8) were placed in 5-ounce plastic cups, and 1 ml. of 2,4-DC (50 μg. per ml.) in acetone was pipetted into each cup. Similarly, 10-gram samples of this soil were prepared with the addition of 1 ml. of 4-(2,4-DB) (500 μg. per ml.) in acetone. An equal number of untreated soil samples were prepared and treated with 1 ml. of acetone to serve as controls. After evaporation of the acetone, water was added to each cup to give 10% soil moisture, and the contents were thoroughly mixed. The cups were covered with aluminum foil and incubated at 21° C. At regular intervals, a cup of treated and a cup of control soil were analyzed by electron affinity gas chromatography by the procedure described earlier (*2*).

The method involved an acetone–phosphoric acid extraction of the total soil sample, followed by direct boron trifluoride methylation of the evaporated acetone extract and partitioning of the methyl esters into hexane. Up to 10 μl. of the hexane was then chromatographed on a 6-foot column of 5% silicone grease on Chromosorb W at 200° C. The analyses were performed with a Barber-Colman, Model 10 gas chromatograph equipped with a battery-operated, radium-226 electron affinity detector. Nitrogen (40 cc. per minute) was the carrier gas.

Results and Discussion

Figure 1 shows chromatograms of (*A*) soil 7 days after addition of 5 p.p.m. of 2,4-DC and (*B*) control soil. The retention times for 2,4-D and 2,4-DC methyl esters were about 4 and 11 minutes, respectively. The recovery of 1 p.p.m. of 2,4-D added to the soil was 83, 87, and 79%. The recovery of 1 p.p.m. of 2,4-DC added to the soil was 81%. The 2,4-DC peak showed slight tailing in both standards and samples. The retention times for 2,4-D and 2,4-DC standards were identical with those observed for the corresponding peaks in the soil sample chromatograms. In several instances, hexane solutions of the methylated standards and methylated soil samples were deliberately mixed and then injected. The resulting chromato-

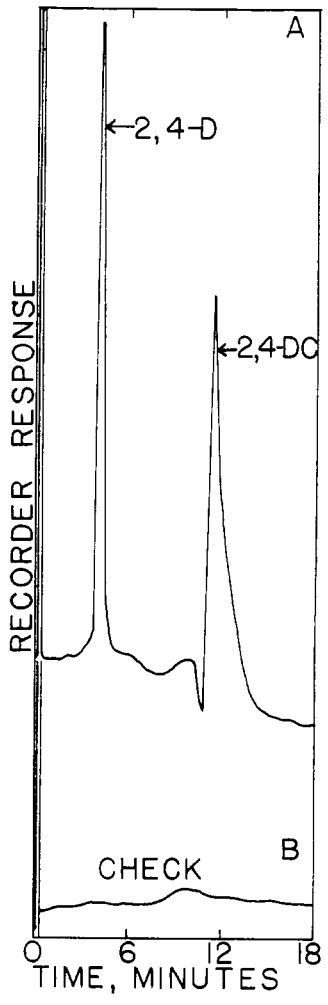

Figure 1. Chromatograms of (*A*) Canfield silt loam 7 days after addition of 5 p.p.m. of 2,4-DC and (*B*) untreated soil

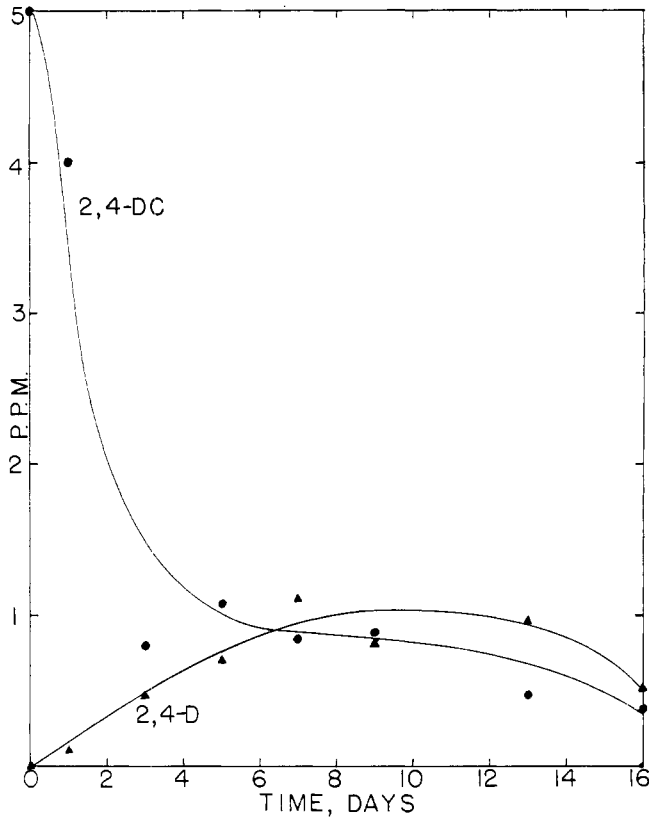

Figure 2. Disappearance of 2,4-DC, and formation and disappearance of 2,4-D in Canfield silt loam

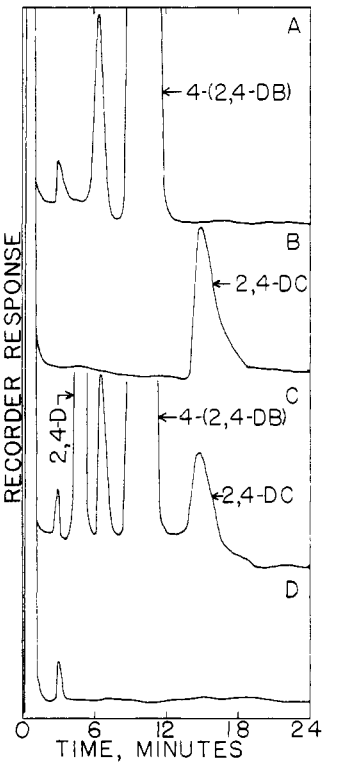

Figure 3. Chromatograms of (A) 4-(2,4-DB) standard, (B) 2,4-DC standard, (C) 4-(2,4-DB)-treated soil, and (D) control soil

grams simply showed a proportional increase in peak height for 2,4-D and 2,4-DC, thus illustrating further that the compounds were identical. Figure 2 shows the disappearance of 2,4-DC, and the resultant formation and disappearance of 2,4-D with time in the Canfield soil. These curves resemble the disappearance of 4-(2,4-DB) and formation of 2,4-D in this soil (*3*).

Figure 3 shows chromatograms of (*A*) 0.05 μg. of 4-(2,4-DB) standard injected, (*B*) 0.0005 μg. of 2,4-DC injected, (*C*) soil 8 days after treatment with 4-(2,4-DB), and (*D*) control soil. The chromatogram in 3*C* shows the expected peak for 2,4-D (at about 5 minutes) and the peak corresponding to 2,4-DC at about 15 minutes. Both standard 2,4-DC and that in the soil chromatogram show the same characteristic tailing. The recovery of 0.25 p.p.m. of 2,4-DC added to soil ranged from 79 to 84%. The method was sensitive to about 0.05 p.p.m. of 2,4-DC in the soil. This compound is thus about 4 times as sensitive to electron affinity detection as 4-(2,4-DB). The large amount of 4-(2,4-DB) standard (chromatogram 3*A*) was injected to show that the 2,4-DC peak observed in the treated soil was not due to an impurity in the standard. The retention times for 2,4-D and 2,4-DC are not identical to those shown in Figure 1 because another gas chromatographic column (although identically prepared) was used. These small differences with replicate columns are commonly observed.

The concentrations of 2,4-DC (based on peak height measurement) found in the soil after 2, 4, 6, 8, and 12 days of incubation were 0.44, 0.20, 0.25, 0.25, and 0.22 p.p.m., respectively. This peak was not observed in the 4-(2,4-DB)-treated soil studies previously (*3*), probably because the concentration of 4-(2,4-DB) added (5 p.p.m.) was too low.

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