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Carbaryl Decomposition to 1-Naphthyl Carbamate by *Aspergillus terreus*

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The metabolic transformation of the insecticide carbaryl (1-naphthyl *N*-methyl carbamate) by *Aspergillus terreus* resulted in the formation of several hydroxylated products. Since approximately 20% of carbaryl was hydroxylated at the carbamate *N*-methyl group, it was attempted to clarify the pathway of the side chain. 1-Naphthyl *N*-hydroxymethyl carbamate was chemically identified, and its further biological transformation to 1-naphthyl carbamate could be established. This intermediate was further degraded to 1-naphthol, but it is not known if biological or chemical activity is responsible for it. 1-Naphthol was evidently further metabolized by the fungus.

INTRODUCTION

The major concern of environmental toxicology about pesticidal pollution of soil is in the persistence or residual effects of the applied chemical. Despite the increasing use of carbaryl (1-naphthyl *N*-methyl carbamate; Sevin) in recent years, its persistence and toxicological properties in soil—and its possible decomposition products—remains unclear. Several factors are known which influence its chemical decomposition (1, 2), but microbial degradation is considered of equal importance for its disappearance from soil. However, only a few recent reports describe transformation mechanisms of carbaryl by soil microbes (3–6). Bollag and Liu (3) found a hydrolysis mechanism whereby carbaryl was degraded to 1-naphthol which in turn could be further transformed by isolated microorganisms. Another detoxication process was described with the fungus *Gliocladium roseum* which hydroxylated the pesticide molecule at different sites (5). Three metabolites were isolated from the fungal culture solution and were identified as 1-naphthyl *N*-hydroxymethyl carbamate, 4-hydroxy-1-naphthyl methyl carbamate, and 5-hydroxy-1-naphthyl methyl carbamate. The same products were also found with a number of other fungal isolates, but the quantitative accumulation of the metabolites varied among the different fungi species (4). The hydroxylation mechanism of carbaryl which is well known in higher plants and insects (7), as well as animals (8) seems also to be an important transformation reaction for soil fungi.

Since the fate of the hydroxylated carbaryl derivatives by soil fungi or other microorganisms is not known, an investigation was undertaken in order to clarify this question. This paper describes the formation of 1-naphthyl *N*-hydroxymethyl carbamate by *Aspergillus terreus*, and the subsequent transformation to 1-naphthyl carbamate.

MATERIALS AND METHODS

A strain of *Aspergillus terreus* was isolated from carbaryl-treated soil by enrichment culture techniques. The fungus was grown

in a nutrient medium containing carbaryl, 0.1 g; nutrient broth (Difco), 8.0 g; yeast extract, 1.0 g; dextrose, 10.0 g; $NaH_2PO_4 \cdot H_2O$, 6.0 g; and Na_2HPO_4, 0.5 g; in 1 liter distilled water (final pH 6.0). Radiolabeled carbaryl (0.02 μCi per 1 ml) was added as 1-naphthyl *N*-methyl (carbamate-^{14}C) (Nuclear Chicago Corp., Ill.), or ring-^{14}C labeled carbaryl which was synthesized by reacting 1-naphthol-1-^{14}C with methyl isocyanate at 140°C for 4 hr (9), and subsequent purification by thin-layer chromatography.

If the fungus was grown in media containing 1-naphthyl *N*-hydroxymethyl carbamate, 1-naphthyl carbamate, or 1-naphthol, a concentration of 30 ppm was used. All these substrates were added to the autoclaved medium after sterilization by membrane filtration (0.22 μ pore size, Millipore Corp., Bedford, Mass.).

The media were inoculated with spore suspensions harvested from one-week-old cultures grown on Czapek Agar (Difco), and incubated on a rotary incubation-shaker (250 oscillations/minute) at 28°C. It was essential that a concentrated spore suspension (3×10^8/milliliter) was used as inoculum, since only during vigorous growth of the fungus was a significant formation of 1-naphthyl carbamate observed. At different growth periods samples were removed from the fungal culture medium, and the culture filtrate was extracted with ether for identification of the metabolites.

Aliquot samples of the concentrated ether extract were analysed by thin-layer chromatography as previously described (5). An additional solvent system was elaborated which resulted in a better separation of the important metabolites in the present investigation; this solution was composed of toluene–benzene–acetone–acetic acid (75:20:8:2). If the metabolites were isolated for further identification, the thin-layer plates were not treated for visualization. Each isolated product was purified twice by thin-layer chromatography and recrystallized from anhydrous ether.

Radioactive compounds on thin-layer plates were measured by scraping the entire spot into a scintillation solution containing 4 g Omnifluor (98% PPO, and 2% bis-MSB, New England Nuclear Corp., Boston, Mass.), and 40 g Cab-O-Sil (Thixotropic suspension powder) in 1 liter of toluene. Aqueous samples were counted in a Bray solution.

Infrared spectra of the metabolites were taken with a Model 621 Perkin-Elmer spectrometer using a KBr disk technique, and uv-spectra were obtained with a Bausch and Lomb Spectronic 505 spectrometer. Mass spectra were taken with a Model 902 Mass spectrometer (Associated Electrical Industries, Ltd., England).

Carbaryl, 1-naphthyl *N*-hydroxymethyl carbamate, and 1-naphthyl carbamate were obtained from the Union Carbide Corp., South Charleston, W. Va.

RESULTS

From the culture medium of *A. terreus* it was possible to separate four metabolites (metabolites A, B, C, and D) by thin-layer chromatography using ^{14}C-methyl and ^{14}C-ring-labeled carbaryl. Metabolites A, B, and C still have the label resulting from the methyl-^{14}C carbaryl, but metabolite D carries radioactivity only when the ring-^{14}C-tagged carbaryl is used. Metabolites B and C which were formed only in minute amounts could be tentatively identified on thin-layer chromatography as 4-hydroxy- and 5-hydroxy-1-naphthyl methyl carbamate. These two intermediates indicate ring-hydroxylation and they were not followed further, since the major concern in the present investigation was the fate of the predominately formed metabolites which showed changes in the side chain.

The appearance of metabolite A and D, and the disappearance of carbaryl from the

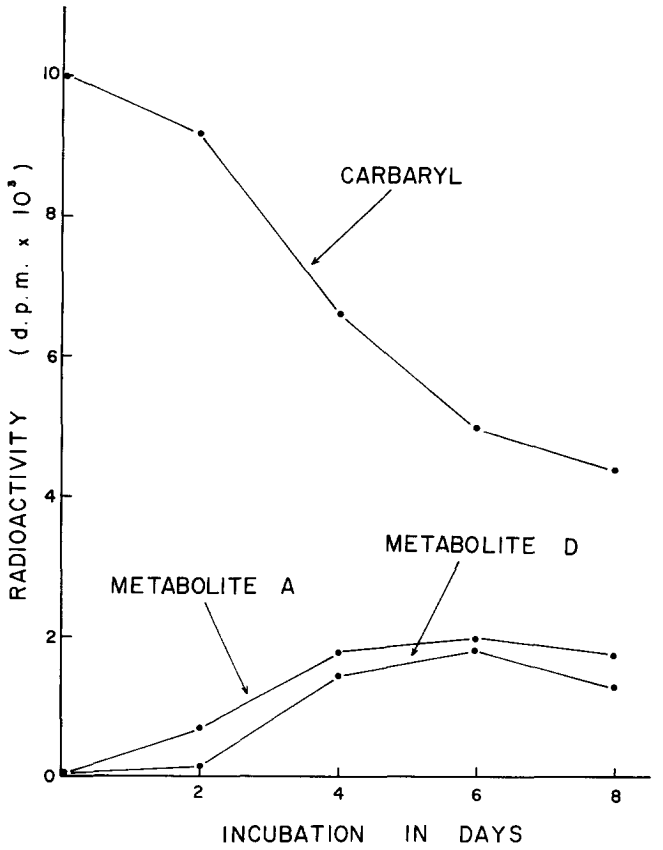


FIG. 1. *Formation of metabolite A and D from carbaryl during the growth of* A. terreus (*radioactivity of chemicals was measured after separation with TLC*).

fungal cultures were followed by thin-layer analysis (Fig. 1). The formation of metabolites A and D increased after 2 days of incubation, and reached its maximum after 6 days; approximately 20% of the added radioactive label was recovered as metabolite A, 18% was detected as metabolite D, and 50% remained unchanged as carbaryl. No attempt was made in this experiment to account for all radioactivity originally applied, but the recovery of 88% and 75% of the total radioactivity as carbaryl, metabolite A and D after 6 and 8 days, respectively, indicated the essential transformation of the pesticide molecule. It was assumed that metabolite D was a further decomposition product of metabolite A, and in order to clarify this assumption, the fungus was grown in media containing the following different substrates: 1-naphthyl *N*-hydroxymethyl carbamate, 1-naphthyl carbamate, and 1-naphthol. A thin-layer chromatogram indicating the transformation by *A. terreus* of carbaryl and the tested substrates during a 6-day growth period is presented in Fig. 2. *A. terreus* metabolized carbaryl and showed clear formation of metabolites with R_f values corresponding to 1-naphthyl *N*-hydroxymethyl carbamate, and 1-naphthyl carbamate. 1-Naphthyl *N*-hydroxymethyl carbamate as substrate was decomposed to 1-naphthyl carbamate and 1-naphthol. A weak spot with the same R_f value as metabolite D was also noticed in the uninoculated control medium, and this indicates that a chemical decomposition

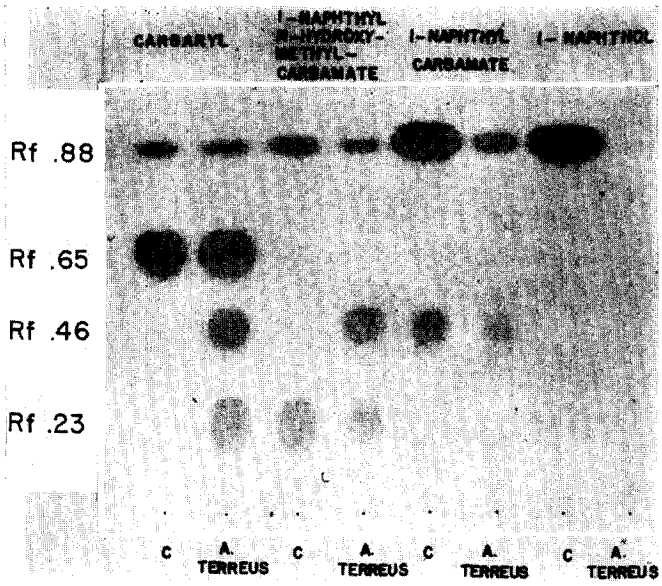


FIG. 2. *Metabolism of carbaryl, 1-naphthyl N-hydroxymethyl carbamate, 1-naphthyl carbamate, and 1-naphthol by* A. terreus; *C represents the control spot of the authentic chemical. The thin-layer plate was developed with ether-hexane (4:1). R_f .88: area of 1-naphthol; R_f .65: area of carbaryl; R_f .46: area of 1-naphthyl carbamate; R_f .23: area of 1-naphthyl N-hydroxymethyl carbamate.*

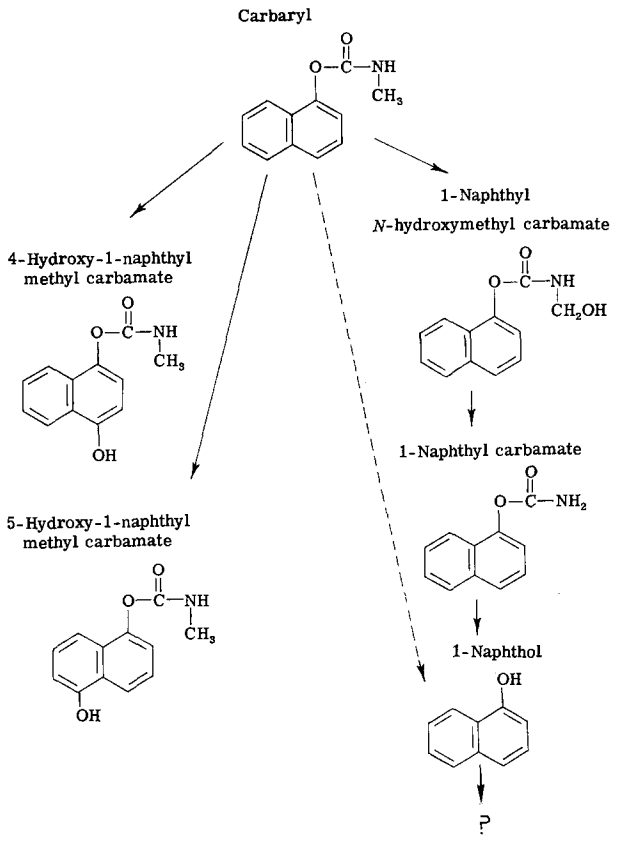
may also occur, but the chemical process is much weaker than the biological activity provoked by the fungus. All tested chemicals were also chemically transformed to 1-naphthol under the applied experimental conditions, but there was always a clear difference to the culture extract from the fungus. The 1-naphthol spot resulting from the chemical decomposition of the control medium o 1-naphthyl carbamate is much stronger than in the fungal culture solution and this in turn indicates that *A. terreus* is active in degrading 1-naphthol. This assumption was further strengthened by showing that 1-naphthol as substrate disappears when inoculated with the fungus.

Metabolite A formed by *A. terreus* was chemically identified as 1-naphthyl *N*-hydroxymethyl carbamate after chromatographic purification by establishing the following characteristics with the authentic chemical: mp 137–139°C; uv-maximum in ether at 282 nm, and by infrared spectrum (Fig. 3). Mass spectral analysis showed metastable peaks at *m/e* 217, 187, 144, 116, and 115 (Table 1).

Since metabolite D has a similar R_f value as 5-hydroxy-1-naphthyl methyl carbamate in the methylene chloride–acetonitrile (4:1), or ether–hexane (4:1) solvent, the use of the solvent system toluene–benzene–acetone–acetic acid (75:20:8:2) separated clearly metabolite D (R_f 0.27) from metabolite C (R_f 0.19). Isolation on TLC ether extraction and evaporation resulted in the formation of white shiny crystals. The following characteristics were identical of metabolite D and 1-naphthyl carbamate: mp 145–147°C; uv-spectra; the mass spectral data established the molecular weight to be 187 ± 0 and indicated the metastable peaks at *m/e* 187, 144, 116, and 115 (Table 1).

DISCUSSION

The isolation and identification of metabolites formed by *Aspergillus terreus* in a culture solution suggests the following breakdown of carbaryl:



It was possible to demonstrate that 1-naphthyl *N*-hydroxymethyl carbamate is a breakdown product of carbaryl by this fungus as was demonstrated for other soil fungi (4, 5), but *A. terreus* metabolizes it further to 1-naphthyl carbamate and, subsequently, to 1-naphthol.

The metabolizing activity of *A. terreus* was found to be the strongest of the fungi investigated (4). In one week approximately half the amount of carbaryl was transformed to other products. A continuous degradation of the pesticide was observed during incubation in the fungal growth medium, but an accumulation—at least a temporary one—of the intermediates could easily be established. This fact needs further study if there is concern about the effect of formed detoxication products from an organism on its influence in the ecosystem.

The formation of 1-naphthyl *N*-hydroxymethyl carbamate could always be observed during growth of *A. terreus*, but its quantitative accumulation varied. If the development of the fungus was slow, the accumulation of the metabolite was larger than during fast growth which could be promoted by a larger amount of spores used as inoculum. However, during more active growth conditions, the fungus transformed 1-naphthyl *N*-hydroxymethyl carbamate more rapidly to 1-naphthyl carbamate than during poor growth; in the latter case 1-naphthyl carbamate was hardly detectable.

1-Naphthyl carbamate was never reported as a biological decomposition product of carbaryl. There is no doubt that 1-naphthyl *N*-hydroxymethyl carbamate can be chemically decomposed to 1-naphthyl carbamate (10), but the present study demonstrated

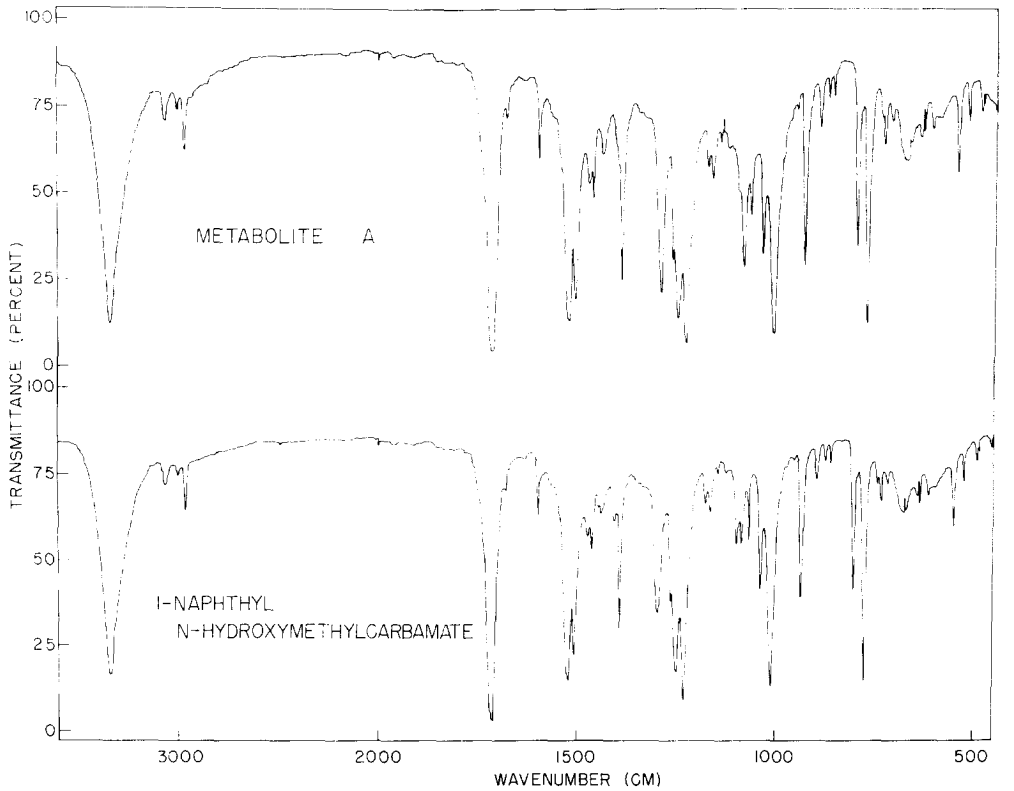


FIG. 3. *Infrared spectra of metabolite A and authentic 1-naphthyl N-hydroxymethyl carbamate.*

TABLE 1

Relative intensities of certain peaks in the Mass spectra of carbaryl and its metabolites formed in the culture solution of A. terreus.

m/e	Relative Intensity, %			
	Carbaryl	Metabolite A (1-naphthyl *N*-hydroxy-methyl carbamate)	Metabolite D (1-naphthyl carbamate)	1-Naph-thol
217		2.0		
201	5.2			
199		1.7		
187		2.4	5.0	
144	100.0	100.0	100.0	100.0
116	26.3	24.6	33.0	33.3
115	44.7	58.0	60.0	60.0

that through the biological activity the formation of this product can be considerably increased.

Previously it was reported (3) that 1-naphthol is a decomposition product of various microorganisms. In this study there was no attempt made to clarify whether the formation of 1-naphthol was due to biological or chemical degradation of 1-naphthyl carbamate, but there were clear indications that 1-naphthol is metabolized further in the presence of *A. terreus*.

The specific mechanism involved during the metabolism of carbaryl by *A. terreus* is not clear. It is of interest to note that 1-naphthyl *N*-hydroxymethyl carbamate was formed by most of the fungi tested (4), but the production of 1-naphthyl carbamate could only be observed with *A. terreus*, and to a certain extent with *Aspergillus flavus*.

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