Degradation of Carbaryl and 1-Naphthol by Marine Microorganisms

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Carbaryl (1-naphthyl N-methylcarbamate), a carbamate insecticide with a wide range of activity and relatively low degree of toxicity to animals, is one of the most commonly used pesticides. It may enter the estuarine environment as a result of run-off from agricultural lands or through its use in the control of estuarine pests. Among the various factors which determine the fate of a pesticide, microbial transformation is one of the most important. The metabolism of carbaryl by soil microorganisms has been reported (BOLLAG and LIU, 1971; LIU and BOLLAG, 1971a; LIU and BOLLAG, 1971b; BOLLAG and LIU, 1972a; KAZANO et al., 1972). It has also been shown that carbaryl is readily hydrolyzed to 1-naphthol non-biologically in sea water (KARINEN et al., 1967; LAMBERTON and CLAEYS, 1970). 1-Naphthol has been reported to be more toxic to fish (STEWART et al., 1967) and microorganisms (BOLLAG and LIU, 1971) than the parent compound (carbaryl); therefore, it becomes important to have information on its further transformation in order to have a comprehensive knowledge of the fate of carbaryl. The degradation of carbaryl and 1-naphthol by marine microorganisms has received little attention. This investigation was undertaken to test the ability of selected species of marine bacteria, yeasts and filamentous fungi to degrade the two chemicals.

MATERIALS AND METHODS

Chemicals: ^{14}C-1-naphthyl-labeled carbaryl (specific activity, 6.56 mCi/mmole) was provided by Union Carbide Corp. and 1-naphthol-1-^{14}C (specific activity, 15.2 mCi/mmole) was purchased from Amersham Searle Corp.

Effect of pH on the stability of carbaryl and 1-naphthol in the growth media: The growth media buffered with 0.05 M MES were adjusted to pH 5.0, 5.5, 6.0 and 6.5. ^{14}C-carbaryl or 1-naphthol were added to the sterile medium at a concentration of 1 ppm. After 3 days of incubation in the dark, the medium was acidified to pH 1 with 12 N HCl and extracted with ether. The amount of ^{14}C in the ether and aqueous fractions was determined using a liquid scintillation counter. The ether extract was concentrated to a suitable volume and spotted on thin-layer silica-gel plates. The chromatograms were developed in solvent systems consisting of chloroform-methanol (98:2) and ether-hexane (4:1). The chromatograms were scanned for radioactivity using a Nuclear Chicago Actigraph. Authentic reference compounds were co-chromatographed for comparison with the unknowns.

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Studies with bacteria, yeasts and filamentous fungi:

Culture Methods: The following organisms orginally insolated from the marine environment were used in these studies: Brevibacterium sp., Flavobacterium sp., Serratia marina and Spirrillum sp. (bacteria); Candida parapsilosis, Rhodotorula glutinis and Trichosporon fermentans (yeasts); Aspergillus fumigatus, Culcitalna achraspora, Halosphaeria mediosetigera, and Humicola alopallonella (filamentous fungi). The bacteria were grown in Difco marine broth (9) while the yeasts and filamentous fungi were grown in a medium containing 5 g glucose, 2.4 g NH_4NO_3, 1.0 g yeast extract per liter of artificial sea water prepared from Rila marine mix (Rila Products, Teaneck, N.J.). This medium (hereafter referred to as fungal medium) is similar to that described by SGUROS et al. (1962), except that 2-(N-morpholino) ethanesulfonic acid (MES) and artificial sea water were used instead of Tris (hydroxymethyl) aminomethane (Tris) and natural sea water, respectively. To minimize the chemical degradation of carbaryl or 1-naphthol, the pH of the growth medium was adjusted to 5.5 except for Spirrillum sp., which was grown in a medium with an initial pH of 6.0. The medium was buffered with 0.05 M MES. The organisms were incubated with the chemicals on a rotary shaker at a temperature of 25°C in the dark, due to the instability of 1-naphthol in the light.

Degradation of carbaryl and 1-naphthol: The organisms were grown in 15 ml of nutrient medium containing 1.7 ppm of ^{14}C-carbaryl or 1.0 ppm of 1-naphthol. The three fungi, Humicola alopallonella, Halosphaeria mediosetigera and Culcitalna achraspora were incubated with the two chemicals for seven days on account of their slow growth, whereas the remaining organisms were incubated for three days. After the necessary incubation period, 5 ml of acetone was added to the culture solution which was acidified to pH 1-1.5 with 0.2 ml of 12 N HCl and then extracted twice with ether. The ^{14}C in the ether extract and the aqueous phase was determined using a liquid scintillation counter. The ^{14}C in the ether extract was analyzed by thin-layer chromatography as described previously. The portions of the silica-gel adsorbent corresponding to the resolved radioactive compounds were scraped, placed in a scintillation solution containing 4% "Cab-o-Sil" (thixotropic gel suspension powder) and the radioactivity was determined by liquid scintillation counting.

RESULTS AND DISCUSSION

Effect of pH on the chemical conversion of carbaryl and 1-naphthol: Since the stability of both carbaryl and 1-napthol varies with the pH of the solution (KARINEN et al., 1967; LAMBERTON and CLAEYS, 1970), experiments were done to study the chemical conversion of the two chemicals in the growth media maintained at pH values ranging from 5.0 to 7.0. The purpose was to determine the physiological pH at which the non-biological conversion of carbaryl and 1-naphthol in the growth medium was minimal.

Table 1 shows the amount of ^{14}C in the ether and aqueous fractions after three days of incubation of ^{14}C-carbaryl and 1-naphthol in the sterile growth media maintained at different pH values. The conversion of carbaryl to water-soluble products was extremely small at pH 5.0-6.5. The amount of ^{14}C-water-soluble product(s) resulting from carbaryl degradation was slightly higher at pH 7.0. At pH 5.0-7.0, more than 90% of the initial ^{14}C was present in the ether phase. Thin-layer chromatographic analysis of the ether extract showed that all of the ^{14}C in this fraction was present as carbaryl. The results indicate that carbaryl is quite stable in the growth media maintained at pH values ranging from 5.0 to 6.5.

TABLE I

Distribution of ^{14}C in the ether and aqueous fractions after three days incubation of ^{14}C-carbaryl and 1-naphthol in the sterile growth media maintained at different pH values.

		Percent of initial ^{14}C			
		Carbaryl		1-naphthol	
pH	Medium	Ether-Sol.	Water-Sol.	Ether-Sol.	Water-Sol.
7.0	Difco Marine broth	91.0	4.0	--	--
6.5	Difco Marine broth	94.0	1.8	71.7	9.2
6.0	Difco Marine broth	94.8	1.1	73.8	7.7
	Fungal Medium	92.2	0.6	77.2	1.9
5.5	Difco Marine broth	95.7	1.1	72.8	4.3
	Fungal Medium	97.3	0.8	74.1	1.6
5.0	Fungal Medium	95.8	0.8	78.5	1.5

1-Naphthol was relatively less stable than carbaryl in the growth media at the above pH range. After three days, 70-80% of the initial ^{14}C was recovered in the ether phase. TLC analysis showed that all of the ^{14}C in the ether phase consisted of 1-naphthol. The water-soluble metabolites resulting from chemical degradation of 1-naphthol increased slightly with an increase in the pH of the marine broth from 5.5 to 6.5. It was noticed that the amount of ^{14}C-water-soluble

metabolites varied with the type of the medium. At pH 6.0 and 5.5, a greater amount of 1-naphthol was converted to water-soluble metabolites in the marine broth than in the fungal medium. The results suggest that the pH of the growth medium should be maintained below 6.5 to minimize non-biological degradation of 1-napthol.

Metabolism of carbaryl by microorganisms: Table II shows the amount of ^{14}C in the ether and aqueous fractions after extraction of the acidified culture solution with ether. A major portion of the radioactivity in the culture solution of all the organisms was present in the ether extract. Thin-layer chromatography of the ether extract on silica gel plates in chloroform-methanol (98:2) system showed that all the ^{14}C in this fraction was present as carbaryl (Rf 0.56). All the organisms included in the study, except Brevibacterium, Flavorbacterium and Spirrillum, were able to convert carbaryl to water-soluble products.

TABLE II

Distribution of ^{14}C among the ether-soluble and water-soluble products in the culture solution of various microorganisms incubated with ^{14}C-carbaryl

Organisms	Percent of Initial ^{14}C	
	Ether-soluble Products (carbaryl)	Water-soluble Products [a]
Brevibacterium sp.	96.1	-
Flavobacterium sp.	95.0	-
Serratia marina	90.2	6.8
Spirrillum sp.	79.4	-
Candida parapsilosis	93.2	2.1
Rhodotorula glutinis	93.5	2.9
Trichosporon ferments	94.2	5.1
Aspergillus fumigatus	94.7	4.2
Culcitalna achraspora [b]	87.7	9.9
Halosphaeria mediosetigera [b]	86.1	7.8
Humicola alopallonella [b]	88.8	6.5

[a] Corrected for non-biological conversion.

[b] These organisms were incubated with carbaryl for 7 days while the others were incubated for 3 days.

The amount of ^{14}C-water-soluble products varied with the organism. The two fungi, Culcitalna and Halosphaeria were relatively more effective in converting carbaryl to water-soluble products than the other organisms. The ^{14}C-products in the aqueous fraction did not move from the origin on the thin-layer plate and were not identified. These findings suggest that the organisms included in this study were not able to metabolize carbaryl to a significant extent. In this regard they differ from soil microorganisms which have been reported to transform carbaryl into a number of metabolites including 1-naphthol; 1-naphthyl N-hydroxymethyl-carbamate; 4-hydroxy-1-napthyl methylcarbamate; and 5-hydroxy-1-naphthyl methylcarbamate (BOLLAG and LIU, 1971; LIU and BOLLAG, 1971a; LIU and BOLLAG, 1971b; BOLLAG and LIU, 1972a; KAZANO et al., 1972). Hydroxylation of carbaryl, which is an important reaction in its transformation in several soil fungi (BOLLAG and LIU, 1972a), appears to be absent in the marine fungi tested.

Metabolism of 1-naphthol: The microorganisms differed greatly in their ability to degrade 1-naphthol; they also produced different types and amounts of metabolites from 1-naphthol (Table III). The cultures of Brevibacterium, Candida, Rhodotorula and Trichosporon incubated with ^{14}C-1-naphthol produced only small amounts of water-soluble metabolites from 1-naphthol. The remaining organisms were able to convert 1-naphthol to both ether-soluble and water-soluble metabolites. Culcitalna, Halosphaeria, Humicola and Aspergillus were more effective than Flavobacterium, Spirrillum, and Serratia in converting 1-naphthol to its water-soluble metabolites. In the first four species, ^{14}C in the ether phase had decreased to less than 20% of the original ^{14}C in the medium. This decrease was accompanied by an increase in the amount of ^{14}C-water-soluble metabolites which accounted for a major portion of the original ^{14}C. Humicola was found to be the most effective in converting 1-naphthol into water-soluble metabolites. About 15% of the initial ^{14}C was present as water-soluble metabolites in cultures of Serratia and Spirrillum, whereas Flavobacterium, had converted only about 5% of ^{14}C-1-naphthol to water-soluble metabolites. BOLLAG and LIU (1972b) and KAZANO et al. (1972) also observed that soil microorganisms like Fusarium and Pseudomonas converted a significant amount of 1-naphthol into unidentified water-soluble metabolites.

In the cultures of Flavobacterium, Serratia and Spirrillum. 85-90% of the ^{14}C in the ether phase consisted of 1-naphthol; the remaining radioactivity was present in the form of a compound(s) which did not move from the origin on the thin-layer plate. Thin-layer chromatographic analysis of the ether extracts from Aspergillus, Culcitalna, Halosphaeria and Humicola cultures revealed the presence of four spots besides 1-naphthol. The Rf values of these metabolites were 0.0, 0.10, 0.31 and 0.64 in the solvent system consisting of chloroform-methanol (98:2). However, the proportion of 1-naphthol and the various metabolites in the ether extract varied from species to species. The concentrations of the ether-soluble metabolites were too small to be identified. Water-soluble metabolites

TABLE III

Distribution of ^{14}C in the culture solution of various microorganisms incubated with ^{14}C-1-naphthol

Organism	Percent of Initial ^{14}C						
	Ether-Soluble Products						Water Soluble Products [a]
	Rf-values						
	0	0.09	0.31	0.39 (1-naphthol)	0.64	Total	
Brevibacterium sp.	-	0	0	77.8	0	77.8	3.6
Flavobacterium sp.	10.6	0	0	72.2	0	76.8	4.7
Serratia marina	5.7	0	0	59.4	0	65.1	15.8
Spirrillum sp.	7.9	0	0	57.1	0	65.0	14.3
Candida parapsilosis	0	0	0	74.7	0	74.7	7.5
Rhodotorula glutinis	0	0	0	73.0	0	73.0	8.0
Trichosporon ferments	0	0	0	71.8	0	71.8	9.6
Aspergillus fumigatus [b]	2.4	2.4	6.9	0	1.1	12.8	74.8
Culcitalna achraspora [b]	4.0	2.1	3.2	0.3	3.1	12.7	70.7
Halosphaeria mediosetigera [b]	1.7	3.0	11.6	2.8	0	19.1	70.7
Humicola alopallonella [b]	0	2.1	2.8	0	0.9	5.8	90.2

[a] Corrected for non-biological conversion.

[b] These organisms were incubated with 1-naphthol for 7 days while the others were incubated for 3 days.

produced as a result of microbial action did not move from the origin on the chromatogram in the solvent systems used.

These studies have shown that in comparison to carbaryl, 1-naphthol is more susceptible to degradation by the marine microorganisms included in the study. Among the various organisms tested, filamentous fungi appear to possess a greater ability to degrade 1-naphthol than bacteria or yeasts.

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