Pakistan J. Sci. Ind. Res., Vol. 14, Nos. 4–5, August–October 1971

CARBARYL DEGRADATION BY PSEUDOMONAS PHASEOLICOLA AND ASPERGILLUS NIGER

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(Received August 10, 1970)

The hydrolytic cleavage of Carbaryl to yield 1-naphthol as a result of the detoxication activity of a bacillus, *Pseudomonas phaseolicola* and a fungus *Aspergillus niger* is indicated in the following studies. In addition, the degradation activity of the bacillus yields a minor metabolite possibly of a phenolic character. Such hydrolytic activity as described above is of significance and of methyl carbamates has been demonstrated for the first time involving soil microflora.

Metabolic pathways of the insecticide Carbaryl (1-naphthyl *N*-methylcarbamate) have been reported in mammalian tissues, their liver microsomal fractions, insects and certain higher plant species[3,5,7] The detoxication of Carbaryl yields both the hydrolysis products such as 1-naphthol as well as certain oxidation products. Boush and Matsumura reported the metabolism of carbaryl by an obligate symbiote *Pseudomonas melophthora* (Allen and Rikker) of apple maggot.[2] Production of 1-naphthol and a polar unidentified metabolite were recognized. The potential of microorganisms, in general, to metabolize organic compounds particularly when they are adapted to them has been expressed.[1,2]

A bacillus *Pseudomonas phaseolicola* (Burkholder) and a fungus *Aspergillus niger* (Van Tieghem) were used. Both were isolated from local soil and the cultures were maintained in these Laboratories. The microorganisms were incubated with the carbamate and after appropriate time the incubation mixture was subjected to extraction by chloroform. The organosoluble fraction was purified and analyzed by column and thin layer chromatography and a colorimetric procedure.

Materials and Methods

Pseudomonas phaseolicola was grown in peptone broth and was standardized by an optical density method. 100 ml of the suspension was incubated with 100 mg of Carbaryl at 37°C with constant shaking 5 ml aliquots of this mixture were drawn at 0,1, 2,6,24 and 48 hr, each aliquot extracted twice with 5 ml of chloroform, dried (Na_2SO_4) and evaporated at room temperature. The extract was analyzed as to the amount of 1-naphthol in the mixture. Each extraction was treated with 1 ml of 6% methanolic acetic acid followed by 0.5 ml of 1% methanolic *p*-nitrobenzenediazonium fluoborate. The naphtholic colour thus developed was read at the wavelength of 485 nm (adapted from Miskus *et al.*[6]). In another experiment the bacilli were homogenized by ultrasonic vibrations in MSE instrument at 1.75 amp and then exposed to Carbaryl. For chromatography purposes the bacillus culture was allowed to metabolize the chemical for 18 hr because this time provided metabolites sufficient for further clean-up process. It was then extracted with chloroform as indicated above. Because of the tissue interference, direct thin layer chromatography was not feasible. Silica gel column of 1.5×30 cm was used. The elution was performed with the following solvent system in the listed order: hexane 50 ml, 1:1 hexane–ether 100 ml, ether 50 ml and methanol 50 ml. Ten ml fractions of the eluate were collected from the column. Portions of these were subjected to 1-naphthol analysis, formaldehyde analysis[4] and thin layer chromatography. For TLC, 4:1 ether–hexane solvent system and silica gel G plates were used. In order to identify naphtholic derivatives on the chromatograms, fluoborate reagent was used after spraying the plates with 15% potassium hydroxide.[9] The chromogenic portions of silica gel adsorbent on these plates were scraped with a metallic blade and extracted separately into methanol. The methanol extracts were read in a colorimeter at the wavelength of 485 nm.

A. niger was grown in the Czapek's media and exposed to Carbaryl in distilled water. Similar procedures as above were observed. Efforts to break the spores were not successful.

Results and Discussion

In the studies involving *P. Phaseolicola*, the production of 1-naphthol increased over a period of 48 hr. At one hour of the incubation, 2 μg equivalent of Carbaryl per sample was found as 1-naphthol; at 2 hr 4.5 μg, at 6 hr 10.5 μg at 24 hr 270 μg and at 48 hr 310 μg of the 1-naphthol were produced. On column, 1-naphthol eluated mostly in the hexane fraction and was identified by IR spectra and thin layer chromatograms. Based on TLC some of the unmetabolized Carbaryl appeared in the hexane fraction and the rest in the next solvent fraction. Except for methanolic

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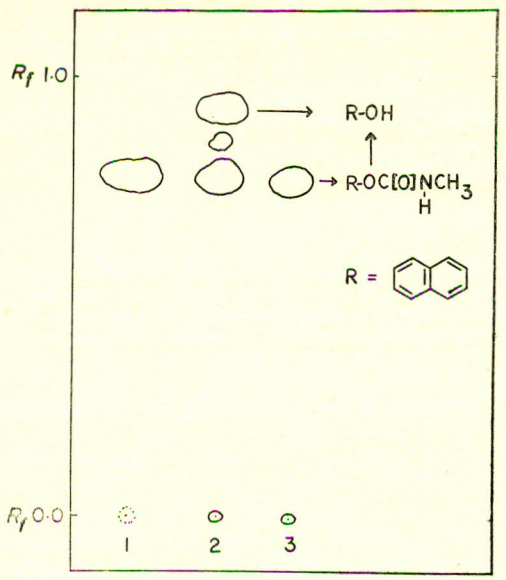

Fig.—1

fraction from the column, no other column eluates yielded 1-napthol. However, on TLC of the hexane fraction, an intermediate compound (R_f=0.86) was detected between unmetabolized Carbaryl (R_f=0.78) and 1-naphthol R_f=0.93) (Fig. 1). We did not find an indication of the minor, polar metabolite of Carbaryl reported from the studies involving bacterial symbiote of the apple maggot.[2] This may be because of the highly sensitive radioautographic techniques employed by these authors. Colorimetric estimation of the methanolic extract of the TLC chromogenic spots from the hexane fraction of the column showed the following composition, 1-naphthol 30%, intermediate metabolite 3% and unmetabolized Carbaryl about 65%. However, considering the total quantity of Carbaryl in samples drawn from the incubation mixture the maximum 1-naphthol production did not exceed 6% at the end of 48 hr. In the case of homogenized bacilli, the expected intermediate compound was not found and the production of 1-napthol was much higher than in the previous case.

In the studies involving *Aspergillus niger*, evidence of 1-naphthol production was found in column eluates. Because of interfering materials, TLC did not provide enough information and several solvent systems failed to separate the components. Some indication of the production of formaldehyde on acid hydrolysis of the chloroform extract has been reported.[8] Even though an oxidation product of Carbaryl may be present we would like to propose an artifact effect due probably to the impurity of sulphuric acid used in Zubairi's reports.

The results indicate considerable ability by two representatives of rather abundant groups of microflora to degrade an extensively used insecticidal chemical, Carbaryl. This is the first demonstration of the degradation of a methyl carbamate by soil microflora. The significance of such an activity lies in the fact that large quantities of Carbaryl are now being used on agricultural crops all over the world. Its stability in the field may well depend considerably on the activity of soil microorganisms.

Acknowledgements.—Thanks are due to Dr. A. Kamal and Dr. A.H. Khan of these Laboratories for certain facilities.

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