Metabolism of Methylcarbamate Insecticides in Soils

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The persistence and metabolism of ^{14}C-carbonyl-labeled carbaryl (1-naphthyl methylcarbamate) and 3,5-xylyl methylcarbamate were studied in five different soil types at two concentrations. Persistence was influenced by soil type and $^{14}CO_2$ evolution varied from 2.2 to 37.4% of initial radioactivity during 32 days of incubation. Hydrolysis was the main pathway of degradation, since very low concentrations of ^{14}C-carbonyl metabolites were detected. $^{14}CO_2$ evolution from ^{14}C-1,4,5,8-ring-labeled naphthol in soil was only 8.2% after 60 days. More than 70% of radioactivity was found to be linked to humic substances. Four metabolites, one of which was coumarin, were produced from ring-labeled naphthol by a soil pseudomonad.

Metabolism of methylcarbamate insecticides has been studied in a number of biological systems. The metabolic pathways of methylcarbamate insecticides in animals (Dorough, 1970), plants, and insects (Kuhr, 1970) have been reviewed. The metabolism and degradation of methylcarbamate insecticides in soil, however, have not yet been extensively investigated. Johnson and Stansbury (1965) examined the half-life of soil-incorporated carbaryl (1-naphthyl methylcarbamate) by colorimetric analysis. They reported a half-life in soil of approximately 8 days under normal conditions, at three concentrations. In every case, carbaryl appeared to be completely degraded within 40 days. Karinen *et al.* (1967) investigated the persistence of carbaryl in the marine estuarine environment and showed complete disappearance, with 43% conversion to 1-naphthol. The decline of both carbaryl and 1-naphthol was more rapid in the hydrosoil, and both compounds were adsorbed by the soil where decomposition continued at a slower rate. The fate of 1-naphthol was studied by Lamberton and Claeys (1970). Microorganisms formed CO_2 from 1-naphthol and produced numerous other products, one of which was a reddish-brown precipitate having a molecular weight of 454. Kaufman *et al.* (1970, 1971) reported that degradation of certain phenylcarbamate and acylanilide herbicides was affected by methylcarbamates in soil.

Liu and Bollag (1971) isolated three metabolites of carbaryl: 1-naphthyl *N*-hydroxymethylcarbamate; 4-hydroxy-1-naphthyl methylcarbamate; and 5-hydroxy-1-naphthyl methylcarbamate produced by the soil fungus *Gliocladium roseum*. A ring fission mechanism involving naphthalene by a soil pseudomonad has been proposed by Davies and Evans (1964). Naphthalene undergoes ring cleavage through 1,2-dihydro-1,2-dihydroxynaphthalene to 1,2-dihydroxynaphthalene to *cis*-hydroxybenzalpyruvate. Coumarin was reported to be an artifact formed from *cis*-hydroxybenzalpyruvate, the ring fission product.

The present investigation examined the rate of breakdown of carbaryl, 3,5-xylyl methylcarbamate, and 1-naphthol in five rice-producing soils from Japan.

MATERIALS AND METHODS

Methylcarbamate and Naphthol. ^{14}C-Carbonyl-labeled carbaryl, specific activity 8.9 μCi per mg, and unlabeled carbaryl, 99.85% analytical grade, were furnished by the Union Carbide Corp. ^{14}C-Carbonyl-labeled 3,5-xylyl methylcarbamate was synthesized by Tomizawa *et al.* (1971), according to the method described by Krishna *et al.* (1962). Its specific activity was 0.45 μCi per mg. Unlabeled 3,5-xylyl methylcarbamate was furnished by Hodogaya Chemical Co. Ltd., Tokyo, Japan. ^{14}C-1,4,5,8-Ring-labeled 1-naphthol was prepared from labeled naphthalene. As shown in Figure 1, 1-^{14}C-labeled naphthalene was sulfonated, followed by alkali fusion, to obtain ring-labeled naphthol which was a mixture of 1-, 4-, 5-, and 8-^{14}C-labeled 1-naphthol (specific activity, 0.017 μCi per mg). This was purified twice by tlc and cochromatographed with authentic 1-naphthol by thin-layer chromatography (tlc) (silica gel, with ethyl ether–*n*-hexane 1:1, v/v) and glc (FID, 6-ft column packed with 10% methylvinyl silicone gum rubber on diatoport S 80–100 mesh, temperature, 150°C).

Soil Properties. Five soil types were selected from typical paddy rice fields in Japan. Four of these soils were similar to those used by Chisaka and Kearney (1970) for metabolic studies on propanil. The fifth soil, designated F, is a loamy sand from Chiba. The soil properties are shown in Table I.

Soils were kept moist during storage and microbiologically active by growing oats and returning the shoots to the soils before starting the experiments.

Metabolism of Methylcarbamate Insecticides in Soils. Experiments were conducted to determine the rates of methyl carbamate degradation among soils by monitoring $^{14}CO_2$ evolution. ^{14}C-Carbonyl-labeled carbaryl and 3,5-xylyl methylcarbamate were dissolved in acetone, and added to duplicate samples (50 g) of each soil at the rate of 2 and 200 ppm on a dry weight basis. Concentrations were selected on the basis of conventional rates of application and 100 times normal rates of the granular formulation of carbaryl (2 ppm). Soils were thoroughly mixed after evaporation of the solvent, placed in biometric flasks (Bartha and Pramer, 1965), and incubated at 25°C for 32 days. Soil moisture content was adjusted to 80% of field capacity.

Ten milliliters of 0.1 *N* KOH was used as the CO_2 trapping solution in the side-arm. The solution was removed and replaced every other day. All determinations of radioactivity were performed by a Nuclear Chicago Mark I liquid scintillation counter. All measurements were corrected for background and quenching.

A 1-ml sample of CO_2 trapping solution was added to 15 ml of scintillation solution (PPO, 12 g, POPOP, 0.6 g, naphthalene, 60 g, and 2-ethoxyethanol, 200 ml, in scintillation grade 1,4-dioxane, 1000 ml), and the radioactivity was measured by liquid scintillation.

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Table I. Properties of Five Soil Types from Paddy Rice Fields in Japan

	Soils		Sand (%)	Silt (%)	Clay (%)	Organic matter, %	pH	CEC, mequiv/100 g
A[a]	Konosu (Saitama)	Clay (c)	31	29	40	5.8	5.2	18.7
C[a]	Utsunomiya (Tochigi)	Loam (l)	44	35	21	12.8	6.1	30.2
D[a]	Fukuyama (Hiroshima)	Sandy loam (sl)	52	29	19	1.5	6.3	10.0
E[a]	Chikugo (Fukuoka)	Clay loam (cl)	40	32	28	4.1	5.6	20.3
F[b]	Chiba (Chiba)	Loamy sand (ls)	83	6	11	3.3	5.5	9.8

[a] Chisaka and Kearney (1970). [b] Kobayashi (1971).

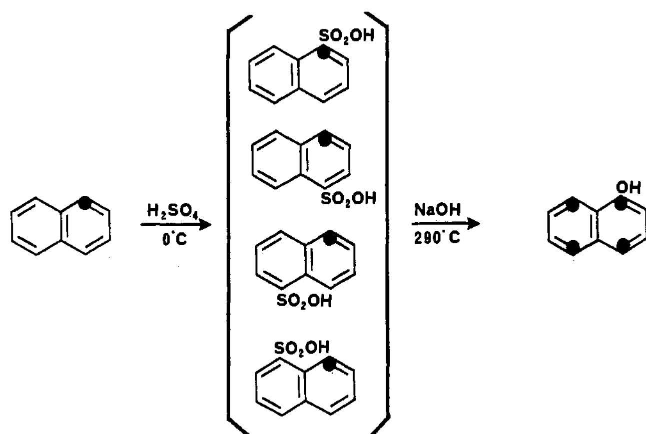

Figure 1. Synthetic pathway of ^{14}C-1,4,5,8-labeled 1-naphthol

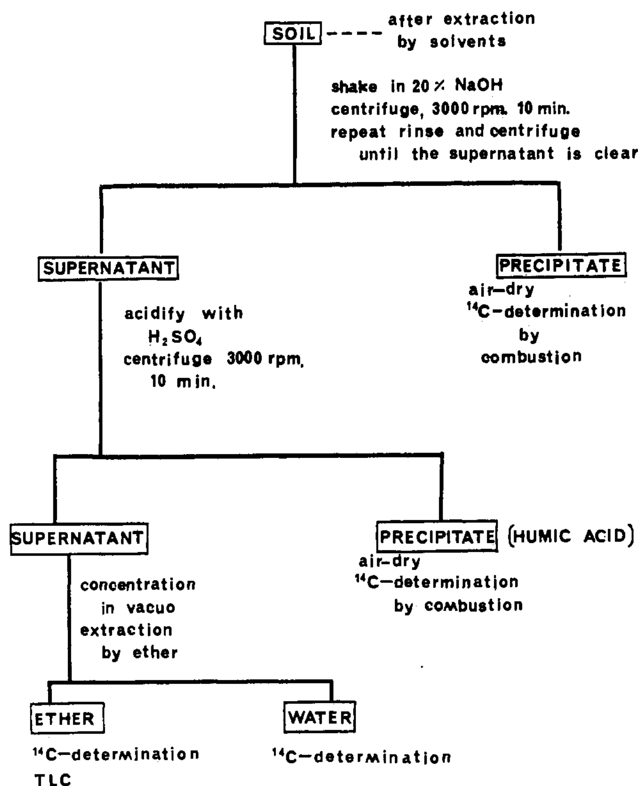

Figure 2. Alkali treatment of soil for the separation of humic acid

At the completion of the incubation period, 5 g of soil from each biometric flask was extracted with ethyl ether, followed by ethanol. Extracted radioactivity was determined by liquid scintillation.

Both ether and ethanol extracts were reduced to a suitable volume and thin-layer chromatography was performed on plates 20 × 20 cm coated with silica gel GF. Chromatograms were developed with ethyl ether and *n*-hexane (4:1, v/v). Chromatograms were exposed to no-screen X-ray film for 1 month and then developed.

Approximately 200 mg of each soil sample was combusted to determine the amount of ^{14}C in soils before and after extraction. $^{14}CO_2$ from combusted soil was trapped in 10 ml of 2-methoxyethanol and monoethanolamine (7:1, v/v), and a 5-ml aliquot of this CO_2 trapping solution was added to 10 ml of scintillation solution (PPO, 5 g, and POPOP, 0.15 g, in toluene, 1000 ml) for measuring radioactivity.

Metabolism of 1-Naphthol in Soils. The rate of $^{14}CO_2$ evolution from ^{14}C-1,4,5,8-ring-labeled 1-naphthol during a 60-day incubation period and distribution of ^{14}C after incubation were examined in soils D and E.

These soils were selected because $^{14}CO_2$ evolution from carbonyl-labeled insecticides was larger than that in the other three soils. 1-Naphthol was added in ethanol solution to provide 2 and 200 ppm in duplicate samples of soil. Monitoring methods for $^{14}CO_2$ evolution, extraction of radioactive compounds, thin-layer chromatography, and ^{14}C determination in soils were performed as above.

After solvent extraction, soils were treated with 20% NaOH solution by shaking overnight, followed by the procedures shown in Figure 2. Each fraction was assayed for radioactivity.

Metabolism of 1-Naphthol by Soil Bacterium. A *Pseudomonas* sp. isolated from Lakeland sandy loam by incubating soils in mineral salt medium containing 100 ppm of carbaryl (w/v) was used to study metabolites.

Duplicate flasks with 100 ml of culture medium containing 100 ppm of carbaryl were inoculated with the pseudomonad. Cells were harvested by centrifugation (5000 rpm, 10 min) after 7 days of incubation with shaking at 25°C, washed twice with cold phosphate buffer, resuspended in 50 ml of distilled water, and transferred into the biometric flask.

An ethanol solution of radioactive naphthol was added to a final concentration of 100 ppm. The biometric flask was set on a rotary shaker at 25°C and $^{14}CO_2$ evolution was examined every hour for 7 hr.

The inoculum was then acidified with 12 *N* HCl and extracted with ethyl ether. Radioactivity in the ether extract and water was measured as described above. Thin-layer chromatography was performed with ether–*n*-hexane (4:1, v/v) used for the first development and methylene chloride–acetonitrile (4:1, v/v) for the second.

RESULTS AND DISCUSSION

Metabolism of Methylcarbamate Insecticides in Soil. The production of $^{14}CO_2$ from carbonyl-labeled carbaryl and 3,5-xylyl methylcarbamate in soil is shown in Figure 3. $^{14}CO_2$ evolution was a function of the chemical, concentration, and soil type, varying from 2.2% (soil F, carbaryl 200 ppm) to

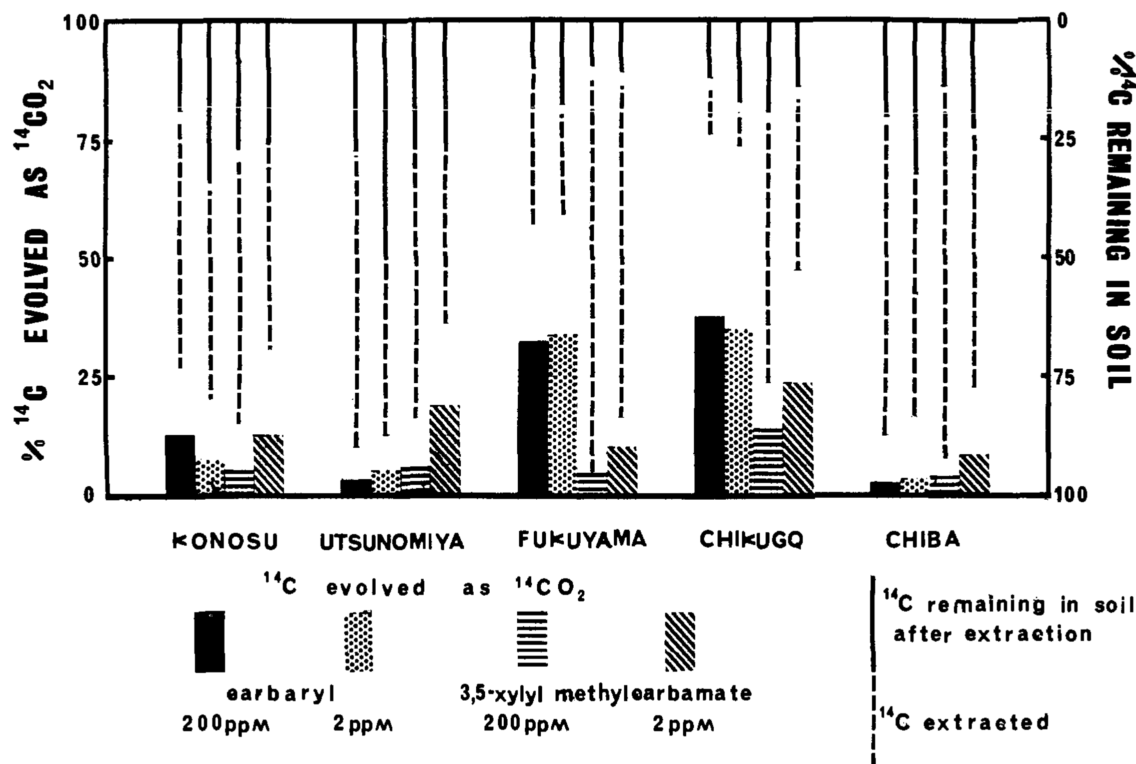

Figure 3. $^{14}CO_2$ evolution from carbonyl-^{14}C carbaryl and 3,5-xylyl methylcarbamate in soils receiving 2 and 200 ppm

37.4% (soil E, carbaryl 200 ppm). The greatest degradation was observed in Chikugo clay loam, and the least in Chiba loamy sand for both insecticides. These findings would appear to contradict the results of Johnson and Stansbury (1965), in which the half-life of soil-incorporated carbaryl was reported to be 8 days, with complete degradation within 40 days as determined by a colorimetric procedure. Differences in soil conditions exist between two experiments and may explain the differences in persistence. At both 2 and 200 ppm of methylcarbamates, the order of $^{14}CO_2$ production was E > D > A > C > F for carbaryl and E > C > A > D > F for 3,5-xylyl methylcarbamate. The degradation of 3,5-xylyl methylcarbamate was influenced by the initial concentration of the insecticide. Total recovery of ^{14}C (CO_2 + combustion) varied from 60 to 100%, and was low when the production of $^{14}CO_2$ was high.

As shown in Figure 3, a considerable amount of radioactivity still existed in soil even after the extractions with ethyl ether and ethanol. The amount of residual ^{14}C in soil was roughly proportional to soil organic matter content. Chisaka and Kearney (1970) observed that some 3,4-dichloroaniline derived from propanil in soil was bound to soil particles, which caused low recoveries. Bartha (1971) digested soil-bound ring-labeled propanil with caustic soda, followed by steam distillation, extraction of the distillate with ether, and identified 3,4-dichloroaniline in this distillate. Formation of a humus–3,4-dichloroaniline complex in soil was suggested. A similar mechanism is apparently operative with the methylcarbamate insecticides in soil. Alkali treatment was not performed in this case, lest it should split the ester linkages, and hence liberate ^{14}C as $^{14}CO_2$ simultaneously with the separation of humic acid.

Radioactivity in the solvent extracts is the result of intact methylcarbamate insecticides, since only the parent material was detected on autoradiograms of thin-layer plates. Some differences may exist in the mechanism by which carbamates

Table II. Radioactivity Extracted from Soils Receiving 2 ppm of Carbaryl by Ether and Ethanol

	% ^{14}C of initial activity			
Soil	as $^{14}CO_2$	Extracted with 50 ml of ether	Extracted with 50 ml of ethanol	In soils after extraction
A	7.6	30.8	14.8	34.7
D	33.9	15.7	7.9	17.4
E	35.2	6.4	3.2	17.2
F	3.0	21.8	29.9	32.1
C	5.0	24.5	9.7	49.0
C[a]	5.0	25.2		57.0

[a] Extracted with 100 ml of ether.

are adsorbed in soil, since a fraction of the ^{14}C-carbamates not removed with ether was extracted with ethanol, as shown in Table II. In the case of carbaryl, ether extracts of soils D, E, and F showed several faint radioactive spots other than carbaryl. These spots, which contained only 0.25% of the radioactivity, corresponded to 1-naphthyl *N*-hydroxymethylcarbamate, 4-hydroxy-1-naphthyl methylcarbamate, and 5-hydroxy-1-naphthyl methylcarbamate. Owing to the low level of radioactivity, it was impossible to confirm their identity. The main pathway of degradation in soil is probably hydrolysis of the carbamate linkage, producing CO_2 and the corresponding phenols, although it is possible that hydroxylation of the ring or the methyl carbon precedes hydrolysis.

Metabolism of 1-Naphthol in Soil. $^{14}CO_2$ evolution from ^{14}C-1,4,5,8-1-naphthol in soil is shown in Figure 4. Soil type and concentration influenced $^{14}CO_2$ evolution. 1-Naphthol decomposed more rapidly in soil E than in soil D. Most of the radioactivity (83 to 92%) was recovered in soils after 60 days of incubation. Approximately 10% of the radioactivity was extracted with ethyl ether and ethanol.

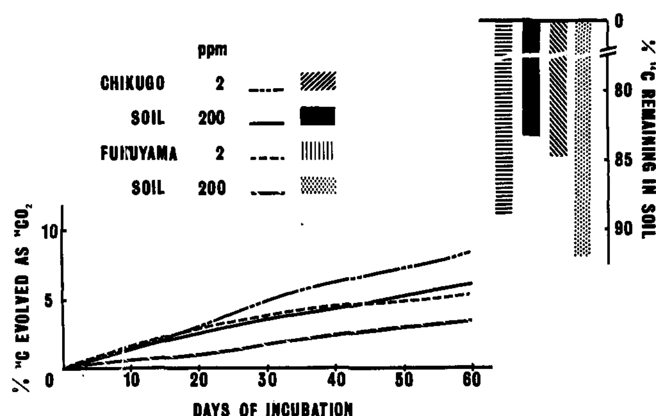

Figure 4. $^{14}CO_2$ evolution from ^{14}*C*-1,4,5,8-1-naphthol in two soils receiving 2 and 200 ppm

Table III. Radioactivity Extracted with Ether and Ethanol from Soils Receiving 2 and 200 ppm of ^{14}*C*-1,4,5,8-naphthol

Soil	Rate of treatment, ppm	Initial activity, 1000 cpm[a]	% ^{14}C of initial activity: as $^{14}CO_2$	Extracted with ether	Extracted with ethanol	In soils, after extraction
D	2	99	5.8	2.9	7.9	77.5
D	200	106	3.3	6.6	3.9	81.5
E	2	95	8.2	2.2	8.9	78.1
E	200	107	5.4	5.7	4.1	73.4

[a] cpm = count per minute.

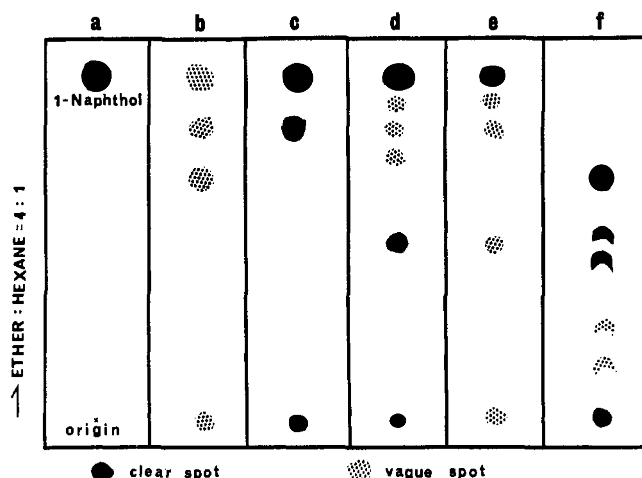

Figure 5. Distribution of radioactive spots on thin-layer chromatograms from ^{14}*C*-1,4,5,8-1-naphthol in soil E. a: authentic naphthol; b: ether extract of incubated soil (2 ppm); c: ether extract of incubated soil (200 ppm); d: ethanol extract of incubated soil (2 ppm); e: ethanol extract of incubated soil (200 ppm); f: ether extract of the supernatant after separating humic acid

As shown in Table III, the amount of radioactivity in ethanol was larger than that in ether at 2 ppm, and ether-soluble radioactivity was dominant at 200 ppm. Bartha (1971) reported that high concentrations favored the formation of solvent-extractable products and lower concentrations favored immobilization of radioactivity derived from ^{14}C-ring-labeled propanil in soil. In this experiment, no clear relationship between the initial concentrations and immobilization of 1-naphthol was observed.

Autoradiograms of solvent extracts showed several spots as unknown metabolites of 1-naphthol. The distribution

Table IV. Distribution of Radioactivity after Alkali Treatment of Soils Receiving 2 and 200 ppm of ^{14}*C*-1,4,5,8-naphthol

Soil	Rate of treatment, ppm	% ^{14}C of initial activity — Extracted by alkali treatment — In supernatant after separation of humic acid: Extracted with ether	In aqueous layer	Precipitated with humic acid by sulfuric acid	In residual soils after alkali treatment
D	2	14.4	12.0	15.0	28.0
D	200	8.7	8.9	21.8	34.9
E	2	10.7	9.6	39.3	15.1
E	200	5.6	13.9	28.0	36.2

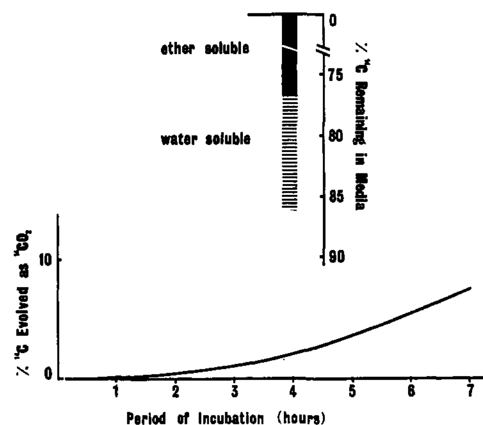

Figure 6. $^{14}CO_2$ evolution from ^{14}*C*-1,4,5,8-1-naphthol by a *Pseudomonas* and distribution of ^{14}C after solvent extraction

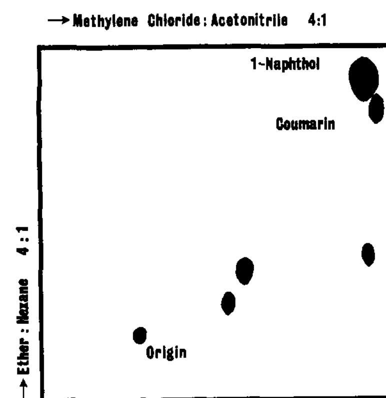

Figure 7. Distribution of radioactive spots on thin-layer chromatograms from ^{14}*C*-1,4,5,8-1-naphthol metabolites by soil pseudomonads

of radioactive metabolites on thin-layer chromatograms is shown in Figure 5. Whether these metabolites arise from ring cleavage or still have the intact naphthol ring could not be determined. No attempts were made to identify these metabolites because of the low radioactivity in these compounds.

Distribution of radioactivity after alkali treatment was shown in Table IV. Fifteen to 35% of the radioactivity remained in the residual soil fraction which was composed of soil minerals and humin, the nonextractable part of soil organic matter with alkali (Hurst and Burges, 1967). More than half of the radioactivity not extracted by organic solvents was solubilized with 20% of NaOH. Fifteen percent (soil D, 2 ppm) to 40% (soil E, 2 ppm) of the initial radioactivity was precipitated with the humic acid fraction by acidifying with sulfuric acid. No radioactivity was extracted from humic

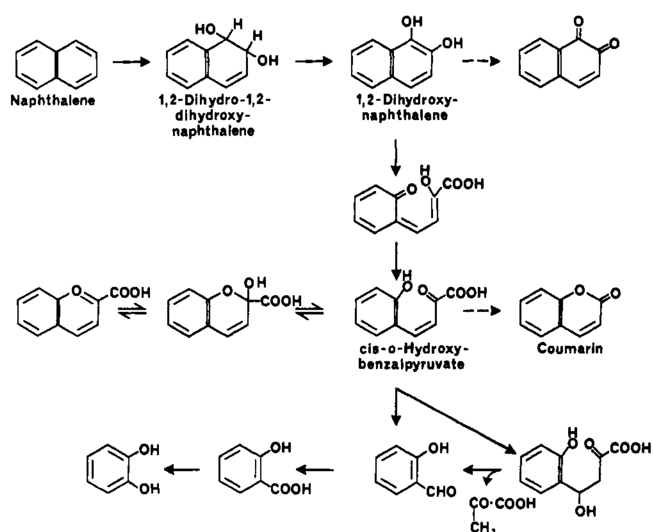

Figure 8. Metabolic pathway of naphthalene (Davies and Evans, 1964)

acid by ethyl ether. These results strongly suggest that naphthol is immobilized on humic substances in soil not by mechanical adsorption but by chemical bonding as found by Bartha (1971) with humus–3,4-dichloroaniline complex. The supernatant solution after separation of humic acid contained 17.6% (soil D, 200 ppm), 19.5% (soil E, 200 ppm), 20.3% (soil E, 2 ppm), and 26.4% (soil D, 2 ppm) of radioactivity. About half of the radioactivity in the supernatant solution was extracted with ether.

The thin-layer chromatogram of the ether extract showed no 1-naphthol (Figure 5). Unlike propanil, which is hydrolyzed to dichloroaniline by alkali, 1-naphthol is rather stable to alkali. The radioactive metabolite in the ether-soluble fraction of soil hydrolyzates appeared to arise in soil, and not by hydrolysis of 1-naphthol with alkali.

Metabolism of 1-Naphthol by Soil Bacteria. Evolution of $^{14}CO_2$ from ^{14}C-1,4,5,8-1-naphthol solution by the *Pseudomonas* sp. and distribution of radioactivity after ether extraction of the inoculum are shown in Figure 6. Only 7.4% of initial radioactivity was recovered as $^{14}CO_2$. Distribution of metabolites on thin-layer chromatograms from the ether extract is shown in Figure 7. There were four metabolites besides 1-naphthol. One metabolite was identified as coumarin by cochromatography in two solvent systems [ether–*n*-hexane (4:1) and chloroform–acetonitrile–cyclohexane (8:1:1)]. The concentrations of the other three metabolites were too small to be identified. Water-soluble metabolites did not move from the origin on thin-layer plate with either of these solvent systems.

Metabolic pathways for the hydroxylation and subsequent ring opening of naphthalene have been proposed by Davies and Evans (1964), as shown in Figure 8. According to this pathway, naphthalene is oxidized to 1,2-dihydroxynaphthalene through 1,2-dihydro-1,2-dihydroxynaphthalene before ring cleavage.

It is assumed similar pathways are operative in the *Pseudomonas* sp. isolated from soil, based primarily on the isolation of coumarin. Coumarin is not regarded as an intermediate in the degradative pathway of 1-naphthol, but an artifact resulting from oxidative decarboxylation and cyclization of the *cis-o*-hydroxylbenzolpyruvate. The latter intermediate is a known metabolite in the naphthol degradation pathway. It is interesting to note that several of the other labeled metabolites detected by tlc could not be cochromatographed with several of the authentic intermediates shown in Figure 8.

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