Bacterial Metabolism of 1-Naphthol

Jean-Marc Bollag,* Edward J. Czaplicki,[1] and Robert D. Minard

Bacterial isolates from river water metabolized 1-naphthol by different pathways. After 60 hr of incubation with 1-naphthol-*1*-^{14}C, 44% of the radiolabeled material was trapped as $^{14}CO_2$, 17% remained in the growth medium, and 22% was recovered in the bacteria. The medium in which the bacteria were grown was extracted with diethyl ether and analyzed. The main product of the ether extract was isolated by thin-layer chromatography and identified by infrared, nuclear magnetic resonance, and mass spectroscopy as 4-hydroxy-1-tetralone [4-hydroxy-3,4-dihydro-(2*H*)-naphthalenone (1)]. The formation of this product and its metabolic implications for a probable pathway are discussed.

The fate in the environment of 1-naphthol, which is formed by chemical as well as biological hydrolysis of the insecticide carbaryl or other synthetic compounds and constitutes a major hydroxylation product of naphthalene, has been elucidated to a very limited extent. This fact has to cause concern since 1-naphthol appears to be a product which can be more toxic to organisms in various ecosystems than the compound from which it originates. Stewart *et al.* (1967) reported that 1-naphthol in sea water was twice as toxic to fish at a concentration of 1.3 μg/ml than carbaryl, and Bollag and Liu (1971) found that various soil microorganisms are inhibited in their growth with lesser amounts of 1-naphthol than carbaryl. However, in studies with human cells in tissue culture it was found that 1-naphthol showed toxicity but to a lesser degree than its parent compound carbaryl (Litterst and Lichtenstein, 1971).

Lamberton and Claeys (1970) investigated the fate of 1-naphthol in a simulated marine estuarine environment. They found that microorganisms produced CO_2 and other products from 1-naphthol, and its degradation in the alkaline environment of sea water is simultaneously affected by light and oxygen. One product generated from 1-naphthol formed a reddish-blue precipitate with a molecular weight of 454 amu, but its identity could not be determined. Bollag and Liu (1972) found that the soil fungus *Fusarium solani* metabolized 1-naphthol. While 1-naphthol disappeared only partially during the fungal growth, a prepared cell extract degraded it completely and more than 80% of the substrate was converted to CO_2. The active enzymatic system involved seems to be constitutive inasmuch as 1-naphthol was metabolized with a cell extract from cells which were not cultured on the inducing substrate. Kazano *et al.* (1972) isolated a *Pseudomonas* sp. from soil and observed that during a cell suspension experiment 7.4% of the initial radioactivity from 1-naphthol-*1,4,5,8*-^{14}C was recovered as $^{14}CO_2$ and three metabolites were separated by thin-layer chromatography, but not identified.

The purpose of the present study was to investigate the metabolism of 1-naphthol as caused by bacteria and to establish the probable pathways.

MATERIALS AND METHODS

Microorganisms capable of degrading 1-naphthol were isolated from river water. Water samples which had a pH of 8.45 were taken from Spring Creek near State College, Pa., and were stored at 2°. Erlenmeyer flasks (125 ml) containing 50 ml of river water were supplemented with 1-naphthol which was added in 0.25 ml of ethanol after membrane filtration (0.22-μ pore size Millipore filter) for a final concentration of 20 ppm. Autoclaved river water was used as a control. The samples were incubated on a rotary shaker (150 oscillations/min) at 25° for 2–4 weeks. Decomposition of 1-naphthol during incubation was followed by colorimetric analysis (Miskus *et al.*, 1959) and thin-layer chromatography as previously described (Liu and Bollag, 1971). An aliquot from the flasks in which 1-naphthol disappeared rapidly was transferred into other flasks which contained autoclaved river water and 20 ppm of 1-naphthol. After additional transfers the incubated river water was streaked on nutrient agar (Difco) and single colonies were selected and further purified using the dilution plate method.

The bacteria which were very active in metabolizing 1-naphthol were chosen for this study. Attempts to identify the bacteria were not successful, but the following morphological and physiological characteristics were established: *bacterium A:* Gram negative, aerobic rod; no flagella as indicated by electron microscopy; glucose was not fermented, but growth on sucrose, lactose, and maltose caused an alkaline shift in the test medium; *bacterium B:* Gram negative, aerobic rod; one to four polar flagella as seen by electron microscopy, motile, glucose was not fermented, but growth on sucrose, lactose, and maltose caused an alkaline shift in the test medium.

The isolated bacteria were grown in a basal salt medium containing K_2HPO_4 (1.6 g), KH_2PO_4 (0.4 g), NH_4NO_3 (0.5 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), $CaCl_2 \cdot 2H_2O$ (0.025 g), and $FeCl_3 \cdot 6H_2O$ (0.0025 g) in 1 l. of distilled water (final pH 7.4). Unlabeled 1-naphthol—at concentrations as indicated under Results—and 1-naphthol-*1*-^{14}C, specific activity 10 mCi/mmol (International Chemical and Nuclear Corp., Calif.), were added—if not otherwise specified—in an ethanol solution after membrane filtration, resulting in a final ethanol concentration in the growth medium of 0.5%. Most cultures were grown in 125-ml erlenmeyer flasks containing 50 ml of media and they were incubated under conditions as previously described. Growth was measured turbidimetrically at 550 nm. At least two replicates were included in every test, and each experiment was repeated two or three times.

For routine thin-layer chromatography analysis, 5 ml of the growth medium from which the cells were removed by centrifugation was extracted with an equal volume of diethyl ether. Subsequently, the extract was concentrated under a gentle stream of air in a water bath at about 40° to a small volume which was applied on the thin-layer plate. Precoated tlc plates (Brinkmann Instruments Inc., Westbury, N.Y.) with a thickness of 0.25 and 0.50 mm of silica gel F-254 were used for the routine tlc analyses and for the preparative separations, respectively. Ether–hex-

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Table I. Transformation of 1-Naphthol (20 ppm) by Bacteria A and B Grown in Basal Salts Medium

	Time of incubation											
	0 days			2 days			3 days			5 days		
	Total	Ether phase	Aq phase	Total	Ether phase	Aq phase	Total	Ether phase	Aq phase	Total	Ether phase	Aq phase
Control (noninoculated)	6710[a]	6540	50	6290	6240	80	6230	6030	100	6250	6160	140
Bacterium A	6740	6710	60	1110	410	770	1150	370	650	1170	370	790
Bacterium B	6620	6600	50	1100	400	720	1150	350	720	1140	320	750

[a] Radioactivity expressed as disintegrations per minute per milliliter of medium.

ane (4:1, v/v) was used as a solvent system for separation of the metabolites from the ether extract of the growth medium. All compounds were made visible with ultraviolet light at 254 nm. Phenol type compounds were detected by spraying the plate with a reagent composed of 50 mg of *p*-nitrobenzenediazonium fluoborate dissolved in 20 ml of methanol and 20 ml of acetic acid or with the ferric chloride test. 2,4-Dinitrophenylhydrazine was used for detection of ketones and the Jones reagent (chromic acid in acetone) served as an indicator of alcohols (Pasto and Johnson, 1969).

Radioactive zones on thin-layer plates were detected by autoradiography using an X-ray film (Kodak RD Royal X-Omat, Rochester, N.Y.). Radioactive samples were measured in a modified Bray solution composed of 8 g of Omnifluor (New England Nuclear Corp., Boston, Mass.), 60 g of naphthalene, and 100 ml of methanol in 1 l. of dioxane. Radioactive compounds from tlc were counted after scraping the entire spot in the scintillation solution, but Cab-O-Sil (Thixotropic suspension powder, Cabot Corp., Boston, Mass.) at a concentration of 6% was added. Radioactivity was determined with a Nuclear-Chicago Isocap-300 liquid scintillation counter.

Breakdown products from 1-naphthol were isolated from the centrifuged media by dual extraction with equal volumes of ether. The remaining aqueous phase was acidified with 10 *N* H_2SO_4 and again extracted twice with ether. The combined ether extracts were then evaporated to a small volume using a flask evaporator at 40° and separated on a preparative thin-layer plate. For further purification, the compound being isolated was removed from the plate and separated by tlc wth the following solvent systems: benzene–dioxane–acetic acid (180:50:8, v/v), methylene chloride–acetonitrile (4:1, v/v), and ether–hexane (4:1, v/v).

Carbon dioxide evolved from the growth medium was trapped by passing the air under slight pressure through a 1 *N* NaOH solution. To measure the trapped $^{14}CO_2$, aliquots of the NaOH solution were analyzed in Bray solution supplied with Cab-O-Sil.

Radioactivity in bacteria was determined after digestion of the cells, which were previously washed several times, in NCS tissue solubilizer (Amersham/Searle, Arlington Heights, Ill.).

Ultraviolet spectra were obtained with a Bausch and Lomb Spectronic 505 spectrometer. Infrared spectra were taken with a Model 621 Perkin-Elmer spectrometer using a KBr disk technique.

Mass spectra were determined using an AEI MS-902 mass spectrometer at an ionization potential of 70 eV using the direct insertion probe. Fourier transform nmr spectra were taken at *ca.* 35° on a JEOL PS-100-FT high-resolution spectrometer coupled with a Nicolet Model 1080E data acquisition system. A repetition time of 8 sec and 8K points were collected over a sweep width of 1000 cycles.

RESULTS

The initial indication for disappearance of 1-naphthol from river water was obtained by using a colorimetric method. After 4 weeks incubation with 20 ppm of 1-naphthol a slight turbidity in the river water was observed and approximately 60% of the added chemical disappeared. Simultaneously a decrease of almost 30% of 1-naphthol in an autoclaved sample was found which indicates that nonbiological degradation also occurred after extended incubation under the experimental conditions.

The two bacteria capable of metabolizing 1-naphthol were isolated from river water using an enrichment culture technique with 1-naphthol as substrate. In a culture medium containing 1-naphthol at a concentration of 25, 50, or 100 ppm, growth of the isolated bacteria was observed as indicated by turbidity between 1 to 2, 2 to 3, and 5 to 7 days of incubation, respectively. Concentration higher than 100 ppm completely inhibited the growth of bacteria A and B.

When 1-naphthol was introduced in a basal salt medium—without the addition of ethanol—it was determined that growth of bacteria A and B occurred which demonstrates that 1-naphthol can serve as the only carbon and energy source. After 5 days incubation of the two bacteria in a medium containing ^{14}C-labeled and nonradioactive 1-naphthol at a total concentration of 20 ppm, approximately 17% of the total radioactivity remained in the medium (Table I). There was a rapid decrease of the total radioactivity within 48 hr of incubation, but no further change occurred thereafter. The remaining radioactivity was partitioned by ether extraction into an organic and an aqueous phase at a ratio of approximately 35% to 65%, respectively. The ether extract was analyzed by tlc and the distribution of the radioactive compounds was followed, but no attempt was made to characterize or identify the composition of the products in the aqueous phase. In the uninoculated control samples there was a slight decrease in total radioactivity which was probably due to volatilization or chemical oxidation of 1-naphthol, but tlc of the ether extract and subsequent autoradiography did not show another spot than the one resulting from authentic 1-naphthol-*1*-^{14}C.

Since the two isolated bacteria appeared to metabolize 1-naphthol in a similar way, only bacterium B was selected for further studies. In a growth experiment it was observed that with the decrease of radioactivity from the culture medium, it was possible to trap a considerable amount of ^{14}C-labeled carbon dioxide (Figure 1). The most intensive loss of radioactivity and formation of $^{14}CO_2$ were noticed between 22 and 33 hr after inoculation, but the major growth rate of the bacteria occurred after this time period as indicated by increasing turbidity. Despite the intensive growth after 40 hr of incubation, the remaining radioactivity in the growth medium accounting for 15–20% of the original labeled substrate was not changed and the formation of labeled CO_2 ceased.

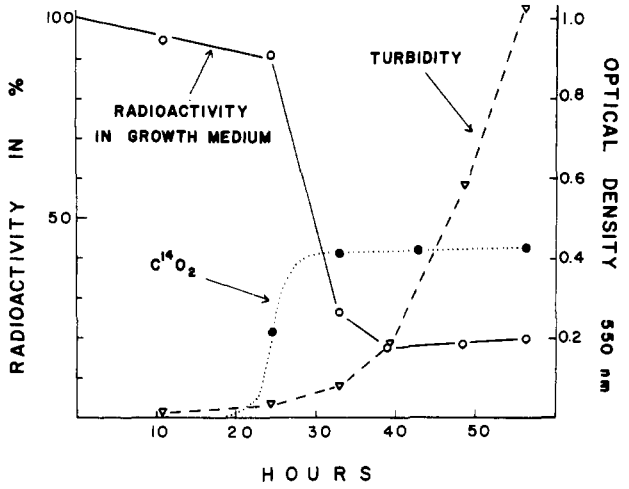


Figure 1. Decrease of radioactivity and trapping of $^{14}CO_2$ during growth of bacterium B in a growth medium containing 20 ppm of 1-naphthol.

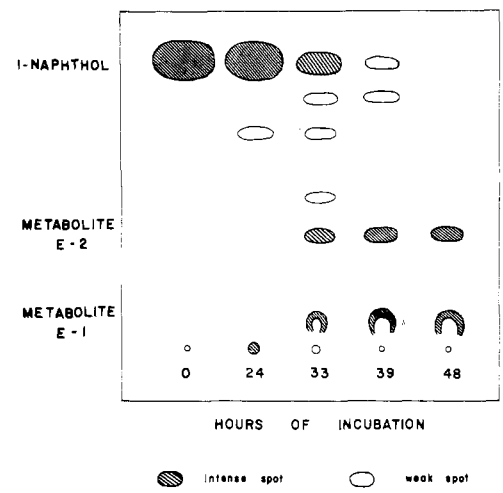


Figure 2. Tlc; autoradiogram of ether extract from bacterial growth medium containing 1-naphthol-1-^{14}C. Samples were taken after time intervals designated.

Table II. Distribution of Radioactivity (%) after 60 hr of Incubation of Bacterium B in the Growth Medium Containing 20 ppm of 1-Naphthol

	Growth medium	Ether phase	Aq phase	Trapped $^{14}CO_2$	In bacteria	Total recovery
Bacterium B	16.5	5.5	11.0	43.5	22.0	82.0
Noninoculated control	100.0	97.5	3.0	0		100.5

The distribution of the radioactivity after 60 hr of incubation is depicted in Table II. Forty-three per cent of the radioactivity was trapped as $^{14}CO_2$, 22% was contained in the bacterial cells, and approximately 17% of the initial radioactivity was still in the growth medium.

When the bacterial growth medium was extracted with ethyl ether after different time intervals and analyzed by tlc, autoradiograms of the thin-layer plates revealed the production of various metabolites (Figure 2). After 2 days of incubation no radioactivity was found in the R_f area of 1-naphthol. Concurrent with the decrease of the original substrate, the formation of two major metabolites, E-1 (R_f 0.08) and E-2 (R_f 0.33), was observed which accumulated during growth and reached a maximum level after 48 hr. These two metabolites accounted for approximately 33% of the radioactivity remaining in the growth medium; metabolite E-1 consisted of approximately 10–15% and metabolite E-2 of 60–65% of the radioactivity in the ether phase. A small amount of radioactivity was detected at the origin of the thin-layer plate. Between 24 and 39 hr of incubation, weak spots of other labeled compounds were detected, but they disappeared after 48 hr. These labeled metabolites as well as 1-naphthol gave a positive reaction (purple color) in a test with *p*-nitrobenzenediazonium fluoborate. Metabolites E-1 and E-2 did not react with the fluoborate reagent indicating that they do not have a phenol group.

Metabolites E-1 and E-2 apparently were not further metabolized and attempts were made to isolate and purify these compounds by preparative tlc using different solvent systems. The two isolated metabolites gave a negative reaction when tested with the $FeCl_3$ reagent which is usually characteristic of phenols. The 2,4-dinitrophenylhydrazine test, which indicates aldehydes and ketones, was positive in as far as an orange-red precipitate was obtained in a reaction with the two metabolites.

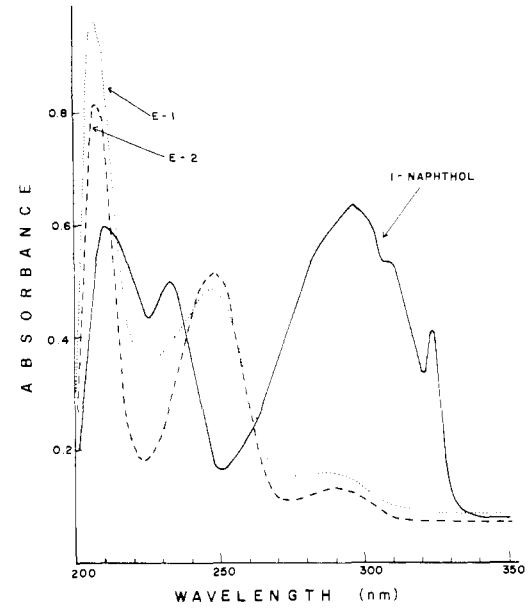


Figure 3. Ultraviolet absorption spectra of 1-naphthol, metabolite E-1, and metabolite E-2.

Ultraviolet absorption spectra of the two metabolites in methanol were essentially the same with three major peaks at 207, 247, and 285 nm for E-1 and 207, 247, and 289 nm for E-2, respectively (Figure 3). Both spectra differed clearly from that of 1-naphthol. It is noteworthy that the uv spectrum of α-tetralone (1-tetralone) with three major peaks at 206, 249, and 292 nm is very similar to the spectra of E-1 and E-2.

High-resolution mass spectra of the isolated metabolites at 200° showed compounds with a molecular weight of 160 for metabolite E-1 and 162 for E-2. The measured masses of the parent ions were consistent with the proposed formulas: metabolite E-1, measd 160.0533, parent ion $C_{10}H_8O_2$, calcd 160.0523; metabolite E-2, measd 162.0711, parent ion $C_{10}H_{10}O_2$, calcd 162.0680.

Only a small amount of metabolite E-1 was obtained in crystalline form, and, therefore, no further tests were performed with it, but metabolite E-2, a slightly yellowish oil, soluble in ethyl ether, benzene, and chloroform, was further analyzed.

Infrared spectroscopy of metabolite E-2 prepared from a

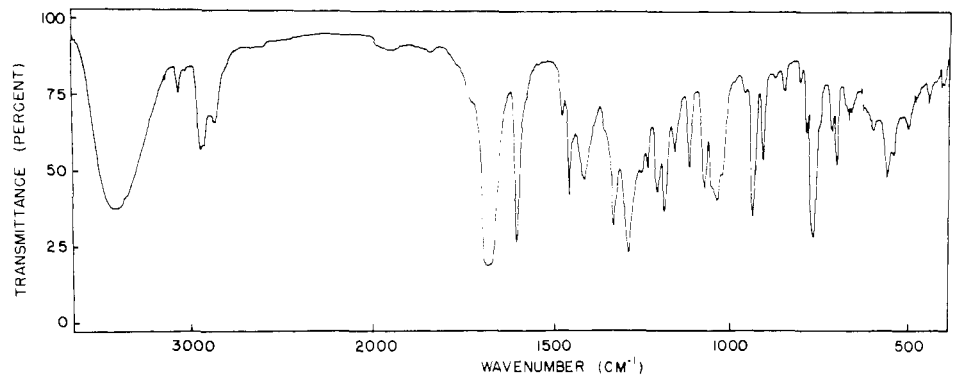


Figure 4. Infrared spectra of metabolite E-2 (4-hydroxy-1-tetralone).

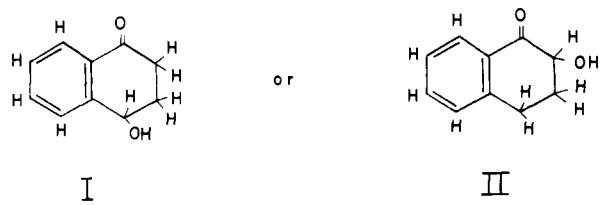
Table III. Mass Measurements of Selected Prominent Fragments of Metabolite E-2

Measd *m/e*	Intensity, %	Possible fragment	Calcd *m/e*
162.0711	58	$C_{10}H_{10}O_2$	162.0680
160.0505	7.0	$C_{10}H_8O_2$	160.0523
145.0653	9.4	$C_{10}H_9O_1$	145.0651
144.0597	58	$C_{10}H_8O_1$	144.0575
134.0755	84	$C_9H_{10}O_1$	134.0731
134.0371		$C_8H_6O_2$	134.0376
117.0726	8.9	C_9H_9	117.0704
116.0616	16	C_9H_8	116.0625
115.0538	42	C_9H_7	115.0547
105.0357	100	C_7H_5O	105.0340
104.0280	7.4	C_7H_4O	104.0261
77.0396	42	C_6H_5	77.0391
76.0307	9.5	C_6H_4	76.0312
51.0235	17.9	C_4H_3	51.0234
50.0157	11.6	C_4H_2	50.0156

film on a KBr pellet showed bands of particular interest at 3400, 2900, 1680, and 770 cm^{-1} (Figure 4). An out of the plane C-H band for four adjacent aromatic hydrogens at 770 cm^{-1} and the absence of a three adjacent aromatic C-H bend band (780–810 cm^{-1}) indicate one ring of 1-naphthol was intact while the other ring had undergone substitution. The intense carbonyl stretch at 1685 cm^{-1} is characteristic of an aromatic ketone.

The C-H stretching at frequencies lower than 3000 cm^{-1} indicated the presence of methylenic hydrogens. The intense, broad band near 3400 cm^{-1} is due to hydrogen bonded OH stretch. The lack of a free (monomeric), weak, but sharp band in the region 3640–3610 cm^{-1} is probably due to overtones of a carbonyl peak also appearing in the OH stretching region, as well as to a medium intensity broad carbonyl band at *ca.* 3430 cm^{-1} (Nakanishi, 1962). Such a strong, broad absorption is the only one observed as a polymeric intermolecular hydrogen bond.

Special data (ir, uv, mass) are consistent with either structure I or II for metabolite E-2. However, careful in-



I II

terpretation of these data coupled with results from oxidative studies of metabolite E-2 tend to favor structure I.

High-resolution mass spectra of metabolite E-2 revealed, in addition to a relatively strong parent ion at *m/e* 162, prominent fragments as presented in Table III. The measured masses of these fragments are consistent with the proposed fragmentation pattern of a compound with structure I (Figure 5).

The ion at *m/e* 134 ($C_8H_6O_2$) presumably arises *via* a retrograde Diels–Alder mechanism and implies a 1,4 relation between the carbonyl and the hydroxyl. The prominent ion *m/e* 105 is a characteristic fragment of aromatic ketones and usually accounts for their base peak. Loss of CO from this fragment results in the "phenyl" ion *m/e* 77. Bowie *et al.* (1965) studying mass spectra of 1,4-naphthoquinones found that ion *m/e* 104 was a prominent peak in the mass fragmentation pattern. In the case of metabolite E-2, the ion can result from *m/e* 160 ion by loss of a C_3H_4O unit (Figure 5).

In the nmr data, one finds four absorption areas (Figure 6). The four methylenic hydrogens and the hydroxyl proton produce the complex multiplet between 1.81 and 2.75. If the hydroxyl would be in the 2 position, its geminal proton would be shifted downfield due to the combined effects of the ketone and the hydroxyl. It could also be split into at least four peaks by the adjacent secondary protons and the hydroxyl proton. Therefore, the peaks centered around 4.67 could be attributed to this proton. However, based on the nmr data for α-tetralone (Jackman and Sternhell, 1969), the pair of benzylic protons present in this structure (II) could be expected to appear no further upfield than 2.92 ppm, especially with the additional deshielding by the hydroxyl. No absorption was apparent in this region in the nmr spectra of E-2.

With the hydroxyl group in the 4 position, the single benzylic hydrogen should be shifted downfield and, in fact, the absorption exhibited by E-2 is quite consistent with the benzylic hydrogen chemical shifts of other benzylic alcohols (1-tetralol, δ 4.49, and α-methylbenzylic alcohol, δ 4.65). The complex multiplet in the region of δ 1.81 and 2.75 for four methylenic hydrogens is also consistent with the nmr spectrum of α-tetralone.

Additional evidence that the hydroxyl group is in the 4 position was obtained by oxidizing metabolite E-2 with chromic acid in acetone (Jones reagent) to the corresponding quinone. If the OH group is attached at position 2 or 4 to carbonyl of the saturated ring, 1,2- or 1,4-dioxotetralin should be present after oxidation, and if further oxidation occurs the formation of 1,2- or 1,4-naphthoquinone would be anticipated. When metabolite E-2 was oxidized and subsequently analyzed by tlc, a minor spot with an R_f value identical with that of authentic 1,4-naphthoquinone and a major spot with a slightly lower R_f value were found. The uv spectrum of the minor spot corresponded to that of 1,4-naphthoquinone.

Therefore, based on all of the spectrometric and other analyses, it is proposed that metabolite E-2 is 4-hydroxy-

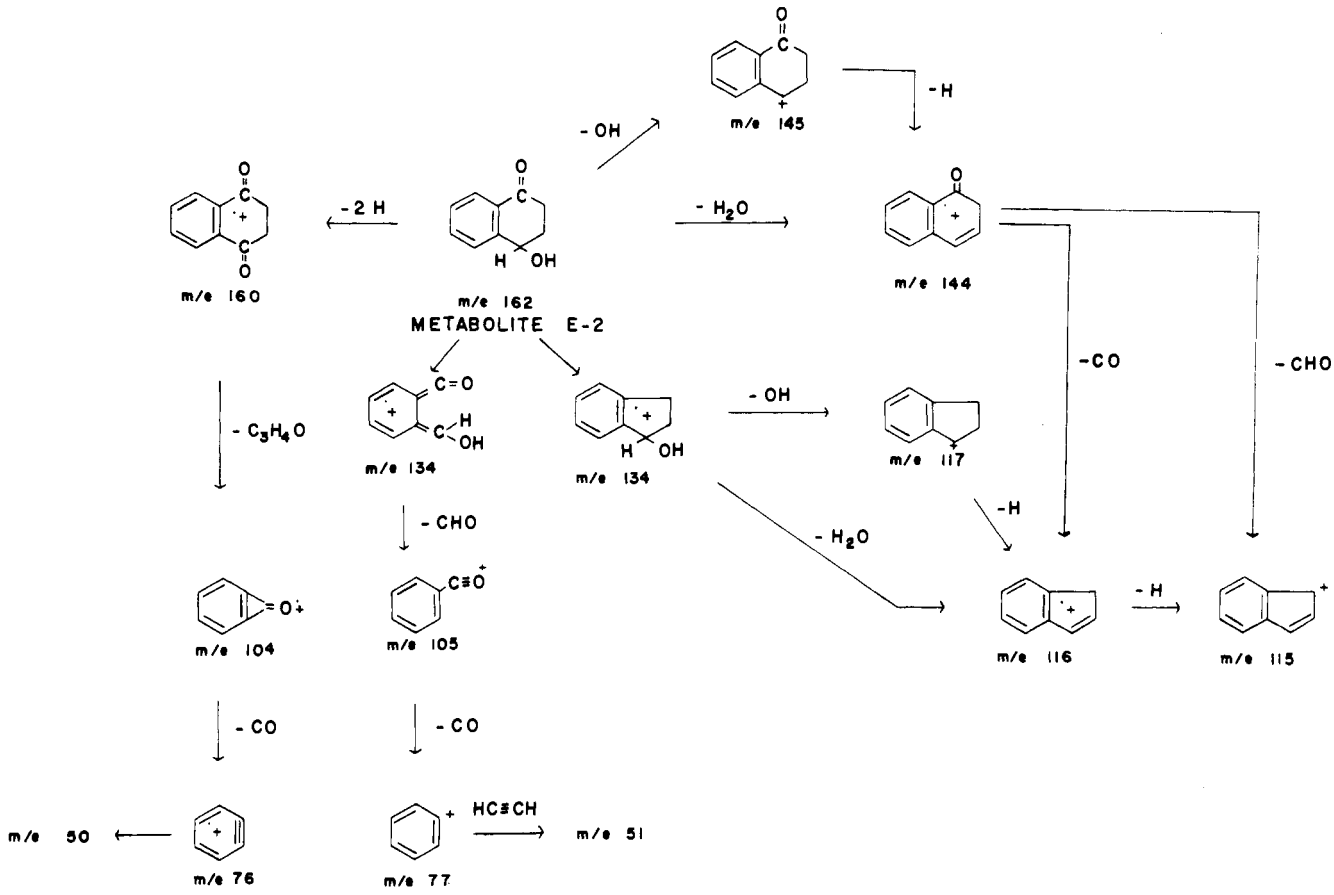


Figure 5. Prominent mass spectral ions and proposed structures arising from the fragmentation of metabolite E-2 (4-hydroxy-1-tetralone).

1-tetralone (synonyms: 4-hydroxy-3,4-dihydrol-(2*H*)-naphthalenone (1); 4-hydroxy-$\Delta^{2,3}$-benzocyclohexanone).

DISCUSSION

Although several studies on the metabolism of 1-naphthol have been conducted, no thorough investigation has been performed to determine the specific pathway of this compound by isolating intermediates or products. Since the metabolic pathway of naphthalene was elaborated with soil bacteria, and the formation of 1,2-dihydro-1,2-dihydroxynaphthalene (Walker and Wiltshire, 1953; Jerina *et al.*, 1971) and 1,2-dihydroxynaphthalene (Fernley and Evans, 1958; Davies and Evans, 1964) could be determined as intermediates prior to ring cleavage, it is often assumed that a similar pathway could also explain the biological transformation of 1-naphthol. However, various researchers investigating the metabolism of naphthalene by soil microorganisms concluded that 1-naphthol is not an intermediate in the breakdown of naphthalene (Tausson, 1927; Murphy and Stone, 1955; Walker and Wiltshire, 1953). Treccani *et al.* (1954) established that washed cells of soil bacteria grown on naphthalene could oxidize 1- and 2-naphthol, but they remained undecided if these compounds can be considered as intermediates in the metabolism of naphthalene.

Recent concern and speculation on the long-range toxic effect of aromatic drugs or their intermediates, especially of arene oxides of polycyclic aromatic hydrocarbons, in relation to carcinogenic activity stimulated intensive work on the metabolism of naphthalene by liver microsomal enzymes (Daly *et al.*, 1972). In studies with the enzymes catalyzing the hydroxylation of an aromatic ring, it was established that naphthalene is converted to naphthalene 1,2-oxide which then can spontaneously isomerize to 1-naphthol (Jerina *et al.*, 1969). The formation of 1-naphthol during bacterial oxidation of naphthalene can also be anticipated, but to our knowledge it has not been shown and no detailed studies have been reported on the further metabolism of 1-naphthol.

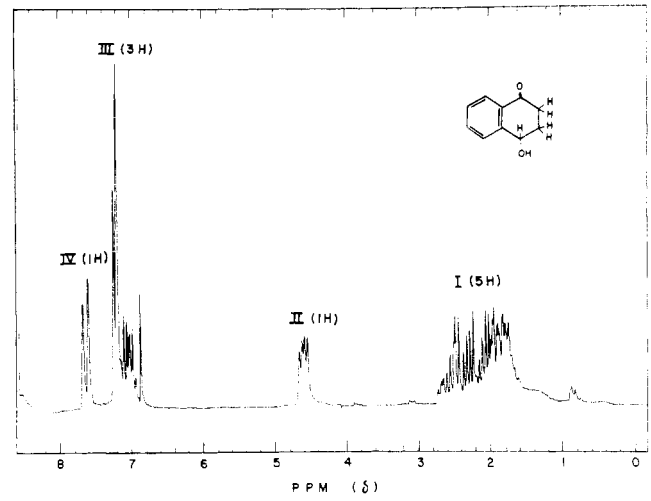


Figure 6. Nmr spectra of metabolite E-2 (4-hydroxy-1-tetralone). The sample was run in deuteriochloroform and chemical shifts are relative to tetramethylsilane. The small amount of undeuterated chloroform present has a peak at δ 7.27 which was in the middle of absorption area III. Its area, corresponding to 1000, was subtracted from area III in order to calculate the number of hydrogens.

The present study provided evidence that 1-naphthol is metabolized by bacteria isolated from river water. Complete degradation of the chemical was clearly indicated by the release of ^{14}C-labeled CO_2 which accounted for almost

50% of the substrate. This is an implicit proof of the rupture of the naphthyl ring. However, it was also found that 15–20% of 1-naphthol which was transformed remained in the growth medium, apparently without further change. This appears to indicate that at least two different pathways are involved in the degradation of 1-naphthol by the bacteria under investigation.

It could be assumed that the 1-naphthol which is completely biodegraded and results in CO_2 formation may be metabolized by the pathway proposed by Davies and Evans (1964), *i.e.*, ortho hydroxylation prior to ring cleavage. The isolation and identification of 4-hydroxy-1-tetralone suggest that at least one alternate pathway involves hydroxylation of the naphthyl ring in the 4 position, and the conversion of an aromatic ring to an aliphatic cyclic compound.

Reduction of the aromatic ring was found in the metabolism of benzoic acid by *Rhodopseudomonas palustris* (Guyer and Hegeman, 1969; Dutton and Evans, 1969), but in this case metabolic activity was only observed anaerobically in the light, and this pathway differs considerably from the known oxidative mechanisms of aromatic ring dissimilation by aerobic microorganisms.

In addition to 4-hydroxy-1-tetralone, it was possible to isolate several other metabolites which are presently under investigation for identification. When the identity of these compounds has been established, it should be possible to suggest the actual pathway and to evaluate its specific features.

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Residues of Leptophos and Its Metabolites following Application to Various Crop Plants

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Leptophos was applied to 13 crops in the early stage of growth and to crucifers in a weekly schedule. Samples were collected at harvest and analyzed for leptophos. Residue levels in beans, cabbage, cauliflower, celery, corn, onions, pea pods, and rutabagas were found to be less than 0.1 ppm where the insecticide had been applied in the early stage of plant growth; higher levels were found in broccoli, lettuce, and carrots; pea vines were found to contain the highest residues at harvest. Where multiple applications of leptophos were made to cabbage, cauliflower, and broccoli on a 7-day schedule, residues at 2 days after the final application ranged from 1.9 to 4.6 ppm. Residues were highest on broccoli and were still present in excess of 1 ppm after 13 days. Residue consisted predominantly of the parent compound with leptophos oxon and 4-bromo-2,5-dichlorophenol accounting for only a small proportion of the total residue recovered.

The insecticide leptophos [*O*-(4-bromo-2,5-dichlorophenyl)-*O*-methyl phosphorothioate] shows promise for control of cutworms and other lepidopterous plant feeders on a number of crop plants. Residues remaining on harvested crops have not been reported except for application to wheat where no residue was found in wheat kernels, although residues of both the O analog and the parent compound remained on straw 63 days after application (Struble and McDonald, 1973). To extend our information on residues, tests were conducted to determine the extent of residues on selected crops if this insecticide were used at the stage of plant growth when cutworms are troublesome in Ontario. In addition leptophos was applied to cabbage, cauliflower, and broccoli in a weekly schedule as required to control the cabbage looper, *Tricloplusia ni* (Hübner), and the imported cabbageworm, *Pieris rapae* (Linnaeus).

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MATERIALS AND METHODS

Two formulations of leptophos, 2.7 lb/gal of emulsifia-