Epoxidation of Aldrin to exo-Dieldrin by Soil Bacteria

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Twenty-two strains of soil bacteria, including representatives of the genera Bacillus, Micromonaspora, Mycobacterium, Nocardia, Streptomycyes, Thermoactinomyces, and Pseudomonas and 10 unidentified gram-negative, motile, rod-shaped bacteria, were shown to degrade aldrin to its epoxide dieldrin. In every case, the exo-stereoisomer of dieldrin was produced exclusively.

The epoxidation of aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo,exo-05,8-dimethanonaphthalene) to dieldrin was among the earliest pesticide conversions described. The first indications that dieldrin could be formed from aldrin by the activity of soil microorganisms were published by Lichtenstein and Schulz (8). Subsequently, the conversion to dieldrin by fungi was demonstrated with pure cultures of two Aspergillus species and three Penicillium species (7). Direct evidence for the metabolism of aldrin to dieldrin by isolated soil bacteria and fungi and a description of the specific microorganisms responsible were published by Tu et al. (14), who found the capability to epoxidize aldrin to be distributed among a number of genera.

It has long been recognized that two stereoisomeric epoxides of aldrin are possible: the exo-epoxide, i.e., exo-dieldrin (1,2,3,4,10,10-hexachloro-endo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene), is the "normal" dieldrin, that insecticide which is commercially synthesized and applied; and the endo-epoxide, i.e., endo-dieldrin (1,2,3,4,10,10-hexachloro-endo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene), which has the epoxide ring in the opposite configuration (Fig. 1). The synthesis of endo-dieldrin has been described (S. B. Soloway, Ph.D. thesis, University of Colorado, Boulder, 1955), but, to the best of our knowledge, no one has attempted to characterize the stereoisomerization of the dieldrin produced metabolically by any of the described organisms. This has no doubt been due to the lack of adequate analytical methods, which distinguish between the two stereoisomers, since exo- and endo-dieldrin behave identically in most common chromatography systems. However, Nitz et al. (12) in their recent discussion of the analytical behavior of endo-dieldrin and endo-photodieldrin have described a gas chromatographic separation of the two stereoisomers.

This paper reports the results of a survey of soil bacteria designed to isolate and describe microorganisms that carry out the conversion of aldrin to dieldrin and to ascertain the stereoconfiguration of the product formed.

MATERIALS AND METHODS

Reagents. All chemicals and solvents were of analytical grade and were obtained from Merck, Darmstadt. n-Hexane was of residue analysis grade purity and was dried carefully before use. All solvents were analyzed for purity by gas chromatography (GC) and were free of contaminants to which the electron capture detector responded.

Aldrin (Merck-Schuchardt, Munich) was purified by column chromatography on alumina, with petroleum ether as the eluent, and by repeated recrystallization from absolute ethanol until a white crystalline preparation was obtained, which was pure as demonstrated by thin-layer chromatography (TLC) in system 2 (see below) and by GC. This preparation was free of any contamination by dieldrin.

exo-Dieldrin was purchased from Fluka, Buchs, Switzerland, and was purified by chromatography on alumina and by recrystallization. Authentic endo-dieldrin was synthesized in this laboratory (12). The chromatographic standards, keto-aldrin (4-oxo-4,5-dihydroaldrin), hydroxyaldrin (exo-4-hydroxy-4,5-dihydroaldrin), and dieldrin hydrul (trans-4,5-dihydroxy-4,5-dihydroaldrin), were analytical samples that had been prepared in this laboratory according to published procedures (9).

Culture media. Cultures were maintained on Standard I Agar slants (Merck, Darmstadt) and were checked periodically for purity. A modification of the medium of Hegemann (6) was used as the basal medium for all aldrin applications. This medium had the following composition per liter: ethylendiaminetetraacetate-sodium, 200 mg; MgSO4·7H2O, 580 mg; CaCl2·2H2O, 67 mg; (NH4)6Mo7024·4H2O, 0.2 mg; FeSO4·7H2O, 2.0 mg; (NH4)2SO4, 1.0 g; KH2PO4, 3.4 g; Na2HPO4, 12H2O, 4.44 g; Tween 80, 1.0 g; trace element solution, 1.0 ml; pH 7.0.
provided by Tu and have been previously described (14). They were: Bacillus sp. 1042, Micromonospora sp. 1040, Streptomyces sp. 1036, and Thermoaotinomyces sp. 1035.

The organisms in the third group were isolated in this laboratory from soils of several origins by means of enrichment on a number of substances, including buturon, naphthalene, and olive oil. Of the 160 isolates tested, only those that were active in producing dieldrin from aldrin will be discussed. Of the effective isolates, all gram-positive bacteria were identified at least to genus, according to the eighth edition of Bergey's Manual (1). Cell wall preparation and investigation of the cell wall components were made according to Engelhardt et al. (3). The characteristics of these cultures are described in Results.

Extraction and cleanup. At the end of the incubation period, each culture, bacterial cells together with medium, was extracted with 2 volumes of chloroform-methanol (1:1) by shaking overnight on a rotary shaker. The chloroform layer was separated either by means of a separatory funnel or, if necessary, by centrifugation. The aqueous layer was then extracted twice with 1 volume of chloroform, and the chloroform layers were pooled. After the extracts had been dried over anhydrous Na₂SO₄, the solvent was removed by careful evaporation on the rotary evaporator at 37°C. Evaporation was stopped just at the point where dryness had been reached. The residue was immediately dissolved in dry hexane and stored in tightly capped vials at −20°C until analysis. If residue insoluble in hexane remained, the residue was triturated three times with dry hexane and the hexane extracts were pooled and concentrated slightly before storing.

Cleanup of the hexane extracts was found to be imperative before subjecting the extracts to GC or TLC analysis. For this purpose, a modification of the cleanup methods described in the Pesticides Analytical Manual was used (4). Florisil (60 to 100 mesh, Merck, Darmstadt) was activated at 250°C before use. For cleanup of extracts from smaller (30 ml) cultures, disposable pipettes served as columns; the resulting column of Florisil was 0.6 by 6 cm, on top of which 1 cm of anhydrous Na₂SO₄ was layered. Each column was washed with 5 ml of dry hexane before applying the hexane extract (1 to 3 ml). Elution with an additional 5 ml of dry hexane removed most of the unreacted aldrin from the Florisil, which facilitated later chromatographic analysis. Dieldrin was eluted from the column by means of a stepwise gradient of acetone in hexane, generally consisting of 5 ml of 2% acetone, 5 ml of 4% acetone, 5 ml of 6% acetone, and 5 ml of 16% acetone in hexane. Extracts from larger-scale incubations (up to 6 liters) were cleaned up using Florisil columns and elution volumes proportional in size to the amount of aldrin applied. The behavior of the Florisil varied from lot to lot but was constant within each batch, and dieldrin could be eluted completely with 2 to 4% acetone in hexane or 2 to 6% acetone in hexane. Elution with 6 to 16% acetone washed a number of pigments and interfering substances from the column.

The trace element solution was a modification of that given by Drews (2), containing, in milligrams per liter: FeCl₃·6H₂O, 1,100; MnCl₂·4H₂O, 10; ZnSO₄·7H₂O, 10.5; LiCl, 0.5; KBr, 2.5; CuSO₄·5H₂O, 0.008; CaCl₂·2H₂O, 1.325; Na₂MoO₄·2H₂O, 1.0; CoCl₂·6H₂O, 9.0; SnCl₂·2H₂O, 0.5; BaCl₂·2H₂O, 0.6; H₃BO₃, 10; ethylenediaminetetraacetic acid-sodium, 20.

Carbon sources were generally added to the basal medium to give a concentration of 1 to 2%. Good growth was obtained for many of the strains tested with either 10 ml of a 1:29 solution of olive oil in ethanol (14) per liter of basal medium added by sterile filtration after sterilization or, alternatively, 10 ml of hexadec(1)-ene per liter added before sterilization. Among the other carbon sources tested were glucose, benzoate, and Tween 80.

A stock solution containing 5 mg of aldrin per ml of absolute ethanol was prepared and stored at −20°C. Dilutions of this solution in 95% ethanol were added to inoculated cultures by means of a syringe fitted with a sterile membrane filter to give an aldrin concentration of 1 mg/liter.

Microorganisms. A large number of microorganisms were examined for their ability to epoxidize aldrin to dieldrin. All strains were first tested for their ability to grow on the test media, and only those organisms that produced heavy growth were selected for testing for dieldrin production. The organisms were of three groups: (i) bacteria from culture collections having known metabolic capabilities; (ii) soil microorganisms known to epoxidize aldrin; and (iii) new isolates of bacteria from a variety of soils.

The organisms in the first group, which were provided by P. Wallnofer (Bayerische Landesanstalt für Bodenkultur, Pflanzenbau und Pflanzenenschutz, Munich), were: Pseudomonas fluorescens 1175, P. fluorescens 1441, P. fragi 1127, P. putida 1065, and Mycobacterium phlei.

The organisms in the second group were kindly
After cleanup, the eluted fractions were analyzed by GC. For each extract from the larger-scale incubations, fractions containing dieldrin were pooled and spotted on silica gel thin-layer plates for confirmation of the GC results.

GC. After cleanup on Florisil, the extracts were analyzed using a Fractovap 2200 gas chromatograph (Carlo Erba, Milan) equipped with a 60Ni electron capture detector. The injection port temperature was maintained at 240°C, and the detector temperature was maintained at 300°C.

Although the endo- and exo-isomers of dieldrin show the same retention time when subjected to gas-liquid chromatography using several common column packing materials, these isomers can be separated using Pyrex glass columns packed with 3% OV-101 on Chromosorb W AW-DMCS, 80 to 100 mesh. A separation of 45 to 60 s can be obtained with a 2-m column of OV-101 operating at 180°C, with a nitrogen carrier gas flow of 30 ml/min. Under these conditions, exo-dieldrin has a retention time of about 12.8 min and endo-dieldrin, about 13.7 min (Fig. 2). Keto-aldrin has the same retention time as exo-dieldrin in this system. Because the retention times vary slightly with age and condition of the column and also from batch to batch of packing material, authentic samples of endo-dieldrin and exo-dieldrin were injected at the beginning and end of each session. All assignments of structure are based on correspondence of retention times within a single session.

TLC. endo-Dieldrin is not separated from exo-dieldrin by means of TLC if the plates are developed with the solvent systems most commonly used for pesticide residue studies. The isomers are separable, however, when applied to activated silica gel thin-layer plates ( precoated Kieselgel 60 F254 plates with a layer thickness of 0.25 mm; Merck, Darmstadt), which are developed in o-xylene (system 1). In this system, R_v values are: exo-dieldrin, 0.50; endo-dieldrin, 0.60; and keto-aldrin, 0.35. This TLC system is sensitive to overloading of the plates, and extracts must be largely free of interfering substances to allow reproducible separations. Keto-aldrin and exo-dieldrin are also separated on silica gel plates developed in chloroform-methanol (9:1) (system 2). After development, plates were sprayed with 1% diphenylamine and irradiated with ultraviolet light (λ = 254 nm) to visualize chlorinated substances. When very small quantities of substances were chromatographed, bands corresponding to authentic substances were scraped off, eluted, and analyzed by GC.

GC-MS. Mass spectra were obtained with an LKB 9000 S gas chromatograph-mass spectrometer (MS) unit. A 2-m Pyrex column packed with 3% OV-101 was used, and all column operating conditions were identical with those for GC analysis, with the exception that the carrier gas used for GC-MS was helium.

RESULTS

Characteristics of isolated cultures. Bacillus cereus UFW-2 was isolated from a local wheat field soil which had been treated earlier in the season with the herbicide CMPP (mecoprop). When isolated, the strain showed a spreading rhizoidal colonial form and produced a yellow-green pigment. Pigment production was lost after several transfers on Standard I Agar, and colonial form was found to be dependent on the growth medium, being either rhizoidal or spreading uniformly over the agar surface to give a frosted-glass appearance. The cells were rod shaped, with elongated central endospores. Long chains of cells predominated. Growth occurred between pH 5 and 9.5; the maximum temperature for growth was 40°C. With basal medium plus hexadecene or olive oil-ethanol, good growth was obtained only with a heavy inoculum. This probably reflected
a growth factor requirement that was satisfied by the nutrient broth in the inoculum.

*B. subtilis* 4 was isolated by means of enrichment on buturon [3-(4-chlorophenyl)-1-methyl-1-(1-methyl-2-propynyl) urea] from a local agricultural soil which had been handled with the herbicide. Colonies on nutrient agar were liquefied up to about 24 h, when they collapsed and became irregular, wrinkled, opaque, and difficult to remove from the agar surface. Microscopically, the cells were motile rods, grouped in short chains, and had elongated central spores. Growth occurred between pH 5 and 9.5. The maximum temperature for growth was 45°C. This strain was capable of growth on basal medium with the carbon sources sucrose, glucose, fructose, mannose, inositol, succinate, acetate, fumarate, aspartate, and glutamate, as well as the experimentally utilized combinations hexadecene-ethanol and ethanol-olive oil.

*Nocardia* sp. UFM-30 was isolated from a local corn field soil on which the herbicide atrazine had been heavily applied during the previous growing season. Colonies of this organism on nutrient agar were bright orange-red, dry and flaky, highly raised, and convoluted. Stationary broth cultures consisted of a fragile pellicle, which was easily disrupted to flakes upon shaking. Cells were highly and irregularly branched in 24-h cultures. In basal medium, growth was supported by sucrose, glucose, fructose, lactate, acetate, fumarate, benzoate, or glycerol as the sole carbon source, but not by malonate, succinate, oxalate, aspartate, glutamate, phenylalanine, tryptophan, and inositol. The organism grew well on either hexadecene or ethanol-olive oil. The pH range for growth was 5 to 9.5. Litmus milk was alkaline after 1 week. The major cell wall constituents of this strain were a peptidoglycan containing cross-linked *meso*-diaminopimelic acid and an arabinogalactan.

*Nocardia* sp. UFM-48 was isolated from the same soil sample as *Nocardia* sp. UFM-30. This organism had orange, round, pulvinate colonies with a smooth, glistening surface and a pasty consistency. Growth in nutrient broth was dispersed. Microscopic examination showed a highly and irregularly branched morphology. Glucose, mannose, fructose, lactate, acetate, ethanol, or Tween 80 served as the sole carbon source in basal medium. Malonate, succinate, oxalate, benzoate, fumarate, inositol, ribitol, and glycerol, however, did not support growth, nor did any of the amino acids tested, i.e., aspartate, glutamate, phenylalanine, alanine, arginine, or tryptophan. Growth occurred between pH 6 and 9.5. The major cell wall constituents of this strain were a peptidoglycan containing directly cross-linked *meso*-diaminopimelic acid and an arabinogalactan. (See Table 1 for properties of gram-positive isolates.)

Nine motile, gram-negative isolates that actively converted aldrin to dieldrin were partially characterized. No attempt was made to analyze the mode of flagellation of these strains, other than to observe the motility in broth cultures with a phase-contrast microscope. On the basis of these gross observations, most, but probably not all, of these strains were members of the genus *Pseudomonas*.

SB-4 was isolated by enrichment on naphthalene from a soil sample obtained from the grounds of a dieldrin-producing factory (cf. reference 10). Colonies on nutrient agar were tan, smooth, raised round, regular, and translucent. Cells were rods, actively motile in a manner suggesting polar flagellation. Broth cultures were heavily turbid. Litmus milk was alkaline after 7 days. No growth occurred in nutrient broth with 5% NaCl. Growth occurred between pH 4.5 and 9. Growth was supported by glycerol, phenylalanine, and tryptophan, in addition to the compounds given in Table 2.

OOM-3 was isolated by enrichment culture on olive oil from the same soil sample as the *Nocardia* species. Colonies on nutrient agar were yellow-tan, irregular, flat, and translucent and tended to have a raised point at the center. Microscopic examination revealed motile rods. Broth cultures were turbid with some viscous sediment. Litmus milk was peptonized.

### Table 1. Characteristic metabolic properties of gram-positive isolates

<table>
<thead>
<tr>
<th>Isolate*</th>
<th>Metabolism</th>
<th>Oxid-&lt;br&gt; &lt;sup&gt; &lt;/sup&gt;dase</th>
<th>Acid from glucose</th>
<th>NO&lt;sub&gt;3&lt;/sub&gt; - to NO&lt;sub&gt;2&lt;/sub&gt; -</th>
<th>Starch hydrolysis</th>
<th>Gelatin liquefaction</th>
<th>Growth on 7% NaCl broth</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em> UFW-2</td>
<td>Oxidative + fermentative</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> 4</td>
<td>Oxidative + weakly fermentative</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Nocardia</em> sp. UFM-30</td>
<td>Oxidative</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Nocardia</em> sp. UFM-48</td>
<td>Oxidative</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*All four strains are catalase positive and grow on Sabouraud-2% glucose broth. None possesses arginine hydrolase, and none is acid fast.*

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completely after 7 days. Good growth was supported by betaine.

OOW-2 was also isolated by enrichment culture on olive oil, but from the same soil sample as B. cereus UFW-2. The colony morphology was very similar to that of OOM-3, but the strains differed microscopically: the cells of OOW-2 were shorter rods. Broth cultures were turbid with a thin, white pellicle. Litmus milk was alkaline after 7 days. Growth occurred in nutrient broth plus 5% NaCl. Growth occurred at pH 4.5 to 9.5. Good growth was observed on a wide variety of compounds, including those given in Table 2, betaine, and glycercol, but not on phenylalanine or tryptophan.

MIM-13 was isolated from the corn field soil sample. Colonies were tan, round smooth, raised, and translucent. Cells were rod shaped and showed a motility that suggested polar flagellation. Broth cultures were turbid. No change was seen in litmus milk after 7 days. Growth occurred in nutrient broth plus 5% NaCl. The pH range for growth was 4.5 to 9.5. Growth was supported by glycercol, betaine, arginine, phenylalanine, and tryptophan.

MNAP-2 was isolated by enrichment culture on napthalene from the same soil sample as the two Nocardia species. Colonies were yellow-tan, irregular, flat, and faintly wrinkled; colonies could be easily removed in toto from the agar surface. Broth cultures showed a light pellicle with some sediment. Microscopic examination showed rods that were motile, but not actively so; some irregularity in size distribution was observed in broth cultures. Litmus milk was alkaline after 7 days. Growth was observed in nutrient broth plus 5% NaCl. In addition to those carbon sources mentioned in Table 2, good growth was supported by glycercol, malonate, betaine, and arginine. The pH range for growth was 5 to 9.5.

The gram-negative strains P-5, P-7, P-9, and P-10 were isolated from the same soil sample as B. subtilis 4. They shared the traits summarized in Table 2 but were distinguishable as different organisms.

The colonies of P-5 were tan, irregular, and opaque. Cells were short rods, actively motile in a manner suggesting polar flagellation. Litmus milk was unchanged after 7 days. No growth occurred in the presence of 5% NaCl. In addition to the compounds given in Table 2, betaine or arginine could serve as the sole carbon source. Growth occurred between pH 5 and 9.

P-7 had beige colonies which were irregular and opaque, with clear margins. The short rods were motile. Litmus milk was slightly alkaline after 7 days. Broth cultures showed a heavy sediment. No growth occurred in nutrient broth plus 5% NaCl. The pH range was 5 to 9.5. Growth was supported by betaine and arginine.

Isolate P-9 had rose-tan colonies which were round, umbonate, and translucent. Microscopic examination showed short rods which were motile, but not actively so. A loose sediment was observed in broth cultures. No change was observed in litmus milk after 7 days. No growth occurred in 5% NaCl. Growth occurred between pH 5 and 9.5. Growth was supported by betaine and arginine.

The colonies of P-10 were pink-tan, round, raised, and translucent. Microscopically, the cells were short rods, actively motile in a manner suggesting polar flagellation. Litmus milk was unchanged after 7 days. No growth occurred in 5% NaCl. Growth was supported by betaine and arginine.

Dieldrin production. Preliminary experiments showed dieldrin production to be depend-

### Table 2. Characteristic metabolic properties of gram-negative isolates

<table>
<thead>
<tr>
<th>Isolate*</th>
<th>Oxidase</th>
<th>Acetate</th>
<th>Glucose</th>
<th>Benzoate</th>
<th>Arginine hydrolase</th>
<th>Gelatin liquefaction</th>
<th>NO₃⁻ to NO₂⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB-4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>OOM-3</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>OOW-2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MIM-13</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>MNAP-2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P-5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>P-7</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>P-9</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Weak</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>P-10</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Weak</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* All strains are gram-negative, motile, rod-shaped organisms occurring singly or in pairs. Metabolism is respiratory. All are catalase positive and can grow on ethanol, succinate, fumarate, lactate, Tween 80, citrate, aspartate, glutamate, or alanine as the sole carbon source. None produces acid from glucose. None hydrolyzes starch. None grows in broth plus 7% NaCl or 0.02% sodium azide. None requires growth factors. The maximum temperature for growth is 37°C.
ent on the culture medium. Three strains known to metabolize aldrin, *Bacillus* sp. 1042, *Streptomyces* sp. 1038, and *Micromonospora* sp. 1040, and one other authentic strain, *M. phlei*, were grown for 3 weeks in nutrient broth, APT (All Purpose Medium with Tween; Merck, Darmstadt) broth, basal medium plus 1% glucose, basal medium plus 1% Tween 80, basal medium plus 1% ethanol-olive oil, and basal medium plus 1% hexadecene. Aldrin was added to each culture by means of a sterile filter syringe to give a final concentration of 1 \( \mu g/ml \). For all of these strains, the highest amount of conversion was observed in basal medium plus ethanol-olive oil or hexadecene. Little or no dieldrin production was observed in most cases with nutrient broth or APT broth, even in the presence of heavy growth (Table 3). Consequently, all authentic strains and newly isolated soil bacteria were screened for their ability to grow in basal medium plus 1% ethanol-olive oil or hexadecene. Only those strains that showed good growth after incubation for 1 week at 30°C were tested for aldrin-epoxidizing activity. These two media were utilized for all aldrin degradation experiments.

For initial testing, 30 ml of growth medium was inoculated with 1 ml of an actively growing (24 to 48 h) nutrient broth culture, and filter-sterilized aldrin solution was added to give a concentration of 1 mg/liter. Cultures were incubated for 3 weeks at 30°C on a rotary shaker in the dark. Using essentially the same conditions, Tu et al. (14) reported a maximum level of dieldrin to be present in their bacterial cultures after 3 weeks. At the end of the incubation period, the cultures were extracted as described in Materials and Methods and subjected to cleanup on Florisil before analysis by GC. About 50% of the strains growing on ethanol-olive oil and 75% of the strains growing on hexadecene produced detectable amounts of dieldrin. A few of the strains produced only small amounts of dieldrin and were inoculated into 400 ml of culture medium containing 1 mg of aldrin per liter, incubated, and worked up as before, in order to obtain sufficient quantities of dieldrin to permit an unequivocal analysis.

Only the exo-isomer of dieldrin was detected (Table 4). All strains able to convert aldrin to dieldrin were inoculated a second time into the same medium, treated with aldrin, and extracted and analyzed as before as an added control. The results were identical in every case.

The results obtained by GC analysis were confirmed by TLC and MS. Large cultures (6 liters) of the strains that converted the largest amounts of aldrin to dieldrin, i.e., *Bacillus* sp. 1042 in ethanol-olive oil, *Nocardia* sp. UFM-30 in hexadecene, and the pseudomonad OOM-3 in both media, were incubated as before with 1 mg of aldrin per liter. Extraction and cleanup were as before. GC again showed the presence of exo-dieldrin only. After preparative TLC in n-hexane, the dieldrin fractions were subjected to TLC in system 1 and GC-MS analysis. No trace

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**Table 3. Aldrin epoxidation in various culture media**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Nutrient broth</th>
<th>APT broth</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus</em> sp. 1042</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Streptomyces</em> sp. 1038</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Micromonospora</em> sp. 1040</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Mycobacterium phlei</em></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 4. Configuration of dieldrin produced by bacterial epoxidation of aldrin**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ethanol-olive oil</th>
<th>Hexadecene</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus</em> sp. 1042</td>
<td>exo</td>
<td>exo</td>
</tr>
<tr>
<td><em>Bacillus</em> cereus UFW-2</td>
<td>exo</td>
<td>exo</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> 4</td>
<td>NT*</td>
<td>exo</td>
</tr>
<tr>
<td><em>Thermoactinomyces</em> sp. 1033</td>
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<tr>
<td><em>Streptomyces</em> sp. 1038</td>
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<tr>
<td><em>Micromonospora</em> sp. 1040</td>
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<tr>
<td><em>Nocardia</em> sp. UFM-48</td>
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<tr>
<td><em>Nocardia</em> sp. UFM-30</td>
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<tr>
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<td><em>Pseudomonas fluorescens</em> 1441</td>
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<tr>
<td><em>Pseudomonas fluorescens</em> 1175</td>
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<td><em>Pseudomonas fragi</em> 1127</td>
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<td><em>Pseudomonas putida</em> 1065</td>
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<td><em>SB-4</em></td>
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<tr>
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<td><em>P-10</em></td>
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* NT, Not tested.
of endo-dieldrin was detected on the thin-layer plates. The mass spectra obtained were identical to those of authentic endo-dieldrin.

DISCUSSION

The ability to epoxidize aldrin to dieldrin is obviously a common trait, widely distributed among soil bacteria. In this survey, we have described a number of strains of gram-positive and gram-negative soil bacteria which share this property and produce exclusively the endo-isomer of dieldrin. Tu et al. (14) reported dieldrin production in 30 gram-positive isolates out of 45 tested, whereas Patil et al. (13) found 11 gram-negative and gram-positive soil bacteria with this trait.

Although the endo-isomer of dieldrin is less stable than the exo-isomer, there seemed to be no a priori reason to expect microbial epoxidation to lead to endo-dieldrin in every case. However, since stereospecific enzyme systems have been described in microorganisms that catalyze the epoxidation of unsaturated steroids (5) or 1,7-octadiene (11), it seemed reasonable to expect the enzymatic epoxidation of aldrin to proceed, with some degree of stereospecificity, to produce only one of the possible stereoisomers. Demonstration that the exo-isomer only is produced in such dissimilar genera as Pseudomonas, Bacillus, and the members of the order Actinomycetales (Streptomycetes, Micromonospora, Thermoactinomyces, Nocardia, and Mycobacterium) suggests that aldrin epoxidation proceeds in bacterial enzyme systems exclusively to endo-dieldrin.

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LITERATURE CITED


