Mineralization of α-, γ-, and β-Isomers of Hexachlorocyclohexane by a Soil Bacterium under Aerobic Conditions

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The degradation of uniformly ^{14}C-labeled isomers (α, γ, and β) of hexachlorocyclohexane (HCH), added separately to a mineral salts medium as a sole source of carbon, by a soil bacterium, *Pseudomonas* sp., under aerobic conditions was studied. During aerobic degradation, about 10–12% of the ^{14}C in α- and γ-HCH was accounted for as $^{14}CO_2$ as compared to 5% from β-HCH. Most of the ^{14}C in α- and γ-HCH accumulated as water-soluble products, while formation of water-soluble products from β-HCH was negligible. Almost all of the ^{14}C in the three isomers of HCH was accounted for in different fractions (CO_2, chloroform–diethyl ether, water phase) following bacterial degradation under aerobic conditions. GC–MS analysis of β-HCH residues in the chloroform–diethyl ether extract suggested the formation of pentachlorocyclohexanol and tetrachlorocyclohexanediol as products of aerobic metabolism of β-HCH.

Keywords: *HCH isomers (α, γ, and β); aerobic degradation; Pseudomonas sp.; pentachlorocyclohexene; pentachlorocyclohexanol; tetrachlorocyclohexanediol*

INTRODUCTION

Hexachlorocyclohexane (HCH), a broad-spectrum insecticide, accounts for nearly 47% of the total pesticides used in India (David, 1992). Commercial formulations of this organochlorine insecticide generally contain α-, γ-, β-, and δ-isomers and other isomers. These isomers have been known for their stability in aerobic environments but undergo fairly rapid degradation in anaerobic ecosystems such as flooded soils and lake sediments (Raghu and MacRae, 1966; Sethunathan et al., 1983).

Anaerobic (strict or facultative) bacteria have been implicated in the rapid degradation of isomers of hexachlorocyclohexane in anaerobic ecosystems (MacRae et al., 1969; Sethunathan et al., 1969; Haider and Jagnow, 1975; Jagnow et al., 1977; Haider, 1979; Sethunathan et al., 1983; Straube, 1991). Recent reports, however, show rapid disappearance of HCH isomers even from aerobic soil systems. Thus, Bachmann et al. (1988a,b) demonstrated rapid degradation of α-HCH in a soil slurry from an HCH-contaminated site under aerobic conditions. Likewise, γ-HCH disappeared rapidly in a re-treated upland soil (Senoo et al., 1989), and suspensions of HCH-treated soils from flooded and nonflooded fields (Bhuyan et al., 1992) effected rapid degradation of γ-HCH under aerobic conditions. A *Pseudomonas paucimobilis*, isolated from HCH-treated upland soil, readily degraded HCH via γ-pentachlorocyclohexene (γ-PCH) to 1,2,4-trichlorobenzene in a mineral salts medium as a sole source of carbon (Imai et al., 1989). α- and δ-isomers of HCH, but not β-HCH, were degraded by this bacterium (Imai et al., 1989). Recently, we found that a *Pseudomonas* sp. (Sahu et al., 1990b), isolated from HCH-treated sugarcane rhizosphere soil, and a *Sphingomonas paucimobilis* (Bhuyan et al., 1993), isolated from a flooded soil, readily degraded not only α- and γ-isomers but also the thermodynamically more stable β-isomer under aerobic conditions. This follow-up study provides further insight into the metabolism of these HCH isomers, β-isomer in particular, by *Pseudomonas* sp. under aerobic conditions.

MATERIALS AND METHODS

HCH Isomers. Technical grade α-, γ-, and β-isomers (99.1% purity) of HCH were obtained from Lachat Chemicals. U-^{14}C-ring-labeled α-HCH (specific activity 50 mCi $mmol^{-1}$), β-HCH (specific activity 33 mCi $mmol^{-1}$), and γ-HCH (specific activity 40 mCi $mmol^{-1}$) were obtained from Radiochemical Centre, Amersham, England. The labeled HCH isomers were dissolved in 100 mL of hexane after the benzene carrier was evaporated.

Growth and Maintenance of Bacterium. The minimal mineral salts medium used was of the following composition: $(NH_4)_2HPO_4$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $FeSO_4 \cdot 7H_2O$, 0.001 g; K_2HPO_4, 0.1 g; $Ca(NO_3)_2$, 0.01 g/L of distilled water; pH 7.0. The *Pseudomonas* sp. used in this study was previously isolated from the rhizosphere soil of HCH-treated sugarcane plants (Sahu et al., 1990b). This bacterium was subcultured and maintained on a mineral salts medium supplemented with 1.6% agar, 1% glucose, and 5 μg mL^{-1} γ-HCH.

Degradation of ^{14}C-Labeled Isomers of HCH. In isotope studies, α-, γ-, and β-isomers of uniformly ring labeled [^{14}C]-HCH in 0.03 mL of hexane were dispensed into separate presterilized 100 mL Erlenmeyer flasks along with 20 μg of technical grade HCH in hexane as carrier. Immediately after evaporation of hexane at room temperature, 10 mL of sterile mineral salts medium was dispensed into each flask. The contents in each flask were then shaken on an orbital shaker for 12 h to bring the HCH isomers into solution. The flasks were inoculated with 0.1 mL of a suspension of *Pseudomonas* sp. (6.5×10^4 cells). Uninoculated flasks served as control.

Assay of $^{14}CO_2$. Incubation flasks were sealed with rubber bungs provided with an inlet and an outlet, which were closed with a pinchcock. The flasks were incubated statically at room temperature (28 ± 2 °C). At appropriate intervals, the inlet

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Table 1. Distribution of Radioactivity during Degradation of Uniformly Ring Labeled [^{14}C]-α-Hexachlorocyclohexane[a] in a Mineral Salts Medium by a *Pseudomonas* Sp. as a Sole Source of Carbon

		radioactivity (%) recovered 10 mL^{-1} of medium			
incubation (h)	treatment	hexane fraction	aqueous fraction[b]	CO_2	total radioactivity
0	uninoculated	96.3 ± 0.3[c]	2.0 ± 0.1	0.05 ± 0	98.3
12	uninoculated	91.7 ± 0.9	2.1 ± 0.1	0.05 ± 0.03	93.8
	inoculated[d]	19.2 ± 7.0	56.5 ± 5.0	4.24 ± 2.20	80.0
24	uninoculated	82.5 ± 1.0	2.6 ± 0.2	0.04 ± 0.14	85.1
	inoculated	8.6 ± 0.2	62.6 ± 2.8	5.61 ± 1.57	76.8
48	uninoculated	65.3 ± 0.2	1.9 ± 0.2	0.05 ± 0	67.2
	inoculated	5.9 ± 0	61.1 ± 2.4	10.00 ± 1.6	77.0

[a] [^{14}C]-α-HCH was added at 1 × 10^5 dpm 10 mL^{-1} medium. [b] Remaining after hexane extraction of the medium. [c] Mean of duplicate estimations ± deviation. [d] Inoculated with *Pseudomonas* sp.

Table 2. Distribution of Radioactivity during Degradation of Uniformly Ring Labeled [^{14}C]-β-Hexachlorocyclohexane[a] in a Mineral Salts Medium by a *Pseudomonas* Sp. as a Sole Source of Carbon

		radioactivity (%) recovered 10 mL^{-1} of medium			
incubation (h)	treatment	hexane fraction	aqueous fraction[b]	CO_2	total radioactivity
0	uninoculated	99.4 ± 3.0[c]	0.94 ± 0.03	0.02 ± 0	100.4
12	uninoculated	100.7 ± 1.0	0.89 ± 0.05	0.02 ± 0	101.7
	inoculated[d]	83.1 ± 1.9	15.41 ± 2.9	0.12 ± 0.03	98.7
24	uninoculated	96.7 ± 1.2	1.10 ± 0	0.04 ± 0	97.9
	inoculated	62.1 ± 2.3	28.20 ± 4.8	1.00 ± 0.1	91.3
48	uninoculated	95.7 ± 0.3	1.04 ± 0.1	0.03 ± 0	96.8
	inoculated	52.2 ± 8.8	41.20 ± 8.6	2.40 ± 0	95.8
72	uninoculated	94.9 ± 0.6	1.23 ± 0.1	0.06 ± 0	96.2
	inoculated	29.8 ± 0.5	63.30 ± 0.6	3.57 ± 0.1	96.6
120	uninoculated	92.0 ± 3.1	1.40 ± 0.1	0.04 ± 0	93.8
	inoculated	14.8 ± 0.7	74.90 ± 1.0	4.14 ± 0.2	93.8
240	uninoculated	91.7 ± 0.7	1.10 ± 0.1	0.02 ± 0	92.8
	inoculated	10.0 ± 0.1	78.40 ± 0.8	4.3 ± 0.2	92.7

[a] [^{14}C]-β-HCH was added at 1.2 × 10^5 dpm 10 mL^{-1} of medium. [b] Remaining after hexane extraction of the medium. [c] Mean of duplicate estimations ± deviation. [d] Inoculated with *Pseudomonas* sp.

was connected to an air generator through a trap containing 25 mL of 2 N KOH solution to remove the $^{14}CO_2$, if any, from the air. The $^{14}CO_2$ that evolved from the [^{14}C]HCH from each of the duplicate flasks containing inoculated or uninoculated medium was purged, after the pinchcock was released into 10 mL of carbon-14 cocktail (repurged with nitrogen) containing pseudocumene (R. J. Harvey Instrument Corp.).

For extraction of ^{14}C residues, the contents in each of duplicate flasks were shaken twice with 10 mL of hexane plus 1 g of sodium sulfate. The hexane fractions were pooled, and 1 mL aliquots were mixed with 5 mL of optiphase Hi-safe II liquid scintillation cocktail (flash point 144 °C) in a 10 mL scintillation vial (PSA Laboratory Suppliers, Loughborough, U.K.). The radioactivity was measured in a Rackbeta liquid scintillation counter, Model 1209 (LKB, Wallac, Finland), with color and chemical quenching correction. To determine the radioactivity in the aqueous phase, 1 mL aliquots of the aqueous phase following hexane extraction were mixed with 10 mL of the same scintillation solution and radioactivity was counted. The counting efficiency was 96.3%.

The residues in the aqueous phase remaining after hexane extraction were re-extracted with 10 mL portions of chloroform–diethyl ether (1:1). One milliliter aliquots of pooled chloroform–diethyl ether fraction were counted.

Radioautograph. The hexane and chloroform–diethyl ether fractions were concentrated to 0.5 mL and then spotted on 300 μm silica gel HP 254 plates (20 × 20 cm). The plates were developed in 5% acetone–hexane for a distance of 15 cm, dried, and then directly exposed to Kodak X-ray film for 15 days.

GC–MS Analysis. The β-HCH residues in the chloroform–diethyl ether extract were subjected to GC–MS analysis after derivatization with acetic anhydride and pyridine. The GC–MS instrument was a Hewlett-Packard MSD (Model 5970). The gas chromatographic portion was equipped with an HP-1, 12 m capillary column. The oven temperature was programmed from 100 to 210 °C at the rate of 10 °C min^{-1}. The flow rate of helium was 20 mL min^{-1}.

RESULTS AND DISCUSSION

The distribution of radioactivity in the hexane fraction and in the aqueous fraction remaining after hexane extraction of the medium during degradation of ^{14}C-labeled HCH isomers (α, γ, and β) by growing cells of *Pseudomonas* sp. as a sole source of carbon under aerobic conditions was examined. In uninoculated samples, the decrease in the radioactivity of hexane extract of the medium was negligible during the incubation period (48 h for α- and γ-isomers and 240 h for β-isomer). However, in all samples inoculated with *Pseudomonas* sp., hexane-extractable radioactivity distinctly decreased with increase in incubation irrespective of the HCH isomer (Tables 1–3). Radioactivity in the hexane fraction reached about 5% of the original level with α- and γ-isomers in 48 h. However, with the β-isomer the decrease in the radioactivity in the hexane fraction was not as rapid as with the α- and γ-isomers; only after 240 h did the radioactivity in the hexane fraction reach about 10% of the original level with β-HCH. A radioautograph of the hexane extract of the inoculated medium at 8 h showed the formation of a metabolite with R_f of 0.19 and 0.17 from α-HCH (R_f 0.93) and γ-HCH (R_f 0.89), respectively. These metabolites from α-HCH and γ-HCH, when eluted from the thin-layer chromatograms in hexane and analyzed by gas–liquid chromatography as described earlier (Sahu et al., 1990b), showed the same retention time as that of α-PCH (1.5 min) and γ-PCH (0.67 min), respectively. Evidently, aerobic degradation of α- and γ-HCH by *Pseudomonas* sp. led to the transitory accumulation of α- and γ-PCH, respectively. A radioautograph of the hexane extract of the inoculated medium amended with β-HCH (R_f 0.85) also revealed the presence of a major metabolite (R_f 0.28) and a minor metabolite (R_f 0.38). This metabolite (R_f 0.28), detected even at 8 h after

Table 3. Distribution of Radioactivity during Degradation of Uniformly Ring Labeled [^{14}C]-γ-Hexachlorocyclohexane[a] in a Mineral Salts Medium by a *Pseudomonas* Sp. as a Sole Source of Carbon

		radioactivity (%) recovered 10 mL^{-1} of medium			
incubation	treatment	hexane fraction	aqueous fraction	CO_2	total radioactivity
0	uninoculated	94.0 ± 3.1[c]	1.3 ± 0.03	0.01 ± 0	95.4
12	uninoculated	95.9 ± 2.1	1.3 ± 0.22	0.02 ± 0	97.2
	inoculated[d]	6.9 ± 0.6	58.2 ± 2.93	5.18 ± 0.9	70.2
24	uninoculated	90.4 ± 2.9	1.7 ± 0.05	0.02 ± 0	92.1
	inoculated	6.6 ± 0.2	58.2 ± 3.32	9.84 ± 0.8	74.6
48	uninoculated	81.9 ± 0.5	1.3 ± 0.11	0.03 ± 0	83.2
	inoculated	3.3 ± 0.1	65.4 ± 0.76	11.52 ± 0.2	80.2

[a] [^{14}C]-γ-HCH was added at 1.1 × 10^5 dpm 10 mL^{-1} of medium. [b] Remaining after hexane extraction of the medium. [c] Mean of duplicate estimations ± deviation. [d] Inoculated with *Pseudomonas* sp.

Table 4. Radioactivity Recovered in Chloroform–Diethyl Ether and Aqueous Fractions during Degradation of α-, β-, and γ-Isomers of Hexachlorocyclohexane (HCH) by a *Pseudomonas* Sp.

		radioactivity (%) recovered 10 mL^{-1} of medium					
		α-HCH		β-HCH		γ-HCH	
incubation (h)	treatment	chloroform–diethyl ether fraction[a]	aqueous fraction[b]	chloroform–diethyl ether fraction[a]	aqueous fraction[b]	chloroform–diethyl ether fraction[a]	aqueous fraction[b]
0	uninoculated	2.1 ± 0.03[c]	0.5 ± 0.02	1.4 ± 0.08	0.4 ± 0	1.1 ± 0.2	0.4 ± 0.1
12	uninoculated	1.1 ± 0.8	0.7 ± 0	1.3 ± 0.1	0.3 ± 0.1	0.9 ± 0.5	0.4 ± 0.04
	inoculated	10.8 ± 0.1	34.3 ± 3.7	15.2 ± 1.3	0.8 ± 0.1	7.0 ± 0.6	32.6 ± 0.1
24	uninoculated	0.5 ± 0.2	1.0 ± 0.1	1.4 ± 0.2	0.5 ± 0.2	1.2 ± 0.2	0.1 ± 0.06
	inoculated	5.7 ± 0.4	40.9 ± 3.5	34.0 ± 2.6	1.3 ± 0.1	3.6 ± 0.7	37.5 ± 3.5
48	uninoculated	1.3 ± 0.4	0.4 ± 0	2.4 ± 0.2	0.4 ± 0	1.0 ± 0.1	0.4 ± 0.05
	inoculated	4.1 ± 0.1	43.7 ± 0.1	30.0 ± 0.3	1.4 ± 0.2	3.5 ± 0.03	41.9 ± 7.9
72	uninoculated	ND[d]	ND	3.1 ± 0.2	0.7 ± 0.1	ND	ND
	inoculated	ND	ND	58.8 ± 0.6	2.5 ± 0	ND	ND
120	uninoculated	ND	ND	2.2 ± 0.4	0.5 ± 0.1	ND	ND
	inoculated	ND	ND	74.6 ± 1.0	2.3 ± 0.2	ND	ND
240	uninoculated	ND	ND	2.5 ± 0.4	0.5 ± 0	ND	ND
	inoculated	ND	ND	81.5 ± 4.9	3.0 ± 0	ND	ND

[a] Residues in aqueous fractions (Tables 1–3) remaining after hexane extraction were re-extracted in chloroform–diethyl ether. [b] Remaining fraction after chloroform–diethyl ether extraction. [c] Mean of duplicate estimations ± deviation. [d] ND, not determined.

inoculation, appeared to be relatively more persistent than the parent β-HCH; however, in 240 h this metabolite almost completely disappeared, indicating its further metabolism.

Assay of $^{14}CO_2$ evolution showed that about 10–12% of the ^{14}C in α- and γ-HCH was evolved as $^{14}CO_2$ after 48 h of inoculation with the bacterium, while only about 5% of the ^{14}C in β-HCH was evolved as $^{14}CO_2$ in 240 h. No further increase in $^{14}CO_2$ evolution was noticed even after extended incubation beyond 48 h for the α- and γ-isomers and 240 h for the β-isomer. This would suggest that a substantial portion of the ^{14}C in HCH isomers is not released as $^{14}CO_2$ during bacterial degradation.

Radioactivity in the aqueous phase remaining after hexane extraction of the inoculated samples supplemented with any of three HCH isomers increased almost in stoichiometric amounts concomitant with a decrease in hexane-extractable radioactivity. Interestingly, β-HCH residues remaining in the aqueous phase after hexane extraction were almost completely partitioned into chloroform–diethyl ether; however, ^{14}C residues of α- and γ-HCH remaining in the aqueous phase after hexane extraction were not extracted by chloroform–diethyl ether (Table 4). Presumably, degradation products of α- and γ-HCH were highly polar. A radioautograph of chloroform–diethyl ether-extractable residues from β-HCH showed a prominent metabolite (R_f 0.17) and a minor metabolite (R_f 0.23).

The β-HCH residues in the chloroform–diethyl ether extract were subjected to GC–MS analysis after acetylation with acetic anhydride and pyridine. The GC–MS analysis showed the presence of two polychlorinated compounds: one at 12.155 min and the other at 13.276 min.

The mass spectrum of the 12.155 min peak (Figure 1A) showed the following sets (A–E) of chlorine-containing mass fragment ions: (A) *m/z* 241 (relative abundance 100%), 243, 245 (area ratio 100:95:33); (B) *m/z* 198 (60%), 200, 202 (100:97:33); (C) *m/z* 181 (16.1%), 183, 185 (100:97:30); (D) *m/z* 156 (48.6%), 158, 160 (99:100:36); and (E) *m/z* 109 (77.5%), 111, 113 (100:87:17). Among these, the sets A–D all demonstrated a typical isotope distribution pattern of trichloro fragment ions (theoretical ratio, M, 100; M + 2, 97; M + 4, 31.7). Peak E seems to correspond to a dichloro fragment ion (theoretical ratio, M, 100; M + 2, 65; M + 4, 10.6). Thus, the 12.155 min compound was assigned to an isomer of monoacetoxypentachlorocyclohexane. Possible mass fragmentation sequences and assignments of ions are as follows: $C_8H_9O_2Cl_5^+$ [*m/z* 312, molecular] → (−Cl, −HCl) → $C_8H_8O_2Cl_3^+$ [*m/z* 241, (A)] → ($-CH_3CO$) → $C_6H_5OCl_3^+$ [*m/z* 198, (B)] → (CHCHO, retro-Diels–Alder) → $C_4H_3Cl_3^+$ [*m/z* 156, (C)] → $C_3H_3Cl_2^+$ [*m/z* 109, (E)], and molecular → ($-CH_3COOH$, −HCl, −Cl) → $C_6H_4Cl_3^+$ [*m/z* 181, (D)] (Figure 2).

The mass spectrum of the second peak (Figure 1B) at 13.276 min indicated the following four principal mass fragment ion peaks (F–I): (F) *m/z* 265 (33.1%), 267, 269 (100:73:14); (G) *m/z* 223 (100%), 225, 227 (100:63:12); (H) *m/z* 163 (18.3%), 165, 167 (100:62:15); (I) *m/z* 145 (27.9%), 147, 149 9100:33:−). Among these, the former three are typical of dichloro fragment ions (theoretical distribution, M, 100; M + 2, 65; M + 4, 10.6). The ion at *m/z* 145 seems to correspond to monochloro fragment ions (theoretical distribution 100:32.5). Thus, the 13.276 min compound was assigned to an isomer of

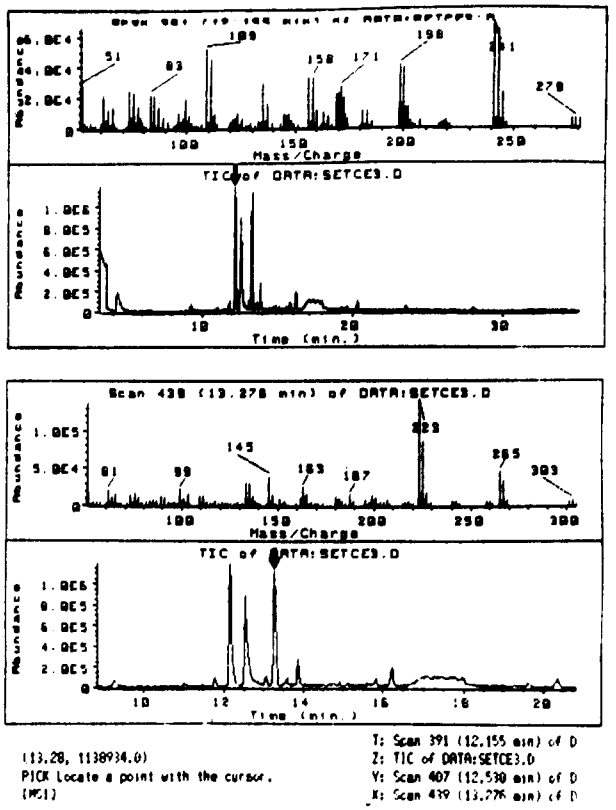

Figure 1. Total ion chromatograms of β-HCH residues in the chloroform–diethyl ether extract after acetylation: (A, top) 12.155 min peak; (B, bottom) 13.276 min peak.

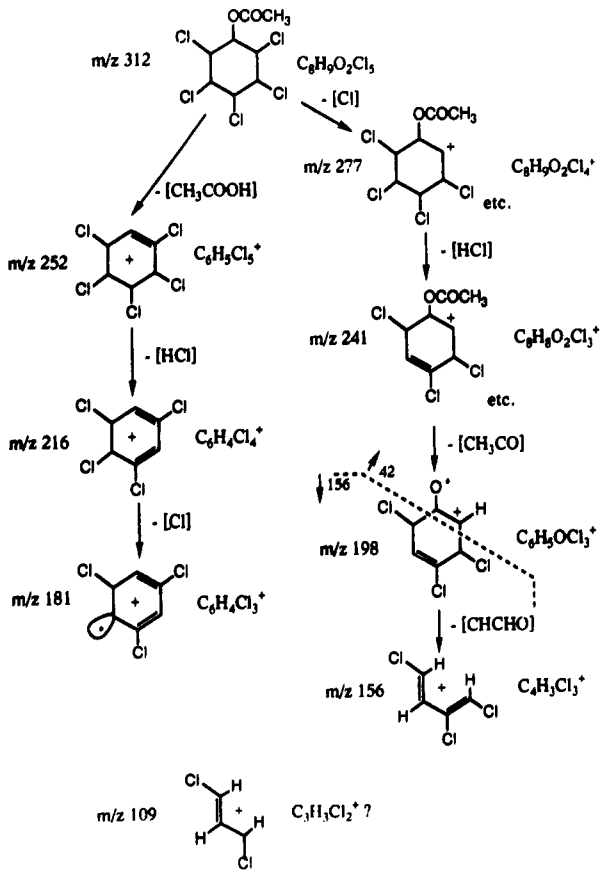

Figure 2. Possible mass fragmentation sequence of 12.155 min MS peak.

diacetoxytetrachlorocyclohexane. Possible mass fragmentation sequences and assignments of ions are as follows: $C_{10}H_{12}O_4Cl_4^+$ (*m/z* 336, molecular) → (−Cl, −HCl) → $C_{10}H_{11}O_4Cl_2^+$ [*m/z* 265, (F)] → (−C_2H_2O = −CH_3CO, +H) → $C_8H_9O_3Cl_2^+$ [*m/z* 223, (G)] → (CH_3-COOH) → $C_6H_5OCl_2^+$ [*m/z* 163, (H)], and (F) → (−C_4H_3-OCl, retro-Diels–Alder) → $C_6H_6O_2Cl^+$ [*m/z* 145, (I)] (Figure 3).

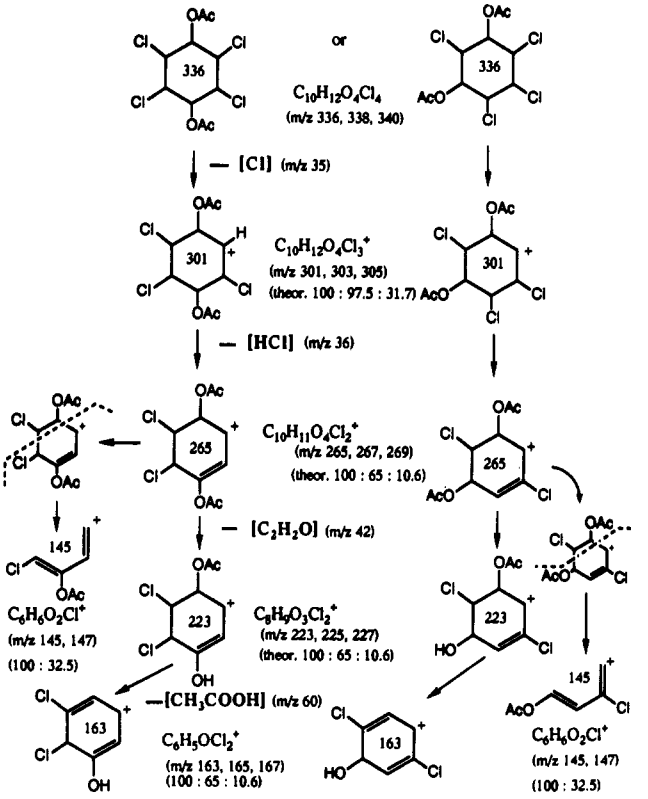

Figure 3. Possible mass fragmentation sequence of 13.276 min MS peak.

Therefore, GC–MS analysis suggested the formation of an isomer of the respective pentachlorocyclohexanol and tetrachlorocyclohexanediol during aerobic degradation of β-HCH by *Pseudomonas* sp.

Evidence for aerobic degradation of HCH isomers has not been convincing until recently. According to earlier studies (Haider, 1979), γ-HCH was degraded in pure cultures of aerobic or facultatively anaerobic bacteria, but rather slowly and only in the presence of an additional carbon source such as glucose, Although HCH isomers including the β-isomer undergo rapid biodegradation in predominantly anaerobic flooded soils, a *Clostridium sphenoides*, isolated from flooded soil, could degrade α- and γ-isomers, but not β- and δ-isomers, of HCH under anaerobic conditions (Sethunathan, 1973). There has been virtually no evidence for degradation of β-HCH in pure cultures of aerobic or anaerobic microorganisms. Recent papers from our laboratory (Sahu et al., 1990b; Bhuyan et al., 1993) and this paper provide convincing evidence for aerobic degradation of not only the γ-isomer but also α-, β-, and δ-isomers of HCH as sole source of carbon in bacterial cultures. Thus, γ-HCH is susceptible to degradation by both aerobic and anaerobic bacteria. However, there exist some distinct differences in the aerobic versus anaerobic metabolism of γ-HCH in bacterial cultures: (i) utilization of γ-HCH as a sole source of carbon for proliferation by aerobic γ-HCH degrading bacterium (Sahu et al., 1990b, 1992) vis-a-vis cometabolism in anaerobic cultures (Ohisa and Yamaguchi, 1978; Ohisa et al., 1980); (ii) transitory accumulation of γ-pentachlorocyclohexene (γ-PCH) as a product of aerobic metabolism of γ-HCH (Sahu et al., 1990b; Imai et al., 1989) and γ-tetrachlorocyclohexene (γ-TCH) as a product of anaerobic metabo-

lism of γ-HCH (Heritage and MacRae, 1977a; Jagnow et al., 1977); (iii) about 80% of the ^{14}C in γ-HCH accounted for, essentially as water-soluble metabolites in aerobic culture (this study) vis-a-vis more than 90% of the ^{14}C in γ-HCH not accounted for during its degradation in anaerobic cultures (Sethunathan et al., 1969; Haider and Jagnow, 1975), probably due to the formation of chlorine-free volatile end products such as benzene (Drego et al., 1990); (iv) mineralization of γ-HCH to CO_2 in substantial amounts (>10%) in aerobic cultures (this study) and not in anaerobic cultures (Sethunathan et al., 1969; Haider, 1979); (v) retention of γ-HCH-degrading ability in cell-free preparations of aerobic cultures (S. K. Sahu, unpublished data) and in the membrane fractions of anaerobic cultures (Heritage and MacRae, 1977b). Interestingly, however, degradation of γ-HCH in both aerobic (Sahu et al., 1990b) and anaerobic (MacRae et al., 1969) cultures led to the release of covalently linked chlorine as chloride almost in stoichiometric amounts.

There are reports of the formation of 2,4,6-trichlorophenol from HCH isomers including the β-isomer by rat liver microsomes in the presence of NADPH and molecular oxygen (Tanaka et al., 1977) but not in microbial cultures. We found that aerobic metabolism of β-HCH by *Pseudomonas* sp. led to the formation of pentachlorocyclohexanol, tetrachlorocyclohexanediol, CO_2 (this paper), and chloride (Sahu et al., 1990b). This is probably the first report of metabolic transformation of β-HCH in pure cultures of an aerobic bacterium. From the observations of this study and the earlier paper (Sahu et al., 1990b), it appears that the great potential of *Pseudomonas* sp. in degrading the HCH isomers including the β-isomer can be exploited in developing a technology for aerobic cleanup of HCH-polluted environments.

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