The Microbial Degradation of Phenylalkanes

2-PHENYLBUTANE, 3-PHENYLPENTANE, 3-PHENYLDODECANE AND 4-PHENYLHEPTANE

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1. Two *Pseudomonas* strains capable of utilizing 2-phenylbutane, 3-phenylpentane and 4-phenylheptane as the sole carbon and energy source were isolated. 2. Two *Nocardia* strains capable of utilizing only 3-phenyldodecane as the sole carbon and energy source were isolated. 3. All the isolated strains were unable to grow on the corresponding phenylalkane-*p*-sulphonates. 4. From liquid cultures of *Pseudomonas* strains utilizing 2-phenylbutane, 2-(2,3-dihydro-2,3-dihydroxyphenyl)butane was isolated and identified. Evidence for a *meta* cleavage of the benzene ring was also obtained. 5. From liquid cultures of *Pseudomonas* strains utilizing 3-phenylpentane, 3-(2,3-dihydro-2,3-dihydroxyphenyl)pentane and 2-hydroxy-7-ethyl-6-oxonona-2,4-dienoic acid were isolated and identified. 6. Evidence for the formation of both a diol and a *meta*-cleavage compound was obtained from liquid cultures of both *Pseudomonas* strains utilizing 4-phenylheptane. 7. Liquid cultures of both *Nocardia* strains utilizing 3-phenyldodecane never formed a diol or a semialdehyde-related compound. 2-Phenylbutyric acid, 3-phenylvaleric acid and 4-phenylhexanoic acid were shown to be present in these cultures.

The microbial metabolism of 1-phenyl-substituted alkanes has been reported previously. Alkyl chains with an odd number of carbon atoms undergo β -oxidation leading to cinnamic acid and benzoic acid, and phenylacetic acid is the metabolic intermediate of alkylbenzenes with an even number of carbon atoms (Webley, 1954; Webley *et al.*, 1955, 1956).

Willetts & Cain (1970) have reported that the metabolic pathway of (1-phenylundecyl)benzene-*p*-sulphonate occurs through the removal of the sulphonate grouping and the formation of a *p*-hydroxyphenylalkanoic acid; this intermediate is further degraded by β -oxidation of the alkyl chain followed by *ortho* cleavage of the aromatic nucleus.

Micro-organisms have been described that are able to grow on phenylalkanes (supplied as the sole carbon and energy source) that differ in the chain length and the phenyl-group position (McKenna & Kallio, 1964); the number of micro-organisms capable of growth on these compounds is lower the further the phenyl group is substituted towards the middle of the carbon chain (Kallio, 1969). However, the degradative metabolic pathway of these phenylalkanes has not been reported. Swisher (1963, 1964), in experiments on the biodegradation of phenylalkane-*p*-sulphonates by a mixed microflora, showed by indirect methods that γ -sulphophenylhexanoic acid and β -sulphophenyladipic acid are metabolic intermediates from 3-phenyldodecane-*p*-sulphonate and 6-phenyldodecane-*p*-sulphonate respectively.

The present paper is concerned with the first steps in the metabolic pathway of 2-phenylbutane, 3-

phenylpentane, 3-phenyldodecane and 4-phenylheptane by pure cultures of micro-organisms. The metabolism of this type of aromatic compound is of increasing importance because they are components of some detergents and herbicides. Preliminary reports of this work have appeared (Treccani, 1971).

Materials and Methods

Micro-organisms

An organism tentatively identified as *Pseudomonas acidovorans* (Stanier *et al.*, 1966) was isolated by enrichment cultures in the presence of 3-phenylpentane. Two *Nocardia* strains (strain A, forming pink colonies, and strain B, forming white colonies) were isolated by elective cultures on 3-phenyldodecane. In addition, a strain of *Pseudomonas putida*, previously isolated by elective biphenyl cultures (Catelani *et al.*, 1970), was used. At present, no micro-organisms able to grow on 5-phenylnonane, 6-phenylundecane or 7-phenyltetradecane have been isolated.

Maintenance and growth of organisms

The isolated strains were maintained on Tausson (1927) or Raymond & Davis (1960) mineral salts media, supplemented with a few drops of the aromatic carbon source. The strains were frequently plated for re-isolation on Difco nutrient agar (Difco Laboratories, Detroit, Mich., U.S.A.). For manometric experiments the organisms were cultured on

mineral salts media solidified with 1.8% (w/v) Difco agar, and supplemented with 0.2% (w/v) sodium succinate for *Pseudomonas* strains or 0.2% (w/v) sodium acetate for *Nocardia* strains. After growth the cells were harvested, washed twice by centrifugation and resuspended in 0.2M-potassium phosphate buffer, pH7, to give a total N content of 0.3mg/ml. A part of this cell suspension was further exposed to the appropriate alkylbenzene for 16h with shaking, to obtain 'induced' cells. The cells were then collected, washed twice by centrifugation and resuspended as described above. The measurement of O₂ uptake was performed in a Warburg apparatus at 30°C.

Spectral analyses

U.v. spectra were recorded in a Zeiss PMQII spectrophotometer; an Infracord model 137 (Perkin-Elmer Ltd.) was used to obtain i.r. spectra (in Nujol). Mass spectra were recorded on a LKB model 9000 spectrometer.

Chemical determinations

The presence of a free aldehyde group was assayed by the Tollens test (English, 1961). Diols were detected as described by Boyland & Wiltshire (1953). Picolinate derivatives were prepared as described by Canonica *et al.* (1966). Methyl esters were prepared from the corresponding acids in methanol, with *p*-toluenesulphonic acid as catalyst.

Chromatography

T.l.c. of phenols was done on silica-gel plates (Eastman Chromagram Sheet no. 6061) that had been activated by heating at 100°C for 30min. The developing solvent system was diethyl ether–light petroleum (b.p. 30–50°C) (1:1, v/v); spots were revealed by spraying with an aqueous 5% (w/v) solution of Fast Blue Salt B (Merck) followed by 0.1M-NaOH.

G.l.c. of phenols was performed by using a C.Erba model G.T. 200 gas chromatograph, with a flame ionization detector. A stainless-steel column (2m × 2mm internal diam.) packed with 10% SE30 on Chromosorb W (60–80 mesh) was used at the following temperatures: column 150°C, detector 190°C, injector 200°C. The carrier gas was N₂ (35ml/min). The flame was fed with H₂ at 70.7kN·m⁻² and air at 120kN·m⁻². For g.l.c. of methyl esters, a stainless-steel column of the same dimensions was used, packed with 5% ethylene glycol succinate (C. Erba LAC 886) on Chromosorb W (60–80 mesh) at the following temperatures: column 180°C, detector 210°C, injector 220°C. The N₂, H₂ and air were supplied as described above.

Chemical syntheses

3-Phenylpentane (b.p. 191°C), 4-phenylheptane [b.p. 225–226°C at 99.3kN·m⁻² (746mmHg)] and 6-phenylundecane [b.p. 122–124°C at 0.4kN·m⁻² (3mmHg)] were obtained by Grignard synthesis (Vogel, 1970, p. 256) from phenylmagnesium bromide and 3-pentanone, 4-heptanone and 6-undecanone respectively. 5-Phenylnonane [b.p. 126–127°C at 1.6kN·m⁻² (12mmHg)] and 7-phenyltetradecane [b.p. 180–181°C at 0.9kN·m⁻² (7mmHg)] were prepared from butylmagnesium bromide and valerophenone, and hexylmagnesium bromide and octanoylphenone, respectively (Vogel, 1970, p. 813). In every case, the tertiary alcohols produced were hydrogenated as described by Canonica *et al.* (1959).

Sodium benzene-*p*-sulphonates were synthesized by the method of Gray *et al.* (1955), starting from the corresponding hydrocarbons: the crude salts were crystallized from anhydrous acetone or methanol.

3-(*o*-Hydroxyphenyl)pentane [b.p. 107–108°C at 1.5kN·m⁻² (11mmHg)] and 3-(*p*-hydroxyphenyl)pentane (m.p. 54–55°C) were prepared as a mixture by alkaline fusion of the crude *p*-(1-ethylpropyl)benzenesulphonate (by the method of Boswell & Dickson, 1918); the reaction product contained about 10% of the *ortho*- and 90% of the *para*-isomer. The two phenols were then separated by steam-distillation.

3-Phenylvaleric acid (m.p. 60–61°C) was obtained by the method of Wotiz *et al.* (1953). 4-Phenylhexanoic acid [b.p. 160–163°C at 0.7kN·m⁻² (5mmHg)] was prepared as described by Kloetzel & Herzog (1950).

All the synthesized compounds were checked for purity and identity by elemental analyses and the g.l.c.–mass-spectrometry technique; their physical constants (b.p. and/or m.p.) were in complete agreement with literature values.

Chemicals

2-Phenylbutane and 3-phenyldodecane were supplied by Eastman-Kodak Co., Rochester, N.Y., U.S.A.; 2-(*o*-hydroxyphenyl)butane and 3-(*p*-hydroxyphenyl)butane from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.; 2- and 3-phenylbutyric acid and 2-phenylsuccinic acid from Fluka AG, Buchs, Switzerland. No impurities were detected by t.l.c. and/or g.l.c.

Results

Growth experiments

Ps. putida and *Ps. acidovorans* grew equally well on 2-phenylbutane, 3-phenylpentane and 4-phenylheptane supplied as the sole carbon and energy

sources, but not on 3-phenyldodecane, 5-phenylnonane, 6-phenylundecane or 7-phenyltetradecane. Both *Nocardia* strains were able to utilize only 3-phenyldodecane. All the isolated strains were unable to grow on the corresponding phenylalkane-*p*-sulphonates.

In addition, we performed growth experiments with some phenylalkanoic acids that could conceivably be intermediates of 2-phenylbutane or 3-phenyldodecane degradation if the microbial attack started from the alkyl side chain. However, 2-phenylbutyric acid, 2-phenylsuccinic acid and 3-phenylbutyric acid did not support growth of *Ps. putida*; both *Nocardia* strains were unable to utilize 2-phenylbutyric acid or 3-phenylvaleric acid. However, cultures of *Ps. putida* and *Ps. acidovorans* incubated with either 2-phenylbutane or 3-phenylpentane or 4-phenylheptane showed a positive test for diols after approx. 16, 14 and 2 h respectively. After prolonged incubation a yellow compound was formed that showed the spectrophotometric features of α -hydroxymuconic semialdehyde (λ_{max} , about 390 nm in alkaline conditions and 325 nm in acidic conditions). The diol test was negative and no evidence for semialdehyde formation was found in *Nocardia* strains cultured with 3-phenyldodecane at different incubation times.

Manometric experiments

Washed suspensions of *Ps. putida* and *Ps. acidovorans* grown on sodium succinate and washed suspensions of both strains grown on sodium succinate and further exposed to 2-phenylbutane, 3-phenylpentane or 4-phenylheptane rapidly oxidized these compounds. Both strains showed a metapyrocatechase (catechol 2,3-oxygenase) activity that was constitutively present, since washed suspensions of cells grown on succinate rapidly oxidized catechol with formation of a yellow compound that had the u.v. spectrum characteristic of 2-hydroxymuconic semialdehyde. Cells of *Ps. putida* exposed to 2-

phenylbutane showed no O_2 uptake in the presence of 2-phenylbutyric acid, 2-phenylsuccinic acid or 3-phenylbutyric acid.

Washed suspensions of both *Nocardia* strains grown on sodium acetate, and washed suspensions of both strains grown on sodium acetate and further exposed to 3-phenyldodecane, showed no significant O_2 uptake in the presence of this substrate and of 2-phenylbutyric acid and 3-phenylvaleric acid. It is noteworthy that *Nocardia* cells grown on benzoic acid slowly oxidized benzoic acid after a lag phase.

These results complicate the elucidation of the metabolic pathway of these phenylalkanes by the method of sequential induction alone.

Metabolism of 2-phenylbutane

2-(2,3-Dihydro-2,3-dihydroxyphenyl)butane. The 16 h cultures of *Ps. putida* incubated with 2-phenylbutane showed a positive test for diols. When the diol concentration in 20-litre cultures was at a maximum, the culture filtrate was adjusted to pH 8 with aq. 5% (w/v) K_2CO_3 . The precipitate of P_1 and cells was removed by filtration and the supernatant was extracted several times with diethyl ether, until the aqueous layer no longer showed a positive diol test. The solvent was dried over anhydrous MgSO_4 and evaporated in the cold under reduced pressure. The crude diol was dehydrated by refluxing for 2 h with conc. HCl -methanol-water (1:5:5, by vol.). The reaction mixture was exhaustively extracted with light petroleum (b.p. 30–50°C), then washed with aq. 5% (w/v) NaHCO_3 and dried over anhydrous MgSO_4 before the solvent was evaporated under reduced pressure. T.l.c. of the residual oil revealed two phenolic spots, the first intense (R_F 0.40) and the second faint (R_F 0.30). Authentic 2-(*o*-hydroxyphenyl)butane and 2-(*p*-hydroxyphenyl)butane under the same conditions had R_F values of 0.40 and 0.30 respectively. G.l.c. gave two peaks with areas in the ratio 95:5, at retention times of 6.0 and 7.3 min, whereas those of authentic *ortho*- and *para*-

Table 1. T.l.c., g.l.c. and mass-spectrometric properties of 2-(*o*-hydroxyphenyl)butane, 2-(*p*-hydroxyphenyl)butane and of the minor and major components of the phenolic mixture obtained by dehydration of the crude diol from 2-phenylbutane

For experimental details see the text.

	R_F	Retention time (min)	m/e ...	Relative abundance (%)						
				150	122	121	107	103	91	77
2-(<i>o</i> -Hydroxyphenyl)butane	0.40	6.0		20	6	100	12	17	17	22
2-(<i>p</i> -Hydroxyphenyl)butane	0.30	7.5		19	10	100	15	11	13	21
Major component	0.40	6.0		20	6	100	13	17	17	21
Minor component	0.30	7.3		28	12	100	12	12	11	13

isomers were 6.0 and 7.5 respectively. The mass spectra confirmed the presence in the mixture of 2-(*o*-hydroxyphenyl)butane as the major component. The minor component ($M^+ = 150$), however, had a spectrum similar to, but not superimposable on, that of 2-(*p*-hydroxyphenyl)butane (Table 1). These results, despite the lack of an authentic sample of 2-(*m*-hydroxyphenyl)butane, are strong evidence for this structure of the minor component. Hence, it is probable that the diol from 2-phenylbutane is 2-(2,3-dihydro-2,3-dihydroxyphenyl)butane (Scheme 1).

Ring-fission product of 2-phenylbutane. In other experiments *Ps. putida* was incubated by shaking in 750-ml Erlenmeyer flasks that contained 120 ml of Tausson (1927) medium, supplemented with a few drops of 2-phenylbutane. After 48–56 h of incubation a yellow colour appeared in the cultures, whereupon samples were taken, made alkaline (pH 12) with 0.1M-NaOH, and filtered. U.v. spectra gave $\lambda_{\max}$ 396 nm, which shifted to 325 nm on acidification to pH 2.5 (keto–enol tautomerism). When the absorption was at a maximum value the culture liquid, collected from several flasks, was made alkaline (pH 9) by adding aq. 5% (w/v) K_2CO_3 , then filtered and extracted three times with light petroleum (b.p. 30–50°C) to remove residual 2-phenylbutane. The aqueous phase was then acidified to pH 3 with conc. HCl and extracted with ether. No definite substance could be characterized in the residue, even when the residue was isolated under N_2 : this suggests that the intermediate was unstable. However, the residue showed a positive $FeCl_3$ test but negative Tollens test. On treatment of the crude residue from the acid ether extraction with ammonium acetate–acetic acid solution (Canonica *et al.*, 1966), the absorption maximum at 325 nm disappeared and a new maximum appeared at 267 nm, characteristic of picolinate derivatives. All these properties are consistent with a structure closely related to α -hydroxymuconic semialdehyde.

Metabolism of 3-phenylpentane

3-(2,3-Dihydro-2,3-dihydroxyphenyl)pentane. A compound that showed a positive diol reaction accumulated during the early growth stages of *Ps. putida* and *Ps. acidovorans* cultures in the presence of 3-phenylpentane. The cultures were incubated by shaking in 750-ml Erlenmeyer flasks with 120 ml of Raymond & Davis (1960) mineral salts medium. When the diol concentration was at a maximum, the culture fluid was processed as described for the diol from 2-phenylbutane. Two phenolic spots were revealed by t.l.c. of the dehydration mixture of the crude diol, and two peaks appeared on g.l.c., with areas in the ratio 95:5. T.l.c., g.l.c. and mass-spectrometric properties of the major component of the mixture were the same as those of the synthetic 3-(*o*-hydroxyphenyl)pentane, whereas the minor component was different from the synthetic *para*-isomer (Table 2).

These results are consistent with the hypothesis that the diol from 3-phenylpentane is 3-(2,3-dihydro-2,3-dihydroxyphenyl)pentane (Scheme 1).

2-Hydroxy-7-ethyl-6-oxonona-2,4-dienoic acid. On prolonged incubation a yellow colour appeared in *Ps. putida* and *Ps. acidovorans* cultures in the presence of 3-phenylpentane. The u.v. spectrum in alkaline conditions (pH 12) had $\lambda_{\max}$ 392 nm, whereas in acidic solution (pH 2.5) the maximum shifted to 316 nm. Three extractions were performed at pH 10 with light petroleum (b.p. 30–50°C) to remove the unmetabolized hydrocarbon; then the medium was acidified to pH 3 and extracted with ether. The ether was dried as usual and was then evaporated under reduced pressure in the cold. The oily residue was treated for 1 min with hot light petroleum (b.p. 30–50°C), then the solvent was decanted and the insoluble fraction was dissolved in diethyl ether (1 ml). The resulting brownish solution was cooled to –20°C and treated with a few drops of *n*-pentane. The yellow crystals that formed were filtered and immediately placed in a container, which was evacuated and then

Table 2. T.l.c., g.l.c. and mass-spectrometric properties of 3-(*o*-hydroxyphenyl)pentane, 3-(*p*-hydroxyphenyl)pentane and of the minor and major components of the phenolic mixture obtained by dehydration of the crude diol from 3-phenylpentane

For experimental details see the text.

	R_F	Retention time (min)	m/e ...	Relative abundance (%)						
				164	136	135	107	105	91	77
3-(<i>o</i> -Hydroxyphenyl)pentane	0.66	6.1		23	5	62	100	20	10	9
3-(<i>p</i> -Hydroxyphenyl)pentane	0.58	7.5		20	10	100	75	—	6	9
Major component	0.66	6.1		22	5	61	100	20	10	9
Minor component	0.40	6.8		23	9	67	100	—	10	12

filled with O₂-free N₂. Under these conditions the substance was stable and could be stored in the cold without any measurable change in properties for 2 or 3 weeks. The crystals showed a positive FeCl₃ test but negative Tollens test. The mass spectrum showed a molecular-ion peak at *m/e* 212, and other peaks at *m/e* 167 (*M* - CO₂H), 141 (*M* - C₅H₁₁), 99 (C₅H₁₁CO⁺) and 95 (C₅H₃O₂⁺) indicating the presence of a carboxyl group and an oxo group attached to a saturated C₅ alkyl chain. The i.r. spectrum showed peaks at 3445 (sharp, enolic hydroxyl group), 2700–2400 (broad, carboxyl group), 1645 (sharp, a conjugated oxo group and a carboxyl group) and 1591 cm⁻¹ (sharp, conjugated double bonds). The u.v. spectrum of the crystals (uncorr. m.p. 92°C, decomp.) showed λ_{max} 392 nm (log ε 4.5) in 0.1M-NaOH and λ_{max} 316 nm (log ε 4.2) in 0.1M-HCl (keto-enol tautomerism) (Found: C, 61.98; H, 7.4; C₁₁H₁₆O₄ requires C, 62.26; H, 7.55%). The compound gave a picolinate derivative that was not isolated: its formation was shown by the absorption band at 267 nm that appeared in ammonium acetate-acetic acid solution after incubation for 1 week at room temperature (Canonica *et al.*, 1966) while the peak at 316 nm disappeared.

Metabolism of 4-phenylheptane

Cultures of *Ps. putida* and *Ps. acidovorans* separately incubated in the presence of 4-phenylheptane, under the same conditions as described for 3-phenylpentane, showed maximum accumulation of a diol after 2 h. The alkaline ether extract also gave a positive test for diols; however, t.l.c. and g.l.c. of the residue showed the presence of different compounds.

On prolonged incubation a yellow colour developed in the cultures, with λ_{max} at 392 nm in alkaline conditions (pH 12), showing the formation of an intermediate having a structure related to α-hydroxy-muconic semialdehydes. This product, however, was present only in low amounts and did not accumulate under the experimental conditions used.

Metabolism of 3-phenyldodecane

Both *Nocardia* strains were separately incubated, by shaking, in 750 ml Erlenmeyer flasks that contained 120 ml of Raymond & Davis (1960) medium supplemented with a few drops of 3-phenyldodecane. At different incubation times samples were taken and the diol test was performed. It was always negative, as also were tests for semialdehydes.

To check the cultures for accumulation of intermediates, extractions were performed when the cultures reached the exponential phase of growth (2 or 3 days). The culture fluid collected from several flasks was made alkaline (pH 8–9) with aq. 5% (w/v) K₂CO₃; the precipitate of inorganic

phosphate and cells was filtered, and then the supernatant was extracted with light petroleum (b.p. 30–50°C) to remove the unmetabolized hydrocarbon. The aqueous phase was then acidified to pH 3 with conc. HCl and continuously extracted with diethyl ether for 24 h. The ethereal solution was dried over anhydrous MgSO₄ and the solvent was evaporated *in vacuo*. The residue was refluxed for 2 h with methanol and a few milligrams of toluene-*p*-sulphonic acid. The reaction mixture was diluted with an equal volume of water and exhaustively extracted with light petroleum (b.p. 30–50°C). The extract was washed with aq. 5% (w/v) NaHCO₃ and then with water. G.l.c. analysis revealed a set of peaks, the retention times of which corresponded to those of a series of homologous compounds. The g.l.c.-mass-spectrometry technique confirmed this assumption, showing molecular weights increasing by constant steps of 14 (=CH₂), i.e. 178, 192, 206 and 220, corresponding to the formula CH₃CH₂CH(C₆H₅)-[CH₂]_{*n*}-COOCH₃ (*n*=0, methyl 2-phenylbutyrate; *n*=1, methyl 3-phenylvalerate; *n*=2, methyl 4-phenylhexanoate; *n*=3, methyl 5-phenylheptanoate). The structures of the first three esters were confirmed by comparison with the mass spectra of authentic compounds. Correlation with these reference spectra allowed us to identify the last methyl ester as methyl 5-phenylheptanoate.

Discussion

Isolated organisms have been shown to grow on phenylalkanes differing in chain length and with the phenyl group attached to C-2, C-3 or C-4. So far we have been unable to isolate organisms that can use phenylalkanes with the phenyl group attached to C-5, C-6 or C-7. The *Pseudomonas* strains able to grow on 3-phenylpentane also utilize 2-phenylbutane and 4-phenylheptane but not 3-phenyldodecane; however, both *Nocardia* strains that are able to grow on 3-phenyldodecane are unable to utilize 3-phenylpentane and the other phenylalkanes tested.

The formation of diols is the first detectable metabolic step in the degradation of 2-phenylbutane, 3-phenylpentane and 4-phenylheptane by *Ps. putida* and *Ps. acidovorans*. The crude diols from 2-phenylbutane and 3-phenylpentane were dehydrated under acidic conditions, giving mixtures that contained two phenolic compounds in the ratio 95:5. The major component in the mixture obtained by dehydration of the crude diol from 2-phenylbutane had the same t.l.c., g.l.c. and mass-spectrometric properties as 2-(*o*-hydroxyphenyl)butane. The mass spectrum of the minor component was similar to, but not identical with, that of *para*-isomer. The dehydration mixture of the crude diol from 3-phenylpentane showed essentially similar features: the major component

showed a mass spectrum wholly superimposable on that of 3-(*o*-hydroxyphenyl)pentane, whereas the minor component was not identical with 3-(*p*-hydroxyphenyl)pentane. Hence, we suggest that the minor components in the dehydration mixtures of crude diols from 2-phenylbutane and 3-phenylpentane are 2-(*m*-hydroxyphenyl)butane and 3-(*m*-hydroxyphenyl)pentane respectively. These results are consistent with the structures of 2-(2,3-dihydro-2,3-dihydroxyphenyl)butane and 3-(2,3-dihydro-2,3-dihydroxyphenyl)pentane for the diols from 2-phenylbutane and 3-phenylpentane respectively. The formation of 2,3-diols was also demonstrated for toluene (Gibson *et al.*, 1970) and biphenyl (Lunt & Evans, 1970; Catelani *et al.*, 1971). A diol was also present in the alkaline ether extract of *Ps. putida* and *Ps. acidovorans* cultures supplied with 4-phenylheptane, but at present we have not succeeded in identifying it.

The mode of cleavage of the benzene ring of 2-phenylbutane, 3-phenylpentane and 4-phenylheptane by *Ps. putida* and *Ps. acidovorans* proceeds via *meta* cleavage, as shown by the formation of yellow compounds with an extinction at about 390 nm in alkaline conditions. We have identified the ring-fission compound of 3-phenylpentane. Its u.v. and i.r. properties, together with the formation of a picolinate derivative, are consistent with a structure related to α -hydroxymuconic semialdehyde. The negative Tollens test and the presence in the mass spectrum of a peak at *m/e* 99, corresponding to a

partial structure $C_5H_{11}CO$, indicate that 2-hydroxy-7-ethyl-6-oxonona-2,4-dienoic acid is the ring-fission product of 3-phenylpentane. It thus appears that ring-fission occurs between the C-1 and C-2 positions (extra-diol vicinal cleavage, in the nomenclature reported by Nozaki *et al.*, 1970), with the 3-amyl group intact on the aromatic ring. A similar cleavage was demonstrated for 3-methylcatechol (Catelani *et al.*, 1968). The corresponding ring-fission compound from 2-phenylbutane was not characterized. However, it may be tentatively identified as 2-hydroxy-7-methyl-6-oxonona-2,4-dienoic acid (Scheme 1) by indirect chemical evidence and by analogy with the structure of the ring-fission product from 3-phenylpentane.

Pseudomonas strains oxidized the tested phenylalkanes by enzymic systems that were constitutively present. However, cells exposed to 2-phenylbutane did not consume O_2 in the presence of 2- and 3-phenylbutyric acid and 2-phenylsuccinic acid. In addition, these phenylsubstituted acids did not support the growth of both *Pseudomonas* strains. These results agree with the biochemical results and confirm that the degradation of 2-phenylbutane occurs at the aromatic ring.

The degradation of 3-phenyldodecane by *Nocardia* strains seems to start from the alkyl side chain. The formation in cultures of a diol or an α -hydroxymuconic semialdehyde-type compound was not observed. The g.l.c.-mass-spectrometry analysis of the acid ether extracts showed the presence of 2-

Scheme 1. Proposed pathway of 2-phenylbutane and 3-phenylpentane degradation by *Ps. putida* and *Ps. acidovorans*

phenylbutyric acid, 3-phenylvaleric acid, 4-phenylhexanoic acid and 5-phenylheptanoic acid. These results are in contrast to the growth experiments: both *Nocardia* strains were unable to utilize the above phenylalkanoic acids when these were supplied as the sole carbon and energy sources. In addition, resting cells of *Nocardia* strains exposed to 3-phenyl-dodecane or benzoic acid did not consume O₂ in the presence of these substrates. Further work with cell-free systems is necessary to clarify these phenomena.

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