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MICROBIAL METABOLISM OF AROMATIC COMPOUNDS

I. DECOMPOSITION OF PHENOLIC COMPOUNDS AND AROMATIC HYDROCARBONS BY PHENOL-ADAPTED BACTERIA

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ABSTRACT

TABAK, HENRY H. (Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio), CECIL W. CHAMBERS, AND PAUL W. KABLER. Microbial metabolism of aromatic carbon compounds. I. Decomposition of phenolic compounds and aromatic hydrocarbons by phenol-adapted bacteria. J. Bacteriol. **87**:910-919. 1964.—Bacteria from soil and related environments were selected or adapted to metabolize phenol, hydroxy phenols, nitrophenols, chlorophenols, methylphenols, alkylphenols, and arylphenols when cultured in mineral salts media with the specific substrate as the sole source of carbon. A phenol-adapted culture (substrate-induced enzyme synthesis proven) was challenged in respirometric tests with 104 related compounds; probable significant oxidative activity occurred with 65. Dihydric phenols were generally oxidized; trihydric phenols were not. Cresols and dimethylphenols were oxidized; adding a chloro group increased resistance. Benzoic and hydroxybenzoic acids were oxidized; sulfonated, methoxylated, nitro, and chlorobenzoic acids were not; *m*-toluic acid was utilized but not the *o*- and *p*-isomers. Benzaldehyde and *p*-hydroxybenzaldehyde were oxidized. In general, nitro- and chloro-substituted compounds and the benzenes were difficult to oxidize.

The dissimilation of phenols and aromatic hydrocarbons by bacteria has been reported by many workers. Results of early studies indicating that microorganisms are able to degrade ring compounds were reported by Buddin (1914), Sen Gupta (1921), Dooren de Jong (1926), Tattersfield (1928), and Gray and Thornton (1928). Stanier (1947) described his technique of simultaneous adaptation for determining metabolic pathways in the bio-oxidation of aromatic compounds and also showed that fluorescent pseudomonads attack many aromatic compounds (Stanier, 1948). Subsequent studies on the mechanism, optimal conditions for degradation, and the intermediate products of the metabolism of aromatic compounds by microorganisms were reviewed by Happold (1950). ZoBell (1946, 1950), in reviewing literature relating to the action of microorganisms on hydrocarbons, mentioned numerous phenolic compounds and hydrocarbons oxidized by bacteria, and the sources and types of organisms involved.

Few studies have been made concerning the effect of chemical structure on the ability of bacteria adapted to a given aromatic compound to oxidize related compounds. Czekalowski and Skarzynski (1948) investigated the relationship between chemical structure and the use of aromatic compounds by one phenol-tolerant strain of *Achromobacter*, and Kramer and Doetsch (1950) used *Achromobacter*, *Micrococcus*, and *Vibrio* species that are capable of utilizing phenols to study their ability to grow on related aromatic compounds.

The purpose of this investigation was to determine the ability of specifically adapted bacteria to degrade phenol and substituted phenols, and to study the relationship between the chemical structure of phenol derivatives and cyclic hydrocarbons and their susceptibility to decomposition by organisms adapted to related aromatic compounds.

MATERIALS AND METHODS

Selection and adaptation of bacteria to utilize phenol and substituted phenols. Organisms were obtained from garden soil, compost, river mud, and sediment from a waste lagoon of a petroleum refinery catalytic cracking plant. Methods used in selecting or adapting organisms to degrade these compounds were soil perfusion, continuous-feed activated sludge, primary enrichment in flasks on a shaker, and enrichment in batch-type

fermentors. In all instances, the material containing organisms subjected to preliminary enrichment was eventually inoculated in baffled Erlenmeyer flasks containing 50 ml of Gray and Thornton's (1928) mineral salts medium to which 0.025 μg of vitamin B_{12} had been added. The specific phenolic compound served as the sole source of carbon. The medium was prepared by aseptically adding concentrates to sterile distilled water. Final pH was 7.0 to 7.2. All cultures were incubated at room temperature on an orbital shaker.

Subcultures in the same medium were made periodically for several weeks or months. All traces of organic nutrients as debris carried in the original inoculum were rapidly lost, and all nonbacterial forms soon disappeared. As soon as the oxidative capacity of the selected bacteria could be demonstrated in media containing a low concentration of the phenolic substrate, 2 to 10 ml of this material were subcultured into media containing progressively increasing concentrations of the same compound. The increase in concentration of the substrate throughout the culture enrichment period ranged from 100 ppm to a maximum of 500 ppm. Compounds used were Eastman Grade (highest purity chemicals suitable for reagent use) or Eastman Practical Grade (suitable for most laboratory syntheses). Occasionally, comparable grades from other sources were used.

Identification of organisms. The mixed cultures were streaked on Tryptose Agar, Trypticase Soy Agar, yeast extract-agar, *Rhizobium* agar, and egg albumin-agar. The pure cultures isolated from these plates were examined for motility; reaction to Gram stain; ability to ferment lactose, glucose, sucrose, salicin, rhamnose, dulcitol, mannitol, maltose, and xylose; capacity to produce hydrogen sulfide, ammonia, indole, and urease; and ability to convert nitrates to nitrites. Their reaction in litmus milk and ability to liquefy gelatin and hydrolyze starch were also determined. The presence of pseudomonad strains was confirmed by the use of the cytochrome oxidase test (Gaby and Hadley, 1957; Gaby and Free, 1958).

Determination of the amount of phenolic substrate remaining. At appropriate intervals during incubation, contents of flasks were restored to the original volume. For analysis of remaining phenolic compound, solids were separated by centrifugation; the supernatant fluid was filtered through a Whatman 50 filter paper. With nitrophenols, degradation was determined spectrophotometrically by measuring the intensity of the yellow color, characteristic of these compounds, at 400 mμ in alkaline solution at a pH higher than 7.0 (Gundersen and Jensen, 1956), and by determining the amount of organic nitrogen converted into nitrite-nitrogen, one of the intermediate products of nitrophenol metabolism (Rider and Mellon, 1946). The 4-aminoantipyrine method (Ettinger, Ruchhoft, and Lishka, 1951; Mohler and Jacob, 1957) was used spectrophotometrically to determine residual concentrations of phenols other than nitrophenols. The color absorbance of aqueous solutions and chloro form extracts was determined at wavelengths of 460 and 510 mμ, respectively. The solutions of test compounds from the respective uninoculated control flasks were also analyzed to verify that any loss of substrate in the test flask was not due to volatilization loss or chemical oxidation. To follow the increase in oxidative ability of organisms and make periodic determinations of the rate of dissimilation of phenols, the 4-aminoantipyrine test was modified for use as a semimicro-method. This test required small amounts of medium and was useful as a rapid screen test for most of the non-nitrogenous compounds used.

Manometric studies. The purpose of the respirometric work was to determine whether organisms that had been adapted to utilize phenol could immediately degrade structurally related compounds to which they had not been adapted. The formation of adaptive enzymes to metabolize phenol was indicated by a marked lag in oxygen uptake in respirometric tests with phenol when organisms were grown in nutrient broth. Standard techniques described by Umbreit, Burris, and Stauffer (1959) were used.

Phenol-adapted bacteria were inoculated in Gray and Thornton's liquid mineral salts medium containing 300 ppm of phenol as the only source of carbon, and incubated on a shaker at room temperature for 16 hr. The cells were then removed by centrifugation, washed several times with buffered dilution water (American Public Health Association, 1960), stored overnight at 5 C in the same buffer, aerated 3 to 4 hr, removed by centrifugation, and resuspended in 0.067 M phosphate buffer at pH 7.2 (Clark, 1920). Each Warburg flask contained an appropriate amount

TABLE 1. *Frequency of occurrence and distribution of bacteria degrading phenolic compounds*

Classes of compounds degraded	Sources of cultures	Generic grouping of culture*			
		Pseudomonas	*Achromobacter*	*Flavobacterium*	*Xanthomonas*
Nitrophenols	Garden soil, compost, and river mud	23	3	14	3
Chlorophenols	Sediment from petroleum refinery wastes lagoon	37	3	3	0
Cresols	Sediment from petroleum refinery wastes lagoon, garden soil, and compost	48	8	4	0
Phenol	Sediment from petroleum refinery wastes lagoon	12	1	1	0
Alkyl phenols	Garden soil, compost, and refinery lagoon sediment	8	0	1	1
Aryl phenols	Garden soil, compost, and refinery lagoon sediment	11	4	1	0
Hydroxy phenols	Garden soil, compost, and refinery lagoon sediment	13	6	1	0
Per cent occurrence†		73.8	12.1	12.1	1.93

* Figures represent number of cultures, each having different biochemical or other characteristics, isolated from media in which a compound in the class indicated served as the sole source of carbon.

† Per cent of a total of 206 isolations falling in the respective genera indicated.

of 0.067 M buffer at pH 7.2 and 0.5 ml of cell suspension in the main compartment, 0.2 ml of 10% KOH solution in the center well, and an amount of stock solution of substrate in the side arm necessary to produce the desired test concentration. The total volume of reagents and cell suspension added to a flask was 3.2 ml. A flask containing substrate without cell suspension was included for each compound to control chemical oxidation, along with an endogenous control and a test with phenol plus cells to confirm that the organisms used in the test had a normally high capacity to utilize phenol. The uniformity of oxygen uptake in the phenol controls indicated there was little variation in the activity of the cell suspensions used in different experiments. All flasks were incubated in a Warburg water bath at 30 C and shaken at a speed of 68 strokes per min. The gas phase was air. Results of 10-min observations of O_2 uptake were averaged for each successive 30-min interval.

Chemical analysis of residual substrate. Whenever the observed oxygen uptake appeared to be significant, the centrifuged supernatant fluid from the respirometric test was analyzed for residual substrate. Phenols were analyzed by the same methods used during culture enrichment. Nitro-aromatic compounds other than nitrophenols were analyzed by the methods of Porter (1955) and Heotis and Cavett (1959).

RESULTS

Degradation of phenol and substituted phenols by cultural methods. Bacteria utilizing phenolic compounds were obtained from all environments sampled. Table 1 shows the original sources of the bacteria and their frequency of occurrence. In the case of the culture utilizing catechol, continued subculture produced a pure culture. In all other instances, enrichment methods yielded mixed cultures of species common to all sample sources. These bacteria were all gram-negative, nonsporulating, aerobic, small to medium sized rods. Optimal growth occurred at 20 to 25 C, or at 35 C. Both motile and nonmotile forms were found. On the basis of colony and cell morphology, motility, Gram's stain, pigment production, and biochemical tests, the following genera were distinguished: *Pseudomonas*, *Flavobacterium*, *Achromobacter*, and *Xanthomonas*. These genera are noted for borderline biochemical reactions, and the findings in this investigation confirm previous reports (Davey, 1961; Haynes, 1951; Rhodes, 1959) regarding the difficulty of defining

these species by any arbitrary selection of morphological and biochemical characters. Data showing the ability of these bacteria to degrade their respective parent substrates are presented in Table 2.

It should be noted that during incubation of nitrophenol-containing culture, the yellow color, characteristic of nitrophenols and chloronitrophenols, progressively faded and finally disappeared from the medium. The color loss coincided with the appearance of abundant growth and disappearance of the substrate from the medium. With the culture enriched on 2,4,6-trinitrophenol, instead of the fading observed with other nitrophenols the color changed from yellow to orange-red; this color persisted in flasks containing the higher concentrations but gradually disappeared in the lower concentrations. The reaction of the medium changed from pH 7.2 to 6.3, and the yellow color was not restored by readjusting to pH 7.2.

Manometric studies with phenol-adapted bacteria. The relationship between molecular structure and ease of degradation was evaluated on a basis of tests with a total of 104 compounds in the following classes: phenols, benzyl alcohols, benzoic acids, benzaldehydes, benzenes, cyclohexane, and heterocyclic compounds. The results obtained with substituted phenols indicated that the phenol-adapted culture had a significant degree of ability to oxidize many of these compounds, while other compounds were relatively resistant. There was no measurable biological oxygen consumption with pyrogallol, the only compound showing a significant oxygen uptake in the chemical oxidation control.

TABLE 2. *Time required for bacteria to utilize 95% of parent substrate*

Compounds degraded in*		
1 to 2 days	3 to 6 days	7 to 10 days
Phenol	*o*-Nitrophenol	2,4-Dinitrophenol
	m-Nitrophenol	
Catechol	*p*-Nitrophenol	2,6-Dimethylphenol, 200 ppm
Resorcinol	2,4,6-Trinitrophenol, 250 ppm	
Quinol		2,4-Dichlorophenol, 200 ppm
Phloroglucinol	2-Chloro-4-nitrophenol	
o-Cresol		2,4,6-Trichlorophenol
m-Cresol	2,6-Dichloro-4-nitrophenol	
p-Cresol		
	m-Chlorophenol, 150 ppm	
	p-Chlorophenol	
	o-Phenylphenol, 100 ppm	
	Thymol, 150 ppm	

* Initial concentration was 300 ppm unless indicated otherwise.

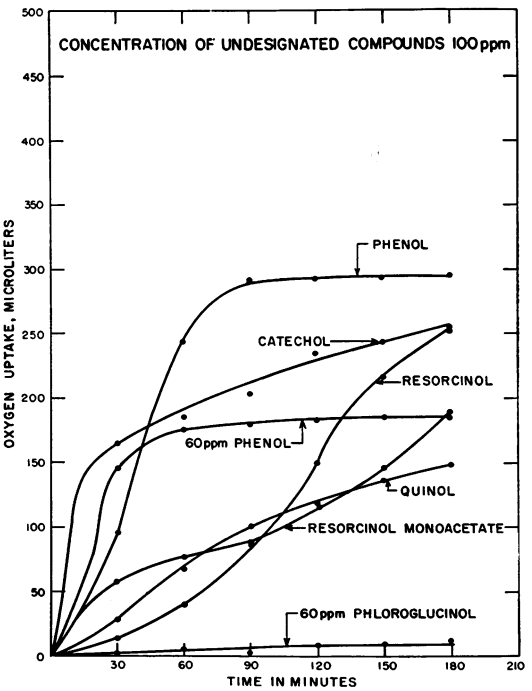

FIG. 1. *Oxidation of hydroxyphenol derivatives.*

Results presented in Fig. 1 indicate that all hydroxyphenols except phloroglucinol and pyrogallol were degraded; Fig. 2 shows that the oxygen uptake with aminophenols, alkylphenols, arylphenols, and chloronitrophenols varied from 50 to 150 μliters. The activity with cresols was higher than with phenol, and orcinol, thymol, and the dimethylphenols were less susceptible to dissimilation than was phenol (Fig. 3). The presence of a chlorine atom increased the resistance of phenol and methylphenols (Fig. 4), and this resistance to decomposition was likewise observed with nitro-aromatic compounds (Fig. 5). Degradation of benzoic and other aromatic acids depended on the nature of the groups

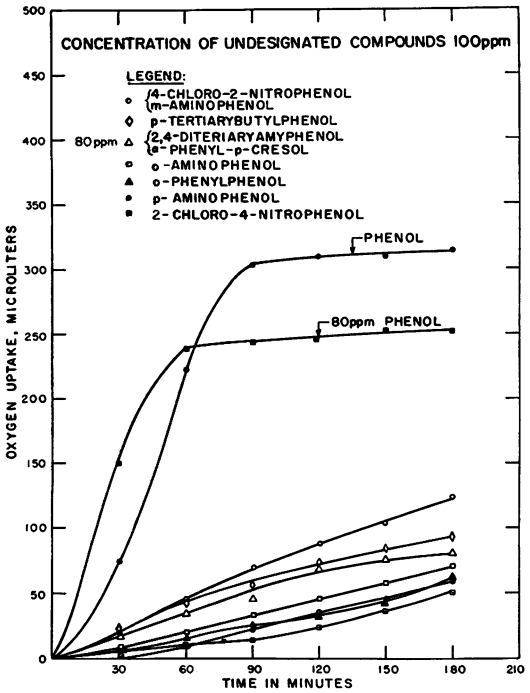

FIG. 2. *Oxidation of alkyl, aryl, chloro-nitro, and aminophenol derivatives.*

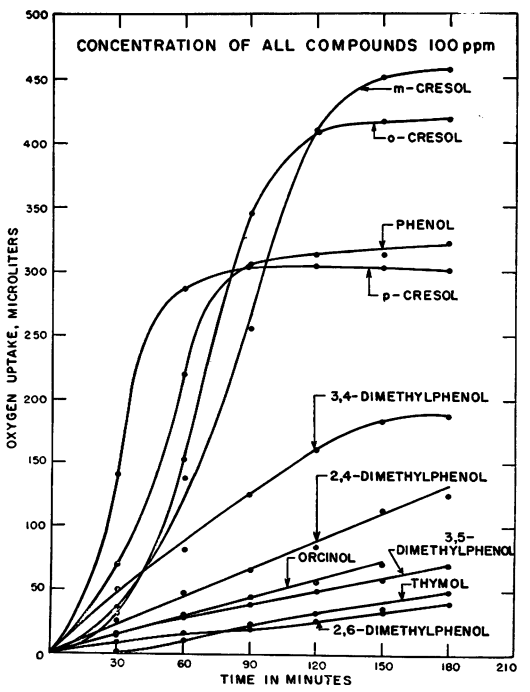

FIG. 3. *Oxidation of cresols and other methylphenol derivatives.*

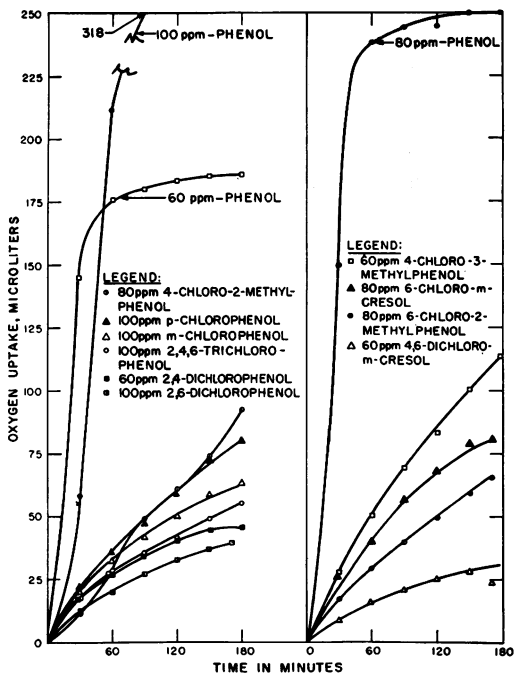

FIG. 4. *Oxidation of chlorophenols and chloromethylphenols.*

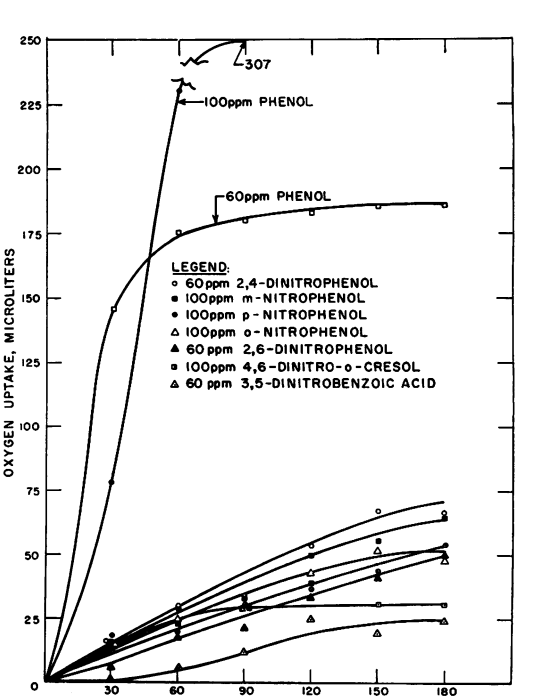

FIG. 5. *Oxidation of nitro aromatic compounds.*

attached to the aromatic ring (Fig. 6). Benzoic acid and the hydroxybenzoic, shikimic, and amino acids had sizable oxygen uptake rates, whereas nitro, chloro, and methoxylated benzoic acids, and benzenesulfonic acids were relatively resistant to decomposition. Activity was high with benzaldehyde and *p*-hydroxybenzaldehyde (Fig. 7), moderate with the methoxylated benzaldehyde, vanillin, and low with the nitrobenzaldehydes and an amide, benzamide. None of the benzenes was rapidly oxidized. High resistance to oxidation was observed with benzene, chlorobenzenes, and nitrobenzenes, whereas nitrotoluenes exhibited a steady but low oxygen uptake (Fig. 7). Although a measurable degree of activity was observed with aniline, the nitroanilines were less susceptible to oxidation. There was little oxygen uptake with either benzyl alcohol or DL-α-methylbenzyl alcohol, but a heterocyclic compound, quinoline, exhibited significant activity.

Quantitative chemical determinations. Analyses at the conclusion of the respirometric tests indicated a definite correlation between oxygen consumption and the depletion of the phenolic substrate. The correlation became more apparent as the oxygen uptake increased, and this reduced

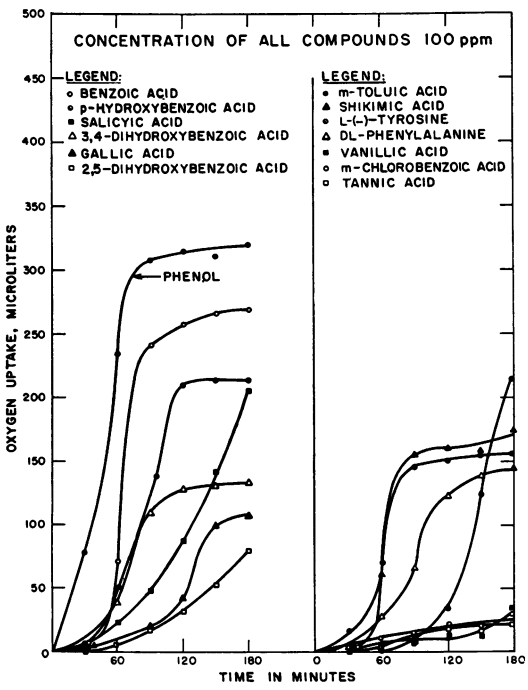

FIG. 6. *Oxidation of benzoic and other acids.*

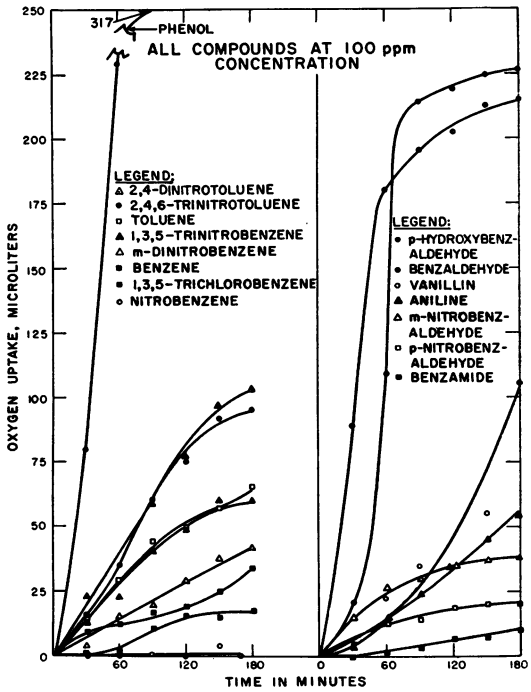

FIG. 7. *Oxidation of benzenes, anilines, and benzaldehydes.*

the possibility that adsorption of substrate by the organisms was a significant factor in removal of the test compound. Data showing the correlation between oxygen uptake and disappearance of substrate are presented in Table 3. Compounds resistant to decomposition are presented in Table 4.

DISCUSSION

A considerable number of the enriched cultures have been screened for adaptive enzyme formation. In all instances, an immediate and rapid oxygen uptake was observed in respirometric tests with the compound used in the original enrichment of the culture if the cells were harvested from a medium containing the same substrate as the sole source of carbon. A marked lag in oxygen consumption was noted in parallel tests when the bacteria were grown in nutrient broth. These findings indicate adaptive enzyme formation (Stanier, 1948).

The results obtained indicate that pseudomonads occur in a variety of natural environments and readily adapt to utilize phenol derivatives. Similar trends were reported by Parr, Evans, and Evans (1959), Simpson and Evans

TABLE 3. *Relationship between O_2 uptake and decomposition of substrate*

Test compound	Test concn		Amt of O_2 consumed* (endogenous corrected)
	Initial ppm	Loss ppm	
			μliters
Phenol	100	99	319
Phenol	80	79	252
Phenol	60	59	186
Catechol	100	97	255
Resorcinol	100	98	252
Quinol	100	86	149
Phloroglucinol	60	3	12
m-Chlorophenol	100	50	66
p-Chlorophenol	100	66	80
2,4-Dichlorophenol	60	18	46
2,6-Dichlorophenol	100	35	39
2,4,6-Trichlorophenol	100	70	56
o-Cresol	100	97	417
m-Cresol	100	97	457
p-Cresol	100	97	306
2,6-Dimethylphenol	100	69	40
3,5-Dimethylphenol	100	37	70
2,4-Dimethylphenol	100	81	126
3,4-Dimethylphenol	100	90	189
Orcinol	100	36	72
Thymol	100	44	48
6-Chloro-*m*-cresol	80	51	81
6-Chloro-2-methylphenol	80	37	66
4-Chloro-2-methylphenol	80	50	90
4-Chloro-3-methylphenol	60	46	113
o-Nitrophenol	100	49	48
m-Nitrophenol	100	39	65
p-Nitrophenol	100	32	54
2,4-Dinitrophenol	60	19	66
2,6-Dinitrophenol	60	8	51
2,4,6-Trinitrophenol	100	28	22
4,6-Dinitro-*o*-cresol	100	60	31
2,4,6-Trinitroresorcinol	60	13	6
2,4,6-Trinitro-*m*-cresol	60	8	14
4-Chloro-2-nitrophenol	100	64	123
2-Chloro-4-nitrophenol	60	7	51
2,6-Dichloro-4-nitrophenol	100	9	35
m-Dinitrobenzene	100	25	42
p-Dinitrobenzene	100	20	32
m-Nitroaniline	100	31	70
2,4-Dinitroaniline	100	39	53
m-Nitrobenzaldehyde	100	27	38
3,5-Dinitrobenzoic acid	100	13	48

* Based on 180 min results with all except orcinol; if endogenous rate was reached sooner, result is that obtained at corresponding time.

(1953), Durham (1957), Rogoff and Wender (1959), Marr and Stone (1961), and Davey (1961).

Differences in resistance to degradation were noted within each of several well-defined groups of phenol derivatives as well as between these classes of compounds as a whole. This relationship was also apparently affected by the position of a group on the ring and by the size and complexity of the substituents.

The results obtained with substituted phenols indicate the possibility of a relationship between structure and susceptibility to bacterial degradation. The presence of more than two hydroxyl groups on the ring appeared to increase resistance to decomposition, e.g., phloroglucinol and pyrogallol, while dihydric phenols seem to be oxidized to about the same or a slightly lesser degree than phenol. Similar results were reported by Happold and Key (1932), Evans (1947), Czekalowski and Skarzynski (1948), Kramer and Doetsch (1950), Stanier (1950), Stanier et al. (1950), and Kilby (1951). Addition of a nitro group to the ring markedly increased the resistance to degradation, and none of the nitrophenols, with the exception of *o*- and *m*-nitrophenol and 2,4-dinitrophenol, had an appreciable oxygen uptake. Simple nitrophenols were degraded by bacteria, whereas those with more complex groups were resistant. These trends are similar to those reported by Kramer and Doetsch (1950) and Czekalowski and Skarzynski (1948). Chlorosubstitution also increased resistance to degradation, dichlorophenols being more refractory than monochlorophenols. Adding a methyl group reduced resistance, as was shown by the high level of activity with cresols and some dimethylphenols. The effect of position of substitution on the ring was illustrated with cresols and dimethylphenols. With cresols there is some indication that substitution of a methyl group in the *para* position resulted in a higher initial oxygen uptake rate but a lower total oxygen uptake than with the *ortho* and *meta* isomers. The same effect on total oxygen uptake, but not on the rate, was observed with *para*-substituted dimethylphenols where a high oxygen uptake was observed with 2,4,- and 3,4-dimethylphenol while the activity was lower with dimethylphenols having methyl groups in other positions. All monochloromethylphenols had a measurable oxygen uptake, whereas dichloro-

TABLE 4. *Compounds resistant to degradation**

Phenol derivatives	Benzoic acids	Benzene derivatives
Phloroglucinol	*o*-Nitrobenzoic acid	Chlorobenzene
Pyrogallol	*m*-Nitrobenzoic acid	*o*-Dichlorobenzene
Dimethyldihydroresorcinol	*p*-Nitrobenzoic acid	1,3,5-Trichlorobenzene
2,6-Dichlorophenol	2,5-Dinitrobenzoic acid	Nitrobenzene
2,6-Dinitrothymol	3,4-Dinitrobenzoic acid	*p*-Dinitrobenzene
2,6-Dinitro-*o*-cresol	3,5-Dinitrobenzoic acid	*p*-Nitroaniline
2,4,6-Trinitrophenol	3,5-Dinitrosalicylic acid	*p*-Phenylenediamine
2,4,6-Trinitro-*m*-cresol	2,4,6-Trinitrobenzoic acid	Benzyl alcohols
2,4,6-Trinitroresorcinol	*o*-Chlorobenzoic acid	Benzyl alcohol
4,6-Dichloro-*m*-cresol	*m*-Chlorobenzoic acid	DL-Methylbenzyl alcohol
2,6-Dichloro-4-nitrophenol	*o*-Toluic acid	Miscellaneous compounds
2,4-Dibromophenol	*p*-Toluic acid	Phthalic acid
m-Phenoxyphenol	Syringic acid	DL-Mandelic acid
Benzaldehydes	Vanillic acid	Anthranilic acid
m-Nitrobenzaldehyde	Benzensulfonic acids	Tannic acid
p-Nitrobenzaldehyde	Benzenesulfonic acid	Benzamide
Benzene	*p*-Toluenesulfonic acid	Cyclohexanol

* Compounds having a maximal endogenous corrected O_2 uptake value below 40 μliters.

methylphenols were more resistant. This indicates that dichlorosubstitution increased the resistance of both chlorophenols and chloromethylphenols. Adding a methoxyl or a phenoxyl group to the ring also increased resistance.

Differences in oxygen uptake related to molecular structure and substitution were also observed with benzoic acids. Activity with benzoic acid was higher than with its dihydroxy derivatives, and the latter had greater activity than did the trihydroxy benzoic acids. In all instances, the hydroxybenzoic acids were more susceptible to degradation than other substituted benzoic acids. The influence of positional substitution was demonstrated by methylbenzoic acids because *m*-toluic acid was more readily oxidized than the *ortho* and *para* derivatives. With methoxylated benzoic acids, increasing the number of methoxyl groups on the ring apparently interferes with oxidation, syringic acid being more resistant to degradation than vanillic acid. The presence of more than one carboxyl group significantly reduced the rate of oxygen uptake by the benzoic acids. In comparing the susceptibility of benzoic and hydroxybenzoic acids with that of nitrobenzoic acids, the number of compounds tested appears to be sufficient to provide some basis for concluding that the former were readily oxidized by phenol-adapted bacteria and the latter were extremely resistant. Similar trends were reported by Kramer and Doetsch (1950), Stanier (1948, 1950), Evans (1947), Evans, Parr, and Evans (1949), Evans et al. (1951), Cain (1958), and Cain, Ribbons, and Evans (1961).

With *p*-hydroxybenzaldehyde, replacement of the hydroxyl by a nitro group blocked the activity. This was demonstrated by the resistance of *p*-nitrobenzaldehyde to decomposition. The resistance of benzamide indicated that the presence of a $CONH_2$ group also increased the resistance.

Results obtained with benzene and its chloro derivatives, regardless of the number or position of the chlorosubstitutions, indicated that, in general, they were resistant to degradation. The effect of a nitro group was significant because such compounds as nitrobenzene and *m*- and *p*-dinitrobenzene showed little activity, particularly the mononitrobenzene whose activity was below the endogenous level. The resistance of the nitrobenzenes to degradation seemed to decrease as the number of nitro groups on the benzene ring increased. Neither the methyl nor amino group appeared to interfere completely with oxidation of benzene, because both aniline and toluene were oxidized to a limited degree. Substitution of a nitro group on the benzene ring of aniline markedly increased the resistance, as was demonstrated with the nitroanilines, *p*- and *m*-nitroaniline. There appeared to be a slight difference in oxidizability between the nitroanilines, which increased in the order of

m-, *p*-, *o*-. Results obtained with an aminoaniline, *p*-phenylenediamine, indicate that adding a second amino group to the benzene molecule increases resistance to oxidation.

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