MICROBIOLOGICAL REVIEWS, Sept. 1979, p. 422-442
0146-0749/79/03-0422/21$02.00/0

Vol. 43, No. 3

Secondary Metabolites of the Fluorescent Pseudomonads

T. LEISINGER* AND R. MARGRAFF†

Mikrobiologisches Institut, Eidgenössische Technische Hochschule, ETH-Zentrum, CH-8092 Zurich, Switzerland

INTRODUCTION

Microbiologists working in the field of secondary metabolism are confronted with the question: What is the role of secondary metabolites in the physiology and ecology of the producing organisms? An answer to this problem would not only enlarge our understanding of an important biological phenomenon, but it might also provide industrial microbiologists with strategies for increasing the yield of economically important microbial products. In recent years it has become obvious that there is no general solution to this enigma, but rather there are as many answers as there are secondary metabolites (153). It has also become apparent that an understanding of the controls affecting the production of a particular secondary metabolite can only be reached by extensive studies on its biosynthetic pathway, on the regulatory mechanisms involved in its formation, and on the genetics and physiology of the producing organism (63). It is obvious to choose secondary metabolites of industrial importance and strains with a high producing capacity for studies on secondary metabolism. In the past this approach has been followed by industrial microbiologists, and, while leading to considerable practical success in increasing the yields of antibiotics, it has failed to reveal the regulatory processes governing the production of secondary metabolites. There are numerous reports offering phenomenological descriptions of the parameters affecting the formation of secondary metabolites, but there is a definite lack of studies at the biochemical and genetic levels. In part this may be due to the complex biosynthetic pathways of many secondary metabolites, to the variety of organisms studied, to the absence of suitable genetic systems, and to the association of the formation of many secondary metabolites with processes of differentiation, adding a further dimension of complexity to the regulatory mechanisms involved.

In the present contribution we summarize information on the production of secondary metabolites by pseudomonads, with special emphasis on the fluorescent pseudomonads. The more important groups of secondary metabolites are discussed and, where available, information on their biosynthesis and their mode of action is reviewed. Pseudomonads represent the major group of non-differentiating microorganisms producing antibiotics. The lack of cytological and physiological differentiation makes it possible to study the effect of various factors on secondary metabolism without interference from genetically programmed development cycles. The pseudomonads may therefore offer experimental advantages over the filamentous fungi, the actinomycetes, and the bacilli for studying certain facets of secondary metabolism.

PATTERN OF SECONDARY METABOLITES LITES PRODUCED BY PSEUDOMONADS

Fluorescent pseudomonads, the most intensively studied group among *Pseudomonas* species, comprise *P. aeruginosa*, the type species of the genus, *P. fluorescens* (four biotypes), *P. putida* (two biotypes), *P. chlororaphis*, *P. aureofaciens*, and the two plant pathogenic species *P. cichorii* and *P. syringae*. The latter species includes a large number of nomenspecies (23).

† Permanent address: Rhône-Poulenc Industries, Centre de Recherches Nicolas Grillet, F-94400 Vitry s/Seine, France.

All fluorescent pseudomonads fall into one of the five "ribonucleic acid (RNA) homology groups" defined by ribosomal RNA-deoxyribonucleic acid competition experiments (109). The guanine-plus-cytosine content of their deoxyribonucleic acid ranges from 58 to 68 mol% (108). In common with the other species of the genus *Pseudomonas*, the fluorescent pseudomonads are gram-negative, strictly aerobic, polarly flagellated rods. The group is quite heterogeneous and, apart from the ability to produce water-soluble fluorescent pigments of unknown chemical structure, there are no phenotypic properties common to all members of the group. In recent years the genetics and biochemistry of pseudomonads, especially of *P. aeruginosa* and *P. putida*, have been explored intensively (20, 59). The wide interest in these organisms stems from the role of *P. aeruginosa* as an opportunistic pathogen in humans, from the enormous catabolic potential of the pseudomonads, from their use in the industrial conversion of organic chemicals, and from the plant diseases caused by some members of the genus.

Apart from the pigments, most secondary metabolites produced by the fluorescent pseudomonads have been detected and investigated because of their antibiotic activity. The practical use of antibiotics from pseudomonads dates back to the period before the "antibiotic era." In 1899 Emmerich and Löw (29) reported that the cell-free culture fluid of *P. aeruginosa*, concentrated to one-tenth of its original volume, killed several kinds of bacteria. Because the preparation exhibited enzymatic activities, it was called pyocyanase. It has been used extensively in the therapy of diphtheria, influenza, and meningitis during the first two decades of this century. Pyocyanase has been produced commercially, but its clinical use was abandoned as the preparations, probably because of strain degeneration, became less potent and varied in efficacy. During the antibiotic era approximately 50 different antibiotic substances from pseudomonads have been discovered (5). Only two of these, pyocyanine and pyrrolnitrin, have been produced on a commercial basis. Although this number may increase in the future, it is clear that the significance of the pseudomonads as producers of industrially important secondary metabolites is very limited.

The known chemical structures of secondary metabolites produced by fluorescent pseudomonads are listed in Table 1. Most of them exhibit antibiotic or phytotoxic activity. These secondary metabolites were detected as a result of their biological activity and, as is the case with other microorganisms, secondary products devoid of antibiotic activity have escaped detection. Table 1, therefore, probably represents a distorted picture of the secondary metabolites from pseudomonads. Nevertheless, it can be said that compared to the variety of antibiotics produced by the *Actinomycetales* and the fungi, antibiotics produced by the pseudomonads cover a more restricted range of chemical structures. Macrolides, aminoglycosides, polyenes, quinone-type antibiotics, oxygen-containing heterocycles, and alicyclic antibiotics have not been found among the secondary metabolites produced by the pseudomonads. Most antibiotics isolated from *Pseudomonas* culture filtrates, namely, phenazines, pyrrolnitrin-type antibiotics, pyo compounds, and indole derivatives, fall into the class of N-containing heterocycles and have been shown to originate from intermediates or end products of the aromatic amino acid biosynthetic pathway. Another substantial class of *Pseudomonas* secondary products comprises unusual amino acids and peptides. In addition to these two major groups of secondary metabolites, some glycolipids, lipids, and aliphatic compounds have been isolated from *Pseudomonas* cultures and are listed in Table 1.

In a few cases it is possible to correlate the pattern of secondary metabolites of pseudomonads with metabolic or ecological properties specific for this group of organisms. Although the presence of most antibiotics from various organisms cannot be demonstrated in the natural environment of the producing organisms (40), it has been demonstrated that some phytotoxins are produced by pseudomonads in infected plants, the natural habitat of the phytopathogenic pseudomonads (102). It thus seems that production of phytotoxins is one of the rare examples where the production of secondary metabolites confers obvious selective advantages to the producing organisms, enabling them, together with other, as yet unknown factors, to colonize specific host plants, i.e., ecological niches otherwise not accessible (111). Slime production by *P. aeruginosa* furnishes another example of a selective advantage due to the formation of a secondary metabolite. From respiratory infections, accompanying cystic fibrosis, mucoid mutants of *P. aeruginosa* can be isolated (31, 41). The production of an alginate-type heteropolysaccharide (8), consisting of β-1,4-linked D-mannuronic acid and L-guluronic acid and containing *O*-acetyl groups (31), leads to increased resistance to carbenicillin and to phage resistance of such strains. Since spontaneously arising nonmucoid revertants have a growth rate advantage, mucoid strains are unstable under laboratory conditions (41, 96), whereas the eco-

TABLE 1. *Secondary metabolites from fluorescent pseudomonads*

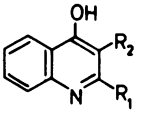
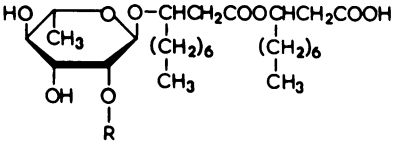
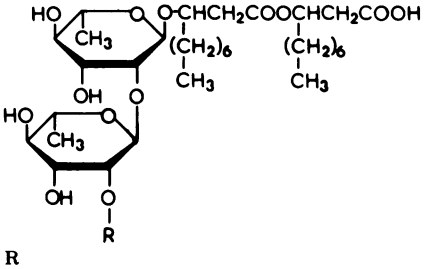
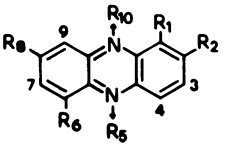
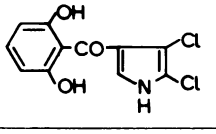
Compound (trivial name)	Structure	Reference
LIPIDS		
Pyo compounds (pseudanes)		
	R_1 / R_2	
2-*n* Pentyl-4-quinolinol	$-(CH_2)_4CH_3$ / $-H$	150
Pyo Ib = pseudane VII	$-(CH_2)_6CH_3$ / $-H$	53
Pyo Ic = pseudane IX	$-(CH_2)_8CH_3$ / $-H$	53
Δ^1-Pseudane VII	$-CH{=}CH(CH_2)_4CH_3$ / $-H$	81
Pyo III = Δ^1-pseudane IX	$-CH{=}CH(CH_2)_6CH_3$ / $-H$	53
2-Alkyl-4-hydroxyquinoline *N*-oxides	$-(CH_2)_nCH_3$, n = 6, 8, 10 (*N*-oxide) / $-H$	21
2-(2-Heptenyl)-3-methyl-4-quinolinol	$-CH_2CH{=}CH(CH_2)_3CH_3$ / $-CH_3$	49
Rhamnolipids		
	R	
Pyolipic acid	$-H$	6
Compound B	$-COCH{=}CH(CH_2)_6CH_3$	152
		
	R	
Jarvis rhamnolipid	$-H$	26, 57, 72, 73
Compound A	$-COCH{=}CH(CH_2)_6CH_3$	152
PHENAZINES		
	R_1 / R_2 / R_5 / R_6 / R_8 / R_{10}	
Pyocyanine	$-OH$ / $-H$ / $-CH_3$ / $-H$ / $-H$ / —	37
Hemipyocanine	$-OH$ / $-H$ / — / $-H$ / $-H$ / —	37
Idoinin	$-OH$ / $-H$ / $-O$ / $-OH$ / $-H$ / $-O$	37
Phenazine-1-carboxylic acid (tubermycin B)	$-COOH$ / $-H$ / — / $-H$ / $-H$ / —	37
Chlororaphin	$-CONH_2$ / $-H$ / $-H$ / $-H$ / $-H$ / $-H$	37
Oxychlororaphin	$-CONH_2$ / $-H$ / — / $-H$ / $-H$ / —	37
Aeruginosin A	$-H$ / $-NH_2$ / — / $-COOH$ / $-H$ / $-CH_3$	58
Aeruginosin B	$-H$ / $-NH_2$ / — / $-COOH$ / $-SO_3H$ / $-CH_3$	54
PYRROLES		
Pyoluteorin		136

TABLE 1—*Continued*

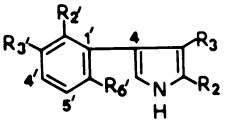
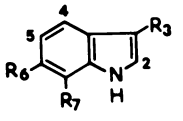
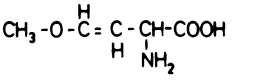
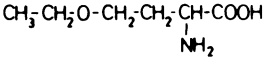
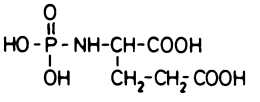
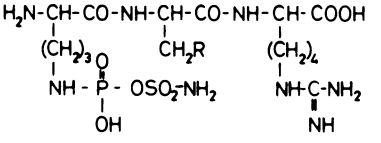
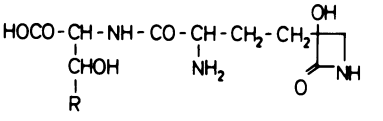
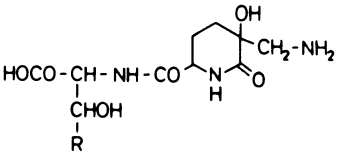
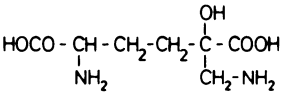
Compound (trivial name)	Structure	Reference
Phenylpyrroles		
	R_2 R_3 $R_{2'}$ $R_{3'}$ $R_{6'}$	
Pyrrolnitrin	—H —Cl —NO_2 —Cl —H	67
Isopyrrolnitrin	—Cl —Cl —NO_2 —H —H	47
2-Chloropyrrolnitrin	—Cl —Cl —NO_2 —Cl —H	45
Aminopyrrolnitrin	—H —Cl —NH_2 —Cl —H	39
Oxypyrrolnitrin	—H —Cl —NO_2 —Cl —OH	48
Monodechloropyrrolnitrin	—H —Cl —NO_2 —H —H	50
4-(2′-Amino-3′-chlorophenyl)pyrrole-2-carboxylic acid	—COOH —H —NH_2 —Cl —H	126
INDOLES		
	R_3 R_6 R_7	
3-Chloroindole	—Cl —H —H	85
Indole-3-carboxaldehyde	—CHO —H —H	150
6-Bromoindole-3-carboxaldehyde	—CHO —Br —H	150
7-Chloroindoleacetic acid	—CH_2COOH —H —Cl	125
Indoleacryloisonitrile	—CH=CH—N≡C —H —H	31
AMINO ACIDS and PEPTIDES		
L-2-Amino-4-methoxy *trans*-3-butenoic acid		124, 128
O-Ethyl homoserine		105
Phaseotoxin A		114
Phaseolotoxins		
	R	
Phaseolotoxin	—H	100
(2-Serine)-phaseolotoxin	—OH	102
Tabtoxins		
	R	
Tabtoxin	—CH_3	131, 140
(2-Serine)-tabtoxin	—H	131, 140
Isotabtoxins		
	R	
Isotabtoxin	—CH_3	131, 140
(2-Serine)-isotabtoxin	—H	131, 140
Tabtoxinine		131

TABLE 1—*Continued*

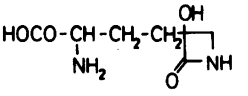
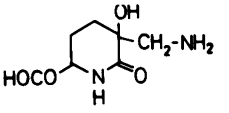
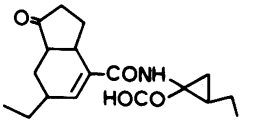
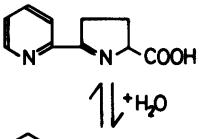
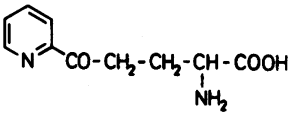
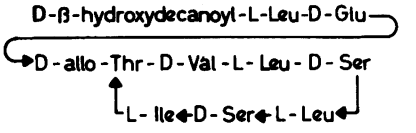
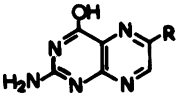
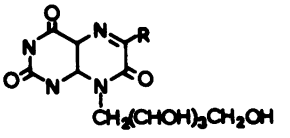
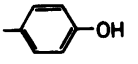
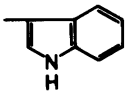
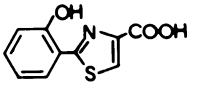
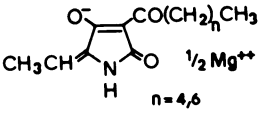
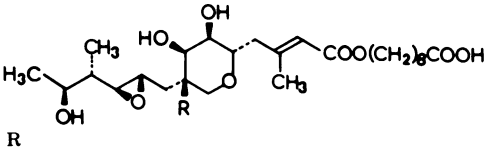
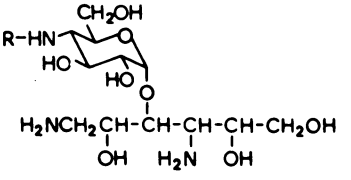
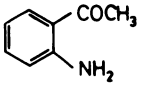
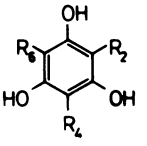
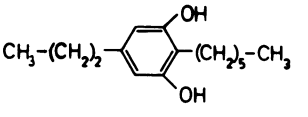
Compound (trivial name)	Structure	Reference
Tabtoxinine-β-lactam		25
Tabtoxinine-δ-lactam		140
Coronatine		66
Proferrorosamine A		115, 116
Pyrimine		129
Viscosin		56
PTERINES		
		
	R	
Pterine	=H	142
6-Aminopterine	$-NH_2$	142
6-Hydroxymethylpterine	$-CH_2OH$	142
Monapterine	$-CHOHCHOHCH_2OH$	135, 141, 143
		
	R	
Ribityllumazine	=H	135
6-Methyl-ribityllumazine	$-CH_3$	134
Putidolumazine	$-CH_2CH_2COOH$	134
6-(*p*-Hydroxyphenyl)-ribityllumazine		134
6-(3-Indolyl)-ribityllumazine		134
MISCELLANEOUS COMPOUNDS		
Cyanhydric acid	HCN	148

TABLE 1—*Continued*

Compound (trivial name)	Structure	Reference
Aeruginoic acid		151
Magnesidin		Hoechst[a]
Pseudomonic acid		
Pseudomonic acid A	R = —H	4, 16, Yoshitomi[b]
Pseudomonic acid B	R = —OH	17
Antibiotic P-2563		
P-2563 (A)	R = —$COCH_3$	103
P-2563 (P)	R = —$COCH_2CH_3$	103
Amino-2-acetophenone		89
C-acetyl phloroglucinols		
	R_2 = —$COCH_3$, R_4 = —H, R_6 = —H	9, 120
	R_2 = —$COCH_3$, R_4 = —$COCH_3$, R_6 = —H	9, 34, 120
	R_2 = —$COCH_3$, R_4 = —$COCH_3$, R_6 = —$COCH_3$	9, 120
Antibotic DB-2073 (alkylresorcinol)		74, 76

[a] A.-G. Hoechst, British patent 1,478,643, July 1977 (Chem. Abstr. 88:168480x, 1978).
[b] Yoshitomi Pharmaceutical Industries Ltd., Japan Kokai 7770,083, June 1977 (Chem Abstr. 87:182603a, 1977).

logical conditions in the infected tissue apparently favor the selection of slime-producing mutants.

LIPIDS AND RELATED COMPOUNDS

In 1945, while reinvestigating the antibiotic substances produced by *P. aeruginosa*, Hays et al. (53) obtained active preparations upon extracting sedimented cells with ethanol. From the ethanol extract they separated a number of antibiotic substances which were named pyo compounds. Four of the five substances obtained, namely, pyo Ib, Ic, II, and III, were shown to be structurally related. The structures of pyo Ib, Ic, and III were determined and confirmed by synthesis (145, 146). All pyo compounds are more active against gram-positive than against gram-negative bacteria.

A group of substances closely related to the pyo compounds was detected in the course of

studies aimed at identifying a streptomycin antagonist excreted by *P. aeruginosa* (133). The factor responsible for this antagonism was isolated (83) and shown to consist of a mixture of 2-*n* heptyl-, 2-*n* nonyl-, and 2-*n* undecyl-4-hydroxyquinoline *N*-oxides (21). These compounds, which probably correspond to pyo II, antagonized the inhibition by streptomycin and dihydrostreptomycin of *Bacillus subtilis* and *Staphylococcus aureus* but not of gram-negative bacteria. They were able to antagonize the action of 1,000 times their weight of dihydrostreptomycin and at higher concentrations inhibited growth of gram-positive bacteria. Although the mode of action of the 2-alkyl-4-hydroxyquinoline *N*-oxides in antagonizing the inhibitory action of dihydrostreptomycin has not been elucidated, it was observed that the compounds inhibited electron transport by cytochromes. This effect was assumed to be the reason for their inhibitory action on bacterial growth (84). Pyo compounds have been rediscovered by Russian workers (80, 81), who named them pseudanes, and more recently they were also detected in a marine pseudomonad (150).

The isolation of anthranilic acid and 2-*n* heptyl-3-oxy-4-quinolene from a "pyo" fermentation broth (137, 138) strongly suggested that the pyo compounds are condensation products between anthranilic acid and a fatty acid precursor according to the scheme proposed by Cornforth and James (21), as depicted in Fig. 1. This scheme was corroborated by following the incorporation into 2-*n* heptyl-hydroxyquinoline of [^{14}COOH]anthranilic acid, [^{15}N]anthranilic acid, [$^{14}CH_3$]acetate, and [^{14}COOH]malonic acid fed to *P. aeruginosa* (122). The distribution of radioactivity in the product was determined after cleaving the molecule by ozonolysis into anthranilic acid, a fatty acid, and carbon dioxide from the C3 of the quinoline ring. The entire molecule of anthranilic acid was incorporated into 2-*n* heptyl-hydroxyquinoline, and the β-keto decanoic acid moiety of the product was shown to originate from four malonate units and one acetate unit according to the pattern shown in Fig. 2.

In addition to its possible role as a precursor of the pyo compounds, decanoic acid forms part of a number of other secondary metabolites from pseudomonads. D-β-Hydroxy-*n*-decanoic acid is a component of pyolipic acid, of the rhamnolipids, of the cyclodepsipeptide viscosin, and presumably of magnesidin (Table 1). This fatty acid has also been identified as a constituent of the lipopolysaccharide layer of the outer membrane of certain gram-negative bacteria, including pseudomonads (121).

Bergström et al. (6) reported the extraction of

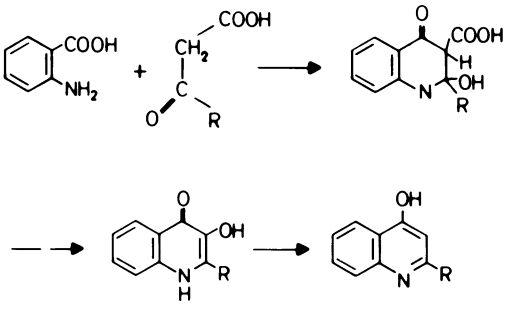


FIG. 1. *Biosynthesis of pyo compounds from anthranilic acid and a β-keto acid intermediate as proposed by Cornforth and James (21).*

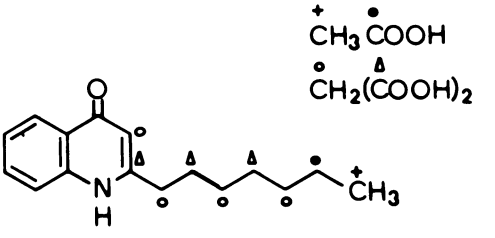


FIG. 2. *Incorporation of ^{14}C-labeled acetate and malonate into pyo Ib (pseudane VII) according to Ritter (122).*

pyolipic acid, an antibiotic active against *Mycobacterium tuberculosis*, from cells of *P. aeruginosa*. By titration, an average molecular weight of 500 was determined for pyolipic acid. Upon acid hydrolysis the substance yielded 27% rhamnose and 73% of a fatty acid fraction consisting mainly of β-hydroxydecanoic acid, with β-hydroxyoctanoic and β-hydroxydodecanic acid representing minor components. Pyolipic acid was thought to be identical to L-rhamnosyl-β-hydroxydecanoic acid, which has a molecular weight of 334 (98). However, the stoichiometry of the components found in the acid hydrolysate of the compound corresponds to L-rhamnosyl-β-hydroxydecanoyl-β-decanoic acid, which has a molecular weight of 502 and contains 74% β-hydroxydecanoic acid.

A similar glycolipid was isolated by Jarvis and Johnson (73) from the culture fluid of *P. aeruginosa* grown on glycerol. It was first identified as L-rhamnosyl-(1,3)-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoic acid. Later it was established that the correct linkage between the two rhamnose moieties was not 1,3 but 1,2 (26). The compound is excreted into the medium during the stationary phase of growth, and the optimal conditions for its formation from various carbon sources have been defined (51, 52). Subsequently the enzymatic synthesis of this rhamnolipid in sonic extracts of *P. aeruginosa* has been studied.

Burger et al. (10) showed that its synthesis proceeds by the following three reactions:

2 β-hydroxydecanoyl-CoA + H_2O
→ β-hydroxydecanoyl-β-hydroxydecanoate + 2 CoA (1)

TDP-L-rhamnose + β-hydroxydecanoyl-β-hydroxydecanoate
→ TDP + L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate (2)

TDP-L-rhamnose + L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate
→ TDP + L-rhamnosyl-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate (3)

where CoA is coenzyme A and TDP is thymidine diphosphate.

Although the exact mechanism of formation of β-hydroxydecanoyl-β-hydroxydecanoate (reaction 1) remains unclear, it has been shown that the synthesis of the rhamnolipid involves two sequential glycosyl transfer reactions (reactions 2 and 3), each catalyzed by a specific rhamnosyl transferase. The latter two enzymes were separated from each other and partially purified (10). L-Rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate, which is identical to pyolipic acid, was isolated from culture broths of *P. aeruginosa* grown on *n*-paraffins (71, 72). It was bactericidal for gram-positive bacteria. Furthermore, both mono- and dirhamnolipids exhibited mycoplasmacidal and antiviral activities in vitro. Because of their emulsifying activities the rhamnolipids stimulated growth of *P. aeruginosa* on *n*-paraffins (57, 72). In a recent study a pseudomonad isolated from oil-soaked soil was shown to produce a new type of rhamnolipid during growth on *n*-paraffins. In addition to one or two molecules of rhamnose and two molecules of β-hydroxydecanoic acid, the compounds also contained α-decenoic acid. They exhibited strong emulsifying activity in an oil-water system, and their use in activated sludge treatment of hydrocarbon-polluted water is under investigation (152).

PSEUDOMONIC ACID

Pseudomonic acid A (Table 1) was isolated in 1971 from a strain of *P. fluorescens* (4). It is responsible for a significant proportion of the antibacterial activity in the culture fluid of this organism. Its structure (16, 17) and its absolute stereochemistry (1) have been determined. The compound is mainly active against gram-positive organisms, whereas gram-negative bacteria, with the exception of *Neisseria* and *Haemophilus*, are relatively insensitive to pseudomonic acid. At low concentrations the antibiotic is bacteriostatic; at higher concentrations it is bactericidal (R. Sutherland, K. R. Comber, L. W. Mizen, B. Slocombe, and J. P. Clayton, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 16th, Chicago, Ill., Abstr. no. 52, 1976). Its low minimum inhibitory concentration for *Neisseria gonorrhoeae* (0.01 μg/ml) may lead to its practical use against penicillin-resistant *Neisseria* strains (J. P. Clayton [Beecham Group Ltd.], German patent 2,746,974, April 1978 [Chem. Abstr. **89**:36919h, 1978].

Pseudomonic acid showed no cross-resistance with a wide range of antibiotics, and its unique structure seems to be correlated with a novel mode of action (64). Protein synthesis in *S. aureus* was shown to be inhibited by pseudomonic acid. The antibiotic also inhibited RNA synthesis, an effect which was prevented by the addition of chloramphenicol to the test organism. This latter observation suggested that pseudomonic acid led to starvation for one or several amino acids or to the inhibition of the aminoacylation of transfer RNA, thereby causing the arrest of RNA synthesis in organisms with stringent control of RNA formation. Chloramphenicol and some other antibiotics acting at the ribosomal level are known to abolish stringent RNA control. The antagonistic effect of chloramphenicol on the inhibition of RNA synthesis by pseudomonic acid might thus be explained by the current views on the regulation of RNA synthesis.

PHENAZINES

The production of phenazine pigments by a variety of procaryotes such as *Streptomyces*, *Streptosporangium*, *Microbispora*, *Brevibacterium*, *Sorangium*, and *Pseudomonas* has been reported. Microorganisms constitute the exclusive source of phenazines in nature, and there is a considerable overlap in the production pattern: several different organisms produce the same compound and a particular microorganism may often produce a variety of phenazines (11, 12, 75). Approximately 30 different phenazine compounds have been described so far (37). Phenazines are isolated by extraction of culture filtrates with organic solvents. Among pseudomonads they are produced by some members of the *P. cepacia* group and by many strains belonging to the fluorescent pseudomonads. Therefore, most studies on the production and biosynthetic

pathways of the various phenazine pigments have been conducted with representatives of the fluorescent pseudomonads, in particular with *P. aeruginosa*, which produces the blue pigment pyocyanine, and with *P. aureofaciens*, which excretes primarily phenazine-1-carboxylic acid into the medium.

The involvement of the aromatic amino acid pathway in the biosynthesis of pyocyanine was first suggested by the incorporation of [^{14}C]shikimic acid and [^{14}C]quinic acid into pyocyanine (87, 99). These early experiments were impeded by the redistribution of the labeled precursors through degradative pathways. In the subsequent studies on pyocyanine biosynthesis various mutants of *P. aeruginosa* have proven useful. Using a mutant blocked in the catabolism of shikimic acid and quinic acid, Ingledew and Campbell (68) were able to show that all carbon atoms of the phenazine nucleus of pyocyanine were derived from shikimic acid. Quinic acid, anthranilic acid, tryptophan, tyrosine, and phenylalanine did not serve as precursor molecules. By analyzing mutants blocked in different steps of aromatic amino acid biosynthesis (13, 86) chorismate was identified as the branch point metabolite leading off the aromatic pathway to pyocyanine. Genetic blocks before shikimate did not affect the formation of pyocyanine on shikimate-supplemented medium. Blocks between shikimate and chorismate abolished pyocyanine production on media containing the required aromatic amino acids and shikimate, whereas lesions in the enzymes of the aromatic amino acid biosyntheses after chorismic acid were without effect on pyocyanine formation.

The pathway from chorismate to the individual phenazines is still obscure. Hollstein and McCamey (61) concluded from incorporation experiments with specifically labeled D-[^{14}C]shikimic acid that a common precursor of the phenazines arises by the symmetrical condensation of two identically substituted molecules of chorismic acid. Although a technique for the recognition of pyocyanine mutants of *P. aeruginosa* has been devised (14), the most recent studies on phenazine biosynthesis using pigmentation mutants have been conducted with *P. phenazinium*, a nonfluorescent pseudomonad of uncertain taxonomical status (11, 12). Two properties of this organism make it a suitable object for studying phenazine biosynthesis: it produces ten different phenazines and, on a minimal medium containing L-threonine as the only source of carbon and nitrogen, it incorporates 25% of the carbon of threonine into phenazines. Under similar growth conditions it incorporates about 70% of the carbon of D-[^{14}C]shikimate, mainly into iodinin. Cross-feeding experiments with pigmentation mutants (11), as well as feeding experiments in which radioactive phenazines isolated from culture media were added to growing cultures of the wild type and of pigmentation mutants, led Byng and Turner (12) to propose a scheme for phenazine biosynthesis, which is represented in Fig. 3. In this hypothetical pathway phenazine-1,6-dicarboxylate is the common precursor of the phenazines produced by *P. phenazinium*. From this branch point compound one route would lead via two consecutive hydroxylative decarboxylations and two *N*-oxidations to 1,6-dihydroxyphenazine 5,10-dioxide (iodinin). Alternatively, phenazine-1,6-dicarboxylate would be decarboxylated to yield phenazine-1-carboxylate, which in turn is viewed as the precursor of the phenazines metabolized to 1,8-dihydroxyphenazine 10-monoxide (Fig. 3). Direct evidence for a pathway with phenazine-1,6-dicarboxylate as a key branch point compound has been obtained by the observations that the dimethyl ester of ^{14}C-labeled phenazine-1,6-dicarboxylate is metabolized by *P. phenazinium* in significant amounts to iodinin (A. M. Messenger and J. M. Turner, personal communication) and by *P. aureofaciens* to phenazine-1-carboxylate (60). Incorporation experiments in which the free acid of phenazine-1,6-dicarboxylate was used instead of its dimethyl ester were unsuccessful, presumably due to permeability problems. The findings of Holliman and collaborators (32, 46, 55) on the formation of phenazines by *P. aureofaciens* and *P. aeruginosa* may be accommodated in the pathway of Fig. 3 by assuming phenazine-1-carboxylate as the second major branch point. This key intermediate would act as the precursor of pyocyanine, 1-hydroxyphenazine and its 10-monoxide, aeruginosins A and B, and 2-hydroxyphenazine and its 1-carboxylate.

PYRROLNITRIN

Pyrrolnitrin, an antifungal antibiotic produced by many fluorescent and nonfluorescent strains of the genus *Pseudomonas*, was first described by Arima et al. (2, 3). Gorman and Lively (39) have reviewed early information on the biosynthetic pathway and the fermentative production of pyrrolnitrin. The antibiotic is primarily used against dermatophytic fungi, especially against members of the genus *Trichophyton*. Isopyrrolnitrin, oxypyrrolnitrin, and monodechloropyrrolnitrin have lower antifungal activities than pyrrolnitrin. Elander et al. (27) surveyed 29 *Pseudomonas* strains classified as *P. chlororaphis*, *P. aureofaciens*, and *P. cepacia* for the production of pyrrolnitrin. Four strains of *P.*

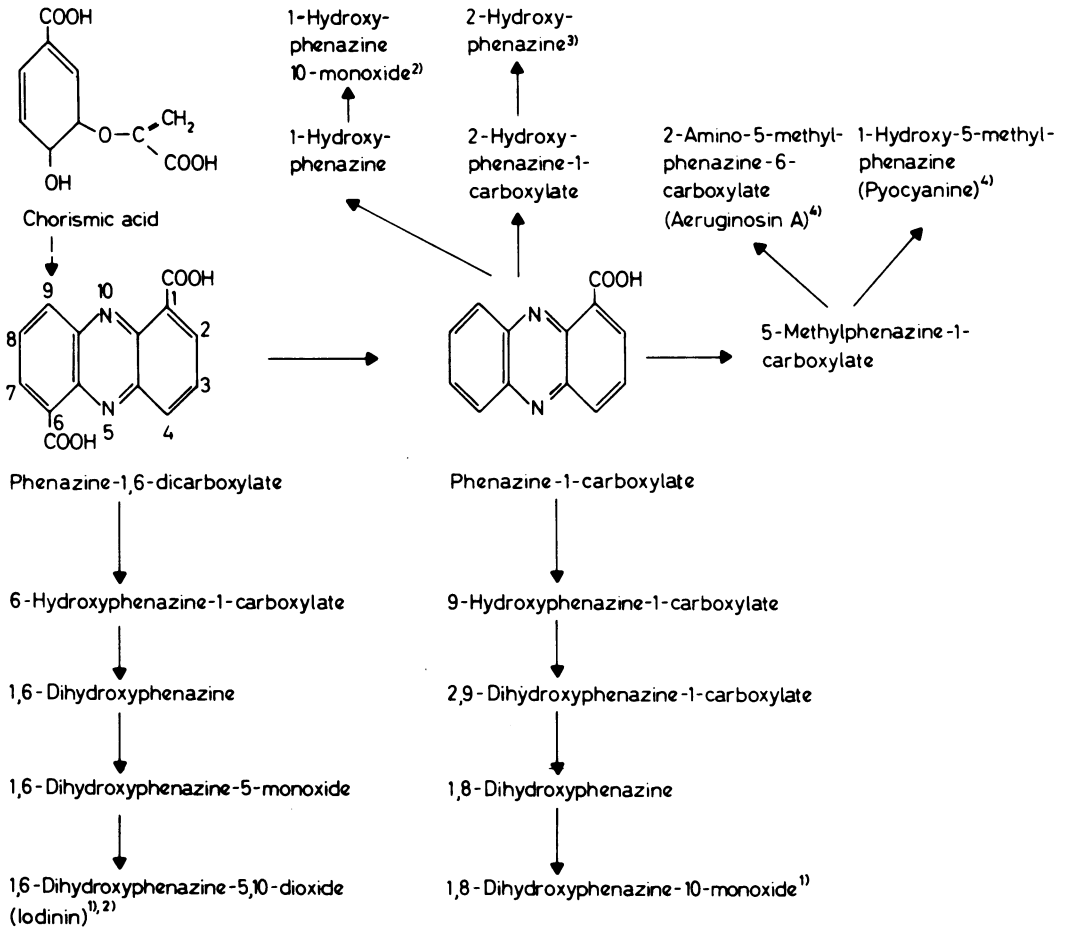


FIG. 3. *Hypothetical scheme for phenazine biosynthesis in Pseudomonas species. Modified after Byng and Turner (12). Incorporation of radioactive precursors according to this scheme has been observed for the end products formed by: 1) P. phenazinium (12), 2) P. iodina (Brevibacterium iodinum) (55), 3) P. aureofaciens (32), 4) P. aeruginosa (32, 46).*

cepacia and four strains belonging to the two fluorescent species excreted pyrrolnitrin into the medium. A maximum level of about 100 μg/ml was reached by a strain of *P. cepacia*.

Whereas it is well established that pyrrolnitrin is formed in several steps from tryptophan, the details of this pathway have not been elucidated. Studies with *P. aureofaciens* have shown that tryptophan is a precursor of pyrrolnitrin. Addition of D-tryptophan to the medium resulted in a marked increase in pyrrolnitrin formation. L-Tryptophan had no effect in this respect, but both labeled enantiomers were incorporated into the product (85). Martin et al. (91), feeding tryptophan isotopically labeled in various positions to *P. aureofaciens*, demonstrated that the amino nitrogen of D-tryptophan gave rise to the nitro group of pyrrolnitrin. The C2 of the indole nucleus was retained and the C3 of the side chain became C3 of the pyrrole. Tritium at the chiral carbon of the amino acid was retained during biosynthesis when L-tryptophan was fed, but not in the case of D-tryptophan. These results supported pathway A (39) shown in Fig. 4.

More recently, Floss et al. (33), using tryptophan labeled with ^{13}C in the 3-position of the chain and with ^{15}N in the amino group, showed that L- rather than D-tryptophan was the immediate precursor of pyrrolnitrin, despite the fact that under any condition tested, ^{14}C-labeled D-tryptophan was incorporated more efficiently into pyrrolnitrin than the L-isomer. The same authors prepared and fed to *P. aureofaciens* a number of presumed precursors of pyrrolnitrin. (Amino-2-phenyl-)-3-pyrrole (Fig. 4) was efficiently incorporated into pyrrolnitrin. This led them to propose a modified pathway (pathway B in Fig. 4) for pyrrolnitrin formation. According

to this proposal chlorine must be introduced into the β-position of the pyrrole ring, although electrophilic substitution of pyrroles normally occurs in the α-position.

The order of introduction of the two chlorine atoms in pyrrolnitrin biosynthesis remained largely speculative until recently, when a new metabolite was isolated at an early stage of a fermentation with *P. aureofaciens* by Salcher et al. (126). (Amino-2-chloro-3-phenyl-)-4-pyrrole-2-carboxylic acid (Table 1), after chlorination and subsequent decarboxylation, yields aminopyrrolnitrin, a direct precursor of pyrrolnitrin. The same authors also isolated 7-chloroindole-3-acetic acid and 3-chloroanthranilic acid from the fermentation broth of the same organism (125). According to these results 7-chlorotryptophan is viewed as a common precursor of 3-chloroanthranilic acid, 7-chloroindole-3-acetic acid, and pyrrolnitrin (pathway C in Fig. 4).

PEPTIDES AND AMINO ACIDS

The phytopathogenic pseudomonads belonging to the species *P. syringae* produce low-mo-

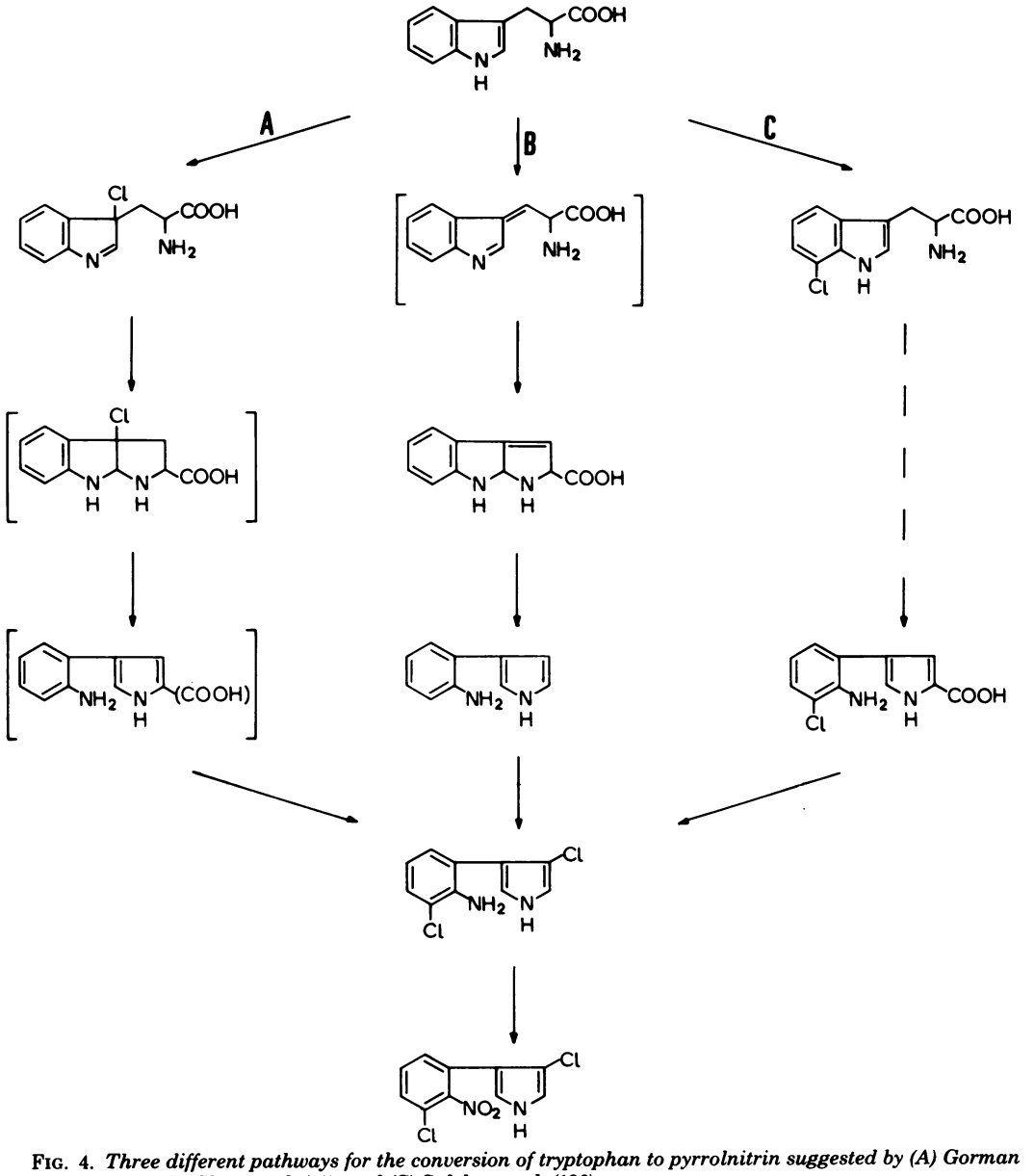


FIG. 4. *Three different pathways for the conversion of tryptophan to pyrrolnitrin suggested by (A) Gorman and Lively (39), (B) Floss et al. (33), and (C) Salcher et al. (126).*

lecular-weight phytotoxins of a peptide or amino acid nature. The role of these toxins in plant disease, their chemistry, and their mode of action have been reviewed by Patil (110) and by Strobel (132). The structure of tabtoxin (Table 1), one of the toxins produced by *P. tabaci*, the causal agent of wildfire disease of tobacco, has been established by Stewart (131). It is a dipeptide consisting of tabtoxinine-β-lactam and L-threonine. All strains forming tabtoxin also produce (2-serine)-tabtoxin (140). Tabtoxin and (2-serine)-tabtoxin isomerize easily to the biologically inactive isotabtoxins by intramolecular translactamization. Tabtoxinine is formed by hydrolysis of tabtoxin or isotabtoxin. In addition to tabtoxin and (2-serine)-tabtoxin, the δ- and β-lactams of tabtoxinine have been detected in culture filtrates of *P. tabaci* (25, 140). Tabtoxin, (2-serine)-tabtoxin, and tabtoxinine-β-lactam have been shown to cause chlorosis in tissues of various plants (25). The biosynthesis of the toxins from *P. tabaci* is unknown, and their mode of action in the plant remains to be determined.

P. phaseolicola is responsible for the disease known as halo blight of beans. It produces a number of extracellular phytotoxins which have been studied extensively in recent years. The role of the halo blight toxins in disease is firmly established. Culture filtrates of *P. phaseolicola* or purified toxin preparations cause the chlorotic symptoms found on naturally infected plants. Furthermore, the accumulation of ornithine typical for chlorotic plant tissue correlated with the specific inhibition of plant ornithine transcarbamylase in vitro by purified toxin(s) (112, 113, 139). The chlorosis in bean leaves was reversed by the addition of citrulline and arginine, which is consistent with the cause of chlorosis being due to an inhibition of ornithine transcarbamylase. Recently, the toxins from culture filtrates of *P. phaseolicola* have been purified and chemically characterized by two research groups. Patil et al. (114) demonstrated the presence of at least four ornithine transcarbamylase-inhibiting compounds separable on diethylaminoethyl-Sephadex. One component, phaseotoxin A, was identified as *N*-phosphoglutamic acid, whereas the structures of the other three components are still unknown. The production of *N*-phosphoglutamic acid by *P. phaseolicola* is the first case of a naturally occurring *N*-phosphorylated primary amine. The unusual structure of phaseotoxin A is strongly supported by the fact that chemically synthesized *N*-phosphoglutamic acid inhibited ornithine transcarbamylase and induced chlorosis in bean leaves (114). Mitchell (100), on the other hand, isolated and characterized the tripeptide (N^5-phosphosulfamyl)-ornithyl-alanyl-homoarginine as the toxin responsible for bean halo blight. This major compound was given the trivial name phaseolotoxin. (2-Serine)-phaseolotoxin was produced as a minor component by the same organism (102). Phaseolotoxin, (N^5-phosphosulfamyl)-ornithyl-alanine, and (N^5-phosphosulfamyl)-ornithine caused chlorosis and ornithine accumulation in bean leaves (100). Since phaseolotoxin is rapidly degraded to (N^5-phosphosulfamyl)-ornithine in plant tissue, Mitchell proposed that the latter compound is primarily responsible for the actual toxic effect on plants (101). The effects of nutritional components, amino acid supplements, and temperature on toxin production by *P. phaseolicola* in liquid culture have recently been investigated (123). Wide variations in toxin yield have been observed, and it was suggested that the conflicting results regarding the chemical composition of the toxin from this organism might be due to the production of different toxins depending on the composition of the culture medium.

Phytotoxins from other phytopathogenic nomenspecies of *P. syringae* are not well characterized. The toxins of *P. coronafaciens*, the pathogen of oats, are related to the tabtoxins, whereas the toxins of *P. glycinea* and *P. tomato*, the pathogens of soybean and tomato, seem to have chromatographic and biological properties similar to those of the bean halo blight toxins (110, 132). *P. syringae*, the pathogen of maize and wheat, produces syringomycin, a wide-spectrum antibiotic that is also phytotoxic (22). The antibiotic has been partially purified and shown to be a small peptide yielding nine different amino acids upon hydrolysis (130). More recently, homogeneous syringomycin preparations have been obtained by thin-layer chromatography and polyacrylamide gel electrophoresis, but the structure of the phytotoxic antibiotic is still unknown (43). It was observed that the acridine orange-induced elimination of a 22×10^6-dalton plasmid from *P. syringae* was correlated with the loss of the ability to produce syringomycin (C. F. Gonzales and A. K. Vidaver, Proc. Am. Phytopathol. Soc. 4:107, 1977). There exists also preliminary evidence for plasmid involvement in the synthesis of phaseotoxin and tabtoxin (62).

L-2-Amino-4-methoxy-*trans*-3-butenoic acid (AMB), an amino acid antimetabolite inhibiting the growth of gram-positive and gram-negative bacteria on minimal media, is produced by *P. aeruginosa* (124, 128). AMB was formed by different strains of *P. aeruginosa* on complex and minimal media containing either glucose or *n*-paraffins as sources of carbon and energy. The maximum levels of antimetabolite produced

were 18 mg/liter. Its minimal inhibitory concentration for *Escherichia coli* on minimal medium was as low as 3×10^{-6} M. Interestingly, the reversal patterns of the growth inhibition caused by AMB were different for different indicator bacteria. In *B. subtilis* growth inhibition was relieved by D- and L-alanine, D- and L-2-aminobutanoic acid, D-glutamic acid, and D-aspartic acid (128), whereas methionine, homocysteine, and cystathionine reversed growth inhibition in *E. coli* (124). A metabolic explanation for the inhibition caused by AMB is still lacking. However, the toxin has been shown to act as an irreversible inhibitor of pyridoxal-linked aspartate-aminotransferase (118, 119).

The enol ether group of AMB is rare among naturally occurring amino acids and has been observed only in two structurally related antimetabolites: rhizobitoxine [2-amino-4-(2-amino-3-hydroxypropoxy)-*trans*-3-butenoic acid] from *Rhizobium japonicum* (107) and L-2-amino-4-(2-aminoethoxy)-*trans*-3-butenoic acid from a *Streptomyces* species (117). In the culture broths of both organisms the saturated inactive compounds dihydrorhizobitoxine (106) and L-2-amino-4-(2-aminoethoxy)butanoic acid (127) were found. Addition of synthetic D,L-2-amino-4-(2-aminoethoxy)butanoic acid to the *Streptomyces* culture medium led to a threefold-enhanced production of the corresponding unsaturated antimetabolite, suggesting that the saturated amino acid serves as a precursor for the antimetabolite (127).

Although the biosynthesis of AMB is not known, preliminary studies have shown that the addition of L-homoserine or synthetic *O*-methyl-D,L-homoserine to the culture medium of *P. aeruginosa* strain PAO1 resulted in a threefold increase in AMB production. This observation and the fact that the label from L-[$^{14}CH_3$]methionine was incorporated with 50% efficiency into the methoxy group of AMB suggest that AMB is formed from homoserine by *O*-methylation and subsequent dehydrogenation (unpublished data).

FLUORESCENT PIGMENTS

Under certain growth conditions all representatives of the fluorescent pseudomonads produce water-soluble, yellow-green, fluorescent pigments which have been called bacterial fluorescein, fluorescein, or pyoverdine. The term "pyoverdine" is preferable to the designations "bacterial fluorescein" or "fluorescein," which are likely to be confused with the chemically synthesized fluorescein (resorcinolphthalein). The pyoverdines formed by different species of fluorescent pseudomonads may be distinguished by a suffix indicating the producing species (94). The excitation maximum of the pyoverdines is around 400 nm, with an emission maximum of around 500 nm. Due to the difficulties encountered during the isolation and purification of the fragile molecules, their chemical structures remain largely unknown, and several hypotheses as to the nature of the fluorescent chromophore have been formulated. Lenhoff (82) and Greppin and Gouda (42) have postulated a pyrrole derivative by analogy with the cytochromes. A riboflavin component of bacterial fluorescein was suspected by Birkofer and Birkofer (7), and a pteridine chromophore was suggested by Giral (38) and Naves (R. G. Naves, Ph.D. thesis, University of Geneva, Geneva, Switzerland, 1955). Chakrabarty and Roy (19) isolated a compound from cultures of *P. fluorescens* with a molecular weight of only 210 which had physiochemical properties similar to those of the pteridines. In these early studies the molecular weights of the fluorescent pigments were presumably underestimated, and the fluorescent compounds isolated may have been degradation products of pyoverdines or other fluorescent metabolites produced by pseudomonads.

More recent studies have revealed that pyoverdines have molecular weights in the range of 1,000. In partially purified preparations, obtained by gel filtration, the fluorescent pigments were found to consist of a peptide associated with a fluorescent chromophore. The peptide from *P. mildenbergii* contained serine, threonine, glutamic acid, and lysine (65, 104), whereas the peptide in the fluorescent pigment of *P. fluorescens* (Migula) consisted of serine, glycine, glutamic acid, ornithine, and lysine (97). A significant contribution to the understanding of the chemistry, biosynthesis, and physiological function of pyoverdine$_{pf}$, the fluorescent pigment of *P. fluorescens*, was made by Meyer and Abdallah (94). They observed that pyoverdine$_{pf}$ formed an extremely stable ferric complex and could be purified in this form by a method developed for the purification of siderochromes (154). *P. fluorescens* produced only one molecular species of pyoverdine with a molecular weight of 1,500, which at slightly alkaline pH was transformed into a number of degradation products. Obviously these derivatives have previously been attributed to different pigment species. Although the chemical structure of pyoverdine$_{pf}$ is not fully established, it probably consists of a quinoline chromophore associated with a cyclic peptide (no free amino group) and a short aliphatic chain (J. M. Meyer, Thèse d'Etat, University Louis Pasteur, Strasbourg, France, 1977). Upon hydrolysis of the peptide

moiety, serine, glycine, alanine, and N^5-hydroxyornithine were detected. The unusual amino acid N^5-hydroxyornithine is a constituent of many siderochromes, including the siderochrome polypeptide ferribatin isolated from cultures of *P. fluorescens* (Migula) (92). In fact, the following evidence has been presented (95) that pyoverdine$_{pf}$ is a typical iron chelator with a specific role in the transport of Fe^{3+} into *P. fluorescens*. (i) Formation of pyoverdine$_{pf}$ occurred only in iron-deficient bacteria and was not influenced by other nutritional factors, the oxygenation rate, the pH, or by illumination. Under iron-limited growth in medium containing 135 μg of Fe^{3+} per liter the amount of pigment produced was almost equal to the cellular dry weight. (ii) Pyoverdine$_{pf}$ formed a very stable ferric complex with a stability constant of the same order as those of other siderochromes. It had a low affinity for ferrous ions. (iii) The rate of iron uptake by *P. fluorescens* increased when purified pyoverdine$_{pf}$ was added to washed cells. Iron transport was an active process.

The physiological function demonstrated for pyoverdine$_{pf}$ has also been described for fluorescent iron transport hydroxamates of unknown chemical structure produced by an unidentified fluorescent pseudomonad (35). The biosynthesis of iron transport compounds by this organism was temperature sensitive: at 20°C 100 μg of Fe^{3+} per liter led to maximal cell yield, whereas at 31°C growth depended on the addition of 3,000 μg of Fe^{3+} per liter, as well as on supplementation of the medium with hydroxamate iron compounds produced by the organism at lower temperatures (35). It will be interesting to see whether a possible common role of the fluorescent pigments from different pseudomonads in iron transport is paralleled by identical or similar chemical structures.

Fluorescent metabolites different from pyoverdines include the ribityllumazines isolated from cultures of *P. putida*, putidolumazine, a possible intermediate in riboflavine biosynthesis (134, 135), 6-hydroxymethylpterine, a precursor of folic acid found in cultures of *P. roseus fluorescens* (142), and D-erythroneopterine formed by *P. putida* (135) (Table 1).

MANIPULATION OF SECONDARY METABOLITE PRODUCTION IN *PSEUDOMONAS*

Little effort has been directed towards increasing the secondary metabolite production of pseudomonads by mutation or by systematic optimization of medium composition and growth conditions. The limited practical use of products from pseudomonads has not stimulated fermentation research programs. Attempts have been made to improve the yields of phenazines and pyrrolnitrin.

Elander et al. (28) reported an example of a rational approach to the selection of mutants of *P. aureofaciens* which produce increased amounts of pyrrolnitrin. Starting from the observation that the addition of 1 g of D,L-tryptophan per liter to the fermentation medium resulted in a doubling of pyrrolnitrin production, they isolated analog-resistant mutants synthesizing higher levels of tryptophan, thereby obviating the need to add exogenous tryptophan. A mutant resistant to 250 mg of 5-fluorotryptophan per liter produced twice as much pyrrolnitrin as the parent strain without D-tryptophan supplementation. In a two-step mutant resistant to 1 g of 6-fluorotryptophan per liter, the level of pyrrolnitrin in a nonsupplemented medium was three times that of the wild type and reached 150 mg/liter. Analog-resistant, high producing strains did not excrete tryptophan and incorporated less exogenous D,L-[^{14}C]tryptophan into pyrrolnitrin than their analog-sensitive parent. As uptake studies with both the D- and the L-isomers of tryptophan revealed no differences between the parent and the analog-resistant mutants, the biochemical basis of analog-resistance and a concomitant increase in pyrrolnitrin formation was thought to be due to a higher pool of endogenous tryptophan, the biosynthetic precursor of pyrrolnitrin. In view of the versatile and efficient amino acid degradative pathways of pseudomonads (20), the production of amino acid-derived secondary metabolites might be increased not only by interfering with the control of the biosynthesis of an amino acid precursor, but also by imposing genetic blocks in the catabolic pathways responsible for its degradation.

The effect of various nutritional factors on the production of phenazines by pseudomonads and, more specifically, on the production of pyocyanine by *P. aeruginosa* have been summarized by Ingram and Blackwood (70) and MacDonald (88), respectively. Pyocyanine production by *P. aeruginosa* starts after the exponential growth phase. However, with the exception of one report (44), the production of pyocyanine with suspensions of resting cells has not been achieved (69). The fact that media on which the organism grows but does not produce pyocyanine have been found in many cases classifies pyocyanine and the phenazines in general as typical secondary metabolites. On defined growth media containing glycerol as a carbon source, leucine or alanine or both as a nitrogen source, and controlled levels of Fe^{2+}, Mg^{2+}, and PO^{4-}, pyocyanine yields of approximately 200 mg/liter have

been achieved (88). The production of pyocyanine required an optimal supply of Fe^{2+} (>0.5 mg/liter) and decreased when iron was the growth-limiting factor of the medium (79).

Several reports stress the importance of phosphate limitation for directing the metabolism of pseudomonads towards the production of phenazines. Ingram and Blackwood (70) proposed that the low phosphate levels reached in production media towards the end of the growth phase are instrumental in triggering pyocyanine synthesis. Addition of high concentrations of phosphate to the culture at this stage stopped pyocyanine production, whereas addition of a limiting amount of phosphate led to an increase in cell mass and pyocyanine formation. Similar observations were made by Ingledew and Campbell (69), who suspended pregrown cells of *P. aeruginosa* in a phosphate-free 2-ketogluconate medium. Pyocyanine synthesis was initiated when the residual phosphate level had dropped below the limit of detection. It stopped when the carbon source was used up and free phosphate, probably originating from ribosome degradation, appeared in the medium. Pyocyanine synthesis thus lends itself as a model for studying "phosphate repression," a phenomenon affecting the production of various antibiotics (90).

Another instance where phosphate repression was studied is the production of hydrogen cyanide, a degradation product of glycine exclusively formed by some species of *Chromobacterium* and *Pseudomonas* among procaryotes. Wissing (148) has proposed that bacterial cyanogenesis from glycine proceeds by two oxidation steps involving flavoproteins, which convert the C—N single bond of glycine to the C≡N triple bond via an imino acid intermediate. Castric (15) has demonstrated that hydrogen cyanide biosynthesis in *P. aeruginosa* has the following characteristics of secondary metabolism. The product was not formed during the lag and exponential phases of growth, active cyanogenesis occurred during the transition from exponential growth to the stationary phase, and limited cyanogenesis was observed in the stationary phase. Cyanide formation was of limited taxonomic distribution. Among 110 *P. aeruginosa* isolates, 74 produced hydrogen cyanide. Among other *Pseudomonas* species tested only one of five strains of *P. fluorescens* was cyanogenic. Cyanide production by growing *P. aeruginosa* had a narrower temperature range than growth, required Fe^{3+} concentrations above 0.6 mg/liter, and was inhibited by inorganic phosphate.

Inhibition of cyanogenesis by phosphate was studied in some detail by Meganathan and Castric (93). Maximum hydrogen cyanide formation occurred between 1 and 10 mM phosphate in a growth medium containing glycine. Above and below this concentration range the rate and the quantity of cyanide production decreased sharply, whereas growth was affected only slightly. Addition of chloramphenicol to a low phosphate medium, prior to a phosphate shift-up triggering cyanogenesis, was inhibitory to cyanide production, indicating that protein synthesis is a prerequisite for phosphate-induced cyanide formation. The demonstration of cyanide production from glycine in a crude particulate preparation from a *Pseudomonas* sp. (149) should allow the biochemical characterization of this process and ultimately the clarification of the role of phosphate in regulating the activity or synthesis, or both, of the enzyme(s) involved.

Knowles (77), in a review on microbial cyanide metabolism, has extended the similarity between cyanide production and the production of secondary metabolites to the possible functions of these compounds for the producing organisms. He pointed out that cyanide excretion could destroy or inhibit growth of neighboring organisms, thereby conferring selective advantages on cyanogenic strains. In this context Weinberg and collaborators (36, 144) have suggested that *P. aeruginosa*, like other microbial cells unable to differentiate, depends on successful completion of secondary metabolism for long-term survival in its natural environment. This view was based on the observation that phosphate concentrations that inhibited pyocyanine formation caused glycerol-grown cells to die early in the stationary phase (36). However, the experiments supporting this interesting hypothesis are inconclusive because the survival rates and the phosphate concentrations in the media were not compared with the quantity and the pattern of secondary metabolites produced. Furthermore, recent experiments by Turner (personal communication) comparing the viability of the wild type and non-pigment-forming mutants of *P. phenazinium* on phosphate-limited media showed that the wild type lost viability more rapidly than the pigmentless mutants after growth was complete.

SUMMARY AND CONCLUSIONS

The first investigations on therapeutically useful metabolites from fluorescent pseudomonads date back some 80 years. Among the considerable number of antibiotic substances that have been isolated since then, only two (pyocyanine and pyrrolnitrin), or possibly three (pseudomonic acid), have reached the stage of practical application. Reports on strain development programs and on efforts to increase secondary metabolite production are scarce, and most in-

vestigations of secondary metabolites of pseudomonads have been abandoned after the isolation of the pure product. With the exception of phenazine and pyrrolnitrin biosyntheses there is little information available on the biosynthesis, genetics, and regulation of secondary metabolites from this group of microorganisms.

Nevertheless, it is important to integrate research on secondary metabolism into the rapidly advancing research on the biochemistry and genetics of *Pseudomonas*. The unicellular mode of growth of pseudomonads, their nutritional versatility, the increasing knowledge on their biochemistry, and the availability of genetic techniques represent experimental advantages frequently missing in other producers of secondary metabolites. Information on the production of secondary metabolites gained in studies with pseudomonads may be applicable to the more complex but industrially more important streptomycetes and bacilli. At the metabolic level the biochemistry and regulation of amino acid biosynthesis and of the multiple amino acid degradative pathways of pseudomonads are being studied intensively. However, the enzymes catalyzing the formation of amino acid-derived secondary metabolites are not known nor are the regulatory mechanisms of the primary pathways permitting the feeding of the secondary pathways. Integration of investigations on secondary metabolite formation into research on amino acid metabolism may lead to a better understanding of the role of catabolic reactions in the formation of certain secondary metabolites and may reveal general regulatory mechanisms affecting both primary and secondary metabolism (24).

At the genetic level the various functions of plasmids in pseudomonads attract much interest (18, 78, 147). Serious attention will undoubtedly be given to the possibility that certain secondary metabolites produced by pseudomonads are plasmid determined. Preliminary evidence indicates that some of the toxic amino acids produced by pseudomonads are plasmid associated (62). If this is confirmed, studies in these organisms may contribute to the analysis of plasmid involvement in the biosynthesis of secondary metabolites. In view of the ideal growth properties and the nutritional versatility of pseudomonads such investigations might also lead to the evaluation of these organisims as hosts for foreign plasmids specifying antibiotic formation.

Finally, the low-molecular-weight phytotoxins produced by the phytopathogenic pseudomonads of the species *P. syringae* are of practical interest because they are considered responsible for the disease symptoms caused by the invading bacteria in host plants (132). Studies on the formation of these secondary metabolites thus will contribute to our knowledge, and ultimately to the control, of certain plant diseases.

ACKNOWLEDGMENTS

We thank Rhône-Poulenc Industries for support of R. M. during his stay at the Swiss Federal Institute of Technology, Zürich.

We are indebted to D. Haas and J. M. Watson for helpful discussions and to J. M. Turner for communicating unpublished data. The expert secretarial help of Hélène Paul is gratefully acknowledged.

LITERATURE CITED

1. **Alexander, R. G., J. P. Clayton, I. Luk, N. H. Rogers, and T. J. King.** 1978. The chemistry of pseudomonic acid. Part 1: The absolute configuration of pseudomonic acid A. J. Chem. Soc. Perkin Trans. 1, p. 561–565.
2. **Arima, K., H. Imanaka, M. Kousaka, A. Fukuta, and G. Tamura.** 1964. Pyrrolnitrin, a new antibiotic substance, produced by *Pseudomonas*. Agric. Biol. Chem. **28**:575–576.
3. **Arima, K., H. Imanaka, M. Kousaka, A. Fukuta, and G. Tamura.** 1965. Studies on pyrrolnitrin, a new antibiotic. I. Isolation and properties of pyrrolnitrin. J. Antibiot. Ser. A. **18**:201–204.
4. **Banks, G. T., K. D. Barrow, E. B. Chain, A. T. Fuller, G. Mellows, and M. Woolford.** 1971. Pseudomonic acid: an antibiotic produced by *Pseudomonas fluorescens*. Nature (London) **234**:416–417.
5. **Bérdy, J.** 1974. Recent developments of antibiotic research and classification of antibiotics according to chemical structure. Adv. Appl. Microbiol. **18**:309–406.
6. **Bergström, S., H. Theorell, and H. Davide.** 1947. On a metabolic product of *Pseudomonas pyocyanea*. Pyolipic acid, active against *Mycobacterium tuberculosis*. Ark. Kemi Mineral. Geol. **23A**:1–12.
7. **Birkofer, L., and A. Birkofer.** 1948. Lactoflavin, eine Komponente des "Bakterien-Fluoresceins." Z. Naturforsch. Teil B **3**:136.
8. **Boyd, J., and J. R. Turvey.** 1978. Structural studies of alginic acid, using a bacterial poly-α-L-guluronate lyase. Carbohydr. Res. **66**:187–194.
9. **Broadbent, D., R. P. Mabelis, and H. Spencer.** 1976. C-acetylphloroglucinols from *Pseudomonas fluorescens*. Phytochemistry **15**: 1785.
10. **Burger, M. M., L. Glaser, and R. M. Burton.** 1963. The enzymatic synthesis of a rhamnose-containing glycolipid by extracts of *Pseudomonas aeruginosa*. J. Biol. Chem. **238**:2595–2602.
11. **Byng, G. S., and J. M. Turner.** 1976. Isolation of pigmentation mutants of *Pseudomonas phenazinium*. J. Gen. Microbiol. **97**:57–62.
12. **Byng, G. S., and J. M. Turner.** 1977. Incorpo-

ration of (^{14}C)shikimate into phenazines and their further metabolism by *Pseudomonas phenazinium*. Biochem. J. **164**:139–145.

13. **Calhoun, D. H., M. Carson, and R. A. Jensen.** 1972. The branch point for pyocyanine biosynthesis in *Pseudomonas aeruginosa*. J. Gen. Microbiol. **72**:581–583.
14. **Carson, M., and R. A. Jensen.** 1974. Phenotypic recognition of pyocyanine mutants in *Pseudomonas aeruginosa*. J. Bacteriol. **117**: 312–314.
15. **Castric, P. A.** 1975. Hydrogen cyanide, a secondary metabolite of *Pseudomonas aeruginosa*. Can. J. Microbiol. **21**:613–618.
16. **Chain, E. B., and G. Mellows.** 1977. Pseudomonic acid. Part 1. The structure of pseudomonic acid A, a novel antibiotic produced by *Pseudomonas fluorescens*. J. Chem. Soc. Perkin Trans. 1, p. 294–309.
17. **Chain, E. B., and G. Mellows.** 1977. Pseudomonic acid. Part 3. Structure of pseudomonic acid B. J. Chem. Soc. Perkin Trans. 1, p. 318–322.
18. **Chakrabarty, A. M.** 1976. Plasmids in *Pseudomonas*. Annu. Rev. Genet. **10**:7–20.
19. **Chakrabarty, A. M., and S. C. Roy.** 1964. Characterization of a pigment from a pseudomonad. Biochem. J. **93**:144–148.
20. **Clarke, P. H., and L. N. Ornston.** 1975. Metabolic pathways and regulation, p. 191–340. *In* P. H. Clarke and M. H. Richmond (ed.), Genetics and biochemistry of *Pseudomonas*. John Wiley & Sons, London.
21. **Cornforth, J. W., and A. T. James.** 1956. Structure of a naturally occurring antagonist of dihydrostreptomycin. Biochem. J. **63**:124–130.
22. **De Vay, J. E., F. L. Lukezic, S. L. Sinden, H. English, and D. L. Coplin.** 1968. A biocide produced by pathogenic isolates of *Pseudomonas syringae* and its possible role in the bacterial canker disease of peach trees. Phytopathology **58**:95–101.
23. **Doudoroff, M., and N. J. Palleroni.** 1974. Genus *Pseudomonas*, p. 217–243. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
24. **Drew, S. W., and A. L. Demain.** 1977. Effect of primary metabolites on secondary metabolism. Annu. Rev. Microbiol. **31**:343–356.
25. **Durbin, R. D., T. F. Uchytil, J. A. Steele, and R. de L. D. Ribeiro.** 1978. Tabtoxine-β-lactam from *Pseudomonas tabaci*. Phytochemistry **17**:147.
26. **Edwards, J. R., and J. A. Hayashi.** 1965. Structure of a rhamnolipid from *Pseudomonas aeruginosa*. Arch. Biochem. Biophys. **111**: 415–421.
27. **Elander, R. P., J. A. Mabe, R. H. Hamill, and M. Gorman.** 1968. Metabolism of tryptophans by *Pseudomonas aureofaciens*. VI. Production of pyrrolnitrin by selected *Pseudomonas* species. Appl. Microbiol. **16**:753–758.
28. **Elander, R. P., J. A. Mabe, R. L. Hamill, and M. Gorman.** 1971. Biosynthesis of pyrrolnitrins by analogue-resistant mutants of *Pseudomonas fluorescens*. Folia Microbiol. (Prague) **16**:156–165.
29. **Emmerich, R., and D. Löw.** 1899. Bakteriolytische Enzyme als Ursache der erworbenen Immunität und die Heilung von Infektionskrankheiten durch dieselben. Z. Hyg. Infektionskrank. **31**:1–65.
30. **Evans, J. R., E. J. Napier, and P. Yates.** 1976. Isolation of a new antibiotic from a species of *Pseudomonas*. J. Antibiot. Ser. A. **29**:850–852.
31. **Evans, L. R., and A. Linker.** 1973. Production and characterization of the slime polysaccharide of *Pseudomonas aeruginosa*. J. Bacteriol. **116**:915–924.
32. **Flood, M. E., R. B. Herbert, and F. G. Holliman.** 1972. Pigments of *Pseudomonas* species. Part V. Biosynthesis of pyocyanine and the pigments of *P. aureofaciens*. J. Chem. Soc. Perkin Trans. 1, p. 622–626.
33. **Floss, H. G., C. J. Chang, O. Mascaretti, and K. Shimada.** 1978. Studies on the biosynthesis of antibiotics. Planta Med. **34**:345–380.
34. **Garagulya, A. D., E. A. Kiprianova, and O. I. Boiko.** 1974. Antibiotic effect of bacteria from the genus *Pseudomonas* on phytopathogenic fungi. Mikrobiol. Zh. (Kiev) **36**:197–202.
35. **Garibaldi, J. A.** 1971. Influence of temperature on the iron metabolism of a fluorescent pseudomonad. J. Bacteriol. **105**:1036–1038.
36. **Gentry, M. J., D. K. Smith, S. F. Schnüte, S. L. Werber, and E. D. Weinberg.** 1971. *Pseudomonas* culture longevity: control by phosphate. Microbios **4**:205–215.
37. **Gerber, N. N.** 1969. New microbial phenazines. J. Heterocycl. Chem. **6**:297–300.
38. **Giral, F.** 1936. Sobre los liocromos característicos del grupo de bacterias fluorescentes. An. Soc. Esp. Fis. Quim. **34**:667–693.
39. **Gorman, M., and D. H. Lively.** 1967. Pyrrolnitrin: a new mode of tryptophan metabolism, p. 433–438. *In* D. Gottlieb and P. D. Shaw (ed.), Antibiotics, vol. 2. Biosynthesis. Springer-Verlag, Berlin.
40. **Gottlieb, D.** 1976. The production and role of antibiotics in soil. J. Antibiot. **29**:987–1000.
41. **Govan, J. R. W., J. A. M. Fyfe, and C. McMillan.** 1979. The instability of mucoid *Pseudomonas aeruginosa*: fluctuation test and improved stability of the mucoid form in shaken culture. J. Gen. Microbiol. **110**:229–232.
42. **Greppin, H., and S. Gouda.** 1965. Action de la lumière sur le pigment de *Pseudomonas fluorescens* Migula. Arch. Sci. **18**:721–725.
43. **Gross, D. C., and J. E. De Vay.** 1977. Production and purification of syringomycin, a phytotoxin produced by *Pseudomonas syringae*. Physiol. Plant Pathol. **11**:13–28.
44. **Halpern, Y. S., M. Teneh, and N. Grossowicz.** 1962. Further evidence for the production of pyocyanine by non-proliferating suspensions of *Pseudomonas aeruginosa*. J. Bacteriol. **83**:

935-936.
45. **Hamill, R., R. Elander, J. Mabe, and M. Gorman.** 1968. Metabolism of tryptophans by *Pseudomonas aureofaciens*. V. Conversion of tryptophan to pyrrolnitrin, p. 388-396. Antimicrob. Agents Chemother. 1967.
46. **Hansford, G. S., F. G. Holliman, and R. B. Herbert.** 1972. Pigments of *Pseudomonas* species. Part IV. *In vitro* and *in vivo* conversion of 5-methylphenazinium-1-carboxylate into aeruginosin A. J. Chem. Soc. Perkin Trans. 1, p. 103-105.
47. **Hashimoto, M., and K. Hattori.** 1966. Isopyrrolnitrin: a metabolite from *Pseudomonas*. Bull. Chem. Soc. Jpn. **39**:410.
48. **Hashimoto, M., and K. Hattori.** 1966. Oxypyrrolnitrin: a metabolite of *Pseudomonas*. Chem. Pharm. Bull. **14**:1314-1316.
49. **Hashimoto, M., and K. Hattori.** 1967. 2-(2-Heptenyl)-3-methyl-4-quinolinol from a *Pseudomonas*. Chem. Pharm. Bull. **15**:718-720.
50. **Hashimoto, M., and K. Hattori.** 1968. A new metabolite from *Pseudomonas pyrrolnitrica*. Chem. Pharm. Bull. **16**:1144.
51. **Hauser, G., and M. L. Karnovsky.** 1954. Studies on the production of glycolipide by *Pseudomonas aeruginosa*. J. Bacteriol. **68**:645-654.
52. **Hauser, G., and M. L. Karnovsky.** 1957. Rhamnose and rhamnolipide biosynthesis by *Pseudomonas aeruginosa*. J. Biol. Chem. **224**: 91-105.
53. **Hays, E. E., I. C. Wells, P. A. Katzman, C. K. Cain, F. A. Jacobs, S. A. Thayer, E. A. Doisy, W. L. Gaby, E. C. Roberts, R. D. Muir, C. J. Carroll, L. R. Jones, and N. J. Wade.** 1945. Antibiotic substances produced by *Pseudomonas aeruginosa*. J. Biol. Chem. **159**:725-750.
54. **Herbert, R. B., and F. G. Holliman.** 1969. Pigments of *Pseudomonas* species. Part II. Structure of aeruginosin B. J. Chem. Soc. C, p. 2517-2520.
55. **Herbert, R. B., F. G. Holliman, and P. N. Ibberson.** 1972. Incorporation of 6-hydroxyphenazine-1-carboxylic acid into iodinin. J. Chem. Soc. Chem. Commun., p. 355-356.
56. **Hiramoto, M., K. Okada, and S. Nagai.** 1970. The revised structure of viscosin, a peptide antibiotic. Tetrahedron Lett. **14**:1087-1090.
57. **Hisatsuka, K., T. Nakahara, N. Sano, and K. Yamada.** 1971. Formation of rhamnolipid by *Pseudomonas aeruginosa* and its function in hydrocarbon fermentation. Agric. Biol. Chem. **35**:686-692.
58. **Holliman, F. G.** 1969. Pigments of *Pseudomonas* species. Part I. Structure and synthesis of aeruginosin A. J. Chem. Soc. C, p. 2514-2516.
59. **Holloway, B. W.** 1975. Genetic organization of *Pseudomonas*, p. 133-161. *In* P. H. Clarke and M. H. Richmond (ed.), Genetics and biochemistry of *Pseudomonas*. John Wiley & Sons, London.
60. **Hollstein, U., G. E. Krisov, and D. L. Mock.** 1976. Biosynthesis of phenazines. The role of phenazine-1,6-dicarboxylic acid. Tetrahedron Lett. **37**:3267-3270.
61. **Hollstein, U., and D. A. McCamey.** 1973. Biosynthesis of phenazines. II. Incorporation of (6-^{14}C)-D-shikimic acid into phenazines-carboxylic acid and iodinin. J. Org. Chem. **38**:3415-3417.
62. **Hopwood, D. A.** 1978. Extrachromosomally determined antibiotic production. Annu. Rev. Microbiol. **32**:373-392.
63. **Hopwood, D. A., and M. J. Merrick.** 1977. Genetics of antibiotic production. Bacteriol. Rev. **41**:595-635.
64. **Hughes, J., and G. Mellows.** 1978. On the mode of action of pseudomonic acid: inhibition of protein synthesis in *Staphylococcus aureus*. J. Antibiot. **31**:330-335.
65. **Hulcher, F. H.** 1968. Activation of 6-phosphogluconate dehydrase by pyoverdine. Biochem. Biophys. Res. Commun. **31**:247-251.
66. **Ichihara, A., K. Shiraishi, H. Sato, S. Sakamura, K. Nishiyama, R. Sakai, A. Furusaki, and T. Matsumoto.** 1977. The structure of coronatine. J. Am. Chem. Soc. **99**:636-637.
67. **Imanaka, H., M. Kousaka, G. Tamura, and K. Arima.** 1965. Studies on pyrrolnitrin, a new antibiotic. III. Structure of pyrrolnitrin. J. Antibiot. Ser. A **18**:207-210.
68. **Ingledew, W. M., and J. J. R. Campbell.** 1969. Evaluation of shikimic acid as a precursor of pyocyanine. Can. J. Microbiol. **15**:535-541.
69. **Ingledew, W. M., and J. J. R. Campbell.** 1969. A new resuspension medium for pyocyanine production. Can. J. Microbiol. **15**:595-598.
70. **Ingram, J. M., and A. C. Blackwood.** 1970. Microbial production of phenazines. Adv. Appl. Microbiol. **13**:267-282.
71. **Itoh, S., H. Honda, F. Tomita, and T. Suzuki.** 1971. Rhamnolipids produced by *Pseudomonas aeruginosa* grown on *n*-paraffin (mixture of C_{12}, C_{13} and C_{14} fractions). J. Antibiot. **24**: 855-859.
72. **Itoh, S., and T. Suzuki.** 1972. Effect of rhamnolipids on growth of *Pseudomonas aeruginosa* mutant deficient in *n*-paraffin-utilizing ability. Agric. Biol. Chem. **36**:2233-2235.
73. **Jarvis, F. G., and M. J. Johnson.** 1949. A glyco-lipide produced by *Pseudomonas aeruginosa*. J. Am. Chem. Soc. **71**:4124-4126.
74. **Kanda, N., N. Ishizaki, N. Inoue, M. Oshima, A. Handa, and T. Kitahara.** 1975. DB-2073, a new alkylresorcinol antibiotic. I. Taxonomy, isolation and characterization. J. Antibiot. **28**: 935-942.
75. **Kanner, D., N. N. Gerber, and R. Bartha.** 1978. Pattern of phenazine pigment production by a strain of *Pseudomonas aeruginosa*. J. Bacteriol. **134**:690-692.
76. **Kitahara, T., and N. Kanda.** 1975. DB-2073, a new dialkylresorcinol antibiotic. II. The chemical structure of DB-2073. J. Antibiot. **28**:943-946.
77. **Knowles, C. J.** 1976. Microorganisms and cyanide. Bacteriol. Rev. **40**:652-680.
78. **Korfhagen, T. R., L. Sutton, and G. A. Ja-**

coby. 1978. Classification and physical properties of *Pseudomonas* plasmids, p. 221-224. *In* D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.

79. **Korth, H.** 1971. Einfluss von Eisen und Sauerstoff auf die Pigmentbildung bei verschiedenen *Pseudomonas*-Spezies. Arch. Microbiol. **77:** 59-64.
80. **Kozlovskii, A. G.** 1975. Alkaloids of microorganisms. Biokhim. Fiziol. Mikroorg., p. 74-77.
81. **Kozlovskii, A. G., M. U. Arinbasarov, G. I. Yakoulev, A. M. Zyklin, and V. M. Adanin.** 1976. 2-(1-Heptenyl)-4-quinolone as a new metabolite of *Pseudomonas aeruginosa*. Izv. Akad. Nauk SSSR Ser. Khim **(5):**1146-1150.
82. **Lenhoff, H. M.** 1963. An inverse relationship of the effects of oxygen and iron on the production of fluorescin and cytochrome C by *Pseudomonas fluorescens*. Nature (London) **199:** 601-602.
83. **Lightbown, J. W.** 1954. An antagonist of streptomycin and dihydrostreptomycin produced by *Pseudomonas aeruginosa*. J. Gen. Microbiol. **11:**477-492.
84. **Lightbown, J. W., and F. L. Jackson.** 1956. Inhibition of cytochrome systems of heart muscle and certain bacteria by the antagonists of dihydrostreptomycin: 2-alkyl-4-hydroxyquinoline N-oxides. Biochem. J. **63:**130-137.
85. **Lively, D. H., M. Gorman, M. E. Haney, and J. A. Mabe.** 1967. Metabolism of tryptophans by *Pseudomonas aureofaciens*. I. Biosynthesis of pyrrolnitrin, 462-469. Antimicrob. Agents Chemother. 1966.
86. **Longley, R. P., J. E. Halliwell, J. J. R. Campbell, and W. M. Ingledew.** 1972. The branchpoint of pyocyanine biosynthesis. Can. J. Microbiol. **18:**1357-1364.
87. **MacDonald, J. C.** 1963. Biosynthesis of pyocyanine. Can. J. Microbiol. **9:**809-819.
88. **MacDonald, J. C.** 1967. Pyocyanine, p. 52-65. *In* D. Gottlieb and P. D. Shaw (ed.), Antibiotics, vol. 2. Biosynthesis. Springer-Verlag, Berlin.
89. **Mann, S.** 1966. Ueber den Geruchsstoff von *Pseudomonas aeruginosa*. Arch. Mikrobiol. **54:**184-190.
90. **Martin, J. F.** 1977. Control of antibiotic synthesis by phosphate. Adv. Biochem. Eng. **6:**105-127. A. Fiechter, and N. Blakebrough (ed.).
91. **Martin, L. L., C.-J. Chang, H. G. Floss, J. A. Mabe, E. W. Hagaman, and E. Wenkert.** 1972. A ^{13}C nuclear magnetic resonance study on the biosynthesis of pyrrolnitrin from tryptophan by *Pseudomonas*. J. Am. Chem. Soc. **94:**8942-8944.
92. **Maurer, B., A. Müller, W. Keller-Schierlein, and H. Zähner.** 1968. Ferribactin, ein Siderochrom aus *Pseudomonas fluorescens* Migula. Arch. Microbiol. **60:**326-339.
93. **Meganathan, R., and P. A. Castric.** 1977. The effect of inorganic phosphate on cyanogenesis by *Pseudomonas aeruginosa*. Arch. Mikrobiol. **114:**51-54.
94. **Meyer, J. M., and M. A. Abdallah.** 1978. The fluorescent pigment of *Pseudomonas fluorescens*: biosynthesis, purification and physicochemical properties. J. Gen. Microbiol. **107:** 319-328.
95. **Meyer, J. M., and J. M. Hornsperger.** 1978. Role of pyoverdine$_{pf}$, the iron-binding fluorescent pigment of *Pseudomonas fluorescens*, in iron transport. J. Gen. Microbiol. **107:**329-331.
96. **Mian, F. A., T. R. Jarman, and R. C. Righelato.** 1978. Biosynthesis of exopolysaccharide by *Pseudomonas aeruginosa*. J. Bacteriol. **134:**418-422.
97. **Michea, M., and M. Greppin.** 1974. Séparation et évolution du complexe pigmentaire de *Pseudomonas fluorescens* Migula. C. R. Seances Soc. Phys. Hist. Nat. Geneve **8:**19-31.
98. **Miller, M. W.** 1961. The Pfizer handbook of microbial metabolites. McGraw-Hill, New York.
99. **Millican, R. C.** 1962. Biosynthesis of pyocyanine. Incorporation of ^{14}C-shikimic acid. Biochim. Biophys. Acta **57:**407-409.
100. **Mitchell, R. E.** 1976. Isolation and structure of a chlorosis inducing toxin of *Pseudomonas phaseolicola*. Phytochemistry **15:**1941-1947.
101. **Mitchell, R. E., and R. L. Bieleski.** 1977. Involvement of phaseolotoxin in halo-blight of beans. Transport and conversion to functional toxin. Plant Physiol. **60:**723-729.
102. **Mitchell, R. E., and E. A. Parsons.** 1977. A naturally-occurring analogue of phaseolotoxin (bean haloblight toxin). Phytochemistry **16:** 280-281.
103. **Nara, K., Y. Sumino, K. Katamoto, S. Akiyama, and M. Asai.** 1977. Chemistry of aminoglycoside antibiotics P-2563 (P) and (A). Chem. Lett., p. 33-36.
104. **Newkirk, J. D., and F. H. Hulcher.** 1969. Isolation and properties of a fluorescent pigment from *Pseudomonas mildenbergii*. Arch. Biochem. Biophys. **134:**395-400.
105. **Oki, T., Y. Nishimura, Y. Sayama, A. Kitai, and A. Ozaki.** 1969. Microbial utilization of non-carbohydrate substances. Part I. Fermentative production of L-glutamic acid from ethanol. (1) Screening of ethanol assimilating microorganisms. Amino Acids Nucleic Acids **19:**73-81.
106. **Owens, L. D., J. F. Thompson, and P. V. Fennessey.** 1972. Dihydrorhizobitoxine, a new ether amino acid from *Rhizobium japonicum*. J. Chem. Soc. Chem. Chem. Commun., p. 715.
107. **Owens, L. D., J. F. Thompson, R. G. Pitcher, and T. Williams.** 1972. Structure of rhizobitoxine, an antimetabolite enol-ether amino-acid from *Rhizobium japonicum*. J. Chem. Soc. Chem. Commun., p. 714.
108. **Palleroni, N. J.** 1975. General properties and taxonomy of the genus *Pseudomonas*, p. 1-30. *In* P. H. Clarke and M. H. Richmond (ed.), Genetics and biochemistry of *Pseudomonas*. John Wiley & Sons, London.
109. **Palleroni, N. J., R. Kunisawa, R. Contopou-**

lou, and M. Doudoroff. 1973. Nucleic acid homologies in the genus *Pseudomonas*. Int. J. Syst. Bacteriol. **23**:333–339.
110. **Patil, S. S.** 1974. Toxins produced by phytopathogenic bacteria. Annu. Rev. Phytopathol. **12**: 259–279.
111. **Patil, S. S., A. C. Hayward, and R. Emmons.** 1974. An ultra-violet induced nontoxigenic mutant of altered pathogenicity. Phytopathology **64**:590–595.
112. **Patil, S. S., P. E. Kolattukudy, and E. A. Dimond.** 1970. Inhibition of ornithine carbamyl transferase from bean plants by the toxin of *Pseudomonas phaseolicola*. Plant Physiol. **46**:752–753.
113. **Patil, S. S., L. Q. Tam, and W. S. Sakai.** 1972. Mode of action of the toxin from *Pseudomonas phaseolicola*. I. Toxin specificity, chlorosis, and ornithine accumulation. Plant Physiol. **49**: 803–807.
114. **Patil, S. S., P. Youngblood, P. Christiansen, and R. E. Moore.** 1976. Phaseotoxin A: an antimetabolite from *Pseudomonas phaseolicola*. Biochem. Biophys. Res. Commun. **69**: 1019–1027.
115. **Pouteau-Thouvenot, M., and M. Barbier.** 1965. Synthèse de l'apoferrorosamine [(pyridyl-2')-2-pyrroline-2]. Bull. Soc. Chim. Fr., p. 3238–3240.
116. **Pouteau-Thouvenot, M., A. Gaudemer, and M. Barbier.** 1965. Sur la ferrorosamine, pigment de *Bacillus roseus fluorescens*. Bull. Soc. Chim. Biol. **47**:2085–2094.
117. **Pruess, D. L., J. P. Scannell, M. Kellett, H. A. Ax, J. Janecek, T. H. Williams, A. Stempel, and J. Berger.** 1974. Antimetabolites produced by microorganisms. X. L-2-Amino-4-(2-aminoethoxy)-trans-3-butenoic acid. J. Antibiot. **27**:229–233.
118. **Rando, R. R.** 1977. Mechanism-based irreversible enzyme inhibitors. Methods Enzymol. **46**: 28–41.
119. **Rando, R. R., N. Relyea, and L. Cheng.** 1976. Mechanism of the irreversible inhibition of aspartate aminotransferase by the bacterial toxin L-2-amino-4-methoxy-trans-3-butenoic acid. J. Biol. Chem. **251**:3306–3312.
120. **Reddi, T. K., Y. P. Khudyakov, and A. V. Borokov.** 1969. *Pseudomonas fluorescens* strain 26.0, a producer of phytotoxic substances. Mikrobiologiya **38**:909–913.
121. **Rietschel, E. T.** 1976. Absolute configuration of 3-hydroxy fatty acids present in lipopolysaccharides from various bacterial groups. Eur. J. Biochem. **64**:423–428.
122. **Ritter, C.** 1972. Zur Biosynthese der Pseudane, p. 545–548. *In* K. Mothes, K. Schreiber and H. R. Schütte (ed.), Biochemie und Physiologie der Alkaloide. Fourth International Symposium, 1969. Akademie-Verlag, Berlin.
123. **Rudolph, K., E. Rasche, and W. Zeller.** 1978. Production of the chlorosis inducing toxin in liquid cultures of *Pseudomonas phaseolicola* (Burkh.) Dowson. Arch. Mikrobiol. **119**:219–226.
124. **Sahm, U., G. Knobloch, and F. Wagner.** 1973. Isolation and characterization of the methionine antagonist L-2-amino-4-methoxy-trans-3-butenoic acid from *Pseudomonas aeruginosa* grown on *n*-paraffin. J. Antibiot. **26**:389–390.
125. **Salcher, O., and F. Lingens.** 1978. Biosynthese von Pyrrolnitrin. Nachweis von 3-Chloranthranilsäure und 7-Chlorindolessigsäure im Kulturmedium von *Pseudomonas aureofaciens*. Tetrahedron Lett. **34**:3101–3102.
126. **Salcher, O., F. Lingens, and P. Fischer.** 1978. Biosynthese von Pyrrolnitrin. Nachweis von 4-(2'-amino-3'-chlorphenyl)pyrrol-2-carbonsäure. Tetrahedron Lett. **34**:3097–3100.
127. **Scannell, J. P., D. L. Pruess, H. A. Ax, A. Jacoby, M. Kellett, and A. Stempel.** 1976. Antimetabolites produced by microorganisms. XIII. The synthesis and microbiological production of a novel amino acid, L-2-amino-4-(2-aminoethoxy) butanoic acid. J. Antibiot. **29**: 38–43.
128. **Scannell, J. P., D. L. Pruess, T. C. Demny, L. H. Sello, T. Williams, and A. Stempel.** 1972. Antimetabolites produced by microorganisms. V. L-2-Amino-4-methoxy-trans-3-butenoic acid. J. Antibiot. **25**:122–127.
129. **Shiman, R., and J. B. Neilands.** 1965. Isolation, characterization, and synthesis of pyrimine, an iron (II)-binding agent from *Pseudomonas* GH. Biochemistry **4**:2233.
130. **Sinden, S. L., J. E. DeVay, and P. A. Backman.** 1971. Properties of syringomycin, a wide spectrum antibiotic and phytotoxin produced by *Pseudomonas syringae*, and its role in the bacterial canker disease of peach trees. Physiol. Plant Pathol. **1**:199–213.
131. **Stewart, W. W.** 1971. Isolation and proof of structure of wildfire toxin. Nature (London) **229**:174–178.
132. **Strobel, G. A.** 1977. Bacterial phytotoxins. Annu. Rev. Microbiol. **31**:205–224.
133. **Sureau, B., E. Arquie, F. Boyer, and M. Saviard.** 1948. Premières données sur la production d'une "streptomycinase" par certaines souches microbiennes. Ann. Inst. Pasteur Paris **75**:169–171.
134. **Suzuki, A., and M. Goto.** 1971. Isolation and characterization of pteridines from *Pseudomonas ovalis*. Bull. Chem. Soc. Jpn. **44**:1869–1872.
135. **Suzuki, A., and M. Goto.** 1972. The structure of a new pteridine compound produced by *Pseudomonas ovalis*. Bull. Chem. Soc. Jpn. **45**:2198–2199.
136. **Takeda, R.** 1958. Structure of a new antibiotic, pyoluteorin. J. Am. Chem. Soc. **80**:4749–4750.
137. **Takeda, R., and I. Nakanishi.** 1959. *Pseudomonas* pigments (VI). Production of anthranilic acid by *Pseudomonas aeruginosa* T 359. J. Ferment. Technol. **37**:54–58.
138. **Takeda, R., and I. Nakanishi.** 1959. *Pseudomonas* pigments (VII). Fraction B produced by *Pseudomonas aeruginosa* T 359. J. Ferment.

Technol. **37**:59–63.

139. **Tam, L. Q., and S. S. Patil.** 1972. Mode of action of the toxin from *Pseudomonas phaseolicola*. II. Mechanism of inhibition of bean ornithine carbamyl-transferase. Plant Physiol. **49**:808–812.
140. **Taylor, P. A., H. K. Schnoes, and R. D. Durbin.** 1972. Characterization of chlorosis-inducing toxins from a plant pathogenic *Pseudomonas* sp. Biochim. Biophys. Acta **286**:107–117.
141. **Viscontini, M., and R. Bühler-Moor.** 1968. Isolierung von L-Monapterin aus *Pseudomonas roseus fluorescens* J. C. Marchal 1937 und seine Konstitutionsaufklärung. Helv. Chim. Acta **51**:1548–1554.
142. **Viscontini, M., and M. Frater-Schröder.** 1968. Isolierung von 6-Hydroxy-methylpterin aus Kulturen von *Pseudomonas roseus fluorescens* J. C. Marchal 1937. Helv. Chim. Acta **51**: 1554–1557.
143. **Viscontini, M., M. Pouteau-Thouvenot, R. Bühler-Moor, and M. Schröder.** 1964. Nouvelles ptéridines et nouvelles porphyrines obtenues à partir du *Pseudomonas roseus fluorescens*. Helv. Chim. Acta **47**:1948–1950.
144. **Weinberg, E. D.** 1971. Secondary metabolism: raison d'être. Perspect. Biol. Med. **14**:565–577.
145. **Wells, I. C.** 1952. Antibiotic substances produced by *Pseudomonas aeruginosa*. Synthesis of Pyo I_b, Pyo I_c, and Pyo III. J. Biol. Chem. **196**:321–330.
146. **Wells, I. C., W. H. Elliott, S. A. Thayer, and E. A. Doisy.** 1952. Ozonization of some antibiotic substances produced by *Pseudomonas aeruginosa*. J. Biol. Chem. **196**:331–339.
147. **Wheelis, M. L.** 1975. The genetics of dissimilatory pathways in *Pseudomonas*. Annu. Rev. Microbiol. **29**:505–524.
148. **Wissing, F.** 1974. Cyanide formation from oxidation of glycine by a *Pseudomonas* species. J. Bacteriol. **117**:1289–1294.
149. **Wissing, F.** 1975. Cyanide production from glycine by a homogenate from a *Pseudomonas* species. J. Bacteriol. **121**:695–699.
150. **Wratten, S. J., M. S. Wolfe, R. J. Andersen, and D. J. Faukner.** 1977. Antibiotic metabolites from a marine pseudomonad. Antimicrob. Agents Chemother. **11**:411–414.
151. **Yamada, Y., N. Seki, T. Kitahara, M. Takahashi, and M. Matsui.** 1970. The structure and synthesis of aeruginoic acid (2-O-hydroxyphenylthiazole-4-carboxylic acid). Agric. Biol. Chem. **34**:780–783.
152. **Yamaguchi, M., S. Sato, and A. Yukuyama.** 1976. Microbial production of sugarlipids. Chem. Ind., p. 741–742.
153. **Zähner, H.** 1977. Some aspects of antibiotics research. Angew. Chem. Int. Ed. **16**:687–694.
154. **Zähner, H., W. Keller-Schierlein, R. Hütter, K. Hess-Leisinger, and A. Deer.** 1963. Sideramine aus Aspergillaceen. Arch. Mikrobiol. **45**:119–135.