# Biosynthesis of Pyocyanin, a Phenazine Microbial Metabolite

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*Summary* Tracer experiments have shown that phenazine-1-carboxylic acid and its 5-methyl quaternary salt are incorporated into pyocyanin by *Pseudomonas aeruginosa* by decarboxylative hydroxylation.

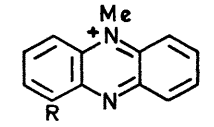
NATURAL products with structures based on the phenazine ring system now number about thirty:[1] they are elaborated exclusively by micro-organisms. The best known of them is pyocyanin (**1**),[2] a metabolite of *Pseudomonas aeruginosa*, other strains of which alternatively produce aeruginosins A (**4**)[3] and B (**5**).[4] Biosynthetic studies on the origins of the phenazine nucleus implicate shikimic acid,[5] and at least one molecule of anthranilic acid has been shown to be involved in the production of chlororaphin, a phenazine-1-carboxamide pigment.[6] With regard to intermediates involved at later stages, it has been shown that iodinin (**6**) may be

derived in high efficiency from phenazine-1,6-diol and the corresponding 5-oxide.[5a] On the other hand, 1-hydroxyphenazine (**7**) is not a precursor of pyocyanin.[7]

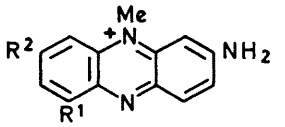
We have already shown that 5-methylphenazinium-1-carboxylate (**2**) can be converted by aqueous ammonia into aeruginosin A (**4**)[8] [which with sulphite ion gives aeruginosin B (**5**)[9]] and by photo-oxidation into pyocyanin,[10] prompting the suggestion that it may represent a common intermediate in the biosynthesis of these metabolites.[10] We now report on the testing of this compound along with 1-carboxyphenazine (**8**), also a microbial metabolite, and phenazine methosulphate (**3**) as precursors of pyocyanin in the living system.

When 1-carboxy-6,7,8,9-tetradeuterio-5-methylphenazinium chloride was administered over the pigment producing period to growing cultures of an appropriate strain of *Ps. aeruginosa*, a 2·4% incorporation into pyocyanin was found. The product was most easily assayed by conversion into 1-hydroxyphenazine (**7**), the mass spectrum of which is simple in the high mass region where, for the undeuteriated material, two prominent peaks are found at *m/e* 196 ($M^+$) and 168 ($M^+ - CO$).† The mass spectrum of the 1-hydroxyphenazine from the feeding experiment showed corresponding peaks of the $^2H_4$ species but not of the $^2H_3$, indicating specific hydroxylation at C-1 with concomitant decarboxylation. A similar result was obtained with 1-carboxy-6,7,8,9-tetradeuteriophenazine (1·4% incorporation). 1,2,3,4-Tetradeuteriophenazine methosulphate was also incorporated into pyocyanin (0·7%) with approximately equal quantities of the $^2H_3$ and $^2H_4$ species being produced.

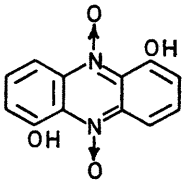
The highly specific hydroxylation of (**2**) and (**8**) and the respective levels of incorporation strongly support the biosynthetic sequence (**8**) → (**2**) → pyocyanin (**1**). On the other hand, the significantly lower level of incorporation of



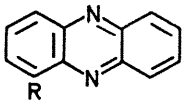

(1) R = $O^-$
(2) R = $CO_2^-$
(3) R = H; methosulphate




(4) $R^1$ = $CO_2^-$, $R^2$ = H
(5) $R^1$ = $CO_2H$, $R^2$ = $SO_3^-$




(6)




(7) R = OH
(8) R = $CO_2H$

phenazine methosulphate (**3**), which, if involved, ought to be the immediate precursor of pyocyanin, is in keeping with it being a non-specific substrate rather than a true biosynthetic precursor.

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† The hydroxyphenazine from the three feeding experiments contained 0·4—2·6% of deuteriated species. Measurements were made at both $M^+$ and $M^+ - CO$ to avoid error from impurity in the sample. The contribution of ions at *m/e* 200, 199, 172, and 171 due to the undeuteriated material was allowed for; reasonable accuracy was assured as they were of low intensity relative to those due to deuteriated species.

[1] N. N. Gerber, *J. Heterocyclic Chem.*, 1969, **6**, 297 and references cited therein; C. D. Tipton and K. L. Rinehart, jun., *J. Amer. Chem. Soc.*, 1970, **92**, 1425.
[2] F. Wrede and E. Strack, *Z. Physiol. Chem.*, 1929, **181**, 58.
[3] F. G. Holliman, *J. Chem. Soc.* (*C*), 1969, 2514.
[4] R. B. Herbert and F. G. Holliman, *J. Chem. Soc.* (*C*), 1969, 2517.
[5] (a) N. N. Gerber, *Biochemistry*, 1967, **6**, 2701; (b) references cited in (a).
[6] R. E. Carter and J. H. Richards, *J. Amer. Chem. Soc.*, 1961, **83**, 495.
[7] L. H. Frank and R. D. DeMoss, *J. Bact.*, 1959, **77**, 776.
[8] G. S. Hansford, R. B. Herbert, and F. G. Holliman, unpublished work.
[9] R. K. Bentley and F. G. Holliman, *Chem. Comm.*, 1966, 312; *J. Chem. Soc.* (*C*), in the press.
[10] M. E. Flood, R. B. Herbert, and F. G. Holliman, *Tetrahedron Letters*, 1970, 4101.