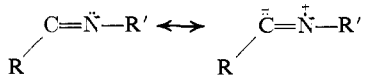
gardless of the substituent attached to the sp^2-hybridized carbon.[12] The radical stabilization was suggested to be derived solely from resonance interaction with the oxygen lone pairs. A similar type of resonance stabilization in iminoyl radicals seems plausible. However, if iminoyl radicals are stabilized by interaction of the unpaired electron with the lone pair of electrons on nitrogen as depicted by the contributing resonance structure **1a**, it is not evident from the magnitude of the nitrogen hyperfine interaction as all the iminoyl radicals reported in Table I exhibit a^N values of only 1.2–1.9 G. It is plausible that the positive spin density at the nitrogen nucleus resulting from a resonance effect as in **1a** may be nearly balanced by a spin polarization mechanism inducing negative spin density at the nitrogen.



1 **1a**

Preliminary INDO calculations support the conclusion that iminoyl radicals are σ species with a nonlinear N=C—C bond. For HN=ĊH the syn configuration is calculated to be slightly more stable than the anti with a moderate barrier to inversion. The calculated a^N values, however, are too large (a^N positive) by about an order of magnitude.

We are continuing our esr investigations of iminoyl radicals as well as determining the chemical behavior of these species and will report additional results at a later date.

(12) R. K. Solly and S. W. Benson, *J. Amer. Chem. Soc.*, **93**, 1592 (1971).

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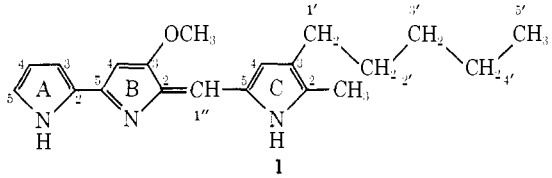
Received July 19, 1973

Biosynthesis of Prodigiosin. Incorporation Patterns of ^{13}C-Labeled Alanine, Proline, Glycine, and Serine Elucidated by Fourier Transform Nuclear Magnetic Resonance[1-3]

Sir:

We have previously reported the biosynthetic origin of ten of the 20 carbon atoms in prodigiosin (**1**) as acetate, using ^{13}C Fourier transform (FT) nmr (Figure 1).[4] We now report our findings on the origin of the remaining carbons of **1** which establish the novel pattern of biosynthesis of this tripyrrole metabolite of *Serratia marcescens*. ^{13}C FT nmr studies have allowed us to elucidate complex patterns of primary *and* secondary pathways of ^{13}C enriched substrate incorporation into **1**, and thus to establish some of the primary metabolic interconversions occurring in *S. marcescens*. We have fed ^{13}C-labeled alanine, proline, glycine, and serine to the bacterium, and determined specific incorporations by observing the ratio (per cent) of the excess ^{13}C at each carbon atom of the isolated prodigiosin to the excess ^{13}C in the fed substrate.[4a] Our results are summarized below.



1

The methoxyl group of prodigiosin is derived from methionine. While the incorporation of [*methyl*-^{14}C]-methionine has been previously established,[5,6] we have fed [CD_3]-D,L-methionine[3,7] (>99% $-CD_3$, 2.0 mmol/l.) to *S. marcescens* and isolated **1** containing 51.0 (±1.0)% $-OCD_3$ as determined by pmr and mass spectral studies.

The pyrrole methyl group in ring C of prodigiosin (C2–*Me*) is derived from the methyl group of alanine. When we fed [1-^{14}C]-, [2-^{14}C]-, and [3-^{14}C]alanine, we observed high molar incorporations of the 2- and 3-carbon atoms into **1** (70–80%), while the carboxyl carbon was not incorporated (<0.1%). When [3-^{13}C]-L-alanine (41.7% ^{13}C, 7.2 mmol/l.) was fed, the isolated **1** showed six sites of primary incorporation by FT nmr, all of approximately equal size (8.0 (±2.0)% ^{13}C). The pattern of labeling was identical with that observed on feeding [2-^{13}C]acetate[4] with one additional, and equal, incorporation at the methyl group on ring C (C2–*Me*) (Figure 1).

The above results can be explained on the following basis: C2 and C2–*Me* in **1** are biosynthetically derived from the 2- and 3- carbon atoms of alanine, respectively, while the additional incorporation of label from [3-^{13}C]alanine arises from its well-established metabolism to acetate *via* reversible transamination to pyruvate which is then oxidatively decarboxylated.[8,9]

Varying conclusions have been drawn from earlier studies of the incorporation of proline into prodigiosin.[11,12] Our own work clearly shows that there is only one site of primary incorporation. When [*carboxyl*-^{13}C]-D,L-proline (31.3% ^{13}C, 6.0 mmol/l.) was fed,[3] there was a single major incorporation of the label at carbon B5 (28.0 (±1.0)% ^{13}C). Mass spectral studies

(1) Biosynthesis of Prodigiosin. II. For the first paper in this series, see ref 4.

(2) Carbon-13 Fourier Transform Nmr. VI. Part V: A. I. Scott, C. A. Townsend, K. Okada, M. Kajiwara, and R. J. Cushley, *J. Amer. Chem. Soc.*, **94**, 8269 (1972).

(3) From the Doctoral Dissertation of R. J. Sykes, Yale University, 1973.

(4) R. J. Cushley, D. R. Anderson, S. R. Lipsky, R. J. Sykes, and H. H. Wasserman, *J. Amer. Chem. Soc.*, **93**, 6284 (1971).

(4a) NOTE ADDED IN PROOF. Measurements were made by comparing the spectrum of the enriched sample with that of an unenriched sample under identical instrumental conditions. The excess ^{13}C was also determined by measuring satellite peaks of pmr spectra where possible and by mass spectral measurements. For detailed procedures see ref 3.

(5) S. M. Qadri and R. P. Williams, *Biochim. Biophys. Acta*, **230**, 181 (1971).

(6) W. K. Tanaka, L. B. deMedina, and W. R. Hearn, *Biochem. Biophys. Res. Commun.*, **46**, 731 (1972).

(7) A. Murray III and D. L. Williams, "Organic Synthesis with Isotopes," Interscience, New York, N. Y., 1958.

(8) The approximately equal levels of incorporation of label from [3-^{13}C]alanine *per se* and *via* its conversion to labeled acetate can be explained if the major source of acetate in *S. marcescens* is the decarboxylation of pyruvate. The bacteria were grown on a medium rich in glycerol (≃135 mmol/l.) which is metabolized to acetate *via* pyruvate.[10] The lack of incorporation of label from [2-^{13}C]acetate at C2–*Me* indicates that in *S. marcescens*, the conversion of alanine to acetate is irreversible.

(9) D. M. Greenberg in "Metabolic Pathways," Vol. III, 3rd ed, D. M. Greenberg, Ed., Academic Press, New York, N. Y., 1969, p 95.

(10) A. Meister, "Biochemistry of the Amino Acids," Academic Press, New York, N. Y., 1965, p 660 ff.

(11) D. M. Shrimpton, G. S. Marks, and L. Bogorad, *Biochim. Biophys. Acta*, **71**, 408 (1963).

(12) L. B. deMedina, Ph.D. Thesis, Iowa State University, 1969.

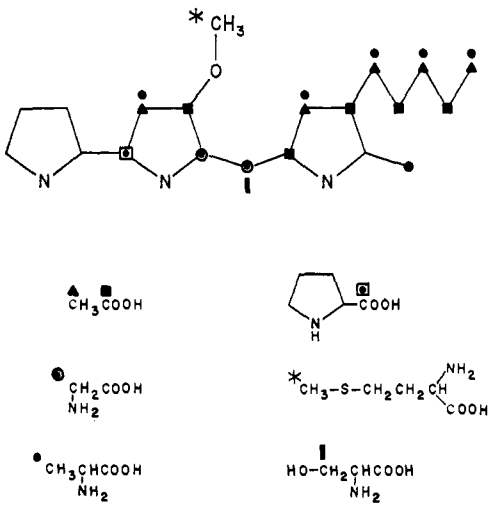


Figure 1. Incorporation patterns of acetate, glycine, alanine, proline, methionine, and serine in prodigiosin.

showed a low level of excess ^{13}C in the acetate-derived carbons of the *n*-amyl side chain (a total of 4.5 (±0.5)% ^{13}C for carbons 2′, 3′, 4′, and 5′) while there was no excess ^{13}C (≤1.0% ^{13}C per carbon) detectable in ring A by FT nmr. The high specific incorporation of the fed proline (>90%) and the low level of scrambling (<10%) provides strong support for the hypothesis that ring A and carbon B5 are derived from a single, intact molecule of proline,[6] or some closely related metabolite such as Δ^1-pyrroline 5-carboxylate.

The incorporation of the glycine methylene carbon into prodigiosin is unusually high.[13] We have regularly recorded molar incorporations of over 300% from a medium containing 10.8 mmol/l. of [2-^{14}C]glycine. (Under the same conditions, incorporation of [1-^{14}C]-glycine was less than 0.1%.) When [2-^{13}C]glycine[3] (27.5% ^{13}C, 10.8 mmol/l.) was fed in the presence of 0.7 mmol/l. L-serine, isolated **1** showed strong enrichment of carbons B2 and 1′′ [14] (21.5 (±1.5)% ^{13}C). Secondary incorporation was found at –O*Me* (7.5 (±1.0)% ^{13}C) and at every carbon atom derived from acetate and alanine (2.5 (±1.0)% ^{13}C).

When [3-^{13}C]-D,L-serine[7] (79.6% ^{13}C, 5.4 mmol/l.) was fed in the presence of 7.5 mmol/l. of glycine, carbon 1′′ became strongly enriched (24.0 (±1.0)% ^{13}C), while there was no detectable enrichment of carbon B2 (<1.0% ^{13}C). Secondary incorporation was seen at –O*Me* (1.5 (±0.5)% ^{13}C) and at carbon atoms derived from the 2-carbon of acetate and the 3-carbon of alanine (7.0 (±1.0)% ^{13}C). Feeding experiments with [1-^{14}C]-, [2-^{14}C]-, and [3-^{14}C]-L-serine showed that the 2-carbon of serine was incorporated as efficiently as the 3-carbon, while the 1-carbon of serine was not incorporated.

The above data show that carbons B2 and 1′′ of prodigiosin are derived from carbons 2 and 3 of serine, respectively, or from the immediate metabolic precursors of serine, *i.e.*, glycine (incorporated as B2) and a one carbon source (incorporated as 1′′). The appearance of label from [2-^{13}C]glycine at –O*Me*, and at each carbon derived from acetate indicates that the

(13) R. Hubbard and C. Rimington, *Biochem. J.*, **46**, 220 (1950).
(14) An AB ^{13}C–^{13}C coupling pattern was observed with $J_{1''-B2}$ = 75 Hz.

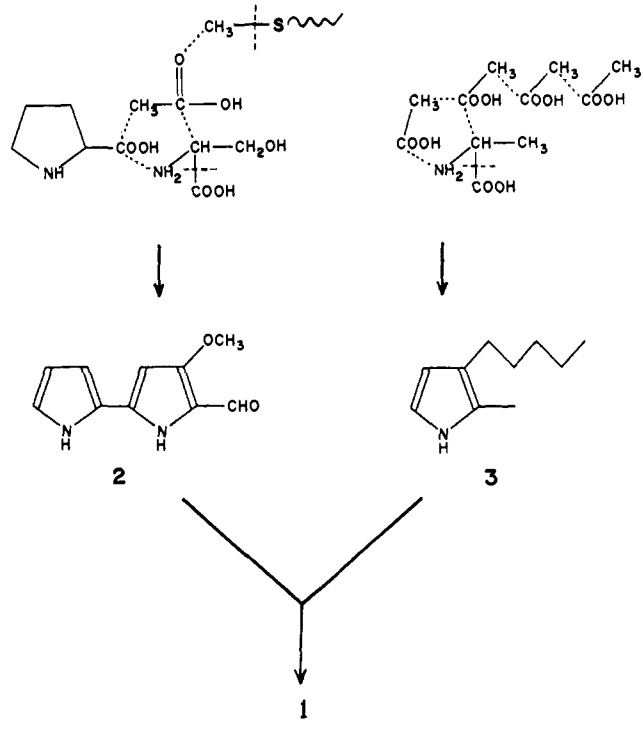


Figure 2. Proposed scheme for the biosynthesis of prodigiosin.

well established glycine–serine interconversion[9,15] (which, in this case, will result in doubly labeled serine) is taking place in *S. marcescens*. The high degree of incorporation of the label at –O*Me* (specific incorporation >25%) in the presence of 10.0 mmol/l. of L-methionine shows that glycine may be the major metabolic source of one carbon units in *S. marcescens*.[16]

Earlier studies have shown that the final step in the biosynthesis of prodigiosin involves condensation of two major components, the methoxybipyrrolecarboxaldehyde **2**, and the methylamylpyrrole **3**.[17] The present work now shows that **2** is constructed from proline, serine, acetate, and the methyl group of methionine, while its partner, **3**, is assembled from alanine and a polyacetate derived moiety (Figure 2). The patterns of pyrrole ring biosynthesis illustrated above for prodigiosin (Figure 2) are without precedent among other naturally occurring pyrroles.

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(15) J. R. Sokatch, "Bacterial Physiology and Metabolism," Academic Press, New York, N. Y., 1969, pp 172, 277.
(16) S. H. Mudd and G. L. Cantoni in "Comprehensive Biochemistry," Vol. 15, M. Florkin and E. H. Stotz, Ed., Elsevier, Amsterdam, 1964, p 16.
(17) (a) M. T. M. Rizki, *Proc. Nat. Acad. Sci. U. S.*, **40**, 1057 (1964); (b) H. H. Wasserman, J. E. McKeon, and U. V. Santer, *Biochem. Biophys. Res. Commun.*, **3**, 146 (1960).

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