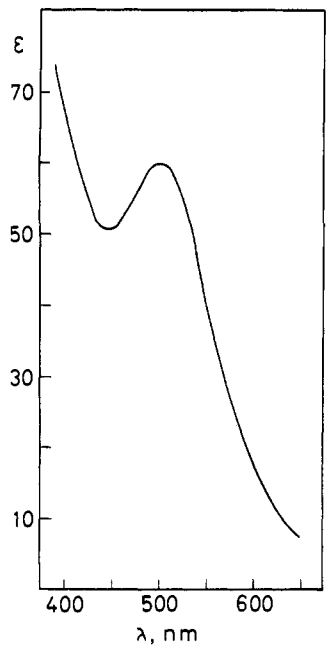


Figure 1. Visible spectrum of Mo(IV) in 1 *M* HPTS.

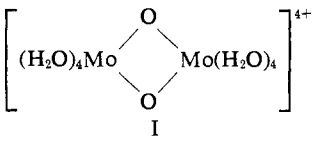
(III). The Mo^{3+} ion was eluted slowly under nitrogen, with 1 *M* HPTS. This eluent failed to remove the red band. A 4 *M* HPTS solution eluted the red band slowly, causing it to move as a single distinct band until it was removed from the column. This ion exchange behavior indicated a cation with a charge greater than +3, presumably +4.

In order to facilitate a proper investigation and identification of the red ion, a more efficient preparative procedure was developed. An equimolar mixture of 0.01 *M* molybdenum(III) and molybdenum(V) in 1 *M* HPTS (molarity given in terms of moles of Mo atoms in both oxidation states) was heated at 90° in an inert atmosphere for 1 hr. During this time the solution turned red. The cooled solution was absorbed on a cation-exchange column. Elution with 0.5 *M* HPTS removed a band of the unreacted dipositive Mo(V) ion.[2] This stage was followed by elution with 1 *M* acid in order to make sure that any unreacted Mo^{3+} is removed from the column. The red ion was finally eluted with 4 *M* acid as described above. The purified solution of the red ion was concentrated by repeated absorption on a short cation-exchange column followed by elution with HPTS (or $HClO_4$). By this procedure solutions approximately 0.04 *M* in molybdenum were obtained. The visible spectrum of the red ion is given in Figure 1.

Aliquots of the red solution were titrated with a standard $KMnO_4$ solution before and after reduction with a Jones reductor[5] to Mo(III). The ratio of permanganate consumed was 2:3, respectively, thus proving that the oxidation number of the red ion is +4. The result of four determinations of the oxidation number was +4.0 ± 0.2.

The red ion was absorbed on a cation-exchange column of known capacity until all of its H^+ ions were replaced by Mo(IV). After rinsing the column with water the red ion was eluted with acid and titrated with $KMnO_4$. The charge per Mo atom was determined by dividing the capacity of the resin by the number of moles of Mo atoms eluted from it. A charge of +2.0 ± 0.1 per molybdenum atom was obtained (average of three determinations).

Since the ion-exchange behavior of the red ion indicates a charge of +4 and since the charge per Mo atom is +2, it is concluded that the red ion is binuclear. The most probable structure of the ion is I. The di-μ-



I

oxo bridge to the Mo atoms is well-known in Mo(V) compounds[2,6] as well as in some salts of Mo(III).[7] An alternative structure with a Mo–Mo bond and two terminal oxygen atoms is much less probable.

The most striking property of the ion is its remarkable stability to air oxidation. In acid solution above 1 *M*, the concentration of Mo(IV) decreases very slowly if the solution is exposed to the atmosphere. The red color of the exposed solutions persists for many days.

The second unexpected property is the resistence of the ion to disproportionation. Even at 90° the ion is quite stable in an inert atmosphere. It should, however, not be concluded from our preparative procedure that molybdenum(IV) is more stable thermodynamically than the mixture of molybdenum(III) and molybdenum(V). Molybdenum(V) is not completely consumed by the formation reaction of molybdenum(IV) and can be recovered by ion-exchange chromatography. It may be that Mo(IV) is formed by the reduction of solvent water by Mo(III), with Mo(V) acting as a catalyst. The mechanism of the formation reaction of Mo(IV) (in the process described above) is now being investigated, as are other oxidation reactions of Mo^{3+} that produce the $Mo_2O_2^{4+}$ ion. We are also investigating the oxidation of $Mo_2O_2^{4+}$ by several one-electron and two-electron oxidizing agents such as Fe^{3+}, Ce^{4+}, and Tl^{3+}.

It is hoped that the discovery of a stable quadrivalent oxidation state of molybdenum in aqueous solution may help to elucidate the mechanism of enzymatic reactions involving molybdenum.

(5) I. M. Kolthoff and R. Belcher, "Volumetric Analysis," Vol. 3, Interscience, New York, N. Y., 1957, p 92.

(6) P. C. H. Mitchel, *Quart. Rev., Chem. Soc.*, 20, 103 (1966).
(7) P. C. H. Mitchel and R. D. Scarle, *J. Chem. Soc., Dalton Trans.*, 1809 (1972).

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Iminoyl Free Radicals. An Electron Spin Resonance Identification of a New Class of σ Radicals[1]

Sir:

Although there are numerous reports in the literature of organic π radicals only a relatively few σ radicals have been identified.[2] A free radical is considered a

(1) Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this work.
(2) For a fairly detailed listing of references to both experimental and theoretical studies on σ radicals, see P. J. Krusic and T. A. Rettig, *J. Amer. Chem. Soc.*, 92, 722 (1970).

π radical if the unpaired electron resides in an orbital with zero probability at the nucleus (most commonly 2p orbitals) while in σ radicals the unpaired electron is localized in an orbital of nonvanishing amplitude at the nucleus.[2,3] Most carbon-centered free radicals are planar (or nearly planar) π radicals because generally it is energetically favorable for a carbon atom to utilize its valence 2s orbital in hybrid bonds to attached atoms or groups.

We wish to report the identification by electron spin resonance (esr) spectroscopy of a new class of σ radicals derived by abstraction of the aldehydic hydrogen of aldimines (eq 1). The esr samples were *ca.* 20% by

$$\text{R—CH=N—R}' \xrightarrow[-t\text{-BuOH}]{t\text{-BuO}\cdot} \underset{\mathbf{1}}{\text{R—}\dot{\text{C}}\text{=N—R}'} \qquad (1)$$

volume of the imine and 20% by volume of di-*tert*-butyl peroxide in cyclopropane. Photolysis with a 2000-W mercury capillary lamp at reduced temperatures directly in the cavity of the esr spectrometer as in our previous studies[4] produced signals of the corresponding iminoyl radicals (**1**). Several spectra are shown in Figure 1; spectral parameters are recorded in Table I.

Table I. Spectral Parameters[a] of Iminoyl Radicals Produced from Corresponding Aldimines and *tert*-Butyl Peroxide at −100°

R′	R	a^N	a_β^H	g[b]
$(CH_3)_3C$	CH_3	1.75[c]	8.55[c]	2.0016
$(CH_3)_3C$	CH_2CH_3	1.85	5.16	2.0016
$(CH_3)_3C$	$C(CH_3)_3$	1.80		2.0016
$CH_3(CH_2)_3$	CH_2CH_3	1.32	5.23	2.0016
$CH_3(CH_2)_3$	$C(CH_3)_3$	1.20		2.0016

[a] Hyperfine splitting constants are reported in gauss and have estimated accuracies of ±0.5% except where noted. [b] Estimated accuracy ±0.0001. [c] Estimated accuracy ±1% due to poor signal-to-noise ratio.

We initially intended to investigate the perturbation nitrogen would exert on allylic-like radicals in which the nitrogen atom would occupy the central or terminal position of the allylic system. However, the multiplicity and intensity ratios of the esr hyperfine coupling patterns actually observed from the imines leave no doubt that iminoyl radicals are produced under the experimental conditions.[5] Moreover, *the esr parameters are consistent with the iminoyl radicals being σ radicals.* The low g values and small β-hydrogen hyperfine interactions are atypical of π radicals but consistent with a nonlinear arrangement about the N=C—C bond with the unpaired electron in a hybrid orbital on carbon. A g value less than the spin-only value of 2.00232 is particularly characteristic of a σ radical.[2,6] Furthermore, the β-hydrogen hyperfine

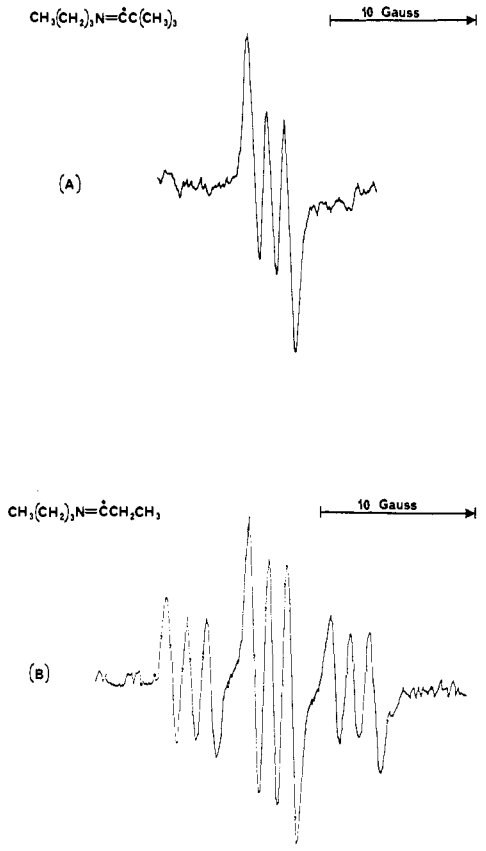


Figure 1. Electron spin resonance spectra of iminoyl radicals derived from (A) $CH_3(CH_2)_3N{=}CHC(CH_3)_3$ and (B) $CH_3(CH_2)_3N{=}CHCH_2CH_3$ at −100°.

splitting of 8.55 G observed in the iminoyl radical $(CH_3)_3CN{=}\dot{C}CH_3$ is of the same magnitude as that observed for the similar acetyl radical, $CH_3\dot{C}O$ (5.1 G).[7] Unfortunately, a sufficiently high steady-state concentration of iminoyl radicals was not available to determine the natural abundance ^{13}C hyperfine coupling which would allow an estimate of the hybrid character of the orbital occupied by the unpaired electron.[8]

The specificity of hydrygen abstraction from the sp^2 carbon is notable. The *tert*-butoxyl radical is a fairly selective reagent, abstracting tertiary, secondary, and primary hydrogens in the respective ratios of 44:12.2:1 at 40°.[9] It also has been determined that esr spectra of carbon radicals derived from removal of the hydrogen having the weakest C–H bond are generally observed when the *tert*-butoxyl radical is used as the abstracting agent.[10] It follows that the weakest C–H bond in the imines must be the C–H bond of the sp^2 carbon. The stabilization of the radical must be due to the adjacent nitrogen atom since vinylic C–H bonds have bond energies of *ca.* 109 kcal mol^{-1}.[11] A similar stabilizing effect has been observed in aldehydes where the C–H bond strength of the sp^2 carbon of aldehydes has been found to be very close to 87 kcal mol^{-1} re-

(3) σ radicals have also been described as radicals in which the spin-bearing orbital is orthogonal to an adjacent π system: R. O. C. Norman and B. C. Gilbert, *J. Phys. Chem.*, 71, 14 (1967).

(4) (a) W. C. Danen and R. W. Gellert, *J. Amer. Chem. Soc.*, **94**, 6853 (1972); (b) W. C. Danen and R. C. Rickard, *ibid.*, **94**, 3254 (1972); (c) W. C. Danen and C. T. West, *ibid.*, **93**, 5582 (1971); (d) W. C. Danen and T. T. Kensler, *ibid.*, **92**, 5235 (1970); (e) W. C. Danen and T. T. Kensler, *Tetrahedron Lett.*, 2247 (1971).

(5) Precedence for removing the hydrogen attached to the sp^2 carbon is available from the fact that benzylidenimines, when allowed to react with diisopropyl peroxycarbonate, form benzonitrile presumably *via* an intermediate iminoyl radical: H. Ohta and K. Tokumuru, *Chem. Commun.*, 1601 (1970).

(6) (a) F. J. Adrian, E. L. Cochran, and V. A. Bowers, *J. Chem. Phys.*, **36**, 1661 (1962); (b) H. Bower, J. McRae, and M. C. R. Symons, *J. Chem. Soc. A*, 2400 (1971); (c) R. W. Fessenden and R. H. Schuler, *J. Chem. Phys.*, **39**, 2147 (1963).

(7) J. E. Bennett, B. Mile, and B. Ward, *Chem. Commun.*, 13 (1968).

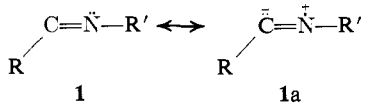
(8) Labeling studies are in progress.

(9) C. Walling and W. Thaler, *J. Amer. Chem. Soc.*, **83**, 3877 (1961).

(10) P. J. Krusic and J. K. Kochi, *ibid.*, **90**, 7155 (1968).

(11) S. W. Benson, "Thermochemical Kinetics," Wiley, New York, N. Y., 1968, p 215.

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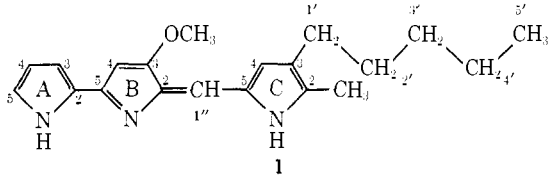
Biosynthesis of Prodigiosin. Incorporation Patterns of ^{13}C-Labeled Alanine, Proline, Glycine, and Serine Elucidated by Fourier Transform Nuclear Magnetic Resonance[1-3]

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).

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