site of the aniline (B) in anhydrous acetic acid (eq 1).

$$B \cdot \mathit{HAc} + HAc(solv) \xrightarrow{k_H} B \cdot HAc + \mathit{HAc}(solv) \quad (1)$$

Values of k_H were obtained by a kinetic analysis of proton exchange rates determined by nmr techniques. In brief, the method requires the measurement of the T_1 and T_2 relaxation times of the carboxyl proton resonance of the solvent for a series of solutions containing the *p*-toluenesulfonate salt (BH^+Ts^-) of the aniline of interest and a variable concentration of *p*-toluenesulfonic acid (HTs).[4,5] The determination of T_2 by the pulsed nmr spin–echo method[3] was simplified considerably by coupling the spectrometer with a Wang Model 700 calculator using an Adams-Smith Model 100-3 interface.[6]

Rate constants for the following processes were derived from the nmr results.[7] Proton exchange ac-

$$\mathit{BH}^+Ts^- + HAc \underset{k_-}{\overset{k_+}{\rightleftharpoons}} \mathit{BH}^+Ac^- + HTs \quad (2)$$

$$\mathit{BH}^+Ts^- + BH^+Ac^- \underset{}{\overset{k}{\rightleftharpoons}} \mathit{BH}^+Ac^- + BH^+Ts^- \quad (3)$$

$$B\mathit{H}^+\mathit{Ac}^- + HAc \xrightarrow{k_e} BH^+Ac^- + \mathit{HAc} \quad (4)$$

cording to eq 4 very probably occurs by a mechanism involving proton transfer (k_{-i}, eq 5), rate-determining

$$B\mathit{H}^+\mathit{Ac}^- \underset{k_i}{\overset{k_{-i}}{\rightleftharpoons}} B \cdot \mathit{HAc} \quad (5)$$

exchange of HAc (eq 1),[3,4] and reverse proton transfer (k_i, eq 5). That is to say, $k_i \gg k_H$, and consequently $k_e = k_{-i}k_H/k_i$. The ratio k_i/k_{-i} is equal to the base ionization constant K_i^B (eq 6), a known quantity.[8] If

$$B \cdot HAc \overset{HAc}{\rightleftharpoons} BH^+Ac^- \quad (6a)$$

$$K_i^B = [BH^+Ac^-]/[B] \quad (6b)$$

this be granted, then the values obtained for k_H are accurate to at least 20% for all our substrates except *p*-anisidine where the error is about 30% (on the basis of the combined errors in k_e and K_i^B).

Results are summarized in Table I. We note in

Table I. Kinetic Summary of Proton Exchange and Hydrogen Bond Replacement for Substituted Anilinium *p*-Toluenesulfonates in Acetic Acid at 30°

Substituent	k_H, sec^{-1}	K_i^B	pK_A (water)[a]	k_-, sec^{-1} M^{-1}	k, sec^{-1} M^{-1}
p-OCH_3	3×10^9	32.8	5.24		
p-CH_3[b]	2.0×10^9	19.2	4.99	7.5×10^9	8.2×10^8
m-CH_3	3.3×10^9	9.56	4.64	8.1×10^9	11×10^8
p-F	2.6×10^9	4.66	4.56	10×10^9	
m-OCH_3	1.8×10^9	4.71	4.15	9.9×10^9	$\sim 9 \times 10^8$

[a] A. I. Biggs, *J. Chem. Soc.*, 2573 (1961). [b] Reference 5.

passing that values obtained for k_- and k are consistent with values reported previously.[4,5]

According to eq 1, the exchange process characterized by k_H involves the breaking of an N·HAc hydrogen bond and the formation of a new one. If hydrogen-bond strength were an important factor, we would expect k_H to increase with decreasing bond strength and hence with decreasing basicity.[9] However, it is clear from the data in Table I that there is no correlation at all between k_H and basicity as measured either by K_i^B in acetic acid or by pK_A in water. Although the variation of k_H is not large (the range of log k_H is 0.3 while that of log K_i^B is 0.85), it seems safe to conclude that factors other than basicity have an overriding effect. This conclusion is consistent with earlier observations of the effect of structure on k_H for amines in water[10] and for *N*-alkylanilines in acetic acid[4,10] where basicity was also shown to be relatively unimportant and where it was indicated that London dispersion forces contribute a dominant interaction. The present communication offers evidence supporting the claim that hydrogen bond strength is not an important consideration for the rate-determining process measured by k_H.

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(6) Adams-Smith, Inc., Needham Heights, Mass.

(7) Required values of the equilibrium constant $K = k_+/k_-$ were taken from C. S. Leung and E. Grunwald, *J. Phys. Chem.*, 73, 1822 (1969).

(8) G. W. Ceska and E. Grunwald, *J. Amer. Chem. Soc.*, 89, 1371 (1967).

(9) We are assuming that the equilibrium constant for H-bond formation is greater than 1. That is, in eq 1, the breaking of the H bond is the rate-determining step for the overall exchange process.

(10) E. Grunwald and E. K. Ralph, *Accounts Chem. Res.*, 4, 107 (1971).

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A ^{13}C Nuclear Magnetic Resonance Study on the Biosynthesis of Pyrrolnitrin from Tryptophan by *Pseudomonas*

Sir:

Previous studies[1,2] with *Pseudomonas aureofaciens* have shown that the antifungal antibiotic pyrrolnitrin (I) is derived from tryptophan. Using various isotopically labeled tryptophan species it was demonstrated[3] that C-2 of the indole nucleus is retained, the amino nitrogen becomes the pyrrole nitrogen, the indole nitrogen gives rise to the nitro group, and tritium from C-2 of the side chain of L- but not D-tryptophan is retained during the biosynthesis. These studies supported the proposed[4] biosynthetic sequence shown in Scheme I, path a. This sequence suggests that the two chlorine atoms found in pyrrolnitrin are introduced late in the biosynthesis, presumably immediately before the last step,[2] the oxidation of the amino to the nitro group. However, this requires introducing chlorine into the 3 position of the pyrrole ring, whereas the normal patterns of electrophilic substitution of pyrroles predict

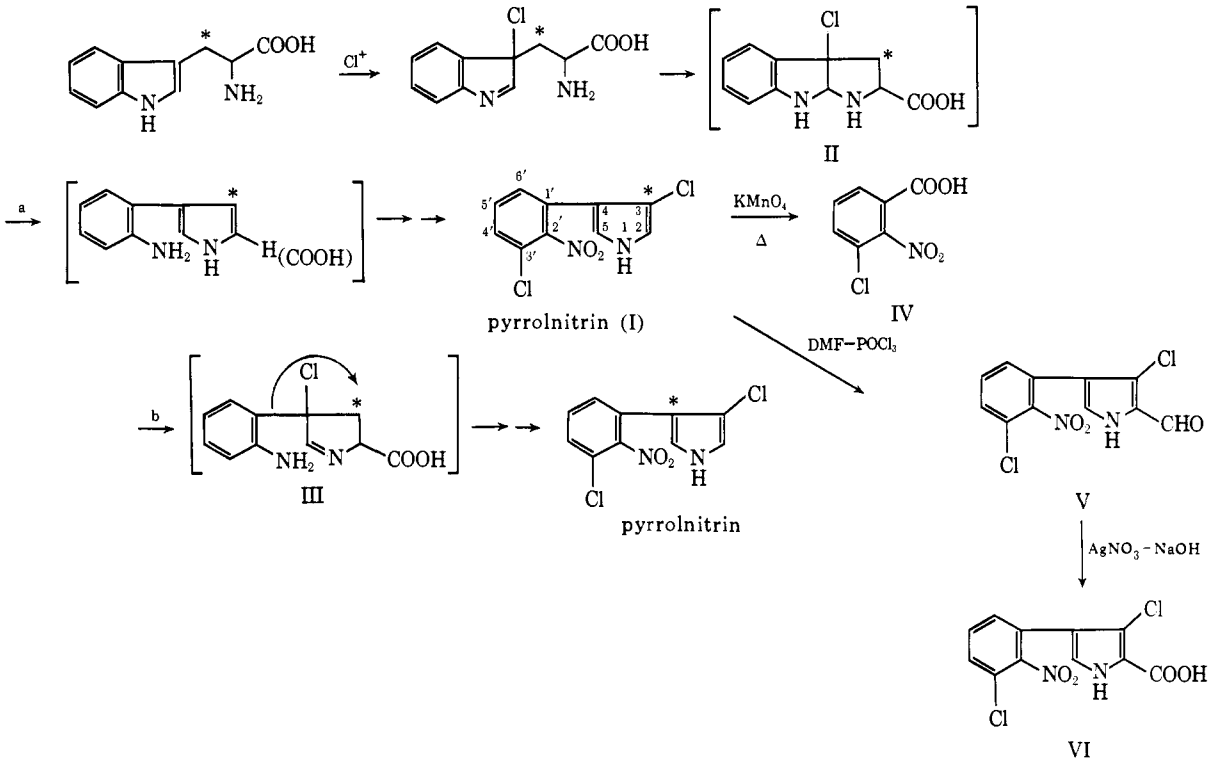
(1) D. H. Lively, M. Gorman, M. E. Haney, and J. A. Mabe, *Antimicrob. Ag. Chemother.*, 462 (1966).

(2) R. Hamill, R. Elander, J. Mabe, and M. Gorman, *ibid.*, 388 (1967).

(3) H. G. Floss, P. E. Manni, R. L. Hamill, and J. A. Mabe, *Biochem. Biophys. Res. Commun.*, 45, 781 (1971).

(4) M. Gorman and D. H. Lively in "Antibiotics," Vol. II, D. Gottlieb and P. D. Shaw, Ed., Springer-Verlag, New York, N. Y., 1967, p 433.

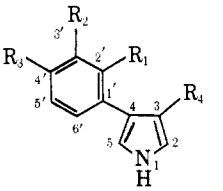
Scheme I



chlorination to occur in the 2 or 5 position. As an alternative, the chlorine atom in the 3 position might have been carried over from an earlier stage of the biosynthesis and would thus be present before the aromatization of the pyrrole ring. This possibility is supported by the fact that several analogs of pyrrolnitrin with varying halogenation patterns are found in *Pseudomonas*,[2] but none without halogen in the 3 position. One way in which chlorine could be carried over into the late stages of the biosynthesis is shown in variant b of Scheme I. The hypothetical intermediate II could be transformed into III followed by a 1,2-aryl shift as in the biosynthesis of isoflavones.[5] Aromatization by dehydrogenation rather than elimination of HCl would then lead to a pyrrolnitrin in which the phenyl substituent is now attached to the carbon atom which originated from C-3 of the tryptophan side chain.

To examine this question and to establish the mode of incorporation of tryptophan into the pyrrole ring of pyrrolnitrin, we synthesized DL-tryptophan-*alanine-3*-^{13}C[6] from $Ba^{13}CO_3$. This material (200 mg, 60.1 atom % excess ^{13}C) was fed to ten cultures of *Pseudomonas aureofaciens* A 10338.5 as described previously[2,3] to give pyrrolnitrin-^{13}C (20 mg, 28.5 atom % excess ^{13}C). The location of the ^{13}C in this pyrrolnitrin sample was then determined by cmr spectroscopy.

Assignments of ^{13}C chemical shifts of the ten carbon atoms of 4-phenylpyrrole (VII) (Table I) were based on studies of pyrrole[7] and biphenyl.[8] Data obtained with



	R_1	R_2	R_3	R_4	
VII	H	H	H	H	
VIII	H	Cl	Cl	H	
I	NO_2	Cl	H	Cl	pyrrolnitrin
IX	NH_2	Cl	H	Cl	aminopyrrolnitrin

Table I. Carbon-13 Chemical Shifts of Substituted Phenylpyrroles[a]

	VII	VIII	I	IX
C-2	118.8	118.1	117.5[b]	117.8[b]
C-3	105.6	106.1	111.7	111.5
C-4	123.8	122.3	115.3	118.7[c]
C-5	114.5	114.9	116.7[b]	117.2[b]
C-1′	135.5	135.9	127.8	120.2
C-2′	124.9	126.4	148.3[d]	141.4
C-3′	128.4	132.2	124.8	119.3[c]
C-4′	124.5	128.4	130.2	130.2
C-5′	128.4	130.2	130.2	116.0
C-6′	124.9	123.1	128.5	128.2

[a] Spectra taken at 15.077 MHz on a Fourier transform spectrometer in $CHCl_3$; chemical shifts are expressed as parts per million downfield from TMS; $\delta_{TMS} = 192.4 - \delta_{CHCl_3}$. [b,c] Values within any vertical column may be reversed. [d] This signal is absent in $CHCl_3$ solution because of the carbon's long relaxation time. By adding $Cr(acac)_3$ to a $CHCl_3$–MeOH (8:1) solution of pyrrolnitrin the signal is observed (R. Freeman, K. G. R. Pachler, and G. N. La Mar, *J. Chem. Phys.*, **55**, 4586 (1971)).

VII and chlorobenzene[9] allowed direct cmr analysis of 4-(3′,4′-dichlorophenyl)pyrrole (VIII). Previous cmr

(5) H. Grisebach in "Biochemistry of Plant Pigments," T. W. Goodwin, Ed., Academic Press, New York, N. Y., 1964, p 233.

(6) F. Weygand and H. Linden, *Z. Naturforsch. B*, **9**, 682 (1954); *cf.* A. Murray, III, and D. L. Williams, "Organic Synthesis with Isotopes," Part I, Interscience, New York, N. Y., 1958, pp 247 and 607.

(7) T. F. Page, Jr., T. D. Alger, and D. M. Grant, *J. Amer. Chem. Soc.*, **87**, 5333 (1965).

(8) T. D. Alger, D. M. Grant, and E. G. Paul, *ibid.*, **88**, 5397 (1966).

(9) P. Lazzeretti and F. Taddei, *Org. Magn. Resonance*, 3, 283 (1971).

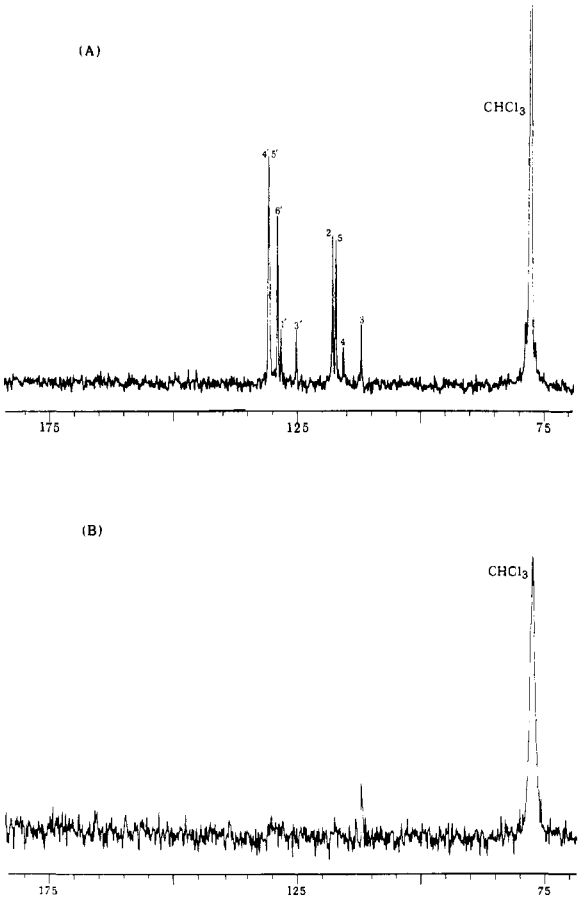


Figure 1. ^{13}C Fourier transform magnetic resonance spectra of pyrrolnitrin: (A) from C-13 natural abundance; (B) from D,L-tryptophan-*alanine-3-*^{13}C.

data for nitrobenzene made the cmr analysis of all carbons except C-2 and C-5 of pyrrolnitrin facile. The most important assignments were the signals for C-3 (111.7 ppm) and C-4 (115.3 ppm) of the pyrrole ring. These assignments were confirmed by checking the effect of reduction of the nitro group. The signal at 111.7 ppm was almost unchanged, whereas the signal at 115.3 ppm was shifted to 118.7 ppm in the spectrum of aminopyrrolnitrin (IX).[2] Finally, in the spectrum of the biosynthetic pyrrolnitrin-^{13}C (*cf.* Figure 1) the signal at 111.7 ppm showed significant enrichment, indicating that C-3 of the pyrrole ring originates from C-3 of the tryptophan side chain.

This conclusion is in agreement with the results of chemical degradations of radioactive pyrrolnitrin samples from earlier feeding experiments. Pyrrolnitrin obtained from tryptophan-*alanine-3-*^{14}C[3] (3140 dpm/μmol) was subjected to a modified permanganate oxidation[10] yielding 3-chloro-2-nitrobenzoic acid (IV) (6.7 dpm/μmol) which contained only 0.21% of the label. Formylation[11] of pyrrolnitrin-^{14}C,^{3}H ($T/^{14}C$ = 2.34) obtained biosynthetically from DL-tryptophan-*alanine-3-*^{14}C*-2-*^{3}H[3] gave the aldehyde V (Scheme I) ($T/^{14}C$ = 0.23) with loss of most of the tritium. Direction of the formylation was confirmed by Ag_2O oxidation[12] of a nonlabeled sample of the aldehyde to the known carboxylic acid VI.[13]

These results establish the labeling pattern of the pyrrole ring of pyrrolnitrin, showing that C-3 of the tryptophan side chain becomes C-3 of the antibiotic and that tryptophan is a direct and specific precursor of pyrrolnitrin. This is important in view of a recent report[14] on the incorporation of δ-aminolevulinic acid-*4-*^{14}C into the pyrrole ring of the antibiotic. Finally, the results rule out the aforementioned rearrangement process involving a 1,2-aryl shift.

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Ferretane. A New Ring System from the Reaction of Diiron Nonacarbonyl and Dibenzosemibullvalene

Sir:

Previously it was reported that reaction of semibullvalene with diiron nonacarbonyl yields a $C_8H_8 \cdot Fe(CO)_3$ complex which possesses the bicyclo[3.2.1]-octyl ring system with π-allyl and σ bonding of the $Fe(CO)_3$ unit to the carbocyclic framework.[1] We now find that reaction of dibenzosemibullvalene (**1**)[2a,b] with diiron nonacarbonyl follows a dramatically different pathway, namely, the iron atom inserts into a σ bond of the three-membered ring to yield a four-membered ring containing iron bonded by two Fe–C σ bonds.[3]

(1) R. M. Moriarty, C.-L. Yeh, and K. C. Ramey, *J. Amer. Chem. Soc.*, **93**, 6709 (1971).
(2) (a) G. F. Emerson, L. Watts, and R. Pettit, *ibid.*, **87**, 131 (1965); (b) E. Ciganek, *ibid.*, **88**, 2882 (1966).
(3) Compound **2** was obtained as yellow crystals, mp 143° dec, in 25% yield by chromatography on silica gel (*Anal.* Calcd for $C_{20}H_{12}O_4Fe$: C, 64.54; H, 3.23. Found: C, 64.80; H, 3.40) and it showed C≡O absorption at 1980–2080 cm^{-1}. The nmr spectrum possessed the same overall appearance as dibenzosemibullvalene, but all resonances were shifted from their positions in the starting material: δ (ppm) 3.04 (H_a, d, 2 H), 4.55 (H_b, H_c, m, 2 H), 7.00 (aromatic protons, m, 6 H), 7.25 (H_d, m, 2 H). The mass spectrum showed the parent molecular ion $C_{16}H_{12}Fe(CO)_4^+$ at *m/e* 372 and $C_{16}H_{12}Fe(CO)_3^+$ at *m/e* 344; $C_{16}H_{12}Fe(CO)_2^+$, 316; $C_{16}H_{12}Fe(CO)^+$, 288; $C_{16}H_{12}Fe^+$, 260; and $C_{16}H_{12}^+$, 204. Compound **4** had mp 158° dec, ν(C≡O) 1900–1935 cm^{-1}.