Table I. Representative ^{13}C and ^{1}H Spin Systems Identified on the Basis of Two-Dimensional ^{13}C–^{13}C and ^{1}H–^{13}C Connectivities

group[a]	carbon atom	chemical shifts[b] (ppm)	
		^{13}C (±0.1)	attached ^{1}H (±0.02)
ribose	1′	52.2	
	2′	71.2	2.79
	3′	74.6	3.60
	4′	71.0	4.21
	5′	63.8	
isoalloxazine ring	5a	139.4	
	6	129.8	
	7	141.9	
	7a	20.3	
	8	152.8	
	8a	23.0	
alanine-A	o	172.8	
	α	51.2	
	β	21.4	
tyrosine-A	o	171.8	
	α	52.3	
	β	34.9	
	γ	127.7	
	δ	131.4[c]	
	ϵ	116.0[c]	
	ζ	156.3	
threonine-A	o	170.8	
	α	59.9	
	β	66.3	
	γ	16.4	

[a] Sequence-specific assignments have not been made yet for the amino acid spin systems. [b] ^{13}C chemical shifts are relative to TMS. ^{1}H chemical shifts are relative to TSP. [c] The two tyrosine $^{13}C_\delta$ and $^{13}C_\epsilon$ carbons appear to have degenerate chemical shifts.

At least 154 of the expected ~210 $^{13}C_0$–$^{13}C_\alpha$ correlations were resolved by using the software package MADNMR.[2] This suggests that uniform ^{13}C labeling will support a heteronuclear approach to sequence-specific resonance assignments. The ^{13}C–^{13}C correlations, in combination with multiple-bond ^{13}C–^{1}H correlations or ^{13}C–^{15}N correlations from dual ^{13}C/^{15}N-labeled proteins, or both, can be used to trace out the peptide backbone connectivities.[1,10,11]

Sensitivity considerations limit the application of the ^{13}C-{^{13}C}DQC experiment to proteins enriched with ^{13}C. Current methods for incorporating stable isotopes into biotechnology derived proteins have begun to alleviate this problem.[12] Carbon-13 enrichment levels of 20–30% represent a good compromise between improved sensitivity and decreased spectral simplicity. Higher enrichment levels might be useful for providing long-range carbon–carbon coupling constants for selectively enriched proteins[1] but would result in increased spectral overlap in a uniformly enriched protein.

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First Direct Observation of Pyridyne: Matrix Infrared Study of the Photolysis Products of 3,4-Pyridine Dicarboxylic Anhydride

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Heteroarynes have been proposed as likely intermediates in many organic reactions, principally those involving cycloaddition or cine-substitution.[1] However, only indirect evidence, based on trapping experiments to verify the presence of heteroaryne intermediacy, has been obtained. The reliability of such inferences is severely limited. Other mechanisms, e.g., addition–elimination, trans-halogenation, or addition ring opening–elimination ring closure(ANRORC), also can account for the formation of observed products.[1] Mass spectrometric analysis following the electron impact or the pyrolytic fragmentation of several heteroarene dicarboxylic anhydrides has been used to conjecture the structure of heteroarynes corresponding to certain m/z peaks.[2–5] Although diazabiphenylene, the dimer of 3,4-pyridyne, has been identified in the time of flight mass spectrometric and kinetic UV spectroscopic analysis of the products formed by flash photolysis of pyridine-3-diazonium-4-carboxylate,[6] no direct observation of any heteroaryne has yet been published.

In this report we present the first infrared spectrum of 3,4-pyridyne (3,4-didehydropyridine), generated via near UV photolysis ($\lambda > 340$ nm) of 3,4-pyridine dicarboxylic anhydride (3,4-PDA) in N_2 or Ar matrices. Similar experiments by Dunkin and McDonald were not successful;[7] apparently the photolytic conditions utilized in that study produced only decomposition products of the desired heteroaryne.

3,4-PDA (obtained from Aldrich and vacuum sublimed before use) was sublimed and codeposited for 2 h with Ar or N_2 (flow rate 2 mmol/min) on the CsI substrate of an Air Products CS202 Displex cryostat. Photolyses were conducted with a 200 W Hg-Xe arc lamp equipped with a water filter and various cutoff filters. Infrared spectra of the precursor and photolyzed products at 13 K were recorded with a BOMEM DA3.01 interferometric spectrometer.

As summarized in Scheme I, mild irradiation ($\lambda > 340$ nm and less than 100 min duration) of 3,4-PDA in N_2 or Ar matrices at 13 K readily fragmented the precursor to form CO, CO_2, and 3,4-pyridyne, which has a strong peak at 2085 cm^{-1} diagnostic of carbon–carbon triple bond formation. Subsequent irradiation with $\lambda > 210$-nm light immediately decomposed 3,4-pyridyne into HCN, diacetylene, acetylene, and cyanoacetylene as a result of alternative two-bond scissions. The infrared spectrum in the 2050–2300 cm^{-1} region prior to and following controlled photolysis (Figure 1) clearly demonstrates the formation of 3,4-pyridyne and its subsequent decomposition. The peak due to 3,4-pyridyne at 2085 cm^{-1} disappears upon shorter wavelength irradiation, and new peaks at 2101 cm^{-1} (HCN), 2181 cm^{-1} (diacetylene) and 2236 cm^{-1} (cyanoacetylene) begin to grow. Ten additional peaks below 2000 cm^{-1} show the same growth and decay pattern as the 2085-cm^{-1} band and are also attributable to 3,4-pyridyne (Table I).

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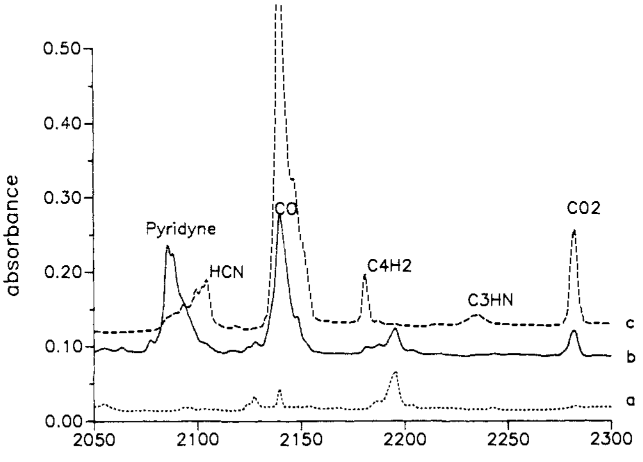


Figure 1. IR spectra of 3,4-PDA and its photolyzed products in the 2050–2300-cm^{-1} region in an N_2 matrix at 13 K: (a) 3,4-PDA; (b) after 100 min photolysis through water and $\lambda > 340$-nm filter (The peak at 2281 cm^{-1} is due to $^{13}CO_2$); (c) following additional 30 min photolysis with $\lambda > 210$ nm.

Table I. Infrared Bands (cm^{-1}) Resulting from Photolysis of 3,4-PDA in an N_2 Matrix at 13 K^d

$\lambda > 340$ nm^a	$\lambda > 210$ nm^b	photolyzed products	*o*-benzyne^c
	2236	cyanoacetylene	
	2181	diacetylene	
	2101	HCN	
2085		3,4-pyridyne	2082
1558		3,4-pyridyne	1596
			1448
1387		3,4-pyridyne	1395
1355		3,4-pyridyne	1355
	1260	polymer	
1216		3,4-pyridyne	
1055		3,4-pyridyne	1055
			1038
996		3,4-pyridyne	
853		3,4-pyridyne	
848		3,4-pyridyne	848
802		3,4-pyridyne	
	751	acetylene	
744	744	acetylene	
			739
	703	polymer	
	673	cyanoacetylene	
648	648	diacetylene	
635	635	diacetylene	
489		3,4-pyridyne	470

^{a}Photolysis of 3,4-PDA. (100 min). ^{b}Additional 30 min photolysis after *a*. ^{c}Reference 9. ^{d}Comparison to *o*-benzyne (last column).

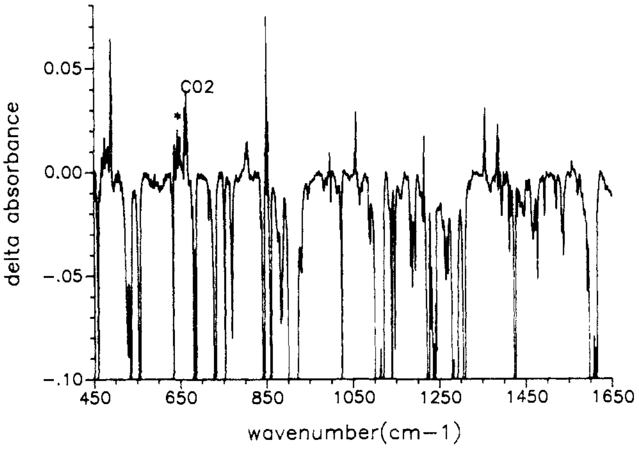
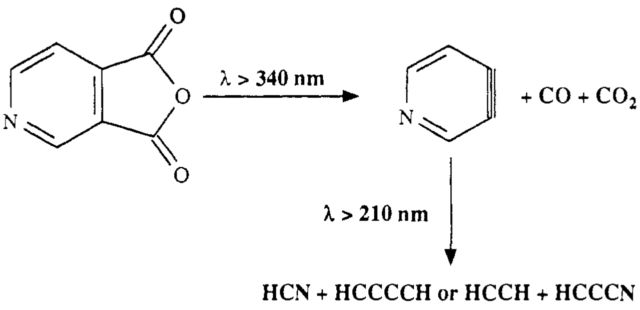


Figure 2. Difference spectrum of 3,4-PDA before and after mild photolysis. * indicates a band due to diacetylene.

Scheme I



The IR frequencies of 3,4-pyridyne indicate that this molecule is remarkably similar to *o*-benzyne in character. The wavenumbers observed for both *o*-benzyne and 3,4-pyridyne in N_2 matrices are collected in Table I. However, 3,4-pyridyne decomposes much faster. Although crude thermodynamic calculations suggest similar ring strain energy for these two molecules (~60 kcal/mol),[8] 3,4-pyridyne has less resonance energy, which may account for its lower stability.

Unlike 3,4-pyridyne, the 2,3-isomer could not be isolated under our experimental conditions. Additional experiments to identify the products of 2,3-PDA photolysis are in progress. The results, plus theoretical calculations of the structures and vibrational frequencies of various heteroarynes, will be reported in a future publication.

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