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Introduction

The ortho-quinone structure of PQQ is famous for its reactivity with nucleophilic species of carbon, nitrogen, and oxygen(Duine et. al. 1987). In fact, the crystal structure of PQQ was solved in the form of the C-5 acetone adduct(Salisbury et. al 1979). The propensity of the ortho-quinone to accept nucleophiles is the chemical basis of the function of PQQ at enzyme active sites. The present study focuses on the NMR of PQQ and various derivatives formed with oxygen and nitrogen nucleophiles. Our goals are to assign the ^{1}H, ^{13}C, and ^{15}N NMR spectra and to rigorously confirm the structures of the adducts. Once the NMR data of the relevant adducts are well defined, we will use ^{13}C and ^{15}N labeled substrates to probe the active sites of PQQ containing enzymes.

Materials and NMR Spectroscopy

Biosynthetic PQQ was isolated(modification of Ameyama et.al. 1984) from the culture broth of *Methylobacterium* AM1 which had been grown on methanol or ethanol in mineral-salts medium(Beardsmore et.al. 1982). Commercial material was purchased from Fluka Chemical Co. For NMR, 25 mg of PQQ was dissolved in 2.3 ml d_6-DMSO and placed in 10 mm NMR tube. Derivatives were prepared by adding a reagent directly to the NMR sample. Spectra were obtained at 50.3 MHz on a Bruker AM200 under the following conditions: 45° pulse, 8200 Hz sweep width, 16K data points, with the decoupler gated off for 2 to 10 sec.

Results and Discussion

Assignment of the ^{13}C NMR spectrum of PQQ. A prerequisite to studies on derivatives was the unambiguous assignment of each of the fourteen 13C NMR signals of PQQ (Table 1). This was achieved by analysis of the ^{1}H-^{13}C coupling patterns ($^{1}J_{CH}$ and $^{3}J_{CH}$) and carbon-carbon correlations. These data agree with the partial assignments made by Duine and coworkers(1981). Carbon-carbon couplings were observed using a sample of [U-^{13}C]PQQ (90+ %^{13}C) isolated from cultures grown on [^{13}C]methanol (99.7%). The complete assignment was achieved by selecting for one-bond ^{13}C coupling interactions ($^{1}J_{CC}$=55 Hz) in ^{13}C COSY experiments.

Reaction of PQQ with oxygen nucleophiles. A solution of PQQ in DMSO was treated with 50% [^{18}O]H_2O (4% v/v). The rate of exchange of ^{18}O into the ortho quinone carbonyls was followed by ^{13}C NMR (Fig 1). substitution of ^{18}O for ^{16}O at either of the ortho quinone carbonyls yields resolvable resonances (0.04 0.05 ppm upfield shift), therefore, the ratio

of signals from ^{18}O and ^{16}O species can be used to monitor the exchange. C-5 equilibrated with H_2O within 20 h, whereas C-4 did not reach equilibrium even after 36 h.

The reaction of methanol with PQQ was also monitored by ^{13}C NMR. A solution of PQQ was treated with methanol (20% v/v in d6-DMSO). The spectrum contained 28 resonances, indicating the sample was a mixture of PQQ and its methanol adduct. The methanol derivative of PQQ had two new resonances (93.7 ppm and at 186.4 ppm.) that could be assigned to C-4 and C-5. The chemical shift at 93.7 is typical of a hemiketal carbon; the resonance at 186.4 would arise from the neighboring carbonyl. To determine which of the possible hemiketals was present, this experiment was repeated using [5,9a-$^{13}C_2$]PQQ. The resulting ^{13}C spectrum contained four resonances: two from PQQ (179.2 and 126.1 ppm) and two from the methanol adduct. The hemiketal carbon was labeled with ^{13}C (93.7 ppm), but the resonance of the downfield carbonyl(186.4 ppm) was not observed. This provides direct physical evidence that the methanol adduct is the C-5 hemiketal.

Reaction with nitrogen nucleophiles. PQQ was treated with a 10% molar excess of d_5-[1-^{15}N]phenylhydrazine·HCl in d6-DMSO. The ^1H NMR of the reaction mixture contained two resonances: the signal of H-8 was shifted 0.05 ppm upfield, and the signal of H-3 was shifted 0.3 ppm downfield from the corresponding signals of PQQ. Only 12 signals were observed in the ^{13}C NMR spectrum, even when using long repetition times(up to 15 sec). No lines in the spectrum showed coupling to ^{15}N and the signals for C-4 and C-5 were not observed. The compound was isolated by reverse-phase flash chromatography (0.005 M HCl followed by 75% aqueous methanol). The ^1H and ^{13}C NMR spectra of the resulting compound were identical to those of the original reaction mixture. Therefore, reaction with phenylhydrazine produces a single derivative of PQQ. The absence of ^{13}C NMR signals from C-4 and C-5 of this adduct is very likely due to azo-hydrazone tautomerism. The azo-hydrazone tautomeric equilibrium has been determined for several systems by ^{15}N NMR(Lycka et.al. 1981 and references therein) and we plan to use such methods to characterize the equilibrium for the PQQ-phenylhydrazone adduct.

Table I. ^{13}C NMR Chemical Shift Assignments of PQQ

Carbon	δ,ppm	$^1J_{CC}$, Hz($^2J_{CC}$, Hz)	$^1J_{CH}$, Hz
1a	136.7	61,62	
2	127.6	65,87	
2'	161.3	87	
3	113.8	61,63	178
3a	123.4	60,63	
4	173.4	58,61	
5	179.2	60(6.5)	
5a	148.1	59	
7	146.5	80,59	
7'	165.4	80	
8	130.3	57,58	170
9	142.2	58,59,65	
9'	167.2	65	
9a	126.1	56(6.5)	

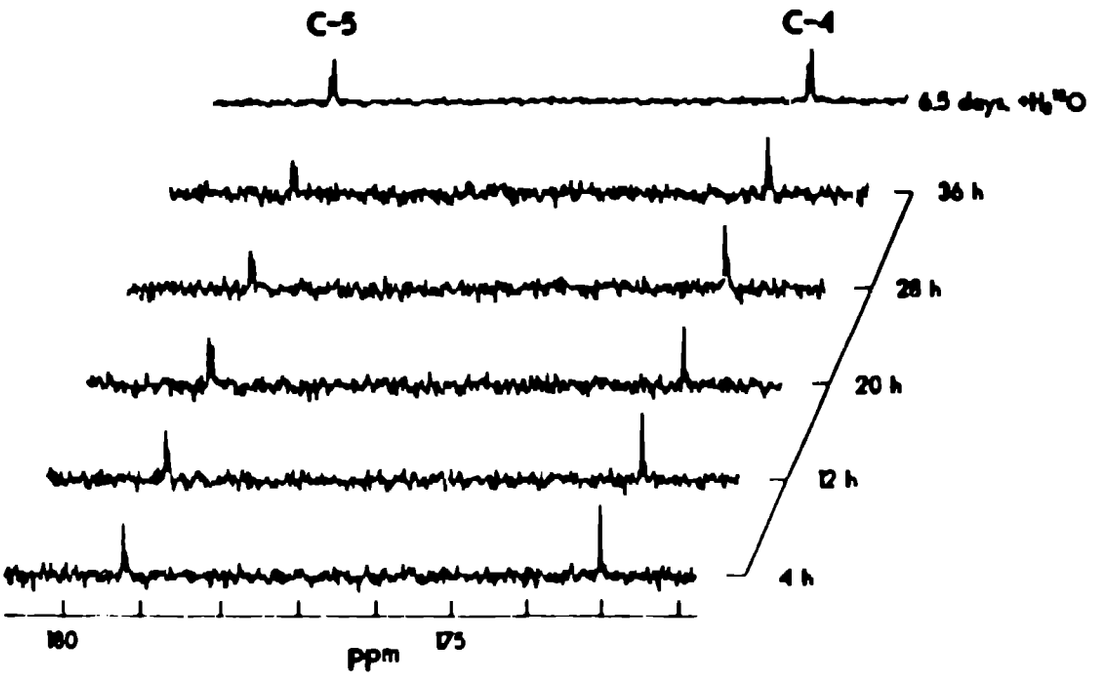

Figure 1. ^{13}C NMR spectra of PQQ detailing the exchange of the o-quinone oxygens with $H_2{}^{18}O$.

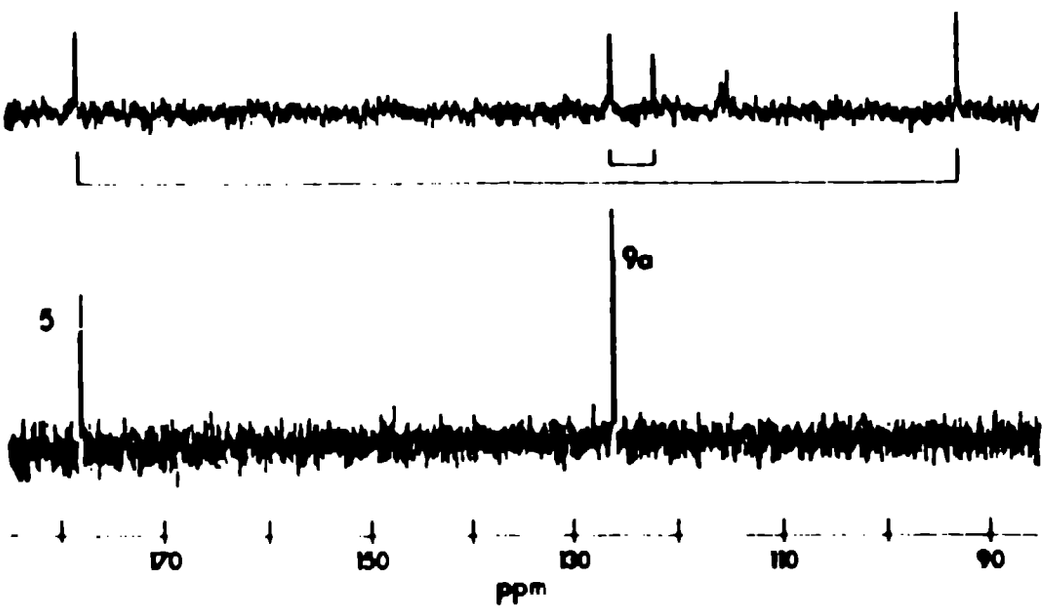

Figure 2. ^{13}C NMR spectra of [5,9a C2]PQQ and its C-5 methanol hemiketal

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