Assignments of Dissociation Constants of Primaquine by ^{13}C-NMR Spectroscopy

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The first and second dissociation constants of primaquine have been determined by titration in acetonitrile-water mixtures and estimated in pure water by using linear regression analysis. The assignments of the dissociation constants were unambigously achieved by studying the ^{13}C nmr spectral data obtained on the mono-, di-, and trihydrochloride salts.

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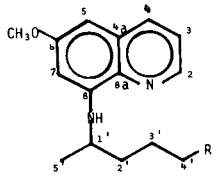
Primaquine (**1**) is an extremely important drug in the treatment of malaria even though its toxic dose is close to its therapeutic dose (1). In spite of the fact that this drug has been utilized for many years, very little is known regarding its metabolism and mechanism of action (2,4). A knowledge of the sites of protonation and p*K*a values in a drug like primaquine is necessary in order to study its effect in biological systems. We report herein the p*K*a values for primaquine and their assignments using ^{13}C nmr spectroscopy.



1 R = $-NH_2$

2 R = $-N(CH_2CH_3)_2$

The p*K*a values for a related drug pamaquine (**2**), have been determined as 10.2, 3.48 and -1.3 (5,6), while one of the p*K*a values for primaquine (**1**) has been determined as 3.2 (7). From the titration of the free base of primaquine, we were able to determine the pK_{a_1} and pK_{a_2} values (Table I) but the pK_{a_3} value was too low to be determined by this procedure. Because of the extremely low water solubility of the free base form, it was necessary to use acetonitrile-water mixtures which have a pronounced effect on the p*K*a values (8). Using linear regression analysis of the p*K*a values and acetonitrile content, the estimated pK_{a_1} value in pure water was found to be 10.39 and a value of 3.20 was found for pK_{a_2}.

Table I

Titrametric Determination of the pK_{a_1}, and pK_{a_2} Values of Primaquine

Solvent	pK_{a_1}	pK_{a_2}
40%	9.82	2.63
30%	9.96	2.80
20%	10.10	2.91
Water	10.39 (b)	3.20 (c)

(a) Acetonitrile in water mixtures. (b) Estimated value using linear regression analysis, correlation coefficient = 0.9999. (c) Estimated value, correlation coefficient = 0.9898.

The assignment of the p*K*a value at 10.39 to the primary amino group was straightforward. However, the assignment of the second p*K*a value at 3.2 was somewhat ambiguous particularly since p*K*a values for aniline, pyridine and quinoline derivatives were quite close together (9). Early work with pamaquine (2) resulted in tentative assignments of the second p*K*a value to the quinoline nitrogen although this was not based on definitive evidence (6). Schulman determined the p*K*a values for 8-aminoquinoline as 3.92 and -0.52 and, utilizing electronic absorption spectral data, concluded that the p*K*a value at 3.92 corresponded to protonation of the quinoline nitrogen rather than the aniline nitrogen (10).

^{13}C nmr spectroscopy appeared to be an ideal method to establish the order of protonation since it is known that when amines are converted to their salt forms, that specific changes in the chemical shifts of certain carbons occur (11) and these can be utilized to determine p*K*a values

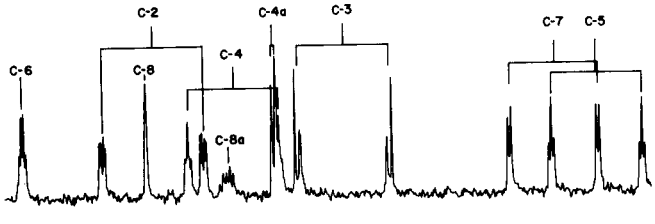


Figure I. Proton-Coupled ^{13}C NMR Spectrum for Aromatic Carbons of Primaquine Free Base Shown in Deuteriochloroform after Exchange with Deuterium Oxide.

Table II

Long-Range Coupling Data for the Aromatic Carbons in Primaquine (a)

	$^1J_{CH}$	$^2J_{CH}$	$^3J_{CH}$
C2	C_2H_2 = 179.2	C_2H_3 = 2.9	C_2H_4 = 7.8
C3	C_3H_3 = 165.6	C_3H_2 = 9.3	—
C4	C_4H_4 = 161.6	—	C_4H_2 = 5.9
			C_4H_5 = 5.9
C4a	—	—	C_4aH_3 = 7.3
C5	C_5H_5 = 160.6	—	C_5H_4 = 4.4
			C_5H_7 = 4.4
C6	—	—	C_6-OCH_3 = 3.9
C7	C_7H_7 = 157.7	—	C_7H_5 = 5.4

(a) The data reported here are for the free base in deuteriochloroform after exchange with deuterium oxide. The proton on the aniline nitrogen long-range couples to C_7 and C_{8a} (11). C_8 appears as a sharp singlet while C_{8a} is a complex signal with extensive long-range couplings.

(12,13). The complete ^{13}C nmr assignments for primaquine (1) have been reported (14) but in order to be certain of being able to make unambiguous carbon assignments after protonation it was necessary to examine the proton-coupled ^{13}C nmr spectrum in detail (see Figure I and Table II). These data agree very closely with those reported for pyridine and quinoline derivatives (15,16).

Since primaquine was commercially available as its diphosphate salt, it was anticipated that by simply adding the appropriate equivalents of acid and/or base that the order of protonation could be studied utilizing deuterium oxide as solvent and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal standard. However, this proved to be unsatisfactory because the diphosphate salt was not very soluble and good proton-coupled data could not be obtained in a reasonable time. Also, the signals for C_5 and C_7 were not present in the proton noise decoupled (PND) spectrum because exchange of H_5 and H_7 with deuterium occurred. This exchange which proved to be very rapid was confirmed by ^1H nmr (50% complete in 15 minutes). The loss of the C_5 and C_7 signals in the PND spectrum was undesirable since these were considered to be important marker carbons. Likewise, since H_5 and H_7 were exchanged with deuterium the valuable long-range coupling information was also lost. To circumvent these problems the much more soluble hydrochlorides were prepared from the free base. A solvent system of water containing 15% perdeuterioacetonitrile with DSS as internal standard proved to be satisfactory for the ^{13}C nmr studies.

The C3′ signal in primaquine monohydrochloride shifted upfield about 5 ppm while the other aliphatic carbon signals remained essentially unchanged when compared with I run in 40% perdeuterioacetonitrile in water (DSS) (Table III). This is exactly what would be expected for protonation of the primary amino group in I (17). There were also some significant shifts of C_2, C_4 and C_{8a}.

Table III

^{13}C Chemical Shifts of Primaquine and its Mono-, Di-, and Trihydrochlorides (a)

Carbon	I (b)	I (c)	I·HCl (d)	I·2HCl (d)	I·3HCl (d)
2	144.3	147.0	145.5	141.4	144.7
3	121.8	124.4	124.3	124.3	125.4
4	134.8	137.7	140.4	147.3	149.3
4a	130.0	132.4	132.6	133.8	134.2
5	91.9	94.7	95.9	97.6	109.4
6	159.6	161.7	161.5	162.9	161.5
7	96.9	99.4	101.6	106.2	123.1
8	145.2	147.3	145.2	141.1	129.9
8a	135.5	137.2	134.0	126.6	128.4
1′	48.1	50.2	50.4	51.4	60.9
2′	34.1	36.0	35.2	34.7	32.0
3′	29.3	31.6	26.3	26.2	25.5
4′	41.7	43.7	42.4	42.4	41.8
5′	20.5	22.1	21.7	21.2	18.6
OCH_3	55.2	57.4	57.8	58.5	59.3

(a) In each case the assignments are based on single-frequency off-resonance decoupling and by examination of the proton-coupled data. (b) The data for the free base in deuteriochloroform (TMS) has been reported previously (14) and is listed here for comparison. (c) I was also run in 40% perdeuterioacetonitrile in water (DDS). (d) The hydrochlorides were run in 15% perdeuterioacetonitrile in water (DSS).

These signals were affected by changes in the electron density at nitrogen which can be explained by the intramolecular hydrogen bond formation between the protons of the amino group and the quinoline nitrogen, a pheno-

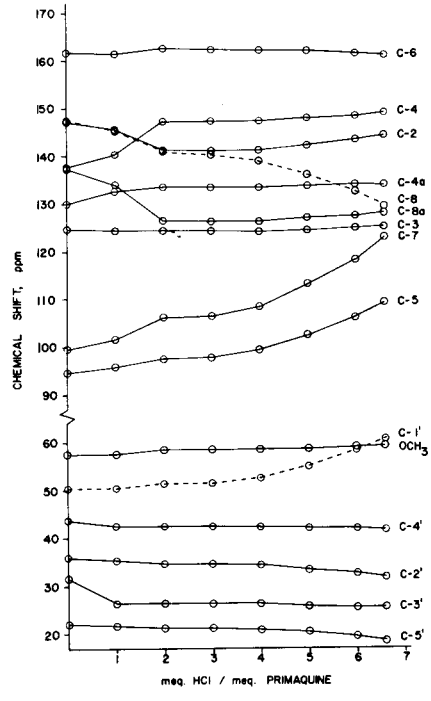


Figure II. Changes of the Chemical Shifts of the Carbon Atoms in Primaquine upon Addition of Hydrochloric Acid.

mena previously reported for pamaquine (18). The proton-coupled spectrum of the monohydrochloride was also obtained and the multiplicities and J values were essentially the same as that for the free base in chloroform.

Primaquine dihydrochloride showed significant shifts of C_2, C_4 and C_{8a} as compared with the monohydrochloride (Table III). The signals for C_2 and C_{8a} shifted upfield while C_4 shifted downfield, data consistent with that reported for pyridine and its hydrochloride (19, 20). These assignments were verified by examination of the proton-coupled spectrum of the dihydrochloride and the data was essentially the same as that for the hydrochloride and free base.

It was necessary to run the ^{13}C nmr spectrum of I in concentrated hydrochloric acid to give complete conversion to the trihydrochloride. The trihydrochloride showed dramatic downfield shifts of C_5 and C_7 and an upfield shift of C-8 (Table III), data consistent for aniline derivatives upon protonation (21,22). Significant shifts for $C_{1'}$ and $C_{5'}$ were also observed. The carbon assignments were verified by examination of the proton-coupled spectrum of the trihydrochloride.

A plot of the number of equivalents of hydrochloric acid added versus the chemical shifts of all carbon atoms in I (Figure II) clearly demonstrates that the order of protonation in primaquine is first the primary amine (pKa = 10.39), followed by protonation of quinoline ring nitrogen (pKa_2 = 3.20) and finally the aniline nitrogen (pKa_3). The third dissociation constant for primaquine was not determined but is estimated to be between -1 and -2 (^{13}C nmr data) and clearly does not represent any biological importance.

EXPERIMENTAL

The ^{13} C nmr spectral data were obtained on a JEOL FX60 instrument operating at 15.03 MHz. Chemical shifts were recorded as ppm downfield from DSS which was used as an internal standard. The proton noise decoupled data were obtained using a 45° flip angle, 5 second repetition rate and 4000 Hz spectral width. The single-frequency off-resonance decoupled spectra were obtained by centering the decoupling frequency 1200 Hz downfield from DSS. The proton-coupled data was obtained using the gated decoupling technique (decoupler off during data acquisition). All spectra were collected with 8K data memory.

The samples were prepared by dissolving 200 mg of primaquine base (obtained by ether extraction of the neutrallized diphosphate, carefully dried and stored at 0° under nitrogen prior to use) in 0.075 ml of perdeuterioacetonitrile, 1 through 6 equivalents of hydrochloric acid (concentrated hydrochloric acid, 0.064 – 0.384 ml) and water (0.36 – 0.04 ml). Solid DSS (~20 mg) was added to each sample. A 200 mg sample of I (base) was soluble in 0.4 ml of 40% perdeuterioacetonitrile in water. A 150 mg sample of I diphosphate could be dissolved in 0.4 ml of water (warming).

In a typical pKa determination, 1.5 meq of primaquine free base was dissolved in 10.0 ml of acetonitrile, then brought to volume with 40.0 ml of water. The solution was then titrated with 0.1N hydrochloric acid and the pH data was recorded using a digital pH meter that had been calibrated at pH 4.0, 7.0, and 10.0 immediately before use. The pKa value for each determination was found using a linear regression analysis of the data using a mathematical model (20) that compensates for the relative values of the primaquine concentration and the hydrogen ion concentration. The correlation coefficients for the pKa determination for a specific acetonitrile content were found to be 0.998 or higher (average r = 0.9992). The pKa values in pure water were then determined by extrapolation of the pKa values obtained for each solvent composition.

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For pKa greater than 10:

$$pH = pKa + \log \frac{B_o - T - [OH^-]}{T + [OH^-]}$$

For pKa less than 4:

$$pH = pKa + \log \frac{B_o - T + [H^+]}{T - [H^+]}$$

Where B_o = initial concentration of solute, T = titrant added.