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Note

Two-dimensional NMR analysis of selected dequalinium analogues having aralkyl linking groups

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ABSTRACT: The ^{1}H signals of the 4-aminoquinolinium moieties and the ^{13}C signals of the title compounds were fully assigned, using the concerted application of one- and two-dimensional NMR techniques, including nuclear Overhauser effect difference, DEPT, COSY, HETCOR and long-range ^{13}C–^{1}H correlation spectroscopy. The effect that quaternization of the endocyclic nitrogen atom has on the resonances of the proton and carbon atoms of the 4-aminoquinoline ring is reported. Atomic charges of the heterocyclic moiety of model compounds such as **4AQ**, **4AQ.HBr** and 4-amino-1-benzylquinolinium bromide (**8**) are shown to be linearly related to ^{13}C chemical shifts.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; COSY; heteronuclear correlation; dequalinium analogues; 1,1′-disubstituted bis(4-aminoquinolinium) dibromides

INTRODUCTION

Dequalinium (**1**) has been shown to block the small conductance calcium-activated potassium ion channel (SK_{Ca}).[1] It has served as a chemical lead for structure–activity exploration, and we have described a series of compounds in which the decamethylene bridge is replaced by benzylic linkers;[2] these have considerable medicinal, chemical and pharmacological interest.

Although the structures of these compounds had been established by standard spectroscopic analysis [^{1}H NMR (CD_3OD), MS], we have not carried out a detailed NMR examination. H-5 and H-8 of 1-alkyl-4-aminoquinolinium salts both have the same coupling pattern (doublet of doublets, dd), which also occurs between H-6 and H-7 (double doublet of doublets, ddd). We have not found any reports of NMR studies on the quaternization products of 4-aminoquinoline (**4AQ**). In view of their biological importance, we have carried out a more detailed NMR study to gain more insight into their molecular structure prior to conducting further structure–activity investigations. This paper reports the unequivocal assignments for the ^{1}H and ^{13}C resonances of the 4-aminoquinolinium moiety and of the complete structures **2**–**7**, using one- and two-dimensional NMR techniques.

The quinolinium rings could interact intramolecularly by folding of the connecting chain [especially for **4**, L = —$(CH_2)_3$— and **5**, L = —$(CH_2)_4$—]. For this reason, data are included for two monocationic model reference compounds, namely protonated 4-aminoquinoline (4-aminoquinoline hydrobromide, **4AQ.HBr**) and 4-amino-1-benzylquinolinium bromide (**8**). We have also established the effects of the 1-substituent on the proton and carbon chemical shifts (CSs) of the 4-aminoquinolinium ring in relation to the free base **4AQ**. Finally, correlations between the atomic charges (ACs) and the ^{13}C chemical shifts of the model compounds have been obtained.

EXPERIMENTAL

Compounds

Compounds **2**–**7** have been described previously.[2] 4-Aminoquinoline hydrobromide (**4AQ.HBr**) was obtained by adding an excess of a 30% solution of hydrogen bromide in acetic acid to the free base. After 5 h, the amorphous white product was filtered in a dry-box after being recrystallized from ethanol by adding diethyl ether to turbidity; yield 78% as monohydrate, m.p. 298–300 °C. Analysis: calculated for $C_9H_9N_2Br \cdot H_2O$, C 44.47, H 4.56, N 11.52; found, C 44.73, H 4.37, N 11.40%. 4-Amino-1-benzylquinolinium bromide (**8**) was prepared (86% yield) by addition of an equimolecular amount of benzyl bromide to a 0.03 M solution of **4AQ** in butanone and heating at 100 °C in a sealed tube. After 24 h, the crystalline white product was collected, washed with chloroform and butanone and recrystallized from ethanol by adding diethyl ether

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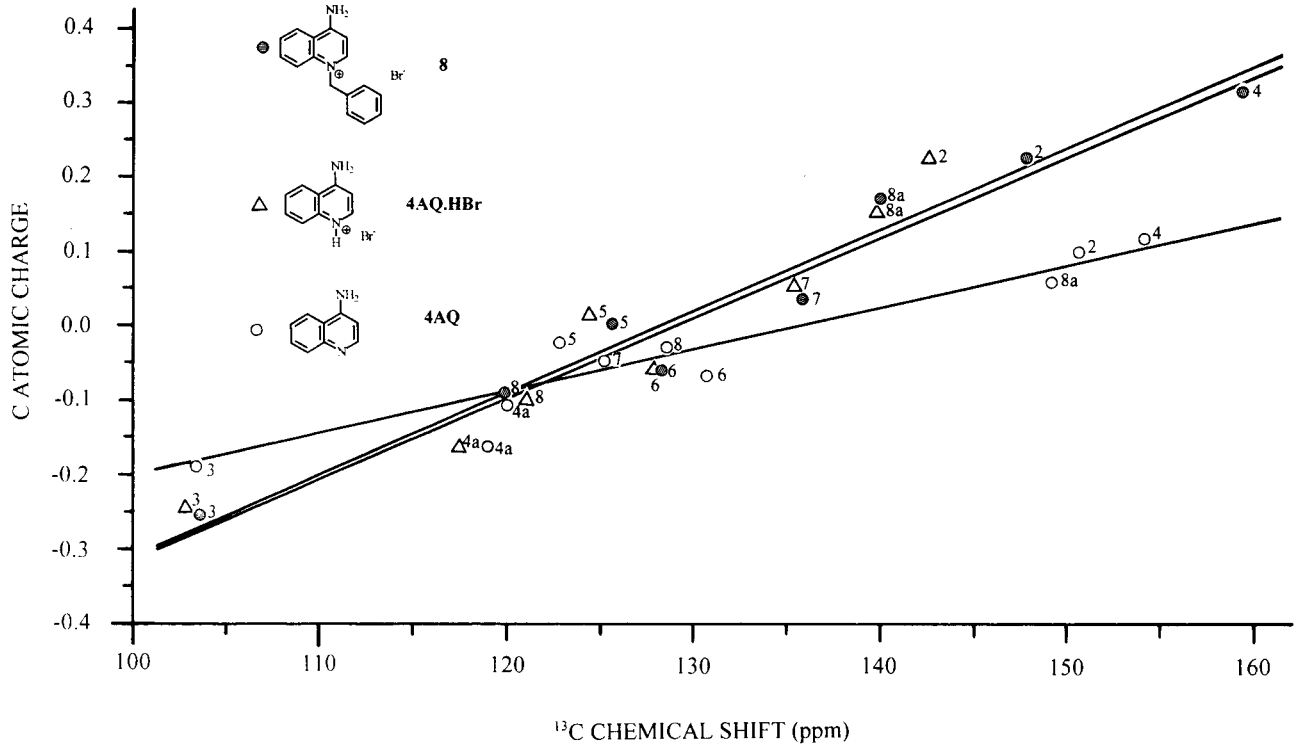


Figure 1. Atomic charges calculated by MOPAC using the MNDO method plotted against the ^{13}C NMR chemical shifts for the compounds indicated.

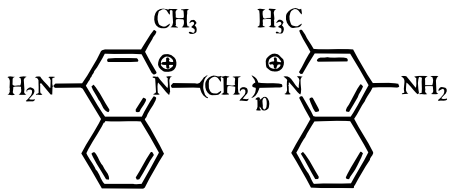
to turbidity; m.p. 259–261 °C. Analysis: calculated for $C_{16}H_{15}N_2Br$, C 60.97, H 4.80, N 8.89; found, C 60.70, H 4.70, N 8.92%.

Atomic charges for **4AQ**, **4AQ.HBr** and **8** were calculated on a Silicon Graphics IRIS 4D workstation, running SYBYL 5.5[3] at the semiempirical level, using the MNDO[4] Hamiltonian of MOPAC (Version 5.0).[5]

Spectra

The ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer operating at 75.479 MHz for ^{13}C and 300.160 for ^1H in CD_3OD for both **4AQ** and the dibromides **2–7** and on a Bruker ARX 400 spectrometer operating at 400.132 MHz for ^1H and 100.623 MHz for ^{13}C for **4AQ.HBr** and **8** (a concentration of *ca.* 27 mg ml^{-1} in all cases). The centre of the peaks of CD_3OD [3.31 ppm (^1H) and 49 ppm (^{13}C)] were used as internal references in a 5-mm $^{13}C/^1H$ dual probe (Wilmad, No. 528-PP). The temperature of the sample was maintained at 297 K. The accuracy of the chemical shifts was better than ± 0.01 ppm.

One-dimensional NOE-difference experiments on **3** were performed by irradiation for 4 s in series of eight scans with alternating on- and off-resonance. The irradiating power was set low to achieve selectivity.



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The following parameters were used for the DEPT experiments: PW (135°), 9.0 μs; recycle time, 1 s; $1/2J(CH) = 4$ ms; 65 536 data points acquired and transformed from 1024 scans; spectral width, 15 kHz; and line broadening, 1.3 Hz.

The ^1H–^1H chemical shift correlation (COSY) utilized the sequence described by Nagayama *et al.*[6] with a mixing pulse of 45°; 16 scans were measured for 128 values of t_1 to give a total data matrix of 128 × 2048 complex points with a sweep width of 2.7 kHz.

The ^1H–^{13}C chemical shift correlation (HETCOR) utilized the sequence described by Rutar[7] and Wilde and Bolton[8] with delays optimized for J(C,H) values of 170 Hz; 32 scans were measured for 128 values of t_1 to give a total data matrix of 128 × 4096 complex points. The carbon F_2 spectral width was 15 kHz and the proton F_1 spectral width was 3.2 kHz.

The long-range ^1H–^{13}C chemical shift correlation was recorded on a Bruker ARX 400 spectrometer and utilized the sequence described by Bax and Morris[9] with delay values optimized for ^3J(C,H) values of 10 Hz; 16 scans were measured for 512 values of t_1 to give a total data matrix of 512 × 8192 complex points. The carbon F_2 spectral width was 20 kHz and the proton spectral width was 4.2 Hz.

RESULTS

In the ^1H NMR spectra of **2–8** and **4AQ**, H-3 shows the largest quinoline shift (upfield), and H-2 behaves in a

similar manner to the α-proton of pyridinium ions[10] (largest downfield).

Irradiation of H-9 of **3** gives rise to an NOE-difference signal for H-2 at 8.57 ppm and for H-8 at 7.95 ppm. Hence the doublet of doublets (dd) centred on 8.40 ppm can be unambiguously assigned to H-5. In order to assign H-6, ^{1}H homonuclear decoupling was applied to H-5. Selective irradiation at the centre of H-5 simplified the double doublet of doublets (ddd) at 7.68 ppm (J = 8.4, 6.8, 1.4 Hz) to a dd (J = 7.2, 1.4 Hz), and the ddd at 7.89 (J = 8.9, 6.8, 1.4 Hz) was reduced to a dd (J = 8.9, 6.7 Hz). Hence the signals at 7.68 and 7.89 ppm were assigned to H-6 and H-7, respectively, with the following coupling constants in Hz: $J(5,6) = 8.4$, $J(5,7) = J(6,8) = 1.4$, $J(6,7) = 6.8$ and $J(7,8) = 8.9$. The COSY experiment corroborates the assignments made on the homonuclear spin decoupling.

DEPT experiments were carried out for the identification of the CH_2, CH and C carbons. Two-dimensional high-field NMR methods were employed to make a complete assignment of the proton and carbon NMR spectra. Assignments of carbons bearing protons for C-2–C-14 are made by reference to the heteronuclear chemical shift correlation spectrum.

It remained to assign the peaks which arise from the quaternary carbon atoms (C-4, C-4a, C-8a, C-10 and C-13). A long-range ^{13}C–^{1}H correlation experiment, with intervals optimized for 10 Hz coupling constants, was then carried out, allowing the identification of connectivities through ^nJ(C,H) coupling. Literature data show that three-bond coupling constants are fairly large in aromatic rings (4–12 Hz) and larger than ^2J(C,H).[11] From the correlation map centred on the aromatic resonances, it can be seen that quaternary carbon resonances were detected in this experiment. Although some one- and two-bond correlations are retained in this spectrum, many long-range cross peaks appear. Cross peaks were identified as 160.42, 143.41, 140.11, 133.71 and 118.90 ppm. The carbon absorbing at 160.42 ppm clearly showed connectivities with H-2 (lower field proton resonance), and a weaker correlation with H-5 (8.40 ppm), so that it can be unambiguously assigned to C-4. The peak at 140.11 ppm was assigned to C-8a from the four correlations with H-2, H-5, H-7 and H-9 and the peak at 118.90 was assigned to C-4a from the three correlations with H-3, H-6 and H-8. Long-range coupling resonances to the other quaternary carbon signals were analysed and assigned in a similar way. Analysis of the spectra of **2** and **4**–**7** was then easily derived. The ^{1}H and ^{13}C NMR assignments of **2**–**8**, **4AQ** and **4AQ.HBr** are reported in Tables 1 and 2, respectively. It should be pointed out that C-11 always appears downfield compared with C-12 in **2**–**6** and **8**, whilst this trend is reversed in **7**, as was demonstrated by the long-range ^{1}H–^{13}C correlation NMR spectroscopy. This arises because C-12 in **7** experiences a paramagnetic deshielding effect associated with the electron circulation around the triple bond.

Intramolecular interactions between the two quinolinium rings are very unlikely, especially in the case of **2**, which has only one methylene group between the two phenyl rings of the linking chain. In the case of **5**, the linker might be folded or partially folded owing to the flexibility of the tetramethylene unit. However, as can be seen from Table 2, the carbon chemical shifts for the 4-aminoquinolinium moiety in CD_3OD for **2**–**7** are all the same and thus show the same behaviour pattern. The lack of intramolecular interactions between the two quinolinium rings is confirmed from the ^{13}C NMR for the 4-aminoquinolinium moiety in **4AQ.HBr** and **8** since the spectra are the same as those of the bis-salts **2**–**7**, with the only exception being C-2 in **4AQ.HBr**.

Although ^{1}H and ^{13}C NMR spectra of **4AQ** (in DMSO-d_6) have been reported previously,[11] one of the proposed ^{13}C shifts was found to be incorrect (probably a typographical error). The value of C-8a, which was reported as being 128.71 ppm (in DMSO-d_6), is actually 148.69 in the same solvent and 149.37 ppm in CD_3OD.

Table 3 presents the average relative chemical shift differences (CSD) measured as the differences between the chemical shifts of **4-AQ** and the dibromides **2**–**7** and the calculated standard deviations for six 1,1′-disubstituted bis(4-aminoquinolinium) salts. Quaternization of the endocyclic nitrogen of **4AQ** deshielded all the quinoline ring protons, as expected (Table 3). In the spectra of the bis-salts **2**–**7**, compared with **4AQ**, the signals of C-2, C-4a, C-6, C-8 and C-8a are shifted upfield, whereas the others are shifted downfield. CSD for C-2, C-3 and C-4 show a similar trend but with lower values than those exhibited for 4-aminopyridine and its hydrochloride in D_2O:[12] C-2, 5.1 against 3.06 for **4AQ**; C-3, −1.0 against −0.03 for **4AQ**; and C-4,

Table 1. ^{1}H chemical shifts (ppm) of the 4-aminoquinolinium moiety in **2**–**8**, **4AQ.HBr** and **4AQ** in CD_3OD

Hydrogen	2	3	4	5	6	7	8	4AQ.HBr	4AQ
2	8.56	8.57	8.58	8.58	8.56	8.59	8.61	8.30	8.26
3	6.90	6.93	6.92	6.92	6.92	6.93	6.93	6.84	6.60
5	8.39	8.40	8.40	8.40	8.40	8.41	8.41	8.33	8.04
6	7.69	7.68	7.68	7.68	7.69	7.71	7.69	7.68	7.40
7	7.89	7.89	7.90	7.89	7.90[a]	7.93[b]	7.91	7.95	7.61
8	7.95	7.95	7.98	7.97	7.90[a]	7.93[b]	7.97	7.84	7.79

[a] H-7 and H-8 overlap in **6**.
[b] H-7 and H-8 overlap in **7**.

Table 2. Carbon chemical shifts (ppm) of the symmetrical 1,1′-disubstituted bis(4-aminoquinolinium) dibromides **2–7** having aralkyl linking groups and the monocationic compounds **8**, **4AQ.HBr**, and **4AQ** in CD_3OD

Carbon	2	3	4	5	6	7	8	4AQ.HBr	4AQ
2	147.74	147.74	147.75	147.73	147.80	147.87	147.85	142.43	150.83
3	103.46	103.44	103.45	103.45	103.52	103.58	103.47	103.11	103.45
4	160.46	160.42	160.43	160.42	160.48	160.59	160.49	160.54	154.20
4a	118.89	118.90	118.90	118.89	118.89	118.92	118.91	117.56	119.92
5	125.46	125.47	125.46	125.47	125.53	125.54	125.49	124.53	122.92
6	127.93	127.93	127.94	127.92	127.97	128.01	127.95	127.89	130.64
7	135.73	135.67	135.71	135.69	135.74	135.86	135.74	135.40	125.32
8	119.88	119.93	119.93	119.93	119.86	119.79	119.92	120.86	128.66
8a	140.12	140.11	140.13	140.12	140.10	140.10	140.13	139.92	149.37
9	58.48	58.60	58.58	58.59	58.58	58.39	58.74		
10	142.91	143.41	144.10	144.40	138.76	136.74	139.19		
11	130.73	130.43	130.32	130.28	130.64	127.83	130.29		
12	127.90	127.71	127.79	127.73	127.64	133.30	127.64		
13	134.01	133.71	133.57	133.44	135.16	124.47	129.58		
—CH_2—	41.84								
—CH_2—C—		38.25							
—CH_2—C—C—			35.91						
—C—CH_2—C—			34.05						
—CH_2—C—C—C—				36.18					
—C—CH_2—C—C—				31.98					
—CH=CH—[a]					131.13				
—C≡C—						90.14			

[a] *cis* compound.

−9.2 against −6.27 for **4AQ**. C-8a is shielded on quaternization. For comparison, C-4 and C-5 of quinacrine[12] (with a 6-chloro-2-methoxy-9-aminoacridine ring system) in D_2O, which have similar environments to **2–7**, show analogous trends, i.e. they shift upfield on protonation on N-10 and on quaternization on N-1, respectively. On the other hand, C-7 of both chloroquine[12] and quinacrine,[12] although bearing a chlorine atom, display a downfield shift on protonation in D_2O, similar to that exhibited by benzylation on N-1 of **4AQ**. The same trend is observed between C-8 of quinacrine and chloroquine in D_2O and the same carbon of the bis-salts **2–7**. The values of the shifts, |CSD|, are largest for C-4, C-7, C-8 and C-8a (6–10 ppm), whereas for the other carbon atoms they are much smaller (1–3 ppm); C-3 practically does not change.

Table 3. Average relative chemical shift differences (CSD) and standard deviations for the 4-aminoquinoline ring protons and for the carbon atoms of 1,1′-disubstituted bis(4-aminoquinolinium) dibromides **2–7** in CD_3OD ($n = 6$)

Quinoline ring position	Average CSD[a] (ppm)	
	^{1}H NMR	^{13}C NMR
2	−0.31 ± 0.01	3.06 ± 0.05
3	−0.32 ± 0.01	−0.03 ± 0.05
4		−6.27 ± 0.06
4a		1.02 ± 0.01
5	−0.36 ± 0.01	−2.57 ± 0.03
6	−0.29 ± 0.01	2.69 ± 0.03
7	−0.29 ± 0.01	−10.41 ± 0.06
8	−0.16 ± 0.03	8.77 ± 0.05
8a		9.26 ± 0.01

[a] CSD = δ(**4AQ**) − δ[bis(4-aminoquinolinium) derivative]. A negative sign indicates a downfield shift.

DISCUSSION

^{13}C chemical shifts for quinoline have been shown[13,14] to be linearly correlated with calculated total charge densities. They agree with previous observations that in nitrogen heterocycles such as pyridine, there is an upfield shift of the ^{13}C chemical shifts of the carbon atoms directly attached to the ring nitrogen atom on protonation.[15]

The results of the calculations on **4AQ**, **4AQ.HBr** and **8** using MNDO[4] suggest the upfield shift in C-2 and C-8a, which are directly connected to the ring nitrogen atom. The ^{13}CSs for **4AQ.HBr** and **8** are similar except for C-2 (5.4 ppm difference) and C-4a (1.4 ppm difference), possibly because C-2 is deshielded from the effect of the benzyl group in **8**.

Good correlations are obtained between atomic charges (ACs) and ^{13}C chemical shifts (CSs) for **4AQ**, **4AQ.HBr** and **8** using the MNDO[4] method to derive MOPAC charges:

$$AC = 0.00554(\pm 0.00054)CS - 0.75146(\pm 0.07177)$$

$$n = 9, \quad r = 0.968, \quad s = 0.026 \quad \text{for } \mathbf{4AQ}$$

$$AC = 0.01093(\pm 0.00102)CS - 1.40115(\pm 0.13335)$$
$$n = 9, \quad r = 0.971, \quad s = 0.048 \quad \text{for } \mathbf{4AQ.HBr}$$
$$AC = 0.01075(\pm 0.00084)CS - 1.38827(\pm 0.11089)$$
$$n = 9, \quad r = 0.979, \quad s = 0.040 \quad \text{for } \mathbf{8}$$

where n is the number of carbon atoms, r is the correlation coefficient, s is the standard deviation and data in parentheses are 95% confidence intervals. Plots of ACs *vs.* CSs for the model compounds are shown in Fig. 1.

In conclusion, the calculations carried out confirm the good correlations between ACs and CSs of the carbon atoms of the nitrogen heterocycles using the MNDO method. The previously anomalous upfield shift in the ^{13}C CSs of carbon atoms directly connected to a protonated endocyclic nitrogen atom is observed in the title compounds.

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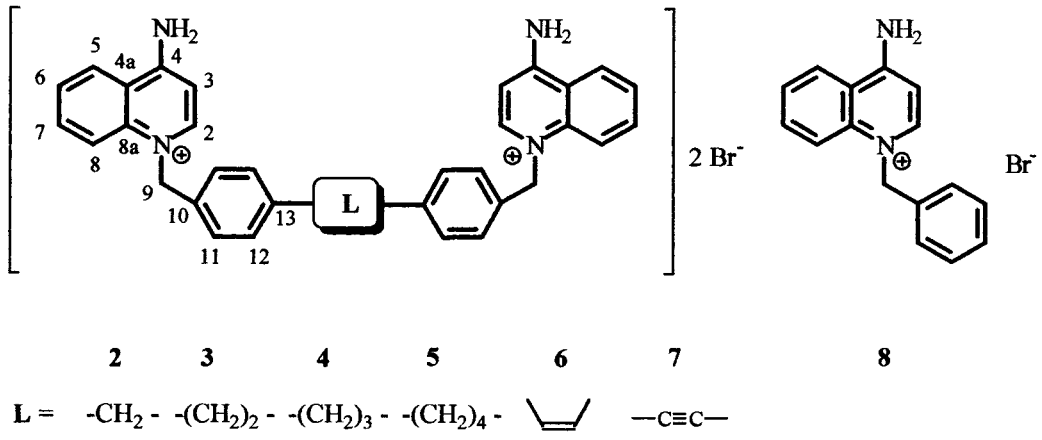

2 **3** **4** **5** **6** **7** **8**

L = $-CH_2-$ $-(CH_2)_2-$ $-(CH_2)_3-$ $-(CH_2)_4-$ —C≡C—