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STUDIES ON CHEMICAL CARCINOGENS—XVII†

STRUCTURE OF CARCINOGENIC 4-HYDROXYAMINOQUINOLINE 1-OXIDE DERIVATIVES

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Abstract—Analysis of proton NMR spectra of N-methyl and O-acetyl derivatives of 4-hydroxyaminoquinoline 1-oxide (**6**) and 4-aminoquinoline 1-oxide revealed that the structure of carcinogenic **6** and its O-monoacetate are 1-hydroxy-4-hydroxyimino-1,4-dihydroquinoline and 1-hydroxy-4-acetoxyimino-1,4-dihydroquinoline, respectively, whereas 4-(N-methylhydroxyamino)quinoline 1-oxide, which is also carcinogenic, has a quinoline N-oxide structure, as have all the 4-amino derivatives examined. Since the structure of O-monoacetate of **6** was determined as such, it is assumed that aminoacyl derivative of **6**, which is supposed to the ultimate carcinogenic form in its carcinogenesis, is formulated as 1-hydroxy-4-aminoacyloxyimino-1,4-dihydroquinoline. PH-dependent UV spectral changes revealed that carcinogenic **6** and its N-methyl derivative are both acidic, the pKa being of similar value in both.

4-Hydroxyaminoquinoline 1-oxide (4HAQO) is a potent chemical carcinogen and the molecular mechanism of its carcinogenicity has extensively been studied.[1-7] It is evident that 4HAQO is metabolically activated for its carcinogenicity with the help of aminoacyl-t-RNA synthetase to aminoacyl derivative of 4HAQO, which is assumed to be the ultimate form in this carcinogenesis.[3-6] It was recently reported[8] that the N-Me derivative of 4HAQO is as carcinogenic as 4HAQO, but aminoacyl-t-RNA synthetase does not participate in its metabolic activation.[8] As one of our serial studies on chemical carcinogenesis by this class of compounds, this paper describes the structures of carcinogenic 4HAQO, its acetyl and N-Me derivatives, studied by proton NMR and UV spectroscopy.

RESULTS

The compounds examined are shown in Table 1.‡ Two of them (**5** and **7**) were newly synthesised. N,O-diMe-4HAQO (**5**) was prepared by methylation of N-Me-4HAQO (**4**) with diazomethane. Mono Ac-4HAQO (**7**) was synthesised by the treatment of the diAc-4HAQO (**8**) dissolved in dimethyl sulfoxide (Me_2SO) with dithiothreitol (DTT), which behaved as the acceptor of one of the acetyl groups in the diacetate molecule. Hence, it became easy to obtain the Me_2SO solution of monoAc-4HAQO (**7**), although DTT and its acetate are present together. Then, an alternative trial was made to prepare **7**, as follows.

When diAc-4HAQO (**8**) was treated with liquid ammonia, monoAc-4HAQO and acetamide were produced quantitatively almost at once. However, the product isolated after evaporation of ammonia was too sensitive to air oxidation to be purified. It was considerably stable in solution of Me_2SO in the presence of DTT as an anti-oxidant. Next, the hydrolysis with dil HCl was examined, expecting that the hydrochloride of monoAc-4HAQO would be stable enough to be isolated. DiAc-4HAQO was partly hydrolysed in 3% HCl at room temperature, but further hydrolysis proceeded gradually at the same time. The monoAc-4HAQO (**7**) solutions obtained from the three procedures described were proved identical with each other by mixing the solutions of each preparation, followed by confirmation of the complete coincidence of all the NMR signals concerned. A sample of monoAc-4HAQO (**7**) therefore became available for NMR measurement, although its isolation failed.

The compounds synthesised for NMR signal assignment were the 2,8-dideuterio derivatives of all the compounds and 5,6,8-trideuterio derivative of 4HAQO.[13]

Structure of free bases. As seen in Table 1 and Fig. 1, chemical shift of protons on the benzene moiety in the molecule of the derivatives **1**–**5** is not dependent on the substituent at the position-4. In contrast, the chemical shift of H-3's of these derivatives is the most affected by the substituent, followed by those of H-2's. This is reasonably explained by taking into account that the electronic effect of the substituent is strongest on H-3

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†Part XVI: *Gann* **70**, 799 (1979).

‡Abbreviations and compound numbers: **1**, 4AQO (4-aminoquinoline 1-oxide);[9] **2**, Me-4AQO (4-methylaminoquinoline 1-oxide);[10] **3**, diMe-4AQO (4-dimethylaminoquinoline 1-oxide);[10] **4**, N-Me-4HAQO (4-(N-methylhydroxyamino)quinoline 1-oxide);[8] **5**, N,O-diMe-4HAQO (4-(N,O-dimethylhydroxyamino)-quinoline 1-oxide); **6**, 4HAQO (4-hydroxyaminoquinoline 1-oxide);[11] **7**, monoAc-4HAQO (4-acetoxyaminoquinoline 1-oxide) **8**, diAc-4HAQO (1-acetoxy-4-acetoxyimino-1,4-dihydroquinoline).[12]

Table 1. Chemical shifts (δ values) of aromatic ring protons of 4-substituted quinoline 1-oxides measured in $(CD_3)_2SO$

Compound	H-2	H-3	H-5	H-6	H-7	H-8
4AQO(1)	8.12	6.51	8.21	7.51	7.72	8.45
Me-4AQO(2)	8.28	6.35	8.28	7.59	7.79	8.55
diMe-4AQO(3)	8.41	6.88	8.15	7.67	7.81	8.59
N-Me-4HAQO(4)	8.52	7.38	8.27	7.68	7.90	8.60
N,O-diMe-4HAQO(5)	8.59	7.36	8.18	7.78	7.92	8.67
4HAQO(6)	7.47	6.17	7.97	7.20	7.50	7.60
monoAc-4HAQO(7)	7.62	6.05	8.07	7.26	7.60	7.60
diAc-4HAQO(8)	7.67	6.21	8.11	7.30	7.61	7.34

then on H-2 and finally on the protons in the benzene moiety. H-3 of diMe-4AQO (**3**) is more deshielded than that of Me-4AQO (**2**), probably due to distortion from the planar structure because of more bulkiness of the substituent. Proton-3 of N-Me and N,O-dimethyl derivatives of 4HAQO (**4** and **5**, respectively) resonated at further lower fields. This may be due to the reduced electron-donating effect of hydroxyamino group, compared with those of amino and alkylamino groups, in addition to the deviation of the molecule from planarity.

H-8 of these derivatives resonated at the lowest field among all the other protons of each molecule. This is considered to be due to the magnetic anisotropy effect of the N-oxide group, as already well documented,[7,14,15] and hence this is evidence for the presence of the N^+-O^- (N oxide) structure in these molecules.

In contrast to the derivatives **1**–**5**, 4HAQO (**6**) and monoAc-4HAQO (**7**) gave a pattern of spectra markedly different from those of **1**–**5**. The spectra of **6** and **7** were very similar to that of diAc-4HAQO (**8**), the structure of which was already determined as 1-acetoxy-4-acetoxyimino-1,4-dihydroquinoline,[12] as formulated in Chart 1. The features characteristic of the N-oxide structure were lost in the spectra of **6** and **7**; chemical shifts of H-8 and H-2 were much higher than those expected for the quinoline 1-oxide structure. It can therefore be concluded that 4HAQO has the structure, in which the 1,4-dihydro tautomer (**6-a**) predominates over the N-oxide tautomer (**6-b**) in Me_2SO solution.

With regard to monoAc-4HAQO (**7**), its structure can be assumed to be 1-hydroxy-4-acetoxyimino-1,4-dihydroquinoline from the shifts caused by monoacetylation of 4HAQO and by the subsequent acetylation of monoAc-4HAQO. Thus, the chemical shifts of the ring protons of 4HAQO were changed by monoacetylation by the following size of the shift $\Delta\delta_{H-2} = +0.15$ ppm, $\Delta\delta_{H-3} = -0.12$, $\Delta\delta_{H-5} = +0.10$, $\Delta\delta_{H-6} = +0.06$, $\Delta\delta_{H-7} = +0.01$, and $\Delta\delta_{H-8} = 0.00$. Those of monoAc-4HAQO shifted in turn by the second acetylation as follows: $\Delta\delta_{H-2} = +0.05$, $\Delta\delta_{H-3} = +0.16$, $\Delta\delta_{H-5} = +0.04$, $\Delta\delta_{H-6} = +0.04$, $\Delta\delta_{H-7} = +0.01$, and $\Delta\delta_{H-8} = -0.28$. In general, acetylation of the substituent is expected to cause a paramagnetic shift (positive values of the shift) of the ring protons due to the electron-withdrawing

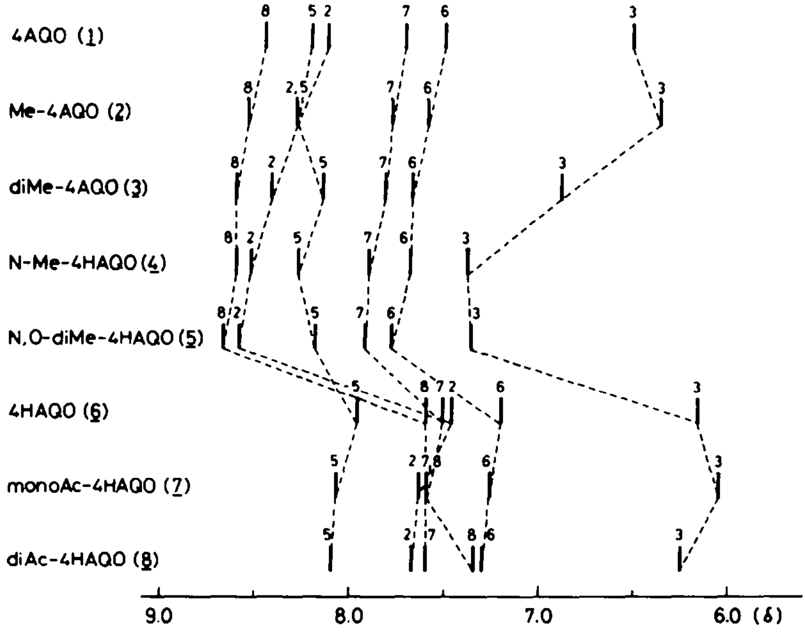


Fig. 1. Schematic presentation of chemical shift (δ value) of the aromatic protons of 4-substituted quinoline 1-oxides (free base form) measured in $(CD_3)_2SO$.

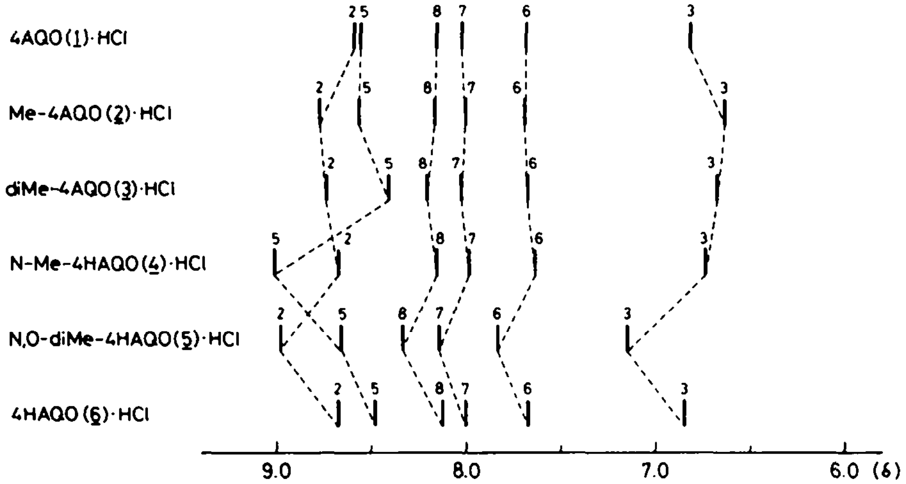
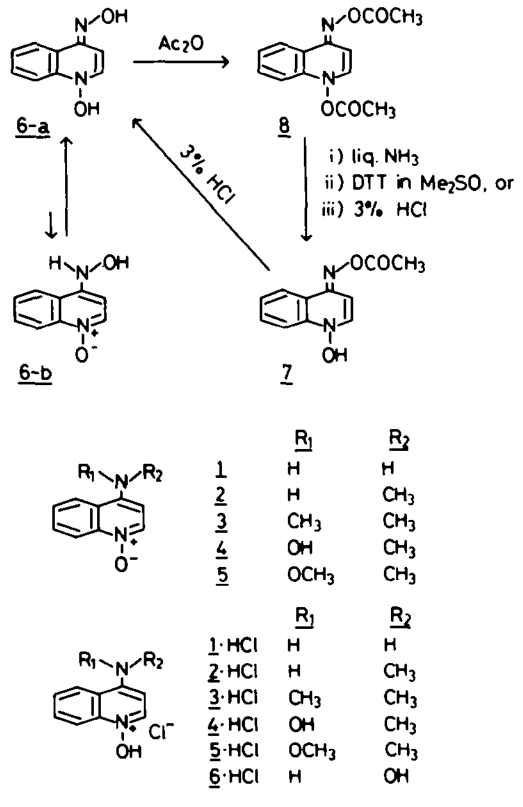


Fig. 2. Schematic presentation of chemical shift (δ value) of the aromatic protons of hydrochlorides of 4-substituted quinoline 1-oxides measured in $(CD_3)_2SO$.

nature of the acetyl group. Attention should be paid on the diamagnetic shift (negative values of the shift) observed by the acetylation, i.e. the shift of H-3 caused by the first acetylation and that of H-8 by the second acetylation. It is considered that these diamagnetic shifts can be produced by spatial magnetic effect of the anisotropic acetyl CO on the closely located proton. It is therefore concluded that the acetylation which affected H-3 should have taken place at the OH group of the substituent and the second which affected H-8 should have occurred at the OH group bonded to the ring nitrogen, as shown in Chart 1.

Structures of hydrochlorides of the derivatives. It is expected that the protonated forms of all the derivatives have a common electronic structure as shown in Chart 1, regardless of the structure of the free bases; the quinoline N oxide form or 1-hydroxy-4-hydroxyimino form. This was strongly supported by the NMR data. Thus, the chemical shifts of any of the protons, except for H-5 of N Me-4HAQO (**4**) hydrochloride, of all the derivatives examined are not appreciably dependent on the kind of substituent present. Even the shift of H-3 was much less dependent on the substituent than in the case of the free bases,



	R1	R2
1	H	H
2	H	CH_3
3	CH_3	CH_3
4	OH	CH_3
5	OCH_3	CH_3

	R1	R2
1·HCl	H	H
2·HCl	H	CH_3
3·HCl	CH_3	CH_3
4·HCl	OH	CH_3
5·HCl	OCH_3	CH_3
6·HCl	H	OH

Chart 1.

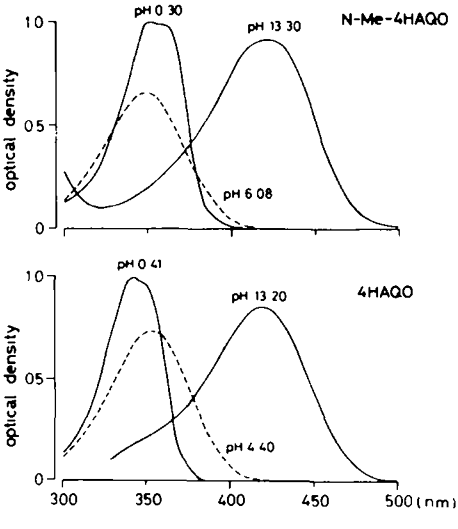


Fig. 3. UV Spectra of N-Me-4HAQO (**4**) and 4HAQO (**6**) in acidic, near neutral, and alkaline media. Those in acidic medium are for their protonated forms and those in alkaline are for deprotonated conjugate bases.

Table 2. Chemical shifts (δ values) of aromatic ring protons of hydrochlorides of 4-substituted quinoline 1-oxides measured in $(CD_3)_2SO$

Compound Hydrochloride of	H-2	H-3	H-5	H-6	H-7	H-8
4AQO(1)	8.60	6.82	8.56	7.68	8.02	8.15
Me-4AQO(2)	8.77	6.65	8.57	7.69	8.01	8.15
diMe-4AQO(3)	8.74	6.90	8.41	7.68	8.02	8.21
N-Me-4HAQO(4)	8.67	6.75	9.01	7.64	7.99	8.17
N,O-diMe-4HAQO(5)	8.98	7.15	8.66	7.83	8.14	8.34
4HAQO(6)	8.68	6.84	8.48	7.67	8.00	8.12

regardless of amino or hydroxyamino and whether bulky or less bulky.† Stronger electron-withdrawing effect of the cationic ring nitrogen must make it possible to overlap p-electrons of the substituent with π-electrons of the aromatic ring, overcoming the energetically unfavourable steric hindrance in the planar structure of the molecule. The only exceptional irregularity found in the NMR data of the salts was a large paramagnetic shift of H-5 of N-Me-4HAQO (**4**) hydrochloride. H-5 of this derivative resonated at the lowest field among the protons of the molecule, whereas, in all the other derivatives, H-2's resonated at the lowest in each molecule. Provided that the salts of all the derivatives are in more or less planar conformation and that the larger N-Me group of the substituent is oriented toward C-3 and the smaller N-OH group toward C-5, it can be assumed that the origin of such a large deshielding of H-5 is attributed to the magnetic anisotropy effect of O-H group, since its O-Me derivative (**5**) gave a regular pattern of spectrum.

UV spectra and Pka values. UV spectra were measured in phosphate buffer of various pH's and absorption maxima are given in Table 3. PKa values, which were evaluated from the pH-dependent spectral changes, are also listed in Table 3. With regard to the pKa's for protonation, they decreased with increasing bulkiness of the substituents; pKa values decreasing in the order of **1**, **2**, and **3** in 4AQO series, and in the order of **6**, **4** and **5** in 4HAQO series.

These results are in a striking contrast to the data from N-substituted anilines, basicity decreasing in the order of N,N-dialkylanilines, N-monoalkylanilines, and aniline. It may therefore be concluded that the pKa's of this series of compounds are governed by thermodynamic stability of the conjugate acids; the more bulky the substituent is, the more unstable the conjugate acid is.

Among these derivatives, 4HAQO (**6**) and its N-Me derivative (**4**) are acidic, their pKa's being almost the same, 9.6[17] and 9.7, respectively. It is rather surprising that these molecules have a similar magnitude of pKa values and that they gave almost the same shape of UV spectra in alkaline solutions above pH 11, which are shown in Fig. 3. The structure of the conjugate base of 4HAQO is reasonably assumed as formulated in Chart 2. The structure of N-Me-4HAQO (**4**) is no doubt formulated as shown in Chart 2. The latter might be stabilised through hybridisation with a kind of the nitrone structure. It should be considered that the pKa values of these derivatives agreed accidentally.

CONCLUSION

It was proved that the structure of carcinogenic 4HAQO is 1-hydroxy-4-hydroxyimino-1,4-dihydroquinoline, whereas its N-Me derivative (**4**), which is also carcinogenic, has the quinoline N-oxide structure as have all the 4-amino derivatives examined. MonoAc-4HAQO (**7**), which can be considered a model compound for the ultimate carcinogen in 4HAQO-carcinogenesis,[7,12] is determined as 1-hydroxy-4-acetoxyimino-1,4-dihydroquinoline.

Table 3. pKa values and UV absorption maxima of 4-substituted quinoline 1-oxides in aqueous solution

Compound	pKa	Absorption maximum(nm) cationic	 neutral	 anionic
4AQO(1)	4.6	335,346s	357	
Me-4AQO(2)	4.4	339,352	365	
diMe-4AQO(3)	3.9	354s,363	369	
N-Me-4HAQO(4)	3.4,9.7	352,363s	350	421
N,O-diMe-4HAQO(5)	2.5	349,361s	348	
4HAQO(6)	3.6,9.6	343,352s	352	418

(s = shoulder)

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†Each proton of **5** resonated in slightly lower field compared with the corresponding protons of all the other derivatives. This seems to correspond to the less basic nature of **5** than the others (Table 3).

Taking into account that the 1-acetoxy group of diAc-4HAQO (**8**) is unstable in the presence of nucleophiles due to its potent acetyl-donating ability, it is assumed that the aminoacyl derivative of 4HAQO, which was assumed to be the ultimate carcinogen,[7,12] has the structure of 1-hydroxy-4-aminoacyloxyimino-1,4-dihydroquinoline. It may be worth noting that carcinogenic derivatives, **4** and **6**, are both acidic, the pKa value being of similar magnitude.

EXPERIMENTAL

Measurements. MNR spectra were measured in $(CD_3)_2SO$ at concentrations of 4-10 mg in 0.4 ml of the solvent at room temp, using a JEOL MH-100 spectrometer, operating at 100 MHz. The chemical shifts described here may include an error of ±0.01 ppm. The spectra were calibrated from the signal of the internal TMS and the chemical shifts were presented in δ value. The spectral assignment was made with comparison with the spectra of 2,8-dideuterated derivatives of each compound and 5,6,8-trideuterated one of 4HAQO.[13] Spectrum of free base of **6** was taken in the presence of a small amount of ascorbic acid. Otherwise, all the signals were broadened probably due to the trace of contaminating free radical. UV spectra were recorded in phosphate buffer soln or soln made acidic with HCl or alkaline with NaOH at room temp, using a Shimazu 210-A spectrometer. Spectra in alkaline soln (pH > 8) were taken in the presence of ascorbic acid (0.2 mg/ml) in order to avoid auto-oxidation of the sample to be measured.[17] PKa's were evaluated based on the spectral changes dependent on the pH-changes of the soln examined.

4-(*N,O-Dimethylhydroxyamino*)*quinoline* 1-*oxide* (**5**). Et_2O soln of CH_2N_2 (20 ml) was added dropwise into a suspension of N-Me-4HAQO (75 mg) in MeOH (5 ml) with stirring for 15 min under cooling. The mixture was gently stirred for about 1.5 hr, until the solid dissolved. The reaction was left in the ice bath for a further 0.5 hr, and then the solvent was evaporated. The brown residue was extracted with $CHCl_3$ (30 ml) and the extract was concentrated *in vacuo*. The residue was chromatographed on a silica gel column (SiO_2, 10 g) and was eluted with $CHCl_3$. The fractions with R_f value of 0.63 on a silica tlc plate (solvent system: 15% MeOH-containing $CHCl_3$), were collected. The residue did not crystallise but its NMR spectrum proved its homogeneity. The absence of NH or OH group was proved by IR spectrum.

MonoAc-4*HAQO* (**7**)

(i) *By acetyl-transfer from diAc*-4*HAQO* (**8**) *to* NH_3. Compound, **8** (20 mg) in liquid NH_3 (0.5 ml) was poured into an NMR tube chilled with Dry Ice–Me_2CO. After the crystalline **8** dissolved, the tube was left chilled for 30 min. The solvent was completely evaporated by being blown with a stream of N_2 gas, leaving a mixture of yellow and white solid material. (The material in the tube colored intensely on exposure to air. From the colored material, acetamide only was isolated in a molar equivalent amount to the starting material.) After evaporation of NH_3, the residue was dissolved in Me_2SO (0.5 ml) containing dithiothreitol (10 mg) under N_2 atmosphere. The soln thus obtained was not coloured. The NMR spectrum of this residue indicated that it was a mixture of molar equivalents of **7** and acetamide. The spectrum of this sample did not change after the sample was left in air for 3 hr at room temp.

(ii) *By acetyl-transfer from* **8** *to dithiothreitol in* Me_2SO. The NMR spectrum of **8** (13 mg) dissolved in $(CD_3)_2SO$ (0.3 ml) containing dithiothreitol (6 mg) was taken at room temp. The signals of **8** gradually decreased and a new set of signals developed. Finally, 30 min after being dissolved, the spectrum became a set of newly developed signals, which were assigned to the protons of **7**.

(iii) *By partial hydrolysis of* **8** *with acid*. DiAc-4HAQO (13 mg) was dissolved in 3% DCl (0.5 ml) in D_2O and its NMR spectrum was measured at 5 min intervals. The spectrum taken after 10 min indicated that it consisted of signals of a mixture of equimolar amount of **7** and **8**. The spectrum after a 30-min indicated that the signals of **8** disappeared and that a part of **7** was further hydrolysed to 4HAQO (**6**). After a 2 hr, **6** was produced in more than 80% yield.

Quinoline [8-D]. After 0.5 g of 5% Pd-C (Kawaken Lab., Tokyo) was pre-reduced in MeOD (10 ml) in D_2 atmosphere, MeOD (20 ml) soln of KOD (2.0 g) and 8-chloroquinoline (5 g) was added and shaken in D_2 atmosphere at room temp. The mixture was filtered when D_2 absorption ceased. The filtrate was concentrated to one-half the original volume and diluted with H_2O. After extraction with $CHCl_3$, the extract was distilled. The residue was chromatographed through a silica gel column which was eluted with hexane containing 35% $CHCl_3$. The yield was 2.7 g (68%). Isotope content determined by NMR was about 85%.

Quinoline[8-D] 1-*oxide*. To a soln of quinoline[8-D] (2.7 g) in AcOH (12 ml), 30% H_2O_2 (3 ml) was added and warmed at 60–70 for 4 hr. Another 30% H_2O_2 (3 ml) was added and the mixture was warmed at the same temp for 5 hr. After the excess of H_2O_2 was decomposed by the addition of Pd-C, the mixture was concentrated and diluted with H_2O. It was made alkaline with Na_2CO_3 and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and evaporated to dryness. The residue was distilled *in vacuo*. b.p. 170° (3 Torr). The yield was 2.2 g (73%).

Quinoline[2,8-D_2] 1-*oxide*. Quinoline[8-D] (2.2 g) was dissolved in D_2O (38 ml) containing NaOD (2.2 g) and warmed at 100° in a sealed tube for 5 hr. The soln was extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and evaporated. NMR spectrum of the crystalline residue (1.8 g) showed that H-2 was replaced with deuterium almost completely.

4-*Nitroquinoline*[2,8-D_2] 1-*oxide*. Quinoline[2,8-D_2] 1-oxide (0.60 g) thus prepared was dissolved in 80% H_2SO_4 (2 ml) and KNO_3 (0.48 g) was added in small portions under stirring. The mixture was kept at this temp for 5.5 hr and poured on ice (20 g). The yellow solid separated and was collected on a sintered glass filter and dissolved in benzene. The benzene soln was washed successively with sat $NaHCO_3$

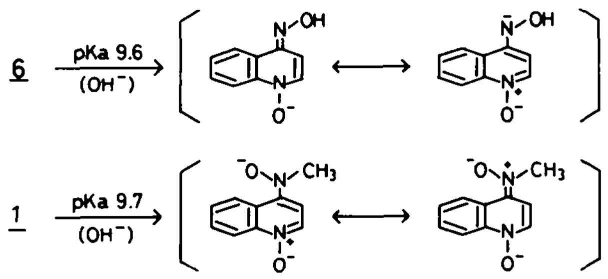

Chart 2

and H_2O. The benzene layer dried over $MgSO_4$ was placed on a small alumina column for chromatography. The column was eluted with benzene and the eluate was collected and evaporated to dryness. The yellow solid formed was recrystallised from Me_2CO. The yield was 0.42 g (56%). The isotope content at 2- and 8-positions were 98% and 85%, respectively.

4-*Chloroquinoline*[2,8-D_2] 1-*oxide*. A soln of 4-nitroquinoline[D_2] 1-oxide (0.40 g) in 5 ml conc HCl was heated at 100° in a sealed tube for 6 hr. The soln was evaporated to dryness *in vacuo* and the residue was recrystallised from Me_2CO. 4-Chloroquinoline[2,8-D_2] 1-oxide was obtained as free base in 89% yield. NMR spectrum showed that no deuterium was lost during the reaction.

4-(N-*Methylhydroxyamino*)*quinoline*[2,8-D_2] 1-*oxide*. A soln of N-methylhydroxylamine HCl (0.2 g) in MeOD (5 ml) was combined with MeOD (5 ml) soln containing KOD (0.12 g). The inorganic salt that precipitated out was eliminated by filtration, and 0.20 g of 4-chloroquinoline[2,8-D_2] 1-oxide dissolved in MeOD (10 ml) was added to this filtrate. The mixture was warmed at 80° for 8 hr with stirring. After evaporation of the solvent, the residue was chromatographed over silica gel (50 g) and was eluted with 1.5% MeOH in $CHCl_3$. Recrystallisation from MeOH–EtOAc gave 0.09 g of **4** [2,8-D_2].

4-*Aminoquinoline*[2,8-D_2] 1-*oxide*. 4-Nitroquinoline [2,8-D_2] 1-oxide (0.10 g) was hydrogenated in EtOH (10 ml) in the presence of 0.10 g of 5% Pd-C. The hydrogenation was continued until the solid that separated dissolved again. After elimination of the catalyst, the soln was evaporated and the residue was recrystallised from EtOH. The yield was almost quantitative. No appreciable deuterium loss was observed.

4-*Hydroxyaminoquinoline*[2,8-D_2] 1-*oxide*. 4-Nitroquinoline[D_2] 1-oxide (0.10 g) was dissolved in EtOH (5 ml) and phenylhydrazine (0.1 ml) was added. The dark colored mixture was warmed at 60° for 3 hr. The separated solids were collected on a sintered glass filter and washed with cold EtOH. The hydrochloride of the product was recrystallised from MeOH–EtOAc. The yield was almost quantitative. No appreciable deuterium loss was observed.

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