PMR Analysis of Nitroquinoline 1-Oxides

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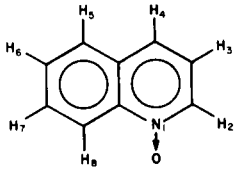
Received May 12, 1972

Chemical shifts and coupling values obtained from the 100 MHz pmr spectra of 12 nitro-substituted quinoline 1-oxide derivatives are presented. Spin-spin decoupling, application of the paramagnetic shift reagent tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione) europium (III), and calculations of theoretical spectra were used in matching signals to protons in the complex portions of the pmr spectra. Precise spectral parameters were obtained by application of an iterative technique provided by a published computer program. The identification of 4- and 5-nitro substituted quinoline 1-oxides by correlation of pmr spectral parameters with chemical structure is illustrated. The preparation and structural elucidation of two potential carcinogenic compounds, 2,6-dimethyl-4-nitroquinoline 1-oxide and 2,7-dimethyl-4-nitroquinoline 1-oxide are described.

The carcinogenic (1), carcinostatic (2) and mutagenic (3) properties of 4-nitroquinoline 1-oxide (4-NQO) are well documented. In addition, there have been reports on the ability of 4-NQO to cause the induction of phage lysis of bacterial cells (4) and on its ability to produce nucleolar segregation in Chang liver cells (5-7). Recently, a model based on molecular orbital calculations and stereochemical considerations for the interaction of 4-NQO with the deoxyguanosine moiety of DNA to form a charge transfer complex has been proposed (8). Based on this model the relative degree of interaction of 4-NQO with DNA and the carcinogenicity of 26 nitroquinoline 1-oxides was predicted. Although evidence has been presented for the physiochemical interaction of 4-NQO and several of its derivatives with DNA (9-11), no systematic quantitative study of the degree of interaction of variously substituted nitroquinoline 1-oxides with DNA and its component nucleosides has been reported. Thus our interest in obtaining appropriately substituted single isomeric 4-NQOs for use in studies related to the interaction of these compounds with DNA has caused us to examine in some detail the methods of preparation, purification and structural verification of these compounds.

When quinoline 1-oxide (Q-1-O) or a substituted Q-1-O is nitrated, the possibility of producing positional nitro isomers exists, *e.g.*, the nitration of Q-1-O yields a mixture of the 4-, 5-, and 8-nitroquinoline-1-oxides (12).



QUINOLINE-1-OXIDE (Q-1-O)

It has been reported that the ratios of the mononitro isomers produced in these reactions are dependent upon the structure of the quinoline 1-oxide, the nature of the nitrating media, the reaction time and the temperature (12-18).

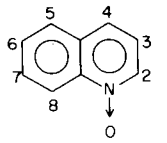
A survey of the literature related to the synthesis and structural elucidation of the variously substituted nitroquinoline 1-oxides revealed that most structrual assignments have been based on converting the mononitro isomers to compounds of known structure, *e.g.*, 4-nitroquinoline 1-oxide (4-NQO) can be reduced to 4-aminoquinoline or converted to a derivative by nucleophilic displacement of the nitro group (19). Polarographic half-wave potentials have been employed in distinguishing positional isomers of the nitroquinoline 1-oxides, *i.e.*, a nitro group in the 4-position of a quinoline 1-oxide is reported to be much more susceptible to electrolytic reduction than is a nitro group in any other position (20).

There have been reports describing the 60 MHz pmr spectra of quinoline 1-oxide (21) and several of its nitro derivatives (22,23). However, due to the complexity and poor resolution of the 60 MHz spectra of these compounds a complete analysis and identification of the nitroquinoline-1-oxides by a systematic pmr study of chemical shifts and coupling values for correlation with structure has not been reported. Clearly the determination of the structure of these compounds by analysis of their pmr spectra has many advantages over previously reported methods.

The purpose of this paper is to illustrate the use of pmr spectral parameters in the determination and verification of substituted NQOs and to report on the synthesis of two potential carcinogenic compounds, 2,6-dimethyl-4-nitroquinoline 1-oxide and 2,7-dimethyl-4-nitroquinoline

TABLE I

Spectral Parameters of some Substituted Quinoline 1-Oxides (a)



Compound	Substituent	δ_2	δ_3	δ_4	δ_5	δ_6	δ_7	δ_8	J_{23}	J_{24}	J_{34}	J_{48}	J_{56}	J_{57}	J_{58}	J_{67}	J_{68}	J_{78}
I	4-NO_2	8.53	8.21		8.74	7.86	7.91	8.82	6.9				8.7	1.9	0.3	7.2	2.0	8.8
II	2-CH_3-4-NO_2	2.72 (b)	8.19		8.66	7.83	7.81	8.72					8.6	1.8	0.4	7.3	1.9	8.6
III	3-CH_3-4-NO_2	8.42	2.52 (b)		7.8 (c)	7.8 (c)	7.8 (c)	8.62							0.3		1.9	8.6
IV	3-Br-4-NO_2	8.64			7.8 (c)	7.8 (c)	7.8 (c)	8.68							0.3		1.9	8.7
V	6-CH_3-4-NO_2	8.44	8.13		8.59	2.64 (b)	7.68	8.64	6.9					1.9	0.3			8.7
VI	6-Br-4-NO_2	8.49	8.25		9.10		7.94	8.65	6.9					2.0	0.3			9.5
VII	6-Cl-4-NO_2	8.48	8.26		8.92		7.82	8.68	6.9					2.0	0.3			9.4
VIII	7-CH_3-4-NO_2	8.43	8.10		8.67	7.67	2.61 (b)	8.51	6.9				8.6		0.4		1.9	
IX	2-CH_3-6-CH_3-4-NO_2	2.67 (b)	8.18		8.53	2.56 (b)	7.66	8.64						1.7	0.4			9.0
X	2-CH_3-7-CH_3-4-NO_2	2.69 (b)	8.15		8.60	7.59	2.58 (b)	8.52					8.6		0.4		1.9	
XI	5-NO_2	8.51	7.54	8.47		8.46	7.53	9.12	6.0	1.2	9.0	0.8				8.0	2.1	9.1
XII	5-NO_2-6-OCH_3	8.44	7.38	7.55		4.06 (b)	7.58	8.89	5.9	1.3	8.9	0.8						9.8
XIII	6-OCH_3	8.48	7.39	7.71	7.23	3.91	7.47	8.66	5.9	1.2	8.8	0.8		2.0	0.4			9.2

(a) Chemical shifts in ppm downfield from TMS; coupling constants in Hz. (b) Denotes chemical shift values of methyl substituents. (c) Due to severe overlap and broadening of signals in this portion of the spectrum a sufficient number of well defined line positions could not be assigned to utilize the iterative feature of the LAOCOON III program.

1-oxide. The 100 MHz pmr spectra of these compounds and of 6 variously substituted quinoline-1-oxides along with the previously unpublished pmr spectral parameters of 12 nitro-substituted quinoline 1-oxide derivatives is presented.

Spin-spin decoupling experiments, application of the paramagnetic shift reagent, tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione) europium (III) abbreviated ($Eu(Fod)_3$ (24-26), as well as stereochemical considerations were used in matching signals to protons. Approximate chemical shifts and coupling constants of quinoline 1-oxides of known structure were used to calculate initial trial spectra of compounds which were difficult to analyze by the usual empirical methods. An iterative technique provided by the published computer program, LAOCOON III (27), was employed to obtain precise spectral parameters.

The chemical shifts and coupling values determined by the analysis of the 100 MHz pmr spectra of twelve nitro substituted quinoline 1-oxides are shown in Table I.

The estimated precision of the chemical shifts shown in Table I is ± 0.01 ppm.; the uncertainty in the coupling values of the carbocyclic ring is ± 0.2 Hz while those of the heterocyclic ring is ± 0.1 Hz.

An inspection of the pmr spectral parameters shown in Table I reveals the protons of the heterocyclic and carbocyclic rings in the nitroquinoline 1-oxides have characteristic spectral parameters. The presence or absence of any of these protons in the nitroquinoline 1-oxide derivatives can be employed in making structural determinations and verifications.

In the nitro substituted quinoline 1-oxides, compounds I, II, V, VI, VII, VIII, IX, X, XI and XII shown in Table I, the protons at positions 5 and 8 absorb at a lower field than the other protons. The H_5 protons of compounds VIII and X absorb at a lower field than the H_8 protons due to the shielding of H_8 by the 7-methyl group. In compounds VI and VII H_5 absorbs at a lower field than H_8 due to the deshielding of H_5 by the 6-halo group.

In the 4-nitroquinoline 1-oxide the H_5 and H_8 protons are nearly chemical shift equivalent. These protons are components of two overlapping four spin systems. The H_6 and H_7 signals which occur at a high field are nearly chemical shift equivalent and are components of two overlapping four spin systems. Spin-spin decoupling by irradiation at H_5-H_8 and at H_6-H_7 was employed to give approximate values for the chemical shifts and coupling values of H_6 and H_7 and of H_5 and H_8. Further evidence supporting the assignment of chemical shift and coupling constant values to the protons of 4-nitroquinoline 1-oxide was obtained by employing the shift reagent $Eu(Fod)_3$. This reagent caused the largest selective downfield shifts in the pmr signals associated with H_2 and H_8 due to the closeness of these protons to the *N*-oxide group. Nitrogen oxide groups interact strongly with $Eu(Fod)_3$, whereas nitro groups do not (25). The shift of H_8 is observed in Figure 1 to be greater than the shift of H_2 in solutions containing up to 50 mole percent of the shift reagent with respect to the nitroquinoline 1-oxide.

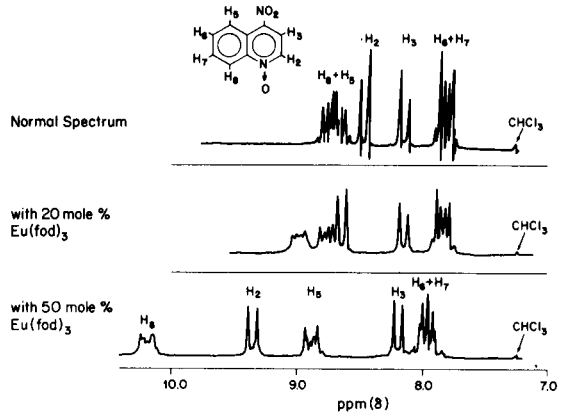


Figure 1. Pmr Spectra of 4-nitroquinoline 1-oxide at at 100 MHz in chloroform-*d*.

The protons in the heterocyclic and carbocyclic rings of the nitroquinoline 1-oxides, with the exception of the H_2 and H_3 protons, have approximately the same coupling constants as do the quinoline 1-oxides (21). The H_2-H_3 coupling constants in the 4-nitroquinoline 1-oxides are in the range of $J_{2,3}$, 6.9 ± 0.1 Hz. The $J_{2,3}$ coupling constants of compounds XI and XII are 6.0 ± 0.1 Hz.

Another characteristic feature in the pmr spectra of 4-nitroquinoline 1-oxides is that the chemical shift of H_3 of the heterocyclic ring absorbs in the range of about 8.1 to 8.3 ppm. In the 4-nitroquinoline 1-oxides, which are unsubstituted in the 2 or 3 positions, H_2 absorbs at a lower field than H_3, and H_2 and H_3 form an AB spin system which is easily recognized.

The nitration of 2,6-dimethylquinoline 1-oxide and 2,7-dimethylquinoline 1-oxide gave good yields of the corresponding 4-nitroquinoline 1-oxides (IX and X). Although the nitration of these 1-oxides might have been expected to produce appreciable amounts of the several possible position isomers, only the 4-nitro isomers IX and X were detected in the crude reaction products by thin-layer chromatography (tlc) and by pmr spectral analysis. The fact that only a single component was indicated by tlc alone was not taken as conclusive that only a single isomer was present since mixtures of closely related compounds are often difficult to distinguish from single pure compounds. The nitroquinoline-1-oxides are particularly difficult to distinguish by their Rf values since these values are practically identical when determined by

either silica-gel tlc or paper chromatography in a variety of solvents. The presence of single isomers was established by an analysis of the pmr peak area integrations. It is estimated that the analysis of peak area ratios for the ring protons would have revealed the presence of other position isomers in the concentration range of 5% to 10% and that position isomers having different methyl group proton absorptions could have been detected at a level of less than 5%. The chemical shifts for the methyl groups associated with methyl substituted quinoline 1-oxides are shown in Table I. The structure of IX and X were assigned by correlating their spectral parameters to the spectral parameters of related compounds in Table I. The aromatic portions of the spectra for 2,6- and 2,7-dimethyl-4-nitroquinoline 1-oxide are illustrated in Figures 2 and 3.

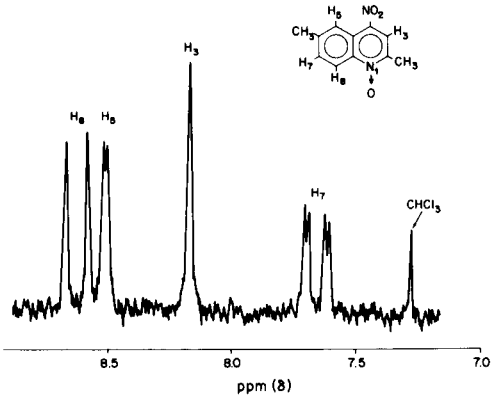


Figure 2. Pmr Spectrum of the aromatic ring protons of 2,6-dimethyl-4-nitroquinoline 1-oxide at 100 MHz in chloroform-*d*.

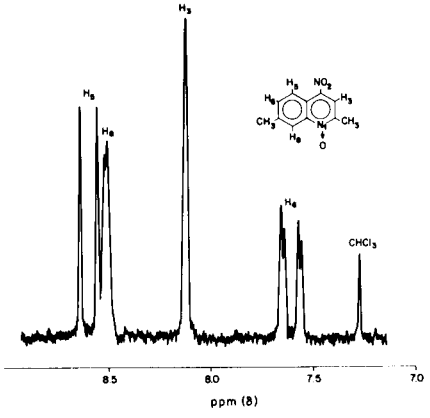


Figure 3. Pmr Spectrum of the aromatic ring protons of 2,7-dimethyl-4-nitroquinoline 1-oxide at 100 MHz in chloroform-*d*.

The pmr spectrum of 3-methyl-4-nitroquinoline 1-oxide (III) shown in Figure 4 reveals an interesting feature. The chemical shift of H_5 which is *peri* to the nitro group appears at a higher field than in 4-NQOs lakcing a substituent *ortho* to the nitro group. It has been suggested that a steric interaction between the methyl group and the nitro group forces the latter into an extreme out-of-plane position with respect to the ring system. The chemical shift of H_5 is explained by noting that the anisotropy effect of the nitro group of H_5 is dependent upon the stereometric relationship of the nitro group with respect to the ring system (23). An examination of space filling molecular models supports this explanation. A nitro group *ortho* to a methyl substituent will be crowded and the nitro group will be greatly twisted out of the plane of the ring to reduce steric interactions. Due to the complexity of the pmr spectrum of compound III, *i.e.*, the severe overlapping and broadening of the H_5, H_6, and H_7 signals, precise spectral parameters for protons H_5, H_6 and H_7 could not be assigned with certainty. Even with the complexity introduced by the severe overlapping of the H_5, H_6 and H_7 signals the position of the nitro group is shown to be at position 4 by comparison of the H_2 singlet in the nitro derivative with the H_2 signal in the starting quinoline 1-oxide. In the starting 1-oxide, H_2 appears as a doublet, $J_{24} = 1.6$. See Figure 5. Further proof that this signal in compound III is due to H_2 was obtained by use of the shift reagent $Eu(Fod)_3$.

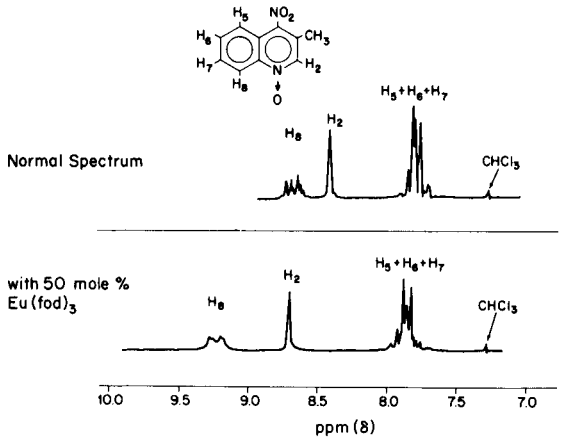


Figure 4. Pmr Spectra of the aromatic ring protons of 3-methyl-4-nitroquinoline 1-oxide at 100 MHz in chloroform-*d*.

The pmr spectrum of 6-methoxy-5-nitroquinoline 1-oxide (XII) shown in Figure 6 is interesting with respect to the H_7-H_8 coupling constant, $J_{78} = 9.8 \pm 0.1$; the H_2-H_3 coupling constant, $J_{23} = 5.9 \pm 0.1$ and the chemical shift of H_4. The value of J_{78} is unusually large for an aromatic coupling constant, and J_{23} has the same value as in the pmr spectrum of 6-methoxyquinoline 1-oxide (XIII)

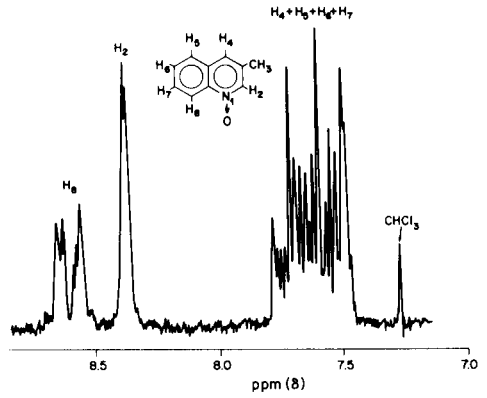


Figure 5. Pmr Spectrum of the aromatic ring protons of 3-methylquinoline 1-oxide at 100 MHz in chloroform-*d*.

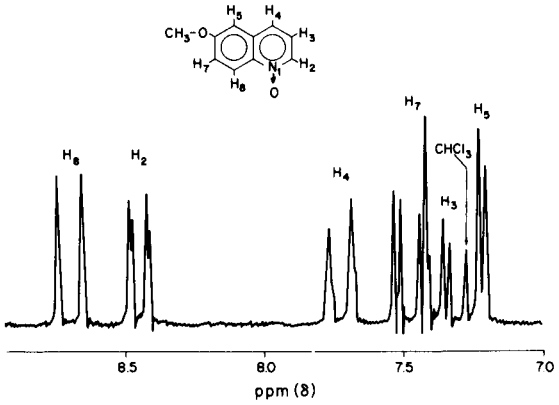


Figure 7. Pmr Spectrum of the aromatic ring protons of 6-methoxyquinoline 1-oxide at 100 MHz in chloroform-*d*.

shown in Figure 7. The chemical shift of H_4 is upfield from where it might have been expected, *i.e.*, H_4 is *peri* to the nitro group at position 5. An examination of space-filling molecular models of (XII) reveals that the steric interaction between the 5-nitro and 6-methoxy group probably results in the nitro group being twisted out of the plane of the ring as was suggested for compound (III). Presumably the twisting of the nitro group out of the plane of the ring prevents the nitro group from exerting its deshielding effect on H_4 as is observed in the pmr spectrum of 5-nitroquinoline 1-oxide (XI). See Figure 8. This explanation is consistent with other reports on the steric effect of the nitro group in aromatic compounds (28). The position of the nitro group is shown to be at position 5 in compound XII by the signal of H_2 which is split by H_3 and H_4.

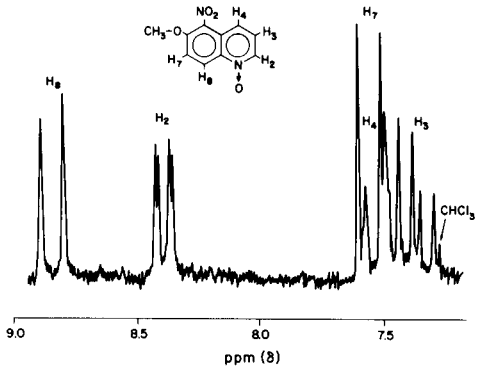


Figure 6. Pmr Spectrum of the aromatic ring protons of 6-methoxy-5-nitroquinoline 1-oxide at 100 MHz in chloroform-*d*.

In this study some evidence for selective solute-solvent interaction was noted. The appearance of the pmr spectra changed as the concentration of the solute or the type of

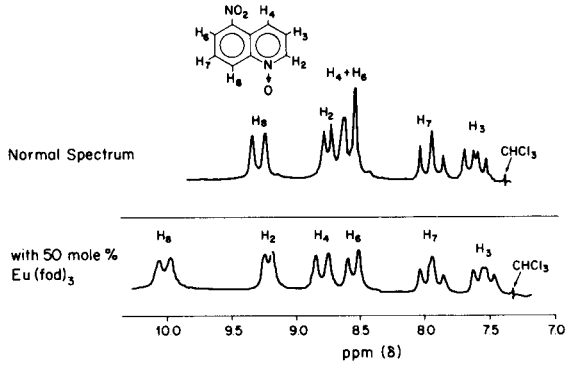


Figure 8. Pmr Spectra of 5-nitroquinoline 1-oxide at 100 MHz in chloroform-*d*.

solvent was changed. The effect of concentration and solvent upon spectrum resolution and upon the chemical shifts of the aromatic ring protons is particularly interesting. Although a quantitative study of the effect of solvent upon chemical shifts was not attempted, it was noted that with benzene as the solvent the proton signals were differentially shifted to a higher field. The effect of solute concentration and of selective effects of solvents on the chemical shifts of the protons of quinoline 1-oxides is not surprising in view of the reports of similar effects observed with substituted quinolines (29-31) and with quinoline 1-oxide (32).

The effect of the lanthanide shift reagent, $Eu(Fod)_3$, on the pmr spectra of nitroquinoline 1-oxides as indicated in Figures 1, 4 and 8 was particularly dramatic, *e.g.* the H_2 and H_8 protons are shifted to a lower field with H_8 being shifted a greater amount than H_2. This effect makes it possible to obtain approximate chemical shift and coupling constants for H_8 and H_5 in spectra where the signals from these protons overlap other signals. The

effect of eruopium complexes on the pmr spectrum of quinoline has recently been reported (24). It is reasonable to suppose that selective solute-solvent and solute-solute effects might aid in the identification of other nitroquinoline 1-oxides and related compounds. Further work is being done in our laboratory in applying both of these effects in determining the structure as well as the purity of variously substituted nitroquinoline 1-oxides.

EXPERIMENTAL

The parameters indicated in Table I were derived from spectra obtained using Varian HA-100 and Jeolco PS-100 nmr spectrometers. Solutions contained 5% or less by weight of the various quinoline 1-oxides in chloroform-*d*. Chemical shift values are in δ units, ppm, with respect to the TMS reference standard. Spectra of compounds I, III and XI were simplified by the use of the paramagnetic shift reagent, $Eu(Fod)_3$ (25,26), *i.e.*, spectra were recorded after successive additions of a chloroform-*d* solution containing 5% by weight of the europium complex until the separation of coincident or overlapping proton signals was observed. Calculated absorption frequencies (lines) and coupling values were matched to experimental lines; iterative calculations based on the least squares criterion brought the calculated line frequencies as close as possible to the experimentally observed lines. The experimental line frequencies used were based on averaging the results of at least three separate determinations. Only the experimental spectra which were visibly resolved in the pmr spectra were matched with calculated lines of the theoretical spectra. Theoretical spectra which resembled experimental spectra were obtained prior to performing the iterative calculations which adjusted the pmr spectral parameters.

All starting reagents employed were obtained from commercial sources. Quinoline 1-oxides were prepared from the corresponding quinolines by modification of the procedure for the *N*-oxidation of pyridine by peracetic acid (33). These compounds were characterized by the agreement of their properties to those reported in the literature.

The nitroquinoline 1-oxides other than compounds IX and X were prepared according to procedures reported in the literature (12,18,23).

Elemental analyses were provided by Atlantic Microlab, Inc. of Atlanta, Georgia.

2,6-Dimethylquinoline 1-Oxide.

To the stirred solution of 31.4 g. (0.20 mole) of 2,6-dimethylquinoline was added 42 ml. (47.5 g., 0.25 mole) of 40% peracetic acid. The peracetic acid was added at a rate sufficient to maintain reflux. After the addition was complete, the reaction mixture was refluxed an additional 3 hours. The progress of the reaction was followed by silica gel thin layer chromatography using benzene-methanol-ethyl acetate (18:1:1 by volume) as the developing solvent. The Rf values of 2,6-dimethylquinoline and 2,6-dimethylquinoline 1-oxide on Eastman 6060 precoated silica gel sheets were 0.91 and 0.79, respectively. These compounds were detected with uv light (2370 Å). The reaction mixture was concentrated by vacuum rotary evaporation and the residue was slurred in 200 ml. of water. The *p*H of the water slurry of the reaction mixture was adjusted to 7.2 by the cautious addition with stirring of an aqueous solution containing about 13 g. of potassium carbonate. After standing overnight the colorless crystalline solid which precipitated was filtered and dried in a vacuum desicator. A yield of 28.9 g. (83.5%) of crude 2,6-dimethylquinoline 1-oxide was obtained. Recrystallization of the crude product from an ether-acetone solution (4:1 by volume) gave 23.3 g. (67.4%) of a crystalline product, m.p. 82.5-82.8°.

Anal. Calcd. for $C_{11}H_{11}NO$: C, 76.28; H, 6.40; N, 8.09. Found: C, 76.22; H, 6.47; N, 8.14.

2,7-Dimethylquinoline 1-Oxide.

The *N*-oxidation of 2,7-dimethylquinoline was conducted by the procedure described for 2,6-dimethylquinoline. The yield of purified 2,7-dimethylquinoline 1-oxide from the *N*-oxidation of 31.4 g. (0.20 mole) of 2,7-dimethylquinoline was 27.1 g. (78.4%) m.p. 73.6-73.9°.

Anal. Calcd. for $C_{11}H_{11}NO$: C, 76.28; H, 6.40; N, 8.09. Found: C, 76.33; H, 6.40; N, 8.90. Found: C, 76.33; H, 6.52; N, 8.01.

2,6-Dimethyl-4-nitroquinoline 1-Oxide (IX).

In a 50 ml. three necked flask equipped with a stirrer and thermometer was placed 30 ml. of 80% sulfuric acid and 5.2 g. (0.03 mole) of 2,6-dimethylquinoline 1-oxide. To the stirred acid mixture 5.0 g. (0.05 mole) of potassium nitrate was added in small portions so that the temperature of the reaction mixture was maintained between 70° and 80°. After the addition of the potassium nitrate the reaction mixture was heated at 75° for an additional 2 hours. The progress of the nitration was followed by silica gel thin-layer chromatography using benzene-methanol-ethyl acetate (18:1:1 by volume) as the developing solvent. After the reaction was complete, which was evidenced by the disappearance of the 2,6-dimethylquinoline 1-oxide (Rf, 0.81) and by the appearance of a product having an Rf value of 0.96, the reaction mixture was poured into 200 ml. of an ice-water slurry. The product, which precipitated as a bright yellow powder, was filtered and washed with three separate 50 ml. portions of cold water. The product was dried in the air and recrystallized from ethanol to yield 4.8 g. (71.8%) of bright yellow needles m.p. 178-179° dec. Chromatography on an alumina column eluting with a chloroform-carbon tetrachloride solution did not reveal the presence of additional isomers.

Anal. Calcd. for $C_{11}H_{10}N_2O_3$: C, 60.55; H, 4.62; N, 12.84. Found: C, 60.69; H, 4.72; N, 12.91.

2,7-Dimethyl-4-nitroquinoline 1-Oxide (X).

The nitration of 2,7-dimethylquinoline 1-oxide was conducted by the same general procedure as described above. The yield of purified product from the nitration of 5.2 g. (0.03 mole) of the starting 1-oxide was 3.7 g. (55.2%) of bright yellow needles m.p. 179-180° dec. Chromatography on an alumina column failed to reveal additional isomers.

Anal. Calcd. for $C_{11}H_{10}N_2O_3$: C, 60.55; H, 4.62; N, 12.84. Found: C, 60.61; H, 4.70; N, 12.96.

Acknowledgments.

This investigation was supported in part by USPHS Grant CA11479-03, Damon Runyon Memorial Fund for Cancer Research Grant DRG-355G, the Southwestern Medical Foundation and USPHS Career Development Award (JSP) (CA-23087-04). The authors also wish to express their gratitude to Dr. Arthur Ihrig of the North Texas State University Chemistry Department and to Dr. Robert Willcott of the University of Houston Chemistry Department for private communications concerning this study.

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