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THE STRUCTURE-CARCINOGENICITY RELATIONSHIP AMONG DERIVATIVES OF 4-NITRO AND 4-HYDROXYLAMINOQUINOLINE 1-OXIDES

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Abstract—Mice were examined for carcinogenic effects of 4-nitro and 4-hydroxylaminoquinoline 1-oxide derivatives which were substituted by an additional group or atom such as a methyl-, halogeno- or nitro-group. It became evident from the carcinogenicity-structure relationship that carcinogenesis of this class of compounds requires the presence of a nitro group at position-4 and an oxide group at position-1 of the quinoline nucleus. However, the proximate structure in the carcinogenic action must be the 4-hydroxylaminoquinoline 1-oxide structure which is formed from the 4-nitro derivatives by metabolic reduction.

AFTER the discovery of the carcinogenic activity of 4-nitroquinoline 1-oxide (Table 1, Ia) by Nakahara *et al.* in 1957,[1] many efforts have been devoted to studies of its biochemical and biological behaviour.[1–7, 14–18, 20–25] This paper deals with the carcinogenic effect of a wide variety of derivatives of this carcinogen on mice and discusses the relationship between the carcinogenic properties and chemical structure.

Although many quinoline derivatives and the probable metabolites of (Ia) have already been assayed for their carcinogenic effect[2–4] (plus numerous unpublished data by Nakahara *et al.*), only 4-hydroxylaminoquinoline 1-oxide, in addition to 4-nitroquinoline 1-oxide from which it can be obtained by reduction, has been shown to possess such activity.[4–7] In order to test in the present study the hypothesis that solely derivatives of these two basic structures produced carcinogenic effects, we synthesized and bioassayed a considerable number of 4-nitro and 4-hydroxylaminoquinoline 1-oxides having various kinds of substituents at different positions of the quinoline nucleus, and compared the influence of such substituents on the carcinogenic activities of the nitro and the hydroxylamino derivatives.

MATERIALS AND METHODS

Compounds

The compounds used in the present study were synthesized by the usual preparative methods and the identification of their structure was carried out by chemical and spectroscopic (infra red, ultra violet and nuclear magnetic resonance) methods without ambiguity. The details have already been reported elsewhere.[8, 9]

Method for bioassay

The compounds to be tested for carcinogenicity were dissolved or suspended in propylene glycol (5 mg/ml) and injected subcutaneously into the left groin of the

mouse in doses of 0·1 ml, the injections being repeated at the same site six times at intervals of 10 days. In the case of compounds that produced too severe local reactions, the dosage had to be cut down by reducing the concentration or by prolonging the interval between injections, or by both procedures.

Normal ddN mice were used in groups of 20 for each test. The mice that developed tumours at the site of injections were recorded and submitted to an autopsy at death. The tumours were histologically diagnosed mostly as fibrosarcomas, with small proportions of rhabdomyosarcomas and squamous cell carcinomas. Compounds were considered as "non-carcinogenic" when none of the treated mice showed tumours by the end of a 300-day period after the first injection.

RESULTS AND DISCUSSION

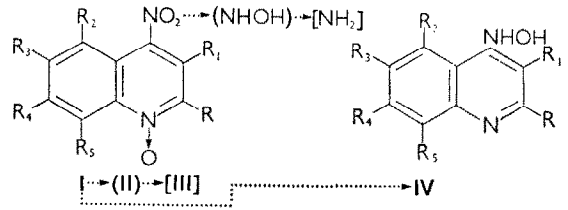
About twenty 4-nitroquinoline 1-oxide derivatives listed in Table 1 (Ia–It) were synthesized by nitration of the corresponding quinoline l-oxides and assayed for their carcinogenic effects. The 4-nitroquinoline l-oxides thus prepared were reduced by three different reduction methods which had been established to be convenient for syntheses of 4-hydroxylaminoquinoline 1-oxides, namely, by the use of phenylhydrazine[10, 11] sodium borohydride[12] or by catalytic hydrogenation.[13] The products isolated from the reduction procedures are given in the penultimate column of Table 1 and the carcinogenic effects of all the compounds are indicated in the second and the last column respectively. The results may be summarized as follows:

1. All the 4-hydroxylaminoquinoline 1-oxides prepared in the present study showed strong carcinogenic activity.
2. Not all of the 4-nitroquinoline 1-oxides which yielded carcinogenic 4-hydroxylamino 1-oxide derivatives proved to be carcinogenic under the conditions described above. Thus, 4,6- and 4,7-dinitroquinoline 1-oxides (Il and Im) did not induce tumours but their corresponding 4-hydroxylamino derivatives (IIl and IIm) were as strongly carcinogenic as other carcinogenic derivatives of the series.
3. None of the 4-nitroquinoline 1-oxides, which did not yield the corresponding 4-hydroxylamino 1-oxides by any of the reduction methods, proved to be carcinogenic.
4. 4-Hydroxylaminoquinoline free bases, one type of the reduction products [Table 1 (IV)], did not exert carcinogenic effects, neither did 4-nitroquinoline, 4-aminoquinoline 1-oxide and 4-aminoquinoline.

From these data, an interesting generality was brought to light concerning the carcinogenicity-structure relationship of this class of carcinogens, namely that the carcinogenic properties of 4-nitroquinoline 1-oxides depend on the production of 4-hydroxylaminoquinoline 1-oxide derivatives. This supports strongly the idea that 4-hydroxylaminoquinoline 1-oxides are biologically the proximate structures in the carcinogenic process of 4-nitroquinoline 1-oxides.

A further piece of evidence for this theory was obtained from the positive or negative carcinogenic properties of 3-halogeno-4-nitroquinoline 1-oxides: 3-chloro-4-nitroquinoline 1-oxide (Io), as well as other 3-substituted 4-nitroquinoline 1-oxides, were not carcinogenic, whereas the 3-bromo derivative (In) showed weak but definite carcinogenic effects. This can be explained by the fact that the latter compound

TABLE 1. CARCINOGENIC ACTIVITIES OF 4-NITRO AND 4-HYDROXYLAMINOQUINOLINE 1-OXIDE DERIVATIVES AND REDUCTION PRODUCTS



Compounds	Carcinogenic activity 4-NO_2	R	R_1	R_2	R_3	R_4	R_5	Reduction products*	Carcinogenic activity 4-NHOH
No Subst. (Ia)	+[1]	H	H	H	H	H	H	IIa, IIIa	+[4, 5, 6, 7]
2-Methyl (Ib)	+[2]	CH_3	H	H	H	H	H	IIb, IIIb	+
5-Methyl (Ic)	+	H	H	CH_3	H	H	H	IIc, IIIc	+
6-Methyl (Id)	+	H	H	H	CH_3	H	H	IId, IIId	+
7-Methyl (Ie)	+	H	H	H	H	CH_3	H	IIe, IIIe	+
8-Methyl (If)	+	H	H	H	H	H	CH_3	IIf, IVf	+
5-Chloro (Ig)	+	H	H	Cl	H	H	H	IIg, IIIg	+
6-Chloro (Ih)	+	H	H	H	Cl	H	H	IIh, IIIh	+
7-Chloro (Ii)	+	H	H	H	H	Cl	H	IIi, IIIi	+
6,7-Dichloro (Ij)	+	H	H	H	Cl	Cl	H	IIj, IIIj	+
6-Carboxyl (Ik)	+[24]	H	H	H	CO_2H	H	H	IIk, IIIk	+
6-Nitro (Il)	−[3]	H	H	H	NO_2	H	H	III,IIII,	+
7-Nitro (Im)	−	H	H	H	H	NO_2	H	IIm. IIIm	+
3-Bromo (In)	+	H	Br	H	H	H	H	IIa†	
3-Chloro (Io)	−	H	Cl	H	H	H	H	IIa‡	
5-Nitro (Ip)	−	H	H	NO_2	H	H	H	(Not identified yet)	
8-Nitro (Iq)	−	H	H	H	H	H	NO_2	IIIq	
3-Methyl (Ir)	−	H	CH_3	H	H	H	H	IIIr, IVr	
3-Methoxy (Is)	−	H	CH_3O	H	H	H	H	IIIs, IVs	
C-Diethylmalony (It)	−	H	CH $(CO_2Et)_2$	H	H	H	H	(Not examined)	

* All compounds gave 4-aminoquinoline derivatives as final reduction products in addition to the listed compounds.
† Dehalogenation took place, producing 4-hydroxylaminoquinoline 1-oxide.
‡ The reduction gave a very poor yield.

undergoes easy debromination to produce 4-hydroxylaminoquinoline 1-oxide itself (IIa), when submitted to usual reduction procedures (see Fig. 1).

In contrast, as it is well known, chlorine as substituent is in general more stable under reductive conditions: 3-chloro-4-nitroquinoline 1-oxide (Io) produced the dechlorinated compound (IIa) only in low yield as compared with the 3-bromo derivative.

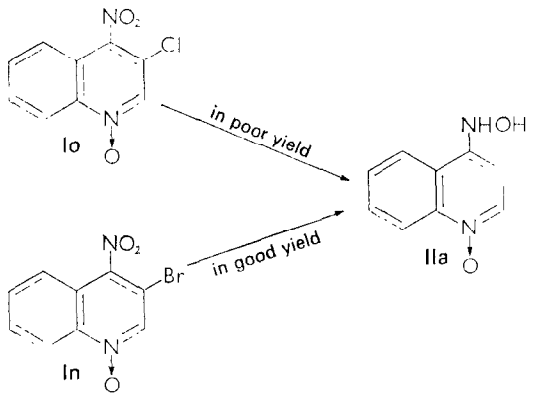


FIG. 1. Chemical reductions of 3-halogeno-4-nitroquinoline 1-oxides.

Another observation provided additional evidence for carcinogenicity-structure relations in this group of compounds: the 4-nitro and 4-hydroxylamino derivatives of 8-methylquinoline 1-oxide (If and IIf) were rather weakly carcinogenic compared with other active derivatives. This is due to the fact that loss of oxygen readily occurs in these derivatives to produce non-carcinogenic 8-methyl-4-hydroxylaminoquinoline (IVf); its 1-oxide (IIf) is so unstable that it could be isolated only in poor yield (see Fig. 2).

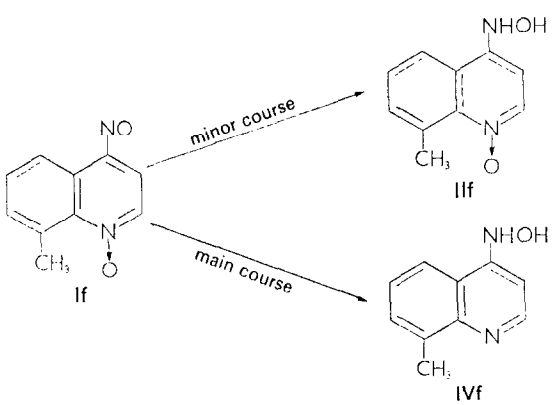


FIG. 2. Chemical reductions of 8-methyl-4-nitroquinoline 1-oxide.

With regard to the non-carcinogenic dinitroquinoline 1-oxides, (Il and Im), whose hydroxylamino derivatives (III) and IIm) are carcinogenic, it could be speculated that they are so reactive toward nucleophiles such as SH-containing compounds[14–16] that they may be easily removed before they are metabolized to the carcinogenic hydroxylamino derivatives.[17, 18] The rate of the nucleophilic replacement reaction of

4,6-dinitroquinoline 1-oxide (II) with thioglicolic acid was reported to be 50 times faster than that of 4-nitroquinoline 1-oxide.[16]

As preliminary experiments indicated, a ten-fold dosage of 4,6-dinitroquinoline 1-oxide did induce tumours in contrast to other derivatives, reported as non-carcinogenic in this paper, which did not show activity, even after a similar increase in the dose level. This seems to prove that the assumption made above is correct (details of these experiments will be reported later).

A brief comment should be offered here on the polarographic reduction potentials of these nitro compounds in connection with the stability of the structure of 4-hydroxylaminoquinoline 1-oxides. In Table 2, the half wave potentials of some

TABLE 2. THE POLAROGRAPHIC HALF WAVE REDUCTION POTENTIALS ($-E_{1/2}$ V) OF 4-NITROQUINOLINE 1-OXIDE DERIVATIVES

		$NO_2 \rightarrow NHOH$	$NHOH \rightarrow NH_2$	$\overset{\diagdown\!\diagdown}{N}\rightarrow O\rightarrow\overset{\diagdown\!\diagdown}{N}$	$\Delta E_{1/2}$*
Non-Substituted (Ia)	(+)†	0·160	0·975	1·245	0·8
2-Methyl (Ib)	(+)	0·180	1·020	1·300	0·8
6-Methyl (Id)	(+)	0·160	0·970	1·230	0·8
6,7-Dichloro (Ij)	(+)	0·130	0·810	1·045	0·7
8-Methyl (If)	(+)	~0·19	?‡	?	0·0 (in main part) 0·7 (in minor part)
3-Methyl (Ir)	(−)	0·275	?	?	0·1
3-Methoxy (Is)	(−)	0·260	?	?	0·0

* The potential difference between the first and the second waves.

† The carcinogenic activity.

‡ The assignments of these waves are still in question.

carcinogenic 4-nitroquinoline 1-oxide derivatives[9, 19] are given. These values indicate that the 4-hydroxylaminoquinoline 1-oxides are sufficiently stable to be isolated from the reduction media, since there exists a large difference in the potentials between the first wave (from nitro 1-oxide to hydroxylamino 1-oxide) and the second one. In contrast to this, non-carcinogenic 3-substituted derivatives gave rather complicated polarograms where the second waves appeared to be very close to the first. It means that 3-substituted 4-hydroxylaminoquinoline 1-oxides readily suffer further reductions. With 8-methyl derivatives, the deoxygenation occurred partly at almost the same potentials as the reduction of the nitro group. These data also indicate the poor stability of the probably carcinogenic 4-hydroxylamino 1-oxide structure in 3- and 8-substituted derivatives and support strongly the theory of the importance of this 4-hydroxylaminoquinoline 1-oxide structure, stabilized in the metabolic medium, for carcinogenicity.

In the case of 3-substituted 4-hydroxylaminoquinolines the possibility of their rearrangement into carcinogenic aminonaphthol structures might have to be taken into account; this ought to be further investigated.

All these discussion statements should remind one first of the recent observation by Sugimura,[20, 21] namely that 4-nitroquinoline 1-oxide was *in vivo* metabolically converted into 4-hydroxylaminoquinoline 1-oxide at the site of injection, and secondly

of a working hypothesis that this group of carcinogens, described and discussed in this paper, may belong to a larger group of active agents, such as N-hydroxylated arylamines (e.g. N-hydroxylaminoazobenzene) and acetylaminofluorenes. This hypothesis could be of value in correlating the carcinogenic mechanism of the quinoline derivatives with that of other classes of chemical carcinogens, and hence in the clarification of the cell cancerization mechanism itself.[4, 6, 22]

Finally, as additional experimental results, a list is given of nitroquinoline 1-oxides and analogues which proved to be non-carcinogenic, thus confirming the theory that for carcinogenecity the nitro group must be at position-4 of the quinoline 1-oxide nucleus, as already predicted by Nakahara:[2, 23] 4-nitroquinoline, 3-, 5-, 6-, 7-, and 8-mononitroquinoline 1-oxides, 4-nitropyridine 1-oxide, 3-hydroxylaminoquinoline 1-oxide, 3-methyl-4-hydroxylaminoquinoline, 8-methyl-4-hydroxylaminoquinoline, 4-hydroxylaminopyridine 1-oxide. Other position isomers of hydroxylamino derivatives are now under investigation.

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