

## 4-Nitrosoquinoline 1-Oxide

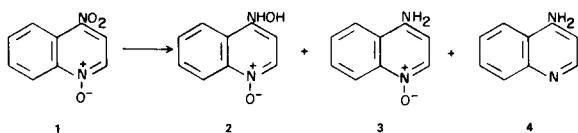
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The title compound has been synthesized by the oxidation of 4-hydroxylaminoquinoline 1-oxide with silver carbonate on celite. Some of its chemical properties have been studied and compared with those of 4-nitrosopyridine 1-oxide. Its mass spectrum and those of related compounds are discussed.

4-Nitroquinoline 1-oxide (**1**) (NQO) has been reported to have carcinostatic (1), carcinogenic (2-4), and mutagenic activity (5-7). Homogenates of liver, kidney, spleen, and lungs of rats are said (8) to be capable of converting **1** to 4-hydroxylaminoquinoline 1-oxide (**2**) and 4-aminoquinoline 1-oxide (**3**). Reduction of **1** by microorganisms give **2**, **3** and 4-aminoquinoline (**4**) (9-13). It has been suggested that the carcinogenicity of **1** may actually be due to **2** (14). When **1** and its derivatives were incubated with bacteriophage-T<sub>4</sub> only **2** was effective in inactivating the transforming activity of DNA (15).

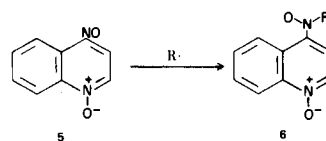


The mode of action of NQO (**1**) and of **2** has been the subject of much discussion. The nitro group in **1** may be displaced by thiol groups in a straightforward S<sub>N</sub>Ar reaction at the activated 4-position in NQO (16), and it has been proposed that the mutagenic and carcinogenic effects of **1** are due to a reaction between an SH group (in a protein) and C<sub>4</sub>, and some histochemical evidence for this has been adduced (17). The carcinogenic effect of **2** is not in accord with this hypothesis since the -NHOH group should not activate C<sub>4</sub> towards nucleophilic attack, nor is it a good leaving group in S<sub>N</sub>Ar reactions.

An alternative hypothesis is based on the finding that NQO (but not 4-nitrosopyridine 1-oxide which lacks the biological activity of NQO) intercalates between the plane of bases of DNA (18). NQO and its analogs give charge-transfer complexes of the π-π and n-π types with DNA, RNA, and purine and pyrimidine bases (19-21). More recently, the reactivity of **1** and **2** with free radicals has been discussed (22). The air oxidation of **2** was visualized as proceeding *via* radical intermediates to give 4-nitroso-

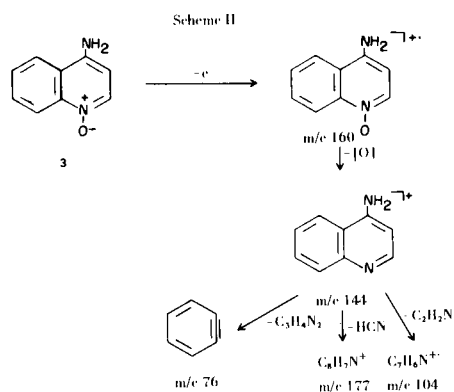
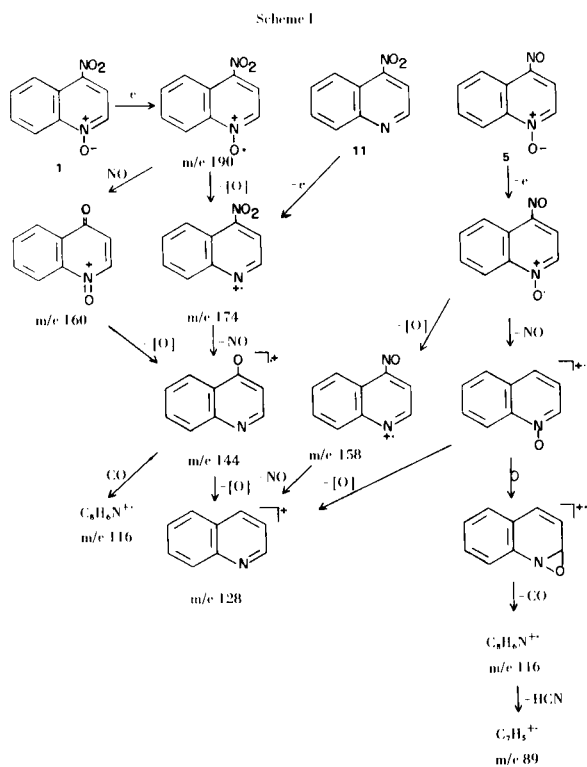
quinoline 1-oxide which, in turn, gave **1** or other products (22).

A possible bridge between **1** and **2** which would account for their carcinogenic activity would be that 4-nitrosoquinoline 1-oxide (**5**) was the actual biologically active species. If *in vivo* oxidation of the hydroxylamino group in **2** occurs to give **5** then this would provide a reasonably good activating and leaving group towards S<sub>N</sub>Ar attack. Support for such an 'alkylation' hypothesis comes from the observation that DNA and RNA form a complex with the carcinogen by the exposure of ascites cell to **2**, the fluorescent portion of the complex appearing to be covalently bound to DNA. No reaction was said to take place under a nitrogen atmosphere (*i.e.* in the absence of atmospheric oxygen) (23). An intriguing possibility could also be that the nitroso group in **5** traps free radical intermediates to form relatively stable nitroxide radicals (6) [see, for example, the reaction of C<sub>6</sub>F<sub>5</sub>NO with Ph· (24)] which could then react with an active site in DNA or RNA, perhaps following intercalation.



It appeared of considerable interest, therefore, to synthesize the as yet unknown 4-nitrosoquinoline 1-oxide (**5**) and to study its chemical and biological properties. We now report this synthesis and some of the reactions of **5**.

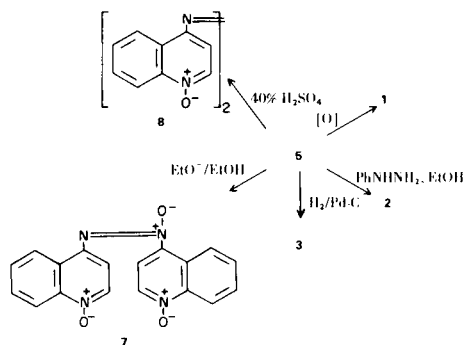
An initial attempt to oxidize **2** under the conditions used to transform 4-hydroxylaminopyridine 1-oxide to the 4-nitroso derivative, *i.e.* KMnO<sub>4</sub> in 20% sulfuric acid (25), was unsuccessful. On the other hand, treatment of **2** in methylene chloride with silver carbonate on celite (26) gave the desired nitroso compound **5**. Its structure was established by microanalysis and by its spectral properties



(see Experimental) and its mass spectrum is outlined below. Confirmation of the structure comes from its oxidation with permaleic acid to **1**. 4-Nitrosopyridine 1-oxide similarly gave 4-nitropyridine 1-oxide. Reduction of **5** with phenylhydrazine in ethanol gave **2**, while catalytic reduction over palladium-on-charcoal gave **3**.

Unlike 4-nitroquinoline 1-oxide (**27**), treatment of **5** with sodium ethoxide in ethanol did not give 4-ethoxyquinoline. Instead, some 4,4'-azoxydiquinoline 1,1'-dioxide (**7**) was isolated. Again, unlike **1** which gives 4-hydroxyquinoline 1-oxide on heating with 40% sulfuric acid at 130-140° (**28**), **5** gave 4,4'-azoxydiquinoline 1,1'-dioxide (**8**).

Nitrosoarenes are known to undergo 'ene' additions to olefins such as tetramethylethylene (**29**). Indeed, 4-nitro-



sopyridine 1-oxide and tetramethylethylene gave the expected hydroxylamine (**9**) together with 4,4'-azopyridine 1,1'-dioxide (**10**). A similar hydroxylamine could not be obtained from **5**, however.

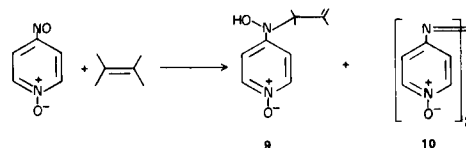


Table I  
Mass Spectra of **1**, **3**, **5**, and **11** (major ions only)

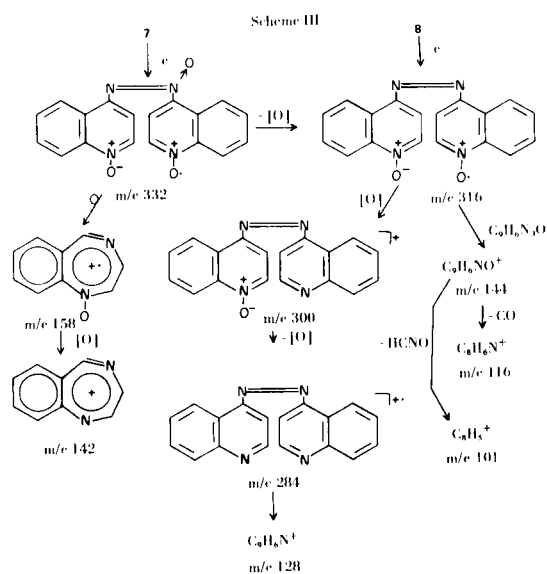
Ion m/e	Relative Abundances			
	<b>1</b>	<b>11</b>	<b>5</b>	<b>3</b>
190	100 (a)			
174	20	52 (a)	51 (a)	
160	55		3	15 (a)
158		21		
144				100
143	4		5	18
128	40	100	45	3
117	10	8	12	28
116	47	59	49	21
114	51		46	
104	18		5	23
102	12	12	10	7
101	41	96	41	5
90	16	5	20	20
89	66	17	100	28
88	31	5	25	6
77	31	6	11	11
76	22	12	15	23
75	45	66	43	18
74	31	33	25	12
63	39	20	35	22
62	29	17	25	11
52	9	12	14	28
51	39	53	38	23
50	30	35	30	20

(a) M<sup>+</sup>

Table II

Mass Spectra of **7** and **8** (main ions only)

Ion m/e	Relative Abundances	
	<b>8</b>	<b>7</b>
332		5 (a)
316	3 (a)	17
300	11	23
284	32	15
158		25
144	14	23
142		32
129	25	33
128	100	100
116	12	23
102	8	28
101	39	57
75	21	31
51	15	25

(a)  $M^+$ 

## Mass spectra.

The major fragment ions and relative abundances for 4-nitroquinoline 1-oxide (**1**), 4-nitroquinoline (**11**), 4-nitrosoquinoline 1-oxide (**5**) and 4-aminoquinoline 1-oxide (**3**) are collected in Table I. Possible fragmentation pathways are outlined in Scheme I for **1**, **11**, and **5**, and in Scheme II for **3**. The mass spectra of 4,4'-azodiquinoline 1,1'-dioxide (**8**) and 4,4'-azoxydiquinoline 1,1'-dioxide (**7**) are compiled in Table II and possible fragmentation patterns are given in Scheme III.

4-Nitrosoquinoline 1-oxide has been submitted to the American Health Foundation, New York, N. Y. for biological testing.

## EXPERIMENTAL

4-Nitrosoquinoline 1-Oxide (**5**).

Silver carbonate on celite reagent (**30**) (5.10 g.) was added to a stirred suspension of 4-hydroxylaminoquinoline 1-oxide (1.02 g.) in methylene chloride (220 ml.). After 1 hour at room temperature the green suspension was filtered through celite. The filtrate was dried (magnesium sulfate) and evaporated *in vacuo* at room temperature to give an orange solid (0.52 g.), m.p. 109° (dec.). It was dissolved in methylene chloride (15 ml.) and filtered through celite into petroleum ether (b.p. 30-60°) (20 ml.) to give 4-nitrosoquinoline 1-oxide as an orange solid (0.32 g.), m.p. 109° dec.; ir (potassium bromide): 3080 (w), 1584 (w), 1555 (w), 1509 (m), 1452 (s), 1425 (s), 1393 (s), 1366 (m), 1304 (s), 1243 (s), 1188 (s), 1140 (s), 1022 (w), 988 (s), 823 (m), 768 (m), 845  $cm^{-1}$  (w). The filtrate was concentrated *in vacuo* to give more nitroso compound (0.17 g.), m.p. 108 dec.

Anal. Calcd. for  $C_9H_6N_2O_2$ : C, 62.07; H, 3.47. Found: C, 62.27; H, 3.62.

Oxidation of 4-Nitrosoquinoline 1-Oxide with Permaleic Acid in Chloroform.

4-Nitrosoquinoline 1-oxide (0.15 g.) in chloroform (20 ml.) was treated with permaleic acid [from maleic anhydride (0.35 g.), 90% hydrogen peroxide (0.25 g.) and chloroform (8 ml.)] at 0° to give a brown solution which became pale yellow. After 15 hours at room temperature a saturated potassium carbonate solution was added until the solution had pH 4-6, the chloroform layer was separated and the acidic layer was extracted with chloroform (3 x 20 ml.). The dried (magnesium sulfate) chloroform extracts were evaporated *in vacuo* to give 4-nitroquinoline 1-oxide (0.12 g.), m.p. 152-153° (acetone) [lit. (31) 153-154°], identical with an authentic sample.

## Reduction of 4-Nitrosoquinoline 1-Oxide.

## (a) Hydrogen, Palladium/Charcoal.

4-Nitrosoquinoline 1-oxide (0.20 g.) in absolute ethanol (50 ml.) containing 10% palladium-on-charcoal (0.05 g.) was hydrogenated at 45 lb/in<sup>2</sup> at room temperature for 15 hours. The reaction mixture was filtered through celite and the yellow solution was concentrated *in vacuo* to give 4-aminoquinoline 1-oxide (0.18 g.), m.p. 265° dec., [lit. (31,32), 267°], identical with the compound obtained by the atmospheric pressure hydrogenation of 4-nitroquinoline 1-oxide.

## (b) With Phenylhydrazine.

4-Nitrosoquinoline 1-oxide (0.25 g.) in absolute ethanol (10 ml.) was treated with phenylhydrazine (1.0 ml.) to give a reddish suspension. This was heated under reflux for 1 hour, cooled, and filtered to yield 4-hydroxylaminoquinoline 1-oxide (0.22 g.), identical with an authentic sample.

## Reaction of 4-Nitrosoquinoline 1-Oxide with Sodium Methoxide in Methanol.

4-Nitrosoquinoline 1-oxide (0.22 g.) in methanol (15 ml.) at room temperature was treated with 10% sodium methoxide in methanol (5 ml.) to give 4,4'-azoxydiquinoline 1,1'-dioxide (0.10 g.), m.p. 298° dec., [lit. (22), > 300°]; ir (potassium bromide): 3600-3200 (br), 3050 (w), 1575 (m), 1561 (m), 1505 (m), 1441 (m), 1417 (m), 1392 (s), 1308 (s), 1238 (m), 1218 (m), 1140 (m), 1050 (m), 899 (w), 830 (w), 778 (m), and 758  $cm^{-1}$  (s).

Reaction of 4-Nitrosoquinoline 1-Oxide with 40% Sulfuric Acid.

4-Nitrosoquinoline 1-oxide (0.44 g.) in 40% sulfuric acid (5 ml.) was heated to 130-140° for 6 hours. The cooled reaction mixture was made basic with a saturated solution of sodium carbonate and then filtered to give 4,4'-azodiquinoline 1,1'-dioxide (0.08 g.), m.p. 258° (dec.) [lit. (22), 260°]; ir (potassium bromide): 3600-3100 (bs), 3050 (m), 1573 (s), 1535 (w), 1050 (s), 1450 (w), 1396 (s), 1302 (s), 1254 (s), 1173 (m), 1154 (m), 1134 (w), 1082 (w), 1042 (w), 845 (w), 777 (s), and 768 cm<sup>-1</sup> (s). The filtrate was acidified with acetic acid and filtered to give a brown solid (0.069 g.), m.p. 245°.

Oxidation of 4-Nitrosopyridine 1-Oxide with Permalleic Acid in Chloroform.

4-Nitrosopyridine 1-oxide (0.50 g.) in chloroform (20 ml.) was treated with permalleic acid [from maleic anhydride (0.70 g.) and 90% hydrogen peroxide (0.50 g.) in chloroform (15 ml.)] at 0°. The pale green solution turned yellow within 1 hour. After 15 hours at room temperature, a saturated potassium carbonate solution was added to pH 4-6, the chloroform solution was removed and the acidic solution was extracted with chloroform (3 x 20 ml.). The combined dried (magnesium sulfate) chloroform extracts were evaporated *in vacuo* to give 4-nitrosopyridine 1-oxide (0.45 g.), m.p. 153-155° (acetone) [lit. 33, 157-159°], (infrared spectrum identical to that of an authentic sample).

Reaction of 4-Nitrosopyridine 1-Oxide with Tetramethylethylene.

4-Nitrosopyridine 1-oxide (0.30 g.) in dichloromethane (30 ml.) was degassed and treated with tetramethylethylene (1 ml.) to give a deep green solution. After 20 hours under a dry nitrogen atmosphere the reaction mixture was concentrated *in vacuo* to give a dark green semisolid (0.46 g.) which was treated with dichloromethane (10 ml.) and filtered to give 4,4'-azoxypyridine 1,1'-dioxide (0.04 g.), m.p. 235° [lit. (25), 236-237°]. The filtrate was diluted with anhydrous ether to give *N*-[3-(2,3-dimethyl-1-butenyl)]-4-hydroxylaminopyridine 1-oxide as a yellow-green solid (0.27 g.); ir (potassium bromide): 3600-3300 (w), 3000-2900 (w), 1624 (m), 1485 (s), 1375 (w), 1330 (w), 1203 (s), 1175 (m), 1148 (s), 1045 (w), 1022 (w), 885 (w), 821 (s), 764 (w), and 645 cm<sup>-1</sup> (w); nmr  $\delta$  (deuteriochloroform): 1.45 (s, 6, 2, CH<sub>3</sub>), 1.80 (s, 3, CH<sub>3</sub>C=CH<sub>2</sub>), 5.03 (d, 2, CH<sub>2</sub>=C), 7.00 (d, 2, C<sub>3</sub>H, C<sub>5</sub>H), 7.85 (d, 2, C<sub>2</sub>H, C<sub>6</sub>H); mass spectrum: m/e 208 (M<sup>+</sup>, 0.5), 192 (M-O, 4), 126 (8), 124 (23), 110 (38), 94 (19), 83 (76), 78 (29), 67 (43), 39 (100).

Anal. Calcd. for C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>: C, 63.44; H, 7.74. Found: C, 63.36; H, 7.82.

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#### REFERENCES

- (1) S. Sakai, K. Minoda, G. Saito, S. Akagi, A. Ueno, and F. Fukuoka, *Gann*, **46**, 605 (1955).
- (2) H. Endo, M. Ishizawa, and T. Kamiya, *Nature*, **198**, 195 (1963).
- (3) A. Nakahara, *Arzneim-Forsch.*, **14**, 842 (1964); *Chem. Abstr.*, **61**, 13714e (1964).
- (4) J. Kamahora and T. Kakunaga, *Tampakushitsu Kakusan, Koso*, **15**, 458 (1970); *Chem. Abstr.*, **73**, 64236p (1970).
- (5) T. Okabayashi, *Chem. Pharm. Bull. (Tokyo)*, **10**, 1127 (1962); *Chem. Abstr.*, **60**, 8377c (1964).
- (6) T. Okabayashi, M. Ide, A. Yoshimoto, and M. Otsuho, *Chem. Pharm. Bull. (Tokyo)*, **13**, 610 (1965); *Chem. Abstr.*, **63**, 6045a (1965).
- (7) M. Ishizawa and H. Endo, *Mutat. Res.*, **9**, 134 (1970); *Chem. Abstr.*, **72**, 52151f (1970).
- (8) T. Sugimura, K. Okabe, and H. Endo, *Gann*, **56**, 489 (1965); *Chem. Abstr.*, **64**, 1190e (1966).
- (9) T. Okabayashi, A. Yoshimoto, and M. Ide, *Chem. Pharm. Bull. (Tokyo)*, **12**, 257 (1964); *Chem. Abstr.*, **61**, 1001f (1964).
- (10) N. Toriyama, *Nichidai Igaku Zasshi*, **24**, 423 (1965); *Chem. Abstr.*, **66**, 112465z (1967).
- (11) T. Kumakura, *Nichidai Igaku Zasshi*, **26**, 76 (1967); *Chem. Abstr.*, **70**, 64656w (1969).
- (12) T. Okabayashi and A. Yoshimoto, *Chem. Pharm. Bull. (Tokyo)*, **10**, 1221 (1962).
- (13) H. Endo and F. Kame, *Naturwiss.*, **50**, 525 (1963); *Chem. Abstr.*, **59**, 10594a (1963).
- (14) T. Ono, *Tampakushitsu Kakausan Koso*, **9**, 62 (1964); *Chem. Abstr.*, **64**, 2323d (1964).
- (15) M. Ishizawa and H. Endo, *Biochem. Pharmacol.*, **16**, 637 (1967); *Chem. Abstr.*, **66**, 113111t (1967).
- (16) H. Endo, *Gann*, **49**, 151 (1958).
- (17) Y. Hayashi, *Gann*, **50**, 219 (1959).
- (18) C. Nagata, N. Kodama, V. Tagashira, and A. Imamura, *Biopolymers*, **4**, 409 (1966).
- (19) M. Kodama, Y. Tagashira, and C. Nagata, *J. Biochem.*, **64**, 167 (1968).
- (20) T. Okano, A. Niitsuya, A. Takadate, and K. Uekama, *Gann*, **60**, 97 (1969); T. Okano, K. Uekama, and E. Taguchi, *ibid.*, **97**, 295 (1969); *Chem. Abstr.*, **71**, 77346r (1969).
- (21) S. Kawashima and M. Tomoeda, *Chem. Pharm. Bull. (Tokyo)*, **18**, 620 (1970).
- (22) T. Kosuge, H. Zenda, H. Sawanishi, and Y. Suzuki, Abstracts of Papers presented at 3rd International Congress of Heterocyclic Chemistry, Sendai, Japan, Aug. 1971, Abstract No. B-26-1, p. 203. T. Kosuge and M. Yokota, *Yakugaku Zasshi*, **85**, 69 (1965); *Chem. Abstr.*, **62**, 10405a (1965).
- (23) T. Takahashi, M. D. Anderson Symposium on Fundamental Cancer Research, Abstracts (1969); M. Tada, M. Tado, and T. Takahashi, *Biochem. Biophys. Research Commun.*, **29**, 469 (1967).
- (24) R. A. Abramovitch and S. R. Challand, *J. Heterocyclic Chem.*, **10**, 683 (1973).
- (25) F. Parisi, P. Bovina, and A. Quilico, *Gazz. Chim. Ital.*, **90**, 903 (1960).
- (26) J. A. Maasen and Th. J. de Boer, *Recl. Trav. Chim.*, **90**, 373 (1971).
- (27) E. Ochiai, M. O. Ishikawa, and Z. Sai, *Yakugaku Zasshi*, **63**, 280 (1943).
- (28) T. Okamoto, *Yakugaku Zasshi*, **71**, 297 (1951).
- (29) R. A. Abramovitch and S. R. Challand, *Chem. Commun.*, 1161 (1972); G. T. Knight, *ibid.*, 1016 (1970).
- (30) M. Fetizon and M. Golfier, *C. R. Acad. Sci. Ser. A, Paris*, **267**, 900 (1968).
- (31) E. Ochiai, "Aromatic Amine Oxides", Elsevier Publishing Company, New York, N.Y., 1967, p. 225.
- (32) S. Kamiya, *Chem. Pharm. Bull. (Tokyo)*, **10**, 471 (1962); *Chem. Abstr.*, **58**, 4545b (1963).
- (33) R. A. Abramovitch and E. M. Smith, "Pyridine and its Derivatives: A Supplement," (R. A. Abramovitch, Ed.), Vol. 2, Ch. IV. Interscience Publishers, New York, 1974, p. 97.