Oxidation of *N*-Naphthylhydroxylamines to Nitrosonaphthols by Air

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N-2-Naphthylhydroxylamine is oxidised by air in neutral or alkaline solution to 2-nitroso-1-naphthol, dibenzo[*a*,*h*]-phenazine, and 2,2'-azoxynaphthalene. *N*-1-Naphthylhydroxylamine is similarly oxidised to 4-nitroso-1-naphthol. 2-Nitrosonaphthalene is oxidised to 2-nitroso-1-naphthol in the presence of an aerated solution of *N*-1-naphthylhydroxylamine or of *N*-biphenyl-4-ylhydroxylamine. *N*-2-Naphthylhydroxylamine reacts with thiols in neutral solution.

SMALL quantities of 4- and 2-nitroso-1-naphthol are produced by aeration in neutral and alkaline solutions of the carcinogenic compounds *N*-1- and 2-naphthylhydroxylamine, respectively. The nitrosonaphthols may be formed by conversion of the naphthylhydroxylamines into nitroso-compounds, followed by hydroxylation by hydroxyl radicals, which are probably produced in arylhydroxylamine oxidations.[1] Thus, 2-nitrosonaphthalene was converted into 2-nitroso-1-naphthol in aerated solutions of *N*-1-naphthylhydroxylamine or of *N*-biphenyl-4-hydroxylamine. However, the related compounds 2-nitronaphthalene and 2-acetamidonaphthalene did not yield hydroxy-derivatives in aerated naphthylhydroxylamine solutions. The oxidation of arylhydroxylamines is reported to give principally azoxy-compounds [2] by a process involving radicals or radical ions.[3] E.s.r. studies [4] have shown that solutions of 1- and 2-naphthylhydroxylamine give free radicals with air. Hence it is possible that the formation of such species as $ArN\dot{O}H$ may be the initial step towards formation of the nitrosonaphthols, taking place either by oxidation of the hydroxyamino-group or by mutual oxidation and reduction in the mixed arylhydroxylamine–nitroso-compound systems. Mesomeric forms of the 1- or 2-$C_{10}H_7\dot{N}OH$ radicals could undergo oxidation at the 4- or 1-positions, respectively to give nitrosonaphthols. *N*-2-Naphthylhydroxylamine reacted with two thiols in air under neutral conditions to yield *S*-(2-amino-1-naphthyl) derivatives; this may likewise depend on the formation of a radical from the arylhydroxylamine. After reaction of the thiol at the 1-position there must be a reductive step involving unchanged thiol to give the amino-group. 2-Nitrosonaphthalene yields the same products with the thiols under neutral anaerobic conditions.[5] Here also the thiol may have a dual function, as a nucleophile and as a reducing agent. The reaction of *N*-2-naphthylhydroxylamine with thiols under neutral conditions differs from the acid-catalysed reaction [6] in that the latter probably involves an intermediate quinol imide ion, and the amino-group is formed by a prototropic change.[7] Similarly, the formation of the aminonaphthols and 2-amino-1-naphthyl dihydrogen phosphate under acidic conditions (this paper) is probably due to nucleophilic attack of water and phosphate ions. The yield of phosphoric ester obtained by treatment of *N*-2-naphthylhydroxylamine with orthophosphoric acid was small compared with that obtained by oxidising 2-naphthylamine with peracetic acid in acetone in the presence of orthophosphoric acid,[8] where the reaction may involve an azomethine intermediate.[9]

[1] H. Tanooka, Y. Kawazoe, and M. Araki, *Gann*, 1969, **60**, 537.
[2] I. T. Millar and H. D. Springall, 'Sidgwick's Organic Chemistry of Nitrogen,' 3rd edn., Oxford University Press, Oxford, 1966, p. 306.
[3] D. F. Bowman, J. L. Brokenshire, T. L. Gillan, and K. U. Ingold, *J. Amer. Chem. Soc.*, 1971, **93**, 6551 and references therein.
[4] C. Nagata, Y. Ioki, M. Inomata, and A. Imamura, *Gann*, 1969, **60**, 509.
[5] E. Boyland, P. L. Grover, and D. Manson, *Reports British Empire Cancer Campaign*, 1966, **44**, 2.
[6] E. Boyland, D. Manson, and R. Nery, *J. Chem. Soc.*, 1962, 606.
[7] H. E. Heller, E. D. Hughes, and C. K. Ingold, *Nature*, 1951, **168**, 909.
[8] E. Boyland and D. Manson, *J. Chem. Soc.*, 1957, 4689.
[9] D. Manson, *J. Chem. Soc.* (*C*), 1971, 1508.

The formation of dibenzo[*a*,*h*]phenazine from 2-naphthylhydroxylamine (and probably from the 1-isomer) is not unexpected, as this is an oxidation product of both 1- and 2-naphthylamine.[10,11]

The major product of the oxidation of *N*-biphenyl-4-ylhydroxylamine was 4,4′-azoxybiphenyl. A nitrosophenol was not detected, although aeration of a solution containing *N*-biphenyl-4-ylhydroxylamine and 2-nitrosonaphthalene gave 2-nitroso-1-naphthol.

Radical formation from 2-naphthylhydroxylamine may contribute to its conversion into *N*-acetyl-*S*-(2-amino-1-naphthyl)cysteine and into 2-amino-1-naphthol derivatives in rabbits,[12] a species in which these metabolites were either absent or formed to only a minor extent after dosing with 2-naphthylamine.[12,13] It is also of interest that ascorbic acid, an antioxidant, diminishes the uptake of 2-naphthylamine metabolites from the urinary bladder.[14]

EXPERIMENTAL

Starting materials and reference compounds related to 2-naphthylamine were synthesised as described by Boyland and Manson.[12] The remaining compounds were of commercial origin or obtained as given in the text. Chromatography was carried out on Whatman No. 1 paper with butan-1-ol–propan-1-ol–0·1N-ammonia (2 : 1 : 1) [solvent (a)] and on silica gel thin layers in light petroleum (b.p. 40—60°)–acetone [(b) 4 : 1 v/v and (c) 7 : 3 v/v]. The reagents employed were (A) cobalt chloride (1% w/v in water), (B) phosphomolybdic acid (20% w/v in ethanol), (C) titanous chloride (15% w/v in water), (D) *p*-dimethylaminocinnamaldehyde (1% w/v) in ethanol–6N-hydrochloric acid (1 : 1), (E) aqueous ammonia (sp. gr. 0·88), and (F) N-hydrochloric acid followed by sodium nitrite (0·5% w/v)

R_F Values and colour reactions on paper and thin-layer chromatograms

Compound	R_F Value with solvent * (a)	(b)	(c)	Colour and reagents *
2,2′-Azoxynaphthalene		0·70	0·80	Brownish red (B); red (D) after (C)
2-Nitroso-1 naphthol	0·62	0·13	0·20	Mauve (A); orange-red (D) after (C); yellow (E)
1-Nitroso-2-naphthol	0·70	0·42	0·42	Orange-red (A); orange-red (B); orange (D) after (C); yellow (E)
4-Nitroso-1-naphthol	0·83	0·55	0·70	No reaction with (A); grey-blue (B); orange-red (D) after (C); yellow (E)
1-Amino-2-naphthol	Oxidised	0·35	0·65	Grey-green (A); blue (B), orange-red (D); blue-green (F)
4-Amino-1-naphthol	Oxidised	0·21	0·42	Blue (A); blue (B); red (D); blue (F)

* For solvents and reagents, see text.

and hexylresorcinol (0·5% w/v) in 2N-sodium hydroxide. Reaction products were identical with authentic compounds with respect to m.p., i.r. spectrum, and chromatographic behaviour. R_F Values are given in the Table.

Oxidation of N-2-*Naphthylhydroxylamine*.—2-Naphthylhydroxylamine (1 g) in acetone (100 ml) and phosphate buffer (pH 7·0; 0·1M-KH_2PO_4–K_2HPO_4; 100 ml) was stirred at room temperature in an open vessel for 3 h. Acetone was added as required to maintain homogeneity. A precipitate was collected, dried, and extracted with hot acetone. Acetone-insoluble material was crystallised from acetic acid to give dibenzo[*a*,*h*]phenazine (30 mg), m.p. 280—282° (Found: C, 85·4; H, 4·5; N, 9·7. Calc. for $C_{20}H_{12}N_2$: C, 85·7; H, 4·3; N, 9·9%). A chromatographic system in which it did not tail was not found for this sparingly soluble compound, but it gave a characteristic greenish yellow fluorescence after treatment with dilute hydrochloric acid and an orange colour with reagent (B). The residue from evaporation of the acetone extract crystallised from ethanol to yield 2,2′-azoxynaphthalene (60 mg), m.p. 166—168° (Found: C, 80·6; H, 5·0; N, 9·4. Calc. for $C_{20}H_{14}N_2O$: C, 80·5; H, 4·7; N, 9·4%). The original aqueous acetone filtrate from the precipitate was evaporated *in vacuo* to 50 ml. A solid was collected which was mainly 2-naphthylamine but which contained 2-nitrosonaphthalene [12] and unidentified compounds. The filtrate was acidified (2N-hydrochloric acid) and extracted with ether. The residue from evaporation of the extract was extracted with hot water. The latter extract, when cooled, gave needles (10 mg) of 2-nitroso-1-naphthol, m.p. 162—164° (Found: C, 69·6; H, 4·2; N, 8·3. Calc. for $C_{10}H_7NO_2$: C, 69·4; H, 4·1; N, 8·1%).

A solution of 2-naphthylhydroxylamine under nitrogen remained unchanged for 3 h. Aeration of 2-nitrosonaphthalene, 2-nitronaphthalene, and 2-naphthylamine gave no nitrosonaphthol. Ethanol could be substituted for acetone as the reaction solvent. In alkali (Me_2CO–2N-NaOH), 9 mg of 2-nitroso-1-naphthol was obtained from 0·5 g of arylhydroxylamine. *N*-Acetyl-*N*-2-naphthylhydroxylamine [12] was unaffected by aeration.

The oxidation of 2-naphthylhydroxylamine (1 g) under neutral conditions was repeated in the presence of glutathione (1 g). After removal of products similar to those obtained in the first experiment the final aqueous solution was neutralised and evaporated to dryness *in vacuo*. The residue was separated into its constituents on Whatman 3MM paper in butan-1-ol–acetic acid–water (2 : 1 : 1), with *S*-(2-amino-1-naphthyl)glutathione [15] as marker. The product corresponding to this was eluted with aqueous methanol and the residue obtained from evaporation was triturated with ethanol–ether to give a solid (5 mg) identical (i.r. spectrum and chromatographic properties) with authentic *S*-(2-amino-1-naphthyl)glutathione. Other unidentified aromatic amino-derivatives and ninhydrin-positive compounds were present on the chromatograms. T.l.c. of the original reaction mixture showed coloured oxidation products [12] of 2-amino-1-naphthol. These were not present in the product from the reaction without glutathione. Glutathione reduced 2-nitroso-1-naphthol to the amino-compound. 2-Naphthylhydroxylamine and *N*-acetylcysteine gave material identical in chromatographic properties with *N*-acetyl-*S*-(2-amino-1-naphthyl)cysteine.[6,12]

For the reaction of 2-naphthylhydroxylamine under acidic conditions 2-naphthylhydroxylamine (1 g) in acetone (20 ml) and water (40 ml) containing orthophosphoric acid

[10] G. A. Swan and D. G. I. Felton, ' Phenazines,' Interscience, New York, 1957, p. 379.
[11] B. C. Saunders and J. Wodak, *Tetrahedron*, 1966, **22**, 505.
[12] E. Boyland and D. Manson, *Biochem. J.*, 1966, **101**, 84.
[13] F. Dewhurst, *Brit. J. Cancer*, 1963, **17**, 365.
[14] B. S. Alam, F. E. Jaramillo, J. U. Schlegel, and T. A. DeRouen, *Proc. Soc. Exp. Biol. Med.*, 1972, **141**, 1008.
[15] D. Manson, *Chem.-Biol. Interactions*, 1972, **5**, 47.

(1 ml) was stirred at room temperature for 30 min. The mixture gave a colour reaction indicating the presence of 2-amino-1-naphthol,[12] and t.l.c. showed oxidation products of this compound. Dibenzo[*a*,*h*]phenazine (80 mg) and 2,2′-azoxynaphthalene (15 mg) were isolated as before. The filtrate was evaporated at 50° and a solid (300 mg) was collected. T.l.c. of the solid showed about 12 products reacting with reagent (B). The filtrate contained 2-naphthylamine, 2-amino-1-naphthol, and 2-amino-1-naphthyl dihydrogen phosphate,[8] but not 2-nitroso-1-naphthol. In a separate experiment without active aeration, 2 g of arylhydroxylamine yielded 50 mg of the phosphoric ester. 2-Amino-1-naphthol and its phosphoric ester were both formed in the absence of air, although the formation of 2-amino-1-naphthol was more apparent in the aerated solution because of its oxidation to coloured products.

Oxidation of N-1-*Naphthylhydroxylamine*.—1-Naphthylhydroxylamine [16] (4 g) was treated at pH 7·0 in the same way as the 2-isomer. 4-Nitroso-1-naphthol was isolated (9 mg), m.p. 190—193° (from benzene) (Found: N, 8·2. Calc. for $C_{10}H_7NO_2$: N, 8·1%). 1-Nitroso-2-naphthol was not detected. The alkali-insoluble fraction was tarry but paper chromatography in solvent (a) showed material with a greenish yellow fluorescence after treatment with acid, possibly dibenzo[*a*,*h*]phenazine. After treatment with orthophosphoric acid, 1-naphthylhydroxylamine gave, with or without aeration, 4-amino-1-naphthol but not 1-amino-2-naphthol. The last-named compound is readily oxidised in air on chromatograms.

1-Nitroso-2-naphthol and 4-nitroso-1-naphthol were not found. A trace of slow-running diazotisable compound separated in solvent (a), possibly a phosphoric ester of the aminonaphthol.

Oxidation of 2-*Nitrosonaphthalene by Aeration of* N-1-*Naphthylhydroxylamine*.—1-Naphthylhydroxylamine (100 mg) and 2-nitrosonaphthalene (10 mg) were aerated together for 3 h at pH 7·0. Paper and thin-layer chromatography of the alkali-soluble fraction showed the presence of 4-nitroso-1-naphthol and 2-nitroso-1-naphthol.

Aeration of N-1- *and* 2-*Naphthylhydroxylamine in the Presence of* (i) 2-*Acetamidonaphthalene*, (ii) 2-*Nitronaphthalene, and* (iii) *Nitrosobenzene*.—Aeration at pH 7·0 gave 4-nitroso-1-naphthol or 2-nitroso-1-naphthol according to the arylhydroxylamine used but *N*-, 1- or 6-hydroxy-derivatives of (i), 2-nitro-1-naphthol from (ii), or *p*-nitrosophenol from (iii) were not detected.

Aeration of N-*Biphenyl*-4-*ylhydroxylamine*.—No evidence for the formation of a nitrosophenol was obtained after aeration under neutral or alkaline conditions but 4,4′-azoxybiphenyl was formed in about 60% yield. Aeration in the presence of 2-nitrosonaphthalene gave 2-nitroso-1-naphthol. In the presence of orthophosphoric acid, 4′- or 3-hydroxy-4-aminobiphenyl did not form and no evidence for a phosphoric ester was found.

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