Tetrahedron, 1966, Vol. 22, pp. 505 to 508. Pergamon Press Ltd. Printed in Northern Ireland

STUDIES IN PEROXIDASE ACTION—XIII*

THE OXIDATION OF 2-NAPHTHYLAMINE

B. C. Saunders and J. Wodak
University Chemical Laboratories, Lensfield Rd., Cambridge

(*Received* 21 *June* 1965; *in revised form* 29 *September* 1965)

Abstract—The oxidation of 2-naphthylamine by the system peroxidase plus hydrogen peroxide has been examined. The reaction is complex, but two oxidation products have been isolated, namely dibenzo(a,h)-phenazine and a compound that appears to be 5-(2-naphthylamino-)-dibenzo[a,h]-phenazine.

The oxidation of 2-naphthylamine is of particular interest because it is carcinogenic. Parke and Williams, reviewing the subject,[1] pointed out that in susceptible animals (e.g. dogs) the product of the metabolism of 2-naphthylamine is 2-amino-1-naphthol, whereas in the rabbit, which is resistant, it is 6-amino-2-naphthol. Haddow[2] discusses bladder tumours in man following exposure to 2-naphthylamine and emphasizes concern over the heavy casualties caused among chemical workers who have handled this substance.

It therefore seemed important that the action of peroxidase (a widely distributed enzyme) on 2-naphthylamine should be investigated.

The fact that 2-naphthylamine is oxidized by the peroxidase system has previously been noted,[3] but the products were not investigated. There is also reference in the literature to another enzymic oxidation (possibly by a peroxidase in rice plant roots) of this compound,[4] but again without analysis of the products. In this preliminary investigation of the oxidation using peroxidase and hydrogen peroxide, two compounds have been isolated in low yield; the identity of one has been established, and a structure has been proposed for the second.

A difficulty associated with the enzymic oxidation was that of obtaining a suitable solution. Oxidations of amines by the peroxidase system are usually carried out using a 2% solution of the amine in aqueous acetic acid at pH 4·5–5·0,[5] to which hydrogen peroxide and peroxidase are added. Under these conditions, however, the solubility of 2-naphthylamine is less than 0·5 g/l., and thus only small quantities could be oxidized. An alternative method was to oxidize a well-stirred suspension, but this led to the recovery of much starting material, which hindered the separation of the products. In some reactions the medium contained up to 15% of ethanol which

---

* Paper XII. B. C. Saunders and B. P. Stark. *Tetrahedron* **4**, 169 (1958).

[1] D. V. Parke and R. T. Williams, *Ann. Reports* **55**, 381 (1958).

[2] A. Haddow, *New Scientist*, **430**, 348 (1965).

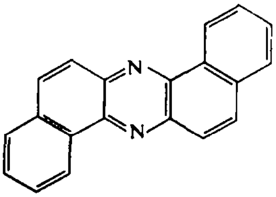
[3] F. G. Mann and B. C. Saunders, *Practical Organic Chemistry* (4th Edition), p. 519. Longmans, Green (1960).

[4] S. Matsunaka, *J. Biochem., Tokyo* **47**, 820 (1960).

[5] B. C. Saunders, *Royal Inst. of Chem., Lectures, Monographs and Reports* No. 1 (1957); B. C. Saunders, A. G. Holmes-Siedle and B. P. Stark, *Peroxidase, The Properties and uses of a versatile enzyme and some related catalysts* p. 10. Butterworths (1964).

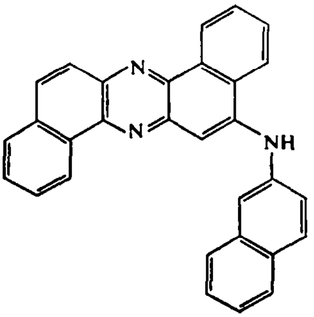
improved the solubility of the amine, but had no apparent effect on the nature of the products.

Oxidation of suspensions or of dilute solutions of 2-naphthylamine gave the colour changes recorded by Mann and Saunders:[3] on addition of the enzyme and peroxide solutions, an immediate brown colouration with a pale blue fluorescence was observed. Later a brown solid separated, part of which was insoluble in chloroform, and was polymeric; the remainder was chromatographed on alumina, giving four readily eluted bands. The fourth band was unchanged starting material, and the second band gave only a trace of a red substance, so attention was focussed on the remaining two bands. The first of these gave a fluorescent yellow solution, which, on evaporation, gave a yellow solid in 5–10% yield; and proved to be dibenzo[a,h]phenazine (I).

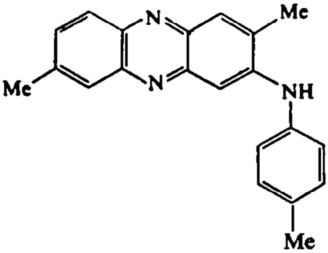


(I)

The third band gave a fluorescent red solution which on evaporation and sublimation gave a trace of yellow solid, to which the fluorescence was due, and a purple compound in ca. 3% yield. Mol. wt. determinations indicated the presence of three amine residues in the purple compound. The IR spectrum was relatively simple; the only peaks above 1500 cm^{-1} could be assigned to a secondary amine grouping, and ring C=C and C=N absorptions. It is provisionally proposed that the structure of this new substance is 5-(2-naphthylamino-)-dibenzo[a,h]phenazine, (II).



(II)



(III)

If this suggestion is correct then II is the analogue of III, which we had previously obtained from the oxidation of *p*-toluidine with Fenton's reagent.[6] We therefore investigated the reaction between 2-naphthylamine and Fenton's reagent (hydrogen peroxide + Fe^{2+}) and indeed obtained II in 9% yield identical in every respect with the enzyme product, together with 2% of dibenzo[a,h]phenazine.

An attempt was made to confirm the proposed structure further by an unambiguous synthesis. 5-Methyldibenzo[a,h]phenazine was prepared, with the object of converting

[6] D. G. H. Daniels, F. T. Naylor and B. C. Saunders, *J. Chem. Soc.* 3433 (1951).

the methyl group to carboxyl and thence to the amino-group; the 5-aminodibenzo-[a,h]phenazine thus obtained should then react with 2-naphthylamine hydrochloride[5] to give the desired product. However, no oxidizing agent was found which would give the acid in significant yield, and the synthesis was abandoned. In spite of this, the proposed structure still seems logical particularly as work on the oxidation of aromatic amines with the peroxidase system reveals that a characteristic product is obtained by a 1,4-condensation of two amine residues with a third.[5] Additional ring closure would then be a consequence of the reactivity of the 1-position of the naphthalene nucleus. It is noteworthy that II has not been reported before from studies of the oxidation of 2-naphthylamine with various inorganic and free radical oxidizing agents, although dibenzo[a,h]phenazine is quite a common product.[7]

EXPERIMENTAL

Oxidation of 2-naphthylamine by peroxidase system

2-Naphthylamine (100 mg) was dissolved in water (500 ml) and acetic acid (5 ml) and the pH adjusted to 4·5. Hydrogen peroxide (20 volume, 2 ml) and peroxidase solution (the extract prepared according to the directions given by Mann and Saunders,[3] 5 ml) were added. On addition of the latter there was an immediate colour change, the solution becoming brown with a pale blue fluorescence. After some time a brown solid separated. Further quantities of peroxide and enzyme were added at 2 hr intervals until the total volume of the former was 10 ml.

A variation on the above method was to dissolve the amine (0·5 g) in acetic acid (10 ml), and dilute with warm water (1000 ml), the pH again being adjusted to 4·5. As the solution cooled down to room temp, the excess of amine formed a fine suspension, and the mixture was stirred throughout the reaction.

For some reactions purified enzyme was used. This was obtained (a gift from the late Professor D. Keilin) in solutions having a PN of 142. These were diluted ×50, and the diluted solutions used 1 ml at a time.

Examination of product. The crude product from several oxidations was bulked together before the mixture was worked up. The solid material was filtered off, and the filtrate extracted with $CHCl_3$. The resulting aqueous solution had absorption maxima at 321 and 500 mμ. The solid material was also extracted with $CHCl_3$, in which about half the product was soluble. The insoluble brown solid was discarded, and the extract, after concentration, was chromatographed on alumina using a mixture of $CHCl_3$ and CCl_4 (b.p. 66–68°) as eluant. Four bands which were easily eluted were obtained, together with a number of strongly adsorbed dark bands which were not examined. On elution, these gave, in order, (i) a fluorescent yellow solution, with a characteristic absorption spectrum, (ii) a light red solution, (iii) a dark red fluorescent solution, and (iv) a dark brown solution which proved to be mainly starting material. The examination of eluate fractions (i) and (iii) is described below.

Examination of fraction (i). The fluorescent yellow solution, on evaporation, gave a yellow solid with an almost featureless IR spectrum. When sublimed, it had m.p. 282°. As dibenzo[a,h]phenazine was considered to be a likely product from this reaction, a sample was synthesized by the method of Maffei and Pietra;[8] chromatographic purification followed by sublimation gave light yellow crystals, m.p. 282°, lit. 282°. (Found: C, 85·3; H, 4·4; N, 9·8. Calc. for $C_{20}H_{12}N_2$: C, 85·7; H, 4·3; N, 10·0%.)

Mixed m.p. with enzymic product: 282°. Visible UV absorption spectrum: (EtOH solution)

---

[7] H. J. Teuber and G. Jellinek, *Chem. Ber.* **87**, 1841 (1954); G. R. Clemo and E. C. Dawson, *J. Chem. Soc.* 1114 (1939); K. H. Pausacker, *Ibid.* 1989 (1953); E. Boyland, D. Manson and P. Sims, *Ibid.* 3623 (1953); G. B. Barlin, K. H. Pausacker and N. V. Riggs, *Ibid.* 3122 (1954); K. H. Pausacker and J. G. Scroggie, *Ibid.* 4003 (1954); S. F. D. Orr, P. Sims and D. Manson, *Ibid.* 1337 (1956); A. P. Terent'ev and Ya. D. Mogilyanskii, *Dokl. Akad. Nauk SSSR* **103**, 91 (1955); *Chem. Abstr.* **50**, 4807 (1956).

[8] S. Maffei and S. Pietra, *Boll. sci. Fac. Chim. ind. Bologna* **8**, 104 (1950).

λ_{max}: 219, 243, 263, 293, 298, 372, 392, 405, 416 mμ. $\log_{10} \varepsilon$: 4·63, 4·55, 4·18, 4·84, 4·82, 3·84, 4·25, 4·14, 4·46.

Examination of fraction (iii). Evaporation of the third fraction followed by sublimation gave two components: a trace of a yellow crystalline material, m.p. 185°, whose alcoholic solution showed a strong blue fluorescence, with absorption maxima at 291, 353, 362, 372, 382, 392 and 410 mμ, and a fibrous red solid, apparently not crystalline, m.p. 244° (dec). The IR spectrum of the latter had peaks at (cm^{-1}): ca. 3300 (secondary amine N—H) and 1660–1500 (six peaks, aromatic C=C and C=N). This compound [*5-(2-naphthylamino)-dibenzo*[a,h]*phenazine*] had identical m.p. and IR spectrum with the compound obtained by oxidizing 2-naphthylamine with Fenton's reagent (see below). (Found: C, 85·5; H, 4·9; N, 9·6. Mol. wt. (Rast), 425. $C_{30}H_{19}N_3$ requires: C, 85·5; H, 4·5; N, 10·0% Mol. wt. 421.)

Absorption spectrum ($CHCl_3$ solution) λ_{max} 486 mμ, $\log_{10} \varepsilon$, 3·81.

Oxidation of 2-naphthylamine with Fenton's reagent

2-Naphthylamine (10 g) was dissolved in a mixture of glacial acetic acid (50 ml) and water (1000 ml) by heating (80°). To the hot solution were added H_2O_2 (20 vol, 2 ml) and fresh $FeSO_4$aq (5%, 2 ml). There was an immediate colouration and the solution rapidly became opaque. Further quantities of peroxide were added every 15 min, and more $FeSO_4$ every ½ hr. No further heat was applied to the reaction mixture, but when the addition of reagents ceased, after 4 hr, it was still warm (30°).

After cooling, the product was filtered off and examined by the procedure used for the peroxidase product. The same four products were obtained, the major constituents being as before, (i) dibenzo[a,h]phenazine, 200 mg., 2%; (iii) 5-(2-naphthylamino)-dibenzo[a,h]phenazine, 440 mg., 9%; (ii) and (iv), mere traces.

Attempted synthesis of 5-(2-naphthylamino)-dibenzo[a,h]*phenazine*

5-Methyldibenzo[a,h]phenazine was prepared by a method similar to that used for the parent compound itself, using 4-methyl-α-tetralone instead of α-tetralone itself. *5-Methyldibenzo*[a,h]*phenazine* was obtained as a yellow crystalline solid, m.p. 233°, purified by sublimation. (Found: C, 85·5; H, 4·9; N, 9·65. $C_{21}H_{14}N_2$ requires: C, 85·7; H, 4·8; N, 9·5%.)

No product was obtained when this compound was oxidized with $KMnO_4$ in 25% H_2SO_4 on a waterbath, or by heating under reflux with $K_2Cr_2O_7$ in 40% acid, and only starting material was recovered using $KMnO_4$ in 10% NaOHaq or $K_2Cr_2O_7$ and dil H_2SO_4, both being heated under reflux. A product was obtained, however, using chromic acid in 90% acetic acid, but only in 10% yield. The IR spectrum of this product showed three peaks of approximately equal intensity in the carbonyl absorption region (1760, 1720 and 1680 cm^{-1}), of which only one (the last) corresponded to that of an aromatic carboxylic acid.

One of us (J. W.) is grateful to the Department of Scientific and Industrial Research and the Trustees of the Countess Eleanor Peel Trust for maintenance grants.