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On the Oxidation of Benzidine and *o*-Dianisidine with Hydrogen Peroxide and Acetylcholine in Alkaline Solution

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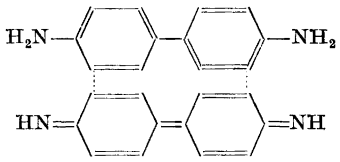
The oxidation of benzidine and *o*-dianisidine with hydrogen peroxide and acetylcholine in alkaline solution at 20°C has been investigated. From benzidine one single oxidation product has been isolated and shown to be identical with synthesized 4-amino-4′-nitrobiphenyl. From the oxidation of *o*-dianisidine three compounds have been isolated. One of these is identical with the previously known *bis* (3,3′-dimethoxy-4-amino)azobiphenyl, whereas the other two have been identified as the previously undescribed compounds, 3,3′-dimethoxy-4-amino-4′-nitrobiphenyl and 3,3′-dimethoxy-4-amino-4′-nitrosobiphenyl.

Benzidine and many of its derivatives have been extensively used as sensitive reagents for several types of oxidizing agents. As examples may serve the detection of lead peroxide [1], phosphomolybdic acid [2], silicomolybdic acid [3], peroxy disulphuric acid [4], peroxide [5] and aldehydes [6].

The methods described for the detection of these oxidants have a common characteristic feature, inasmuch as the oxidation is invariably carried out in a neutral or faintly acidic solution. One of the coloured compounds formed when benzidine is oxidized under these conditions, is the meriquinoid oxidation product "benzidine blue" (I).



(I)

The oxidation of benzidine and its derivatives in alkaline solution, seems to have attracted less interest. In this work we intended to isolate and identify the oxidation products arising from the oxidation with hydrogen peroxide of benzidine and *o*-dianisidine in alkaline solution.

When highly purified reagents are used, it is known that at a moderate temperature (20°C), benzidine and *o*-dianisidine are very sparingly oxidized by hydrogen peroxide in alkaline solution. However, impurities of various kinds are able to promote the reaction. At least when heavy metal ions are involved, the effect is in all probability a catalytic one.

Among organic compounds, acid halogenides, acid anhydrides and esters all greatly accelerate oxidation with alkaline hydrogen peroxide. These agents, however, are consumed in certain stoichiometric proportions, so that their action is not strictly catalytic.

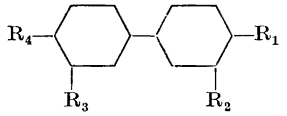
This paper is exclusively devoted to the isolation and identification of the coloured products formed in the oxidation reaction of benzidine and *o*-dianisidine with hydrogen peroxide in alkaline solution, and with acetylcholine as "activator".

REACTION CONDITIONS

An alkaline solution of benzidine or *o*-dianisidine is obtained by using acetone as mixing solvent. The oxidation was carried out at a pH approximately equal to 11. As alkaline buffer solution, dipotassium hydrogen phosphate-potassium hydroxide was used, the reaction being carried out at room temperature.

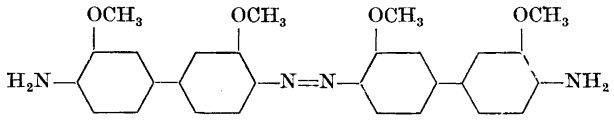
Under these conditions the solution containing benzidine or *o*-dianisidine, alkaline buffer solution, acetone and hydrogen peroxide, stayed colourless for at least one hour, provided that the most carefully purified reagents were used. When acetylcholine was added to this solution in the form of the bromide or the chloride, a rapidly developing colour could be detected after a few seconds. The colour which at first appeared yellow, quickly changed through orange and red, and finally attained a deep red tone when *o*-dianisidine was used, whereas in the case of benzidine the solution remained intensely orange-yellow even after half an hour.

The oxidation products were isolated by chromatographic methods. 4-Amino-4′-nitrobiphenyl was isolated from benzidine as the only oxidation product: II, $R_1 = NO_2$, $R_2 = R_3 = H$, $R_4 = NH_2$)



(II)

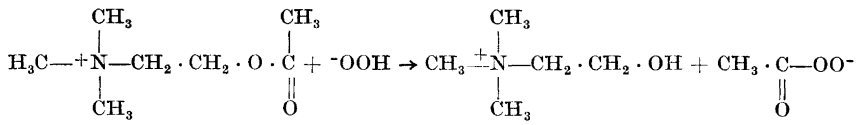
From the oxidation of dianisidine 3 substances were isolated, 3,3′-dimethoxy-4-amino-4′-nitrobiphenyl (II, $R_1 = NO_2$, $R_2 = R_3 = OCH_3$, $R_4 = NH_2$), 3,3′-dimethoxy-4-amino-4′-nitrosobiphenyl (II, $R_1 = NO$, $R_2 = R_3 = OCH_3$, $R_4 = NH_2$), and *bis*(3,3′-dimethoxy-4-amino)azobiphenyl(III).



(III)

DISCUSSION

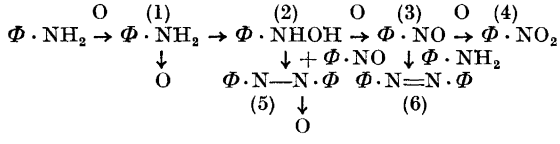
The ability of acetylcholine to enhance the oxidizing action of hydrogen peroxide in alkaline solution, is due to the formation of peracetic acid [7] in a nucleophilic reaction:



In alkaline solution peracetic acid is rapidly decomposed, yielding acetic acid and active oxygen. By volumetric measurement of the oxygen liberation from an alkaline solution of hydrogen peroxide after adding acetylcholine, it was clearly demonstrated that 1 mole oxygen is evolved from every 2 moles of acetylcholine. Peracetic acid has furthermore been qualitatively detected under these conditions [7].

The chemical compositions of the isolated oxidation products of benzidine and *o*-dianisidine supply further evidence in favour of the assumption that oxidation is brought about by oxygen uptake of one of the amino groups.

The series of oxidation products of *o*-dianisidine described in this paper, bears a striking resemblance to another series found earlier by Bamberger [8], who examined the oxidation products of aniline with per sulphuric acid. Bamberger showed that the following mechanism applies in the case of aniline oxidation:



Of these products (1) and (2) are very unstable, and Bamberger has not succeeded in isolating them. The nitroso compound (3) on the other hand, can be isolated provided sufficient care is taken, and products (4), (5) and (6) are relatively easily isolated.

In our case the isolated oxidation products of *o*-dianisidine correspond to the products (3), (4) and (6) in Bambergers aniline series. We have not succeeded in isolating the azoxy compound corresponding to product (5) in Bambergers series, but this might be due to deficiencies in the experimental methods used. However in our opinion the absence of this compound may be due to an even stronger tendency of the hydroxylamine derivative of our series to further oxidation, than is shown by phenylhydroxylamine. In our experiments we have not isolated any product in which both of the two amino groups have been oxidized.

Comparing the product obtained from benzidine with those from *o*-dianisidine, it is to be noted that in the former case only one oxidation product is isolated, *viz.* the nitro compound corresponding to (4) in Bambergers series. We have no reason to believe that the nitroso compound and the azo compound should be present here in detectable quantities and yet have escaped our attention as long as they did not do so in the *o*-dianisidine series. In our opinion, therefore, the two methoxy groups in the *ortho* positions to the amino

groups of *o*-dianisidine may play an important role by stabilizing the nitroso compound of this series, thus retarding further oxidation to the nitro compound.

EXPERIMENTAL

Our experiments were all carried out in the following way, with only slight modifications specified in each particular case.

500 mg *o*-dianisidine hydrochloride (or benzidine respectively) was dissolved in 50 ml water. To this solution 100 ml buffer solution (1.65 g K_2HPO_4 and 1.0 g KOH per 100 ml H_2O) was added, followed by 200 ml acetone, 20 ml 2 % H_2O_2 solution and finally 500 mg acetylcholine bromide in 10 ml H_2O.

The reaction was allowed to proceed for half an hour, and the mixture was filtered, water was added to the filtrate, and extraction effected using approx. 100 ml of chloroform. The extract was washed twice with 0.1 N HCl, then twice with water, dried with Na_2SO_4 and evaporated to dryness *in vacuo* at room temperature. The residue was dissolved in 5 ml benzene and transferred to a chromatographic coloumn packed with activated alumina.

In the case of benzidine, the chromatogram was developed and the product eluted with 50 % benzene and 50 % ether. The eluate was again evaporated *in vacuo*, and the residue dissolved in benzene and rechromatographed twice using 90 % benzene and 10 % ether.

The oxidation products of *o*-dianisidine were first chromatographed using 50 % benzene and 50 % ether as developer, and three coloured zones became visible. The first and the second zone from the bottom (a yellow (I) and a brown (II) zone) were not clearly separated. Above them a red zone (III), relatively well separated from the others, could be collected. The red zone was purified by twice repeated chromatography from benzene, and the two lower zones were separated and purified by chromatographing two or three times from benzene.

Care was taken not to leave the compound from the brown zone (II) on the alumina for any length of time, since purification has to be carried out as fast as possible because this nitroso compound is relatively unstable. Even when purified, passage of the compound through the column leaves behind a brown colouring because of polymerization, so that no sharply defined zone is obtained.

In our experiments we have observed that we can increase the amount of nitroso compound by using acetylcholine chloride instead of the bromide, and at the same time the reaction is shortened to 10—15 min. The best yields of the azo compound are obtained when the unwashed chloroform extract is allowed to stand overnight before proceeding.

Oxidation product from benzidine, *4-amino-4'-nitrobiphenyl:* M. p. 195°C (uncorr.)
Molecular weight 225 (Rast), theory 212.2
Ultraviolet spectrum: abs. max. at 370 mμ (in ethanol).
Infrared spectrum: abs. max. in mμ (NaBr-disk):
NH_2-frequency: 2.98 (s), 2.90 (d)
NO_2- » 6.28 (s), 7.45 (d)

4-amino-4'-nitrobiphenyl was synthesized according to Fittig [9]; m.p. 195°C (uncorr.). Mixed melting point with our substance showed no depression. Ultraviolet and infrared spectrum were identical.

Oxidation products from *o*-dianisidine, *3,3'-dimethoxy-4-amino-4'-nitrobiphenyl (zone 1):*

M. p. 101°C (uncorr.). (Found: C 61.53; H 5.19; N 9.97. Calc. for $C_{14}H_{14}O_4N_2$ (274.27): C 61.30; H 5.15; N 10.20).
Ultraviolet spectrum: abs.max. at 370 mμ (in ethanol).
Infrared spectrum: abs.max. in mμ (NaBr-disk)
NH_2-frequency: 2.98 (s) 2.90 (d)
NO_2- » 6.25 (s) 7.40 (d)

3.3'-dimethoxy-4-amino-4'-nitrosobiphenyl (zone II):
M. p. 148° (uncorr.)
Test for nitroso group according to Feigel [10] is positive.

The substance condenses easily with compounds containing active metylene groups. The substance is easily hydrolyzed by cold alkali [11].

Ultraviolet spectrum: abs. max. at 445 mμ (in ethanol).
Infrared spectrum: abs. max. in mμ (NaBr-disk):
NH_2-frequency: 3.0 (s) 2.92 (d)
NO^- » 7.18 6.28 (?)
The nitroso compound was oxidized with H_2O_2 and acetylcholine to give 3,3'-dimethoxy-4-amino-4'-nitrobiphenyl (see above).

bis (3 3'-dimethoxy-4-amino) azobiphenyl (zone III).
M. p. 240° C (uncorr.).
Ultraviolet spectrum: abs. max. at 435 mμ (in ethanol).
Infrared spectrum: abs. max. in mμ (NaBr-disk):
NH_2-frequency: 2.98 (s), 2.91 (d)
-N=N- — » 6.23

Bis (3 3'-dimethoxy-4-amino) azobiphenyl was synthesized according to Moir [12]. The two substances showed identical melting points identical ultraviolet and infrared absorption spectra. Mixed melting point showed no depression.

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