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Electroörganic Preparations

V. On the Polarographic Determination of Phenols after Treatment with Nitrous Acid

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It is shown that the polarographic wave obtained in the wellknown determination of morphine and other *p*-substituted phenolic compounds after treatment with nitrous acid is due to a nitro derivative rather than a nitroso derivative.

A number of *p*-substituted phenolic compounds and derivatives thereof which are not, themselves, reducible at the dropping mercury electrode can be determined polarographically after treatment with nitrous acid; such compounds are, *e. g.*, morphine [1], codeine [2], cephaeline [3], estrone [4,5], and estrogenic compounds [4,5]. The reaction has generally been formulated as a nitrosation of the phenolic compound to an *o*-nitroso derivative.

The reaction of phenolic compounds with nitrous acid [6] or nitric acid [7] has been applied to the colorimetric determination of phenols and the coloured compound obtained in alkaline solution has been regarded as a salt of the quinone oxime.

Ochiai and Nakamura [8], however, reported that the compound obtained by Wieland and Kapelmeier [9] on treatment of morphine with N_2O_3 was not 2-nitrosomorphine but 2-nitromorphine. Most recently Baggesgaard Rasmussen and Boll [10] have published results which strongly favour the formulation of the compound as 2-nitromorphine.

The formation of a nitro derivative is in agreement with the results of Philpot and Small [11] who examined the action of nitrous acid on *p*-cresol and tyrosine and found, that unless the nitroso compound was trapped as the stable copper derivative, very little of it accumulated, since it was rapidly transformed further. The products of further reaction was found to depend on the concentration of nitrous acid and phenols and on the temperature. At high concentrations of nitrous acid the formation of nitro compounds was suggested, whereas diazo derivatives were formed at somewhat lower concentrations of nitrous acid.

The purpose of the present investigation was to determine the nature of the derivative responsible for the polarographic wave obtained from morphine after treatment with nitrous acid. The investigation consists of a polarographic examination of "2-nitrosomorphine" and related compounds, and a preparative reduction at controlled potential of some of these compounds.

POLAROGRAPHIC INVESTIGATION

In Table 1 are listed the half-wave potentials at a few pH-values of 4-hydroxy-3-nitro-toluene, 4-hydroxy-3-nitroso-toluene, 3-hydroxy-6-nitro-toluene, 3-hydroxy-6-nitroso-toluene, "2-nitroso-morphine", prepared according to Wieland and Kappelmeier [9], and 4-nitro-estrone.

Table 1. Half-wave potentials (*vs.* S.C.E.) at different pH-values of "2-nitroso-morphine" and some reference compounds.

	4-Hydroxy-3-nitro-toluene	4-Hydroxy-3-nitroso-toluene	3-Hydroxy-6-nitro-toluene	3-Hydroxy-6-nitroso-toluene	"2-Nitroso-morphine"	4-Nitro-estrone
pH	0.78	1.32	0.81	0.96	0.80	1.02
$E_{\frac{1}{2}}$	0.14_5	$\begin{cases}+0.22\\-0.09\end{cases}$	0.23	+0.20	0.14	0.15
pH	3.00	3.05	2.93	3.00	3.13	3.63
$E_{\frac{1}{2}}$	0.25_5	$\begin{cases}+0.09_5\\-0.35\end{cases}$	0.39_5	+0.04	0.24	0.31_5
pH	4.65	4.62	4.65	4.58	4.92	5.15
$E_{\frac{1}{2}}$	0.35	$\begin{cases}-0.00_5\\-0.9\end{cases}$	0.51	−0.08	0.34	0.40_5
pH	6.88	6.88	6.90	6.84	7.07	7.44
$E_{\frac{1}{2}}$	0.45	0.15	0.60	0.21	0.52_5	0.52
pH	8.86	9.01	8.89	8.89	9.13	10.0
$E_{\frac{1}{2}}$	0.58	0.28_5	0.79	0.34	0.59_5	0.63
pH	13	13	13	13	13	13
$E_{\frac{1}{2}}$	0.80_5	0.54_5	$\begin{cases}0.99\\1.40\end{cases}$	0.59	0.82_5	0.86_5

The polarographic behaviour of 4-hydroxy-3-nitro-toluene in the medium used was similar to that of *o*-nitrophenol found by Stocesova [12]. The wave obtained in acid and alkaline solution corresponded to a 6 electron reduction, which was verified by a controlled potential reduction at pH 1.5 and 10. This is in disagreement with the results of Astle *et al.*[13] who from the wave height in acid solution reported that 4 electrons were consumed in acid solution. In a pH interval around pH 5 the height of the wave corresponded to a consumption of less than 6 electrons (between 4 and 5) and the main wave was mostly succeeded by a drawn out wave. The sum of the waves nearly corresponded to a 6 electron reduction. The reason for this behaviour, according to Stocesova, is that the *o*-hydroxyphenylhydroxylamine which is primarily formed by a 4 electron reduction is converted to the quinoneimine, which is reduced by a 2

electron reduction. The conversion is acid-base catalyzed, and in a pH-interval around 5 the height of the wave is determined by the rate of the conversion.

The 4-hydroxy-3-nitroso-toluene showed at pH-values higher than 8 a single wave corresponding to a 4 electron reduction, which was verified by a controlled potential reduction at pH 10. In acid solution 2 waves occurred. The height of the first wave corresponded to between 2.5 and 3 electrons and the second one to about 1 electron. The second wave became more and more drawn out at higher pH values and disappeared almost at pH 6. The half-wave potential of the first wave was in the whole pH region 0.25—0.3 V more positive than that of the nitro compound.

The polarographic behaviour of 3-hydroxy-6-nitro-toluene was somewhat different from that of 4-hydroxy-3-nitro-toluene. The compound showed a single wave at pH-values lower than 7 corresponding to a 6 electron reduction, which was verified by a controlled potential reduction at pH 4. At pH 7 the wave was somewhat drawn out and in a small interval about pH 8 the wave split up in 2 waves. From pH 9 to 10 a single 6 electron wave again was found, but at higher pH values the wave split up in two waves. The height of these waves were dependent upon pH, but the nature of the phenomenon was not investigated further.

3-Hydroxy-6-nitroso-toluene was reduced in a single step at pH values higher than 7 and the wave corresponded to a 4 electron reduction, which was verified by a controlled potential reduction at pH 10. In an interval around pH 5 the height of the main wave decreased somewhat and a drawn out, second wave appeared.

"2-Nitroso-morphine" was reduced polarographically very similarly to 4-hydroxy-3-nitro-toluene. At pH-values higher than 7 a single wave was obtained, whereas the main wave decreased somewhat in a pH interval about pH 4 and a second wave appeared, which became more and more drawn out at higher pH-values. The difference between the half-wave potential of 4-hydroxy-3-nitro-toluene and that of "2-nitroso-morphine" was, except at pH about 6, less than 0.02 V.

4-Nitro-estrone was reduced in a single 6 electron wave in the whole pH region, except in a small interval about pH 5. The diminishing of the wave was not very pronounced.

Another series of experiments were performed in which morphine, estrone and a few of their derivatives together with some model compounds were treated with nitrous acid under the conditions used by Baggesgaard Rasmussen *et al.*[1] for the determination of morphine (N HCl, method A) and those described by Gry[4] for the determination of estrone (acetate buffer pH 3.2, method B). After treatment with nitrous acid excess of potassium hydroxide (method A) or ammonium hydroxide (method B) was added. In some cases no nitrite was present. The results are summarized in Table 2, where only the half-wave potentials of the main wave(s) is given.

Morphine treated with nitrite (method A) and "2-nitroso-morphine" without nitrite behaved very similarly; morphine only differed from "2-nitroso-morphine" by a small second wave succeeding the main wave. The height of this wave was about 5 % of the height of the main wave; the half-wave potential was about 1.0 V. The nature of this wave was not investigated further.

Table 2. Half-wave potentials of the main wave(s) obtained on treatment of some phenolic compounds with nitrous acid.

Compound	Method	Nitrite	pH	$E_{\frac{1}{2}}$ V
Morphine	A	+	14	0.84
"2-Nitroso-morphine"	A	–	14	0.84
p-Cresol	A	+	14	0.82_5
p-Cresol	B	+	10	0.76
4-Hydroxy-3-nitroso-toluene	A	–	14	0.56
4-Hydroxy-3-nitroso-toluene	B	–	10	0.36
4-Hydroxy-3-nitroso-toluene	B	+	10	0.75
4-Hydroxy-3-nitro-toluene	A	–	14	0.81
4-Hydroxy-3-nitro-toluene	B	–	10	0.75_5
4-Hydroxy-3-amino-toluene	B	+	10	0.54 1.02
Estrone	B	+	10	0.65
4-Nitro-estrone	B	–	10	0.64_5
m-Cresol	B	+	10	0.44 0.81 1.00
3-Hydroxy-6-nitroso-toluene	B	–	10	0.43
3-Hydroxy-6-nitroso-toluene	B	+	10	0.99
3-Hydroxy-6-nitro-toluene	B	–	10	0.98_5
2,4-Dinitro-phenol	B	–	10	0.69 0.97

Treatment (method A) of *p*-cresol with nitrite yielded a main wave very much like that of 4-hydroxy-3-nitro-toluene under the same conditions without nitrite, but different from the wave of 4-hydroxy-3-nitroso-toluene without nitrite. The main wave of the "nitrosated" *p*-cresol was preceded by a small wave (wave height about 5 % of the height of the main wave) with a half-wave potential about 0.47 V. This wave might have been due to the nitroso derivative or the "diazo-derivative" [11].

Estrone with nitrite in acetate buffer (method B) produced a wave similar to that of 4-nitro-estrone without nitrite.

p-Cresol nitrosated (method B) with potassium nitrite or amyl nitrite, 4-hydroxy-3-nitroso-toluene with nitrite and 4-hydroxy-3-nitro-toluene without nitrite showed very similar waves, which were different from the wave of 4-hydroxy-3-nitroso-toluene under the same conditions without nitrite.

m-Cresol on the same treatment produced 3 waves, a small wave at the same potential as did 3-hydroxy-6-nitroso-toluene without nitrite and a main wave at the same potential as that of 3-hydroxy-6-nitro-toluene. The nature of the third wave at a potential between the other two waves is not known. 3-Hydroxy-6-nitroso-toluene produced on treatment with nitrous acid a wave at the same potential as that of the nitro derivative; only a small part remained as the nitroso compound.

The spectra of the solutions used for polarography (method B) showed, that other compounds besides the nitro derivative were formed from *p*-cresol on treatment with nitrous acid. 4-Hydroxy-3-nitro-toluene showed under

these conditions (pH 10) an absorption maximum about 437 mμ, 4-hydroxy-3-nitroso-toluene (without nitrite) a maximum about 454 mμ and the maximum of the "nitrosated" *p*-cresol varied around 420 mμ.

PREPARATIVE REDUCTIONS

4-Hydroxy-3-nitro-toluene was reduced at pH 1.5 and 10. At pH 1.5 a small second wave was found on the polarograms taken in the strong solutions (5×10^{-2} M) used for the preparative reductions indicating that the rate of the conversion of the primarily formed phenylhydroxylamine derivative was not sufficiently high to secure a single 6 electron wave under these conditions. As the reduction, however, consumed 5.9 ± 0.1 electrons the intermediate formed product must be transformed in the bulk of the solution to a species reducible at the potential used, which is considerably more positive than the half-wave potential of the second wave. Some coloured compounds were formed during the reduction, but apparently not enough to influence the electron consumption to any appreciable degree. At pH 10 6.0 electrons were consumed, and in both cases the expected 4-hydroxy-3-amino-toluene was isolated. The same compound was obtained by the reduction at pH 10 of 4-hydroxy-3-nitroso-toluene. The reduction required 4 electrons. 3-Hydroxy-6-nitro-toluene was reduced at pH 4 consuming 6 electrons and 3-hydroxy-6-nitroso-toluene at pH 10 requiring 4 electrons for the reduction.

"2-Nitroso-morphine" was reduced at pH 10 at —0.85 V. The reduction consumed 5.95 ± 0.1 electrons and an aromatic amine could be detected in the reaction mixture.

DISCUSSION

The polarographic data presented in Tables 1 and 2 substantiate the assumption that *p*-substituted phenols on treatment with excess of nitrous acid form mononitro derivatives as a main product. The reaction, however, is not clean and various amounts of other phenolic derivatives are formed. The importance of these side reactions depends among other things on the structure of the phenolic compound; tyrosine, for instance, forms much more material of unidentified structure than does *p*-cresol under the same conditions. A special method, therefore, has to be developed for a given phenol, if a polarographic determination of the compound is to be worked out.

The question whether the nitro compounds are formed by a direct attack of N_2O_4 on the phenol or by an oxidation of a primarily formed nitroso derivative cannot be answered conclusively, although it can be pointed out, that both 4-hydroxy-3-nitroso-toluene and 3-hydroxy-6-nitroso-toluene on treatment with nitrous acid yield, more or less completely, the nitro derivative.

The investigation of Baggesgaard Rasmussen *et al.*[1] and the data presented here point to the conclusion that the wave obtained on treatment of morphine with nitrous acid is due to the compound formulated by Wieland and Kappelmeier as "2-nitroso-morphine". The reduction wave of this compound is found to represent a 6 electron reduction under conditions, where 4-hydroxy-

3-nitro-toluene consumed 6 electrons and 4-hydroxy-3-nitroso-toluene was reduced by 4 electrons per molecule. As it seems very unlikely that other parts of the molecule than the substituent in the 2 position are involved in the reduction the presented data are taken as strong evidence in favour of the formulation [8,10] of the compound obtained by Wieland *et al.* as 2-nitro-morphine.

The result of the present investigation is that the main polarographic wave obtained on treatment of morphine [1], estrone [4], or *p*-cresol with excess of nitrous acid is due to the reduction of the mononitro derivative rather than the mononitroso derivative.

EXPERIMENTAL

The polarograph was a recording polarograph Radiometer Type PO 3a. The capillary delivered 3.24 mg of mercury per second at a corrected mercury height of 48.5 cm; the drop time 5.82 sec (H_2O, open circuit); the capillary constant $m^{2/3}t^{1/6} = 2.94$ $mg^{2/3}sec^{-1/2}$.

The reference electrode, the buffer solutions and the apparatus used for the controlled potential reductions were the same as described in Part I [14] and III [15]. The medium used in the polarographic investigation was aqueous N KCl, except for the nitroestrone, where the solution contained 40 % alcohol.

4-Hydroxy-3-nitroso-toluene was made according to Philpot and Small [11]. It was recrystallized from petrol ether (b. p. 60°—80°) by cooling in a carbon dioxide-acetone cooling bath, m. p. 56°—58°.

"2-Nitroso-morphine" was prepared according to Wieland *et al.*[9] and the potassium salt purified after recrystallization from alcohol by chromatography [10]. The potassium salt was converted to the hydrochloride. (Found: C 49.75; H 5.94; N 6.90; Cl 9.38. Calc. for $C_{17}H_{19}N_2O_5$, 2 ½ H_2O: C 49.62; H 5.89; N 6.81; Cl 8.63.)

Reduction of "2-nitroso-morphine". 400 mg of the hydrochloride of "2-nitroso-morphine" was dissolved in 120 ml N NaCl containing 5 % sodium bicarbonate and 5 % sodium carbonate, which was deaerated and purified by electrolysis beforehand. The compound was reduced at −0.85 V (*vs* S.C.E.). The reduction was followed polarographically. It required 5.9_5 electrons. After completion of the reduction a small part of the reaction mixture was made acid and treated with sodium nitrite. After destruction of excess of nitrous acid with ammonium sulfamate, naphthylethylenediamine was added, and a violet colour slowly developed indicating the presence of an aromatic amine in the reduction medium. 2-Amino-morphine was found by Wieland *et al.* to couple slowly after diazotation. Attempts to isolate the diamine were not succesful.

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