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Electrochemical Study of the Hallucinogen (\pm) -1-(2,5-Dimethoxy-4-nitrophenoxy)-2-aminopropane

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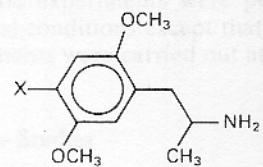
An electrochemical study of the hallucinogen (\pm) -1-(2,5-dimethoxy-4-nitrophenoxy)-2-aminopropane, which differs from similar compounds in that a hydrophilic nitro group replaces hydrophobic substituents on the 4-position of the benzene ring, was carried out using a solvent - buffer system containing pyridine - formic acid and tetramethylammonium chloride solution.

Polarographically and voltammetrically the drug behaves as other structurally related aromatic nitro derivatives, the nitro group being reduced to the hydroxylamine in a single, well defined irreversible, diffusion-controlled and pH-dependent wave. The $E_{1/2}$ versus pH and i_{lim} versus pH relationships were examined and cyclic voltammograms were recorded at different pH values and at different scan rates in order to elucidate the reduction mechanism and to identify relatively unstable intermediate species.

The results of this study suggest that a significant relationship can be established between the electrochemical behaviour of any compound and its molecular - electronic structure, and between molecular structure and pharmaceutical or biological activity, as confirmed recently by quantitative structure - activity relationship studies.

Keywords: (\pm) -1-(2,5-Dimethoxy-4-nitrophenoxy)-2-aminopropane; nitro derivatives; polarography; cyclic voltammetry

1-(2,5-Dimethoxyphenyl)-2-aminopropane, like other structurally related derivatives containing different substituent groups, has proved to be a particularly active hallucinogenic agent. It was found that the introduction of a hydrophobic atom or group such as Br, CH₃ or C₂H₅ at the 4-position in the original molecule provided compounds with both higher hallucinogenic potency and with different effects on the qualitative nature of the drug experienced by humans.¹



1-(2,5-Dimethoxyphenyl)-2-aminopropane derivatives

(\pm) -1-(2,5-Dimethoxy-4-nitrophenoxy)-2-aminopropane (DON; racemic form) is one of many drugs investigated for their hallucinogenic properties. As the nitro group is clearly hydrophilic, and as the drug shows a low octanol - water partition coefficient compared with the 4-CH₃ (DOM) and 4-Br (DOB) analogues,² and as it is not so effective as these on the serotonin receptors of the sheep's umbilical artery,³ it has been suggested that this compound might not be a very potent hallucinogen in humans. However, Coutts and Malicky,⁴ in a rat behavioural assay, demonstrated that DON shows a similar potency to the 4-CH₃ analogue. Recently, quantitative structure - activity relationship (QSAR) studies on indole-alkylamines⁵ and phenylalkylamines⁶ were carried out and the results suggested that DON should be a very potent hallucinogen at low doses, comparable to DOB and DOM. Subsequently, these theoretical and pharmacological presumptions were confirmed experimentally⁷ using racemic DON which

was synthesised as the nitrate salt following a previously reported procedure⁸ and then characterised spectroscopically.⁹

It is reasonable to assume that the biological activity of any type of drug depends on its molecular structure, and the spectrum of activity and selective toxicity can be explained by the fact that the receptor possesses systems with which the drug can interact. It is also evident that the key to the activity of these compounds is the position and nature of the substituents. On the other hand, the nature and position of the substituents in structurally related compounds are not only decisive with respect to the biological or pharmaceutical activity but also in the electrochemical behaviour of such compounds. Hence a significant correlation may be established between QSAR studies and electrochemistry.

Great attention has been paid to electrochemical studies of the nitro derivatives, which are frequently prescribed for therapeutic purposes and widely used. The pattern of action is a consequence of the ability of these drugs to accept electrons, which makes the reduction of the nitro group possible, these being compounds metabolised *in vivo* to the corresponding amines via nitroso and hydroxylamine intermediates. In this context, electrochemistry permits the investigation of the reduction mechanism and the establishment of its resemblance with the metabolic pathways for the biological degradation of these nitro derivatives.

The aim of this work was to study the effect of the nature and position of the substituents on the reduction of the nitro group in DON and to establish a comparison with the electrochemical behaviour of the nitro-containing heterocyclics and other aromatic nitro derivatives of biological importance when a dropping mercury or solid electrode is used. Further, a rapid polarographic method for the determination of DON is proposed that is applicable in overdose cases where a relatively high concentration of the free drug remains in a particular body fluid, provided that chloramphenicol is absent.

A survey of the literature indicated that no electrochemical study on this drug has been reported to date.

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Experimental

Reagents

All chemicals used were of analytical-reagent grade unless stated otherwise.

DON was synthesised following a recent procedure.⁸ Stock solutions (10^{-2} – 10^{-3} M) were prepared by dissolving the appropriate amount of DON in freshly distilled, de-ionised water. Gelatin solution (0.5%) was used as a maximum suppressor. The supporting electrolyte contained 0.1 M tetramethylammonium chloride (TMAC) solution and pyridine-formic acid buffer and was prepared as described previously.¹⁰

Apparatus

Polarographic assays were performed using a Polariter PO₄ instrument (Radiometer, Copenhagen). A dropping mercury electrode was used as a working electrode and a saturated calomel electrode (SCE) as the reference electrode.

Cyclic voltammetric experiments were carried out using a CV-27 voltammograph (Bioanalytical Systems, Lafayette, IN, USA). A three-electrode assembly was used for all measurements. A glassy carbon electrode was employed as a working electrode, an SCE as the reference and a platinum coil as the counter electrode.

An Orion Research Digital Ion-Analyzer 701 with glass and saturated calomel electrodes was used for pH determinations.

General Procedure

Aliquots of the stock solutions were diluted in 10 ml of the supporting electrolyte, 1 ml of gelatin solution was added and the solution was purged with oxygen-free nitrogen for 10 min, then subjected to polarography in the d.c. mode.

Cyclic voltammetric experiments were performed under identical experimental conditions except that gelatin was not added. All measurements were carried out at $25 \pm 1^\circ\text{C}$.

Diffusion Dependence Studies

Polarograms of DON solutions (10^{-4} – 10^{-5} M) were recorded at various heights of the mercury column (h) ranging from 30 to 65 cm. The corresponding diffusion currents (i_d) obtained were plotted against $h^{1/2}$ and the values of $i_d h^{-1/2}$ were calculated.

The diffusion-controlled character of the current and its dependence on the depolariser concentration were also established.

Cyclic voltammograms of DON were recorded at scan rates between 0.02 and 0.6 V s⁻¹ and the current function $i_p/Cv^{1/2}$ was determined.

pH Dependence Studies

The effect of pH on the half-wave potentials and diffusion-limited current for DON at a concentration of 0.289 mM was studied over the pH range 1–14. The best defined and differentiated waves for analytical purposes were obtained at pH < 6. The corresponding cyclic voltammograms were recorded under identical conditions.

Calibration Graphs

Aliquots of the pure drug dissolved in distilled, de-ionised water were diluted in the supporting electrolyte and polarographed. The standard additions method was employed in all instances and the results were used in the preparation of the limiting current versus concentration graphs.

Results and Discussion

Polarograms of DON recorded in the above supporting electrolyte (pH 4.5) exhibit one well defined wave with a half-wave potential of -0.39 V, corresponding to the reduction of the nitro group.

The nature of the wave was found to be diffusion-controlled, as shown by the linear dependence of the limiting current on $h^{1/2}$ and on the depolariser concentration. The relationship between limiting current and concentration of DON was found to be linear over a wide range of concentrations (Table 1). Above 10^{-3} M the value of i_d/C was not constant, probably owing to adsorption of the drug at the mercury electrode. However, the data in Table 1 are perfectly reproducible, and imply that DON can be determined by d.c. polarography over the entire concentration range from about 5.88×10^{-5} M to the limit of detection of 1.24×10^{-6} M.

Under the same experimental conditions DON behaves polarographically as chloramphenicol and other structurally related compounds, which are reduced to the corresponding hydroxylamine in a four-electron reduction process. When equimolar solutions of DON and chloramphenicol are subjected to polarography, the ratio of the wave heights is 0.93 ± 0.05 , indicating a similar electrode process; this corresponds to the well known four-electron reduction of the nitro group to hydroxylamine^{10–15} (Fig. 1).

Reduction in a four-electron process to give hydroxylamine as the final product depends on the nature and position of the substituents. The absence of π electrons in the substituent at the 1-position and the weak donor properties of the methoxy groups located at the 2- and 5-positions on the benzene ring inhibit the homogeneous and very fast chemical reaction coupled to the electrode process, avoiding the transformation of the hydroxylamine into the highly reducible intermediate imine and the subsequent reduction of the latter to the primary amine.

On the other hand, it has been observed that aromatic nitro compounds having strong π -donor substituents, such as

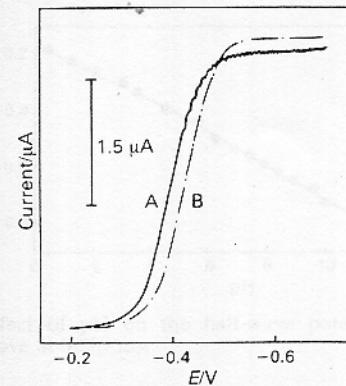


Fig. 1. Polarographic reduction waves of DON and chloramphenicol at 0.289 mM. (A) DON: $E_{1/2}$, -0.39 V and i_d , 3.38 μA . (B) Chloramphenicol: $E_{1/2}$, -0.42 V and i_d , 3.60 μA . V_i , -0.20 V

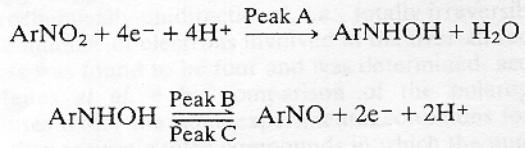
Table 1. Effect of DON concentration on i_d values

Concentration (C) 10^{-6} M	$i_d/\mu\text{A}$	i_d/C
1.24	0.015	12.09
2.44	0.029	11.88
3.61	0.042	11.63
12.4	0.146	11.77
24.4	0.284	11.64
36.1	0.423	11.72
58.8	0.687	11.68
124	1.450	11.69
361	4.190	11.61
588	6.821	11.60

p-nitrophenol, *p*-nitroaniline^{10,16,17} and nitrofuran derivatives,^{15,18} are reduced directly to the primary amine via the unstable imine intermediate.

Cyclic voltammograms of DON were recorded under identical conditions in order to identify the intermediate species. Fig. 2 shows a typical cyclic voltammogram of DON at pH 3. On the first cathodic scan initiated at 0.0 V, one wave (A) is observed with $E_p = -0.58$ V, corresponding to the reduction of the nitro group to hydroxylamine. On reversal of the potential scan at *ca.* -0.95 V, no reverse current is indicated for the oxidation of the reduction product. Instead, a new reversible redox couple (B, C) is observed with an anodic $E_p + 0.16$ V on the second and all subsequent scans. Wave clipping, that is, reversal of the scan direction before peak A, causes peaks B and C to disappear, indicating that this redox couple arises from the hydroxylamine previously formed in the reduction of the nitro group (peak A).

The shape of the cyclic voltammogram of DON is essentially the same as that obtained in the reduction of nitrobenzene¹⁹ and chloramphenicol^{14,15} and is in keeping with the following known electrode reactions previously described for these compounds^{14,15,19}:



It must be stressed that the current function $i_p/Cv^{1/2}$ was fairly constant (Table 2) and independent of the scan rate, which implies that the electrode reaction is a simple irreversible electron transfer and that it is neither preceded nor followed by a slow chemical reaction.²⁰

Effect of pH

The effect of pH on the reduction was examined by polarography and cyclic voltammetry.

As the pH is gradually increased, the half-wave potential, $E_{1/2}$, of the DON wave shifts towards more negative values.

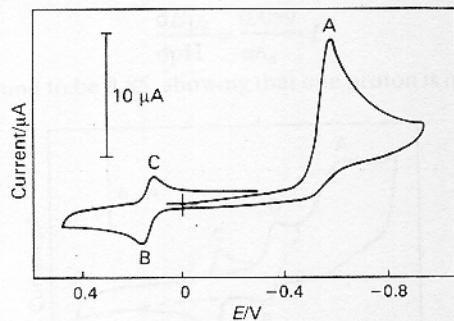


Fig. 2. Cyclic voltammogram of DON at 0.219 mM, pH 3; glassy carbon electrode; scan rate, 0.1 V s⁻¹. Peak A corresponds to the reduction of the nitro group to hydroxylamine; peaks B and C denote the hydroxylamine - nitroso reversible redox couple

Table 2. Voltammetric data for reduction of 0.426 mM DON in pyridine - formic acid with TMAC as supporting electrolyte

Scan rate (v)/ V s ⁻¹	i_p/c / μA	$i_p/(Cv^{1/2})$
0.020	8.8	146
0.050	13.6	143
0.100	20.0	148
0.200	26.4	139
0.300	32.4	139
0.400	36.8	137
0.500	41.2	137
0.600	44.4	135

The $E_{1/2}$ versus pH graph (Fig. 3) shows three linear portions with a slope of -48.4 mV at $0 < \text{pH} < 4.7$ and -61.2 mV at $4.7 < \text{pH} < 11$, whereas it tends almost to zero at $\text{pH} > 11$. The first break on the graph is a direct consequence of the potential-dependent changes in the structure of the mercury-solution interface. Because electron transfer is a heterogeneous process, it can be affected substantially, sometimes even profoundly, by changes in the composition of the interfacial region. A similar behaviour for nitrobenzene reduction in aqueous solution has been reported.²¹

The other break observed in the $E_{1/2}$ versus pH graph was examined together with the effect of pH on the diffusion-limited current. It was found that the diffusion-limited current is also dependent on pH. It remains essentially constant from pH 0 to 6, and then slowly begins to decay. At about pH 11 the height of the polarographic wave falls sharply and it breaks up into two waves (Fig. 4). This fall is accompanied by a change in the slope of the $E_{1/2}$ versus pH graph (Fig. 3), indicating that a different electrode process occurs. Therefore, for DON the best defined and differentiated waves for analytical purposes were obtained at $0 < \text{pH} < 6$. The scission of the polarographic wave and the change in the slope of the $E_{1/2}$ versus pH graph observed at pH 11 can be related to the cyclic voltammetric behaviour.

In the pH range 0–10 no difference was observed in the shape of the cyclic voltammetric waves (Fig. 2), except that the potentials were shifted cathodically as the pH increased, indicating a similar electrode process over this pH range. However, above approximately pH 10 a different process occurs. At these pH values, a one-electron reduction peak appears at -0.85 V, corresponding to the reversible reduction of the nitro group to a nitro-anion radical. This peak precedes the main reduction wave, which is shifted to a more negative potential (Fig. 5). Wave clipping at a potential between peaks D and A (see Fig. 5) causes peaks B and C to disappear, indicating that peak E corresponds to the oxidation of the anion radical formed in D. Because this one-electron transfer does not need hydrogen ions and occurs only at pH > 11, the slope of the $E_{1/2}$ versus pH graph, at these pH values, tends almost to zero.

The existence of this relatively stable anion radical in alkaline media has recently been reported in the reduction of

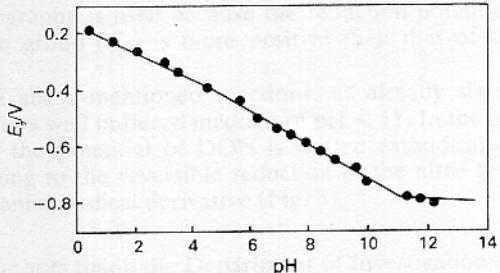


Fig. 3. Effect of pH on the half-wave potential of the DON reduction wave at 0.289 mM

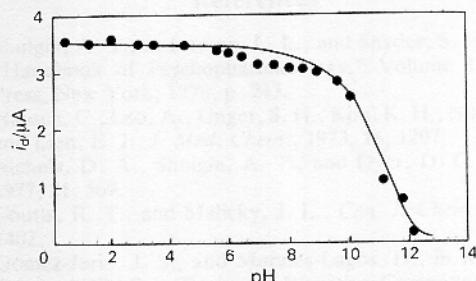


Fig. 4. pH dependence of the diffusion-limited current of 0.289 mM DON

nitrobenzene when platinum, gold and glassy carbon electrodes were used¹⁹ and also in the reduction of 5-nitrofuran derivatives at pH > 8.5.¹⁵

Mechanism of the Electrode Process

The irreversible nature of the wave was confirmed by logarithmic plots and it was found that the slope of the graph of E versus $\log[i/(i_d - i)]$ appreciably exceeded $59.2/n$ mV, where n is the number of electrons involved in the over-all reduction process. Further, the fact that the numerical value of $E_{1/2} - E_{3/4}$ of the polarographic wave exceeds $56.4/n$ mV also confirms the irreversible nature of the wave.²² A closer inspection of the cyclic voltammograms reveals that the current at the foot of the cathodic wave is independent of the scan rate. Such behaviour, originally noted by Reinmuth,²³ strongly suggests that the electron transfer to DON is electrochemically unidirectional, *i.e.*, totally irreversible.

The number of electrons involved in the over-all reduction process was found to be four and was determined, according to Meites *et al.*,²⁴ by comparison of the polarographic responses under the same experimental conditions for DON and other aromatic nitro compounds in which the number of electrons involved in the reduction process is known (Table 3).

The αn_a values and the number of protons (P values) corresponding to the rate-determining step were calculated at selected pH values. In the pH range 1–10, αn_a was found to be 0.95 ± 0.04 . An attempt to calculate n_a gave a value of 2 because for totally irreversible systems, as in this instance, α should be less than 0.5. However, according to Meites,²⁵ only a single electron can be transferred at a time during the course of the electrode reaction, and a value of n_a exceeding 1 would merely mean that successive steps are too close together to be distinguished on the time scale implicit in the polarographic measurements.

From the equation

$$\frac{dE_{1/2}}{dpH} = \frac{0.059}{\alpha n_a} \cdot P$$

P was found to be 0.85, showing that one proton is involved or

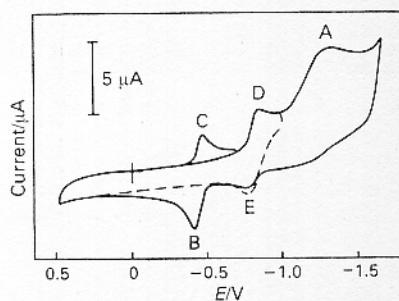


Fig. 5. Cyclic voltammogram of 0.219 mM DON in alkaline medium; pH 12; glassy carbon electrode; scan rate, 0.1 V s^{-1} . Peaks A, B and C as in Fig. 2 but shifted negatively. Peak E denotes the oxidation of the nitro-anion radical formed in D. Broken line obtained when sweep reversed after peak D

Table 3. Polarographic data for the reduction of different nitro compounds (0.124 mM) in pyridine-formic acid (pH 4.5) with TMAC as supporting electrolyte

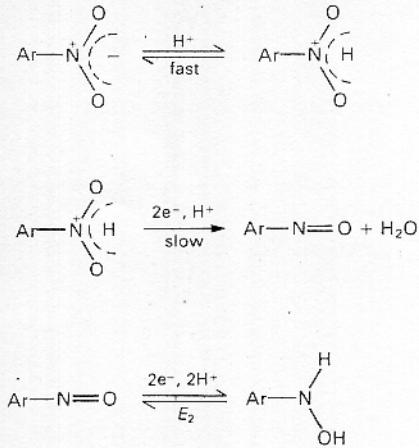
Compound	No. of runs	$E_{1/2}/\text{V}$	$i_d/\mu\text{A}$	n
Nitrofurantoin	8	-0.16	2.27	6
Nitrofurazone	7	-0.18	2.30	6
Furazolidone	7	-0.17	2.29	6
Chloramphenicol	9	-0.41	1.62	4
Nitrazepam	5	-0.34	1.62	4
Flunitrazepam	6	-0.28	1.55	4
Clonazepam	7	-0.28	1.58	4
Parathion	8	-0.32	1.64	4
DON	6	-0.39	1.45	4

precedes the rate-determining step of the reaction over the pH range 1–10.

The formation of a nitroso intermediate in the rate-determining step has been reported for different aromatic nitro compounds independent of the nature of the ultimate reduction product.^{10,12,15,26,27}

The nitroso intermediate formed is rapidly reduced to the hydroxylamine derivative, which is stabilised at this stage because the fast chemical reaction that would allow the transformation of the hydroxylamine into the unstable imine is inhibited.^{15,16} This inhibition takes place when the substituents do not have strong π -donor properties, in which event a four-electron reduction occurs (CEE reaction).

After establishing the stoichiometry of the rate-determining step, *i.e.*, $n_a = 2$ and $P = 1$, the following mechanism can be proposed for the polarographic reduction of DON to the corresponding phenylhydroxylamine derivative:



The nitroso-hydroxylamine reversible couple was detected by cyclic voltammetry (Fig. 2), and it must be stressed that the nitroso intermediate is not observed when normal d.c. polarography is used because the reduction potential of the nitroso group (E_2) is more positive than that of the nitro group.

The above-mentioned reactions, as already stated, take place in a well buffered medium of pH < 11. In the pH range 11–14 the potential of DON is shifted cathodically, corresponding to the reversible reduction of the nitro group to a nitro-anion radical derivative (Fig. 5).

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