Voltammetric Study of 7-Nitro-1,4-benzodiazepin-2-ones and Their Acid Hydrolysis Products, 2-Amino-5-nitrobenzophenones

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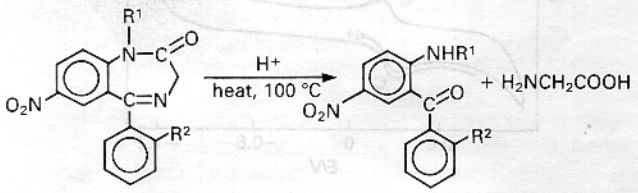
The electrochemical reduction of 7-nitro-1,4-benzodiazepin-2-ones and of their acid hydrolysis products, 2-amino-5-nitrobenzophenones, was studied by polarography and cyclic voltammetry in a solvent - buffer system containing pyridine, formic acid and tetramethylammonium chloride solution in order to elucidate the effect of the nature and position of the substituents on the reduction of the nitro group. It was found that these two types of compounds can be polarographically and voltammetrically distinguished and that their reduction mechanisms differ owing to a structural change in substituents located at a *para*-position relative to the nitro group. Based on polarographic and cyclic voltammetric data, reduction mechanisms for these two species are proposed in which the donor - acceptor properties of the substituents and the importance of the chemical reactions associated with the electron-transfer steps are indicated.

Keywords: *7-Nitro-1,4-benzodiazepin-2-one; 2-amino-5-nitrobenzophenone; polarography; cyclic voltammetry*

Nitrazepam (**I**), flunitrazepam (**II**) and clonazepam (**III**) are 1,4-benzodiazepines which contain a nitro group in the 7-position. Several workers have studied the polarographic behaviour of these compounds. In the acidic range of Britton - Robinson buffers, two polarographic reduction waves are observed. The first corresponds[1–3] to the reduction of the 7-nitro group to a hydroxylamine derivative and the second to a simultaneous reduction of the hydroxylamine and the azomethine groups. In a phosphate buffer of pH 6.9 the reduction occurs in two waves, the second being attributed[4] to a single two-electron reduction of the azomethine group.

The nitro derivatives of the 1,4-benzodiazepines **I–III**, which have a common molecular skeleton, show similar electrochemical behaviour and cannot be distinguished owing to the proximity of their half-wave potentials. However, **II** and **III** have been determined in blood and urine by acid hydrolysis to the corresponding 2-amino-5-nitrobenzophenones followed by gas - liquid chromatography with electron-capture detection.[5] Compound **II** can alternatively be determined in the presence of its analogues based on differences in the hydrolysis rates.[2] Compounds **I–III** can be hydrolysed quantitatively in acidic media to the corresponding benzophenones according to the following reaction:



R^1	R^2		
H	H	Nitrazepam (**I**)	2-Amino-5-nitrobenzophenone (**IV**)
CH_3	F	Flunitrazepam (**II**)	2-Methylamino-5-nitro-2′-fluorobenzophenone (**V**)
H	Cl	Clonazepam (**III**)	2-Amino-5-nitro-2′-chlorobenzophenone (**VI**)

Whereas the electrochemical reduction of the nitro group in **I–III** has been widely studied,[1–4,6] no information is available concerning the reduction of the nitro group in their hydrolysis products **IV–VI**. The reduction in **IV–VI** is altered owing to the presence of an amino group in the *para*-position.

In this study the electrochemical behaviour of **I–III** was compared with that of **IV–VI**.

Experimental

Reagents

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

Compounds **I–III** were obtained from La Roche (Basilea, Switzerland) and used as received. The benzophenone derivatives were synthesised by the method of Sternbach *et al.*[7] [m.p. 158 °C (**IV**), 118 °C (**V**) and 183 °C (**VI**)]. Stock solutions (1×10^{-2} M) were prepared by dissolving the appropriate amount of each compound in dimethylformamide (DMF). Gelatin solution (0.5%) was used as a maximum polarographic suppressor. The supporting electrolyte was prepared by adding 1 ml of pyridine (12.3 M) and 0.5 ml of formic acid (98–100%) to 100 ml of 0.1 M aqueous tetramethylammonium chloride. The resulting solution had a pH of 4.5. On varying the ratio of formic acid to pyridine the pH could be varied over the range 2.5–5.1. Extension of the pH range was achieved by using dilute hydrochloric acid or sodium hydroxide solution.

Apparatus

Polarographic curves were recorded using a Polariter PO4 instrument (Radiometer, Copenhagen, Denmark). A dropping mercury electrode (DME) was used as the working electrode (mercury flow-rate = 2.360 mg s^{-1}, drop time = 3.15 s) and a saturated calomel electrode (SCE) as the reference electrode.

Cyclic voltammograms were recorded using a CV-27 voltammograph (Bioanalytical Systems, Lafayette, IN, USA) with a three-electrode assembly. The working electrode was glassy carbon, the counter electrode a platinum coil and the reference electrode an aqueous SCE. Before each experiment the glassy carbon electrode was polished with CF-1050 polishing alumina (BAS) on a felt wetted with water or ethanol and finally cleaned with acetone on a fluff-free tissue.

Procedures

Aliquots of the stock solution were diluted with 15 ml of the supporting electrolyte to yield 0.184 mM solutions (unless stated otherwise), 1 ml of 0.5% gelatin solution was added, the solution was purged with oxygen-free nitrogen for 10 min and d.c. current - voltage curves were recorded at a DME.

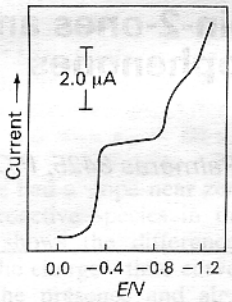


Fig. 1. Polarographic reduction waves of 0.184 mM nitrazepam in 0.24 M DMF at pH 4.5. The buffer consisted of 0.11 M pyridine and 0.12 M formic acid

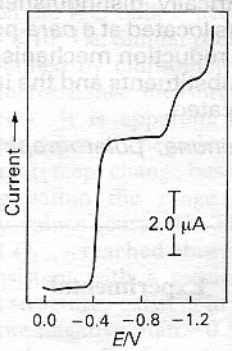


Fig. 2. Polarographic reduction waves of 2-amino-5-nitrobenzophenone at 0.184 mM. Conditions as in Fig. 1

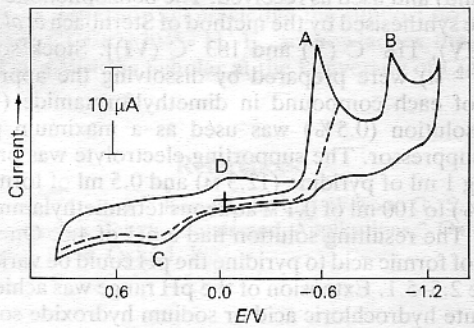


Fig. 3. Cyclic voltammogram of 0.196 mM nitrazepam in pyridine - formic acid buffer (pH 4.5), 0.25 M DMF, with a glassy carbon electrode; scan rate, 0.1 V s^{-1} from $0.0 \rightarrow -1.3 \rightarrow +0.8 \rightarrow -0.5$ V. The broken line was obtained when the sweep was reversed after peak A, from $0.0 \rightarrow -0.7 \rightarrow +0.8$ V. For details see text

Cyclic voltammetric experiments were performed under identical conditions except that gelatin was not added. All measurements were carried out at 25 ± 1 °C.

A linear dependence of the d.c. limiting current on the concentration of the nitro compounds in the range 1×10^{-4}–1×10^{-5} M and on $h^{\frac{1}{2}}$ (where h is the height of the mercury column) proved that diffusion control applied.

Results and Discussion

Most substituted nitrobenzenes in protic media are reduced in a four-electron wave to arylhydroxylamines, the protonated forms of which are reduced in acidic media in a further two-electron step.[8] For nitrobenzenes substituted in the *ortho*- or *para*-position by a hydroxy or amino group[9,10] or for 5-nitrofurans bearing such substituents in position 2 on the furan ring,[11–14] the limiting current is a function of pH and in acidic and alkaline media reaches a value corresponding to a six-electron reduction. This has been attributed[9] to an acid- or base-catalysed dehydration of the hydroxylamino derivative,

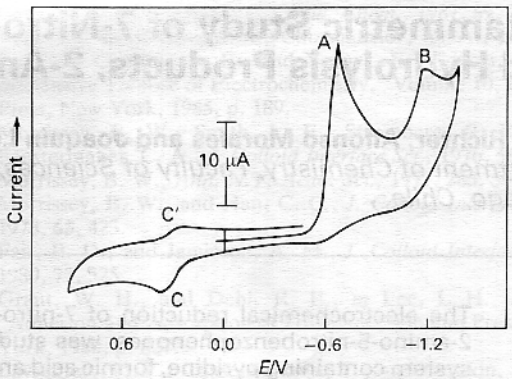


Fig. 4. Cyclic voltammogram of 0.196 mM 2-amino-5-nitrobenzophenone. Conditions as in Fig. 3 except that the pH 4.5 buffer consisted of 0.018 M formic acid and 0.102 M formate. For details see text

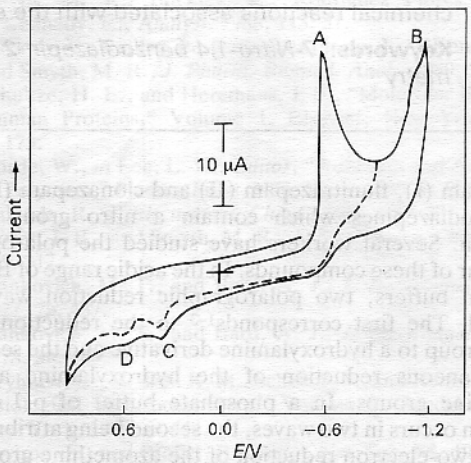


Fig. 5. Cyclic voltammogram of 0.196 mM 2-amino-5-nitrobenzophenone. Conditions as in Fig. 3. The broken line was obtained when the sweep was reversed after peak A. For details see text

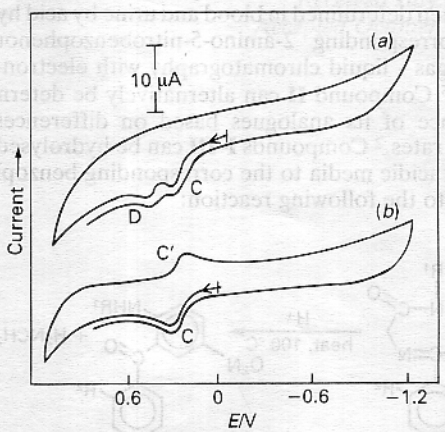


Fig. 6. Cyclic voltammogram of 0.196 mM 1,4-diaminobenzene in (*a*) pyridine - formic acid buffer (pH 4.5) (composition as in Fig. 1) and (*b*) formic acid - formate buffer (composition as in Fig. 4). Conditions for recording i - E curves as in Fig. 3. For details see text

yielding a quinone or a quinoneimine. This easily reducible species is immediately reduced in a two-electron step at the potential at which it is formed. The driving force for the dehydration of the hydroxylamine is the stabilisation of the quinonoid compound formed.

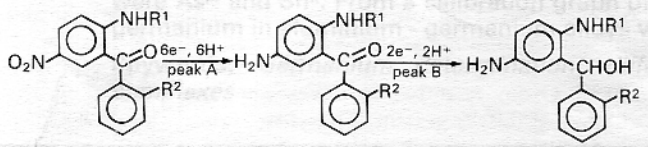
Among the compounds studied, the 7-nitro-1,4-benzodiazepin-2-ones **I–III** are reduced at pH 4.5 in a four-electron wave corresponding to the reduction of the nitro group to the

hydroxylamino derivative. At this pH, the protonation of the hydroxylamino group is insufficiently fast and the wave at more negative potentials (Fig. 1) corresponds to the reduction of the azomethine group. This interpretation is in agreement with those proposed for the reduction of 7-nitro-1,4-benzodiazepin-2-ones by other workers in other supporting electrolytes.[1–4,6]

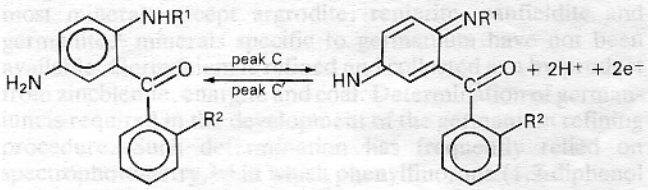
The hydrolysis products **IV–VI** show, on the other hand, a six-electron reduction of the nitro group followed by a two-electron reduction of the benzophenone carbonyl to the corresponding alcohol (Fig. 2). Evidently, the presence of the NHR^1 group in the *para*-position relative to the hydroxylamino group facilitates dehydration and reduction of the quinonediimine formed.

Cyclic voltammograms support this interpretation; reduction of the nitrobenzodiazepines **I–III** (Fig. 3) in peak A yields an arylhydroxylamine that is oxidised in peak C to a nitroso compound, which in a second cycle is reduced in peak D. The height of peak C is unaffected by the reduction in peak B (compare the full and broken lines in Fig. 3).

Reduction of the 2-amino-5-nitrobenzophenones **IV–VI** results in a six-electron reaction peak A (Fig. 4) representing a 2,5-diaminobenzophenone; the carbonyl group is then reduced in peak B:



The 2,5-diaminobenzophenone is oxidised in peak C to a quinonediimine, which is reversibly reduced in peak C′:



The oxidation potentials of the 2,5-diaminobenzophenone in **IV–VI** are unfortunately close to those of the phenylhydroxylamine group in the structurally related **I–III** (compare Figs. 3 and 4), which prevents the fraction of arylhydroxylamine which did not undergo dehydration (if any) from being distinguished.

The presence of pyridine results in a separation of the anodic peak C into two and the disappearance of the reduction peak C′ (Fig. 5). Analogous effects of pyridine were observed in the oxidation of 1,4-diaminobenzene, used as a model compound (Fig. 6). These effects can be attributed either to a rapid nucleophilic addition of pyridine to the quinonediimine formed or to an effect of an adsorbed pyridine film. The separation of peaks C and D disappears at scan rates higher than 500 mV s^{-1}, probably owing to an insufficient rate of either nucleophilic addition or film formation. This problem will be studied further.

The reduction potentials of the nitrobenzodiazepines **I–III** and their hydrolysis products **IV–VI** are sufficiently different for polarography to be used as an analytical method in future studies of the hydrolysis of **I–III**.

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