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N-HYDROXY-*N*-ARYLACETAMIDES. VI* CHEMICAL AND ELECTROCHEMICAL OXIDATION OF *N*-HYDROXY-*N*-ARYLACETAMIDES

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The electrochemical behaviour of seven *N*-hydroxy-*N*-arylacetamides ($RNOHCOCH_3$, R = phenyl, 4-chlorophenyl, 3,4-dichlorophenyl, 4-ethoxyphenyl, 4-biphenylyl, 2-fluorenyl and 2-phenanthryl) was investigated by cyclovoltammetry. Each compound showed two different oxidation potentials, attributed to a quasi-reversible one-electron transfer in the range between 0·55 and 0·63 V and an irreversible transfer of a second electron between 0·80 and 1·20 V. Since the one-electron oxidation of *N*-hydroxy-*N*-arylacetamides gives the corresponding nitroxides, the kinetics of the self-reaction of these radicals and the concentration dependence of the product pattern were also studied.

INTRODUCTION

N-Hydroxy-*N*-arylacetamides (*N*-arylacetohydroxamic acids) play an essential role in the metabolic activation of *N*-arylacetamides due to cytochrome P-450-dependent *N*-hydroxylation.[1,2] Since *N*-arylacetamides are also formed after incorporation of several aromatic amines and nitroarenes (which can be reduced to amines *in vivo*), a greater number of chemicals may be considered to enter this metabolic pathway. Further oxidation of *N*-hydroxy-*N*-arylacetamides *in vivo* and *in vitro* has been suggested by several workers in connection with the mechanism of chemical carcinogenesis[3] and in the catalytic oxidation of haemoglobin.[4,5] Therefore, we were interested in the oxidation–reduction potentials of these compounds in order to obtain more information about the probability of the formation of secondary nitroxides from *N*-hydroxy-*N*-arylacetamides in biological systems.

We also report kinetic data for the decay of several acetyl arylnitroxides in toluene and methanol which may be suitable for elucidating some of the properties of these radicals.

EXPERIMENTAL

Chemicals. The preparation and properties of *N*-hydroxyacetanilide, *N*-hydroxy-4-chloroacetanilide, *N*-hydroxy-3,4-dichloroacetanilide, *N*-hydroxyphenacetin, *N*-hydroxy-4-acetylaminobiphenyl and *N*-hydroxy-2-acetylaminofluorene were described in Part II.[6] *N*-Hydroxy-2-acetylaminophenanthrene (m.p. 177–178 °C) was prepared by reductive acetylation of 2-nitrophenanthrene according to Poirier *et al.*[7] 4-Chloronitrosobenzene and 4-chloroacetanilide were prepared as described.[6]

4-Chloronitrobenzene, lead dioxide and all solvents (analytical-reagent grade) were purchased from Merck (Darmstadt, FRG).

Methods. UV–visible absorption spectra were measured with a Varian Cary 118 spectrophotometer.

Voltammetric measurements were performed on a Polarecord E 506 from Deutsche Metrohm (Filderstadt, FRG). All electrodes were purchased from Deutsche Metrohm. The working electrode was a glassy carbon electrode (EA 276/2) and the reference electrode was an Ag/AgCl (3 M KCl) (EA 441/5) together with a Pt-helping electrode (EA 282/1).

EPR measurements were performed on a Varian E 109 spectrometer.

For the determination of *N*-hydroxy-4-chloroacetanilide and its oxidation products, an HPLC system from Waters (Milford, MA, USA) was used with a μ Bondapak C_{18} column and methanol–water–acetic acid (60 : 30 : 10, v/v/v) as the mobile phase. The resolved metabolites were detected by their UV absorbance at 254 and 280 nm and

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quantified by comparing the areas under the peaks with those of known amounts of the authentic compounds.

Melting points were determined with a Dr Tottoli apparatus from Büchi Laboratoriums-Technik (Flawil Switzerland) and corrected.

Solutions of acetyl aryl nitroxides in toluene or methanol were obtained by oxidation of 10^{-3} M solutions of the corresponding *N*-hydroxy-*N*-arylacetamides with lead dioxide. After rapid filtration from the oxidizing agent, the solutions were used to record the EPR spectra and to determine the reaction rate of the self-reaction of the nitroxides by monitoring the maximum absorption band in the visible region.

RESULTS

Voltammetric determination of the oxidation–reduction potentials of various *N*-hydroxy-*N*-arylacetamides

The voltammetric investigation of seven *N*-hydroxy-*N*-arylacetamides (*N*-hydroxyacetanilide, *N*-hydroxy-4-chloroacetanilide, *N*-hydroxy-3,4-dichloroacetanilide, *N*-hydroxyphenacetin, *N*-hydroxy-4-acetylaminobiphenyl, *N*-hydroxy-2-acetylaminofluorene and *N*-hydroxy-2-acetylaminophenanthrene) showed a very close relationship of the electrochemical behaviours of these compounds.

In Figure 1 the voltammogram of *N*-hydroxy-4-chloroacetanilide is given, showing the current vs potential characteristics typical of all of the seven hydroxamic acids apart from the differences in the heights of the potentials. The interpretation of the three distinct potentials as shown in Figure 1 is based on the following observations.

Potential (1) is not observed in the first run in the anodic direction, but appears as soon as the potential difference of potential (2) is applied to the working electrode. Therefore, it is obvious that potential (1) does not arise from the hydroxamic acid itself, but is caused by an oxidation product. Such as assumption could easily be verified by comparison with the voltammogram of 4-chloronitrosobenzene or *N*-hydroxy-4-chloroaniline, both of which showed the same characteristic reversible potential.

Potential (2) displays the characteristics of a quasi-reversible electron transfer process, as might be expected for the formation of an unstable oxidation product. Since it is known that *N*-hydroxy-*N*-arylacetamides can be oxidized to the corresponding secondary nitroxides,[8,9] we assume that this one-electron transfer is involved in the observed potential (2). The value of 0·61 V for this potential is in agreement with the observation that only very strong oxidizing agents allow the formation of the nitroxides.

Potential (3) is characterized by the missing potential wave during the run in the cathodic direction, thus giving the typical shape of an irreversible oxidation

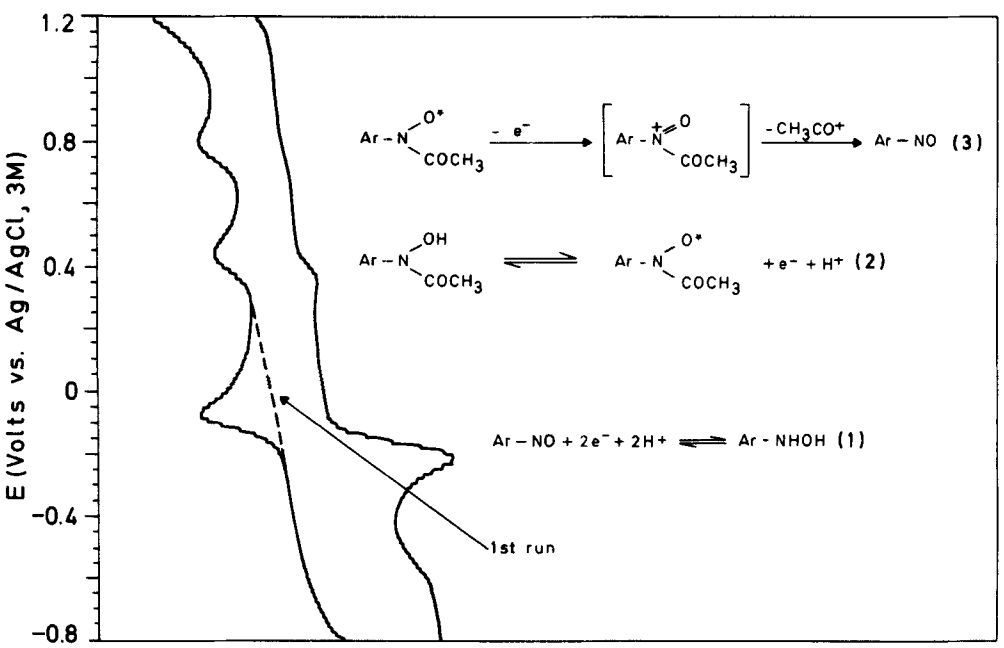

Figure 1. Voltammogram of a 10^{-4} M solution of *N*-hydroxy-4-chloroacetanilide in 0·05 M phosphate buffer (pH 7·4)–methanol (9 : 1, v/v) at 22 °C. Working electrode, glassy carbon; reference electrode, Ag/AgCl (3 M KCl); scan rate, 20 mV s^{-1}

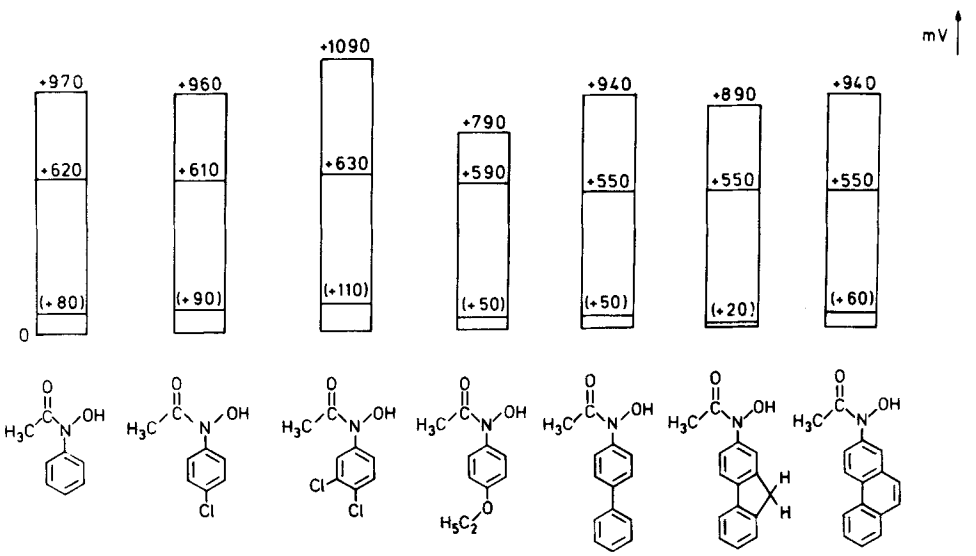

Figure 2. Oxidation–reduction potentials (vs the normal hydrogen electrode, NHE) of seven *N*-hydroxy-*N*-arylacetamides determined by voltammetric measurements. The lowest potential (1) between 20 and 110 mV is not caused by the hydroxamic acid itself, but results from the formation of the corresponding nitrosoarenes

process. Therefore, we assume that the *N*-hydroxy-*N*-arylacetamide looses a second electron and forms an unstable intermediate that is considered to decay under *N*-deacetylation (see Figure 1).

Differences in the positive potentials due to different aromatic substituents are apparent from Figure 2. Since we used an Ag/AgCl electrode as a reference electrode, the values given in Figure 2 were obtained by adding 210 mV to each of the original potential values.

Figure 3 shows how the potential values vary with the pH of the solution. The highest oxidation potential could not be determined at pH > 9, because the glassy carbon electrode is not stable under these conditions. The pH dependence of the lowest potential, which is due to the arylhydroxylamine–nitrosoarene couple, displays a slope of 0·059 V pH^{-1}, which is in agreement with the theoretical value (i.e. the Nernst factor), thus providing further evidence for the interpretation of this potential. Potential (2) for the one-electron transfer shows the same slope as does potential (1) up to pH ≈ 8 and roughly half the slope from pH 8 to 12. This can be explained as the transition from the non-ionized to the ionized state of the hydroxamic acid, since the pK_a value of *N*-hydroxyacetanilide was determined to be 8·34 ± 0·02 (at 25 °C) by Monzyk and Crumbliss.[10]

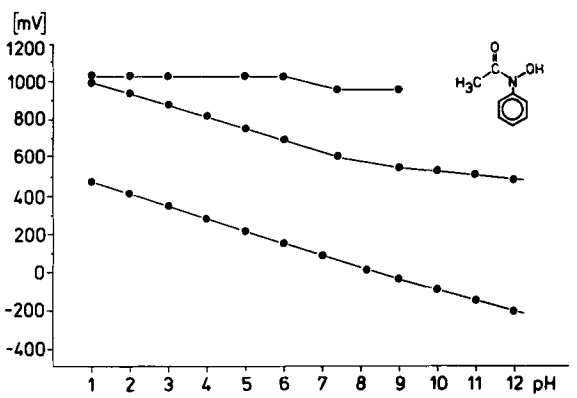

Figure 3. pH dependency of the oxidation–reduction potentials of *N*-hydroxyacetanilide (potentials referred to the NHE)

The third potential does not depend on the pH from pH 1 to 6. This is in agreement with the assumption that an electrically neutral molecular species, i.e. the secondary nitroxide, is oxidized at this potential. So far we have no interpretation for the small variations between pH 6 and 9.

Chemical oxidation of *N*-hydroxy-*N*-arylacetamides

In order to obtain some information on the stability of the primary oxidation products of *N*-hydroxy-*N*-arylacetamides, i.e. acetyl arylnitroxides, we studied the decay of these radicals in toluene and methanol. All the nitroxides produced by oxidation of the corresponding hydroxamic acids with lead dioxide showed absorption bands in the visible region as shown in Figure 4. Because of the rapid decay of the nitroxides, we did not determine any molar absorptivities. Figures 5 and 6

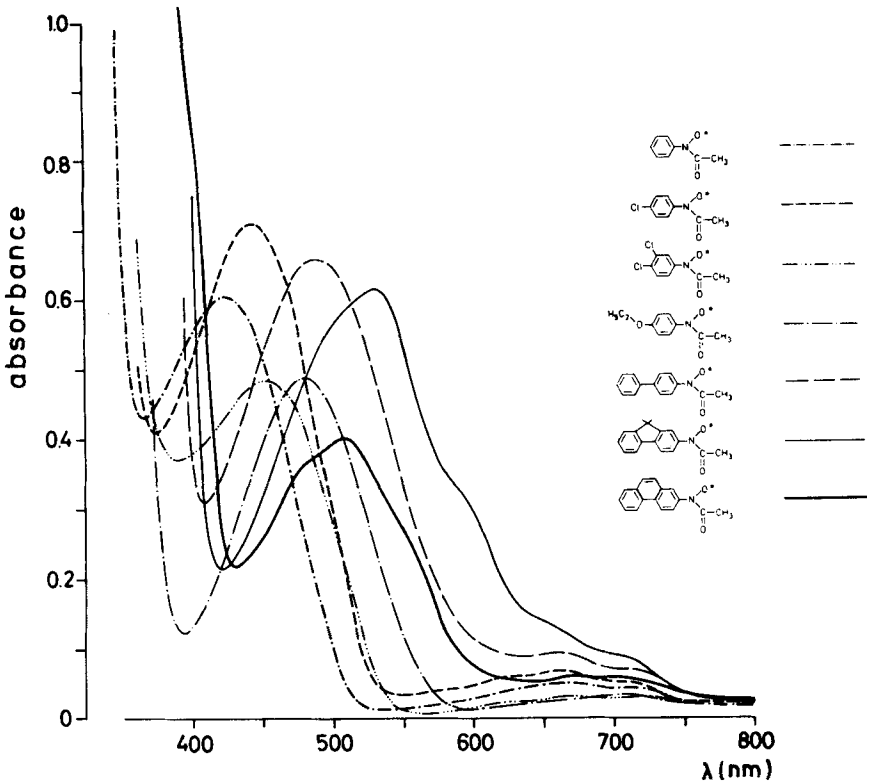

Figure 4. Electronic spectra of several acetyl aryl nitroxides in toluene. Volumes of 10 ml each of 10^{-3} M solutions of the hydroxamic acids in toluene were shaken at room temperature for 10 s with 4 ml of 10^{-3} M $KMnO_4$, the organic phase was removed and the absorbance was scanned between 800 and 350 nm. The relative absorbances shown here do not reflect the real differences in the molar absorptivities

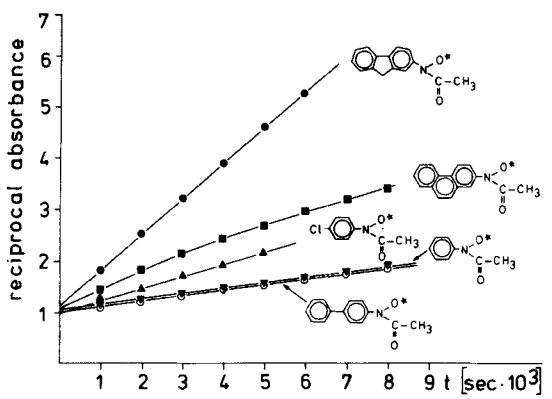

Figure 5. Kinetics of the decay of several acetyl aryl nitroxides in toluene. Solutions (10^{-3} M) of the hydroxamic acids in toluene were oxidized at room temperature with 10^{-3} M $KMnO_4$ solution and the organic phase was monitored at the maximum absorption band in the visible region every 5 min for 2 h. On plotting reciprocal differences in absorbance vs time, straight lines were obtained, indicating second-order decay kinetics

show the kinetics of the radical decay obtained by monitoring the maximum wavelength of each nitroxide. The kinetic data for acetyl 4-ethoxyphenyl nitroxide and acetyl 3,4-dichlorophenyl nitroxide are not shown here, because they did not obey second-order kinetics but displayed a formal order of reaction of about 2·5. We have not yet analysed this in detail. Acetyl 4-chlorophenyl nitroxide is also missing from Figure 6, because its decay in methanol followed a first-order rate equation.

Table 1 gives the apparent rate constants determined for the second-order decay reaction of several acetyl aryl nitroxides. Although the rate constants of the decay reactions are 15–58 times higher in methanol than in toluene, the order remains the same for both solvents: acetyl 2-fluorenyl nitroxide > acetyl 2-phenanthryl nitroxide > acetyl 4-biphenylyl nitroxide = acetyl phenyl nitroxide. We have no explanation for the surprising observation that the rate constants of the last two compounds are the same in both methanol and toluene.

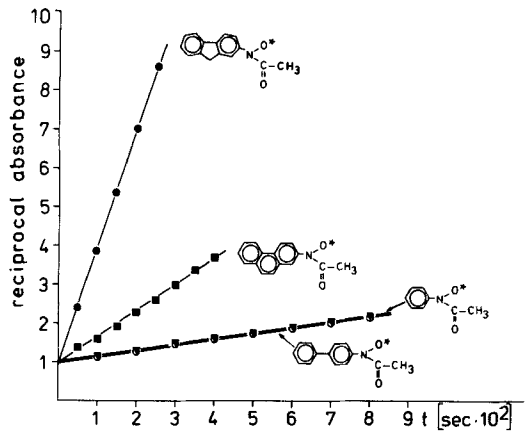

Figure 6. Kinetics of the decay of several acetyl aryl nitroxides in methanol. Solutions (10^{-3} M) of the hydroxamic acids in methanol were shaken with 50 mg of PbO_2 in a 10-ml syringe. After 3 s the solution was ejected through a membrane filter (Minisart P) into the cuvette in order to remove PbO_2 and monitored at the maximum absorption band in the visible region 1 s later. On plotting reciprocal differences in absorbance vs time, straight lines were obtained for four acetyl aryl nitroxides, indicating second-order decay kinetics

EPR spectra of several acetyl aryl nitroxides

In order to confirm our assumption about the nature of the primary oxidation products of *N*-hydroxy-*N*-arylacetamides, we recorded the EPR spectra of the nitroxides generated in toluene by oxidation with lead dioxide. The spectra shown in Figure 7 are in agreement with the structure of acetyl aryl nitroxides. The hyperfine splitting constants (h.f.s.c.) of acetyl phenyl nitroxide are similar to those reported by Aurich and Baer.[8] The h.f.s.c. of acetyl 4-chlorophenyl nitroxide were reported in a previous paper.[5] Although the EPR spectra of compounds 3,4 and 6 are not complex, their h.f.s.c. could not be determined because the spectra could not be better resolved.

Table 1. Decay kinetics of various acetyl arylnitroxides[a]

Compound	Apparent rate constants ($l\ mol^{-1}\ s^{-1}$) in Toluene	Methanol
Acetyl phenyl nitroxide	0·11	1·6
Acetyl 4-chlorophenyl nitroxide	0·22	[b]
Acetyl 4-biphenylyl nitroxide	0·11	1·6
Acetyl 2-fluorenyl nitroxide	0·88	51·3
Acetyl 2-phenanthryl nitroxide	0·34	9·3

[a] Acetyl aryl nitroxides were formed from the corresponding *N*-hydroxy-*N*-arylacetamides either by oxidation of the toluene solutions with aqueous $KMnO_4$ or by oxidation of the methanol solutions with PbO_2. Decay kinetics were determined by monitoring the maximum absorption band in the visible region and plotting reciprocal differences in absorbance vs time. Second-order rate constants were read from the straight lines.

[b] The decay of acetyl 4-chlorophenyl nitroxide in methanol apparently followed first-order kinetics, with $k = 1{\cdot}4\ s^{-1}$.

Concentration dependence of the product pattern obtained by chemical oxidation of *N*-hydroxy-4-chloroacetanilide

In a previous paper[5] we reported on the kinetics of the self-reaction of acetyl 4-chlorophenyl nitroxide in water. Since the decay of this radical led to the formation of *N*-acetoxy-4-chloroacetanilide and 4-chloronitrosobenzene, we proposed an intermolecular acetyl transfer as the first step of the radical degradation. From the additional formation of 4-chloroacetanilide and 4-chloronitrobenzene, a 'spin-trapping analogue' reaction of the nitroxide with 4-chloronitrosobenzene has been assumed as a parallel reaction. As additional proof for this mechanism, originally suggested by Forrester *et al.*,[9] we were interested in the effect of dilution on the product pattern.

Since 4-chloronitrosobenzene is a volatile compound, the determination of the products had to be carried out without any concentration of the solution. Hence our method of product determination by direct HPLC analysis was limited to concentrations $\geqslant 10^{-5}$ mol l^{-1} (referred to *N*-hydroxy-4-chloroacetanilide before the oxidation with PbO_2). The results given in Table 2 show that the decay of acetyl 4-chlorophenyl nitroxide indeed depends on its original concentration. Lowering the radical concentration gradually diminished the relative amount of *N*-acetoxy-4-chloroacetanilide, but raised the relative amount of 4-chloronitrosobenzene. This observation can be explained by the increasing probability of an acetyl transfer to the solvent. Table 2 also shows that the relative amount of 4-chloroacetanilide decreased with increasing dilution, as

Table 2. Dependency of the product pattern (mol l^{-1}) on the concentration of *N*-hydroxy-4-chloroacetanilide in aqueous solution[a]

	N-Hydroxy-4-chloroacetanilide (mol l^{-1})		
	10^{-3}	10^{-4}	10^{-5}
4-Chloronitrosobenzene	$2{\cdot}5 \times 10^{-4}$	$6{\cdot}3 \times 10^{-5}$	$7{\cdot}7 \times 10^{-6}$
4-Chloronitrobenzene	$3{\cdot}2 \times 10^{-4}$	$0{\cdot}4 \times 10^{-5}$	$1{\cdot}1 \times 10^{-6}$
N-Acetoxy-4-chloroacetanilide	$3{\cdot}5 \times 10^{-4}$	$2{\cdot}5 \times 10^{-5}$	$0{\cdot}5 \times 10^{-6}$
4-Chloroacetanilide	$0{\cdot}4 \times 10^{-4}$	$0{\cdot}2 \times 10^{-5}$	$0{\cdot}1 \times 10^{-6}$

[a] Different concentrations of *N*-hydroxy-4-chloroacetanilide in water were oxidized by PbO_2 at room temperature and the product pattern determined by HPLC as described under Experimental.

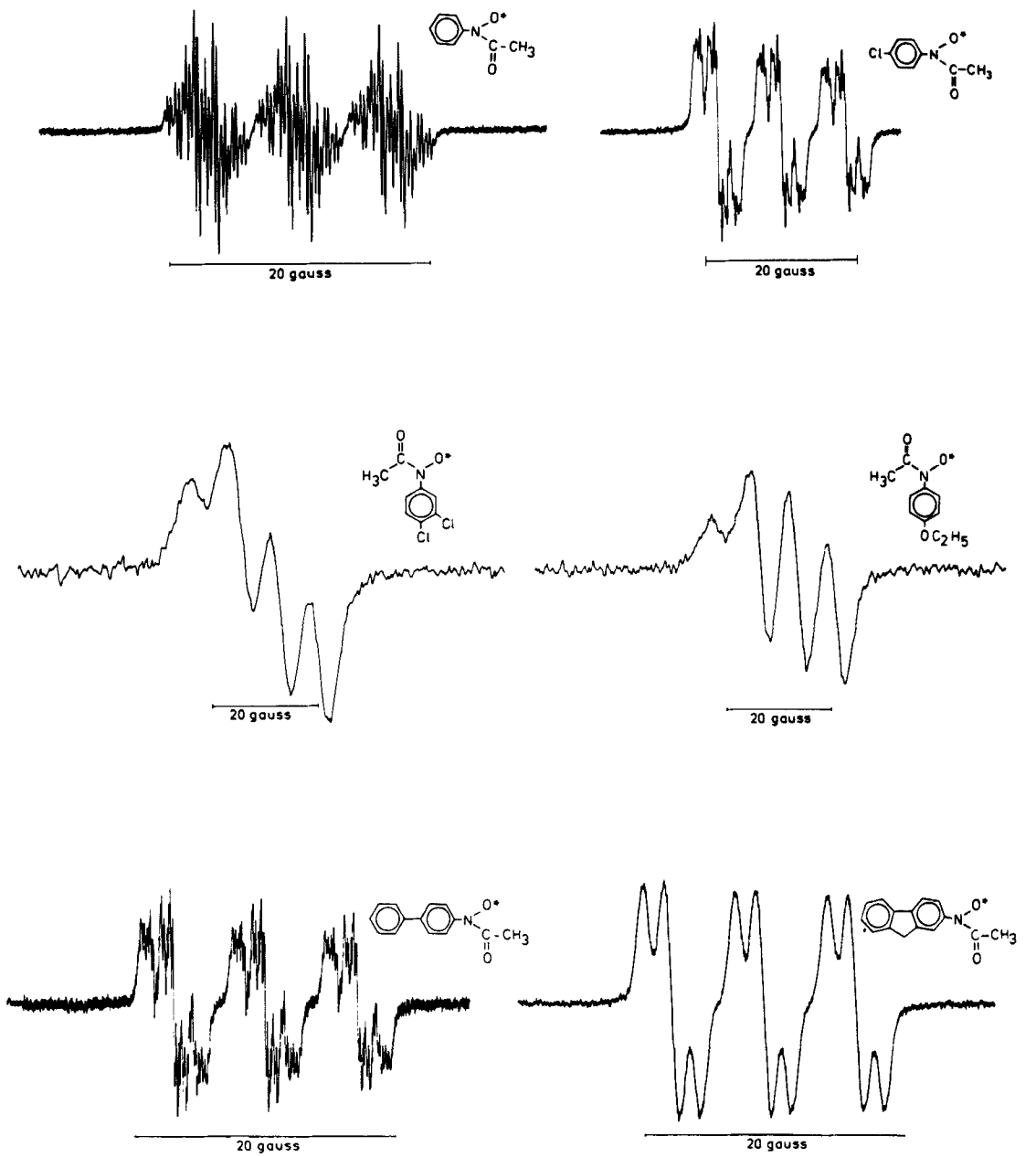

Figure 7. EPR spectra of six acetyl arylnitroxides in toluene. The nitroxides were generated by lead dioxide oxidation of 10^{-3} M solutions of the corresponding *N*-hydroxy-*N*-arylacetamides. EPR conditions: magnetic field, 3300 G; microwave frequency, 0·15 GHz; microwave power, 2 mW; modulation frequency, 10 kHz; modulation amplitude, 0·05 G

expected for an intermolecular reaction mechanism. This provides further evidence that 4-chloroacetanilide is not formed from N-hydroxy-4-chloroacetanilide by reduction, but rather as a consequence of an oxidative process.

DISCUSSION

Oxidation–reduction potentials of N-hydroxy-N-arylacetamides

The potential values for the one-electron oxidation of the seven N-hydroxy-N-arylacetamides [=potential (2)] measured were between 550 and 630 mV, i.e. they varied only within a small range of 80 mV, in contrast to the variation of potential (3) of 300 mV between 790 and 1090 mV. This clearly shows that the enthalpy required for the withdrawal of a second electron depends much more on the electronic properties of the aromatic system than it does for the one-electron transfer. This can be understood if the transition state of the nitroxides's oxidation is assumed to have a structure close to the positively charge state $[ArN^+(=O)COCH_3]$, because the electron-donating effect of the aromatic ring system acts more specifically stabilizing on a charged transition state than it does on a neutral molecule.

Hence the electron-donating effect of the ethoxy group in a *para*-position caused a decrease in potential (3) of 180 mV (from 970 to 790 mV), but a decrease of only 30 mV (from 620 to 590 mV) in the case of the one-electron potential (2). In a similar way, the lower electron density in the aromatic system due to 3,4-dichloro substitution is responsible for a rise in the two-electron oxidation potential (3) of 120 mV from 970 to 1090 mV, whereas the one-electron oxidation potential (2) showed only a very small increase from 620 to 630 mV. Compared with the relatively small effects of electron-donating and -withdrawing substituents on the one-electron oxidation, the nature of the aromatic system itself seems to be of greater significance, since all the hydroxamic acids containing a polycyclic aromatic ring system have a one-electron oxidation potential of 550 mV, which is 70 mV lower than the potential of the unsubstituted monocyclic compound (i.e. N-hydroxyacetanilide). Whether such a difference has any implications for the toxicity of hydroxamic acids or may be useful in explaining the observation that many polycyclic compounds of this type are chemical carcinogens, whereas most of the monocyclic analogues obviously are not, will be a matter of further investigation.

This is consistent with reports of Crumbliss and coworkers for the acidity of C- and N-substituted hydroxamic acids,[10] substituted N-phenylacetohydroxamic acids[11] and substituted N-methylbenzohydroxamic acids,[12] and for the coordination chemistry of subsituted hydroxamic acids.[13–16] They presented evidence that changes in the N-substituent will not strongly influence electron density at the N-hydroxy groups, but will influence the electron density in the C—N and C = O bonds through stabilization of a positive charge on the nitrogen. This supports the relative changes in redox potential with N-substituents shown in Figure 1.

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