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Substituent effect on the redox potential of substituted (aryl)(2-nitrobenzo[*b*]thiophen-3-yl)amines

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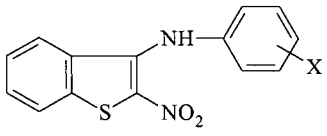
Abstract—The electronic effect of some *meta*- or *para*-substituents on the reduction of the title compounds has been investigated. The reversible reduction potential values of these compounds have been evaluated by cyclic voltammetry at a mercury electrode in 0.1 M tetraethylammoniumtetrafluoroborate, dimethylsulfoxide solutions. The substituent effect depends on both its nature and its position. The reduction potential values of the derivatives studied have been correlated with the Hammett substituent constants.

1. Introduction

It has been recently reported that several *N*-substituted 3-amino-2-nitrobenzo[*b*]thiophenes show analgesic, anti-exudative and/or anti-inflammatory properties.[1] Some of these compounds display activities comparable to that of phenylbutazone, though they exhibit lower toxicity and gastric lesivity.[1] Because of the concern associated with the problem of gastrointestinal tolerance of anti-inflammatory agents, a factor that has increased the interest towards non-acid and non-steroid drugs,[2] we have addressed our attention to the understanding of general chemical and biological properties of these amines. Thus, investigating their syntheses, we observed the occurrence of a novel kind of nucleophilic substitution with rearrangement,[3] the mechanism of which has been investigated through the use of a ^{13}C-labelled substrate.[4] Moreover, we have analysed the ^{13}C NMR chemical shifts in a series of differently *meta*- and *para*-substituted (aryl)(2-nitrobenzo[*b*]thiophen-3-yl)amines **1**, gaining information on the electron density distribution and on the transmission of substituent effects.[5] For example, the plot of substituent-induced chemical shift (SCS) for C-2 (the carbon to which the nitro group is bound) vs. Hammett substituent constants gave good relationships for *meta*- and *para*-substituted derivatives with high susceptibility constants [ρ_m 7.2(0.5), ρ_p^- 7.9(0.7)], providing evidence for a significant effect of the substituent on the electron density.



1

a: X = H	**j**: X = *m*-Me
b: X = *m*-F	**k**: X = *p*-Me
c: X = *p*-F	**l**: X = *m*-OH
d: X = *m*-Cl	**m**: X = *p*-OH
e: X = *p*-Cl	**n**: X = *m*-OMe
f: X = *m*-Br	**o**: X = *p*-OMe
g: X = *p*-Br	**p**: X = *m*-NMe_2
h: X = *m*-CF_3	**q**: X = *p*-NMe_2
i: X = *p*-CF_3	**r**: X = *p*-NEt_2

As compounds **1** are nitroheterocyclic drugs, and therefore of great pharmacological and toxicological interest in human and veterinary medicine,[6] we have also estimated their mutagen activity (genotoxicity may represent a limit to the programme of drug development) by performing the screening of compounds **1** in the Ames test[7] with different isogenic strains of *Salmonella typhimurium*.[8] This provided evidence that compounds **1**, not withstanding the nitro

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group which is usually considered to lead to genotoxicity, generally show low mutagenicity[8] and some of the compounds examined (**1a**, **c**, **e**, **f**, **q** and **r**) did not show any activity in TA98.[7] Interestingly, **1c** and **e** (non-mutagenic compounds) were indicated[1b] as promising anti-inflammatory drugs.

As the intracellular activation of nitro derivatives appears to occur by bacterial nitroreductase, nitrosoreductase and O-acetyltransferase, that cause their conversion[9] into the ultimate mutagenic species,[8,10] we are now carrying out a study of their electrochemical reduction. Both electrochemical and biological (nitro)reductions depend on the structure of the organic skeleton to which the nitro group is linked, therefore, the results of a cyclic voltammetric study of the thermodynamics of the reduction of compounds **1** can give a preliminary insight into the mechanism of reduction.

2. Results and discussion

The series of compounds studied includes several *meta*- and *para*-substituted (2-nitrobenzo[*b*]thiophen-3-yl)phenyl amines (**1a**–**r**): the aim of our work was that of evaluating only the electronic effects of a given substituent. *ortho*-Substituted amines have not been taken into account, in order to avoid the superimposition of steric effects. Thus, besides the unsubstituted compound **1a**, i.e. the reference compound, we have tested derivatives containing halogens (**1b**–**g**: F, Cl and Br), trifluoromethyl (**1h**, **i**), methyl, hydroxy and methoxy (**1j**–**o**), dimethyl- and diethylamino (**1p**–**r**) groups. The electronic properties of the chosen substituents[11] range from that of the strongly electron-withdrawing trifluoromethyl (σ_m +0.46; σ_p +0.53) to those of the strongly electron-donating *para*-methoxy (σ_p −0.28), *para*-hydroxy (σ_p −0.38) and *para*-dialkylamino (σ_p −0.63) groups. Additional substituents with intermediate effects have also been examined: moderately electron-withdrawing substituents like halogens (σ_m and σ_p from +0.06 to +0.37), *meta*-hydroxy and *meta*-methoxy groups (σ_m +0.13 and +0.10, respectively), moderately electron-donating substituents like *meta*-dimethylamino (σ_m −0.10) and *meta*- and *para*-methyl (σ_m −0.06 and σ_p −0.14, respectively) groups.

The redox properties of the examined compounds were studied by cyclic voltammetry at different potential scan rates (v), cycling the potential between −0.6 and −1.2 V. All the compounds tested displayed reversible cathodic–anodic peak systems when observed at high enough potential scan rates, varying at varying the nature of the compound.

The half-wave potential $E_{1/2}$, which is an excellent approximation of the formal potential $E^{\circ\prime}$, and, consequently, of the standard potential, E°, of the redox couple involved in the one-electron reduction reaction, could be hence obtained as the half-sum of the cathodic and anodic peaks involved in the process (see Table 1). The reported values are the averages of four repeated tests, actually of eight measurements, also considering the half-sum of maximum and minimum values of the responses deconvoluted from diffusion,[12]

Table 1. Reduction potentials obtained from CV for the tested compounds and substituent constants used for the LFER

Substituent (σ)[a]	$E_{1/2}$ (mV) at v=500 mV s^{-1}	$E_{1/2}$ (mV) at v=200 mV s^{-1}
X=H (0.00)	−898.0	Not estimated
X=*m*-F (0.34)	−858.0	−856.0
X=*p*-F (0.36)	−901.0	−901.5
X=*m*-Cl (0.37)	−858.0	−856.0
X=*p*-Cl (0.22)	−875.5	−874.0
X=*m*-Br (0.37)	−853.0	Not estimated
X=*p*-Br (0.22)	−873.5	Not estimated
X=*m*-CF_3 (0.46)	−848.0	−850.0
X=*p*-CF_3 (0.53)	−829.0	−825.5
X=*m*-Me (−0.06)	−905.0	−904.0
X=*p*-Me (−0.14)	−921.0	−920.5
X=*m*-OH (0.13)	−898.5	−901.5
X=*p*-OH (−0.38)	−965.5	−967.0
X=*m*-OMe (0.10)	−889.0	−889.0
X=*p*-OMe (−0.28)	−946.5	−945.0
X=*m*-NMe_2 (−0.10)	−919.0	−921.0
X=*p*-NMe_2 (−0.63)	−983.0	−984.0
X=*p*-NEt_2 (−0.63)	−988.0	−988.0

[a] Substituent constants from Ref. 11.

that could be directly computed by the software available on the electrochemical instrument. As an example, Fig. 1 shows the I vs. E and the relevant semidifferential responses for the case of **1l**. When the peak current ratio was equal to one, the values from the tests performed at 200 mV s^{-1} potential scan rate were also taken into account, together with those computed at 500 mV s^{-1} scan rate: a student's t test between $E_{1/2}$ at v=500 and 200 mV s^{-1} indicated an excellent agreement between the thermodynamic estimates computed under the two different conditions. It should be noted that the standard deviation of the data samples resulted in each case in the order of 1 mV.

On the basis of these $E_{1/2}$ values, one can see that the tendency to reduction is strongly dependent on the electronic nature of the substituent. For instance, the $E_{1/2}$ value of **1o** bearing a strong electron donor group like *p*-OMe is more negative by about 50 mV with respect to the potential value of the reference, unsubstituted compound, providing evidence for the increased difficulty of the nitro group reduction; on the contrary, substitution with a withdrawing substituent like *p*-CF_3 (**1i**) leads to an $E_{1/2}$ value more positive by about 70 mV.

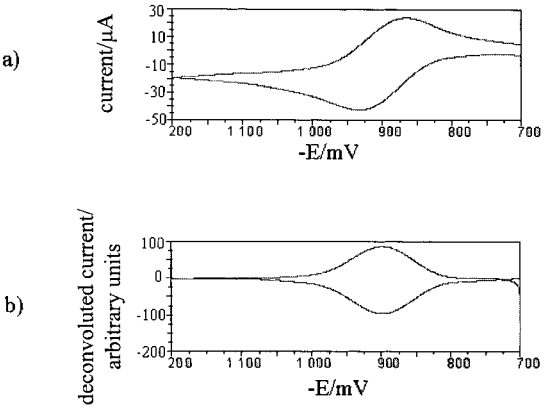

Figure 1. Cyclic voltammogram (a) and relevant semidifferential response (b) obtained for compound **1l**.

These results agree with previous literature data.[13–17] The polarographic reduction of several series of nitroaromatics as a function of the present substituents has been studied widely, observing the occurrence of linear free energy relations (LFERs) with a positive susceptibility constant (the so called reaction constant: i.e. $\rho>0$). In particular, Tirouflet et al. investigated the polarographic behaviour of some series of nitroheterocycles,[13–16] developing techniques that were also used largely for analytical purposes. Thus, they studied some 2-X-4-(*meta*-like) and -5-nitrothiophenes (*para*-like), pointing out that half-wave potentials could be linearly correlated with those of *meta*- and *para*-substituted nitrobenzenes.[13b] Similar results were obtained for other nitroheterocycles, e.g. pyrroles,[14–16] pyridines[13a]. By plotting $\Delta E_{1/2}$ $[(E_{1/2})_X-(E_{1/2})_H$; in mV] of nitrothiophenes, determined by Tirouflet,[13b] vs. Hammett substituent constants (σ_m or σ_p), we calculated for *meta*- and *para*-like isomers, a poor [ρ_m 189(61), *i* 8(25), *r* 0.872, *n* 5] and an acceptable [ρ_p 276(41), *i* 14(17), *r* 0.941, *n* 8] relationships, both with high susceptibility constants. Recently, Squella et al.[17] reported an electrochemical study of the electronic effect of different substituents on the reduction potential of a series of β-nitrostyrenes, pointing out the occurrence of a LFER.

The calculated $\Delta E_{1/2}$ measured by us at v=500 mV s^{-1}, have been plotted vs. Hammett constants (σ_m and σ_p). The results obtained in the voltammetric reduction lead to an excellent LFER, according to:

$$\Delta E_{1/2} = 134(4)\sigma_{m,p} - 7(1),\ r = 0.9935,\ n = 18.$$

Taking into account the relatively distant position of the substituents with respect to the nitro group, the calculated susceptibility constant value appears rather high (a ratio <2 has been observed with nitrothiophenes containing the *meta* or *para* substituent and the nitro group in the same ring).[13b] This observation confirms the ability of the –NH– bridge to powerfully transmit the substituent effects, as already pointed out by ^{13}C NMR data.[5]

Moreover, $\Delta E_{1/2}$ values can be related to SCS, calculated for C-2 of **1**, giving excellent [*s* 16(1), *i* −2(1), *r* 0.9938, *n* 9] and rough [*s* 19(3), *i* −12(6), *r* 0.93, *n* 10] cross-correlations for *meta*- and *para*-substituted amines **1**, respectively. This observation agrees with the fact that $\Delta E_{1/2}$ of *meta*- and *para*-substituted amines **1** led to excellent LFERs with Hammett substituent constants (σ_m and σ_p); in contrast, SCS values showed a dual behaviour: *meta*-substituted amines gave good LFER with Hammett substituent constants (σ_m) and *para*-substituted amines with modified Hammett substituent constants (σ_p^-, through-resonance being operative), respectively.

At first glance, the occurrence of such LFERs seems in contrast with the results observed for mutagenic activity, where no significant FER can be found. However, this can be related with the complexity of the multi-step biological processes, for which the physico-chemical properties of the active product 'are directly related to the intermolecular forces involved in the drug–receptor interactions, as well as to the transport and distribution properties of drugs.'[18] Inversely, the chemical processes studied are comparatively simple. Indeed, one can argue that modifications in the chemical structure of the organic skeleton linked to the nitro group differently affect the several stages of the biological reduction, thus determining the absence of any FER. It is well-known that, while the chemical reactivity is usually dominated by the occurrence of one- or two-parameters FERs, biological activity often follows quantitative structure activity relationships (QSAR), requiring multi-parameter regressions, often including lipophilicity, polarisability and topological parameters, besides electronic and steric constants.[18] Therefore, the redox potential measurements of compounds **1** must be considered the first step towards understanding their biological activity.

3. Experimental

3.1. Chemicals

All chemicals were of reagent grade and were used without further purification. Anhydrous 99.8% pure dimethylsulfoxide (DMSO) and tetraethylammoniumtetrafluoroborate ($TEABF_4$) were obtained from Aldrich. Before use, $TEABF_4$ was dried under vacuum at 45°C for 48 h. The synthesis of the compounds **1** tested in this study has been reported previously.[1,5]

3.2. Electrochemical measurements

All the electroanalytical measurements were performed by using an Autolab PGSTAT20 (Ecochemie, Utrecht) instrument, interfaced with a personal computer, using the GPS software (Ecochemie) for waveform generation and data acquisition and elaboration. The experiments were carried out in a single-compartment three-electrode cell under nitrogen atmosphere, at room temperature, in DMSO solution with 0.1 M $TEABF_4$ as supporting electrolyte, containing the compound under investigation at 3–4 mM concentration. Cyclic voltammetric tests were performed with an Hg working electrode, obtained by dipping an Au disk electrode, 0.071 cm^2 geometric area, sealed inside a teflon tube, into three-times distilled Hg. A platinum wire was the counter electrode and an aqueous KCl saturated calomel electrode (SCE) was the reference electrode. All potential values (in mV) reported are quoted vs. SCE, and the same reference electrode was used in any test. The cyclic voltammograms were recorded at different potential scan rates, from 50 to 1000 mV s^{-1}, performing the iR drop compensation by positive feedback.

Electrochemical reversibility was ascertained on the basis of (i) the ratio of cathodic to anodic peak currents and (ii) the value of the potential difference between forward and associated backward peaks. Deviations of the peak current ratio from unity are indicative of homogeneous kinetics following the charge transfer, and ΔE_p values higher than 60 mV, one electron per molecule being involved in the reduction, are typical for a non-reversible character of the charge transfer. Actually, in the absence of iR drop compensation, the peak potential separation increases gradually, as the scan rate increases, but application of positive feedback compensation makes ΔE_p values decrease within the range 54–64 mV, i.e. to values diagnostic for a Nernstian reaction. As to the peak current ratio, it was necessary in some

cases, to increase the potential scan rate up to 500 mV s^{-1}, in order to achieve the conditions of 'chemical reversibility', which means that the reduced product does not react significantly on the time scale of the experiment. Finally, the cathodic peak currents (I_{pc}) were found to increase linearly with $v^{1/2}$ (v=potential scan rate), indicating that the reduction process is under diffusion control.[19]

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