Tetrahedron Vol. 49, No. 33, pp. 7317-7324, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

An EPR Study of the Radicals from 5-Nitrothiophenecarboxamides: a Novel Group of Direct Acting Mutagens

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(*Received in UK* 4 *June* 1993)

Key words: Radical anions, nitrothiophenecarboxamides, EPR.

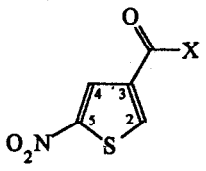
Abstract: The title compounds have been reduced to the corresponding radical anions by means of potassium *tert*-butoxide in dimethylsulphoxide solution, and their EPR spectra have been recorded. In the instance of *meta*-like isomers **1-7** the spectrum of initially formed radical anion is replaced by that of a second radical which after some time remains the only observable species. On the basis of the spectral parameters it is suggested that the secondary species are the radical anions of 3-carbamoyl-5-nitrothiophene-2-carboxylates substituted at the amidic function.

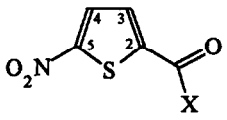
Metronidazole (1-hydroxyethtyl-2-methyl-5-nitroimidazole) is one of the best drugs for the treatment of several protozoal diseases and of infections due to anaerobic bacteria.[1-3] Antiprotozoal and antibacterial activity is also exerted by a variety of nitroheterocyclic compounds whose use as drugs is now well established,[4] but a drawback of these compounds when used as chemotherapeutic agents is their mutagenic activity.[5] The pharmacological activity of several thiophenes has also been tested and some of them are presently being used as drugs.[6]

A new series of recently synthesized 5-nitrothiophene-3-carboxamides is now undergoing pharmacological tests and their direct-acting mutagenicity in Salmonella typhimurium has been checked,[7] indicating that the mutagenic activity is largely dependent on bacterial nitroreductase.

Nitroaromatic compounds, because of their characteristic high electron affinity, readily undergo redox processes when put in a physiological environment. These processes usually lead to the initial formation of negatively charged radical species of the nitrocompounds, which may subsequently evolve to differently functionalized derivatives such as nitroso compound, hydroxylamines and their acetoxyderivatives, amines or nitrenium ions.[8,9] In order to investigate the chemical course of the reduction process of the active mutagens 5-nitrothiophene-3-carboxamides[7] and more generally to ascertain the effects of the amidic nitrogen atom

substituents on the electronic properties and the reactivity of the corresponding radical anions, we have therefore carried out an EPR spectroscopic study on 5-nitrothiophene-3- (**1-7**) and -2-carboxamides (**8**) as well as on 5-nitrothiophene-2-carboxylic acid (**9**).





1; X = piperidin-1-yl
2; X = morpholin-1-yl
3; X = NHPh
4; X = NMePh
5; X = NHC_6H_4-2'-NO_2
6; X = NHC_6H_4-3'-NO_2
7; X = NHC_6H_4-4'-NO_2
8; X = piperidin-1-yl
9; X = OH

RESULTS AND DISCUSSION

The substituted 5-nitrothiophenes **1-9** were reduced to the corresponding radical anions either by treatment with potassium *tert*-butoxide (^{t}BuOK) in dimethylsulphoxide (DMSO) solution or by photolyzing deoxygenated methanol/dimethoxyethane (MeOH/DME) solution (1:1, v/v) of the nitrocompounds containing sodium methoxide (MeONa, ca. 1M). In the latter case the reaction sequence leading to the radical anion is exemplified below:

$$ArNO_2 \xrightarrow{h\nu} ArNO_2^*$$
$$ArNO_2^* + MeOH \longrightarrow ArN(O\bullet)OH + \bullet CH_2OH$$
$$ArN(O\bullet)OH + MeO^- \longrightarrow ArNO_2^{-\bullet} + MeOH$$

The EPR spectra recorded in DMSO solution just after mixing the reactants show with compounds **1-7** the presence of two radical species (see Fig. 1), one being the expected radical anion (most likely the same species formed in the biological nitroreduction process) and showing coupling of the unpaired electron (see Table 1) with the nitrogen of the nitrogroup (ca. 9-10 G) and with the two protons in position 4 (ca. 5.8 G) and 2 (ca. 4.7 G), in agreement with previous studies on related derivatives.[10] In the second radical, whose amount increases with time and which eventually (1 hour or less) remains the only observable species, the coupling with the proton in position 2 is missing and the nitrogen splitting is ca. 1 G smaller than that of the primary radical. This indicates that substitution has occurred in position 2 by an electron withdrawing group which does not have hydrogens coupled with the unpaired electron. This behaviour appears to be typical of 5-nitrothiophenes containing an amido substituent in position 3; in fact the reduction of 2-nitrothiophene (**10**) itself under similar experimental conditions only affords the corresponding radical anion. The presence of an amido-group in position 2, on the other hand, does not seem to induce the formation of secondary species, since upon reduction of compound **8** a clean spectrum of the corresponding radical anion, which lasts for at least 24 hours, is observed (see Fig. 2). The same behaviour has been observed for 5-nitrothiophene-2-carboxylic acid (**9**).

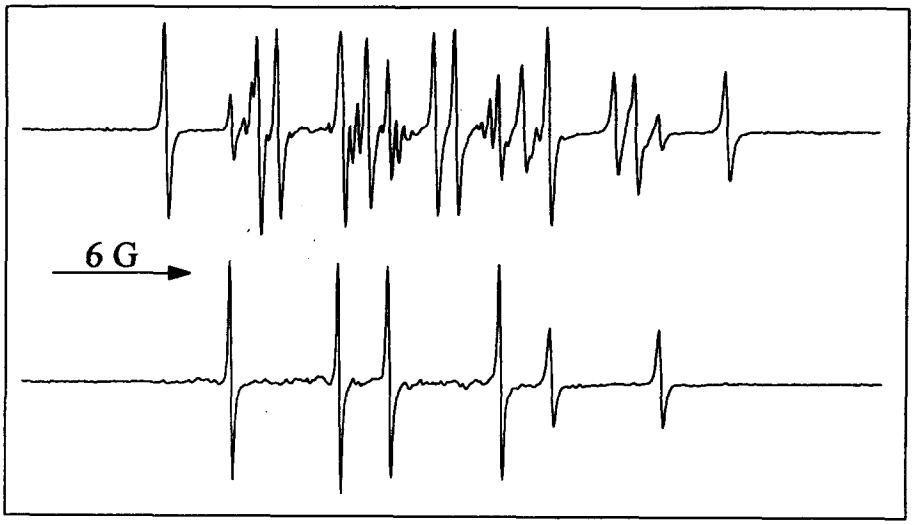
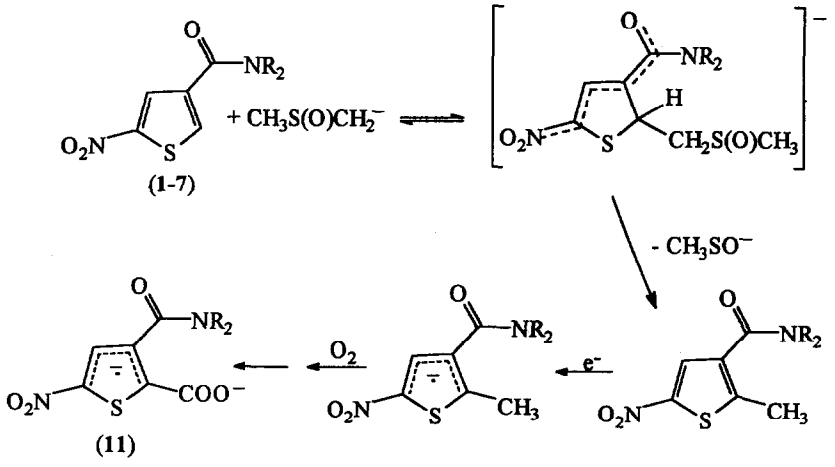


Fig. 1. EPR spectra obtained by reducing 1 with ^{t}BuOK in DMSO soon after mixing the reactants (upper trace) and after 40 minutes from mixing.

The spectral parameters do not allow an unambiguous identification of the secondary radical anion; however, in view of the experimental conditions, we suggest that it results from nucleophilic attack at position 2 of the starting compound, which is strongly activated owing to the simultaneous presence of two strongly electron withdrawing groups in conjugated positions with respect to C-2. Consistently with the mechanism proposed by Russell *et al.*[11] for the formation of secondary species in the reaction of easily reducible compounds with ^{t}BuOK in DMSO, we suggest that the attacking nucleophile is the methylsulphinyl carbanion, $CH_3SOCH_2^-$, and that the observed secondary species is the radical anion **11**, presumably formed as shown in Scheme 1. As far as the last steps in Scheme 1 are concerned, oxidation of the radical anions of nitroarenes bearing a methyl

SCHEME 1



substituent in a conjugated position to the anion of the corresponding acid is a well established process.[12]

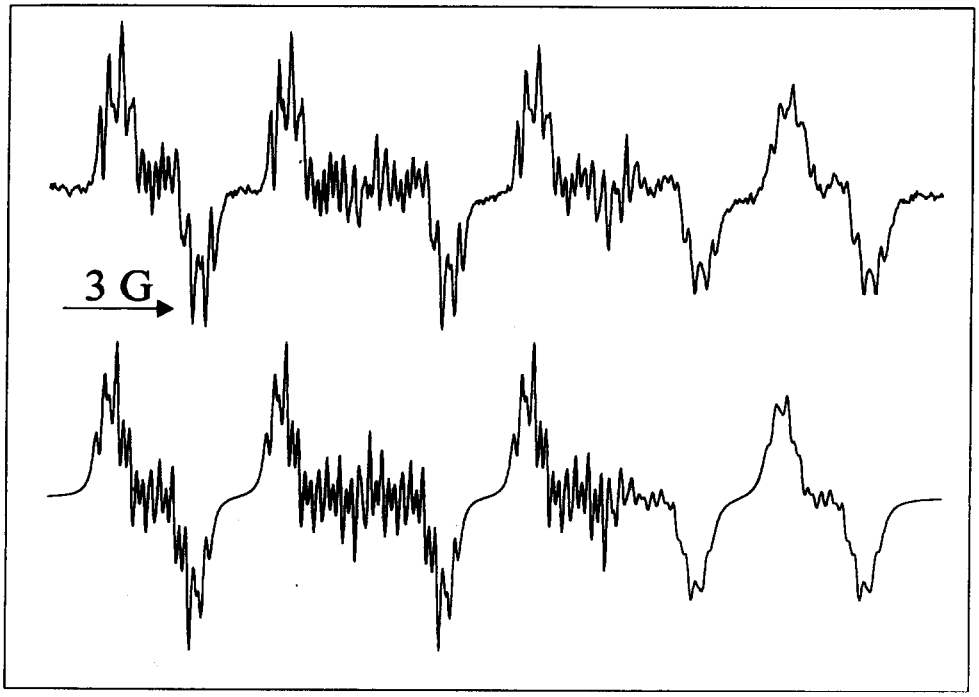
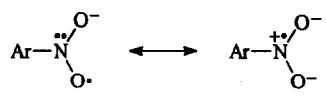


Fig. 2. Experimental (upper trace) and simulated EPR spectrum of the radical anion from **8** in DMSO.

The presence of a carboxylate group in position 2 of the secondary radical is consistent with the value of the nitrogen hyperfine splitting which is smaller by 1-1.3 G than that of the primary radical. Indeed, introduction of a carboxylic group in position 2 induces a decrease of the nitrogen splitting from 10.52 in the radical anion of 2-nitrothiophene (**10**) to 8.94 G in that of 5-nitro-2-thiophenecarboxylic acid (**9**) (see Table 1).

When the reduction is carried out photolytically in alkaline MeOH/DME solutions, only the radical anions of the starting compounds are observed. This means that nucleophilic substitution does not occur, presumably because the attack of MeO^- at the position 2 is highly reversible, since MeO^- is a better leaving group than $CH_3SOCH_2^-$.

An examination of the Tables shows that the proton hyperfine splittings are almost independent on the medium while the nitrogen splittings are larger in MeOH/DME solutions than in DMSO. This is a very well known effect and is to be attributed to the formation of hydrogen bonds in the former medium, which greatly stabilize the mesomeric structure B, where the unpaired electron is delocalized on nitrogen, with respect to A.



(A) (B)

It may also be noticed that the introduction of the electron withdrawing amidic function in 2-nitrothiophene induces a decrease of the nitrogen hfs constant, the effect being very significant if substitution takes place at the *para*-like conjugated position, and smaller but still noticeable if the *meta*-like non-conjugated position is involved. In addition, the decrease of the nitrogen coupling is also quite sensitive to the nature of the amido group, being smaller for C(O)NHR than for $C(O)NR_2$ groups. This reduction of a_N caused by an amido group in the *meta*-like position seems essentially due to inductive effects, since no delocalization of the unpaired electron on the substituent was observed. On the other hand, when the amidogroup is in the *para*-like position, conjugative effects are also important as proved by the detection of hyperfine splittings at the nitrogen and at the two adjacent equatorial and axial protons of the piperidinic unit in the radical anion from **8**.

Table 1. EPR Spectral Parameters (hfs Constants in Gauss) for the Radical Anions of Substituted 5-Nitrothiophenecarboxamides and of 5-Nitrothiophene-2-carboxylic Acid in DMSO/^{t}BuOK.

Compound	Species	a(N)/G	$a(H_4)$/G	$a(H_2)$/G	a_{other}/G	g
1	I	9.03	5.69	4.69	----	2.0050_0
1	II	8.07	5.51	----	----	2.0051_0
2	I	9.11	5.82	4.79	----	2.0050_0
2	II	8.00	5.52	----	----	2.0051_7
3	I	10.05	5.85	4.71	----	2.0049_6
3	II	8.76	5.45	----	----	2.0049_6
4	I	9.10	5.71	4.62	----	2.0050_2
4	II	7.98	5.46	----	----	2.0048_8
5	I	10.03	5.90	4.77	----	2.0049_4
5	II	8.76	5.51	----	----	2.0049_4
6	I	9.89	5.88	4.75	----	2.0050_2
6	II	8.58	5.43	----	----	2.0050_3
7	I	9.73	5.80	4.66	----	2.0049_9
7	II	8.46	5.39	----	----	2.0050_1
8	I	7.32	4.85	----	1.22(H3)[a]	2.0051_8
9	I	8.94	5.42	----	1.34(H3)	2.0051_7
10	I	10.52	5.72	4.77	1.23(H3)[b]	2.0050_8

[a] Additional splittings from 1N 0.41 G, 2H 0.34 G and 2H 0.22 G. [b] The proton hyperfine splitting constants of the 2-nitrothiophene radical anion are reported following the same order of compounds **1-9**, i.e. correspond to 5-nitrothiophene.

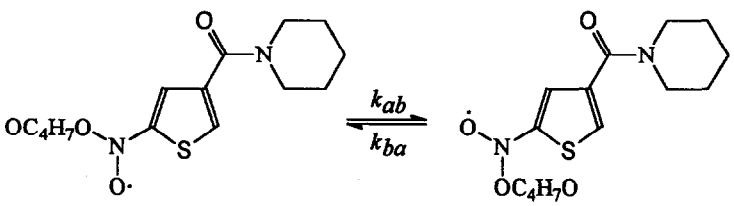
It is also worth pointing out that the hyperfine splitting constants in the radical anions of **5** - **7** are essentially the same as those of the radical anion of **3**. This indicates that reduction occurs preferentially at the nitrothiophene moiety and that the presence of a nitrosubstituted aromatic ring in the amidic group does not affect to any significant extent the spin density distribution. However, the second nitrogroup in **5** - **7** has some

effect on the stability of the radical anions which give EPR spectra much weaker than those of the radical anions containing only the nitrogroup attached to the thiophene ring. This may be due to the fact that these radicals can undergo further reduction to the diamagnetic dianions.

Table 2. EPR Spectral Parameters for the Radical Anions of 5-Nitro-thiophene-3-carboxamides in MeOH/ DME/ MeONa.

Compound	$a(N)$/G	$a(H_4)$/G	$a(H_2)$/G	g
1	11.25	5.77	4.55	2.0049_7
2	11.15	5.77	4.52	2.0049_3
3	11.98	5.90	4.59	2.0048_5
4	11.35	5.83	4.44	2.0049_3
5	12.04	5.88	4.59	2.0049_0
6	12.16	5.90	4.58	2.0048_0
7	12.00	5.92	4.64	2.0048_6

In order to investigate the effect exerted by the amidic function in position 3 on the conformational mobility of the present radicals, we have studied the strictly related 2-tetrahydrofuranyl adduct (**12**), obtained by direct photolysis of compound **1** in tetrahydrofuran solution. The nitroxide **12** may exist in two different conformations **12a** and **12b** giving origin to two distinct EPR spectra.



12a **12b**

$a(N)$	= 13.50 G	$a(N)$	= 13.61 G
$a(H_4)$	= 5.49	$a(H_4)$	= 5.37
$a(H_2)$	= 4.51	$a(H_2)$	= 4.19
$a(H_{THF})$	= 0.40	$a(H_{THF})$	= 0.43

In fact at or below room temperature we could observe signals from both the rotational isomers, in the approximate ratio 3:2. We identified **12a** and **12b** as the more and less abundant species, respectively, on the basis of the relative value of their proton splittings and by analogy with previous data for the related nitroxides from 2-nitrothiophene.[13]

By simulation of the experimental spectrum at 293K we could determine the rate constant for the conformational exchange as $k_{ba}^{293} = 1.2 \times 10^6$ s^{-1}. A comparison with the data available for the triethylsilyloxy nitroxide from 2-nitrothiophene (ratio a/b = 1/0.8, $k_{ba}^{293} = 3.2 \times 10^6$ s^{-1}),[13] indicates that the conformational

stability and the rate constant for isomerization are quite similar in the unsubstituted radicals and in those substituted at the *meta*-like position.

EXPERIMENTAL

Materials - 5-Nitrothiophene-2-carboxylic and 5-nitrothiophene-3-carboxylic acids were prepared by standard procedures,[14] 2-nitrothiophene was purchased from Aldrich and crystallized from ethanol before use, while all other compounds were synthesized as described below.

Synthetic procedures - Compounds **3** - **7** were prepared as reported.[7,15] Compounds **1** (colourless, recrystallized from methanol, mp 100°C), **2** (light yellow, recrystallyzed from methanol-dioxane, mp 158-159°C) and **8** (light yellow, recrystallized from methanol-dioxane, mp 86-87°C) were synthesized following the same procedure from the corresponding 5-nitrothiophenecarboxylic acid chloride and the appropriate amine. The obtained amides gave correct elemental analysis (C, H, N, S), MS, ^{1}H and ^{13}C NMR spectra.

Spectroscopic studies - The radicals from the nitrocompounds were generated either by reacting a DMSO solution of the nitrocompound with dry potassium *tert*-butoxide in a capillary tube (i.d.= 1 mm), or by photolyzing a nitrogen purged MeOH/DME solution of the nitrocompound containing some MeONa in a quartz tube (i.d.= 2 mm) inside the cavity of the EPR spectrometer at room temperature. The spectra were recorded by means of a BRUKER ESP 300 EPR spectrometer, equipped with a NMR gaussmeter (field calibration), a Hewlett-Packard frequency counter (*g*-factor determination), and standard variable temperature accessories. The UV light from a Hanovia 500 W high pressure mercury lamp was used to irradiate the samples when necessary.

Acknowledgement - Financial support from CNR and from MURST (Rome) is gratefully aknowledged by two of us (G.F.P. and D.S.)

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