

***gem*-Nitronitroso Dianion Radicals $R-C'(NO_2)(NO)^{2-}$ Formed in the Reaction between Sodium Nitrite/Ascorbate and the Sulfur Compounds Methionine, (Methylthio)acetic Acid and *S*-Methylcysteine Studied by EPR Spectroscopy**

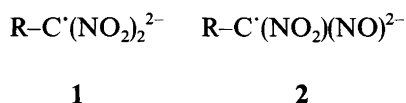
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gem-Nitronitroso dianion radicals $R-C'(NO_2)(NO)^{2-}$ ($R = \text{alkyl}$) are formed in the reaction between sodium nitrite and the sulfur compounds methionine, (methylthio)acetic acid and *S*-methylcysteine dissolved in acid–water solutions containing a small amount of ascorbic acid. The radicals appeared in the dark at room temperature when the reaction mixtures had been made alkaline. The EPR spectra indicated an interaction of the unpaired electron with two non-equivalent nitrogen nuclei and hydrogen nuclei of the parent substances. The reaction involves degradation of the parent substances with cleavage of carbon–sulfur bonds and nitrosation on the carbon atom in juxtaposition to the sulfur atom. This leads to intermediates of the type $R-C(NO_2)(NO)^-$. These intermediates are reduced to the observed radicals by ascorbate when the reaction mixtures are made alkaline.

In a series of papers, it has been reported that dianion radicals of type **1** and **2**, are formed by reduction in alkaline solution of substances such as *gem*-dinitroalkanes and of the corresponding *gem*-nitronitroso compounds, respectively.^{1–6}



In these series R was equal to groups such as NC or NO_2 in addition to alkyl groups.

In the course of EPR studies of radicals formed during photochemical reactions of the nitrite ion with a number of organic species, signals were observed which were attributed to dianion radicals of both types **1** and **2** during the dark reaction between sodium nitrite and a number of carbonyl compounds. These radicals were formed when, with exclusion of light, the parent substances were dissolved with sodium nitrite in aqueous acid containing a small amount of ascorbic acid, and the reaction was then made alkaline.⁷ Radicals were obtained

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with substances such as acetaldehyde, benzaldehyde, furfural, cyclopentanone, cyclohexanone, δ -valerolactam, ϵ -caprolactam, ascorbic acid, glycylglycine, alanyl-glycine, alanylalanine and insulin. The EPR spectra indicated an interaction of the unpaired electron with two nitrogen nuclei. It was considered that the reaction involved nitration–nitrosation on the carbonyl carbon which constituted the central carbon atom in the radicals **1** and **2**. Intermediately formed *gem*-dinitro and *gem*-nitronitroso monoanions were formed, which were reduced to the observed radicals by ascorbate. Cleavage of the carbon–carbon bond or dissociation of a hydrogen atom must be involved in the reactions to obtain the planar sp^2 -hybridization on the central carbon atom which constituted the carbonyl carbon of the parent substances. The EPR spectra indicated that the two nitrogens were non-equivalent in the radicals derived from substances such as acetaldehyde or glycylglycine (nine-line system), i.e., the formation of $R-C'(NO_2)(NO)^{2-}$ (**2**) but equivalent in the radicals derived from substances such as cyclopentanone (five-line system), i.e., formation of $R-C'(NO_2)_2^{2-}$ (**1**).

It has now been found that *gem*-nitronitroso dianion radicals of a structure similar to **2** are formed in the dark

in the reaction between sodium nitrite–ascorbic acid and the sulfur compounds methionine, (methylthio)acetic acid and *S*-methylcysteine. The reaction conditions were the same as those used for the production of the *gem*-dinitro and *gem*-nitronitroso dianion radicals **1** and **2** formed from the carbonyl compounds.⁷ Thus the parent substances were dissolved in H₂O–HCl together with sodium nitrite and a small amount of ascorbic acid. The radicals appeared in the dark at room temperature when the reaction mixtures had been made alkaline. The present paper details the observations obtained with sulfur compounds.

Experimental procedure

Electron paramagnetic resonance (EPR) spectra were recorded using a Varian E-9 spectrometer at 20 °C with a microwave power of 5 mW and a 100 kHz modulation amplitude of 0.02–0.05 mT. The samples were contained in a flat aqueous solution cell. Hyperfine splitting constants were measured by comparison with the splittings of Fremy's radical ($a_{N14} = 1.3$ mT).

Chemicals were obtained from Aldrich and were used as supplied. Na¹⁵NO₂ was from ICON Inc.

N-Acetyl-*S*-methylcysteine was prepared by acetylation of *S*-methylcysteine with acetic anhydride in acetic acid at room temperature, m.p. 157–158 °C.⁸

Preparation of the radicals. The radicals were obtained at room temperature in the dark by suspending 0.1–0.5 mmol of the parent substances and 0.5–1 mmol of NaNO₂ together with ca. 0.1 mmol of ascorbic acid in 50–100 μl of H₂O. Subsequently, 200 μl of 4 M HCl were added followed by 400–500 μl of 4 M NaOH in H₂O after the samples had been allowed to stand for ca. 30 s.

Results

The coupling constants of the radicals are collected in Table 1. All spectra showed line broadening in the high-field part which originated from anisotropy of the *g*-values and coupling constants combined with relatively slow tumbling of the radicals.

Radicals from methionine. Fig. 1(a) shows the EPR spectrum obtained with methionine HOOC–CH(NH₂)–

Table 1. Coupling constants of the radicals in mT.

Parent compound	a_{N14}^1	a_{N14}^2	a_H
Methionine	1.17	0.585	0.169
<i>N</i> -Acetylmethionine	1.17	0.585	0.169
<i>S</i> -Methyl thioacetate	1.21	0.57	—
<i>S</i> -Methylcysteine	1.14	0.61	0.037
	1.41	0.61	0.13
	1.41	0.61	0.28
<i>N</i> -Acetyl- <i>S</i> -methylcysteine	1.05	0.58	0.04
	1.05	0.58	0.1
	1.05	0.58	0.22

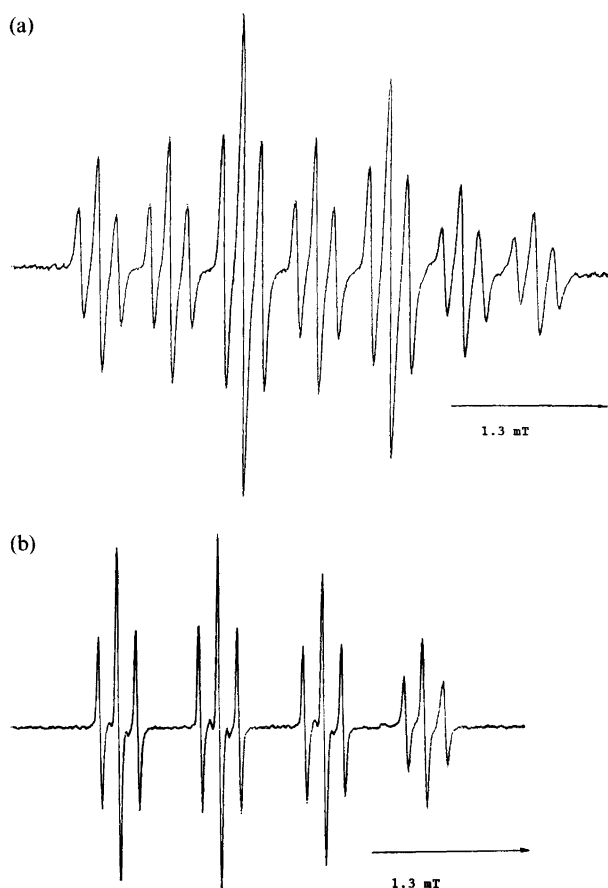


Fig. 1. The EPR spectrum of the radicals formed in the reaction between sodium nitrite and methionine dissolved in an acid–water solution containing a small amount of ascorbic acid. The radicals appeared in the dark at room temperature after the reaction mixture had been made alkaline: (a) with Na¹⁴NO₂; (b) with Na¹⁵NO₂.

CH₂–CH₂–SCH₃. The spectrum consists of seven groups of triplets (1:2:1). The intensity distribution of the seven groups is 1:1:2:1:2:1:1, an arrangement that indicates an interaction of the unpaired electron with two non-equivalent ¹⁴N nuclei, the coupling constants of which are related as 2:1. The coupling constants are $a_{N14}^1 = 1.17$ mT (1 N); $a_{N14}^2 = 0.585$ mT (1 N); $a_H = 0.169$ mT (2 H). When the reaction was carried out with Na¹⁵NO₂, the seven-group spectrum of Fig. 1(a) was replaced by four triplet groups of equal intensity which indicated an interaction with two non-equivalent ¹⁵N nuclei, Fig. 1(b). The coupling constants are $a_{N15}^1 = 1.66$ mT (1 N); $a_{N15}^2 = 0.82$ mT (1 N); $a_H = 0.169$ mT (2 H). These values are consistent with those obtained with Na¹⁴NO₂, i.e., $a_{N15}^1 = 1.4 \times 1.17 = 1.64$ mT; $a_{N15}^2 = 1.4 \times 0.585 = 0.82$ mT, where the factor 1.4 is the ratio between the coupling constants of ¹⁵N and ¹⁴N. These findings indicate that both of the non-equivalent nitrogen atoms are derived from the nitrite ions and none of them from the amino nitrogen of the parent substance.

Radicals from N-acetylmethionine. Spectra similar to those observed with methionine were obtained with

N-acetylmethionine. However, the yield was lower than that obtained with methionine.

Radicals from (methylthio)acetic acid. The spectra obtained with this substance, $\text{HOOC-CH}_2\text{-SCH}_3$, exhibited a nine-line spectrum with $\text{Na}^{14}\text{NO}_2$, and a four-line spectrum with $\text{Na}^{15}\text{NO}_2$, Figs. 2(a) and (b). The coupling constants were $a_{\text{N}^{15}}^1 = 1.70$ mT (1 N); $a_{\text{N}^{15}}^2 = 0.80$ mT (1 N). No secondary splittings were observed. The yield of radicals was high.

Radicals from S-methylcysteine. Whereas the EPR spectra obtained with methionine and (methylthio)acetic acid each showed a single species, the spectra observed with *S*-methylcysteine, $\text{HOOC-CH}(\text{NH}_2)\text{-CH}_2\text{SCH}_3$, exhibited complicated overlaps of several species, the relative proportions of which changed with time of observation. However, the main groups, seven or nine lines with $\text{Na}^{14}\text{NO}_2$ and four lines with $\text{Na}^{15}\text{NO}_2$, were preserved, Figs. 3(a) and (b), whereas the secondary splittings of the main groups changed. After some time the secondary splittings became less complicated. Figure 3(b) shows the spectrum observed ca. 15 min after mixing of the reagents. The secondary splittings consist of a predominant doublet with $a_{\text{H}} = 0.13$ mT, and further doublets of smaller amplitude with $a_{\text{H}} = 0.037$ mT and $a_{\text{H}} = 0.28$ mT. The secondary splittings were almost identical with $\text{Na}^{14}\text{NO}_2$ and $\text{Na}^{15}\text{NO}_2$. The nitrogen coupling constants were: $a_{\text{N}^{15}}^1 = 1.59$ mT; $a_{\text{N}^{15}}^2 = 0.85$ mT.

Radicals from N-acetyl-S-methylcysteine. This substance gave rise to seven–nine-line spectra (^{14}N), the secondary structure of which was dominated by a narrow doublet splitting, $a_{\text{H}} = 0.037$ mT, Fig. 4. This splitting corresponds to that observed with *S*-methylcysteine. The spectrum of Fig. 4 was obtained immediately after mixing of the reagents, and did not change during the time of observation (ca. 20 min).

The g -value of the radicals of this series was ca. 2.0043 as evaluated by comparison with the g -value of the ascorbic radical ($g = 2.0043$).⁹

No radicals were obtained with substances such as diethyl sulfide, possibly because of its low solubility in water solutions.

Reaction mechanism. It is considered that the radicals formed in the reaction between sodium nitrite–ascorbic acid and the sulfur compounds of this series are dianion nitronitroso radicals $\text{R-C}(\text{NO}_2)(\text{NO})^{2-}$. They are of the same type as those formed in the reaction between sodium nitrite–ascorbic acid and a number of carbonyl compounds as described above. The reaction which gave rise to the radicals $\text{R-C}(\text{NO}_2)(\text{NO})^{2-}$ from the sulfur compounds is suggested to involve degradation of the parent substances with *S*-nitrosation^{10–12} and breaking of carbon–sulfur bonds. The reaction which gave rise to the radicals from methionine can be formulated as follows.

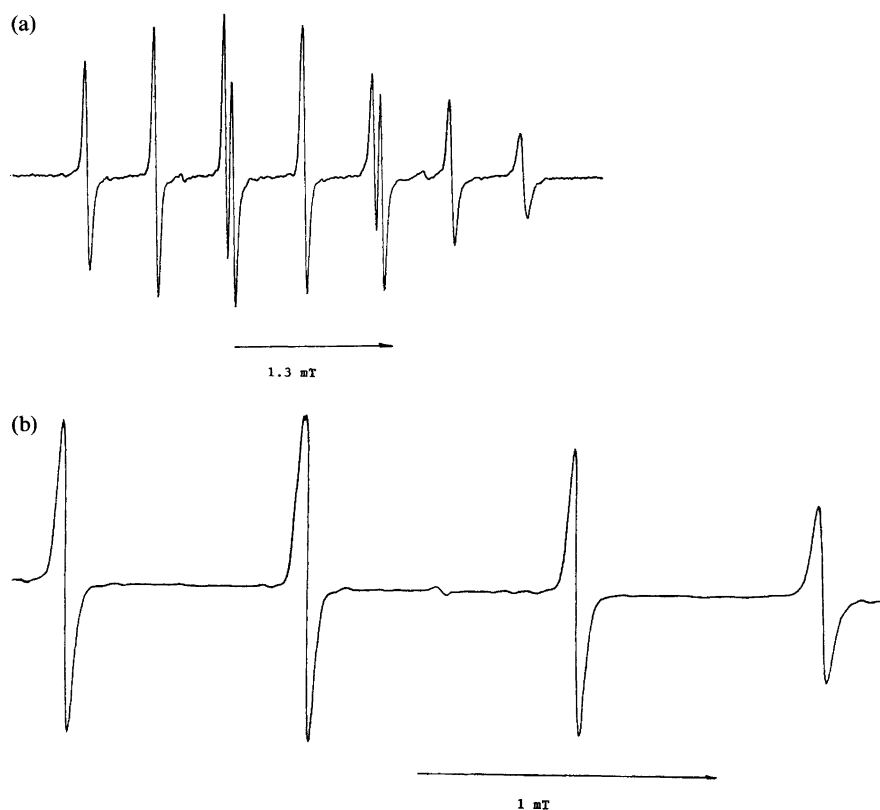


Fig. 2. The EPR spectrum obtained with (methylthio)acetate: (a) with $\text{Na}^{14}\text{NO}_2$; (b) with $\text{Na}^{15}\text{NO}_2$.

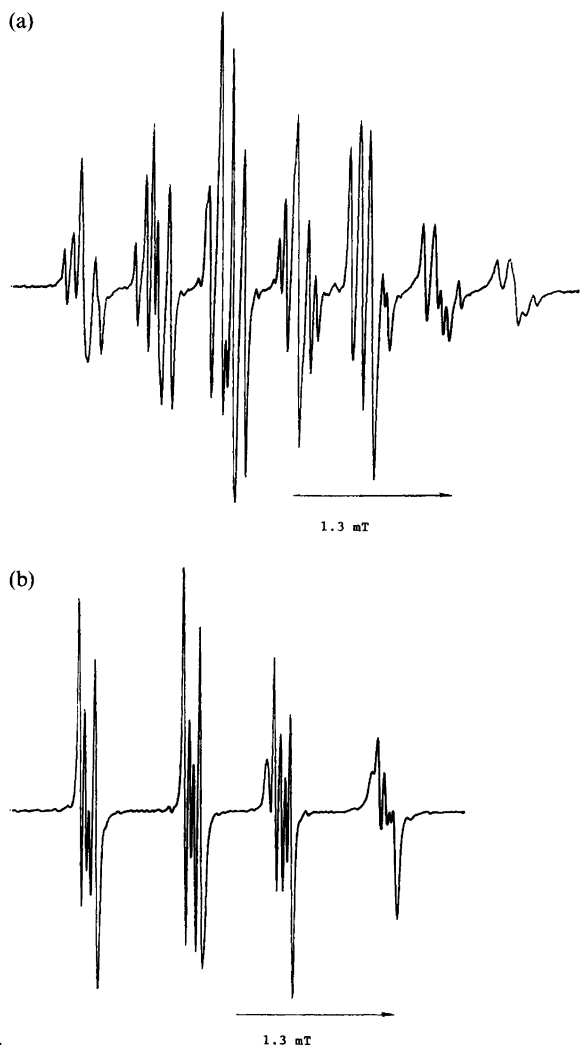
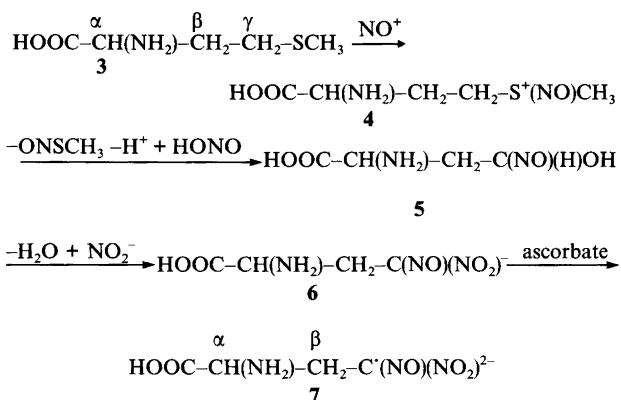


Fig. 3. Spectrum obtained with *S*-methylcysteine: (a) with $\text{Na}^{14}\text{NO}_2$ ca. 4 min after mixing of the reagents; (b) with $\text{Na}^{15}\text{NO}_2$ 15 min after mixing.



The reaction involves *S*-nitrosation of **3**, followed by cleavage of the bond between sulfur and the carbon atom in γ -position. Addition of HONO followed by elimination of water and addition of NO_2^- gives the *gem*-nitronitroso monoanion **6** which is reduced by ascorbate

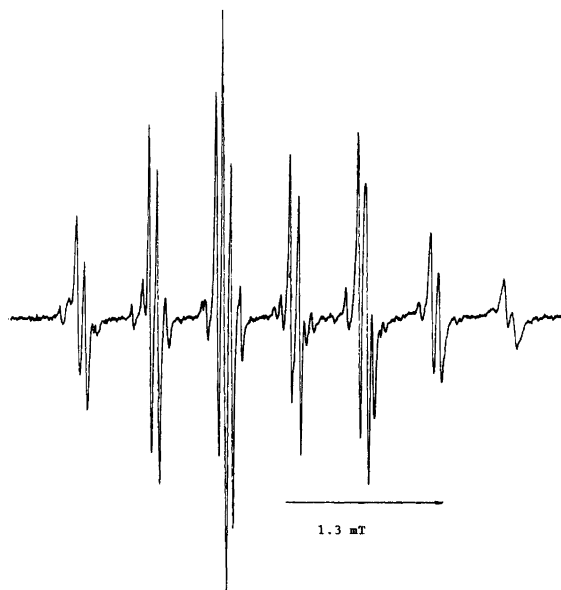


Fig. 4. Spectrum obtained with *N*-acetyl-*S*-methylcysteine and $\text{Na}^{14}\text{NO}_2$.

in the alkaline reaction to produce the observed radical **7**. The analogous formulae for the radicals derived from (methylthio)acetic acid **8** gave the radical **9**.



As expected no secondary splittings were observed.

The interpretation of the radical spectra obtained with *S*-methylcysteine is more complicated. All spectra [Figs. 3(a) and (b)] observed with $\text{Na}^{14}\text{NO}_2$ or $\text{Na}^{15}\text{NO}_2$ at different times after preparation of the reaction mixtures, indicated an interaction with two non-equivalent nitrogen nuclei, i.e. seven- or nine-line groups with ^{14}N and four groups with ^{15}N . This means that the structural element $-\overset{\alpha}{\text{C}}(\text{NO}_2)(\text{NO})^{2-}$ is preserved in the radicals derived from *S*-methylcysteine. The secondary spectral splittings associated with the main seven-nine or four groups are considered to originate from interactions with nuclei in the α -position. It is suggested that the α -amino group and the α -hydrogen now in juxtaposition to the central carbon carrying the nitro and nitroso groups are involved in a number of reactions with the nitrite reagents including formation of transient diazonium ions and deamination that leads to the secondary splittings in the spectra, Figs. 3(a) and (b). These spectra and the spectrum of Fig. 4 might indicate the presence of three different species, each of which gives rise to a doublet splitting. However, the structures of such species or a possible mutual connection between these secondary splittings are not known.

Similar reactions are also expected to take place with methionine, but are not recognized in the spectra, since the methylene group in the β -position is preserved, the

hydrogens of which constitute the secondary triplet splittings shown in spectra of Figs. 1(a) and (b).

Alternative structures should also be considered, such as the formation of diazenyl radicals of the type $A-N\equiv N\cdot$. However, such radicals are unstable above ca. -100°C .¹² Furthermore, these radicals are characterized by a very low g -value, ca. 2.0002. Therefore, the formation of any diazenyl radicals can be ruled out.

Conclusions

The present and earlier⁷ experiments have shown that *gem*-dinitro and *gem*-nitronitroso dianion radicals $R-C'(NO_2)(NO)^{2-}$ and $R-C'(NO_2)(NO)^{2-}$ are formed in the reaction between sodium nitrite-ascorbic acid and a number of carbonyl and sulfur compounds. The radicals are formed at room temperature in the dark when the reactants are dissolved in acid-alkaline solutions. The reaction involved degradation of the parent compounds with cleavage of carbon-carbon or carbon-sulfur bonds.

The radicals discussed here are of the same type as those produced by reduction of the corresponding univalent anions.¹⁻⁶ The nitrogen coupling constants of all these radicals are similar, i.e., for the dinitro radicals: $a_{N14} = 0.90-1.20$ mT (2 N), and for the nitronitroso radicals: $a_{N14}^1 = 1.00-1.20$ mT (1 N) and $a_{N14}^2 = 0.50-0.60$ mT (1 N). The EPR spectra of the radicals concerned often exhibit secondary splittings characteristic of the parent substances by interactions with hydrogen and nitrogen nuclei of the group R (1 and 2).

S-Methylcysteine gave rise to spectra with complicated secondary EPR splittings with a number of overlaps from different species which changed their proportions during the time of observation. At this time it is not possible to assign these splittings reliably to structures.

Finally, it should be emphasized that the nitro-

nitrosation involved the methylene group in juxtaposition to the sulfur atom in the reaction of the sulfur compounds, whereas the carbonyl carbon was considered to give rise to the central carbon atom in the reaction of the carbonyl substances.⁷ In view of the results obtained with the sulfur compounds, it seems possible that methylene groups in an α -position to the carbonyl group might be involved at least in some of the reactions of the carbonyl substances. Further experiments are needed to elucidate this problem.

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