PERKIN 2

gem-Dinitro and *gem*-nitronitroso dianion radicals formed in the reaction between sodium nitrite–ascorbate and carbonyl compounds such as aldehydes, alicyclic ketones, lactams and peptides. An EPR study

Carl Lagercrantz†

Department of Medical Physics, University of Göteborg, S-413 90 Göteborg, Sweden

***gem*-Dinitro and *gem*-nitronitroso radical dianions $R–C^{\bullet}(NO_2)_2^{2-}$ or $R–C^{\bullet}(NO_2)(NO)^{2-}$ (R = alkyl, aryl) are formed in the reaction between sodium nitrite and carbonyl compounds such as acetaldehyde, benzaldehyde, furfural, cyclopentanone, cyclohexanone, lactams, some dipeptides or insulin dissolved in aqueous acid 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 nitrogen nuclei, and also with either a hydrogen or a further nitrogen nucleus in the parent substances. The two nitrogens were non-equivalent in the radicals derived from substances such as acetaldehyde or glycylglycine (nine-line system) owing to the formation of $R–C^{\bullet}(NO_2)(NO)^{2-}$, but equivalent in the radicals from substances such as cyclopentanone (five-line system) owing to the formation of $R–C^{\bullet}(NO_2)_2^{2-}$. The reaction involves nitration/nitrosation of the carbonyl carbon in acid solution with cleavage of C–C bonds or dissociation of a hydrogen ion leading to intermediate nitronitroso or dinitro compounds which are reduced by ascorbic acid to give the observed radicals when the reaction mixtures are made alkaline.**

It has been reported in a series of papers that reduction in alkaline solutions of *gem*-dinitroalkanes, and of the corresponding nitro–nitroso compounds, results in the formation of long-lived radical dianions of the type **1** and **2**, respectively.[1-6] In this series, the substituent group R was H–, D–, H_3C–, $H_3CCOCH_2CH_2$–, $H_2NCOCH{=}CH$–, NC–, H_2NCO–, C_2H_5OOC–, NO_2– or aryl. Radicals **1** and **2** were assumed to be π-electron radicals with planar sp^2-hybridization at the central carbon atom.

$R–C^{\bullet}(NO_2)_2^{2-}$
1

$R–C^{\bullet}(NO_2)(NO)^{2-}$
2

Recently, in the course of EPR studies of paramagnetic species formed during photochemical reactions of the nitrite ion with a number of organic species, signals have been observed which are attributed to radical dianions of both types **1** and **2** during the dark reaction between sodium nitrite and a number of carbonyl compounds. These radicals are formed when, with the exclusion of light, the parent substances are dissolved in sodium nitrite in aqueous acid containing a small amount of ascorbate and the solution is then made alkaline. The present paper details these observations and suggests structures for the species observed.

Radicals were obtained with substances such as acetaldehyde, benzaldehyde, furfural, cyclopentanone, cyclohexanone, δ-valerolactam, ε-caprolactam, ascorbic acid, glycylglycine, alanylglycine, alanylalanine and insulin. The EPR spectra indicated an interaction of the unpaired electron with two equivalent, or two non-equivalent ^{14}N nuclei. It is believed that the reaction involves nitration/nitrosation of the carbonyl carbon which will constitute the central carbon atom in radicals **1** and **2**. *gem*-Dinitro and *gem*-nitronitroso mono-anions are formed as intermediates and are reduced by ascorbate to the observed radicals. Cleavage of C–C bonds or dissociation of a hydrogen atom must be involved in the reactions. Alternative structures to **1** and **2** have been considered. However, no such structures were found to constitute a realistic interpretation of the observed EPR spectra.

Experimental

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 0.05 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 ($a_N = 1.30$ mT).

The chemicals used were obtained from Aldrich and were used as supplied. $Na^{15}NO_2$ was from ICON Inc. Bovine pancreas insulin was from Sigma. 2,5-Dimethylcyclohexanone was from Pfalz & Bauer.

Results and discussion

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 $NaNO_2$, together with *ca.* 0.1 mmol of ascorbic acid in 50–100 µl of H_2O (1 µl = 1 mm^3). Subsequently, 200 µl of 4 mol dm^{-3} HCl was added followed by 400–500 µl of 4 mol dm^{-3} NaOH in H_2O after the samples had been kept standing for *ca.* 30 s. Immediately after the samples had been prepared, the EPR spectra showed the presence of several radical species with complicated overlaps. However, after a few minutes one single radical species was generally found to dominate. The coupling constants of the radicals are collected in Table 1. The EPR spectra are shown in Figs. 1–8.

EPR spectra

The EPR spectra indicated an interaction of the unpaired electron with two nitrogen nuclei. Some of the spectra exhibited seven or nine lines which indicated non-equivalence of the nitrogens in those radicals, *i.e.* the radicals obtained with acetaldehyde, benzaldehyde, butan-2-one, 3-methylbutan-2-

---

† Present address: Änggårdsplatsen 2, S-413 19 Göteborg, Sweden.

Table 1 Coupling constants of the radicals $R–C^{\bullet}(NO_2)_2{}^{2-}$ and $R–C^{\bullet}(NO_2)(NO)^{2-}$ formed in the reaction between sodium nitrite–ascorbate and some carbonyl compounds

Parent compound	a^1_N/mT	a^2_N/mT	a_H or a_N/mT	Number of main groups
Acetaldehyde	1.19	0.60	0.26 (1 H)	9
Benzaldehyde	1.18	0.57	—	7
Furfural	1.17	0.60	—	7
Butan-2-one	1.11	0.62	0.26 (1 H)	9
3-Methylbutan-2-one	1.11	0.64	0.26 (1 H)	9
Cyclopentanone	0.99	0.99	0.20 (2 H)	5
Cyclohexanone	0.95	0.95	0.20 (2 H)	5
2,5-Dimethylcyclohexanone	0.95	0.95	0.20 (2 H)	5
ε-Caprolactam	1.22	0.60	0.17 (2 H)	7–9
δ-Valerolactam	1.20	0.57	0.20 (2 H)	7
Ascorbic acid	1.20	0.52	—	9
Glycylglycine	1.10	0.63	0.04 (1 N)	9
Alanylglycine	0.95	0.95	0.07 (1 N)	5
Alanylalanine	0.94	0.94	0.07 (1 N)	5
Insulin	1.14	0.63	—	9

one, δ-valerolactam, ε-caprolactam, ascorbic acid and glycylglycine. The radicals obtained with cyclopentanone, cyclohexanone, 2,5-dimethylcyclohexanone, alanylglycine and alanylalanine gave rise to five-line spectra which indicated equivalence of the two nitrogens. The g-values of the radicals of this series were *ca.* 2.0046, as evaluated by comparison with the g-value of the ascorbate radical ($g = 2.0046$).[7]

Generally, the radicals were rather unstable and recording could not be performed for more than *ca.* 10 min. The somewhat lower spectral amplitudes at higher magnetic field are in part due to decay of the radicals during the recording from low to high field, but are also connected with line-broadening produced by anisotropy of g-values and coupling constants.

Structure and formation of the radicals

The radicals of the present series are considered to have the same structure as those of the series of divalent radical anions $R–C^{\bullet}(NO_2)_2{}^{2-}$ **1** and $R–C^{\bullet}(NO_2)(NO)^{2-}$ **2** formed by reduction of the corresponding monovalent anions with glucose when dissolved in 1 mol dm^{-3} NaOH, as described in the introduction.

The nitrogen coupling constants of the earlier described radicals, **1** and **2**, and those of the present study are very similar, *i.e.* $a_{N14} = 0.8–1.4$ mT (2 N) for the dinitro radicals and $a^1_{N14} = 1.0–1.2$ mT (1 N) and $a^2_{N14} = 0.5–0.6$ mT (1 N) for the nitronitroso radicals.

The present radical series is an extension of the former series to a direct, or *in situ*, formation of *gem*-dinitro or *gem*-nitronitroso radical dianions by nitration/nitrosation of the parent carbonyl compounds followed by reduction with ascorbate. Evidently, such reactions must involve cleavage of C–C bonds or dissociation of a hydrogen atom to obtain the planar sp^2-hybridization at the carbon atom, *i.e.* the carbonyl carbon, with three groups attached to this atom. Tentative reaction formulae might be as follows where R^1, R^2 are H, alkyl or aryl.[8–10]

$$R^1R^2C{=}O \xrightarrow[+HONO]{+Acid} R^1R^2C(OH)NO_2 \qquad (1)$$

$$R^1R^2C(OH)NO_2 \xrightarrow[+NO_2^-]{-OH^-} R^1R^2C(NO_2)_2 \qquad (2)$$

$$R^1R^2C(NO_2)_2 \xrightarrow[-HOR^1]{+OH^-} R^2C(NO_2)_2^- \qquad (3)$$

$$R^2C(NO_2)_2^- \xrightarrow{+e^-} \underset{\mathbf{1}}{R^2C^{\bullet}(NO_2)_2^{2-}} \qquad (4)$$

The reactions involve addition of HNO_2 to the parent compound [eqn. (1)] followed by exchange of OH for the second nitro group, [eqn. (2)]. The C–C cleavage or dissociation of a hydrogen atom takes place when the reaction mixture had been made alkaline [eqn. (3)], a reaction in which the monovalent anions are formed. Finally, the divalent radical anions are formed by reduction with ascorbic acid [eqn. (4)]. The *gem*-nitronitroso compounds might be produced by reduction of one of the nitro groups. It is also possible that the second nitrogen-containing group is introduced as a nitroso group to give the *gem*-nitronitroso compound directly.

Alternatively, a methylene carbon in juxtaposition to the carbonyl group could be converted into the central carbon atom of the radicals. Such a reaction might be considered for the radicals produced from the alicyclic ketones and lactams (see below). However, it was found that 2,5-dimethylcyclohexanone gave rise to a five-line main system with each of the lines split into a triplet (1:2:1) due to interaction with two methylene hydrogens. This finding supports the statement that the carbonyl carbon is converted into the central carbon atom and not the methylene carbon in juxtaposition to the carbonyl carbon. The latter alternative should have given rise to a secondary doublet splitting and not to a triplet which was actually found. The reaction with alanylalanine $CH_3CH(NH_2)CONHCH(CH_3)CO_2H$ and sodium nitrite gave rise to a five-line system with each of the main groups split into a triplet 1:1:1. This finding supports the statement that the carbonyl carbon of the amide bond is converted to the central carbon of the radical since no $-CH_2-$ group is present in this dipeptide.

An alternative structure of the observed radical species might involve the formation of diazenyl radicals of the type $Ar–N{=}N^{\bullet}$.[11] However, such radicals are unstable over *ca.* −100 °C. Their ^{14}N coupling constants a_1 and a_2 are non-equivalent. Furthermore, these radicals are characterized by a very low g-value, *ca.* 2.0002. Therefore, the formation of any diazenyl radicals could be ruled out.

EPR spectra and structure of the radicals formed from some carbonyl compounds

It should be pointed out that the structure of the 'tails' of the group R formed after the cleavage of C–C bonds discussed below is rather uncertain for some of the species.

Radicals from acetaldehyde

Fig. 1 shows the EPR spectrum of the radicals obtained from acetaldehyde. The main radical species gives rise to a 9 × 2 line system which indicates interaction with two non-equivalent nitrogen nuclei and one hydrogen. The structure is believed to

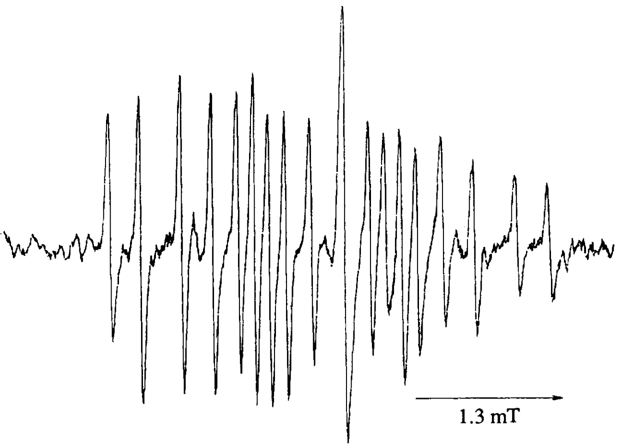


Fig. 1 EPR spectrum of the radicals formed in the reaction between sodium nitrite and acetaldehyde dissolved in aqueous acid containing a small amount of ascorbic acid. The radicals were formed in the dark at room temperature after the reaction mixture had been made alkaline.

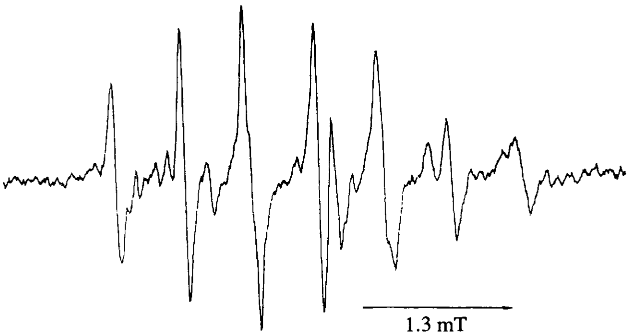


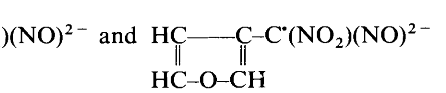
Fig. 2 EPR spectrum obtained with furfural

be H–C$^{\bullet}$(NO$_2$)(NO)$^{2-}$ which infers that the parent acetaldehyde had been degraded with loss of the methyl group.

Radicals from benzaldehyde and furfural

Fig. 2 shows the spectrum obtained with furfural. The spectrum consists of seven lines which indicates that the nitrogens are non-equivalent, with the nitrogen coupling constants in a ratio of nearly 2:1. A similar spectrum was observed with benzaldehyde. It is suggested that the hydrogens of the aldehydes are dissociated and that the structures of the radicals are

Ph–C$^{\bullet}$(NO$_2$)(NO)$^{2-}$ and


$$\begin{array}{l} HC\text{——}C\text{–}C^{\bullet}(NO_2)(NO)^{2-} \\ \,\| \qquad\ \ \| \\ HC\text{–}O\text{–}CH \end{array}$$

The coupling constants a_N^1 and a_N^2 obtained with benzaldehyde (*cf.* Table 1) are very close to those observed for the secondary radical dianions obtained in the reduction of Ph–C(NO$_2$)$_2$$^-$, *i.e.* 1.19 and 0.602 mT.[3]

Radicals from butan-2-one and 3-methylbutan-2-one

The radical spectra observed with butan-2-one (CH$_3$CO-CH$_2$CH$_3$) and 3-methylbutan-2-one [CH$_3$COCH(CH$_3$)$_2$] are almost identical and are also very similar to the spectrum obtained with acetaldehyde (Fig. 1). It is likely that the parent ketones are degraded and that the observed radicals are identical to those observed with acetaldehyde.

Radicals from cyclopentanone, cyclohexanone and 2,5-dimethylcyclohexanone

The radicals obtained with cyclopentanone gave rise to the spectrum shown in Fig. 3. The five-line system indicated that the two nitrogens were equivalent. The triplet splitting (1:2:1) originated from an interaction with two equivalent hydrogens, very probably those of a methylene group. The formation of the radicals is considered to involve cleavage of the C–C bond between the carbonyl carbon and one of the carbon atoms in juxtaposition, *i.e.* the structure of the radicals is R–(CH$_2$)$_2$–CH$_2$–C$^{\bullet}$(NO$_2$)$_2$$^{2-}$ where R is the end-standing group formed after cleavage of the C–C bond. Very similar spectra were obtained with cyclohexanone and 2,5-dimethylcyclohexanone.

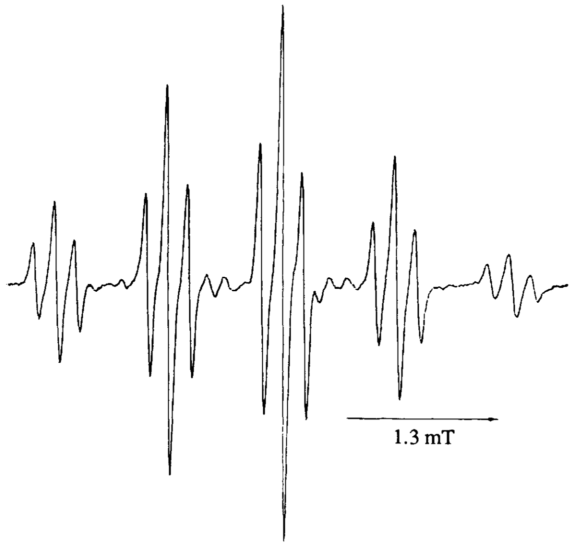


Fig. 3 EPR spectrum observed with cyclopentanone

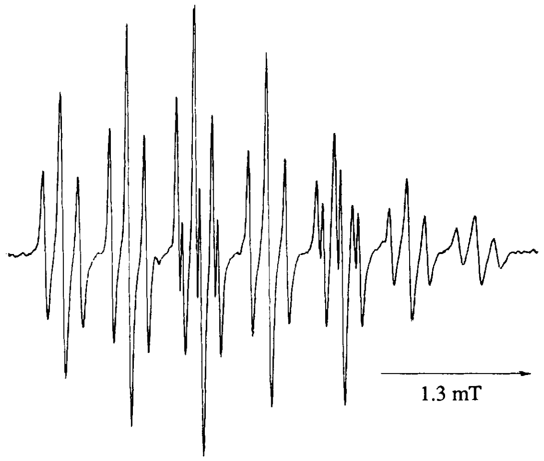


Fig. 4 EPR spectrum observed with ε-caprolactam

Radicals from ε-caprolactam and δ-valerolactam

The spectrum obtained with ε-caprolactam [$\overline{\text{CH}_2\text{–CO–NH–(CH}_2)_3\text{–CH}_2}$] exhibited seven main groups, each split into triplets 1:2:1 (Fig. 4). The groups 3 and 5 are incompletely split into doublets. This arrangement originates from the interaction with two non-equivalent nitrogen nuclei, the coupling constants of which are nearly related in a 2:1 ratio. No splittings could be resolved from the ring nitrogen of the parent ε-caprolactam. Therefore, it seems very likely that the formation of the radicals includes hydrolysis of the lactam ring to give the radical H$_2$N–(CH$_2$)$_4$–CH$_2$–C$^{\bullet}$(NO$_2$)(NO)$^{2-}$. The hydrogens of the methylene group in the α-position give rise to the triplet splittings.

Radicals from ascorbic acid

In higher concentrations, ascorbic acid was found to react also with sodium nitrite as a carbonyl compound (lactone) just like

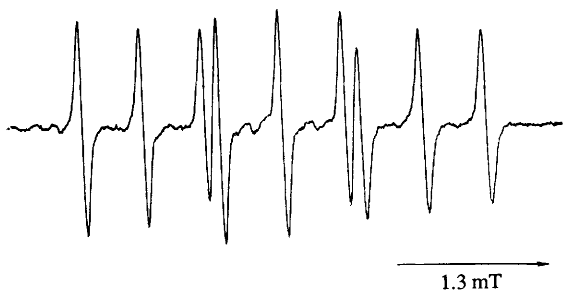


Fig. 5 EPR spectrum obtained with ascorbic acid as the parent compound

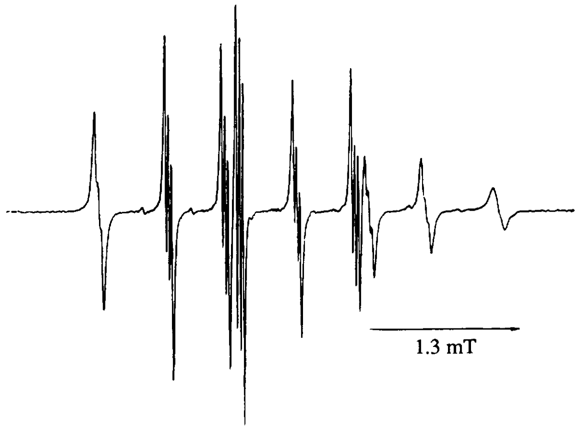


Fig. 6 EPR spectrum observed with glycylglycine

the other parent substances of this series. The radical species formed gave rise to a nine-line spectrum, Fig. 5, which indicated non-equivalence of the two nitrogens. The radical formation is considered to include hydrolysis of the lactone ring. The structure of the radical is $HOCH_2-[CH(OH)]_2-C(OH)=C(OH)-C^{\bullet}(NO_2)(NO)^{2-}$. Evidently, ascorbic acid acts here both as a parent carbonyl compound and as a reducing reagent for the radical production. In view of these findings, the amount of ascorbic acid should be kept as low as possible when used as a reducing reagent. So far it has not been possible to replace ascorbic acid with some other reductant such as glucose used in the earlier experiments.[1-6]

Radicals from glycylglycine and alanylglycine

Fig. 6 shows the spectrum obtained with glycylglycine, $H_2N-CH_2-CO-NH-CH_2-CO_2H$. The nitrogens are non-equivalent and give rise to a nine-line system, with each of the groups split into a triplet 1 : 1 : 1 due to the interaction with the amide nitrogen of the parent dipeptide. The spectrum exhibited linewidth alternations with the spectral components $M_I(+1, +1)$, $M_I(0, -1)$, $M_I(-1, 0)$ and $M_I(-1, -1)$ broadened.

The spectrum observed with alanylglycine $CH_3-CH(NH_2)-CO-NH-CH_2-CO_2H$, showed equivalence of the nitrogens with each of the five main lines split into triplets 1 : 1 : 1 by the amide nitrogen of the parent peptide, Fig. 7.

Since the amide nitrogens are preserved in the radicals, the reaction must involve removal of an H_2N-CH_2 and a $CH_3CH(NH_2)$ fragment. The structure of the radical produced with glycylglycine is $HO_2C-CH_2NH-C^{\bullet}(NO_2)(NO)^{2-}$ and with alanylglycine $HO_2C-CH_2-NH-C^{\bullet}(NO_2)_2{}^{2-}$.

Radicals from alanylalanine

The spectrum obtained in the reaction between $NaNO_2$–ascorbic acid and alanylalanine (see above) gave rise to a 5 × 3 line spectrum which was almost identical to that of alanylglycine (Fig. 7).

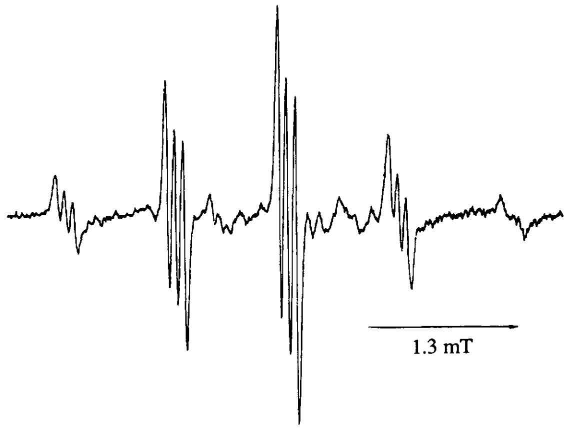


Fig. 7 EPR spectrum obtained with alanylglycine

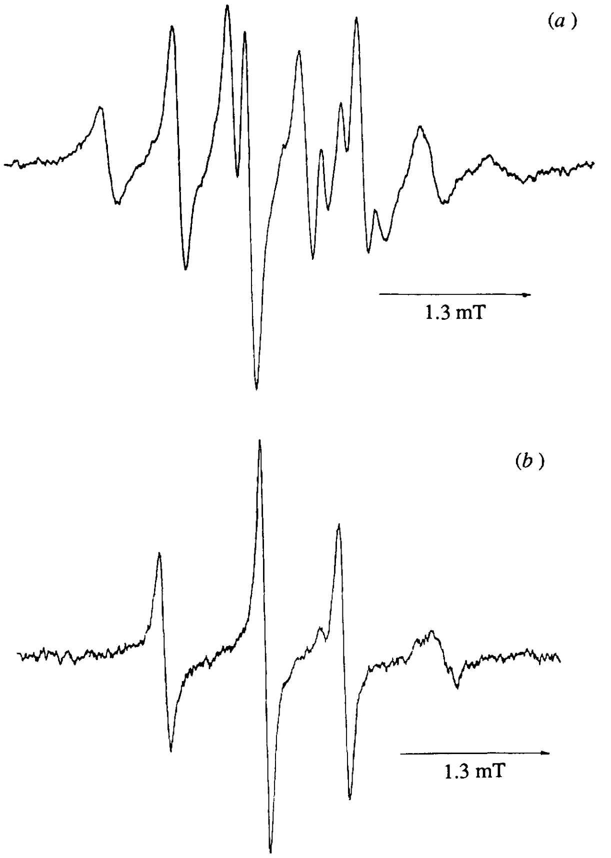


Fig. 8 EPR spectrum obtained with bovine pancreas insulin and sodium nitrite. Insulin (20 mg), sodium nitrite (25 mg) and ascorbic acid (10 mg) in 250 µl of H_2O, 50 µl of 1 mol dm^{-3} HCL and 250 µl of 2 mol dm^{-3} NaOH: (*a*) with $Na^{14}NO_2$ and (*b*) with $Na^{15}NO_2$.

Radicals from insulin

Fig. 8(*a*) shows the EPR spectrum obtained in the reaction between $Na^{14}NO_2$–ascorbate and the polypeptide insulin. There are about nine rather broad main lines which are distorted compared with those of the spectra from the other members of this series. Such an appearance is expected since the reaction involves formation of radicals from a large number of peptide linkages. The orientation of the dinitro or nitronitroso radicals to each other and relative to the rest of the insulin fragments is certainly very different with restricted motion, all factors which contribute to the complicated overlap spectrum of Fig. 8(*a*).

Fig. 8(*b*) gives the spectrum obtained with insulin and $Na^{15}NO_2$. This spectrum is much simpler and exhibits four main lines which indicate an interaction with two non-equivalent ^{15}N nuclei. The coupling constants are $a^1_{N15} = 1.60$ and $a^2_{N15} = 0.88$ mT (1 N). These values correspond to $a^1_{N14} =$

$1.60/1.4 = 1.14$ and $a^2_{N14} = 0.88/1.4 = 0.63$ mT, where the factor 1.4 is the ratio between the coupling constants for ^{15}N and ^{14}N.

The results show that the main part of the radicals formed with the polypeptide insulin consists of nitronitroso radicals $R–C^{\bullet}(NO_2)(NO)^{2-}$, where R is the remaining part of the polypeptide fragments.

Conclusions

The reaction between sodium nitrite–ascorbic acid and a number of different carbonyl compounds including aldehydes, ketones, lactones, lactams and substances with peptide linkages in strong acid/alkaline solutions, gives rise to dinitro or nitronitroso dianion radicals $R–C^{\bullet}(NO_2)_2{}^{2-}$ or $R–C^{\bullet}(NO_2)(NO)^{2-}$. Although the reactions involve degradation of most of the parent compounds, or dissociation of a hydrogen ion to produce the planar sp^2 hybridization of the central carbon atom, many of the EPR spectra exhibited hydrogen or nitrogen interactions characteristic of the parent compounds. It could not be decided whether the nitronitroso radicals were primary products or were formed by reduction of the dinitro species. However, the nitronitroso radicals seem to be the products obtained in most of the reactions. It was not evident why some parent substances gave rise to nitronitroso radicals, whereas the reaction with some others gave rise to dinitro radicals.

The results are in agreement with earlier experiments in which dinitro and nitronitroso radicals of the same types were produced by one-electron reduction of the corresponding monoanions.[1–6] The direct reaction method considered here makes it possible to produce the *gem*-dinitro and *gem*-nitronitroso radicals from a number of substances without access to their parent dinitro monoanions.

Acknowledgements

The author is indebted to the referees for valuable suggestions. This work was supported by grants from Adlerbertska Forskningsfonden and The Royal Society of Arts and Sciences in Gothenburg.

References

1 C. Lagercrantz, *Acta Chem. Scand.*, 1964, **18**, 382.
2 C. Lagercrantz, *Acta Chem. Scand.*, 1964, **18**, 1384.
3 C. Lagercrantz, K. Torssell and S. Wold, *Arkiv Kemi*, 1966, **25**, nr. 53, 567.
4 A. Hudson, C. Lagercrantz and G. R. Luckhurst, *Mol. Phys.*, 1966, **11**, 321.
5 K. Torssell, C. Lagercrantz and S. Wold, *Arkiv Kemi*, 1968, **29**, nr. 19, 219.
6 C. Lagercrantz, S. Forshult, T. Nilsson and K. Torssell, *Acta Chem. Scand.*, 1970, **24**, 550.
7 L. H. Piette, I. Yamazaki and H. S. Mason, in *Free Radicals in Biological Systems*, eds. M. S. Blois, Jr., H. W. Brown, R. M. Lemon, R. O. Lindblom and M. Weissbluth, Academic Press, New York, 1961, ch. 14, pp. 195–208.
8 O. Touster, *Organic Reaction*, **VII**, John Wiley, New York, 1953, pp. 327–377.
9 J. March, *Advanced Organic Chemistry: Reaction, Mechanisms, and Structure*, MacGraw-Hill, New York, 1968, p. 34.
10 A. T. Nielsen, *The Chemistry of the Nitro and Nitroso Groups. Part 1*, ed. H. Feuer, Interscience, New York, 1969, ch. 7, pp. 349–486.
11 T. Suehiro, S. Masuda, T. Tashiro, R. Nakausa, M. Taguchi, A. Koike and A. Rieker, *Bull. Chem. Soc. Jpn.*, 1986, **59**, 1877.

Paper 5/02601F
Received 24*th April* 1995
Accepted 1*st September* 1995