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SEPARATION AND CHARACTERIZATION OF SPIN-ADDUCTS IN A γ-IRRADIATED AQUEOUS SOLUTION OF 2-METHYL-2-NITROSOPROPANE BY A HIGH PERFORMANCE LIQUID CHROMATOGRAPH EQUIPPED WITH AN ESR SPECTROMETER

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Spin-adducts produced in a γ-irradiated aqueous solution of 2-methyl-2-nitrosopropane were investigated by means of high performance liquid chromatography and ESR spectroscopy. The existence of five kinds of spin-adducts was found. Three of them were assigned to the structures: ${}^{t}BuN(O\cdot)-CH_3$, ${}^{t}BuN(O\cdot)-CH_2C(CH_3)_2NO$ or ${}^{t}BuN(O\cdot)-CH_2C(CH_3)_2-N^+(O^-)=N^+(O^-){}^{t}Bu$ and ${}^{t}BuN(O\cdot)-{}^{t}Bu$.

In a previous paper[1)], it was demonstrated that 2-methyl-2-nitrosopropane (MNP) is a useful spin-trap to convert short-lived radicals to relatively stable ones, spin-adducts, especially when it is used in an aqueous solution. The spin-trap has been used mainly for investigating radicals produced in γ-irradiated aqueous solutions of biomolecules.[2)] To investigate these radicals by means of the spin-trapping method using MNP, it is indispensable to solve a problem of what spin-adducts are produced in a γ-irradiated aqueous solution containing only MNP, because the γ-irradiation combined with the simultaneous spin-trapping of aqueous solutions containing both biomolecules and MNP produces not only spin-adducts formed by the reaction of MNP with radicals due to biomolecules but also those by the reaction of MNP with radicals due to MNP. However, previous investigations by the spin-trapping method using MNP in aqueous solutions have dealt almost exclusively with the application of the method. Therefore, the present investigation was undertaken in order to work out this unsolved essential problem.

We reported in a previous paper[3)] that spin-adducts produced by the reaction of MNP with radicals due to DL-methionine can be separated by a high speed liquid chromatograph equipped with an ESR spectrometer. In this study, we also attempted the similar procedure to solve the problem. The equipments used here were almost identical with those described previously except that a longer column of IEX-210SC (60 cm in length, TOYO-SODA) was utilized for higher resolution, and the operating procedure of the chromatograph was also identical. About 10 cm^3 of an aqueous solution containing ca. 10 mg of MNP was irradiated with ^{60}Co γ-rays to a total dose of 3×10^5 rad and then this sample solution was immediately injected to a high performance liquid chromatograph, HLC-803 of TOYO-SODA.

The sample solution just after the irradiation exhibited an ESR spectrum shown in Fig.1 which may be assigned to those of several spin-adducts. Chromatogram obtained from the sample solution is shown in Fig.2. Five peaks with characters from A to E appeared there. The peak intensities in the chromatogram are not really proportional to the concentration of radicals as described previously. ESR spectra obtained from the separated fractions are shown in Fig.3. Abbreviations from (a) to (e) in Fig.3 correspond to the ESR spectra of the fractions from A to E in Fig.2, respectively.

The ESR spectrum shown in Fig.3(a) consists of triple-triplet. As this spectrum with regular sharp and broad splitting patterns exhibits line width alternation, this assignment is believed to be reasonable. Though the dominant splitting is attributed to a ^{14}N nucleus and the secondary splitting is to two β-protons(a_N= 16.2 G, a_H=10.1 G) and it was assured that this spectrum was due to a spin-adduct formed by the reaction of MNP with a radical produced from a chemical derivative of MNP, an exact structure of the spin-adduct was not determined in this study.

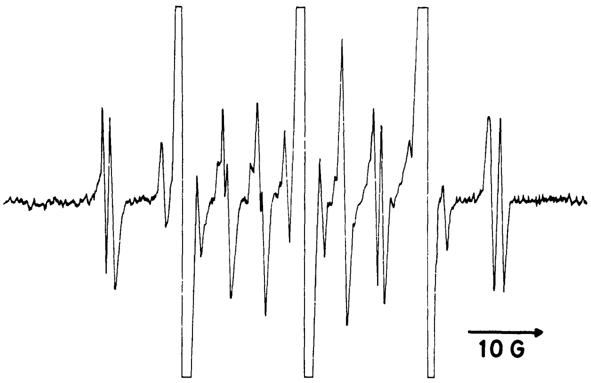

Fig.1 ESR spectrum of γ-irradiated aqueous solution of MNP.

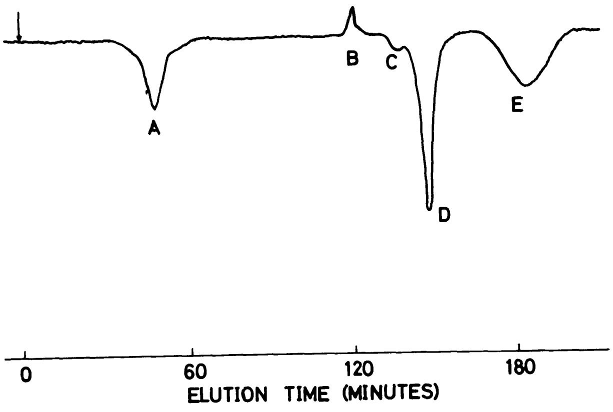

Fig.2 Chromatogram obtained from γ-irradiated aqueous solution of MNP.

The fraction of B gave an ESR spectrum shown in Fig.3(b). As it consists of a ^{14}N triplet and a quartet due to three β-protons(a_N=17.3 G, a_H=14.2 G) and is consistent with the spectrum of a spin-adduct of a methyl radical described in the paper of Rustgi et. al.$^{4)}$, this ESR spectrum was assigned to be due to a spin-adduct of a methyl radical formed by the cleavage of the CH_3-C bonding in a t-butyl group of MNP. The fraction of C gave an ESR spectrum shown in Fig.3(c). It consists of a ^{14}N triplet and a triplet due to two β-protons(a_N=16.6 G, a_H=11.1 G) and also exhibits line width alternation. Considering the elution time of the spin-adduct, this spectrum is believed to be produced by the spin-adduct due to H-abstraction by OH from a methyl group of MNP, because radicals with hydrophobic groups tend to be eluted much later than those with hydrophilic groups in the column used here and MNP has only hydrophobic groups. Consequently, the spectrum was assigned to the

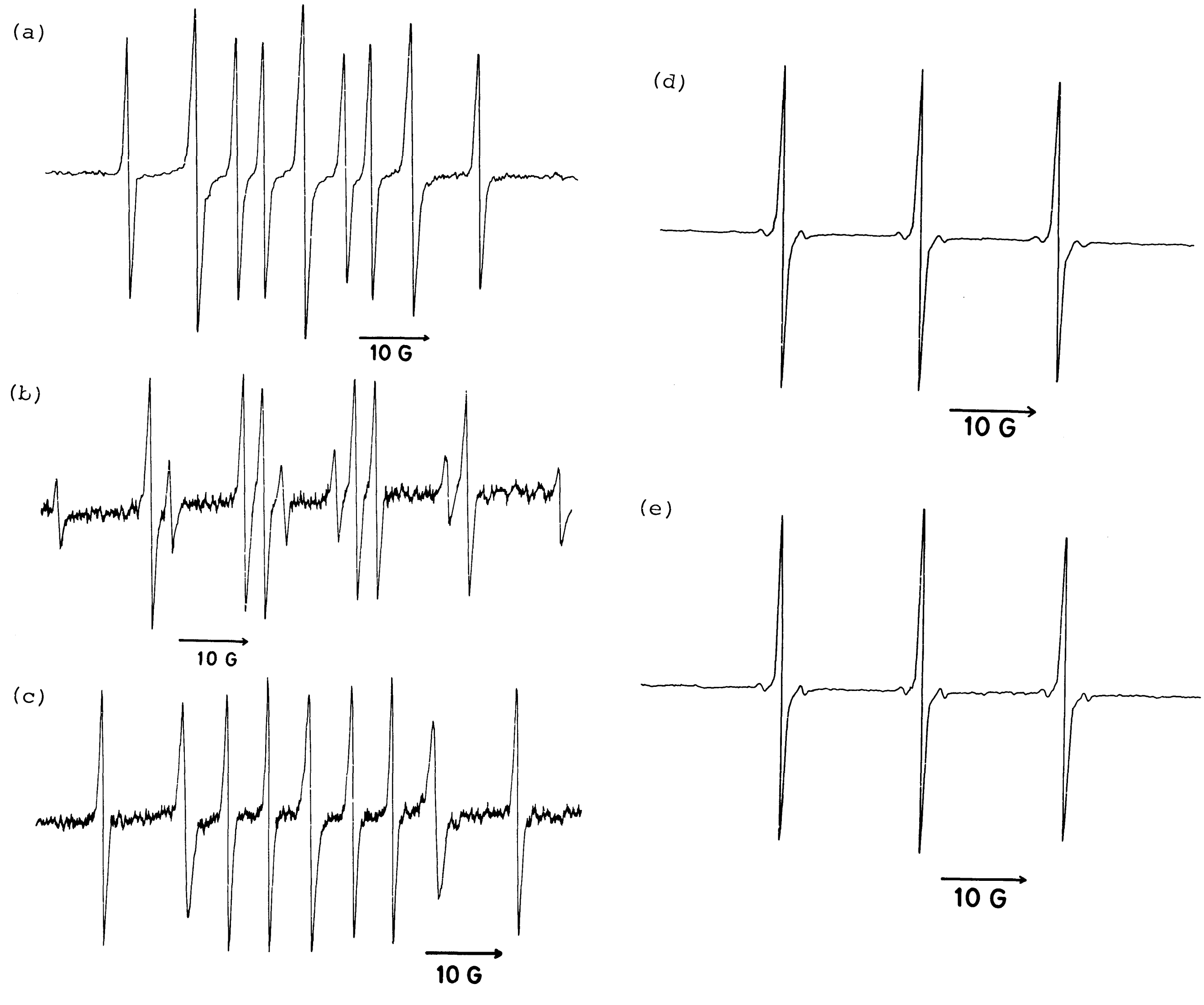

Fig.3 ESR spectra of the separated spin-adducts.

exact structure: ${}^{t}BuN(O\cdot)-CH_2C(CH_3)_2NO$ due to monomeric MNP or ${}^{t}BuN(O\cdot)-CH_2C(CH_3)_2-N^{+}(O^{-})=N^{+}(O^{-})-{}^{t}Bu$ due to dimeric MNP. Both spectra shown in Fig.3(d) and (e) consists of simple triplets due to only ${}^{14}N$ nuclei(a_N(d)=16.8 G, a_N(e)=17.2 G). One of them is supposed to be due to ${}^{t}BuN(O\cdot)-{}^{t}Bu$. In order to determine which spectrum is due to ${}^{t}BuN(O\cdot)-{}^{t}Bu$, the radical was synthesized according to the direction of Brière and Rassat.5) The hyperfine coupling constant of the synthesized radical was 17.2 G and was in good agreement with that of the spin-adduct exhibiting the spectrum shown in Fig.3(e). On the basis of the result, it seems most reasonable to assign the spectrum shown in Fig.3(e) to ${}^{t}BuN(O\cdot)-{}^{t}Bu$. The 16.8 G triplet shown in Fig.3(d) was unidentified in this study.

In conclusion, five kinds of spin-adducts are produced in the γ-irradiated aqueous solution of MNP and they can be identified by means of high performance liquid chromatography and ESR spectroscopy.

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