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The Structure of a Free Radical produced from Questiomycin A. Photochemistry of Antibiotics. II[1]

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Questiomycin A (3-aminophenoxazone-(2)) showed stable ESR spectra in both solid state and organic solvents. The structure of the free radical was discussed by analysis of the hyperfine structure of the radicals produced from questiomycin A and the isotope compounds and simulation using an electronic computor.

It was concluded that the spin density of the compound mainly existed in the nitrogen atom of the amino group.

In our previous paper[3] it was reported that questiomycin A was produced from *o*-aminophenol by photooxidative coupling, and that an ESR signal was obtained during photoirradiation of the *o*-aminophenol. In the course of studies on the photooxydative coupling reaction mechanism it has been observed that questiomycin A (the photoproduct) shows stable ESR spectra.

Questiomycins[4] show bacteriocidal properties, and actinomycins[5] have antitumor activities. The 3-aminophenoxazone system chromophore is common to both antibiotics. It may be of interest that questiomycin A shows the stable ESR spectra.

In the present paper we report that questiomycin A showed ESR signals in both the solid state and in solution. The free radical structure was determined by analysis of hyperfine structure using isotope compounds and simulation using an electronic computor.

Experimental

Compound——Questiomycin A (3-aminophenoxazone-(2)) was made by the method described in the previous paper.[3] N^{15}-replaced questiomycin A was prepared from N^{15}-replaced *o*-aminophenol by the same procedure. The isotope compound showed one spot on a thin-layer chromatogram and the same *Rf* values with the N^{14}-compound.

Deuterium questiomycin A was prepared by repeated treatment of questiomycin A with D_2O in anhydrous dioxane solution.

ESR Measurement——ESR spectra were measured in dioxane or benzene solutions using a Japan Electron Optics Laboratory's JES-3BX spectrometer with 100 kc/sec field modulation.

UV irradiation of the samples was carried out at a distance of 30 cm, using the 100 watt high pressure mercury lamp (Ushio Denki Co. Ltd., UN-102) attached to the ESR spectrometer.

Spectral Simulation——The simulated spectra were plotted by a Japan Electron Optics Laboratory's RA-514 Spectrum Accumulator using hyperfine splitting constants and a Lorentzian linesharpe, line-width at half-height=1.15 gauss.[6]

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1) Part 1: T. Ikekawa, N. Uehara and T. Okuda, *Chem. Pharm. Bull.* (Tokyo), **16**, 1705 (1968).
2) Location: *Tsukiji 5-chome, Chuo-ku, Tokyo.*
3) T. Ikekawa, N. Uehara and T. Okuda, *Chem. Pharm. Bull.* (Tokyo), **16**, 1705 (1968).
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Results and Discussion

Questiomycin A (3-aminophenoxazone-(2)) showed stable ESR spectra both in solutions and in the solid state. The g value of the spectra was 2.009. The signal intensity increased by photoirradiation at room temperature, but even without irradiation the ESR spectra of the compound were observed. When the sample in dioxane solution was degassed below 10^{-4} mmHg by oil diffusion pump and thereafter sealed in a quartz tube, the ESR signal intensity was very weak, but it increased gradually after the sealed tube was opened. This fact suggested that a trace of oxygen was necessary for formation of the free radical, which was produced by removal of one hydrogen atom from the questiomycin A molecule.

As shown in Fig. 1, the ESR spectrum of questiomycin A in dioxane solution consisted of fourteen lines. The spectrum was obtained from 0.01 mole/liter solution and observed with 1.5 gauss field modulation amplitude. The total width of the observed pattern was 25.7 gauss. This spectrum was well elucidated, assuming that an unpaired electron interacted with one nitrogen atom and three protons with the coupling constants shown in Table I, respectively. The simulated spectra using the coupling constants shown in Table I was obtained by the above assumption. Fig. 1 and 2 showed that the simulated curve was almost completely in agreement with the observed curve for the photoirradiated dioxane solution of questiomycin A.

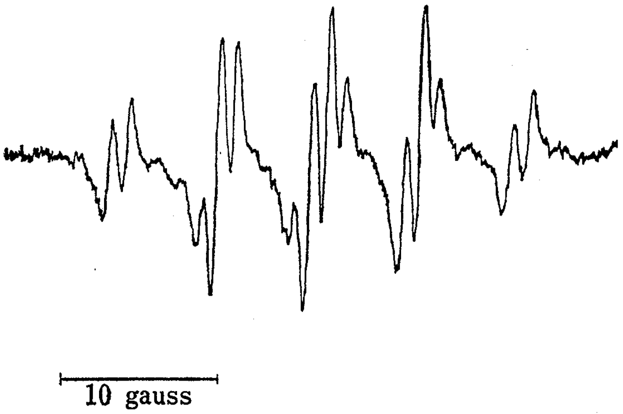

Fig. 1. ESR Spectrum obtained from Questiomycin A ($N^{14}H$) in Dioxane

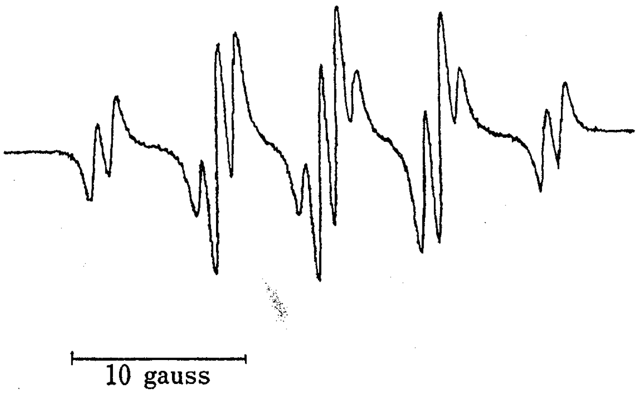

Fig. 2. Simulated Spectrum using Coupling Constants

$a^{N^{14}}=5.64$ gauss $a^{H_2}=6.58$ gauss
$a^{H_2}=6.58$ gauss $a^{H_3}=1.00$ gauss

Since N^{15} and D have different spin numbers (I) and magnetogyric ratio (γ) from those of N^{14} and H, respectively, the replacement of N^{14} by N^{15} and that of H by D are expected to

Table I. Coupling Constants of Questiomycin A (Gauss)

Observation	NH-compound	$N^{15}H$-compound	ND-compound
a^N	5.60	7.53	
a^{H_1}	6.76	6.33	
a^{H_2}	6.76	6.33	
a^{H_3}	1.00	1.06	
Simulation			
a^N	5.64	7.77	5.64
a^{H_1}	6.58	6.58	1.01
a^{H_2}	6.58	6.58	6.58
a^{H_3}	1.00	1.00	1.00

lead to significant changes in the ESR signal patterns and the values of the coupling constants. In this study N^{15}- and D-replacement were carried out in order to elucidate the radical structure of questiomycin A.

The hyperfine structure of the free radical produced from N^{15}-replaced compound consisted of ten broad lines as shown in Fig. 3 and the total width of the pattern was 21.25 gauss. N^{15}-replaced compound had two heavy nitrogen in the molecule, because of the synthesis from oxydative dimerization of N^{15}-replaced *o*-aminophenol. From analysis of the spectrum, only one of the two nitrogen atoms of the molecule seemed to have the effect to change the ESR spectrum by isotope replacement.

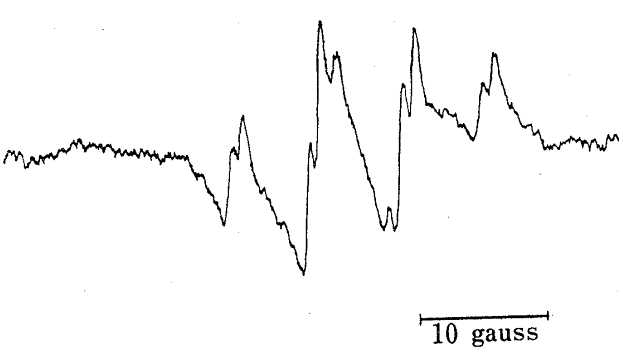

Fig. 3. ESR Spectrum obtained from N^{15}–Isotope Compound of Questiomycin A in Dioxane

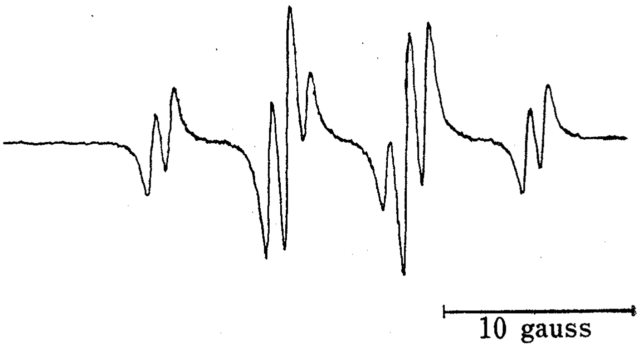

Fig. 4. Simulated Spectrum using Coupling Constants

$a^{N^{15}}=7.77$ gauss $a^{H_1}=6.58$ gauss
$a^{H_2}=6.58$ gauss $a^{H_3}=1.00$ gauss

Fig. 4 showed the simulated curve by the above assumption, using the same coupling constants as N^{14}-compound for three protons and that for N^{15} atom obtained from the ratio $A^{N^{15}}/A^{N^{14}}$ of 1.403.

Though the observed spectrum showed broader lines than the simulated, these two spectra were well matched as shown in Fig. 3 and 4.

The hydrogen exchange of an amino group to deuterium was made by treatment with heavy water. It was confirmed by NMR and IR spectra that no exchange occured in the ring hydrogens.

Fig. 5 showed that the replacement of H by D caused a significant spectral change. From this finding it was confirmed to be the amino nitrogen, not the ring nitrogen, that had

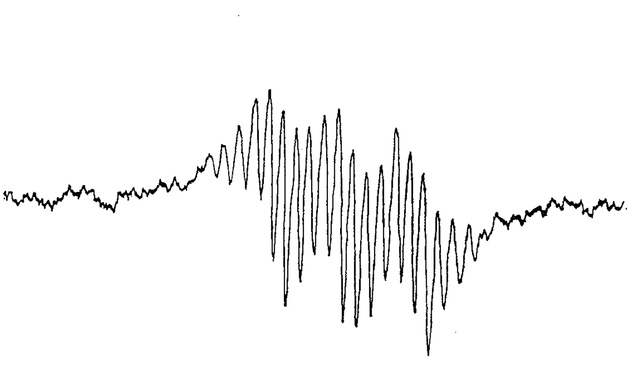

Fig. 5. ESR Spectrum in Dioxane obtained from D–Replaced Compound of Questiomycin A

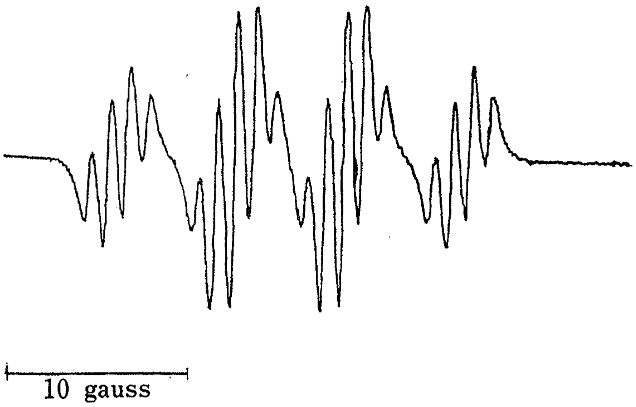

Fig. 6. Simulated Spectrum using Coupling Constants

$a^{N^{14}}=5.64$ gauss $a^{D}=1.01$ gauss
$a^{H_2}=6.58$ gauss $a^{H_3}=1.00$ gauss

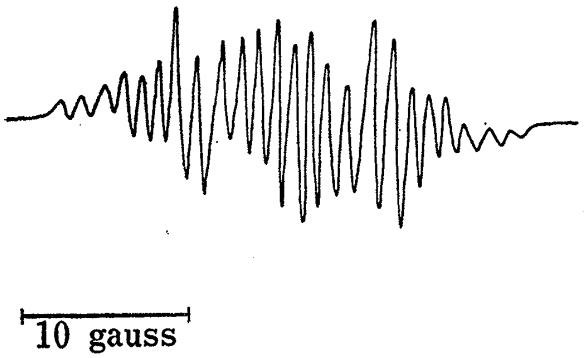

Fig. 7. Summation of Simulated Spectra of Fig. 2 and 4

a large effect on the spectrum. It was also suggested that one of three protons constituting the spectrum was the proton of the amino group.

The numerical values of each coupling constant of the three protons could be obtained by the ratio of $A^{\mathrm{D}}{}_{i}/A^{\mathrm{H}}{}_{i}$ of 0.154 (i=1, 2 or 3). By joining the simulated curve of ND-compound obtained by the replacement of $A^{\mathrm{H}}{}_{1}$ to $A^{\mathrm{D}}{}_{1}$ to that of the NH-compound, the pattern shown in Fig. 7 was obtained, and it showed an agreement with the observed spectrum of the ND-compound (Fig. 5). Therefore, the ESR spectrum in Fig. 5 was well elucidated by considering it as a mixture of NH- and ND-compounds.

The radical produced by photoirradiation of the dioxane solution of questiomycin A might be represented as shown in chart 1.

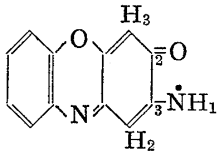

Chart 1

The spin densities on the carbon atoms could be estimated from the coupling constants for protons attached to the carbon atoms by the following equation:

$$A_{\mathrm{H}}=Q\rho_{\mathrm{c}},$$

where A_{H} is the coupling constant due to a proton and Q is the constant which is connected with the δ–π interaction. Usually, Q is taken to be 22.5 gauss.[7] With regard to the nitrogen atom, the relation between the coupling constant and the spin density might be represented as follows:

$$A_{\mathrm{N}}=Q\rho_{\mathrm{N}},$$

where Q was taken to be 25.3 gauss.[8]

In questiomycin A, therefore, a spin density of approximately 0.22 exists on the amino nitrogen atom and densities of approximately 0.30 and 0.04 on two carbon atoms in the ring attach to hydrogen atoms.

From the above facts, it is suggested that spin densities existed on the amino nitrogen and two carbon atoms attached to hydrogen atoms. The position of the carbon atoms can not be exactly determined from the experimental results, although a 4-position may be involved as suggested by a spin densities calculation.

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