

WAVELENGTH-DEPENDENT PEROXIDE FORMATION UPON IRRADIATION
OF all-trans RETINAL IN AN AERATED SOLUTIONKazuo TSUJIMOTO,* Hiroshi HOZOJI, Mamoru OHASHI,
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Irradiation of an aerated solution of all-trans retinal in acetonitrile gave the 5,8-peroxyretinal in a quantum yield of 0.03 together with the retinal isomers. The formation of the peroxide was predominant on the irradiation with light at wavelength shorter than 400 nm.

In vision research it is well-known that 11-cis retinal is bound to the opsin resulting in the reconstitution of rhodopsin.¹⁾ The preparation of 11-cis retinal has so far been performed by photochemical isomerization of all-trans retinal in polar solvents, especially in acetonitrile,²⁾ following the HPLC separation.

In the course of experiments in the photoisomerization of all-trans retinal we have found that a side reaction competing with the isomerization reduces the efficiency of the isomerization. The reaction turned out to be photooxygenation of retinals which is wavelength-dependent.

An aerated solution (3 cm³) of all-trans retinal (2.1 mmol/dm³) in acetonitrile in a quartz cell was irradiated with the Okazaki Large Spectrograph³⁾ at an appropriate wavelength for 17 min. The purity of the light can be estimated that the maximum deviation at the selected wavelength is 5 nm on the basis of the lens used (9 cm in diameter). The irradiation wavelengths were chosen from 340 nm to 500 nm at an interval of 20 nm.

The HPLC chromatogram of the reaction mixture upon irradiation at 360 nm is shown in Fig.1. The last fraction in the chromatogram of 340 nm irradiation was collected on a preparative scale to be identified with all-trans 5,8-peroxyretinal.⁴⁾ The 5,8-peroxyretinal was authentically prepared by the reaction of all-trans retinal with singlet oxygen which was generated from Methylene-Blue-sensitization with 650 nm-excitation as previously reported by Lerner et al.⁴⁾ The characterization of this compound was carried out in detail.⁵⁾ It is most effective in confirming the structure of the peroxide to analyze the

C-13 NMR spectrum including INEPT as shown in Fig. 2. Since the chemical shifts of carbons 5 and 8 around 80 ppm are close to each other, the carbon 5 and 8 atoms are possibly bridged by hetero atoms.⁶⁾

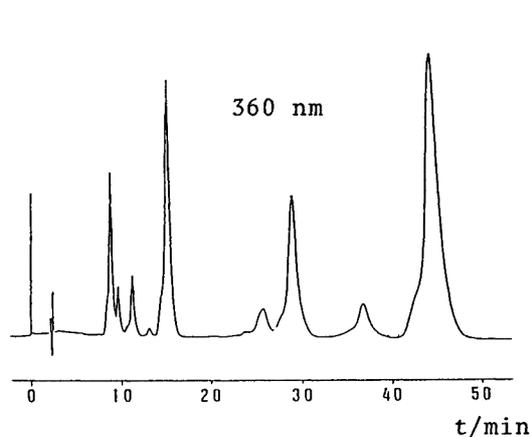


Fig. 1. HPLC chromatogram of the reaction mixture upon irradiation of an aerated solution of all-trans retinal.

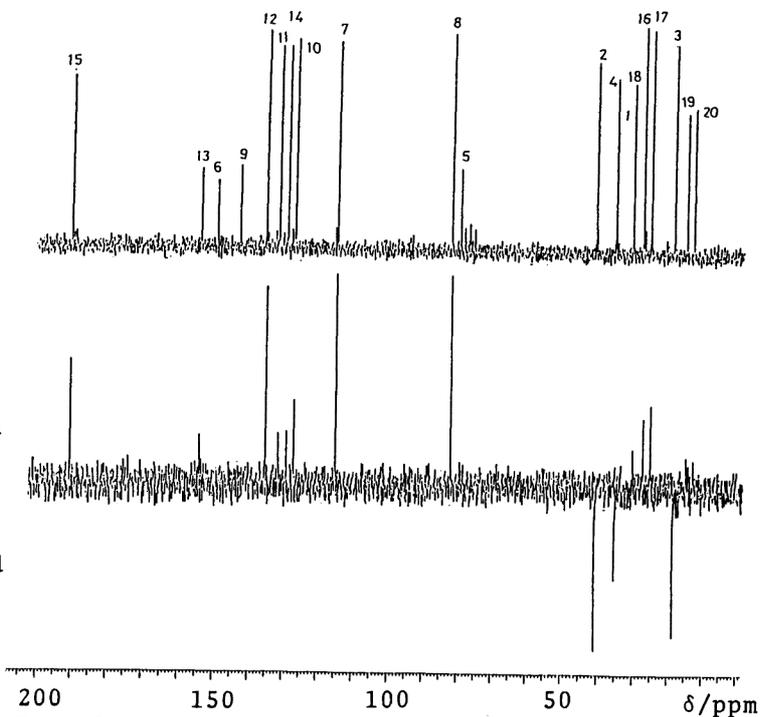


Fig. 2. C-13 NMR spectrum of all-trans 5,8-peroxyretinal (upper) and its INEPT spectrum. ($\Delta=3/4J$, lower)

In Fig. 1, a set of several peaks at the retention time of 25 to 35 min correspond to the isomers of 5,8-peroxyretinal as judged by the UV (λ_{\max} 320 nm) and MS data.

The change of absorption spectra of the irradiated solution also suggests the formation of the peroxides as shown in Fig. 3. The peak at 380 nm which is the characteristic absorption of all-trans retinal decreased its intensity upon irradiation and shifted to 320 nm. When the same solution was irradiated under oxygen-free conditions, the peak at 380 nm diminished in intensity but shifted slightly to 360 nm as shown in Fig. 3b. In the latter case the photoisomerization of all-trans retinal could be confirmed to be predominant on the basis of HPLC analyses of the retinal isomers in the irradiated solution.

Thus, in preparing retinal isomers by irradiation of all-trans retinal it is necessary to remove oxygen from the solution for avoiding the formation of the peroxide (q.y.=0.03),⁷⁾ since this value is comparable to that for the isomerization (q.y.=Ca. 0.1).⁸⁾

To shed light on factors which control the reaction modes, photooxygenation and photoisomerization, we examined the action spectra of the photochemical transformation of all-trans retinal. As shown in Fig. 4, the oxygenated products were predominantly obtained when the solution was irradiated at shorter wavelengths than 400 nm, whereas the retinal isomers were given as the major products upon the irradiation at longer wavelengths.

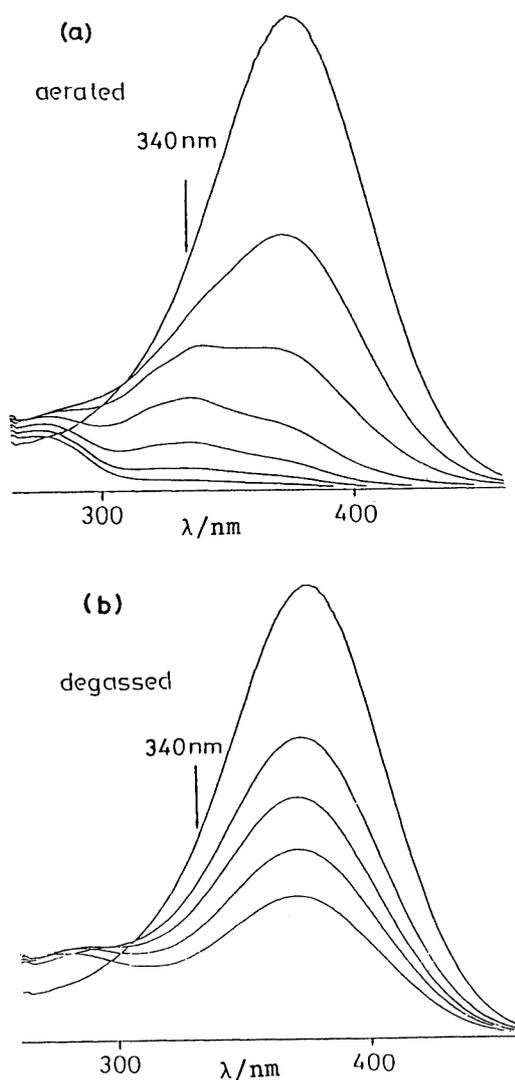


Fig. 3. Spectral changes during irradiation of all-trans retinal at an interval of 5 min.

Effect of wavelength on the photoreaction has been well-documented so far in the photolysis of thiobenzophenone.⁹⁾ Generally speaking, the wavelength-dependent photoreaction suggests that there are two states which can react before internal conversion occurs.¹⁰⁾ In the electronic spectra of retinal three kinds of transitions are usually assigned.¹¹⁾ Although a main band (I) around 380 nm has been well-characterized as a transition from which photoisomerization proceeds, the others, 280 nm and 250 nm (II and III) still remain unclarified, especially in the chemical behavior.

Consequently, it is conceivable that as a possible mechanism the singlet oxygen which is generated by retinal-sensitization (triplet energy of retinal: 38 kcal/mol)¹²⁾ can react with retinal in the ground state to give the oxygenated products. We have found that the photooxygenation of all-trans retinal is wavelength-dependent. Further studies are now in progress.

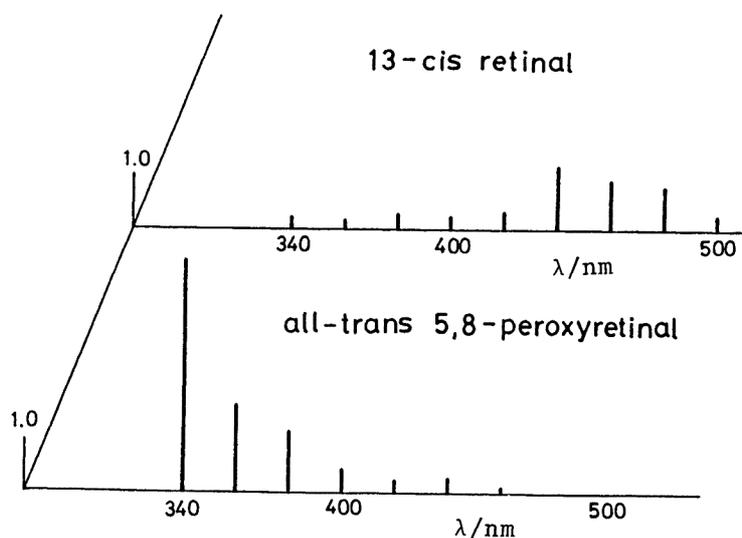
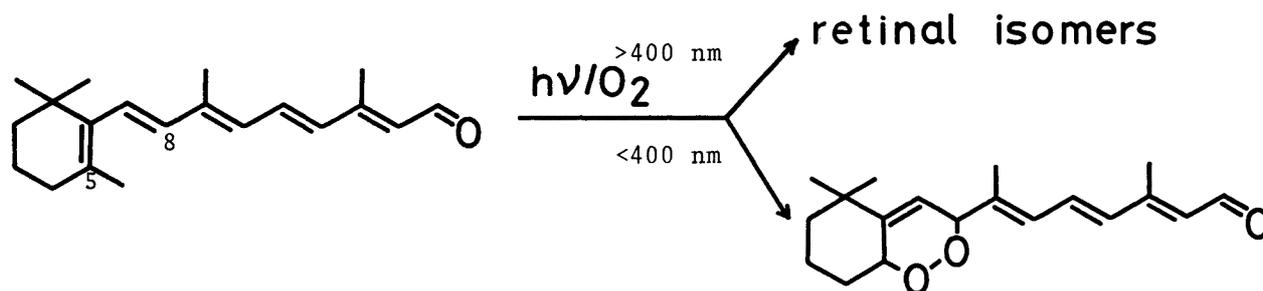


Fig. 4. The action spectra of the photochemical transformation of all-trans retinal. Ratios of the intensities of the products on the basis of the amount of all-trans retinal were shown in the vertical scale.



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