Chemical Stability of Ethyl Icosapentate Against Autoxidation. II. Effect of Photoirradiation on Oxidation Kinetics

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The effect of ultraviolet (UV) or visible light (VIS) irradiation on the chemical stability of ethyl icosapentate [ethyl-(all-cis)-5,8,11,14,17-icosapentaenoate] (EPA) was investigated at 45°C by means of HPLC and by measuring the peroxide value (POV). EPA was oxidized to peroxides after an induction period by photoirradiation, and the peroxide subsequently degraded to secondary products. The autoxidation of EPA followed consecutive reaction kinetics including an induction period, and the kinetic parameters of the oxidation were calculated based upon the consecutive reaction model by computer curve fitting. The results of the degradation rate constant, k, and the induction period obtained by HPLC showed that the radical and the peroxide formation rates are affected by UV, but not by VIS light irradiation. The formation rate constant of peroxide, k1, and its degradation rate constant to secondary products, k2, obtained from the POV under UV light irradiation, increased with irradiation intensity, during which the induction period decreased. On the other hand, k1, k2 and the induction period by VIS light irradiation did not change significantly. The relationship between the induction periods obtained by HPLC and POV and the UV light irradiation energy were superimposed in the plots, indicating that these parameters depended on the UV irradiation energy. The relationship between k1/k2 ratio and the UV irradiation energy suggested that the formation of secondary products was more remarkably accelerated by UV energy than that of peroxide.

KEY WORDS: ethyl icosapentate; autoxidation; photoirradiation; degradation kinetics analysis; HPLC analysis; peroxide value; chemical stability.

INTRODUCTION

Preformulation studies are important in the rational development of the dosage forms of labile drugs against some environmental factors. To design a dosage form, the inherent stability of the drug against stimuli, such as heat, humidity, light and others must be investigated. Therefore, there are many reports concerning the stability (1) of organic compounds under various conditions of temperature and humidity (2). Photolabile drugs have been adequately protected from photolytic degradation by light-resistant packaging (3). However, it is better to use stable pharmaceutical bulk drugs for photochemical reactions without a light-resistant system.

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Ethyl icosapentate [ethyl(all-cis)-5,8,11,14,17-icosapentaenoate] (EPA) is prepared from fish oil, and clinically used as an anti-thrombotic agent in patients with arteriosclerosis. Since EPA is a polyunsaturated fatty acid ester containing five double bonds, it is very labile at high temperatures and its autoxidation follows consecutive reaction kinetics including an induction period (4). The autoxidation of unsaturated fatty acids consists of chain-initiating, -propagating and -terminating steps (5), and these rapid reactions under specific conditions are affected by photoirradiation. Since the final products of oil and fat degradation, namely hydroperoxides and epoxides resulting from autoxidation, present serious toxic problems for humans and other animals, the prevention of oxidation is important for pharmaceutical and food formulations (6–13). Since the initiating step of autoxidation is the period in which the free radical concentration (5) is reached and which is required to start the propagating step, autoxidation is affected by the free radical obtained during photoirradiation. On the other hand, though EPA is very photolabile, the effect of photoirradiation on its oxidation has not yet been clarified. Therefore, we investigated the dependence of the autoxidation rate of a drug under near ultra violet fluorescent (UV) and conventional white fluorescent (VIS) lamps at various photoirradiation intensities, using a kinetic method to provide basic information for the pharmaceutical design of EPA preparations.

MATERIALS AND METHODS

Materials EPA, $C_{22}H_{34}O_2$, Mw. 330.51, (lot No. T-0615B) was provided by the Mochida Pharmaceutical Co., Ltd., Tokyo, Japan. All other reagents were of analytical grade unless otherwise stated.

Chemical stability under various photoirradiation conditions The degradation rate was measured to estimate the chemical stability of EPA as follows: Fifty milligrams of EPA was placed in a glass beaker with a 2-cm inner diameter. The beakers were rotated on a turn table under UV or VIS lamps in a photostability tester (Light-Tron, LT-120, Nagano Science Co., Osaka, Japan) at 45 ± 0.5°C. UV and VIS irradiation intensities were automatically controlled in the tester by a photometer with filters at regions of 300–400 nm and 400–700 nm respectively. The irradiation energies of both lamps (310–400 nm) at the sample surface was independently measured with a UV intensity meter (Model UVR-365, Tokyo Optical Instruments Co., Tokyo, Japan).

High-performance liquid chromatography (HPLC) EPA was analyzed by HPLC consisting of a solvent delivery system (model 600, Waters Associates), an automatic injector (model 710B, Waters Associates), integrator (CR-4A, Shimadzu), and a variable-wavelength UV absorbance detector (Lambda-Max model 481, Waters Associates) operated at 213 nm. The prepacked column (Superspher Si 60, 4 μm; 125 × 4 mm i.d., Merck Co.) was operated at 27°C with the mobile phase at a flow rate of 1.8 ml/min. The mobile phase consisted of a solvent system of n-hexane-diethylether (92:8). A solution of 2,4-dinitrochlorobenzene in chloroform (3.25 mg/ml) was used as the internal standard. After the storage study, 5 ml of the internal standard was added to the

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sample, and the mixture was diluted 60 fold. The sample (2 μl) was injected into the chromatograph to determine the concentration of unchanged EPA.

Measurement of the peroxide value The peroxide value (POV) (14) of the drug was measured as follows: After irradiation, the sample container was placed in a 100-ml beaker containing 25 ml of acetic acid-chloroform (3:2), and mixed with a magnetic stirrer for 10 min after adding 1.0 ml of saturated aqueous potassium iodide. The liberated iodine was titrated by rapidly adding 0.01 N sodium thiosulfate using an automatic titrator (Comtite-101; Hiranuma Sangyo Co.). All experimental procedures were performed under protection from light. The POV (meq/kg) of the sample was calculated from the volume of consumed sodium thiosulfate.

$$POV = F(V - B)/W \qquad \text{eq. 1}$$

F is factor for 0.01 N sodium thiosulfate, V is volume (ml) of the 0.01 N sodium thiosulfate in the sample, B is the volume of 0.01 N sodium thiosulfate in the blank test and W is the weight (kg) of the sample.

Kinetic interpretation of EPA oxidation The degradation profiles of EPA were determined by HPLC and POV measurement as described above. The degradation kinetic data obtained by HPLC were analyzed using the least-squares method. The computer program MULTI (15) was also used to perform non-linear least-squares analysis of the degradation kinetics data obtained from the POV measurement. The kinetic parameters were calculated using the Gauss-Newton method. A weight of unity was employed in this analysis.

RESULTS AND DISCUSSION

Mechanisms of EPA autoxidation under photoirradiation

The oxidation of unsaturated fatty acids starts after the number of initiator radicals reaches a constant concentration (13). Since EPA has four active oxidation sites where hydrogen atoms are attached to bis-allylic carbon atoms (16) as reported previously (4), it is assumed that the autoxidation of the drug consists of three processes: an induction period of radical formation, peroxide formation and its subsequent degradation to secondary products, such as acids and aldehydes as follows:

$$\underset{}{\text{EPA}} \xrightarrow[\text{Ri}]{} \underset{}{\text{EPA}^*} \xrightarrow[\text{k1}]{} \underset{}{\text{Peroxide}} \xrightarrow[\text{k2}]{} \underset{}{\text{Secondary products}}$$

where EPA* is the activated drug containing enough radicals to start the radical chain reaction, Ri is the radical formation rate, k1 is the formation rate of peroxide and k2 is the transformation rate of peroxide to secondary products.

The kinetic parameters of oxidation were calculated by equations 2, 3 and 4.

$$[EPA] = [A]o \exp\{-k1(t-to)\} \qquad \text{eq. 2}$$

$$[PO] = [A]o \{k1/(k2-k1)\}[\exp\{-k1(t-t_o)\} - \exp\{-k2(t-t_o)\}] \qquad \text{eq. 3}$$

$$[SP] = [A]o [1 + 1/(k1-k2)][k2 \exp\{-k1(t-t_o)\} - k1 \exp\{-k2(t-t_o)\}] \qquad \text{eq. 4}$$

$$Ri = 1n[R]c/t_o \qquad \text{eq. 5}$$

where [EPA], [PO], and [SP] are the concentrations of EPA, peroxide and the secondary products at time t, [A]o is the initial concentration of EPA, t_o is the induction period, and [R]c is the radical concentration required to initiate the autoxidation.

The theoretical maximum POV [A]o in equation 3 was defined as 24200, based on the assumption that four sites of active hydrogen were oxidized, since the hydrogen atoms bonded to bis-allylic carbons were easily attacked by the radicals and transformed to peroxides (16).

Photostability of EPA determined by HPLC

Figure 1 shows the effects of UV photoirradiation on the time course of the EPA remaining and their POV. All degradation processes (Fig. 1-A) of EPA identified by HPLC showed straight lines on the semilogarithmic plot, and they all crossed with that at 100% remaining. Therefore, the in-

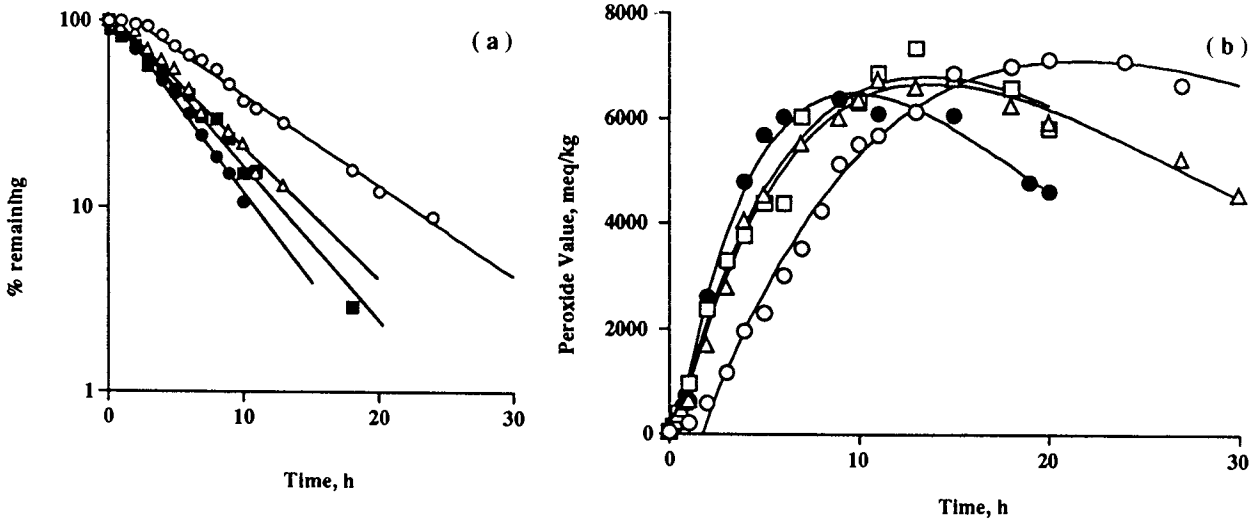

Fig. 1 Effect of UV lamp irradiation intensity on the time course of remaining EPA and the POV (a), Measured by HPLC; ○, intensity at 0 μW/cm²; △, intensity at 300 μW/cm²; ■, at 500 μW/cm²; ●, at 1000 μW/cm²; (b), measured by POV; ○, intensity at 0 μW/cm²; △, at 300 μW/cm²; □, at 500 μW/cm²; ●, at 1000 μW/cm²;

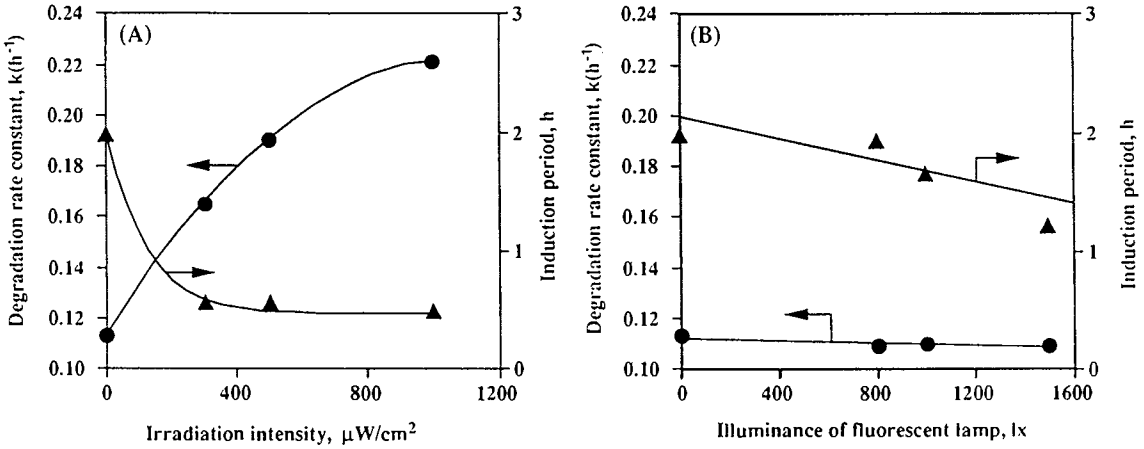

Fig. 2 Effect of photoirradiation intensity on the degradation rate constant and the induction period measured by HPLC. (A), UV irradiation; (B), VIS irradiation.

duction period defined the intercepted time at 100% remaining, according to the straight line. This suggested that the degradation of the drug followed first-order kinetics including the induction period. The percent of remaining EPA under UV and VIS irradiation decreased with time. The degradation rate constant and the induction period were estimated from the semilogarithmic plots by the least-squares method. The degradation profiles under UV lamp irradiation were changed, but they were unaffected even at 1500 lx by VIS lamp irradiation, indicating that the degradation of EPA was accelerated by UV, but not by VIS light irradiation.

Figure 2 shows the effect of UV and VIS light irradiation on the degradation rate constant and the induction period. The degradation rate constant under UV light increased with the irradiation intensity, being twofold higher at 1000 $\mu w/cm^2$, with an induction period 1.5 h shorter than that without irradiation. However, the degradation rates under VIS light irradiation were constant at about 0.115 h^{-1} at all intensities, and the induction period slightly decreased with increasing intensity. These results show that the radical and the peroxide formation rates were affected by UV, but not by VIS light irradiation.

Photostability of EPA as indicated by the peroxide value

The effects of UV photoirradiation on the time course of the EPA peroxide value are shown in Fig. 1b. The POV of all samples irradiated by UV light rapidly increased at the initial stage after the induction period, and the rate of the increase gradually reduced at the latter stage due to the transformation to secondary products. The initial peroxide formation rate under UV light increased with irradiation intensity, and the time required for reaching the peak value decreased, but the maximum POV was almost constant at 7000. However, the initial peroxide formation rate, the time required for reaching the maximum value and the maximum POV under VIS light irradiation were not significantly different.

The POV profiles were also analyzed using the kinetic model based on equation 3. The theoretical values (shown by the solid lines in the figure) were in good agreement with the observed values under all experimental conditions.

Effect of photoirradiation intensity on the kinetic parameters for EPA autoxidation

The effects of photoirradiation intensity on the kinetic

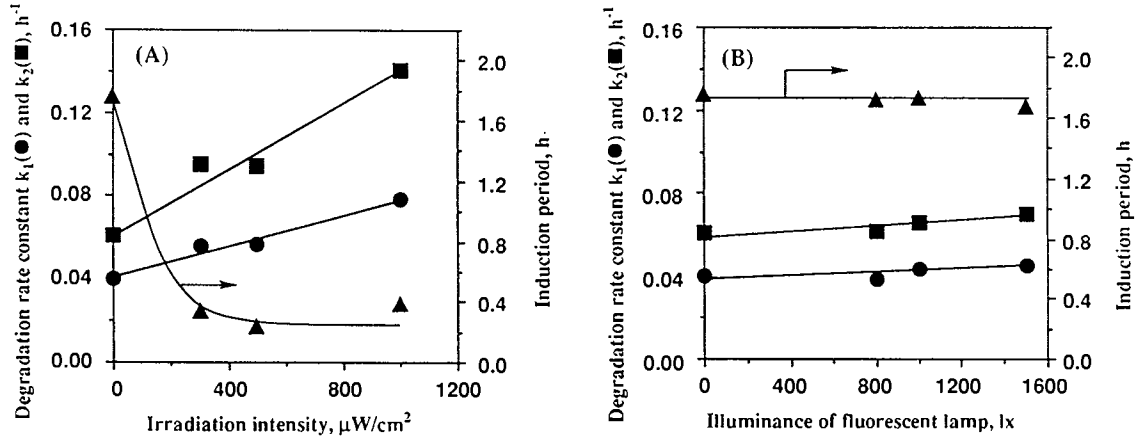

Fig. 3 Effect of photoirradiation on the degradation rate constants and the induction period measured by POV. (A), UV irradiation; (B), VIS irradiation.

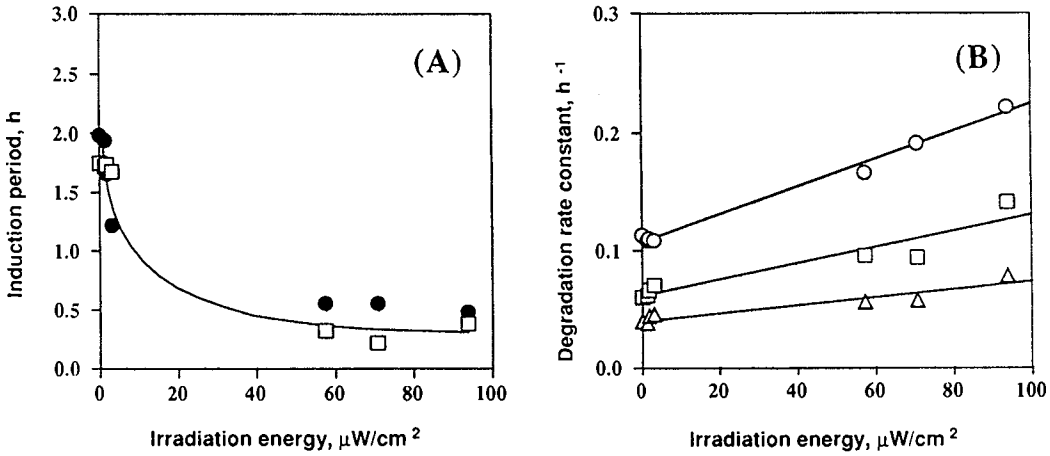

Fig. 4 Effect of UV irradiation energy (310–400 nm) on the degradation rate and the induction period by UV and VIS irradiation. (A), Induction period; (●), measured by HPLC; (□), measured by POV: (B), degradation rate constant; (○), k obtained by HPLC; (△, □), k1 and k2 obtained by POV, respectively.

parameters for the oxidation are shown in Fig. 3. The k1 and k2 under UV light irradiation increased and the induction period decreased with the irradiation intensity. On the other hand, the k1, k2 and the induction period under VIS light irradiation did not increase significantly with increasing intensity. This suggested that the autoxidation of EPA was accelerated by UV, but not by VIS light irradiation.

We reported that the degradation of nifedipine powder was accelerated more by mercury vapor lamp irradiation than by VIS lamp irradiation (17), indicating that a shorter wavelength was more effective upon EPA degradation. Since the VIS lamp irradiated in very wide region from 400 to 700 nm (17), the photoirradiation energy attributable to the ultra violet region was very low. On the contrary, the main spectral distribution of the UV lamp was between 300 and 400 nm (18). Therefore, we measured the irradiation energy (310–400 nm) as an index of UV irradiation energy using a UV intensity meter in this study.

Figure 4 shows the relationship between the UV irradiation energy and the kinetic parameters for photodegradation of EPA by UV and VIS lamp irradiation. The relationship between the induction period obtained by HPLC and UV energy were superimposed on that obtained by POV in the plot, indicating that the induction period depended on the UV irradiation energy. On the contrary, all relationships between degradation rate constants obtained by UV and VIS irradiation and UV energy increased with increasing UV irradiation energy and individually they were straight lines. However, k obtained by HPLC was not superimposable on k1 by POV in the plot, and k was larger than the k1. Because EPA has four active oxidation sites, HPLC can separate EPA and degraded EPA, but POV measures amount of peroxide in EPA and degraded EPA, so the physicochemical meaning of these kinetic parameters are different.

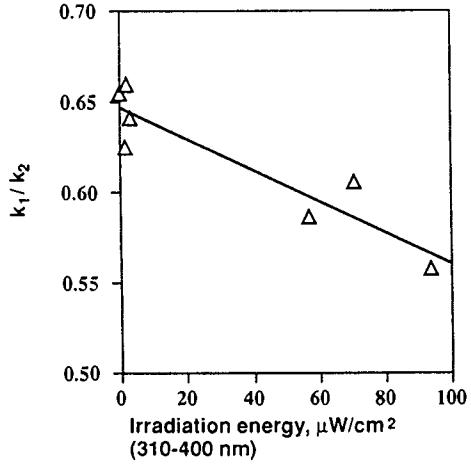

Fig. 5 Effect of UV irradiation energy (310–400 nm) on the k1/k2 ratio of EPA degradation by UV and VIS irradiation.

Figure 5 shows the effect of photoirradiation energy on the k1/k2 ratio of the oxidation rate constants obtained by UV and VIS irradiation. The plot of the k1/k2 ratio against temperature was a straight line and the k1/k2 ratio decreased with increasing temperature as reported previously (4). However, the k1/k2 ratio for degradation decreased significantly with increasing of UV irradiation energy. This suggested that the formation of secondary products was more accelerated by the UV irradiation energy than that of peroxide, because the EPA degradation products (peroxide) still had active sites for photoirradiation, since it has four active oxidation sites.

In conclusion, the photodegradation of EPA was accelerated by UV, but not by VIS light irradiation. The photodegradation of EPA followed consecutive reaction kinetics including an induction period. All kinetic parameters obtained by HPLC and POV were dependent on the UV irradiation energy. The acceleration of photoirradition-induced EPA autoxidation was affected by the UV irradiation energy. The information obtained here from HPLC and POV should be important for quality assurance of autoxidation susceptibility.

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