Chemical Stability of Ethyl Icosapentate Against Autoxidation. I. Effect of Temperature on Oxidation Kinetics

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INTRODUCTION

To maintain the stability of oils and fats, additives are used for the prevention of oxidation (1). The autoxidation of unsaturated fatty acid consists of chain-initiating, -propagating, and -terminating steps (2). Since the products of oil and fat degradation, i.e., hydroperoxides and epoxides, formed by autoxidation can cause serious toxicity in humans and animals, the prevention of oxidation is important. Harle and Thomas (3) found that relatively stable diaryl nitric oxide radicals (nitroxides) are produced during inhibition of autoxidation with diarylamines. Subsequently, there have been many reports on the inhibition of oxidation of oil and fat compounds (4–9) with various antioxidants. The induction period of autoxidation is the initiating step, during which free radicals (2) reach the concentration required to initiate the propagating step. Therefore, the rate of autoxidation is generally retarded by antioxidants which destroy the propagation of free radical intermediates.

Ethyl icosapentate [ethyl(all-*cis*)-5,8,11,14,17-icosapentaenoate; EPA] is prepared from fish oil and is used clinically as an antithromobotic agent for arteriosclerosis. Since the drug is a polyunsaturated fatty acid containing five double bonds, as shown in Fig. 1, it is very labile. The dosage form of the commercial preparation is a soft elastic gelatin capsule containing α-tocopherol to prevent oxidation. However, the quantitative relationship between the oxidation of EPA and anti-oxidants has not yet been clarified. Accordingly we investigated the autoxidation rate of the drug at various temperatures for the pharmaceutical design of the preparation.

MATERIALS AND METHODS

Materials

EPA, $C_{22}H_{34}O_2$, MW 330.51 (Lot No. T-005) was provided by Mochida Pharmaceutical Co. Ltd., Tokyo. All other reagents used were of analytical grade unless stated otherwise.

Chemical Stability at Various Temperatures

To estimate the chemical stability of EPA, the degradation rate was measured as follows: 50 mg of EPA was placed in a glass beaker with a 2-cm inner diameter. The beakers were covered with aluminum foil and placed on a rotating table in a photostability tester (Light-Tron, LT-120, Nagano Science Co.) without light irradiation at 25, 35, and 45 ± 0.5°C.

High-Pressure Liquid Chromatography (HPLC)

EPA was analyzed by HPLC. The HPLC apparatus consisted of a solvent delivery system (Model 600, Waters), automatic injector (Model 710B, Waters), integrator (CR-4A, Shimadzu), and variable-wavelength UV absorbance detector (Lambda-Max Model 481, Waters) operated at 213 nm. The prepacked column (Supersphere 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 *n*-hexane:diethyl ether (92:8). A solution of 2,4-dinitrochlorobenzene in chloroform (3.25 mg/ml) was used as the internal standard. After storage, 5 ml of the internal standard solution was added to the sample, and the mixture was diluted 60 times. Two microliters of the sample solution was injected into the chromatograph to determine the concentration of EPA remaining. Figure 2 shows a typical chromatogram of the sample obtained after storage at 45°C for 3 hr. All values are reproducible and represent the average of two runs.

Measurement of Peroxide Value

The peroxide value (POV) (10) of the drug was measured as follows: The sample was placed in a 100-ml beaker containing 25 ml of acetic acid–chloroform (3:2) solvent and was mixed with a magnetic stirrer for 10 min after adding 1.0 ml of saturated aqueous solution of potassium iodide. The liberated iodine was titrated by rapidly adding 0.01 *N* sodium thiosulfate with an automatic titrator (Comtite-101, Hiranuma Sangyo Co.). All experimental procedures were carried out in a light-protected area. The POV (mEq/kg) of the sample was calculated from the volume of consumed sodium thiosulfate. All values are reproducible and represent the average of two runs.

Kinetic Interpretation of EPA Oxidation

The degradation kinetic data obtained by HPLC were analyzed using the least-squares method. The Gauss–Newton method in the MULTI computer program (11) was also used to perform nonlinear least-squares analysis of the degradation kinetics data obtained from the POV measurement and to estimate the kinetic parameters. A weight of unity was employed in the computer analysis.

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$C_{22}H_{34}O_2$
M.W.: 330.51
Fig. 1. Chemical structure of EPA.

RESULTS AND DISCUSSION

Mechanisms of EPA Autoxidation

There are four possible oxidation sites in EPA where the hydrogen atoms are attached to bis-allylic carbon atoms. Niki (12) reported that the active hydrogens attached to the bis-allylic carbons of polyunsaturated fatty acids containing more than three double bonds, e.g., arachidonic acid, were attacked by radicals and transformed to peroxy radicals. The oxidation of unsaturated fatty acids begins after the number of radicals regarded as the initiator increases and reaches a constant concentration (12). It is therefore assumed that the autoxidation of a drug such as EPA consists of three processes: (i) the induction period, during which radicals are formed; (ii) the peroxide formation process; and (iii) the subsequent process of peroxide degradation to secondary products, namely, acids, aldehydes, etc., as follows:

$$\text{EPA} \xrightarrow[R_i]{} \text{EPA}^* \xrightarrow[k_1]{} \text{Peroxide} \xrightarrow[k_2]{} \text{Secondary products}$$

where EPA* is the activated drug containing enough radicals to start the radical chain reaction. Thus, the oxidation of the drug followed consecutive reaction kinetics, including an induction period, and the kinetic parameters of oxidation were calculated by Eqs. (1)–(4).

$$[\text{EPA}] = [A]_o \exp(-k_1(t - t_o)) \quad (1)$$

$$[\text{PO}] = [A]_o\{k_1/(k_2 - k_1)\}[\exp(-k_1(t - t_o)) - \exp(-k_2(t - t_o))] \quad (2)$$

$$[\text{SP}] = [A]_o\{1 + 1/(k_1 - k_2)\}\{k_2 \exp(-k_1(t - t_o)) - k_1 \exp(-k_2(t - t_o))\} \quad (3)$$

$$[R]_c = \text{constant}$$

$$R_i = \ln[R]_c/t_o = k(1/t_o) \quad (4)$$

where [EPA], [PO], and [SP] are the concentrations of EPA, peroxide, and secondary products at time t, $[A]_o$ is the initial concentration of EPA, k_1 is the formation rate constant of peroxide, k_2 is the transformation rate constant of peroxide to secondary products, t_o is the induction period, R_i is the radical formation rate, and $[R]_c$ is the radical concentration required to initiate autoxidation. The theoretical maximum POV $[A]_o$ in Eq. (2) was estimated to be 24,200. This was based on the assumption that four sites of active hydrogen were oxidized, since it has been shown that the hydrogen atoms bonded to bis-allylic carbons were easily attacked by radicals and transformed to peroxides (12).

Chemical Stability of EPA Determined by HPLC

The percentage of remaining EPA determined by HPLC analysis decreased exponentially over time, with 74.4, 38.1, and 10.4% remaining after storage for 20 hr at 25, 35, and 45°C, respectively. Hence, the degradation of EPA was accelerated with increasing temperature. Figure 3 shows a semilogarithmic plot of the EPA degradation profiles at various temperatures. Straight lines were obtained at each temperature, including in the induction period, indicating that degradation of the drug followed first-order kinetics. The degradation rate constant and the induction period were estimated from the data of a linear part of the plots by the least-squares method. Table I shows the effects of temperature on the degradation rate constants and the induction period, as assessed by HPLC. The degradation rate constant at 45°C was 3.3 times higher than that at 25°C, and the induction period at 45°C was 5 hr shorter than that at 25°C.

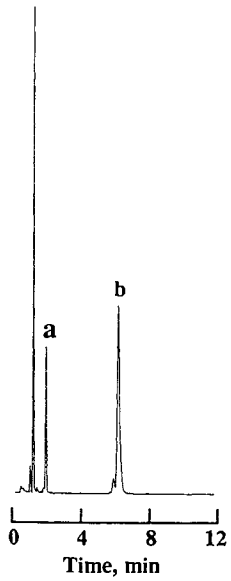

Fig. 2. Typical HPLC chromatogram of EPA. (a) EPA; (b) 2,4-dinitrochlorobenzene (IS).

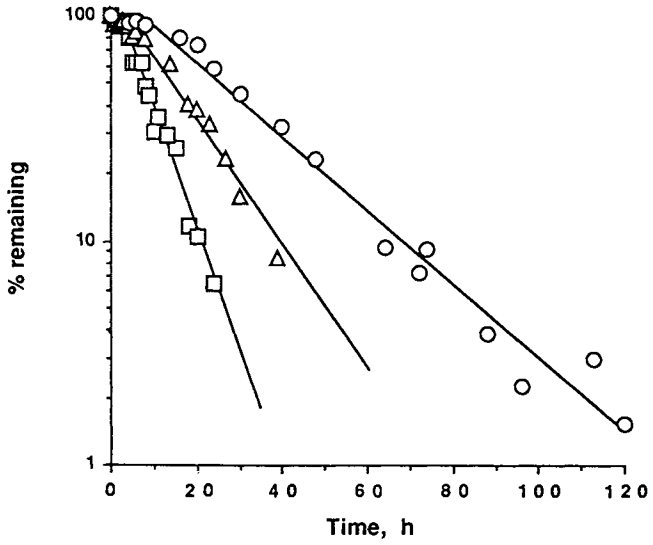

Fig. 3. Effects of temperature on apparent first-order degradation profiles of EPA, determined by HPLC: (○) 25°C; (△) 35°C; (□) 45°C.

Table I. Radical, Peroxide, and Secondary Product Formation Rate Constants, and Induction Period, Obtained by HPLC and POV Methods

Temperature (°C)	k_1, hr^{-1}[a] (SD)	k_2, hr^{-1}[b] (SD)	k_1/k_2	t_o, hr[c]	$1/t_o$, hr^{-1}	SS $\times 10^6$[d]
HPLC method						
25	3.75×10^{-2}	—	—	6.85	0.145	—
35	6.42×10^{-2}	—	—	3.13	0.319	
45	12.30×10^{-2}	—	—	2.00	0.500	—
POV method						
25	1.24×10^{-2}	1.39×10^{-2}	0.892	6.53	0.153	1.71
	(5.06×10^{-4})	(5.22×10^{-4})	—	(0.63)	—	—
35	2.18×10^{-2}	2.79×10^{-2}	0.781	2.66	0.376	2.17
	(9.98×10^{-4})	(1.33×10^{-4})	—	(0.37)	—	—
45	4.74×10^{-2}	7.22×10^{-2}	0.657	1.28	0.781	2.98
	(3.17×10^{-3})	$(.79 \times 10^{-3})$	—	(0.33)	—	—

[a] Peroxide formation ratio.
[b] Secondary product formation rate.
[c] Induction period.
[d] Residual sum of squares.

These results show that the radical and peroxide formation rates were affected by the storage temperature.

Chemical Stability of EPA by Measurement of the Peroxide Value

Figure 4 shows the effect of temperature on the time course of the peroxide value of EPA. The POV of all samples rapidly increased during the initial stage after the induction period, and, after reaching a maximum, slowly declined in later stages because of transformation to secondary products. While the initial peroxide formation rate increased at higher temperatures, the maximum POV and the time required for reaching the peak value decreased, indicating that transformation to secondary products was accelerated, since the peroxide is less stable at high temperatures. The maximum POV at all temperatures exceeded 6050, which was the theoretical value of one active reaction site, and all POV were within the range of 7000–8000. This result suggests that the maximum theoretical value $[A]_o$ of POV represented more than two sites. The POV profiles were also analyzed by the kinetic method based on Eq. (2). The calculated kinetic parameters are summarized in Table I. The results of fitting the index parameter (the residual sum of squares; SS) supported the applicability of these equations to the present degradation process. The theoretical values, shown in Fig. 4, were in good agreement with the observed values under all experimental conditions. The effect of temperature on the kinetic parameters of oxidation is shown in Table I. The k_1 at 25°C was almost the same as the k_2, and both increased with the elevation of temperature. However, k_2 was affected more remarkably by temperature than k_1. In contrast, the length of the induction period decreased with elevations of temperature, as did the k_1/k_2 ratio (Table I). This result suggested that the transformation to secondary products was more rapidly accelerated by the elevation of temperature than by the formation of peroxide.

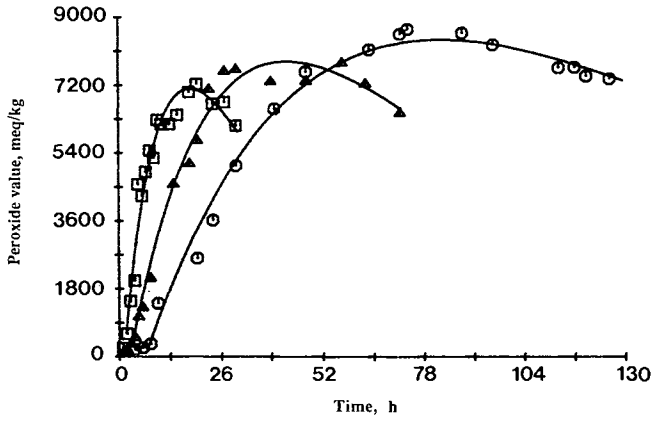

Fig. 4. Effects of temperature on the time course of the POV: (○) 25°C; (△) 35°C; (□) 45°C. Solid lines represent the theoretical curves.

Thermodynamic Parameters of EPA Autoxidation Processes

The activation energies (E) of the EPA autoxidation process were estimated from the Arrehenius plots for the EPA degradation processes as measured by HPLC and POV. All degradation processes were straight lines on the Arrehenius plot, and E values were estimated from the slopes by the least-squares method (Table II). The radical formation rate

Table II. Activation Energies Required for the Processes of Radical, Peroxide, and Secondary Product Formation from EPA Obtained by HPLC and POV

Reaction process	HPLC E[a] (kJ/mol)	HPLC r[b]	POV E (kJ/mol)	POV r
Radical formation	48.6	0.991	64.3	0.999
Peroxide formation	46.7	0.997	52.6	0.994
Secondary product formation	—	—	64.7	0.994

[a] Activation energy.
[b] Correlation coefficient.

constants were calculated from the induction period (t_o), using Eq. (4), based on the assumption that the radical concentration required to initiate the radical chain reaction was constant. The reciprocal of t_o may therefore be a parameter of the radical formation rate [Eq. (4)] on the Arrehenius plot. All of the activation energies of the radical and peroxide formation processes (E_{0H} and E_{1H}) measured by HPLC were lower than those (E_{0P} and E_{1P}) measured by POV. Since there are more than two active oxidation sites in EPA, the peroxides at all active sites were measured by the POV method. The intact drug concentration, in contrast, was measured by HPLC, indicating that the weakest reaction site for oxidation was measured. Therefore, the activation energy estimated by HPLC was the most active site in the molecule, whereas the activation energies measured by POV were the mean values of all active sites. E_{0H} was slightly larger than E_{1H}. E_{1P} was 11.9 kJ/mol smaller than E_{0P}, and E_{2P} was 12.1 kJ/mol larger than E_{1P}. The radical formation process was not consecutive, whereas peroxide formation and the secondary product transformation processes were. Thus, the stability of EPA depended on the radical formation process. On the other hand, the POV increased at the initial step of autoxidation, since secondary product transformation was more difficult to achieve than peroxide formation.

CONCLUSION

The information obtained from HPLC is important for evaluation of the therapeutic effects of preparations of EPA from the pharmaceutical perspective. On the other hand, the information obtained by the POV method is important from the toxicological point of view, since peroxide has serious toxic effects in humans. It is thus important that the analytical methods used for the assessment of various products be selected appropriately.

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