Kinetic Studies of the Photochemical Decomposition of Alkannin/Shikonin Enantiomers

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The photodegradation of alkannin/shikonin (A/S) was studied as a function of solvent polarity, pH and ionic strength. This process follows an apparent first-order kinetic reaction. The photodegradation rate is inversely proportional to the solvent polarity in the order of chloroform > dichloromethane > 2-propanol > ethanol > methanol. The rate-pH profile reveals that A/S is more stable in an acidic condition: marginally subject to specific acid or base catalysis and is affected by two ionizable groups on the molecule. Ionic strength does not affect the photochemical decomposition rate at pH 5, 9 or 12.

Key words alkannin/shikonin enantiomer; shikon; photochemical decomposition; photodegradation

Alkannin and its enantiomer shikonin are the main pharmacological components of Shikon. Alkannin and shikonin have no significant difference in terms of their pharmacological activities[1]; these activities include anti-inflammatory,[1,2] antibacteria,[3-5] wound healing,[2,6,7] and antitumor[8-10] effects. In Japan and China, Shikon has been used as a major ingredient to prepare "Shiunko" ointment, which is frequently used for the treatment of wounds, skin diseases and burns.[2,6,10,11]

Despite the use of Shiunko ointment in the Orient for hundreds of years, very little research has been performed on the chemistry of A/S or its derivatives. Since most of the pharmaceutical preparations containing Shikon extract are for dermatological use, light exposure is inevitable during the treatment regimen. In our previous study,[12] we demonstrated that the oxidation at C-13 position is the main pathway leading to the photodecomposition of A/S (Fig. 1). The objective of the present study was to further investigate the photochemical decomposition of A/S *via* a kinetic approach. The results demonstrated that the photodecomposition of A/S was affected by solvent polarity and pH but not by ionic strength.

Experimental

Materials Methanol, ethanol, and 2-propanol (LC grade) were purchased from Lab-Scan (Dublin, Ireland). Chloroform, dichloromethane (LC grade), *n*-hexane and chloroform (extra pure grade) were purchsed from E. Merck (Darmstadt, Germany). Mefenamic acid (Mf) was purchased from Sigma Chemicals (St. Louis, MO), and Cosmosil reversed-phase HPLC column was from Nakalai Tesque (Kyoto, Japan). Water was purified through a Barnstead (Boston, MA) NANO pure water system before use. A/S was extracted from *Macrotomia euchroma* as previously described.[12]

HPLC Assay Condition for Kinetic Studies A Jasco 880-PU HPLC system equipped with a linear 206 PHD photo-diode array detector was used to analyze the photodegraded samples on a Cosmosil $5C_{18}$-AR analytical column (i.d. 4.6 mm × 15 cm) with a Cosmosil $5C_{18}$-AR guard column (i.d. 4.6 mm × 1 cm) at 280 nm. A mixture of MeOH–0.02 M phosphate buffer pH 3.0 (3 : 1, v/v) was used as the eluent. Flow rate was set at 1 ml/min. Mf was used as the internal standard at a final concentration of 40 μg/ml.

Sample Preparation for Kinetic Studies A 100 μg/ml A/S solution was prepared in methanol, ethanol, 2-propanol, dichloromethane or chloroform to study the solvent polarity effect. To study the pH and ionic strength effect, a 200 μg/ml of A/S ethanol solution was diluted with equal volume of individual buffer solution of different pH (2 to 12) at various levels of ionic strength (μ=0.1 to 0.3). Three ml of one of the above solutions was transferred to an individual 10 ml clear container and then placed under fluorescent light (NEC-FL20SSEX-N-18F). The remaining A/S in each solution was measured by HPLC at each pre-determined checkpoint.

Data Analysis The apparent first-order degradation rate constant was calculated by linear regression analysis using Sigmaplot, Version 5.0 software from Jandel Scientific (San Rafael, CA).

Results and Discussion

Photochemical Decomposition of A/S In our previous study, we demonstrated that photo-oxidation at C-13 of A/S is the main degradation process.[12] To further characterize this process, 100 μg/ml A/S ethanol or chloroform solution in a clear bottle was either wrapped with aluminum foil or replaced with an amber bottle and exposed to fluorescent light for one week. The data presented in Table 1 clearly shows that no photodegradation occurred when A/S solution was protected from exposure to light by aluminum foil. Furthermore, this process slowed down significantly when amber glass bottles were used ($p<0.01$). These results indicate that this degradation process is a photochemical reaction and that light is absolutely essential to initiate it.

Solvent Polarity Effect on the Photochemical Decom-

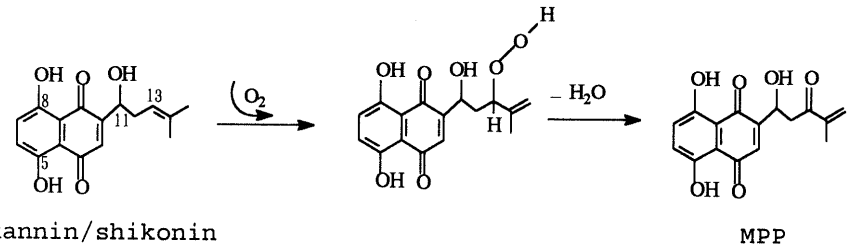

Fig. 1. Postulated Photo-Oxidation Mechanism of A/S to a Major Photolytic product (MPP)[12]

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position of A/S The influence of the solvent on the photochemical decomposition of A/S was investigated in five different solvent systems: methanol, ethanol, 2-propanol, dichloromethane and chloroform. The percentage of A/S remaining in each solvent *vs.* time was plotted to ascertain the kinetic order (Fig. 2). Plots of the logarithm of the percentage parent compound remaining *vs.* time were linear ($r>0.991$), indicating that this photochemical decomposition followed an apparent first-order reaction. The rate constants (k_{obs}) were calculated by linear regression analysis of the apparent first-order plots (Table 2). The results indicated that the photochemical decomposition rate decreased with increasing dielectric constant, *i.e.*, in more polar solvents. Polar solvents generally accelerate reactions that form products having higher polarity than the reactants.[13] Since the photolytic products of A/S were more polar than A/S, as indicated in the reversed-phase HPLC chromatogram with shorter retention time (data not shown), our kinetic results were contrary to the general rules. This suggests that a free radical reaction was involved in the degradation process. Non-polar solvents such as chloroform and dichloromethane stabilize free radicals, and thus facilitate the degradation reaction.[14]

pH Influence on the Photochemical Decomposition of A/S The pH effect on the photochemical decomposition of A/S is shown in Fig. 3. A/S was more stable below pH 6.0 and the degradation rate rose to a maximum between pH 7.5 and 10. The bell-shape curve of rate-pH profile indicated that the photochemical decomposition of A/S was only marginally subject to specific acid or base catalysis but was drastically affected by two ionizable groups on the molecule. A/S thus appears to be a dibasic acid with its mono-anionic form being the most reactive form. Though the exact pK values of A/S are not available in the literature, it is stated in the Merck Index that the aqueous solution of A/S is red at pH 6.1, purple at pH 8.8, and blue at pH 10.0,[15] suggesting that pK_1 is in the range of 6 to 10. This is in good agreement with our observation that maximum degradation of A/S occurs between pH 7.5 and 10 when A/S is in its mono-anionic form.

Ionic Strength Effect on the Photochemical Decomposition of A/S Since the photodecomposition of A/S was most active in its mono-anionic form, the ionic strength effect on the degradation was investigated. The results, as presented in Table 3, indicated that ionic strength did not affect the photochemical decomposition of A/S at either pH 5, 9 or 12, regardless of the ionic form of A/S present in the solution. Ions in aqueous solution tend to exert

Table 1. Percentage of A/S Remaining in Ethanol or Chloroform Solution after Exposure to Fluorescent Light for One Week ($n=3$)

Solvent system	Clear bottle		Amber bottle
	Aluminum foil wrapped	Not aluminum foil wrapped	
Ethanol	101.3±0.7	36.0±1.2[a)]	72.2±0.3*[,a)]
Chloroform	99.0±1.3	13.3±0.7[a)]	47.3±1.3*[,a)]

a) There is a statistic difference between the % of A/S remaining in an aluminum foil wrapped bottle and that in a not aluminum foil wrapped bottle or an amber bottle by Student's t test ($p<0.01$). * There is a statistic difference between the % of A/S remaining in a not aluminum foil wrapped bottle and that in an amber bottle by Student's t test ($p<0.01$).

Table 2. Observed First-Order Photochemical Decomposition Rates of A/S in Five Different Solvents

Solvent	Dielectric constant	k_{obs} (h^{-1})
Chloroform	4.8	1.3×10^{-2}
Dichloromethane	8.9	8.6×10^{-3}
2-Propanol	18.3	6.6×10^{-3}
Ethanol	24.3	5.9×10^{-3}
Methanol	32.8	5.7×10^{-3}

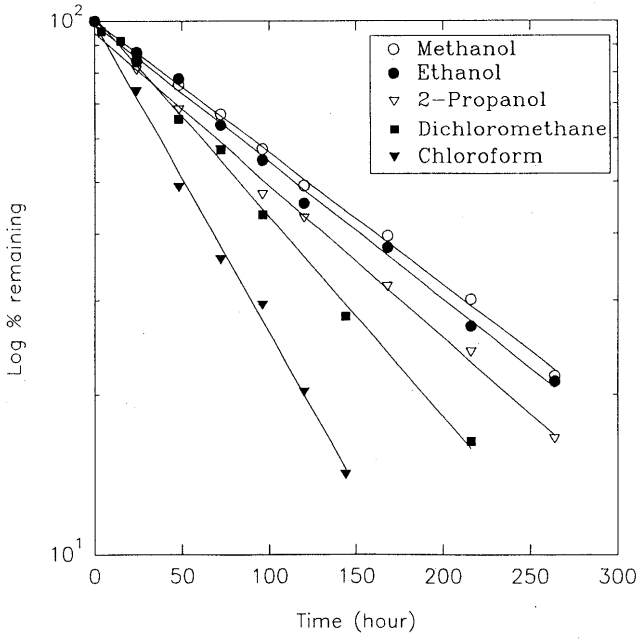

Fig. 2. A Linear Regression Plot of the log % of Remaining A/S *vs.* Time for the Photochemical Decomposition of A/S in Five Different Solvents

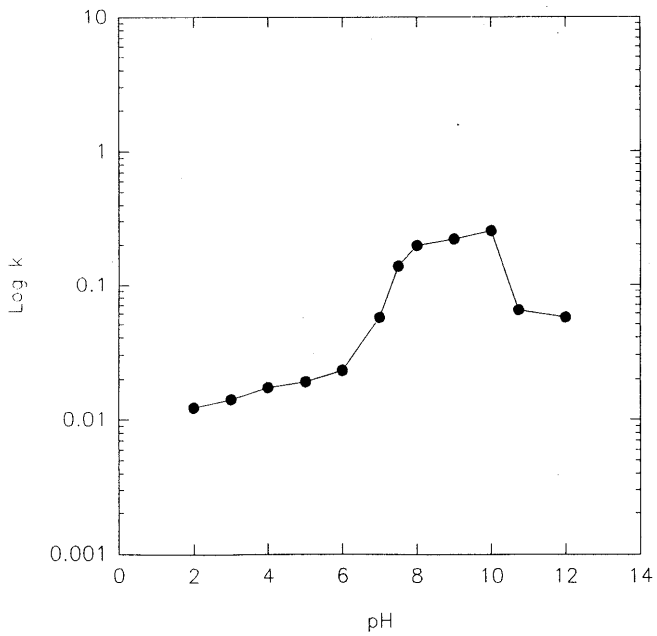

Fig. 3. Rate-pH Profile for the Photochemical Decomposition of A/S
All buffers were kept at an ionic strength of 0.1.

Table 3. Observed First-Order Photochemical Decomposition Rates of A/S Buffer Solutions with Different Ionic Strengths at pH 5, 9 and 12

pH	Ionic strength (μ)	k_{obs} (h^{-1})
5	0.1	1.9×10^{-2}
(Acetate)	0.2	1.9×10^{-2}
	0.3	1.9×10^{-2}
9	0.1	2.2×10^{-1}
(Phosphate)	0.2	2.2×10^{-1}
	0.3	2.1×10^{-1}
12	0.1	5.6×10^{-2}
(Phosphate)	0.2	5.6×10^{-2}
	0.3	5.4×10^{-2}

their effect mainly at the ionized groups. The results from the ionic strength effect study also indirectly support our hypothesis of a free radical reaction involvement in the photochemical decomposition of A/S.

In conclusion, kinetic study on the photochemical decomposition of A/S shows that it is facilitated by non-polar solvent and alkaline pH and is not affected by the ionic strength of the solution. From a practical point of view, our findings suggest that using polar solvent to formulate pharmaceutical preparations containing A/S or shikon extract in an acidic condition is an effective means to enhance the photostability of the final products.

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References

1) Tanaka S., Tajima M., Tsukada M., Tabata M., *J. Nat. Products*, **49**, 466 (1986).
2) Hayashi M., *Folia Pharmacol. Jpn.*, **73**, 193 (1977).
3) Honda G., Sakakibara F., Yazaki K., Tabata M., *J. Nat. Products.*, **51**, 152 (1988).
4) Tabata M., Tsukada M., Fukui H., *Planta Med.*, **44**, 234 (1982).
5) Tabata M., Mizukami H., Naoe S., Konoshima M., *Yakugaku Zasshi*, **95**, 1376 (1975).
6) Seto Y., Motoyoshi S., Nakamura H., Imuta J., Ishitoku T., Isayama S., *Yakugaku Zasshi*, **112**, 259 (1992).
7) Papageorgiou V. P., *Experienta*, **34**, 1499 (1978).
8) Sankawa U., Otsuka H., Kataoka Y., Iitaka Y., Hoshi A., Kuretani K., *Chem. Pharm. Bull.*, **29**, 116 (1981).
9) Sankawa U., Ebizuka Y., Miyazaki T., Isomura Y., Otsuka H., Shibata S., Inomata M., Fukuoka F., *Chem. Pharm. Bull.*, **25**, 2392 (1977).
10) Konoshima T., Kozuka M., Koyama J., Okatani T., Tagahara K. Tokuda H., *J. Nat. Products*, **52**, 987 (1989).
11) Hayashi M., *Folia Pharmacol. Jpn.*, **73**, 205 (1977).
12) Cheng H. W., Chen F. A., Hsu H. C., Chen C. Y., *Int. J. Pharm.*, **120**, 137 (1995).
13) Martin A., "Physical Pharmacy," 4th ed., Lea & Febiger, PA, London, 1993, p. 299.
14) Ellis G. P., "Modern Textbook of Organic Chemistry," 1966, p. 74.
15) Windholz M., "The Merck Index," 10th ed., 1983, p. 39.