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Analysis of the Stability and Degradation Products of Triptolide

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Abstract

Triptolide is the major active ingredient of the Chinese herbal remedy *Tripterygium wilfordii* Hook F. (TwHF). As triptolide content is used to estimate the potency of preparations of TwHF, assessment of its stability is warranted.

The accelerated stability of triptolide was investigated in 5% ethanol solution in a light-protected environment at pH 6·9, within a temperature range of 60–90°C. The observed degradation rate followed first-order kinetics. The degradation rate constant (K_{25}°C) obtained by trending line analysis of Arrhenius plots of triptolide was $1{\cdot}4125 \times 10^{-4}$ h^{-1}. The times to degrade 10% ($t_{1/10}$) and 50% ($t_{1/2}$) at 25°C were 31 and 204 days, respectively. Stability tests of triptolide in different solvents and different pH conditions (pH 4–10) in a light-protected environment at room temperature demonstrated that basic medium and a hydrophilic solvent were the major factors that accelerated the degradation of triptolide. Triptolide exhibited the fastest degradation rate at pH 10 and the slowest rate at pH 6. In a solvent comparison, triptolide was found to be very stable in chloroform. The stability of triptolide in organic polar solvents tested at both 100% and 90% concentration was greater in ethanol than in methanol than in dimethylsulphoxide. Stability was also greater in a mixture of solvent : pH 6 buffer (9 : 1) than in 100% solvent alone. An exception was ethyl acetate, which is less polar than the other solvents tested, but permitted more rapid degradation of triptolide. Two of the degradation products of triptolide were isolated and identified by HPLC and mass spectroscopy as triptriolide and triptonide. This suggested that the decomposition of triptolide occurred at the C12 and C13 epoxy group and the C14 hydroxyl. The opening of the C12 and C13 epoxy is an irreversible reaction, but the reaction occurring on the C14 hydroxyl is reversible.

These results show that the major degradation pathway of triptolide involves decomposition of the C12 and C13 epoxy group. Since this reaction is very slow at 4°C at pH 6, stability is enhanced under these conditions.

Triptolide has been characterized as one of the most important biologically active components of the Chinese traditional remedy, *Tripterygium wilfordii* Hook F. (TwHF). Two kinds of oral preparations of TwHF, a chloroform methanol extract termed T2 and an ethyl acetate extract, have been used in China for many years. They have been shown to be effective in a number of autoimmune and inflammatory diseases (Lipsky & Tao 1997). Of the many components of TwHF, the diterpenoid, triptolide, is thought to be the most potent anti-inflammatory and immunosuppressive agent (Tao et al 1995). Due to its activity, triptolide content has been used to evaluate the quality of all the preparations of TwHF extracts (Lipsky & Tao 1997). However, triptolide is known to be unstable (Zhang & Deng 1991) and therefore, the extraction procedure or subsequent storage can alter the content of triptolide. Previous studies on the relationship of structure and activity of triptolide demonstrated that the 9,11-epoxide and the 14β-oriented-hydroxyl groups are necessary for the anti-leukaemic and immunosuppressive activity (Kupchan et al 1972; Tao et al 1995). The α, β-unsaturated lactone and other oxane moieties of

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triptolide are also thought to contribute to its activity (Kupchan et al 1971). It is possible that instability of these functionally active portions of triptolide may contribute to the tendency of triptolide to undergo degradation. However, no previous studies have documented the degradation products of triptolide and the reaction mechanisms involved.

Therefore, we have carried out a series of experiments to examine these issues. The stability of triptolide in different conditions was studied using an HPLC detection method and the stability kinetics and the degradation rates of triptolide were estimated by classical chemical kinetic methods. The major degradation products were isolated and identified by combining HPLC with mass spectrometry and UV spectroscopy.

The results of these studies could provide the basis for the reproducible manufacture and storage of triptolide and its effective use in the treatment of autoimmune diseases.

Materials and Methods

Chemicals

All the diterpenoids were isolated from TwHF as described by Mao et al (1998). Triptolide, tripdiolide, triptonide, 16-hydroxytriptolide and triptophenolide were identified by UV, IR, MS, H NMR and ^{13}C NMR and the structural results were compared with published data (Kupchan et al 1972; Deng et al 1982; Ma et al 1991). Two-dimensional NMR confirmed the structure of 16-hydroxytriptolide. Triptriolide was characterized by UV, MS, H NMR and^{13}C NMR, and the results compared with published data (Ma et al 1991).

Reagents

Acetophenone, as an internal standard, was HPLC grade (Sigma Chemical Co., St Louis, MO). Acetonitrile was HPLC grade (Aldrich Chemical Company, Milwaukee, WI). Potassium phosphate monobasic sodium hydroxide buffer (pH 4–10) came from Fisher Scientific (Pittsburgh, PA). Ethyl acetate, ethanol, methanol, dimethylsulphoxide (DMSO) and chloroform were reagent grade (E. M. Science Chemicals, Gibbstown, NJ). Water was prepared with a Millipore Milli-Q Plus device (Millipore, Bedford, MA).

Instruments

The model 188 constant-temperature bath (Precision, Chicago IL) was used for kinetic measurements. The HPLC used to identify diterpenoids was equipped with two model 510 pumps, a U6K injector, and a 486 detector (Waters Assoc.). Nova-Pak C_{18} stainless steel columns (300 mm × 3·9 mm i.d. and 150 mm × 3·9 mm i.d., particle size 4 μm) were used for analysing diterpenoids and identification of degradation products of triptolide. A Nova-Pak C_{18} column (100 mm × 25 mm i.d.) was used for the isolation of degradation products of triptolide (Waters, Milford, MA). Eluates were monitored at 218 nm. All procedures were conducted at room temperature. The HPLC data were processed with Millennium software, Version 2.01 (Chromatography Manager, Millipore Corporation, Milford, MA). Regression analysis of kinetic stability data was calculated with Cricket Graph version 1.3.2. The electrospray mass spectra were obtained with a model VG Quattro II instrument (Micromass, Manchester, UK).

HPLC methods

Method 1. The method reported by Cai et al (1994) was used. The column was 150 mm × 3·9 mm, the mobile phase was acetonitrile–water (19 : 81), and the flow rate was 1 mL min^{-1}. The concentration of triptolide was calculated by comparison with the internal standard.

Method 2. A 300 mm × 3·9 mm stainless steel column was used, the mobile phase was acetonitrile–water (35 : 65), the flow rate was 1 mL min^{-1} and the injection amount was 10 μL. Peak area was used for calculating and comparing the concentration of triptolide. A standard curve was prepared for correlation of determination error.

Method 3. A 300 mm × 3·9 mm stainless steel column with a flow rate of 1 mL min^{-1} was used for the determination and comparison of degradation products. The mobile phase was acetonitrile–water (28 : 72). Peak area was used for calculating and comparing the concentration of triptolide.

Stability kinetics procedure

Assay preparation is based on the solubility of triptolide and the injection requirement; 5% ethanol in water was used as the solvent in the stability kinetics test of triptolide. Approximately 5 mg triptolide was weighed accurately, dissolved in 4 mL ethanol, and then diluted to 80 mL with water to produce a 61·32 μg mL^{-1} concentration. Samples of 0·5 mL triptolide solution were added to 2 mL amber glass ampoules, which were flame sealed

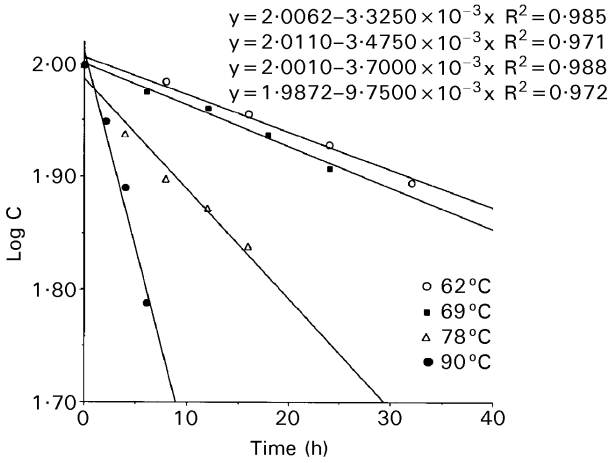

Figure 1. The degradation of triptolide in 5% ethanol maintained at different temperatures.

Table 1. The experimental mean constant k of triptolide.

t (°C)	T (t + 273) °C	k ($\times 10^{-3}$)	r
62	335	6·92	0·9965
69	342	8·57	0·9940
78	351	0·225	0·9859
90	363	0·626	0·9980

and maintained in a constant-temperature bath in which the appropriate temperature (Table 1) had been adjusted in a light-protected environment. The "zero time" samples were maintained at −20°C. Ampoules were withdrawn from the water bath at suitable time intervals (Figure 1), and immediately frozen at −20°C until analysis. All the analyses were carried out in duplicate.

Before analysis, the samples were taken from the freezer and warmed to room temperature. One millilitre of 24·5 μg mL^{-1} acetophenone methanol solution was added accurately to the samples of triptolide for the HPLC analysis (method 1). The injection volume for each sample was 8 μL.

The standard solutions, containing 59·464, 29·732, 14·866 or 7·434 μg mL^{-1} triptolide and 25·5 μg mL^{-1} acetophenone (as an internal standard for triptolide), were prepared in acetonitrile–water (19 : 81). The linear calibration of triptolide was obtained by comparing the ratio of the HPLC peak areas of triptolide with the internal standard (HPLC method 1).

Stability of triptolide at different pH

Triptolide was dissolved in chloroform at the concentration of 54·42 μg mL^{-1}. Samples of 200 μL triptolide chloroform solution were evaporated to dryness at room temperature and dissolved in 200 μL of each of the different pH buffer solutions (Figure 3) in amber bottles. Eight samples were prepared and analysed for each pH. All samples were maintained at 50°C in constant-temperature water until analysed. Two samples were analysed at each time point. After evaporation to dryness, samples were stored at −20°C until quantitative determination. Each sample was analysed by HPLC method 2 in duplicate.

Stability test of triptolide in different solvents

Stability in chloroform solution. Triptolide was dissolved in chloroform solution (44·6 μg mL^{-1}) and stored at room temperature in a light-protected environment for three years. Samples were analysed quantitatively for triptolide and its degradation products initially, and then at yearly intervals for up to three years.

Stability in other solvents. Triptolide was dissolved in chloroform (51·78 μg mL^{-1}) and 200-μL samples were evaporated to dryness at room temperature and then dissolved in 200 μL of either ethyl acetate, DMSO, methanol, ethanol, DMSO : pH 6 buffer (9 : 1), methanol : pH 6 buffer (9 : 1) or ethanol : pH 6 buffer (9 : 1). Four samples dissolved in each buffer were prepared. After maintaining the solutions at room temperature in amber bottles for three days, the samples were evaporated to dryness and stored at −20°C until analysis. For analysis, 200 μL methanol was added to each sample before HPLC determination. Each sample was analysed in duplicate by HPLC method 2.

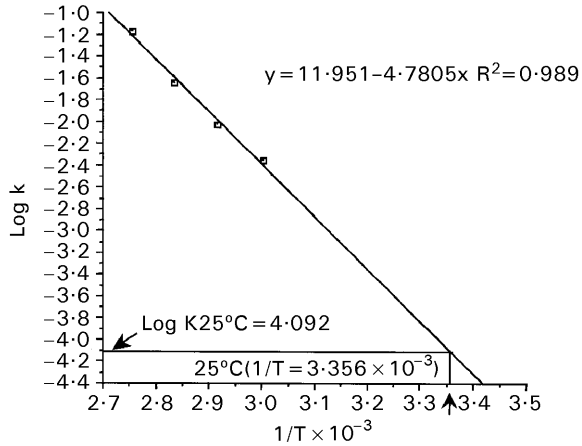

Figure 2. Arrhenius plot of triptolide decomposition.

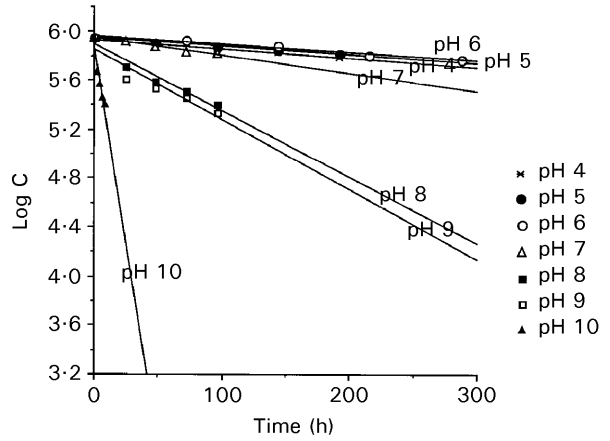

Figure 3. The effect of pH on triptolide stability at 50°C.

Table 2. The change in triptolide concentration in different solvents.

	Triptolide concentration ($\mu g\,mL^{-1}$)			
	Ethyl acetate	Ethanol	Methanol	DMSO
100% solvent	48·95	50·90	50·66	49·90
90% solvent		51·63	51·11	50·16

Other stability tests of triptolide and the degradation products of triptolide

To determine the stability of triptolide more precisely, the following experiments were performed. For these experiments, samples were analysed by HPLC method 3. Firstly, samples of triptolide in aqueous solution (70·054 $\mu g\,mL^{-1}$) were stored at 4 and 25°C in a light-protected condition for 20 days (n = 5). Secondly, triptriolide and triptonide were dissolved in methanol (4·04 $\mu g\,mL^{-1}$) and stored at room temperature for two days (n = 3). Thirdly, triptolide was dissolved in pH 7·95 or 5·14 buffer (70·054 $\mu g\,mL^{-1}$), and stored at 4°C in a light-protected condition for six months. Samples were analysed every two months (n = 3).

Identification of degradation products of triptolide

For the analysis of the degradation products, the remaining stability kinetic samples of triptolide were incubated at 70°C for 22 h, combined and then stored at −15°C for two months, in a light-protected environment.

For HPLC identification, a 3·9 mm × 300 mm column was used. Chromatograms were generated in both the gradient and the isocratic modes. Mixtures of six authentic standards of triptriolide, 16-hydroxy-triptolide, tripdiolide, triptolide, triptonide

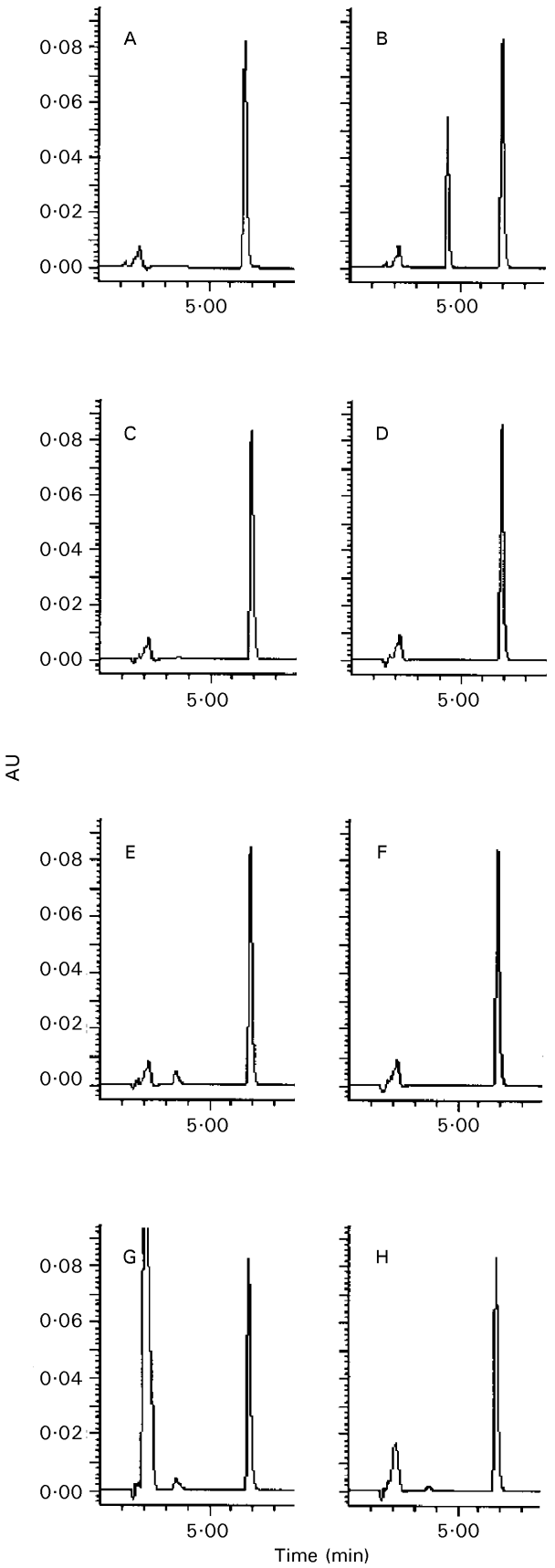

Figure 4. Triptolide (A) stability in ethyl acetate (B), ethanol (C) ethanol (D), methanol (E), 90% methanol (F), DMSO (G) and 90% DMSO (H).

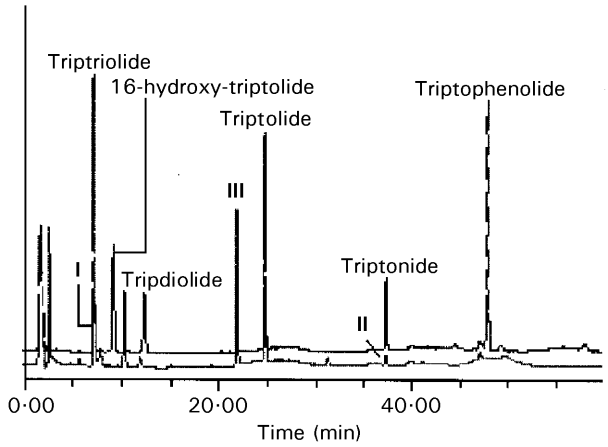

Figure 5. Overlay plot of analytical chromatograms of six authentic standards (upper plot) and triptolide and its degradation products (lower plot).

Gradient table

Time	Flow	%H_2O	%CH_3CN	Curve
0	1·0	83	17	0
10	1·0	82	18	6
28	1·2	65	35	6
43	1·2	50	50	6

and triptophenolide were prepared by dissolving the individual compound in 50% methanol to produce a concentration of approximately 4·4 $\mu g\,mL^{-1}$. The authentic standard solutions of triptolide, triptriolide and triptonide were dissolved in methanol at a concentration of approximately 5 $\mu g\,mL^{-1}$, respectively. The retention times of degradation products of triptolide were compared with the authentic standards using the same HPLC conditions.

For HPLC isolation of degradation products of triptolide, a 25 mm × 100 mm column was used. The liquid phase was acetonitrile : water (35 : 65) and the flow rate was 8 mL min^{-1}. The eluants containing degradation products of triptolide were warmed moderately to remove most of the acetonitrile, and then passed through a C_{18} cartridge. The cartridge was eluted with methanol. After evaporation of the eluates to dryness, one white crystalline (fraction I) and two white powder fractions (fractions III and II) were obtained for HPLC, UV and MS spectroscopic identification.

Results and Discussion

Stability kinetics of triptolide

Calibration graphs. The peak area values at selected wavelengths were linearly correlated with concentrations in the range of 59·472–475·712 $\mu g\,mL^{-1}$ for triptolide. The correlation coefficients of 0·9999 indicated good linearity of the calibration graphs.

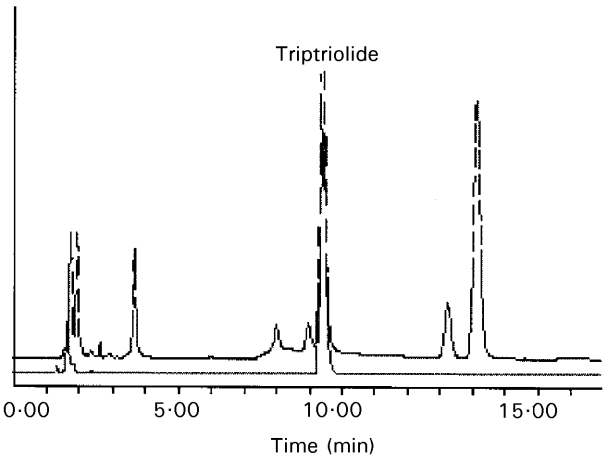

Figure 6. Overlay plot of chromatograms of an authentic standard of triptriolide (solid line) and degradation products of triptolide (dashed line). The mobile phase was acetonitrile–water (15 : 85) and the flow rate was 1 mL min^{-1}.

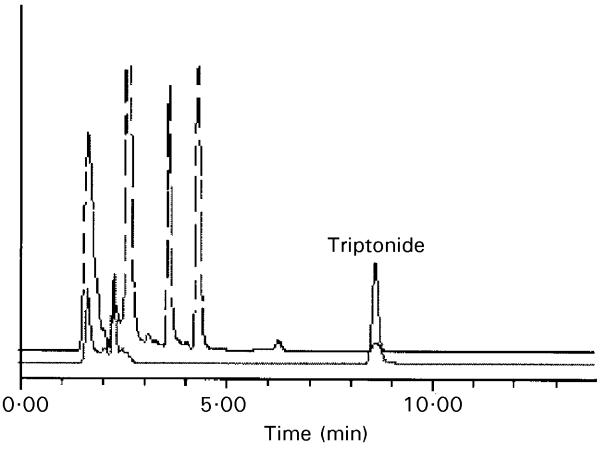

Figure 7. Overlay plot of chromatograms of an authentic standard of triptonide (solid line) and degradation products of triptolide (dashed line). The mobile phase was acetonitrile–water (44 : 56) and the flow rate was 1 mL min^{-1}.

Reaction order and rate constants. After HPLC assay, the results of the stability kinetics of samples of triptolide were analysed theoretically. The reaction order was determined graphically by plotting the logarithm of the sample concentrations (log C) vs time. Over the temperature range of the experiment (60–90°C), the degradation of triptolide demonstrated first-order hydrolysis rates, fitting Arrhenius kinetics (Figure 1). The average experimental rate constant k for triptolide at different temperatures is shown in Table 1.

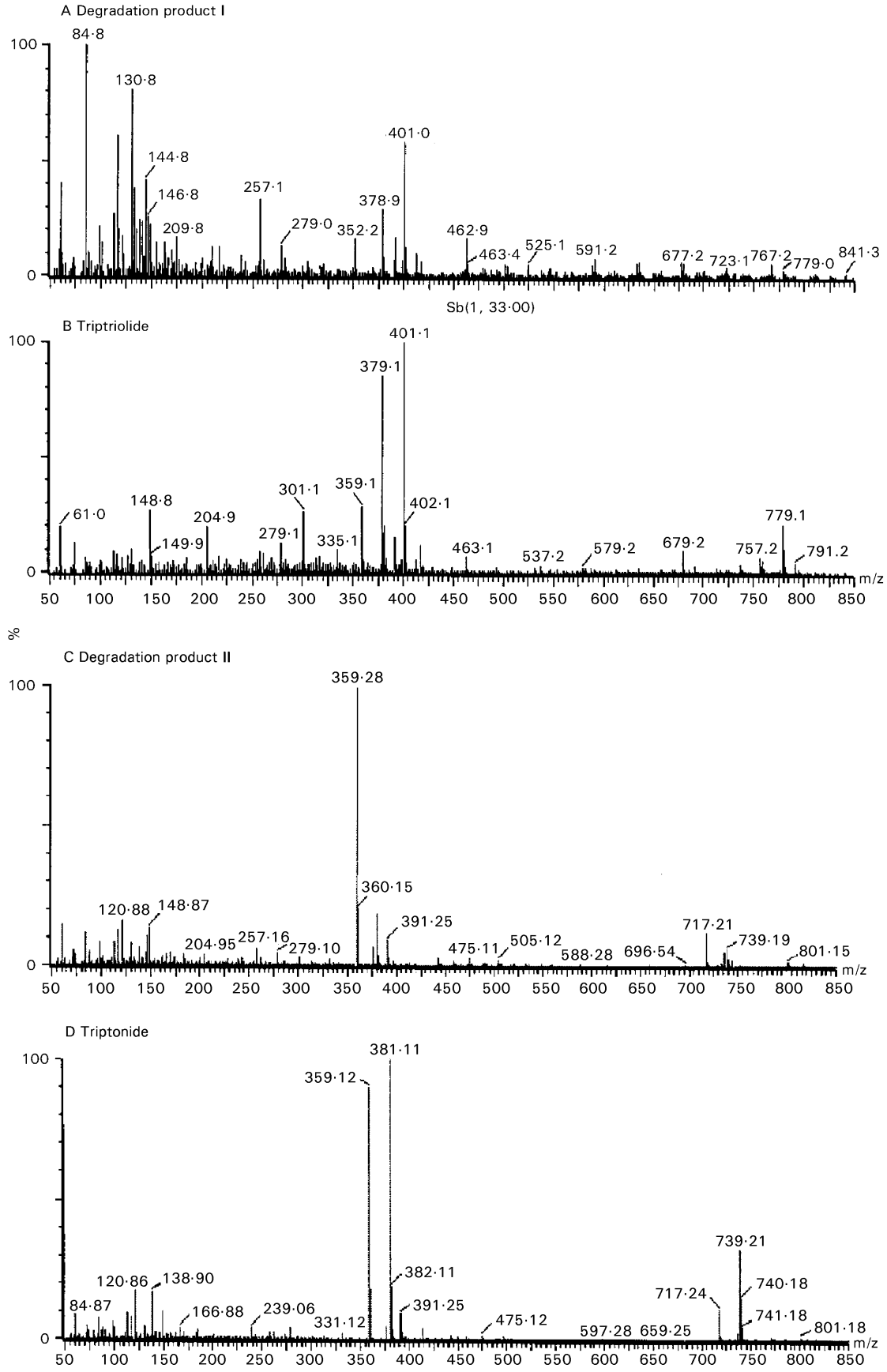

Figure 8. Mass spectra of degradation product I (A), triptriolide (B), degradation product II (C) and triptonide (D).

Plotting log k vs the reciprocal of absolute temperature (1/T), gave an Arrhenius plot for triptolide (Figure 2). Extrapolating the regression curves to 25°C, the k_{25}°C of triptolide was determined as $1{\cdot}4125 \times 10^{-4}\,h^{-1}$. Using appropriate equations, the times taken to degrade triptolide by 10% ($t_{1/10} = 0{\cdot}105/k_{25}$°C) and 50% ($t_{1/2} = 0{\cdot}693/k_{25}$°C) at 25°C were calculated as 31 and 204 days, respectively.

Triptolide stability at different pH

Zhang & Deng (1991) compared the stability results of triptolide in different pH buffer solutions and reported that pH 4–6 was the stable range for triptolide. The current studies have expanded upon this finding and have shown that basic medium can markedly accelerate the degradation of triptolide. When triptolide was dissolved in different pH buffers (pH 4–10), it had the fastest degradation rate at pH 10, and the slowest degradation rate at pH 6. Triptolide was more stable in an environment of slight acidity (pH 6 > pH 5 > pH 4) than in a pH 7 or an alkalized environment (pH 7 > pH 8 > pH 9 > pH 10) (Figure 3).

Triptolide stability in different solvents

Triptolide was stable in chloroform solution. After storage for three years, no degradation products were detected by HPLC. In addition, no obvious change was observed in the physical appearance of the triptolide chloroform solution.

The stability of triptolide in other organic solvents exhibited the following order from most to least stable: ethanol > methanol > DMSO and 90% ethanol > 90% methanol > 90% DMSO (Table 2, Figure 4). These results suggested that the stability of triptolide decreased with increasing hydrophilicity of the solvent. The finding that solvents containing 10% pH 6 buffer increased the stability of triptolide compared with 100% organic solvents further indicated that a pH 6 environment was important to keep triptolide stable. An exception was that triptolide was very unstable in ethyl acetate solution, the most commonly used solvent in the extraction of triptolide from TwHF. The major degradation product of triptolide in ethyl acetate solution was different than that appearing with other solvent solutions. Instead of hydrolysis, an esterification of triptolide appeared to occur in ethyl acetate solution (Figure 4).

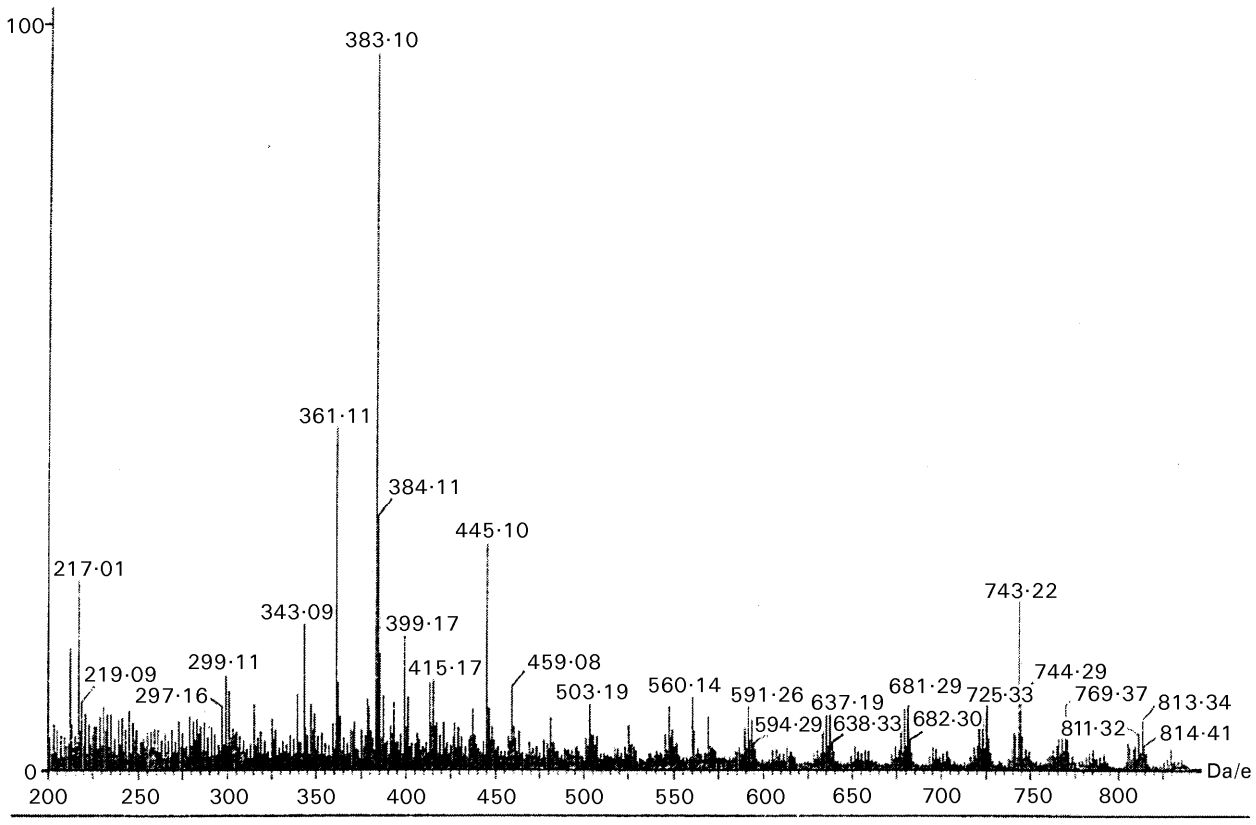

Figure 9. Mass spectra of degradation product III.

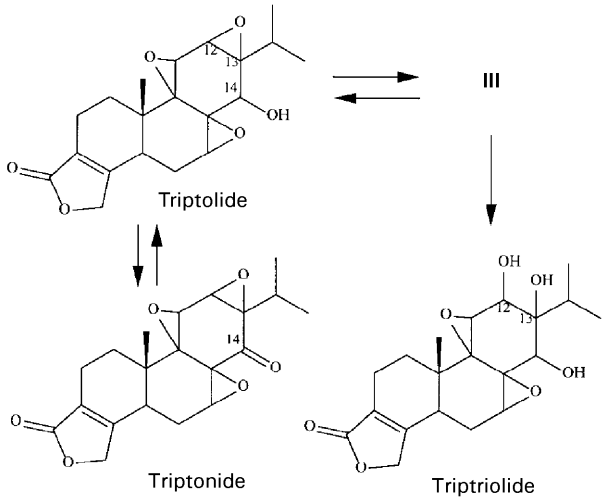

Figure 10. Degradation scheme of triptolide.

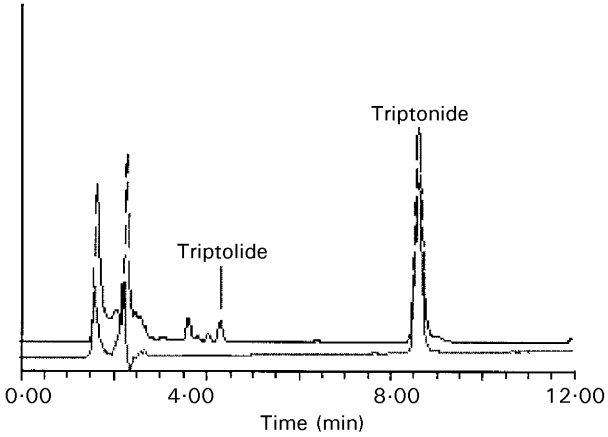

Figure 11. Overlay plot of chromatograms of authentic triptonide. The upper plot shows triptonide stored in methanol at room temperature for two days. The lower plot shows triptonide detected immediately after being dissolved in methanol. The mobile phase was acetonitrile–water (44 : 56) and the flow rate was 1 mL min^{-1}.

Identification of degradation products of triptolide

Figure 5 shows the analytical HPLC chromatograms of triptolide and its degradation products determined by gradient elution. Two peaks of degradation products of triptolide, labelled I and II were detected with retention times of 7·283 and 37·450 min, matching the authentic standards triptriolide and triptonide, respectively. The isocratic mode of HPLC was used to confirm the results: peak I with a retention time of 9·467 min, matched the retention time of triptriolide (Figure 6). The retention time of peak II was 8·667 min, matching the retention time of triptonide (Figure 7). Peak III did not match any authentic standard. None of the triptolide degradation products were identified as 16-hydroxytriptolide, tripdiolide or triptophenolide.

The electrospray mass data of I and II and the FAB mass data of III were obtained with a solvent system of methanol : water (1 : 1) + 0·01% formic acid. The spectrum of I showed an ion at m/z 378·9 (compared with authentic triptriolide m/z 379·1) representing $(M+H)^+$. The spectrum of II had an ion at m/z 359·28 (compared with authentic triptonide m/z 359·12) representing $(M+H)^+$. Other ions were consistent with the proposed structures of I and II as triptriolide and triptonide, respectively (Figure 8). The spectrum of III showed an ion peak at m/z 361·11 and 383·10, representing $(M+H)^+$ and $(M+Na)^+$, respectively (Figure 9). This pattern is similar to triptolide ($M^+ = 360{\cdot}16$, Kupchan et al (1972)). The H NMR and C^{13} NMR results of III (unpublished data) provided additional evidence that III had the same backbone as triptolide. Therefore, we suggest that III is an isomer of triptolide. UV spectra with 50% methanol showed maximum absorption at 218 nm for I, II and III.

Based on the MS spectra and HPLC retention times that matched the authentic standards, triptolide degradation products I and II were tentatively assigned as triptriolide and triptonide. It is apparent that a hydrolysis reaction occurred at the C12–C13 epoxy group and a mild oxidization reaction occurred at the C14 hydroxyl group of triptolide under the reported test conditions (Figure 10).

Other stability tests

The results of the other stability tests of triptolide and its degradation products are shown in Table 3. The behaviour of triptolide at two different temperatures indicated that both triptriolide and III were major degradation products of triptolide in water solution at 25°C, but more III and only a trace of triptriolide were found in the same samples stored at 4°C for 20 days. This difference suggested that the hydration of triptolide to triptriolide required more energy than the reaction of triptolide to III.

The formation of III under mild hydrolysis conditions is noteworthy because it confirmed that the decomposition of triptolide involved isomerization and suggested that the isomerization reaction of triptolide required less energy than its hydrolysis. III is an energy-rich compound with low stability; thus its structure analysis could not be completed. III was quantitatively converted to triptriolide after two-dimensional NMR identification with methanol : chloroform (1 : 1) as the solvent. One expla-

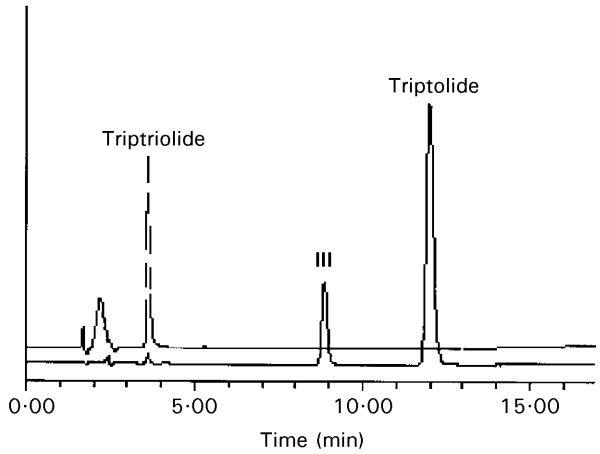

Figure 12. Chromatograph of triptolide at 4°C. The upper plot shows authentic triptriolide. The lower plot shows triptolide stored at 4°C for six months.

nation for this phenomenon is that III might be an intermediate in the conversion of triptolide to triptriolide. At low temperature, it is relatively stable, but at high temperature, III is likely to convert to triptriolide.

Analysis of degradation products of triptriolide and triptonide indicated that both were unstable in methanol. After storage of triptriolide at room temperature for two days, several degradation products were detected but none were identified as triptolide. This suggests that the transformation of triptolide to triptriolide is irreversible. When triptonide was dissolved in methanol and stored at room temperature for two days triptolide was identified as the major degradation product (Figure 11). This indicated that triptolide and triptonide may interconvert under certain conditions. The reaction rate of the conversion of triptolide to triptonide appears to be much slower than its reverse reaction.

An equilibrium reaction between triptolide and III was observed at a pH range of 5·14–7·95 at 4°C. The reaction reached equilibrium in less than two months, by which time the concentration ratio of triptolide to III was about 100 : 27·5 (Table 4). Only traces of triptriolide were found in these samples after they were stored for prolonged times (Figure 12).

Table 4. The ratio of triptolide/III produced from triptolide maintained at different pH.

Length of storage at 4°C	pH 5·14	pH 7·95
	Triptolide : III	Triptolide : III
2 months	100 : 28·6	100 : 27·05
4 months	100 : 27·53	100 : 27·81
6 months	100 : 27·95	100 : 27·49

The α, β-unsaturated lactone of triptolide has been thought to be an unstable structure. However, this structure has proven to be relatively stable in the conditions tested here. There was no indication of a change in this portion of triptolide.

In conclusion, triptolide degradation followed first-order hydrolysis rates, fitting Arrhenius kinetics. The decomposition of triptolide in a light-protected environment involved a series of complex reactions, including hydrolysis, isomerization and oxidation. The observed hydrolysis occurred at the C12–c13 epoxy group and the oxidation occurred at the C14 hydroxyl group. At 4°C, isomerization was the primary degradation reaction, and the transformation between triptolide and iso-triptolide III was reversible, reaching equilibrium when the peak area of III at $\lambda = 218$ nm was 27·5% of that of triptolide. Hydroxyl ions were the most important accelerators of triptolide degradation. pH 6 was the optimum condition for triptolide stability. The polarity of the solvent and temperature were the other factors affecting the degradation of triptolide. Triptolide was stable in chloroform. Hydrolysis from triptolide to triptriolide was an irreversible reaction and it was the major degradation pathway of triptolide exhibiting first-order degradation kinetics. This reaction was very slow at 4°C at pH 6. Therefore, the degradation of triptolide was limited under these conditions.

Table 3. Comparison of the degradation products of triptolide, triptriolide and triptonide.

Test	Diterpenoids	Solvent	Storage days	Storage temp.	Degradation results
1	Triptolide	Water	20	4°C	Trace of triptriolide; III was the major degradation product
			20	25°C	Triptriolide and III were the major degradation products
2	Triptriolide	Methanol	2	25°C	No triptolide was detected
	Triptonide	Methanol	2	25°C	Triptolide was one of the major degradation products
3	Triptolide	pH 7·95	60–180	4°C	Both samples had trace of triptriolide, the peak area ratio of triptolide : III was 100 : 27·5
		pH 5·14	60–180	4°C	

References

Cai, J. J., Tao, X. L., Lipsky, P. E. (1994) High-performance liquid chromatographic determination of triptolide and tripdiolide in an ethyl acetate extract of Tripterygium Wilfordii Hook F. J. Liq. Chromatogr. 17: 4479–4487

Deng, F. X., Zhou, B. N., Song, G. Q., Hu, C. Q. (1982) Studies on the chemical constitutions of Tripterygium wilfordii Hook F. Yao Xue Xue Bao 17: 148–150

Kupchan, S. M., Eakin, M. A., Thomas, A. M. (1971) Tumor inhibitors. 69. Structure–cytotoxicity relationship among the sesquiterpene lactones. J. Med. Chem. 14: 1147

Kupchan, S. M., Court, W. A., Dailey, R. G., Christopher, J., Gilmore, J., Bryan, R. F. (1972) Triptolide and tripdiolide, novel anti-leukemic diterpenoid tripoxides from Tripterygium wilfordii. J. Am. Chem. Soc. 94: 7194–7195

Lipsky, P. E, Tao, X. L. (1997) A potential new treatment for rheumatoid arthritis: Thunder God Vine. Semin. Arthritis Rheum. 26: 713–723

Ma, P. C., Lu, X. Y., Yang, J. J., Zhang, Q. T. (1991) The isolation and structure studies of 16-hydroxyl-triptolide. Yao Xue Xue Bao 26: 759–763

Mao, Y. P., Cai, J. J., Tao, X. L., Lipsky, P. E. (1998) High-performance liquid chromatographic determination of triptonide, triptriolide and triptophenolide in ethyl acetate extract of tripterygium wilfordii Hook F. J. Liq. Chromatogr. Rel. Technol. 21: 2699–2714

Tao, X. L., Cai, J. J., Lipsky, P. E. (1995) The identify of immunosuppressive components of the ethyl acetate extract and chloroform methanol extract (T2) of tripterygium wolfodii Hook F. J. Pharmacol. Exp. Ther. 272: 1305–1312

Zhang, H. L., Deng, K. Q. (1991) The stability studies of triptolide and its injection. Zhong Guo Yao Xue Zha Zhi 36: 3–4