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Stability of Antineoplaston A10 in Aqueous Solution

In Joon Oh, Jeong Min Lee, Yong Bok Lee, Sang Chul Shin and Bo Gil Choi

College of Pharmacy, Chonnam National University, Gwangju, 500-757, Korea

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The analysis method and stability test of antineoplaston A10, a new anticancer drug candidate, were established. A10 and phenylacetyl- L-glutamine, one of the degradation products, can be detected by high-performance liquid chromatography. The degradation kinetics of antineoplaston A10 in aqueous solutions from pH 1 to 10 buffers were carried out at 40, 50 and 60℃. Pseudo-first order kinetics were obtained throughout the entire pH ranges studied. The pH-rate profiles showed that antineoplaston A10 was very unstable in alkaline conditions and most stable at pH 4.

Key words : Antineoplaston A10, Stability, Hydrolysis, Anticancer agent

INTRODUCTION

Antineoplaston A10, 3-(N-phenylacetylamino)-2,6-piperidinedione is a natural compound isolated from human urine. This compound is known to have antitumor activity and a lack of toxicity (Burzynski *et al.*, 1984). Antineoplastons are small peptides and amino acid derivatives produced by the living organism and constitute a natural biochemical defense system against development of neoplastic growth by a nonim-munological progress which does not significantly inhibit the growth of normal cells (Burzynski,1986). The size and shape of antineoplaston A10 is similar to a DNA base pair and may intercalate ste reospecifically between base pairs in double helical DNA (Burzynski *et al.*, 1984). Antineoplaston A10 interacts very closely with adenosine units in nucleic acids and enzymes, which may interfere with protein synthesis in neoplastic cells (Michalska, 1990). Antineoplaston A10 has higher effects and lower toxicity towards most human cancers. Before the development of dosage forms with a new drug candidate, it is essential that certain fundamental phy sical and chemical properties of the drug molecule and other derived properties of the drug are determined. This information will dictate many of the subsequent events and possible approaches in formulation development. Antineoplaston A10 is poorly soluble in water and fairly resistant to acid hydrolysis at room temperature. But the detail information of stability data has not been reported. In this paper, the rapid determination of antineoplaston A10 in solution using a suitable chromatographic column and mobile phase were carried out. And the stability test of antineoplaston A10 was undertaken at various temperatures and pH.

Correspondence to: In Joon Oh, College of Pharmacy, Chonnam National University, 300 Yongbong-dong, Gwangju, 500-757 Korea

MATERIALS AND METHODS

Materials

Antineoplaston A10 and phenylacetyl-L-glutamine, one of the degradation products of antineoplaston A 10 were synthesized. The synthesis and characterization were described elsewhere (Seo, 1994). High performance liquid chromatography (HPLC)-grade acetonitrile and purified water by Milli-Q system were used to prepare the mobile phase. All other materials were analytical grade and used as received.

Instrumentation

A Suntex Model SP-7 digital pH meter equipped with a Ingold Model U457-S7 combination electrode was used to measure the pH of the solution. HPLC was performed using a Waters 501 pump equipped with a Waters 484 variable wavelength UV detector, a Waters U6K injector, and a Youngin D520A integrator.

Separation of antineoplaston A10 from its degradation products

A reverse-phase HPLC method has been developed for the quantitation of A10. The method employs a Waters-Bondapak C18 10μm 3.9 x 300mm column,

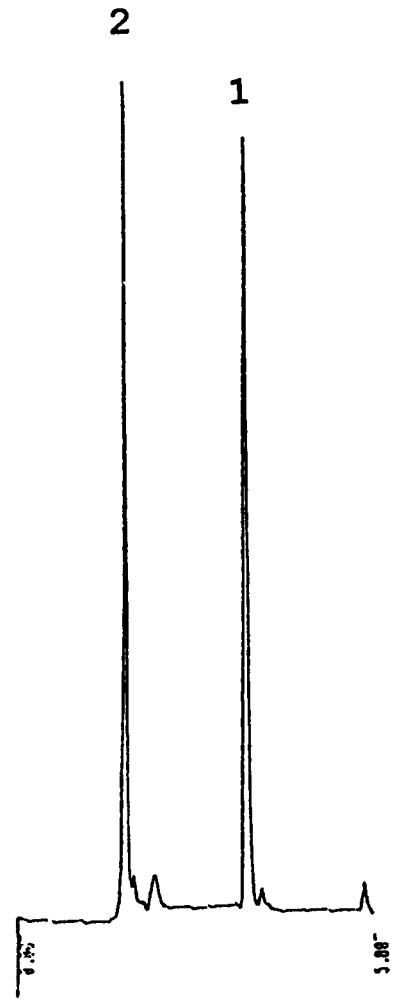


Fig. 1. HPLC chromatogram of 1, antineoplaston A10 and 2, phenylacetyl-L-glutamine.

a flow rate of 1.00ml/min, and UV detection at 254nm. The isocratic method employs a mobile phase of (50 : 50 v/v %) acetonitrile/pH4 water and gives excellent linear responses (r=0.997) with injection sizes of A10 in the range of 0.5 to 50μg/ml.

Stability test of antineoplaston A10

A proper amount of antineoplaston A10 was solubilized in 50ml of distilled water at 50°C for 4 hours using a hot plate magnetic stirrer. The solution was then filtered through a membrane filter (0.45μm). This solution (40ml) was added into respective buffer solutions (pH 1-10) (Perrin and Dempsey, 1977) which used HCl (pH 1, 2), chloroacetic acid (pH 3), formic acid (pH 4), acetic acid (pH 5, 6), phosphoric acid (pH 7, 8), boric acid (pH 9), and carbonic acid (pH 10). And then, drug solution was incubated at constant temperature (40, 50 and 60°C) and sampled at various intervals and assayed by using the HPLC method. The buffer capacities were sufficient to maintain constant pH value as demonstrated by no observed pH change for all the solutions throughout the entire period of study. The sample solution for HPLC was prepared by adding the solution to 5ml of acetonitrile. The quantities of antineoplaston A10 remained were analyzed by HPLC.

RESULTS AND DISCUSSION

The information about the HPLC analysis of antineoplaston A10 has not been reported. So analysis conditions of A10 were established by the HPLC method. Antineoplaston A10 was separated from its degradation pro-

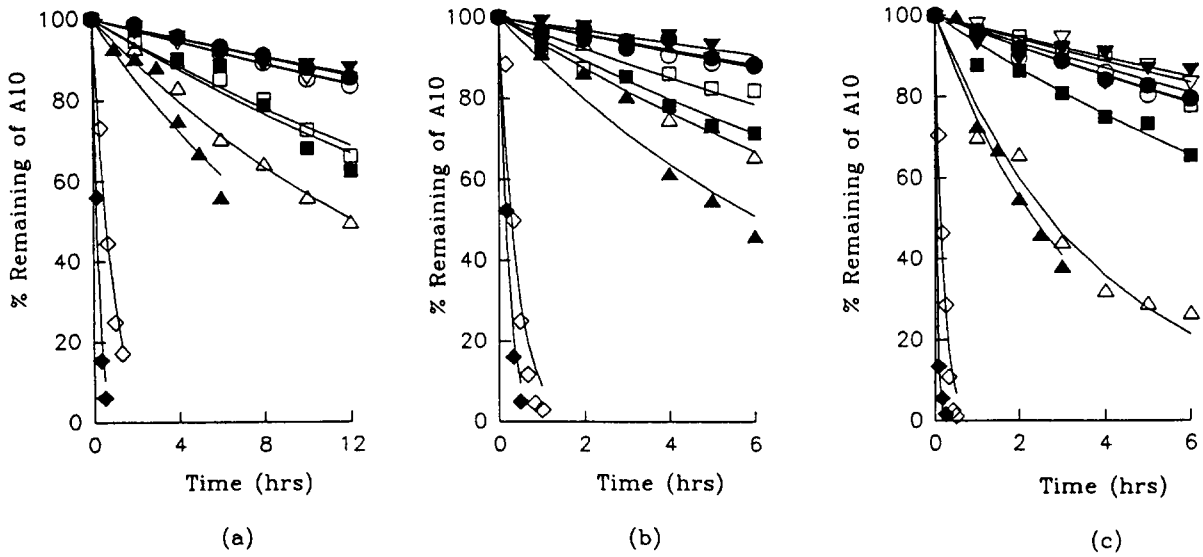


Fig. 2. Stability of antineoplaston A10 at various buffer solutions. (a) 40, (b) 50 and (c) 60°C. Keys: (○) pH 1, (●) pH 2, (▽) pH3, (▼) pH 4, (□) pH 5, (■) pH 6, (△) pH 7, (▲) pH 8 (◇) pH 9 and (◆) pH 10.

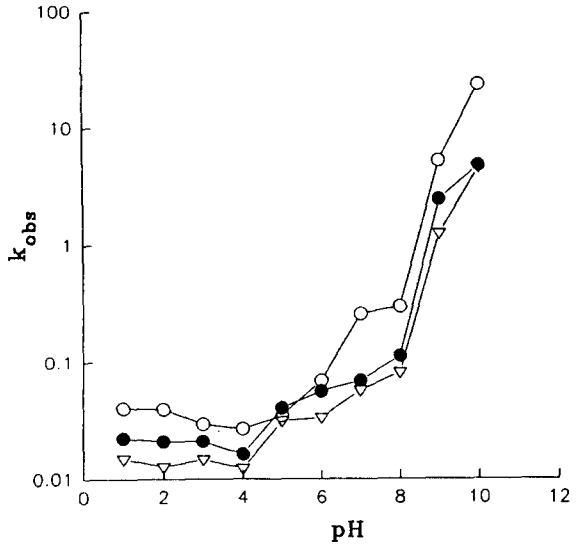
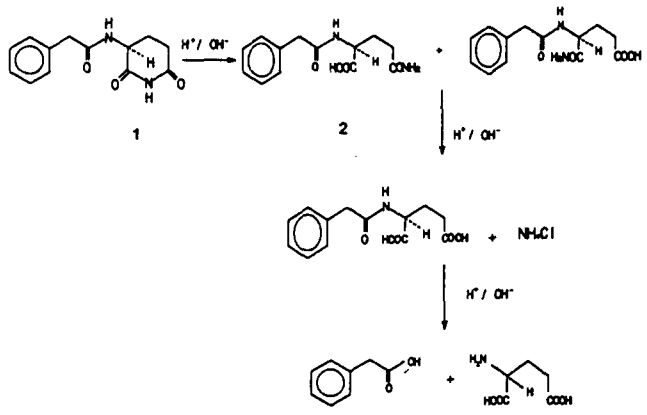


Fig. 3. pH-rate profiles for the degradation of antineoplaston A10 at various pHs and temperatures. Keys: (▽) 40, (●) 50 and (○) 60°C.



Scheme I. Possible mechanism of basic and acidic hydrolysis of antineoplaston- A10. Number 1 and 2 denote antineoplaston A10 and phenylacetyl-L-glutamine, respectively.

ducts as shown in Fig. 1. The peak of antineoplaston A10 in the HPLC chromatogram appeared at 3.6 minutes. One of the products of hydrolysis of antineoplaston A10 is N-phenylacetyl-L-glutamine which has a retention time of 1.7 minutes. The stability study of a drug is important in pharmacy because stability relates to the formulation, shelf-life and ultimately therapeutic efficacy of a drug (Taylor and Shivji, 1987). In the present study, the stability of antineoplaston A10 depending on the temperature and pH was tested at 40, 50 and 60°C and from the pH 1 to 10. The degradation patterns were displayed as semi-log plots of the residual amounts of antineoplaston A10 versus time as shown in Fig. 2. The mathematical bases (Connors, *et al.*, 1986) are C=Co e-kt, where C is the remaining concentration at time t, Co is the initial concentration and k is the reaction constant. The observed rate constants were obtained from the slopes of the semi-log plots of concentration versus time by statistical regression analysis. The results indicate that the observed degradation reaction rates approximately followed first order kinetics. And from these results, antineoplaston A10 is very unstable in alkaline conditions and stable in acidic conditions. Fig 3 shows the pH rate profiles for the degradation of antineoplaston A10 at various temperatures and pH values. This result indicates that antineoplaston A10 is most stable at pH4 and very unstable in alkaline conditions. The hypothetical mechanism of basic and acidic hydrolysis of antineoplaston A10 is shown in Scheme I. By Wesolwski(Wesolwski, *et al.*, 1968) the degradation of glutethimide in aqueous solutions appeared to be a base-catalyzed reaction. A mechanism is proposed which involves direct attack by a hydroxyl ion on the unhindered carbonyl of the glutethimide, followed by cleavage of thering to 4-ethyl-4-phenyl glutamic acid. In this reaction, A10 was hydrolyzed by H^+ or OH^- resulting in cleavage of the piperidinedione ring. It is assumed when hydrolysis was carried further, the products of reaction included phenyl acetic acid, glutamic acid and ammonia. But this reaction has not been continued

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