Stability Kinetics of Ginsenosides in Aqueous Solution

ETSUKO MIYAMOTO *, SHIZUO ODASHIMA ‡x, ISAO KITAGAWA §, and AKIRA TSUJI ¶

Received July 7, 1982, from the **School of Pharmacy, Hokuriku University, Kanagawa-machi, Kanazawa 920-11, Japan,* ‡*the Department of Pathology, Kanazawa Medical University, Uchinada-machi, Kahoku-gun 920-02, Japan,* §*the Faculty of Pharmaceutical Sciences, Osaka University, 1-6, Yamada-oka Suita, Osaka 565, Japan, and* ¶*the Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan.* Accepted for publication December 21, 1982.

Abstract □ The stability of ginsenosides (Rb_1, Rb_2, and Rg_1) in aqueous solutions of varying pH was studied kinetically at 37°C and an ionic strength of 0.15, using reverse-phase high-performance liquid chromatography. Degradation readily occurred by a proton-catalyzed reaction, the half-lives being only ~30 min at pH 1. However, under neutral intestinal pH conditions, the degradation was almost negligible throughout the experimental period (~40 h).

Keyphrases □ Ginsenosides—stability kinetics, aqueous solutions of varying pH □ Stability—ginsenosides in aqueous solutions of varying pH, kinetics □ Ginseng—stability kinetics of ginsenosides in aqueous solutions of varying pH

Ginseng has been used as a folkloric medicine in traditional herbal remedies for a long time. It has several noteworthy properties such as an antistress or rehabilitative action (1, 2). Many studies suggest that the pharmacological effects of ginseng come from the ginsenosides. Recently, Odashima *et al.* (3) reported the interesting finding that ginsenosides induced morphological changes and phenotypic transformations in cultured Morris Hepatoma cells (MH_1C_1), calling this "reverse transformation."

We have investigated the stability of the ginsenosides in both water and GI fluid since many herbal medicines seem to be effective when given orally. The ginsenosides have been known to degrade in the presence of acid, and the degradation mechanism and structures of the products have been described (4, 5). However, the detailed stability kinetics of ginsenosides have not been investigated. The present study reports the kinetics of degradation of the ginsenosides Rg_1, as 20*S*-panaxatriol, and Rb_1 and Rb_2, as 20*S*-panaxadiol, in water.

EXPERIMENTAL

Materials—The ginsenosides used in this study were prepared from the dried roots of *Panax ginseng* C. A. Meyer (Araliaceae), which were obtained from a commercial source[1], according to the usual procedures (6). The purity of the ginsenosides was confirmed by TLC (6). All other chemicals were of reagent grade and were used without further purification.

Ginsenosides Hydrolysis—Kinetic studies were carried out at 37 ± 0.1°C, at various pH values, and an ionic strength of 0.15. Each ginsenoside was dissolved in buffer solution preheated at 37°C to give a final concentration of 1×10^{-4} to 4×10^{-4} M. The buffer solutions employed were hydrochloric acid and phosphate (NaH_2PO_4) buffer systems, which were adjusted to a constant ionic strength of 0.15 with potassium chloride. The artificial GI fluids of pH 1.22 and pH 6.84 described in the Japanese Pharmacopeia X were also used. At appropriate intervals, aliquots were withdrawn, cooled, neutralized as necessary to quench the reaction with the same volume of phosphate (NaH_2PO_4–Na_2HPO_4) buffer solution (0.1 M, pH 6.8), and then analyzed. The pH of the reaction mixture was measured[2] both before use and at the end of the experiments. No significant change was observed.

Analytical Procedures—The reaction samples were assayed by reverse-phase HPLC. The liquid chromatograph[3] was equipped with a UV detector[4] set at 207 nm and a radial compression system[5] with an inserted reverse-phase cartridge[6]. The mobile phase was 70% (v/v) methanol–water. The instrument was operated at ambient temperature and a flow rate of 1.0 mL/min. A 100-μL sample was injected through an injector[7] on flow; the samples were filtered through a 0.45-μm membrane filter[8] before injection. Peak heights were used for quantification, and the coefficient of variation (*CV*) was 2.9%.

RESULTS AND DISCUSSION

Figures 1 and 2 show typical chromatograms of intact Rg_1 in the reaction mixture at 37°C, an ionic strength of 0.15, and pH 1.81. The peak of Rg_1 (peak 1) with a retention time (t_R) of 9 min decreased as a function of time and several peaks of degradation products appeared as the degradation reaction proceeded (Fig. 2).

All reactions were followed for 1–3 half-lives. The degradation of Rg_1, Rb_1, and Rb_2 followed first-order kinetics. Furthermore, in the pH range studied (pH 1–3), the rate of degradation was found to be first order with respect to hydrogen ion activity and independent of buffer concentration.

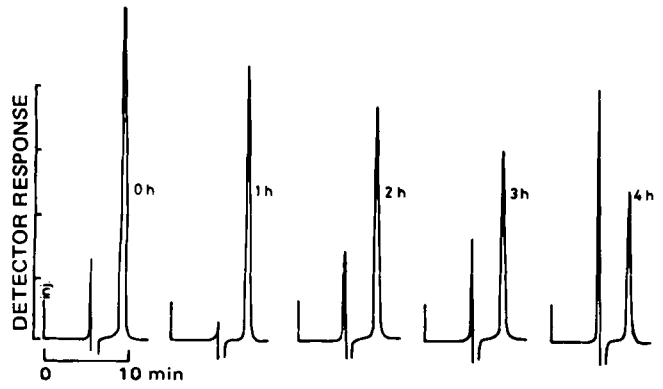


Figure 1—*Chromatograms of Rg_1 in 0.15 M phosphate buffer solution (pH 1.81) at 37°C and an ionic strength of 0.15 with time. t_R in minutes.*

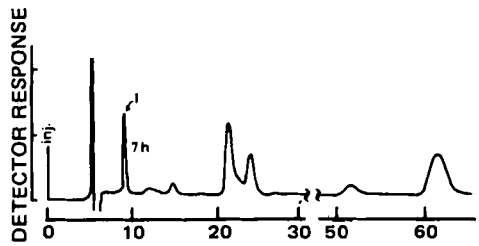
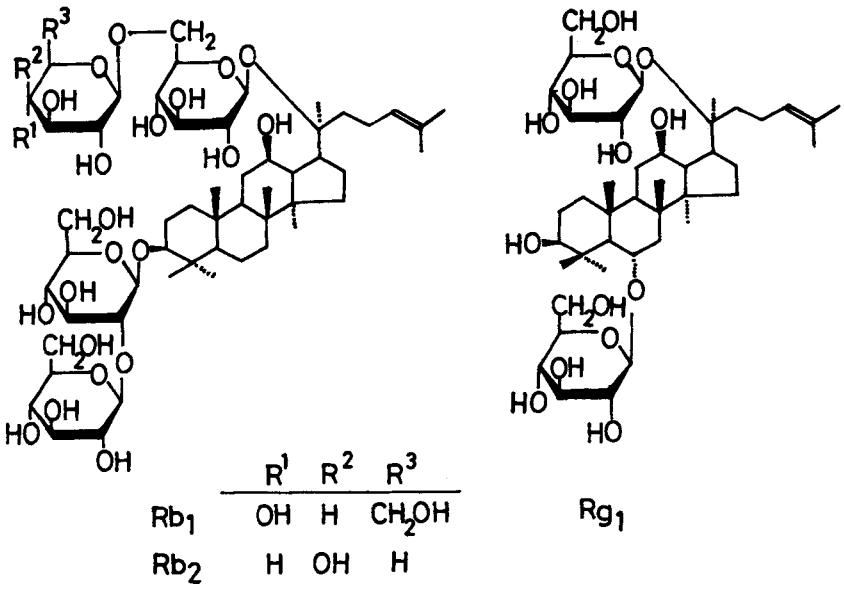


Figure 2—*Typical chromatogram of 80% degraded Rg_1 under the same conditions as in Fig. 1. t_R in minutes.*

[1] Japan and Research Laboratory, C. Koshiro and Co. Ltd., Osaka, Japan.
[2] pH meter, Model HM-18E; TOA Electronics Ltd., Tokyo, Japan.
[3] Waters analytical liquid chromatograph, model 244; Waters Associates, Milford, Mass.
[4] UVIDEC-100-III, UV spectrophotometer; Japan Spectroscopic Co., Tokyo, Japan.
[5] Model RCM-100; Waters Associates, Milford, Mass.
[6] Radial-Pak A cartridge (8 mm i.d. × 10 cm long); Waters Associates, Milford, Mass.
[7] Model U6K; Waters Associates, Milford, Mass.
[8] Sartorious-membranfilter, GmbH; 34 Göttingen, West Germany.

0022-3549/84/0300-0409$01.00/0



The pH dependency of the Rg_1 degradation in the acidic region can therefore be represented by the following:

$$k_{obs} = k_H a_{H^+}$$

where k_{obs} is the observed first-order rate constant, k_H is the specific proton-catalyzed rate constant, and a_{H^+} is the hydrogen ion activity as determined with a glass electrode. The k_H value for each ginsenoside was calculated from the kinetic data, and the observed rate constants and the values of k_H for the respective ginsenosides are listed in Table I. No significant difference among the catalyzed rate constants was observed. If these catalyzed rate constants can be employed to predict the stability of the ginsenosides at constant pH in nonbuffered solution, the half-life at 37°C and pH 1 would be 30 min. At neutral pH, Rg_1 is very stable and the degradation is negligible overnight.

These results suggest that chemical degradation of ginsenosides in the acidic pH region, such as in the stomach, could proceed readily by producing many products (see Fig. 2). On the other hand, the degradation under intestinal pH conditions was negligible.

Table I—Apparent First-Order Rate Constants, k_{obs}, at Various pH Values and Rate Constants, k_H, for the Acid Degradation of Ginsenosides at 37°C and Ionic Strength of 0.15

Ginsenoside	pH	k_{obs} (±*SD*), h^{-1}	k_H, $M^{-1}\,h^{-1}$
Rg_1	0.85[a]	2.096[b]	15.0[c]
	1.22[a]	0.924 (0.012)[d]	
	1.48[a]	0.552[b]	
	1.81[e]	0.224 (0.012)[f]	
	2.04[e]	0.131 (0.006)[f]	
	2.34[e]	0.055 (0.002)[f]	
Rb_1	1.81[g]	0.218[b]	14.0[h]
Rb_2	1.81[g]	0.231[b]	14.9[h]

[a] Hydrochloric acid was used as a buffer solution. [b] Observed value, calculated by the least-squares method using the experimental data. [c] Calculated according to the equation, obtained by the least-squares method using k_{obs} at varying pH. [d] Averaged value, calculated using k_{obs} under the experimental condition of three different initial concentrations of Rg_1. [e] Phosphate buffer solution was used. [f] Averaged value, calculated using k_{obs} under the experimental condition of at least three different buffer concentrations. [g] Phosphate buffer solution (0.15 M) was used. [h] Calculated according to the equation using k_{obs}.

REFERENCES

(1) W. Petkov and D. Staneva, *Arzneim.-Forsch.*, **13**, 1078 (1963).

(2) I. I. Brekhman and I. V. Dardymov, *Annu. Rev. Pharmacol.*, **9**, 419 (1969).

(3) S. Odashima, Y. Nakayabu, N. Honjo, H. Abe, and S. Arichi, *Eur. J. Cancer*, **15**, 885 (1979).

(4) O. Tanaka, M. Nagai, T. Ohsawa, N. Tanaka, K. Kawai, and S. Shibata, *Chem. Pharm. Bull. (Tokyo)*, **20**, 1204 (1972).

(5) M. Nagai, T. Ando, N. Tanaka, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull. (Tokyo)*, **20**, 1212 (1972).

(6) S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull. (Tokyo)*, **22**, 421 (1974).

ACKNOWLEDGMENTS

The data reported here were presented in part at the 15th Symposium on Wakan-yaku, Toyama, Japan, August 1981.