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Note

Hydrolysis of ginsenosides in artificial gastric fluid monitored by high-performance liquid chromatography

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Ginseng saponins, isolated from the root of Panax Ginseng C. A. Meyer (Araliaceae), can be classified into two groups, namely the 20(*S*)-protopanaxatriol group (I) and the 20(*S*)-protopanaxadiol group (II) (Fig. 1).

In spite of the many pharmacological[1] and biochemical studies[2], there is only one report[3] on the degradation of ginsenosides under mild acidic conditions. According to this work, R_{g_1} and R_e ginsenosides were hydrolyzed with 0.1 *M* hydrochloric acid at 37°C for 2 h and the structures of the resulting prosapogenins assigned by ^{13}C NMR spectroscopy. Ginsenoside R_{b_1} representative of group II, was similarly treated, but evidence of the structure of its prosapogenins was not produced.

Recently, the stability of the same saponins at pH 1.81 and 37°C was examined using isocratic high-performance liquid chromatography (HPLC), although the chromatographic data refer only to R_{g_1}[4].

Owing to this lack of information, we applied a previously described[5] HPLC procedure to the kinetics of the hydrolysis of ginsenosides of groups I, II and of Ginseng extracts in artificial gastric fluid at 37°C. The results of this investigation are now reported.

EXPERIMENTAL

Materials and reagents

Ginsenosides R_{b_1}, R_{b_2}, R_c, R_d, R_{g_1} and R_e were obtained from Pharmaton (Lugano-Bioggio, Switzerland). Ginseng extracts were from different commerical sources. Acetonitrile and water were of HPLC grade (Chromasolv, Riedel-de Haën, Hannover, F.R.G.).

Standard stock solutions were prepared by dissolving each ginsenoside (1 mg/ml) and Ginseng extracts (45 mg/ml) in 1 m*M* phosphate (NaH_2PO_4) adjusted to pH 1.2 with 5 *M* hydrochloric acid and to a constant ionic strength of 0.15 with potassium chloride.

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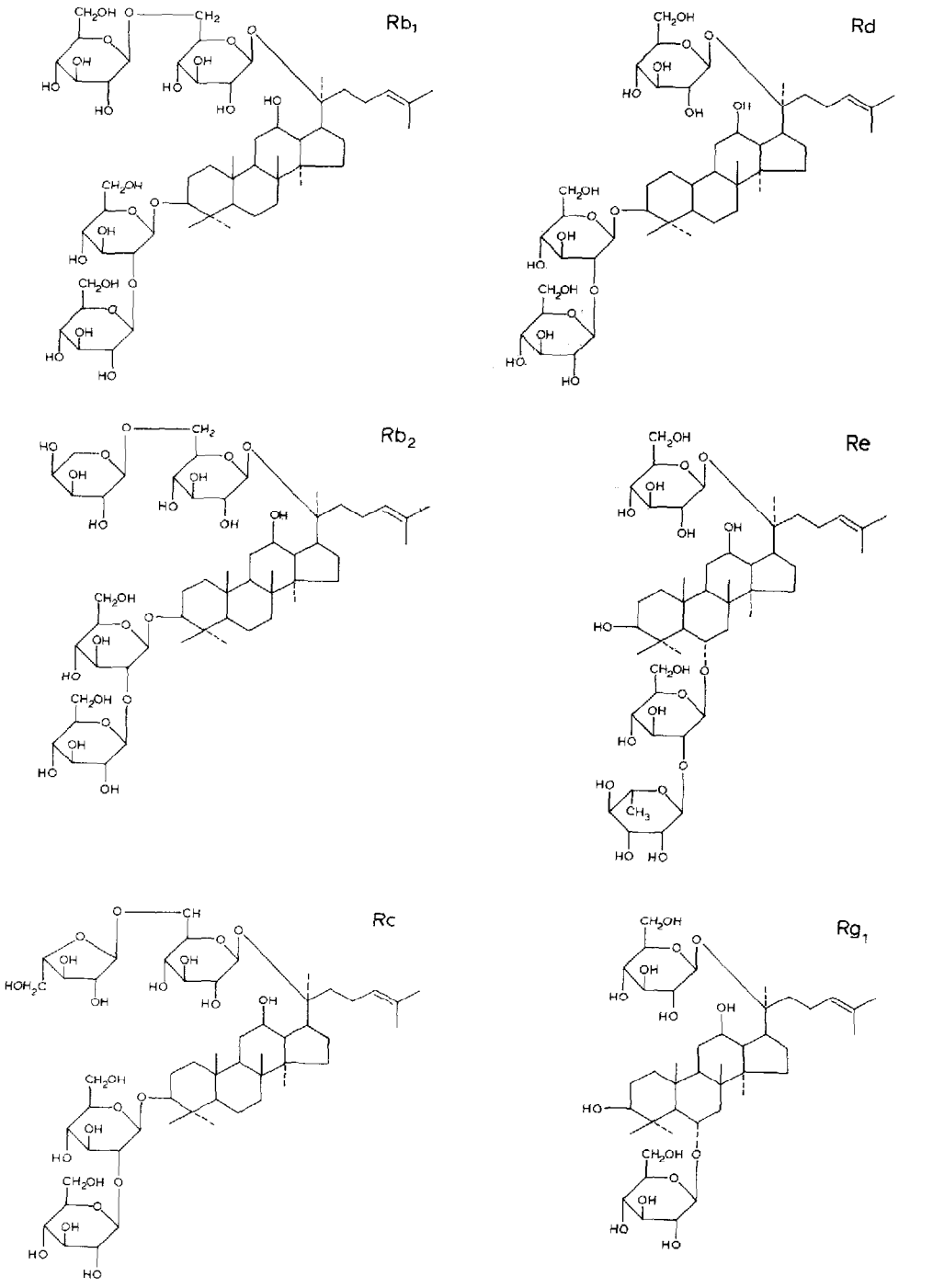

Fig. 1. Chemical structures of the ginsenosides.

Ginsenosides hydrolysis

Aliquots (50 μl) of each stock solution were incubated at 37°C for different times. Every 30 min the appropriate aliquot was cooled, neutralized by addition of 50 μl of 0.1 *M* phosphate (NaH_2PO_4–Na_2HPO_4) buffer, pH 6.8, and then analyzed. Control blanks were performed by analyzing each sample at zero time. The pH of the reaction mixture remained unchanged during the hydrolysis.

Chromatographic conditions

HPLC was performed on a Waters Assoc. liquid chromatograph equipped with a M-590 pump, a M-510 pump, a M-680 gradient controller, a M-U6K universal injector and a M-lambda max 480 ultraviolet detector. The column was a RP-18 Spheri 5 cartridge (100 mm × 4.6 mm, 10 μm) from Kontron (Milan, Italy). The separations were obtained isocratically with acetonitrile–water (28.5:71.5; 20:80, v/v) or by gradient elution, using the eluents 15% acetonitrile (A) and 80% acetonitrile (B) according to the following profile: 0–12 min, 93% A, 7% B; 12–30 min, 72% A, 28% B (curve 5); 30–45 min, 72% A, 28% B (curve 6). The flow-rate was 3 ml/min and the peaks were monitored at 203 nm (0.05 a.u.f.s.).

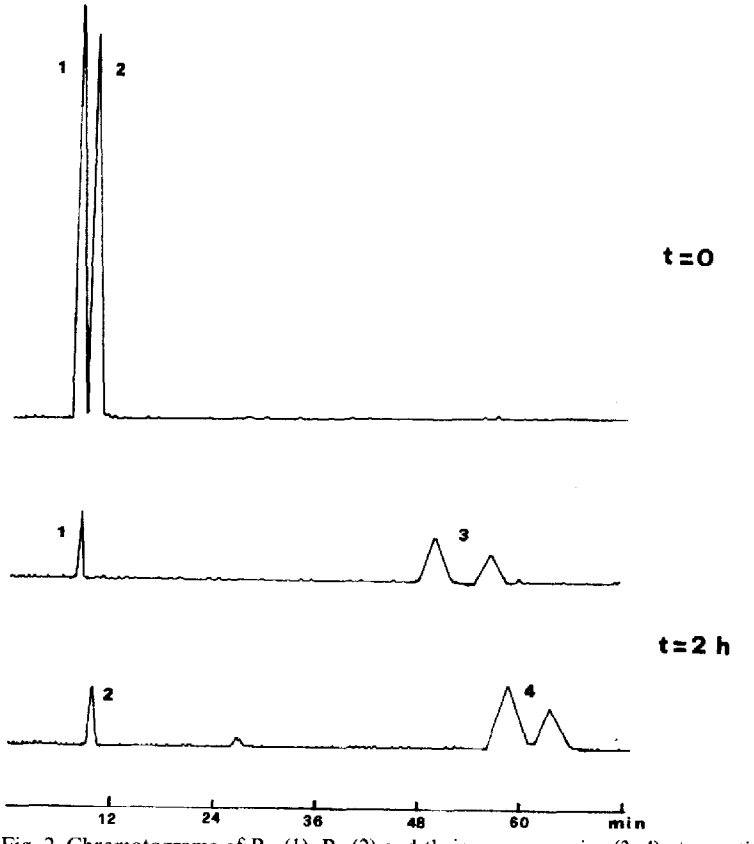

Fig. 2. Chromatograms of R_{g_1}(1), R_e (2) and their prosapogenins (3, 4) at zero time and after hydrolysis for 2 h. Eluent: 20% acetonitrile.

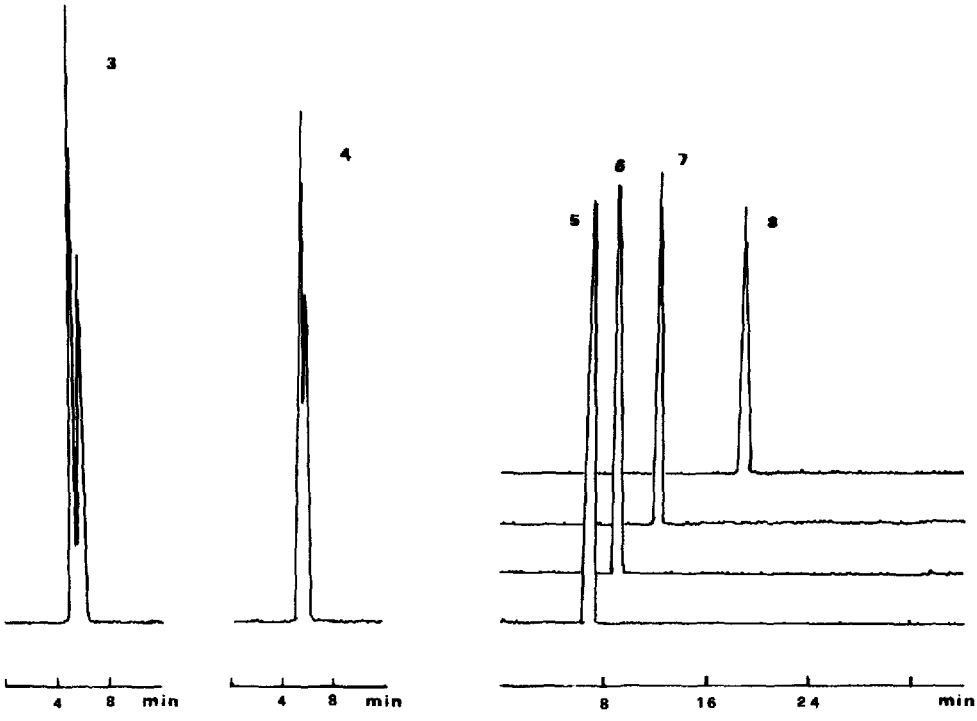

Fig. 3. Chromatograms of R_{g_1} (1) and R_e (2) after hydrolysis for 2 h. Eluent: 28.5% acetonitrile.

Fig. 4. Chromatograms of R_{b_1}(5), R_c (6), R_{b_2}(7) and R_d (8) after hydrolysis for 2 h. Eluent: 28.5% acetonitrile.

RESULTS AND DISCUSSION

Fig. 2 shows typical chromatograms obtained from ginsenosides R_{g_1} and R_e at the initial time and after 2 h at pH 1.2 and 37°C. The R_{g_1} and R_e prosapogenins produced by the cleavage of the C-20 glucosidic bond are eluted after 50 min using 20% acetonitrile, which is the customary solvent for these saponins. 28.5% Acetonitrile elutes intact R_{g_1} and R_e with the "solvent front", while the related prosapogenins yield peaks with retention times of 4.8–5.6 min (Fig. 3). Under these chromatographic conditions, the ginsenosides of group II are eluted within 20 min, but their common prosapogenin is retained on the column, as shown in Fig. 4. To elute this less polar derivative, it has been necessary to apply a gradient (Fig. 5). A chromatogram obtained from ginsenosides R_{g_1} and R_e, and their prosapogenins, using the same gradient is also shown (Fig. 6).

The hydrolysis of Ginseng extracts at pH 1.2 and 37°C has been followed similarly. The peaks of the ginsenosides of group II decrease as the reaction proceeds and a new peak due to R_{g_1} and R_e prosapogenins appears (Fig. 7). To extend these results, the Ginseng extracts have also been monitored using the gradient approach. As Fig. 8 shows, the R_{g_1} and R_e prosapogenins are eluted at 21 min, while the common derivative from the ginsenosides of group II is clearly identifiable at 48 min.

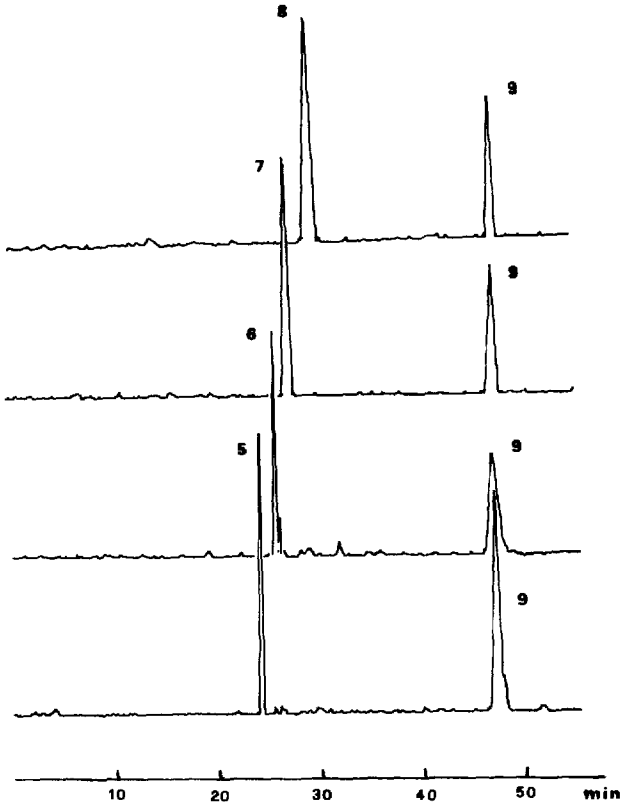

Fig. 5. HPLC with gradient elution of R_{b1}(5), R_c (6), R_{b2}(7), R_d (8) and their common prosapogenin (9) after hydrolysis for 2 h. See text for eluents and profiles.

The hydrolysis of each ginsenoside has been followed up to 70% degradation, and a linear relationship between the reaction time, x (h), and the residual amount of intact ginsenoside (peak area, y) has been found:

Relationship	*R*	*Half-time (h)*	
$y = -3.44x + 6.38$	0.999	0.928	(R_{g_1})
$y = -7.92x + 5.18$	0.997	0.896	(R_e)
$y = -1.13x + 7.52$	0.996	3.31	(R_{b_1})
$y = -1.12x + 7.45$	0.995	3.32	(R_c)
$y = -0.764x + 3.89$	0.995	3.77	(R_{b_2})
$y = -0.584x + 3.89$	0.997	3.33	(R_d)

As is seen, the half-times for the ginsenosides of group II are similar and nearly three times higher than those of R_{g_1} and R_e.

In conclusion, the described procedure allows the kinetics of hydrolysis in artificial gastric fluid of each ginsenoside in Ginseng extracts to be followed by monitoring both intact and degradation products.

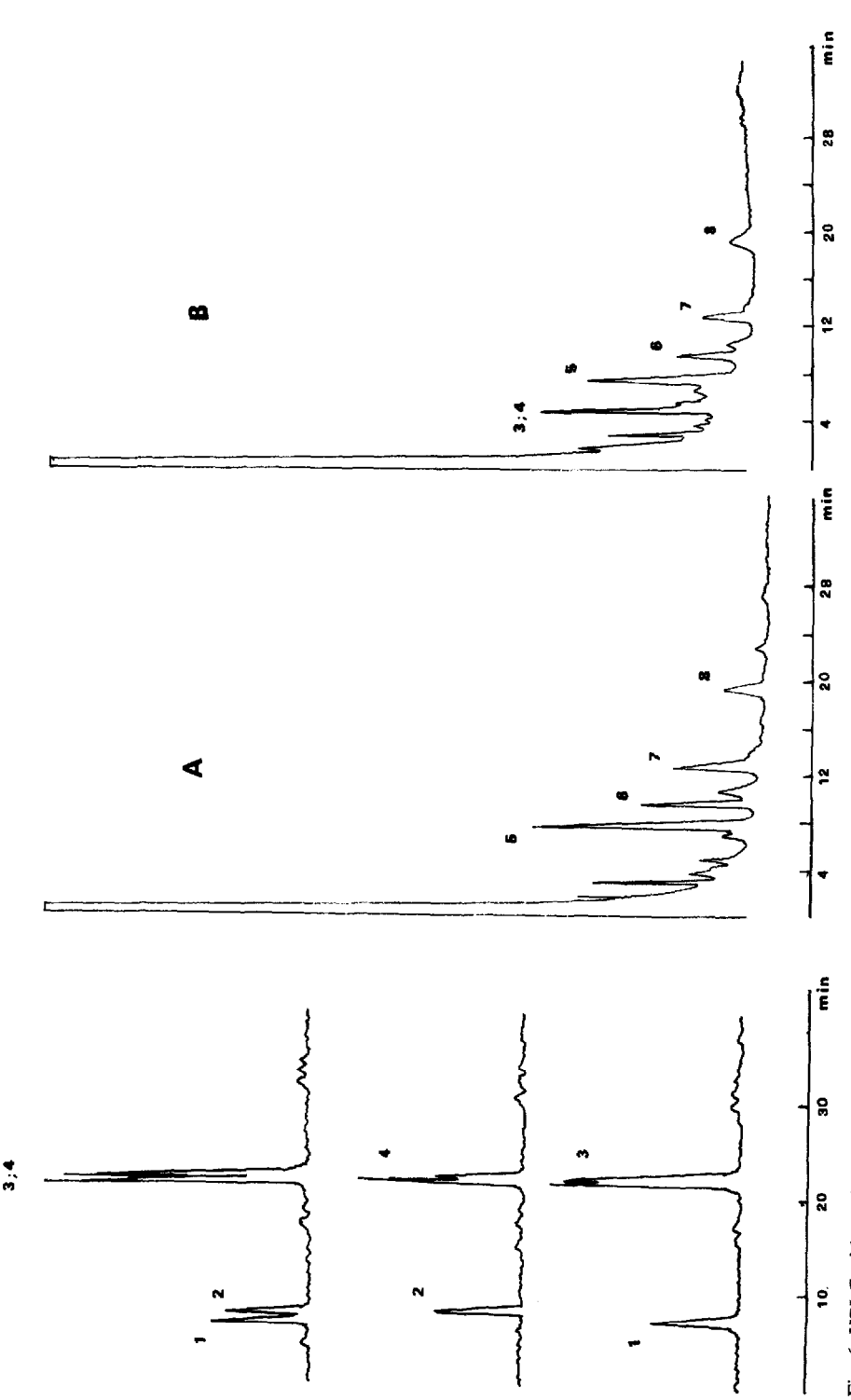

Fig. 6. HPLC with gradient elution of R_{g1} (1), R_c (2) and their prosapogenins (3, 4) after hydrolysis for 2 h. See text for eluents and profiles.

Fig. 7. HPLC with isocratic elution of a Ginseng extract at zero time (A) and after hydrolysis for 2 h (B). Peaks as in Figs. 2 and 4. Eluent: 28.5% acetonitrile.

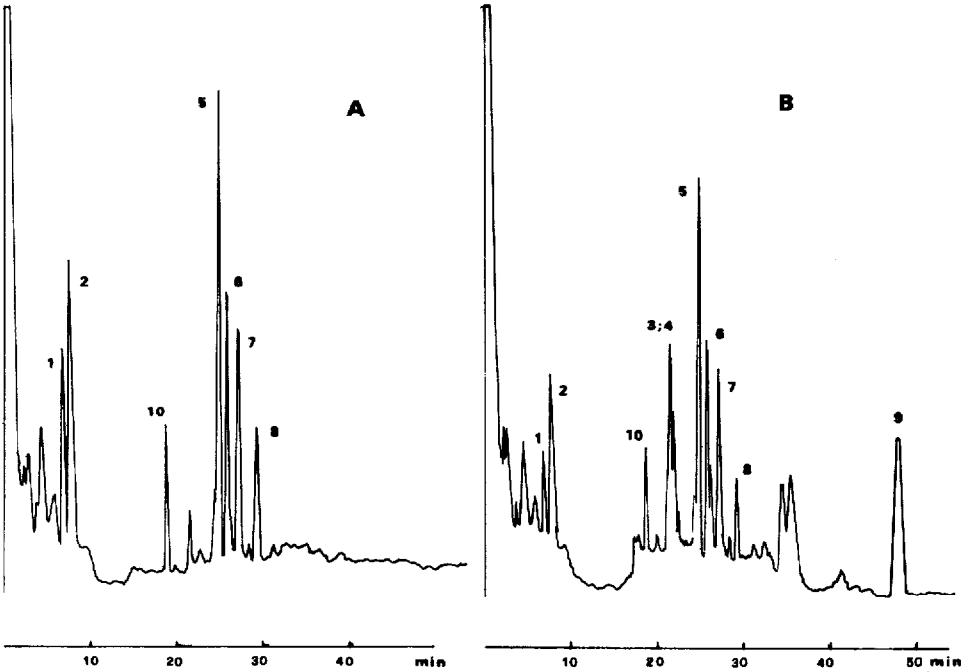

Fig. 8. HPLC with gradient elution of a Ginseng extract at zero time (A) and after 2 h (B). Peaks as in Figs. 2 and 5, except for 10 = R_f. See text for eluents and profiles.

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