AMERICAN GINSENG. II. ANALYSIS OF GINSENOSIDES AND THEIR SAPOGENINS IN BIOLOGICAL FLUIDS[1]

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ABSTRACT.—A gas-liquid chromatography (tlc) method was developed to assay individual ginsenosides and sapogenins in rabbit plasma and urine samples. A flavonoid, panasenoside, and a sterol, stigmasterol, were used as internal standards for ginsenosides and their sapogenins, respectively. Linear relationships of peak height ratio to weight ratio were obtained for ginsenosides (A_1, 20–350 μg; A_2, 20–400 μg; B_2, 20–300 μg; C, 20–500 μg), and sapogenins (panaxadiol or panaxatriol, 10–200 μg) in 0.1 ml of the silylation mixture. The glc assay method developed was sensitive to 0.2 μg of ginsenosides and 0.1 μg of sapogenins.

Ginseng preparations from *Panax* species have been evaluated biologically for antifatigue (1), anti-diuretic (2), and hemolytic or protective (3) properties. These properties may be due to the ginsenosides known to be present in ginseng.

Gas-liquid chromatography (glc) (4, 5, 6) and high pressure liquid chromatography (hplc) (7, 8, 9) methods have been developed to separate the sapogenins (4, 5, 6) and ginsenosides (7, 8, 9). Results of the analysis of ginsenosides and sapogenins in various ginseng preparations by colorimetry (10, 11), droplet counter-current chromatography (12), or thin-layer chromatography (tlc) with flame ionization (13), fluorescence (14), or the spectrodensitometer (15) have been published.

Ginsenoside and sapogenin analyses in biological fluids have been conducted by labeling ginsenosides (16), or by using a tlc-colorimetric procedure (17). The reported methods are either low in sensitivity, interfered with by cholesterol or other compounds contained in the biological samples, or are limited in use because of the scarcity of radioactive ginsenosides. The glc method developed enables an accurate, sensitive analysis in plasma or urine samples of ginsenosides A_1 (pseudo-ginsenoside F_{11}) (18), A_2 (Rg_1), B_2 (R_e), C (Rb_2) and the sapogenins, panoxadiol or panaxatriol (8), when analyzed individually.

EXPERIMENTAL

MATERIALS.—Ginsenosides A_1, A_2, B_1, B_2, C and panasenoside were isolated and identified from American ginseng (*Panax quinquefolius* L. Araliaceae) stems and leaves (8). Panaxadiol and panaxatriol were obtained by the hydrolysis of ginsenosides with methanol-HCl, purification of the hydrolyzate on a silica gel column, and crystallization from ethyl acetate or benzene as colorless needles (8). Stigmasterol was purchased from Sigma Chemical Co., St. Louis, MO. Pyridine and Tri-Sil TBT (Trimethyl silyl imidazole-*N*,*O*-bis-trimethyl-silyl acetamide-trimethyl-chlorosilane 3:3:2) were purchased from Pierce Chemical Co., Rockford, IL.

INSTRUMENTATION.—A Varian Aerograph model 1740–2 glc system equipped with 9.14 m x 6.4 mm glass columns, a non-linear temperature programmer and flame ionization detectors was used. The column was packed with 0.5% OV–101 liquid phase coated on 100/120 mesh Chromosorb W(HP) solid support (Varian Associates, Palo Alto, CA). The flame ionization detector was used with a hydrogen (25 ml/min) and air (240 ml/min) mixture. The carrier gas used was nitrogen (45 ml/min). Chromatograms were recorded on a Varian Aerograph A-25 recorder with a chart speed of 25.4 cm/hr.

ANALYSIS.—Rabbit plasma and urine samples (0.5 ml) collected for pharmacokinetic studies (19) were extracted with water-saturated butanol (2 x 2.5 ml). The butanol layers were combined, and a methanol solution of panasenoside (100 μl, 1 μg/μl) was added to each sample.

[1]Previous articles in series: S. E. Chen and E. J. Staba, *Lloydia* **41**, 316 (1978).

They were taken to dryness on an Evapo-Mix at 50°. Samples were then dissolved in 50 μl of pyridine and derivatized with 50 μl of Tri-Sil TBT in a 50° oven for 10 min. One μl of each reaction mixture was injected (injector temp. 330°) into the glc system. Column temperature was programmed from 220 or 275° to 330° (ginsenosides A_1, A_2 and B_2), and from 275 to 340° (ginsenoside C). Detector temperature was set at 350°. Concentrations of unknown plasma or urine samples were determined by measurement of peak height ratios (ginsenoside/panasenoside) and by reference to their standard curves. Appropriate dilution of some plasma and urine samples was required to avoid exceeding the limits of linear relationship of peak height ratio to weight ratio. Dilutions were also required in urine samples collected over a longer period of time, as the color of concentrated urine affected the assay results. The standard curves of ginsenosides were prepared as above, except that a methanol solution of ginsenoside (20–500 μl, 1 μg/μl) was added and dried with nitrogen before the addition of blank plasma and urine.

Analysis of panaxadiol and panaxatriol was performed in a way similar to that for ginsenosides. A chloroform solution of stigmasterol (50 μl, 1 μg/μl) was used as internal standard and was added to the plasma and urine samples (0.5 ml) before chloroform extraction (2 x 2.5 ml). Column temperature was programmed from 175 to 250° (panaxadiol) or from 220 to 275° (panaxatriol). Standard curves of sapogenins were prepared as above except that a chloroform solution of sapogenin (10–200 μl, 1μg/μl) was also added to 0.5 ml blank plasma or urine.

RESULTS AND DISCUSSION

Water-saturated butanol and chloroform satisfactorily extracted purified ginsenosides and sapogenins from the plasma and urine samples. However, panasenoside, the internal standard of ginsenosides, was not butanol-extractable and had to be added to the butanol extracts of standards and samples. Typical chromatograms of rabbit plasma and urine samples containing ginsenosides are shown in figure 1. No endogenous peak was observed in urine samples. Some endogenous peaks were observed in plasma samples, but they did not interfere

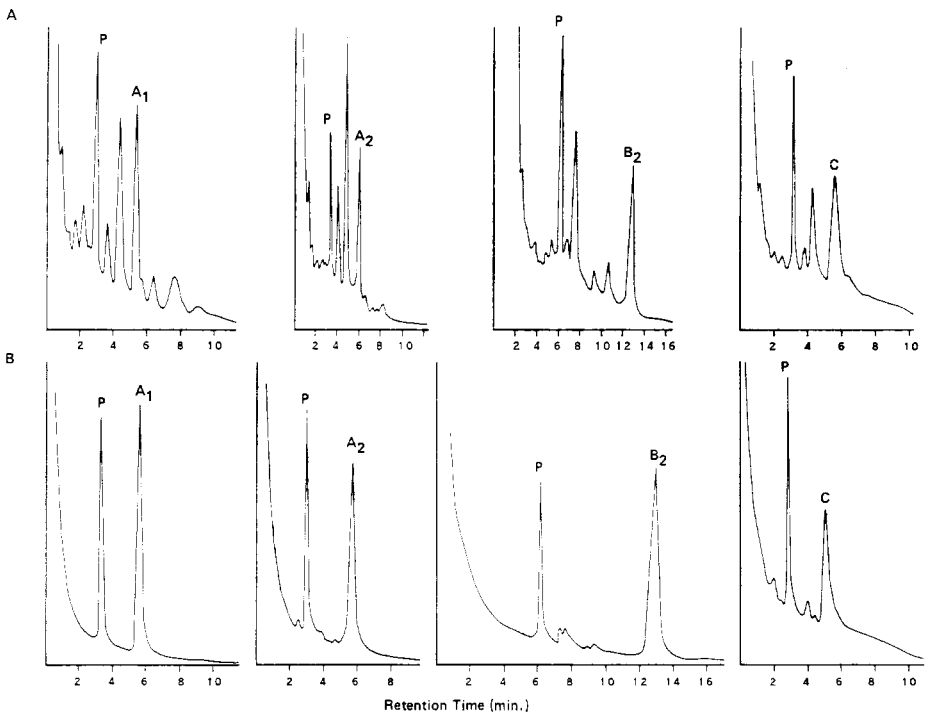

FIG. 1. Chromatograms of plasma (A) and urine (B) extracts contained ginsenosides A1, A2, B2, C and internal standard panasenoside (P). Assay conditions were optimized for each ginsenoside as shown in Table 1.

with the analysis of ginsenosides. Table 1 summarizes the assay conditions and retention times for each ginsenoside and sapogenin.

Linear relationships of peak height ratio to weight ratio were obtained when the amount of ginsenoside assayed was in the range of 20 μg to 300 μg (B_2), to 350 μg (A_1), to 400 μg (A_2), to 500 μg (C), and that of sapongenins was in the range of 10 μg to 200 μg in 100 μl of the silylation mixture. When the concentration of ginsenosides and sapogenins exceeded these limits, peak heights were reduced and their retention times increased. Therefore, concentrations of plasma and urine samples must be adjusted to these limits.

TABLE 1. Gas-liquid chromatography of ginsenoside- and sapogenin TMS derivatives.

Ginsenoside (Sapogenin)	Column temp.[a] (°C)	Retention time (Min.)
A_1	275→330	5.63
A_2	275→330	6.00
B_2	220→330	13.13
C	275→340	5.25
Panaxadiol	175→250	5.44
Panaxatriol	220→275	4.50

[a]Column temperatures were programmed with a non-linear temperature programmer at setting 6.5 on Varian Aerograph Model 1740-2.

The glc assay method introduces stigmasterol and panasenoside as the internal standards for sapogenins and ginsenosides analysis. The procedure of Bombardelli *et al* (6) used an unconventional column length (0.25 m x 2 mm), whereas a conventional column length (1.4 m x 6.4 mm) was used in this study. The method is sensitive to 0.2 μg of ginsenosides and 0.1 μg of sapogenins. A plasma/urine concentration of 40 μg/ml ginsenosides or 20 μg/ml sapogenins can easily be assayed. The assay conditions were optimized for each ginsenoside or sapogenin and is best for assaying a single ginsenoside in these samples. This glc assay is superior to the colorimetric method because of the increased sensitivity and because it is not interfered with by cholesterol and other C 3 hydroxy steroids. Whereas contaminations of ginsenosides with their radiolytic products would make pharmacokinetic studies impractical, the glc method developed enables an accurate, sensitive analysis of ginsenosides and sapogenins in the plasma and urine samples.

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