scraped off and extracted with 10% methanol–ethyl acetate. The contracting activity was found only in the fraction with $Rf=0.08$—0.25. This active fraction showed three major spots on analytical TLC developed with the same solvent system.

Preparative TLC of the Active Fraction——The active fraction was further purified by preparative TLC on eight silica gel plates (E. Merck, 0.25 mm × 20 cm × 20 cm) using the procedure described above. Each plate was divided into five fractions, and bioassay of these fractions showed that only one of them contained the active substance (AS), which corresponded to the middle of the three major spots of the above active fraction. AS had the same *Rf* value (0.17) as $PGF_{2\alpha}$.

Preparation of the Active Substance Methyl Ester (AS-Me)——A solution of AS in methanol (1 ml) was treated with an ethereal solution of CH_2N_2 at room temperature. The solvent was evaporated off after 1 min. The residue was purified by preparative TLC on silica gel, developing with benzene–dioxane (5 : 4), to afford AS-Me, which had the same *Rf* value (0.22) as authentic $PGF_{2\alpha}$ methyl ester.

Trimethylsilylation of AS-Me and Mass Spectrometry——A methanolic solution of AS-Me was transferred to a probe for mass spectrometry and dried sufficiently *in vacuo*. The residue was treated with one drop of bis(trimethylsilyl)trifluoroacetamide at room temperature for 1 hr under a dry atmosphere. The resulting AS-Me trimethylsilyl derivative (AS-Me-TMS) was used directly to obtain a mass spectrum (Hitachi RMU-7M mass spectrometer; ionizing voltage, 70 eV; sample heating temperature, 105°). The spectrum was identical (M^+ 584 and all fragment ions) with that of an authentic $PGF_{2\alpha}$-Me trimethylsilyl derivative obtained from $PGF_{2\alpha}$-Me by the same procedure.

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Effect of Ginseng Saponin on Serine Dehydratase Activity in Rat Liver

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Treatment of rats with saponin (fraction 5) from the roots of *Panax ginseng* C.A. MEYER caused a decrease in the activity of hepatic serine dehydratase (EC 4.2.1.13). Maximum decrease in the enzyme activity was observed 2 hr after the administration of the ginseng saponin, and it was found that this response depended on the amount of fraction 5 administered to rats. In contrast, when the animals were starved, ginseng treatment resulted in an increased level of the enzyme.

Keywords——*Panax ginseng* C.A. MEYER; serine dehydratase; rat liver; enzyme activity; saponin; starvation

As reported previously,[2)] treatment of rats with saponin from the roots of *Panax ginseng* C.A. MEYER produced an increase in the activity of hepatic pyruvate kinase (EC 2.7.1.40) in rats fed on a laboratory pellet chow. In contrast, when glycolysis occurred on a major scale, *i.e.*, on feeding a high carbohydrate diet, ginseng treatment resulted in decreased pyruvate kinase activity. These results suggest that the ginseng saponin may influence the direction of liver carbohydrate metabolism through its effects on enzyme systems.[3–5)] From this point of view, the present paper describes the effects of ginseng saponin on the activity of serine dehydratase (EC 4.2.1.13), which catalyzes the degradation of serine to pyruvate and ammonia.

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Materials and Methods

Animals and Diet——Male rats of the Wistar strain (70—80 g) were maintained in an air-conditioned room with lighting from 6 a.m. to 6 p.m. The room temperature was automatically controlled at 25±1°. A laboratory pellet chow (obtained from Clea Japan Inc., Tokyo; protein 24.0%, lipid 3.5%, carbohydrate 60.5%) and water were given freely, unless otherwise indicated. Starved rats had free access to water.

Saponin——Fraction 5 was prepared from the extract of roots of *Panax ginseng* C.A. MEYER, and was shown to consist of ginsenoside-Rb_1, -Rb_2, -Rc, -Rd, -Re, and -Rg_1.[6]

Enzyme Preparation[7]——To exclude the complication of diurnal variation in liver serine dehydratase,[8] rats were sacrificed by cutting the carotid artery between 1 and 2 p.m. The liver was removed quickly, transferred to liquid nitrogen, and weighed rapidly. Fresh liver was homogenized with 9 volumes (w/v) of 0.1 M potassium phosphate buffer, pH 7.4, containing 10^{-3} M ethylenediaminetetraacetic acid (EDTA) and 0.15 M KCl in a glass homogenizer. The crude homogenate was then centrifuged at $105000 \times g$ for 60 min in the cold, and the supernatant liquid was used for enzyme assay as the crude extract.

Assay of Serine Dehydratase Activity——The enzyme activity of the supernatant was determined according to the method of Bottomley *et al.*,[9] with a slight modification. The assay mixture contained 100 μmol of potassium phosphate buffer (pH 8.0), 0.4 μmol of pyridoxal phosphate, 100 μmol of L-serine, and 0.1 ml of liver supernatant in a total volume of 1.0 ml. The reaction was started by the addition of L-serine after preincubation of the enzyme with pyridoxal phosphate for 5 min, and was stopped by the addition of 1.0 ml of 10% trichloroacetic acid (TCA) after incubation for 5 min at 37°. The blank was incubated without L-serine and 100 μmol of L-serine was added after addition of TCA. The precipitate formed was removed by centrifugation after leaving the mixture to stand for 10 min in an ice-bath. A 0.5 ml aliquot was pipetted into 0.5 ml of 0.033% 2,4-dinitrophenylhydrazine in 2 N HCl in a test tube and the mixture was allowed to stand for at least 5 min at room temperature. Then 2 ml of 2 N NaOH solution was added to the mixture and its optical density was measured at 520 nm, after full color development (about 5 min). One unit of the enzyme was defined as the amount of enzyme catalyzing the formation of 1 μmol of pyruvate per min under the assay conditions.

Determination of Protein——Protein was determined by the method of Itzhaki and Gill,[10] with bovine serum albumin as a standard.

Results and Discussion

Initially an attempt was made to determine the dose-response relationship of the ginseng saponin (fraction 5). Control animals were treated with an equal volume of saline. At 1.5 hr after treatment, the animals were sacrificed by means of a blow on the head and exsanguinated. As shown in Table I, the activity of serine dehydratase in the liver depended on the amount of fraction 5 administered to rats; the administration of 20 mg of the saponin decreased it by 50% of the control level. However, administration of 10 mg of glucose (a major sugar component in the saponin) caused only a 7% decrease in serine dehydratase activity (data not shown). These experimental results suggest that the active principle is saponin.

To obtain further data on the effect of the ginseng saponin on the enzyme activity, the time course was followed. As shown in Fig. 1, maximum decrease in the enzyme activity was observed 2 hr after the administration of fraction 5, but after 4 hr the decrease was not significant. In contrast, administration of the ginseng saponin to rats increased the activity of hepatic pyruvate kinase, the key glycolytic enzyme.[2] Maximum increase in this enzyme activity was observed 1 hr after the treatment. The level was still high 2 hr after the treatment, but the increase was no longer significant after 4 hr. These changes are in reciprocal relation to those of serine dehydratase. This supports the view that the ginseng saponin plays an important role in amino acid and carbohydrate metabolism in rat liver.

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Table I. Dose-Response Relationship in the Effect of Fraction 5 on Serine Dehydratase Activity in Rat Liver

Material	Dose (mg)	Enzyme activity (units/mg protein $\times 10^3$)
Control	—	10.3±0.5(100)
Fraction 5	2.5	9.5±0.7(92)
Fraction 5	5	7.8±1.2(76)
Fraction 5	10	6.5±0.6(63)
Fraction 5	20	5.4±0.4(52)

Fraction 5 was administered intraperitoneally to rats. Control animals were treated with an equal volume of saline. At 1.5 hr after treatment, rats were sacrificed by cutting the carotid artery. Groups of 5 to 6 rats were used for each experiment. The data are presented as means±S.E. Figures in parentheses are percentages with respect to the control value.

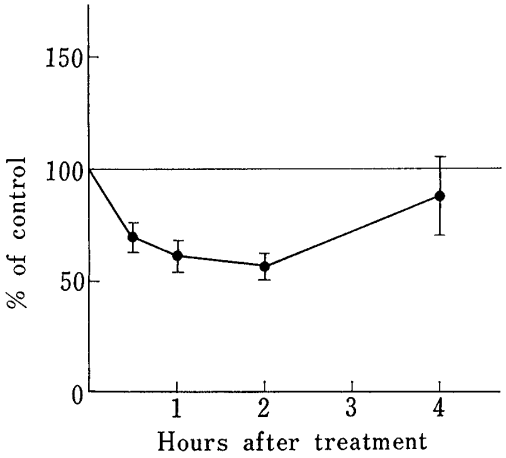

Fig. 1. Time Course of the Effect of Fraction 5 on Serine Dehydratase Activity in Rat Liver

At the indicated time after intraperitoneal administration of fraction 5 (10 mg), rats were sacrificed by cutting the carotid artery. Groups of 5 to 6 rats were used for each experiment. Data are expressed as percentages of the control value. Vertical bars show standard errors.

Table II. Effect of Fraction 5 on Serine Dehydratase Activity in the Livers of Fasted Rats

Fasting time (hr)	Material	Enzyme activity (units/mg protein $\times 10^3$)
0	Control	12.7±1.2(100)
	Fraction 5	7.0±0.7(55)
48	Control	42.7±9.5(100)
	Fraction 5	77.2±9.9(181)

Rats were administered 10 mg of fraction 5 intraperitoneally. Control animals were treated with an equal volume of saline. At 1.5 hr after treatment, rats were sacrificed by cutting the carotid artery. Groups of 5 to 6 rats were used for each experiment. The data are presented as means±S.E. Figures in parentheses are percentages with respect to the control value.

On the other hand, it is accepted that the activity of serine dehydratase in the liver is enhanced in states of increased gluconeogenesis such as starvation, feeding of a high protein diet, or alloxan-induced diabetes, and the resulting pyruvate is important as a carbon source for gluconeogenesis.[7] Table II shows the results of an experiment using starved rats. In the control group, serine dehydratase activity increased about 3.4-fold in the livers of rats which had been starved for 48 hr (12.7 *vs.* 42.7 units/mg protein $\times 10^3$). On treatment with the ginseng saponin, the enzyme activity increased by about 81% over that of the control animals. Thus, ginseng saponin action appears to be compatible with the overall metabolic pattern; when gluconeogenesis is enhanced, ginseng saponin promotes the activity of serine dehydratase. It is conceivable that serine is converted to sugar *via* pyruvate and the resulting ammonia is metabolized *via* the urea cycle. The relation of serine dehydratase level to gluconeogenic activities, however, is poorly understood. According to Weber *et al.*[11] and Henning *et al.*,[12] changes in serine dehydratase are similar to those found for glucose 6-phosphatase (EC 3.1.3.9), hexose diphosphatase (EC 3.1.3.11), and pyruvate carboxylase

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(EC 6.4.1.1), and these changes are in reciprocal relation to those of glucokinase, which catalyzes the first step of glucose utilization.[7]

In the present work, particular care was taken to consider the nutritional states of rats in elucidating the effect of the ginseng saponin on serine dehydratase activity. In summary, it was found that the response of serine dehydratase activity to ginseng saponin administration is dependent on the nutritional status in rats.

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Quinolizidines. III.[1] An Improved Synthetic Route to Stereoisomers of *dl*-2,3-*cis*-Emetine[2]

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A formal synthesis of the four stereoisomers (type **2**) of *dl*-2,3-*cis*-emetine has been effected through the synthesis of the lactam acid **10** from methyl *dl*-*cis*-5-ethyl-2-oxo-4-piperidineacetate (**4**). The steps involved are conversion of **4** into the lactim ether **5** or **6**, N-alkylation of **5** or **6** with 3,4-dimethoxyphenacyl bromide, $NaBH_4$ reduction of the resulting lactam ketone **7** followed by catalytic hydrogenolysis to give the lactam ester **9**, and alkaline hydrolysis of **9**.

Keywords——lactam ester; lactim ether; N-alkylation; $NaBH_4$ reduction; catalytic hydrogenolysis; alkaline hydrolysis; *cis* configuration; *cis*-emetine isomer

The four possible stereoisomers (type **2**) of *dl*-2,3-*cis*-emetine have already been prepared[4] during the course of extensive synthetic studies of the Ipecac alkaloid emetine (**1**).[5] It is known[4d] that none of these isomers has an *in vitro* amoebicidal effect comparable to that of natural *l*-emetine (**1**) or racemic 2,3-dehydroemetine (**3**).[6] In connection with our recent studies on the anti-tumor activities of lactams and pyridones[7] and on the syntheses of the

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