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Chemical Structures and Corticosterone Secretion-inducing Activities of Saikosaponins

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The corticosterone secretion-inducing activities of saikosaponins isolated from the roots of *Bupleurum falcatum* L., their artifact saponins and genuine genins were examined in rats 30 minutes after administration. Intraperitoneal administration of saikosaponin-a (**1**) or -d (**2**) increased the plasma corticosterone level maximally at a dose of 2.5 mg/kg or 1.0 mg/kg, respectively. Saikosaponin-c (**9**) did not increase the plasma corticosterone level at a dose of 100 mg/kg. Saikogenin-F (**3**) and -G (**4**), the genuine aglycones of **1** and **2**, also increased the plasma corticosterone level but their activities were 1/70 to 1/90, respectively, of those of the corresponding saponins on a molar basis. Saikosaponin-b_1 (**5**) and -b_3 (**7**), artifact saponins derived from **1**, showed 1/8 and 1/40 of the activity of **1**, respectively. Saikosaponin-b_2 (**6**) and -b_4 (**8**), derived from **2** through the same structural change as in the case of **1**, showed 1/50 and 1/30 of the activity of **2**, respectively. Oral administration of **2**, which was unstable to acid, increased plasma corticosterone to a maximum level at a dose of 50 mg/kg in fed rats but did not in fasted rats.

Keywords——saikosaponins; saikogenins; artifact saikosaponins; *Bupleurum falcatum*; plasma corticosterone; structure-activity relationship; saikosaponin-d administered orally to fasted rats

Saikosaponin-a (**1**), -d (**2**), -b_1—-b_4 (**5**)—(**8**) and -c (**9**) were isolated from the methanolic extract of *Bupleurum falcatum* L., and their structures were determined.[1] Yamamoto *et al.* reported that **1** and **2** showed both anti-inflammatory activity as detected by the cotton pellet method and metabolic activity, whereas **9** showed neither that.[2] Rao *et al.* reported that **1** showed antiviral activity but that saikosaponin-b (probably **5**) did not.[3]

In the preceding paper,[4] we showed that **1** and **2** stimulated the rat pituitary-adrenocortical system, but **9** did not. In the present work, the corticosterone secretion-inducing activities of saikosaponins and saikogenin-F (**3**) and -G (**4**), the aglycones of saponins **1** and **2**, respectively,[5] were determined.

Materials and Methods

Male Wistar rats weighing 130—150 g were used. They were kept in a 24° room under a light-dark cycle(light phase, 0600—1800 hr) for more than six days. They were given food and water *ad libitum*, unless otherwise specified. To avoid stress-induced release of corticosterone they were "gentled" by daily handling twice a day in the morning and evening for four days. Thirty minutes after the administration of saponin or sapogenin, rats were decapitated between 0900 and 1000 hr. Trunk blood was collected in a chilled tube containing heparin sodium, and centrifuged at 4°, and the plasma was stored at −20°. Corticosterone and glucose were determined by the competitive protein binding method and the glucose oxidase method, respectively, as described previously.[6] Saikosaponins did not affect the determination of corticosterone. Saikosaponin-a (**1**), -b_1—-b_4 (**5**)—(**8**), -c (**9**) and -d (**2**), and saikogenin-F (**3**) and -G (**4**) were kindly supplied by Dr. K. Kawasaki and Dr. H. Ishii of Shionogi and Co., Ltd., Osaka. Saikosaponins were ground and suspended in saline just before use. When administered orally, saikosaponins were suspended in distilled water. Saikogenins were suspended in saline containing 0.8% dimethylsulfoxide.

Results

Effects of Saikosaponins and Saikogenins Administered intraperitoneally to Rats

The activities of saikosaponins were examined 30 minutes after intraperitoneal administr-

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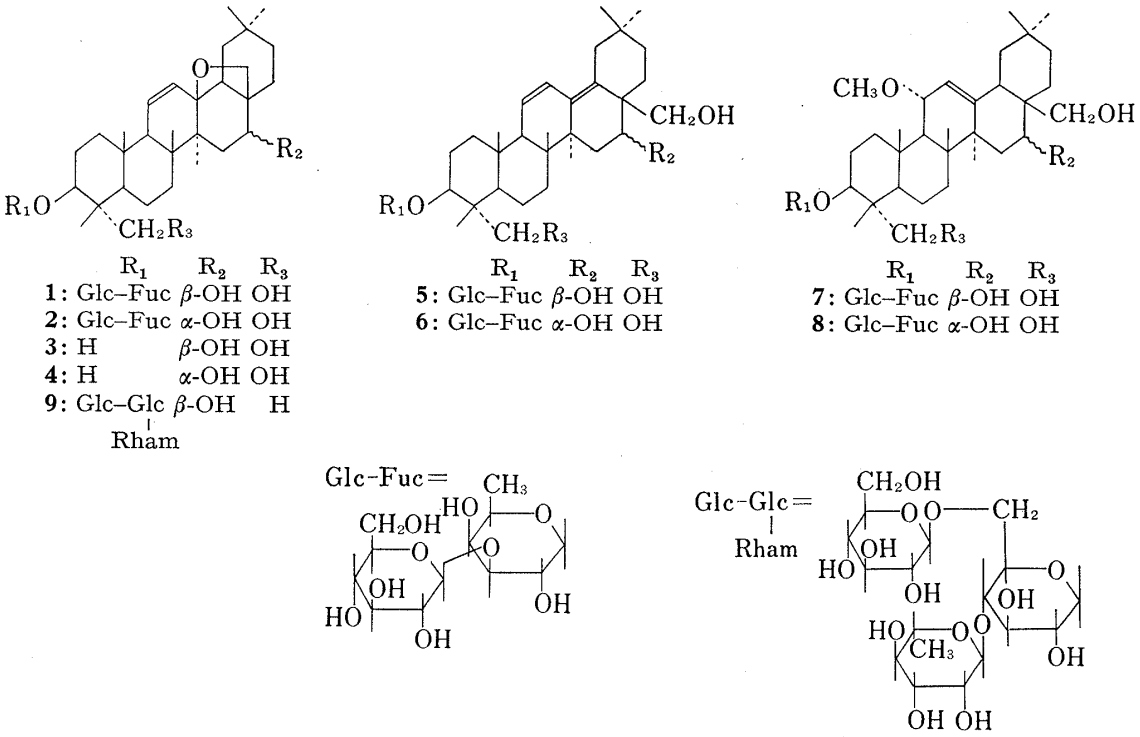

Chart 1. Structures of Saikosaponins and Saikogenins

ation. Saponins **1** and **2** differ from each other in the orientation of C-16 OH. Saponin **1** has a β-equatorial OH group at C-16, but **2** has an α-axial OH group at C-16. As shown in Fig. 1, the increment in plasma corticosterone level became greater with increasing dosages of **1** and **2**. When 2.5 or more mg/kg of **1**, and 1.0 or more mg/kg of **2** were administered, plasma corticosterone was elevated to a maximum level. The 50%-effective dose (ED_{50}) was determined to be 0.9 mg/kg for **1** and 0.3 mg/kg for **2** from the dose-response curves of plasma corticosterone concentration (Fig. 1). Thus, the corticosterone secretion-inducing activity of **2** was three times more potent than that of **1**. Saponin **9** was different from **1** and **2** both in having no OH group at C-23 and in the sugar moiety. Saponin **9** did not increase the plasma corticosterone level even at a dosage of 100 mg/kg.

Sapogenins **3** and **4** significantly increased the plasma corticosterone level at dosages of 50 mg/kg and 25 mg/kg, respectively. ED_{50} was determined to be 35 mg/kg for **3** and 17 mg/kg for **4**. Thus, **4** was twice as potent as **3**. The activities of **3** and **4** were about 1/70 and 1/90 of those of the corresponding saponins on a molar basis.

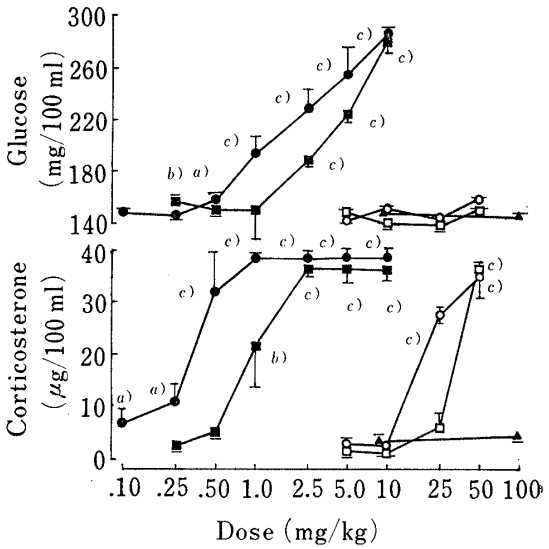

Fig. 1. Effects of Graded Doses of Saikosaponin-a (**1**), -c (**9**) and -d (**2**), and Saikogenin-F (**3**) and -G (**4**) on Rat Plasma Corticosterone and Blood Glucose Concentrations

Plasma samples were taken 30 minutes after intraperitoneal administration of 0.5 ml of saponin or sapogenin suspension. Both plasma corticosterone and blood glucose were assayed in the same sample. Each point represents the mean ± S.E. of 4 to 6 samples.

■: Saikosaponin-a (**1**), ●: saikosaponin-d (**2**), ▲: saikosaponin-c (**9**), □: saikogenin-F (**3**), ○: saikogenin-G (**4**). *a*) $p<0.05$ *vs.* saline control, *b*) $p<0.01$ *vs.* saline control, *c*) $p<0.001$ *vs.* saline control.

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Upon administration of **1** and **2**, blood glucose increased dose-dependently in the dose ranges of over 1.0 and 0.5 mg/kg, respectively. Saponin **9** did not increase blood glucose even at a dose of 100 mg/kg. As for **3** and **4**, no increase in blood glucose level was found at dosages of 5 mg/kg to 50 mg/kg.

Saponins **5**—**8** increased the plasma corticosterone level and blood glucose level dose-dependently. ED_{50} values for the activities of the saponins on plasma corticosterone concentration are shown in Table I. The order of the corticosterone secretion-inducing activities of the saikosaponin-b group was **5**>**8**>**6**>**7**.

Table I. ED_{50} Values of Saikosaponin-a (**1**), -b_1 — -b_4 (**5**)—(**8**) and -d (**2**), and Saikogenin-F (**3**) and -G (**4**) for Corticosterone Secretion-inducing Activity

		16β-OH ED_{50} (mg/kg)	Compound $\frac{ED_{50}}{ED_{50}\text{ of }1}$		16α-OH ED_{50} (mg/kg)	Compound $\frac{ED_{50}}{ED_{50}\text{ of }2}$	$\frac{ED_{50}\text{ of 16β-OH Comp.}}{ED_{50}\text{ of 16α-OH Comp.}}$
Saponin	**1**	0.9	1	**2**	0.3	1	3
	5	7	8	**6**	15	50	1/2
	7	35	40	**8**	9	30	4
Genin	**3**	35	70[a]	**4**	17	90[a]	2

a) Expressed on a molar basis.

Effects of Saikosaponins administered orally

1) **In Fed Rats**——The effect of orally administered **1** was examined at doses of 25, 50 and 100 mg/kg. As shown in Fig. 2A, **1** caused a gradual increase in plasma corticosterone concentration with increasing dose. The effect of **2** was examined at doses of 10, 25 and 50 mg/kg. Saponin **2**, at a dose of 50 mg/kg, significantly increased the plasma corticosterone level. In view of these results, it appears that oral administration required doses about 50 times greater than intraperitoneal administration to cause the maximal increment in plasma corticosterone concentration. Blood glucose level also increased significantly upon administration of 50 mg/kg of **2** ($p<0.02$, 194±11 *vs.* 154±2 mg/100 ml). The effects of **5**—**8** were examined at a dose of 100 mg/kg. As shown in Fig. 2B, the plasma corticosterone concentration after the administration of one of **5**—**8** showed a tendency to increase over that of the control rats. Blood glucose level was not affected by the administration of **5**—**8**. As regards plasma corticosterone-enhancing activity, the order of activities of the saikosaponin-b group administered orally (**5**>**8**>**7**>**6**) was almost the same as the order when they were administered intraperitoneally.

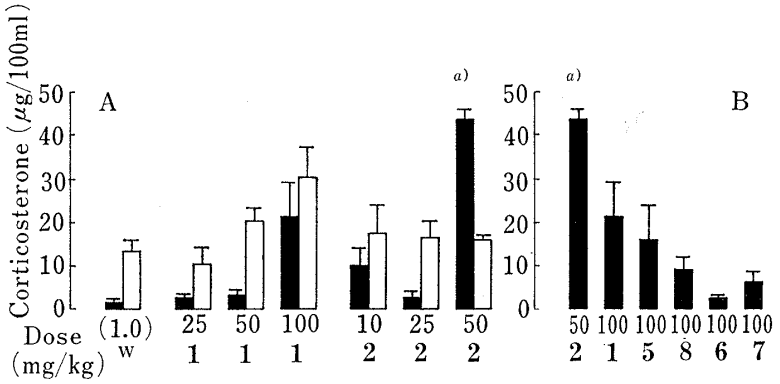

Fig. 2. Effects of orally Administered Saikosaponin-a (**1**), -b_1 — -b_4 (**5**)—(**8**) and -d (**2**) on Rat Plasma Corticosterone Concentration

Plasma samples were taken 30 minutes after oral administration of 1.0 ml of saponin suspension. Data are means±S.E. of 4 to 7 rats. ■: Fed rats, □: fasted (16 hr) rats, w: distilled water. *a*) $p<0.001$ *vs.* water-administered control.

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2) **In Fasted Rats**——The effects of **1** and **2** in rats fasted for 16 hr was examined at the same doses as those used in fed rats. Saponin **1**, as in fed rats, showed a tendency to gradually increase the plasma corticosterone level with increasing dosage. Saponin **2**, however, did not affect the plasma corticosterone level at a dose of 50 mg/kg in fasted rats.

Discussion

The present work indicates that **1** and **2** showed corticosterone secretion-inducing activity, but **9** did not. Further, **3** and **4** also showed corticosterone secretion-inducing activity. These results suggested that the genin moiety of **1** and **2** was responsible for the corticosterone secretion-inducing activity, and that C23–OH in the genin moieties of **1** and **2** was critical. The more potent activity of **1** and **2** as compared with **3** and **4** may be due to the contribution of the sugar moiety to the activity of saikosaponins, for example, by modifying the bioavailability or the potency of the genin moiety.

Saponins **5**—**8** were formed secondarily through the cleavage of the bridged 13β, 28-epoxyoleanene system in saponins **1** and **2**.[1b] The activities of **5**—**8** were 1/8, 1/50, 1/40 and 1/30, respectively, of those of the corresponding genuine saikosaponins. Therefore, it appears that the bridged 13β, 28-epoxyoleanene system in saikosaponin is important to the corticosterone secretion-inducing activity of saikosaponins.

The contribution of C16–OH orientation to corticosterone secretion-inducing activity was assessed in four pairs of compounds. The activity of **2** possessing a 16α-OH group was three times more potent than that of **1** possessing a 16β-OH. Genin **4** (16α-OH) was twice as potent as genin **3** (16β-OH). Saponins **7** and **8** have a methoxyl group at C-11 and a double bond at C-12. The activity of **8** (16α-OH) was four times that of **7** (16β-OH). These results suggested that C16α-OH was two to four times more favorable for corticosterone secretion-inducing activity than C16β-OH. However, in the pair **5** and **6**, which have a conjugated heteroannular diene system, **6** (16α-OH) was 1/2 as potent as **5** (16β-OH). The reason for this exceptional relation is not clear at present.

The anti-inflammatory activities and metabolic activities of **1**, **2** and **9**, reported by Yamamoto *et al.*,[2a] parallel their corticosterone secretion-inducing activities. Rao *et al.*[3] showed that the antiviral activity of saikosaponin-b (probably **5**) was lower than that of **1**. This result was in accordance with our findings that the corticosterone secretion-inducing activity of the saikosaponin-b group was lower than that of genuine saikosaponins. Abe *et al.*[7] investigated the hemolytic activities of **1**, **2**, **5**, **6** and **9**, and showed that the order of activity was **2**>**1**>**5**>**6**>**9**. Although no hemolysis was found in our *in vivo* experiment, the order of the hemolytic activity of saikosaponins was the same as the order of their corticosterone secretion-inducing activity. These results suggest that there may be some common process among the corticosterone secretion-inducing activity, anti-inflammatory activity, antiviral activity and hemolytic activity of saikosaponins.

In the oral administration experiment, the increase of plasma corticosterone level caused by **2** in fed rats did not appear in fasted rats. Saponin **1** administered orally increased the plasma corticosterone level in both fed and fasted rats. On the other hand, **2** was unstable to weak acid and was readily converted to **6**,[1b,8] which was less potent than **2**, whereas **1** was fairly stable to acid. Therefore, it is possible that **2** administered orally was converted to **6** by the gastric acid and did not act as **2** in the fasted condition, whereas **1** administered orally acted as **1** in the fasted condition.

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