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In vitro Degradation of Saikosaponin-a in Physiological Condition

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Abstract—*In vitro* degradation of saikosaponin-a in physiological condition was conducted. Saikosaponin-a in 0.1 N-HCl of 25% 1,4-dioxane was incubated at 37° and the products were analyzed and the time course of degradation was observed. Saikosaponin-a(Sa) was transformed to saikosaponin-b_1(Sb_1) and saikosaponin-g(Sg) in the course of time. Sa was decreased drastically and not detected after 6 hours. Sb_1 and Sg was increased with the time. After 6 hours the ratio of Sb_1 and Sg was about 4:1 and was maintained for 24 hours.

Keywords—Saikosaponin-a • saikosaponin-b_1 • saikosaponin-g • *in vitro* degradation• physiological condition

Degradation of natural products in physiological condition has very important meanings in consideration of the use of natural products as pure state or in the form of preparation. Natural products are expected to be transformed in the course of decoction or in the biological condition. When we consider these seriously, it is important to elucidate the active components of the crude drugs used in single or in the form of prescriptions. Many drugs are known to be transformed to active or inactive form when it is prescribed with other drugs or it was absorbed and metabolized in the physiological condition. But little was known about the transformation of bio-active natural products, especially of saponin drugs.

In crude drugs there are many saponin drugs which have many important physiological activities. But the mechanism of action of saponin remained almost unknown. Which functional group in a saponin structure plays important roles in the manifestation of the action of saponin, sugar moiety or sapogenin moiety? To elucidate the mechanism of action of saponin, the metabolism and the active form of saponin are to be clarified. Han et al.[1] studied *in vitro* degradation of ginseng saponins in the gastric physiological condition. Ginseng saponin was reported to be transformed to some hydroxylated and partially hydrolysed saponins(prosapogenins). Their biological action needs to be investigated for the clarification of the action of ginseng saponins.

Saikosaponin-a(Sa) is one of the active saponin components contained in Bupleuri Radix (the root of *Bupleurum falcatum* L.: Umbelliferae) which is used as one of the chief components in many kinds of prescriptions for its antypyretic, analgetic and sedative action in oriental medicine. Sa has very unstable allyl oxide linkage and is reported to be degraded to heteroannular diene saponin, saikosaponin-b_1

(Sb_1) in weak acidic condition including gastric condition.[2] Sb_1 has none of the actions, whereas Sa has an anti-inflammatory action or preventive effect of liver.[3] There rises some questions including which is actually active components of crude drug, Bupleuri Radix. Considering these, to elucidate the actually active components of Bupleuri Radix, the degradation of saikosaponin-a in *in vitro* gastric physiological condition.

Sa in 0.1N-HCl of 25% 1,4-dioxane was incubated at 37° for 24 hours. Thirty min, 1, 2, 3, 6, 12 and 24 hr after incubation, the reactant was collected and the structure of metabolites and the ratio of the components was elucidated and calculated. Sa is sparingly soluble in water,[4] so that minimal solvent which is thought to be inactive and make the solution clear throughout this degradation reaction, 25% 1,4-dioxane is selected as a solvent. After 6hr the reaction mixture was precipitated when the reaction mixture was cooled to room temperature. Alcohol could not be used as a solvent because of its solvolytic activity. The time course of degradation of Sa was shown in Fig. 1. Each metabolites showed good separation when they are chromatographed with HPTLC RP-2 $F_{254}S$ and MeOH-H_2O(70:30) as solvent system and were separated to each components; unreacted Sa, compound A and compound B. Compound A was found to be saikosaponin-b_1(Sb_1) by direct comparison with Sb_1 by the known method.[2] Compound B has same molecular formula based on elemental analysis. It was suggested to have homoannular diene moiety at C-9(11), 12 on the basis of the observation of UV spectrum (λ_{max} 280 nm). In ^{1}H-NMR spectrum, olefinic protons at δ 5.6 (2H, singlet) were observed. This supported the presence of homoannular diene moiety. In ^{13}C-NMR spectrum, the signals based on the sugar moiety were identical with those of Sa. Thus, the structure of compound B was found to be saikosaponin-g, which was originally derived by reaction of Sa with 1N-H_2SO_4 by Shimizu et al.[5] Scheme 1 showed *in vitro* degradation of Sa.

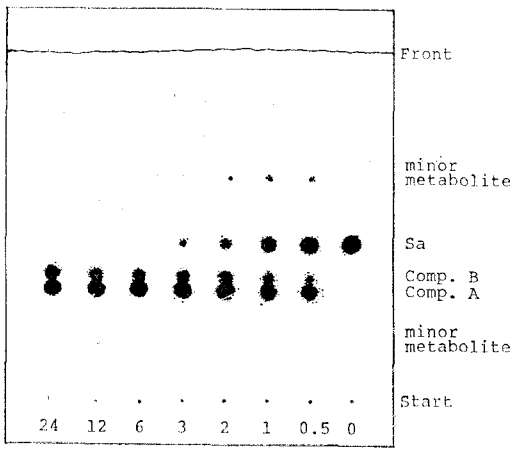


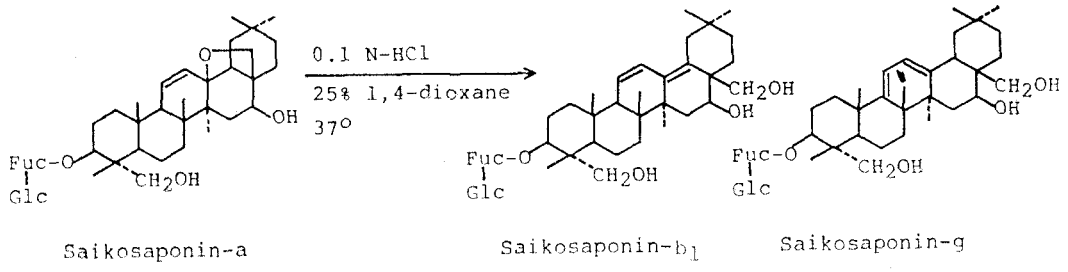
Fig. 1. TLC chromatogram of incubation mixture
TLC condition: HPTLC RP-2 $F_{254}S$, solv. MeOH-H_2O(70:30), coloring: 1% DABA-10% H_2SO_4 (Comp. A: Sb_1, Comp. B: Sg, 0: reaction mix. before incubation, 0.5~24: reaction mix. taken 30 min~24 hr after incubation).

Time course of degradation of Sa was deter-

Table I. Saponin contents of each incubation mixture (mcg)

Compound \ Time(hr)	0.5	1	2	3	6	12	24
Saikosaponin-a	5.10	3.68	2.10	1.11	0.05	0	0
Saikosaponin-b_1	4.01	5.19	6.68	7.02	7.62	7.33	7.59
Saikosaponin-g	0.84	1.13	1.36	1.80	2.02	2.01	2.12

* Each incubation mixture contained 10 mcg of total saponin.



Scheme 1. *In vitro* degradation of saikosaponin-a.

mined and calculated by densitometric analysis. Densitometry was carried out at 254 nm for heteroannular diene saponin(Sb_1), 280 nm for homoannular diene saponin(Sg) and 530 nm after colorization with 1% dimethylaminobenzaldehyde(DABA) and 10% H_2SO_4 for the determination of saponin without any diene moiety(Sa). The results are summarized in Fig. 2 and Table 1. Sa was drastically decreased with the time and was not detected after 6 hr. Sb_1 and Sg was increased with the time and maintained constant after 6 hr upto 24 hours. After 30 min, the reaction mixture consisted of 50% Sa, 40% Sb_1 and 10% Sg. After 6 hr, the ratio of Sb_1 and Sg was 4:1 and maintained for 24 hours.

Sa was found to be degraded completely to Sb_1 and Sg in the ratio of 4:1 after 5 hr under *in vitro* condition which was considered to be almost the same as physiological gastric condition. Then Sb_1 and Sg were also considered absorbed to body fluid together with Sa and to exert some physiological actions. On this point of view the physiological action of Sa comes to be more complex. But when we consider the full understanding of the action of some natural products, the effect of the action of metabolites as well as that of natural product itself must be considered seriously.

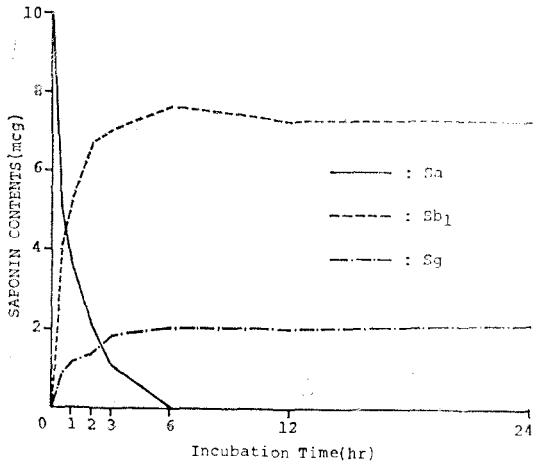


Fig. 2. Time course of degradation of saikosaponin -a. (Sa: saikosaponin-a, Sb_1: saikosaponin $-b_1$, Sg: saikosaponin-g)

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