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# Effects of Escins Ia, Ib, IIa, and IIb from Horse Chestnuts on Gastrointestinal Transit and Ileus in Mice

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**Abstract**—The effects of saponin fraction and its principal constituents escins Ia (**1**), Ib (**2**), IIa (**3**), and IIb (**4**) from horse chestnuts on gastrointestinal transit (GIT) and ileus were investigated in mice. Ileus was induced by acetic acid peritoneal irritation or by laparotomy with manipulation. One hour after the oral administration, the saponin fraction (12.5–100 mg/kg) and **1**–**4** (12.5–50 mg/kg, except for **3** at 12.5 mg/kg) dose-dependently accelerated GIT. The optimal effects of the saponin fraction (25 mg/kg) occurred 5–240 min (applied intervals between the fraction and the charcoal meal) after the oral administration. The fraction (12.5–100 mg/kg) and **1**–**4** (12.5–50 mg/kg, except for **1** and **2** at 12.5 mg/kg) dose-dependently prevented the inhibition of GIT induced by the acetic acid peritoneal irritation. They (12.5–100 mg/kg) also dose-dependently prevented the inhibition of GIT induced by the laparotomy with manipulation. Desacylescins I (**5**) and II (**6**) (50 mg/kg) showed no such effects. These results demonstrated that the saponin fraction and **1**–**4** accelerated GIT and prevented the experimental ileus, and indicate that the 21, 22-acyl groups are essential for the accelerative effects of **1**–**4**. The accelerations of GIT by **1**–**4** were completely abolished by the pretreatment with streptozotocin (100 mg/kg, iv), but not by the pretreatment with capsaicin (75 mg/kg in total, sc) or atropine (10 mg/kg, sc). These results imply that the sympathetic nervous system may be, but neither capsaicin-sensitive sensory nerves nor the cholinergic mechanism, involved in the accelerations of GIT by escins **1**–**4**. 

## Introduction

The saponin mixture 'escin' obtained from the seeds of the horse chestnut tree, *Aesculus hippocastanum* L., is widely used in the therapy of peripheral vascular disorders and in cosmetics for prevention and treatment of cellulitis. But the isolation and structure elucidation of saponin constituents in 'escin' had been uncharacterized. We recently isolated twelve acylated polyhydroxyolean-12-ene 3-*O*-monodesmosides, escins Ia (**1**), Ib (**2**), IIa (**3**), IIb (**4**), IIIa, IIIb, IV, V, and VI and isoescins Ia, Ib, and V, from horse chestnuts and determined their chemical structures.[3] The principal saponins (**1**, **2**, **3**, and **4** as shown in Fig. 1) have been shown to have the inhibitory effects on increased blood glucose or ethanol concentration in oral glucose- or ethanol-loaded rats, on inflammatory responses in rats and mice, and on gastric lesions in rats.[1–6] Furthermore, investigation of the action modes of them for the hypoglycemic activity revealed that they potently inhibited gastric emptying in rats and mice.[7,8]

Gastric emptying and gastrointestinal transit (GIT) are very important functions of the digestive system. Ileus is the common complication induced by various reasons, such as laparotomy with manipulation, peritoneal irritation, etc. Precisely because we lack specific therapy, ileus remains an important clinical problem. Patients with ileus accumulate gas and secretions leading to bloating, distention, emesis, and pain. Prokinetic drugs, including cisapride, metoclopramide, erythromycin, and octreotide, are commonly used for chronic ileus. Unfortunately, in advanced cases, no medical therapy provides impressive relief.[9] Non-steroidal anti-inflammatory drugs, such as indomethacin, are known for their ability to block the prostaglandins biosynthesis and they are widely used for postoperative pain. These medicines are shown to be beneficial in the treatment of postoperative ileus in rodents.[10,11] However, they are also known to cause undesirable side effects.

Previously, we have demonstrated that several oleanolic acid glycosides accelerated GIT and prevented the ileus induced by peritoneal irritation and laparotomy with manipulation.[12] In this report, we investigated the effects of the saponin fraction, escins Ia–IIb (**1**–**4**), their alkaline-hydrolyzed products desacylescins I (**5**) and II (**6**) on GIT and ileus in mice. We also examined the

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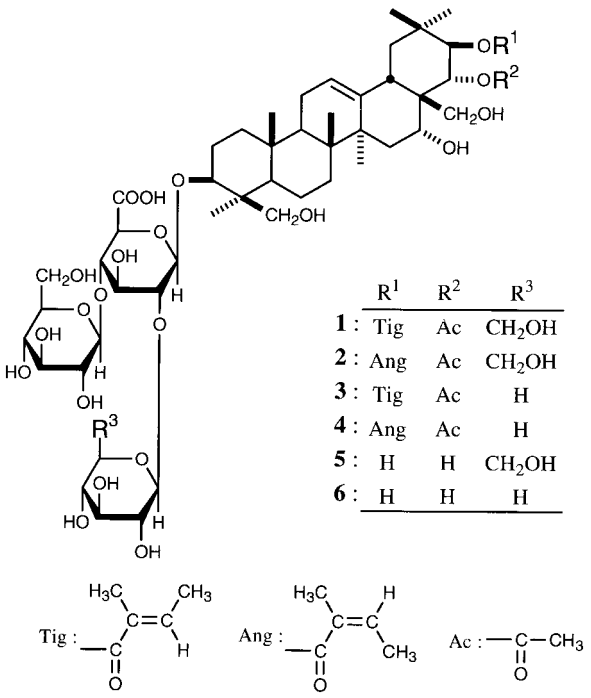


**Figure 1.** Chemical structures of escins Ia (**1**), Ib (**2**), IIa (**3**), and IIb (**4**) and desacylescins I (**5**) and II (**6**).

effects of **1**–**4** on GIT in streptozotocin-, capsaicin-, and atropine-pretreated mice, and discuss the roles of the sympathetic nervous system (SNS), capsaicin-sensitive sensory nerves and the cholinergic mechanism in these effects.

## Results and Discussion

The effects of saponin fraction, its principal saponins (escins Ia (**1**), Ib (**2**), IIa (**3**), and IIb (**4**)), their alkaline-hydrolyzed products (desacylescins I (**5**) and II (**6**)), and reference drugs (metoclopramide and indomethacin) on GIT are summarized in Table 1. One hour after the oral administration, the saponin fraction (12.5–100 mg/kg) and **1**–**4** (12.5–50 mg/kg) dose-dependently accelerated GIT by 14.9–46.5% (Table 1: Normal). But desacylescins I (**5**) and II (**6**) (50 mg/kg) did not. Metoclopramide (25–50 mg/kg) also accelerated GIT by 23.9–25.4%. Indomethacin tended to inhibit GIT. Our previous study showed that **1**–**4** inhibited the rate of gastric emptying.[7,8] In contrast, they accelerated GIT in the present study. These results indicate that **1**–**4** accelerate GIT by accelerating small intestinal transit.

The duration of the GIT acceleration by the saponin fraction (25 mg/kg) is shown in Figure 2. The optimal effects occurred 5–240 min (applied intervals between the fraction and the charcoal meal) after the oral administration of the fraction. The maximal acceleration by 46.5% was achieved by 60 min following the administration, and the effect persisted for 240 min, and disappeared by 300 min.

The intraperitoneal injection of 1% acetic acid (0.2 mL/mouse) potently inhibited GIT (Table 1: Ileus by AcOH). The saponin fraction (12.5–100 mg/kg) and **1**–**4** (12.5–50 mg/kg) dose-dependently prevented the inhibition, except for **1** and **2** at 12.5 mg/kg. The reference drugs, metoclopramide (50 mg/kg) and indomethacin (5–25 mg/kg), also prevented the inhibition. The laparotomy with manipulation also induced the inhibition of GIT (Table 1: Ileus by operation). The saponin fraction and **1**–**4** (25–100 mg/kg) as well as reference drugs, metoclopramide (50 mg/kg) and indomethacin (5–25 mg/kg), prevented the inhibition. But **5** and **6** (50 mg/kg) did not. These results suggest that the saponin fraction and **1**–**4**, but not **5** and **6**, prevent the experimental ileus.

In this study, escins Ia–IIb (**1**–**4**) accelerated GIT in normal mice, and prevented the ileus induced by peritoneal irritation or laparotomy with manipulation. But desacylescins I (**5**) and II (**6**) (50 mg/kg) showed no effect. These results indicate that the 21, 22-acyl groups are important for the effects of **1**–**4**.

SNS plays an important role in the modulation of gastrointestinal motility. Abdominal nociceptive stimulation and major trauma is usually followed by reflex inhibition of gastrointestinal motility. The gastrointestinal motility is inhibited by both sympathoadrenergic and vagal reflexes which may be elicited by nociceptive stimulation of the peritoneum.[13–15] The afferent pathways from the abdomen are mediated via sympathetic fibers to the spinal cord and ascending spinal pathways.[14] The vagal efferents inhibiting gastric motility are shown to be mediated by nonadrenergic and noncholinergic fibers.[16] Ileus in response to surgery or peritoneal irritation is partly due to activation of a neural reflex, and the efferent limb seemed to involve sympathetic adrenergic neurons.[17] Surgically and peritoneal irritation-induced ileus are known to activate an extrinsic inhibitory nervous control of gastrointestinal motility.[18] Hyperactivity of the sympathetic nervous system, which may be elicited by nociceptive stimulation of the peritoneum, plays a prominent role in the genesis of postoperative ileus.[13,15,16,19] Sympathetic activation may enhance the synthesis of prostaglandins (PGs).[20] PGs may be involved in the gastric emptying and the control of the contractions of gastrointestinal smooth muscle.[21–23] Hyperglycemia in STZ-induced hypoinsulinemic rats reduced the activity of SNS.[24] Previous study showed that both of the inhibitions of gastric emptying in mice and the gastroprotections against ethanol-induced gastric mucosal lesions in rats by **1**–**4** were markedly attenuated by the pretreatment with STZ.[8,12] In this study, the GIT accelerations by **1**–**4** were also markedly attenuated in STZ-pretreated mice (Table 2). Furthermore, **1**–**4** prevented the inhibition of GIT induced by the peritoneal irritation or by the laparotomy with manipulation. These results imply that all of their inhibitions of gastric emptying, gastroprotections, and GIT accelerations are mediated via the similar pathway, and the mechanism of SNS may be involved.

Capsaicin-sensitive afferent neurons innervate the digestive tract.[25–28] Capsaicin has been systematically used to ablate all capsaicin-sensitive C fibers to produce

**Table 1.** Effects of the saponin fractions, escins Ia (**1**), Ib (**2**), IIa (**3**), and IIb (**4**) and descaylescins I (**5**) and II (**6**) on GIT in mice[a]

| Treatment | Dose (mg/kg, po) | GIT (%) | | GIT (%) | | GIT (%) | |
|---|---|---|---|---|---|---|---|
| | | *N* | Normal | *N* | Ileus by AcOH | *N* | Ileus by operation |
| Control | — | 11 | 47.5 ± 3.3 | 10 | 12.2 ± 0.9 | 8 | 21.7 ± 2.3 |
| Saponin fraction | 5 | 10 | 55.4 ± 3.0 | 10 | 17.8 ± 2.7 | | — |
| | 12.5 | 10 | 61.1 ± 2.5*[b] | 10 | 27.4 ± 2.5** | 8 | 21.4 ± 2.3 |
| | 25 | 10 | 69.6 ± 1.9** | 10 | 32.7 ± 2.7** | 8 | 31.9 ± 3.9** |
| | 50 | 10 | 63.2 ± 3.4** | 10 | 35.1 ± 1.3** | 8 | 35.3 ± 2.3** |
| | 100 | 10 | 66.3 ± 3.9** | 10 | 40.1 ± 1.9** | 8 | 42.2 ± 3.1** |
| Control | — | 12 | 51.0 ± 2.3 | 12 | 14.5 ± 1.3 | 10 | 20.9 ± 1.7 |
| Escin Ia (**1**) | 12.5 | 10 | 58.8 ± 1.4* | 10 | 14.1 ± 1.3 | 8 | 30.3 ± 1.5* |
| | 25 | 10 | 61.0 ± 1.6* | 10 | 24.5 ± 1.6** | 8 | 36.4 ± 2.2** |
| | 50 | 10 | 62.9 ± 1.4** | 10 | 28.4 ± 1.6** | 8 | 36.8 ± 2.1** |
| Escin Ib (**2**) | 12.5 | 10 | 59.9 ± 1.9* | 10 | 14.9 ± 1.1 | 8 | 35.9 ± 3.9** |
| | 25 | 10 | 65.7 ± 2.3** | 10 | 26.1 ± 3.1** | 8 | 39.5 ± 2.1** |
| | 50 | 10 | 66.1 ± 2.8** | 10 | 33.1 ± 2.9** | 8 | 39.4 ± 2.9** |
| Control | — | 12 | 50.2 ± 2.1 | 12 | 13.1 ± 1.3 | 10 | 22.5 ± 1.1 |
| Escin IIa (**3**) | 12.5 | 10 | 57.7 ± 3.0 | 10 | 24.3 ± 2.8** | 8 | 30.8 ± 2.2* |
| | 25 | 10 | 65.8 ± 2.6** | 10 | 30.4 ± 1.8** | 8 | 41.0 ± 1.3** |
| | 50 | 10 | 66.3 ± 1.7** | 10 | 41.0 ± 3.8** | 8 | 39.6 ± 1.0** |
| Escin IIb (**4**) | 12.5 | 10 | 60.9 ± 2.2* | 10 | 25.2 ± 2.5** | 8 | 30.9 ± 2.2** |
| | 25 | 10 | 66.3 ± 2.5** | 10 | 33.3 ± 1.4** | 8 | 39.0 ± 1.9** |
| | 50 | 10 | 69.7 ± 2.8** | 10 | 36.8 ± 1.8** | 8 | 40.5 ± 1.4** |
| Desacylescin I (**5**) | 50 | 10 | 50.1 ± 2.6 | 10 | 14.6 ± 1.2 | 8 | 25.0 ± 2.4 |
| Desacylescin II (**6**) | 50 | 10 | 48.7 ± 1.9 | 10 | 13.6 ± 1.3 | 8 | 24.3 ± 2.1 |
| Control | — | 10 | 47.2 ± 3.1 | 10 | 14.0 ± 0.8 | 10 | 21.8 ± 2.2 |
| Metoclopramide | 12.5 | 8 | 54.0 ± 2.5 | 8 | 13.6 ± 1.5 | 8 | 22.8 ± 2.1 |
| | 25 | 8 | 58.5 ± 2.7* | 8 | 14.3 ± 1.3 | 8 | 24.4 ± 2.8 |
| | 50 | 8 | 59.2 ± 2.7** | 8 | 21.4 ± 1.9* | 8 | 33.4 ± 1.8** |
| Indomethacin | 5 | 8 | 42.2 ± 2.4 | 8 | 25.5 ± 2.5** | 8 | 35.0 ± 2.0** |
| | 12.5 | 8 | 40.6 ± 3.0 | 8 | 23.4 ± 2.8** | 8 | 36.9 ± 2.2** |
| | 25 | 8 | 38.2 ± 3.6 | 8 | 32.1 ± 2.3** | 8 | 38.4 ± 1.6** |

[a] Values represent the means ± SEM.
[b] Significantly different from the control group, $^{*}p < 0.05$, $^{**}p < 0.01$.

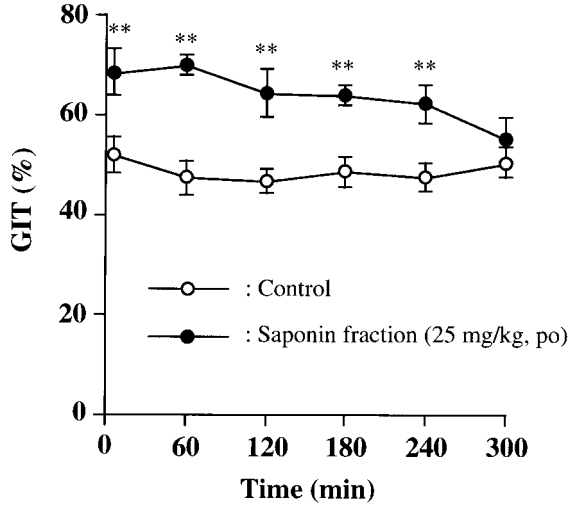


**Figure 2.** Effects of saponin fraction on GIT in mice at different times. The saponin fraction was given orally at different times (5–300 min) prior to administration of the charcoal meal. GIT was determined 30 min after the charcoal meal. Plots represent the mean with SEM. ($N = 10$). Significantly different from the control group, $^{**}p < 0.01$.

a sensory pathway-specific ablation in rats and mice.[29] Hormones, such as corticotropin-releasing factor and secretin, inhibit gastric emptying via capsaicin-sensitive afferent neurons.[30,31] Both of the inhibitions of gastric emptying in mice and the gastroprotections against ethanol-induced gastric mucosal lesions in rats by **1**–**4** have been also shown to be involved in the capsaicin-sensitive sensory nerves.[8,12] But capsaicin-sensitive afferent neurons do not participate in the physiologic control of gastrointestinal propulsion.[17] The peritoneal irritation-induced ileus was not affected by perivagal capsaicin treatment.[32] In the present study, the GIT accelerative effects of **1**–**4** were not attenuated in the capsaicin-pretreated mice (Table 2). The result suggests that, differently from the action mechanism on gastric emptying, capsaicin-sensitive sensory nerves are not involved in the GIT accelerative effects of **1**–**4**.

Muscarinic receptor agonists, such as carbachol, accelerate GIT.[33] Atropine is a selective antagonist of muscarinic receptors. Basal GIT was decreased in mice treated with atropine as shown in Table 2. **1**–**4** still accelerated GIT in atropine-pretreated mice, as compared with the control. The result suggests that the cholinergic mechanisms could not be involved in the GIT accelerative effects of **1**–**4**.

In conclusion, the results in this study demonstrated that the saponin fraction and escins Ia–IIb (**1**–**4**) accelerated GIT in normal mice and prevented the ileus induced by peritoneal irritation or laparotomy with manipulation. As concerns structural requirements of

**Table 2.** Effects of escins Ia (**1**), Ib (**2**), IIa (**3**) and IIb (**4**) on GIT in capsaicin-, STZ-, or atropine-pretreated mice[a]

| Treatment | Dose (mg/kg, po) | *N* | GIT (%) | | |
|---|---|---|---|---|---|
| | | | STZ-pretreated mice | Capsaicin-pretreated mice | Atropine-pretreated mice |
| Normal (naive mice) | — | 8 | 50.3 ± 2.7 | 49.0 ± 2.6 | 49.6 ± 2.8**[b] |
| Control | — | 10 | 54.1 ± 2.4 | 43.8 ± 1.4 | 33.0 ± 1.8 |
| Escin Ia (**1**) | 25 | 10 | 50.2 ± 2.4 | 54.4 ± 1.8* | 54.8 ± 2.6** |
| Escin Ib (**2**) | 25 | 10 | 51.5 ± 3.2 | 61.2 ± 3.3** | 51.6 ± 2.8** |
| Escin IIa (**3**) | 25 | 10 | 50.3 ± 3.3 | 62.2 ± 4.2** | 56.0 ± 2.8** |
| Escin IIb (**4**) | 25 | 10 | 49.8 ± 1.7 | 59.1 ± 3.1** | 54.0 ± 3.6** |

[a] Values represent the means ± SEM.
[b] Significantly different from the control group, $^{*}p < 0.05$, $^{**}p < 0.01$.

**1**–**4**, the 21, 22-acyl groups are necessary for the effects. Though their action mechanisms are still unclear, SNS may be involved. The involvement of SNS and other mechanisms need to be studied further. It is worthy to study their therapeutical effect in the prevention and treatment of the inhibition of GIT, including ileus, in clinic.

## Experimental

### Materials

The saponin fraction and escins Ia (**1**), Ib (**2**), IIa (**3**), and IIb (**4**) were isolated from the seeds of *A. hippocastanum* L., and desacylescins I (**5**) and II (**6**) were obtained by alkaline hydrolysis of **1** and **3**, as described in previous reports.[2] Other reagents were purchased from Wako Pure Chemical Industries, Japan.

### Animals

Male ddY mice, weighing 27–30 g, were purchased from Kiwa Laboratory Animal Co., Ltd., Japan. The animals were maintained at a constant temperature of 23 ± 2°C and were fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Japan) for a week. The animals were fasted for 18–20 h prior to experiments, but were supplied with water ad libitum. The saponins were dissolved in phosphate buffered saline (PBS), and metoclopramide and indomethacin were suspended in 5% acacia/PBS solution, and the solution was orally administered at 10 mL/kg in each experiment, while the vehicle was administered orally at 10 mL/kg in the corresponding control group.

### Measurement of GIT in normal mice

A charcoal meal containing a solution of 1.5% carboxymethyl cellulose sodium salt (CMC-Na) and 5% charcoal as a marker was intragastrically given (0.2 mL/mouse) to conscious mice. Thirty minutes later, mice were sacrificed by cervical dislocation. The abdominal cavity was opened, and the gastrointestinal tract was removed. The traveled distance of the marker was measured and expressed as a percentage of the total length of the small intestine from pylorus to caecum. The test samples were given orally by means of a metal orogastric tube 5, 60, 120, 180, 240 or 300 min prior to the administration of the charcoal meal, respectively.

### Measurement of GIT in peritoneal irritated mice

The peritoneal irritation was induced by a modification of the method described by Riviere et al.[32] Acetic acid saline solution (1%, 0.2 mL/mouse) was peritoneally injected 30 min after the oral administration of the samples. The charcoal meal was administered 30 min after the injection of acetic acid. GIT was determined as described above.

### Measurement of GIT in laparotomized and manipulated mice

The postoperative ileus was induced as the method described by De Winter et al.[34] Briefly, the mice underwent an operation under ether anesthesia. In contrast with pentobarbital anesthesia, ether anesthesia was chosen, whose effect on gastrointestinal motility approximately lasted for only 1 h after the induction of the anesthesia.[35] The abdominal cavity was opened, and then the small intestine and caecum were gently pulled out of the abdominal cavity and unfurled like a fan on two sterile gauzes covering the abdomen of the mouse. After 5 min of gentle manipulation, the small intestine and caecum were replaced in the abdominal cavity and the surgical wound was sutured. After the operation, the mice were allowed to recover for 60 min. Test samples were given orally 30 min before the operation. The charcoal meal was administered 65 min after the operation. GIT was determined as described above.

### Measurement of GIT in STZ-induced hyperglycemic mice

STZ (100 mg/kg, dissolved in 10 mL citrate buffer (pH 4.2), iv) was administered to the 20-h fasted mice. Four weeks later, blood samples were collected from the retro-orbital sinus under the unfasted condition. Serum glucose levels were determined by the glucose-oxidase method (kit reagent: Glucose CII-test Wako, Wako Pure Chemical Industries). Mice with a serum glucose level above 600 mg/dL, considered to be diabetic, were used in this study. The charcoal meal was administered

60 min after the oral administration of the samples. GIT was determined as described above.

### Measurement of GIT in capsacin-pretreated mice

Capsaicin solution was prepared in a solution containing 99.5% ethanol, Tween 80, and saline (2:1:7, v/v/v). Mice were anesthetized with sodium pentobarbital (30 mg/kg, ip), and treated with increasing doses of capsaicin for 2 consecutive days (25 and 50 mg/kg, sc) to deplete neuropeptides in primary afferent neurons as a modification of the method described previously.[29] To counteract any respiratory impairment associated with administration of capsaicin, the mice were pretreated with aminophylline (10 mg/kg, dissolved in 5 mL saline, im) 30 min before capsaicin injection. After 14 days, the efficiency of capsaicin pretreatment was verified by the corneal chemosensory test which consists of monitoring the wiping reflex to ocular instillation of a drop of 0.1% $NH_4OH$ solution. None of the capsaicin-pretreated mice showed a wiping response, indicating effective ablation of primary sensory afferents, whereas wiping reflex was present in vehicle-pretreated mice. The charcoal meal was administered 60 min after the oral administration of the samples. GIT was determined as described above.

### Measurement of GIT in atropine-pretreated mice

Atropine sulfate (10 mg/kg, dissolved in saline, sc) was injected into the back of the mice 30 min before the administration of the samples. The charcoal meal was administered 60 min after the administration of the samples. GIT was determined as described above.

### Statistics

Values were expressed as means ± SEM. One-way analysis of variance following Dunnett's test for multiple comparison analysis was used for statistical analysis. Probability ($p$) values less than 0.05 were considered significant.

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