Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 13, pp. 1611-1616, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00275-8

ANTIINFLAMMATORY EFFECTS OF ESCINS Ia, Ib, IIa, AND IIb FROM HORSE CHESTNUT, THE SEEDS OF *AESCULUS HIPPOCASTANUM* L.

Hisashi Matsuda[1], Yuhao Li[1], Toshiyuki Murakami[1], Kiyofumi Ninomiya[1], Nobuharu Araki[1], Masayuki Yoshikawa[1]* and Johji Yamahara[2]

[1]*Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan*

[2]*Research Institute for Production Development, 15 Morimoto-cho, Shimogamo, Sakyo-ku, Kyoto 606, Japan*

Abstract: We have investigated the effects of escins Ia, Ib, IIa, and IIb isolated from horse chestnut, the seeds of *Aesculus hippocastanum* L. and desacylescins I and II obtained by alkaline hydrolysis of escins on acute inflammation in experimental animal models, and some structure-requirements of escins for the activity was obtained. © 1997 Elsevier Science Ltd.

The saponin mixture "escin" obtained from horse chestnut, the seeds of *Aesculus hippocastanum* L. is widely used in the therapy of peripheral vascular disorders and also cosmetics for prevention and treatment of cellulitism. "Escin" have been reported to show antiinflammatory activity,[1] but no experimental study of antiinflammatory activity using each pure saponin was performed, because the isolation and structure determination of the saponin constituents were imperfect.

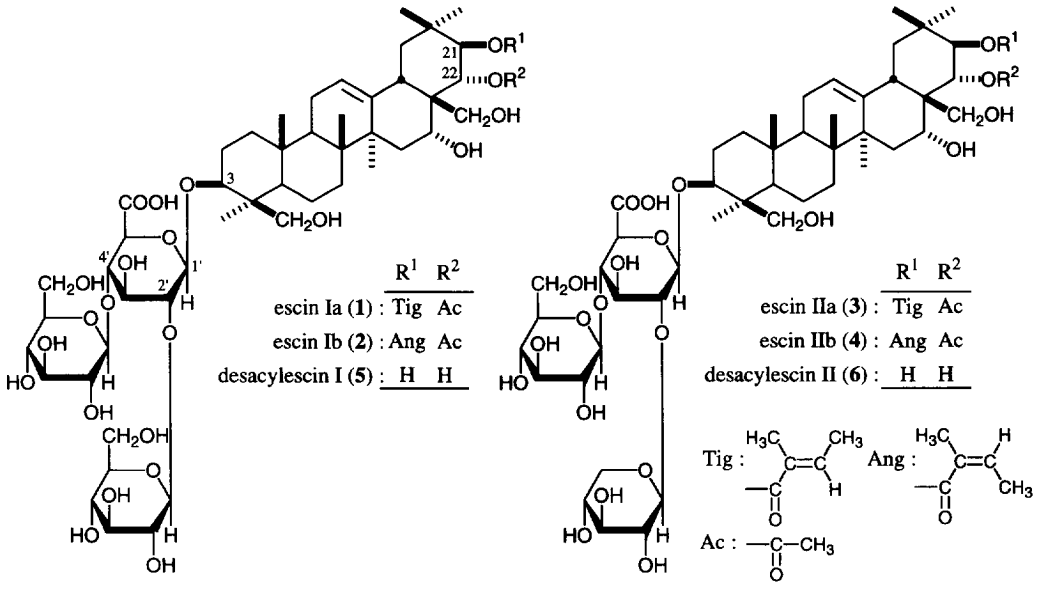

Chart 1

Recently, in the course of our studies on new biological active constituents of natural medicines, we have isolated pure eleven saponins named escins Ia (**1**), Ib (**2**), IIa (**3**), IIb (**4**), IIIa, IIIb, IV, V, and VI and isoescins Ia and Ib from horse chestnut, and determined the chemical structures on the chemical and physicochemical evidence.[2] In this study, we examined antiinflammatory activity of the principal escins (**1**, **2**, **3**, **4**) on various acute inflammatory models, and showed some structure-requirements for the activity.

Methods and Results

Escins Ia (**1**), Ib (**2**), IIa (**3**), and IIb (**4**) (50—200 mg/kg, *p.o.*) dose-dependently inhibited the increase of vascular permeability induced by intraperitoneal injection of acetic acid in mice (Fig. 1). **1**, **2**, **3**, and **4** (200 mg/kg, *p.o.*) also significantly inhibited the hind paw edema induced by carrageenin at first phase in rats (Fig. 2). Escin Ib (**2**) (200 mg/kg, *p.o.*) also showed significant suppressions at second phase. Escins IIa (**3**) and IIb (**4**) showed tendency to inhibit the second phase. **1**, **2**, **3**, and **4** (25—200 mg/kg, *p.o.*) dose-dependently inhibited the scratching behavior induced by subcutaneous injection of compound 48/80 in mice (Fig. 3). **1**, **2**, **3**, and **4** (50—200 mg/kg, *p.o.*) dose-dependently inhibited the increase of vascular permeability induced by intracutaneous injection of histamine in rats (Fig. 4). Escins Ib (**2**), IIa (**3**), and IIb (**4**) (50—200 mg/kg, *p.o.*) also dose-dependently inhibited the increase of vascular permeability induced by intracutaneous injection of serotonin in rats, but escin Ia (**1**) lacked the significant inhibition. Desacylescins I (**5**) and II (**6**) (200 mg/kg, *p.o.*) lacked these effects.

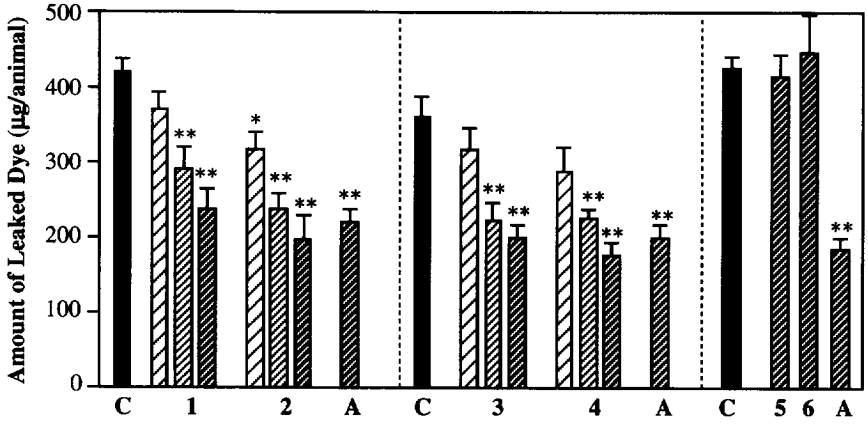

Fig. 1. Effect of Escins (1 - 4) and Desacylescins (5, 6) on Increase of Vascular Permeability Induced by Acetic Acid in Mice

Male ddY mice weighing 27-30g were used. Four percent (w/v) pontamine sky blue solution in saline was injected (10 ml/kg, *i.v.*) 55 min after the administration of a test compound. Five minutes later, 1% (w/v) acetic acid solution in saline was injected (10 ml/kg, *i.p.*), and 20 min later, the mice were sacrificed by cervical dislocation and the abdomen was immediately opened. After washing of the peritoneal cavity with 8 ml of saline, the washed solution was filtered through grass wool, and added 0.1 ml of 1N NaOH. The solution was filled up to 10 ml with saline, and the absorbance was measured at 590 nm. Vascular permeability was assessed by the amount of the dye leaked into the peritoneal cavity.

C : control , **A** : aspirin (200 mg/kg), ▨ : 50 mg/kg, ▨ : 100 mg/kg, ▨ : 200mg/kg
Each value represents the mean with S.E. (n=8 - 12), $^{**}p<0.01$.

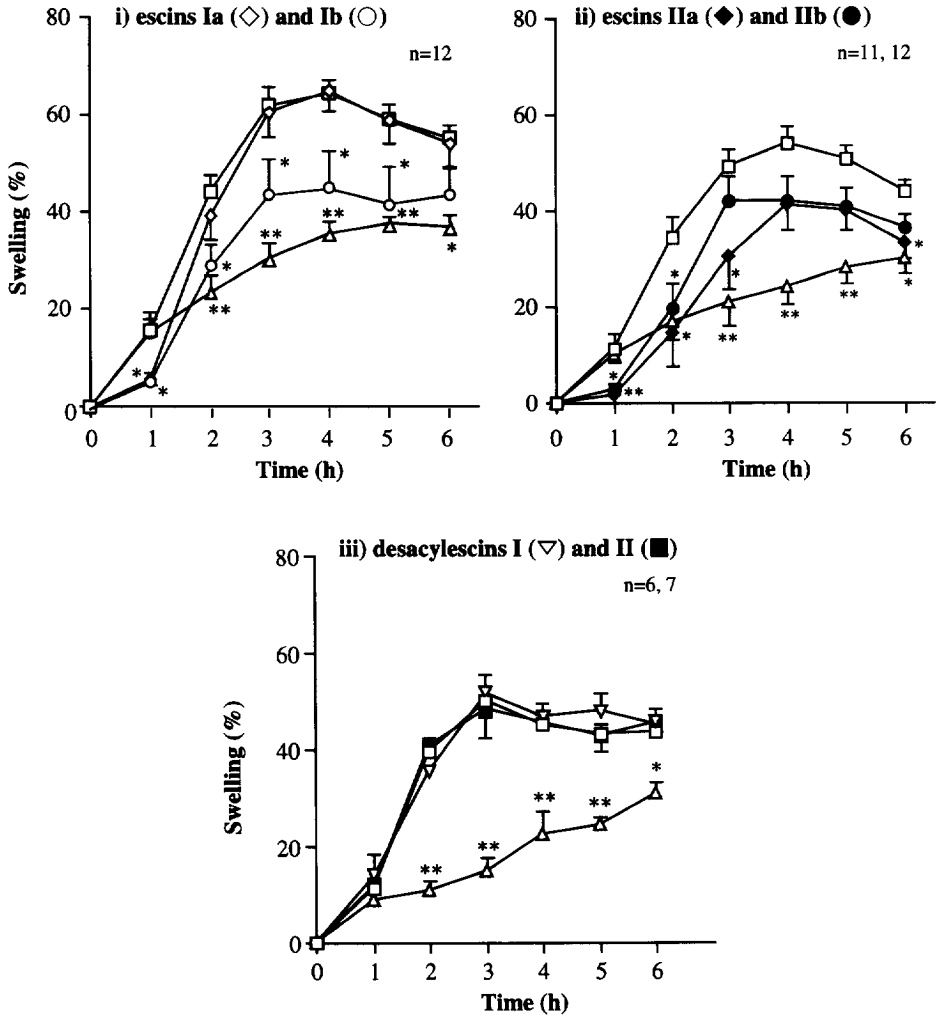

Fig. 2. Effect of Escins (1 - 4) and Desacylescins (5, 6) on Hind Paw Edema Induced by Carrageenin in Rats

Male Wistar rats weighing 140 - 160g were used. The 0.1 ml of 1% carrageenin was injected subcutaneously into the left hind paw 1h after the administration of test samples. The volume of the hind paw was measured by a plethysmometer (model KN-357, Natsume Seisakusyo Co., Ltd., Japan). The results were expressed as swelling (%), which means the percent increase in hind paw volume as compared with the initial volume.

□ control; ◇ escin Ia (**1**), 200 mg/kg; ○ escin Ib (**2**), 200 mg/kg; ◆ escin IIa (**3**), 200 mg/kg; ● escin IIb (**4**), 200 mg/kg; ▽ desacylescin I (**5**), 200 mg/kg; ■ desacylescin II (**6**), 200 mg/kg); △, indomethacin, 20 mg/kg. Each value represents the mean with S.E. (n=6 - 12), $*p<0.05$, $**p<0.01$.

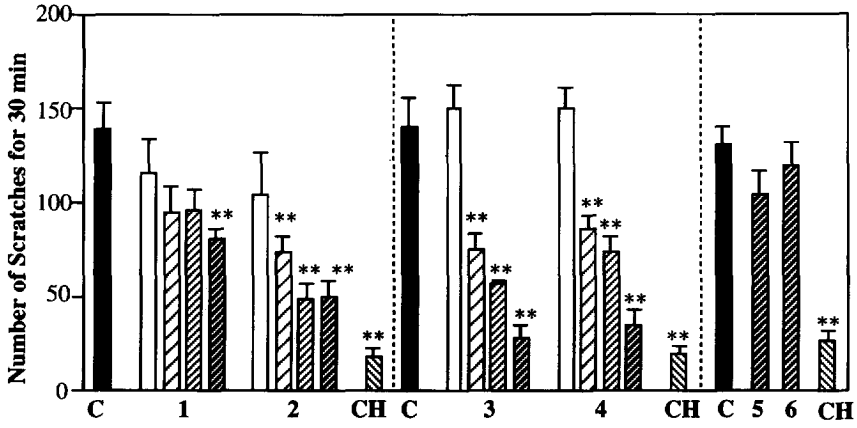

Fig. 3. Effect of Escins (1 - 4) and Desacylescins (5, 6) on Scratching Behavior Induced by Compound 48/80 in Mice

Male ddY mice weighing 26 - 29g were used. Scratching behavior was induced by subcutaneous injection of compound 48/80 at 100μg/site into the back of mice. Scratching on the injected site by the hind paws was counted, and that of other sites such as ears was disregarded. The mice generally showed several scratches for about 1s and a series of these behaviors was counted as one incident of scratching. Each test sample was given orally 1 h before the compound 48/80 injection.

C: control, **CH**: cyproheptadine HCl (5 mg/kg), □: 25 mg/kg, ▨: 50mg/kg, ▨: 100 mg/kg, ▨: 200 mg/kg. Each value represents the mean with S.E. (n=6 - 10), $^{**}p<0.01$.

Discussion

The development of edema induced by carrageenin and the increase of vascular permeability induced by acetic acid are known to correspond to the early exudative stage of inflammation, one of the important process of inflammatory pathology.[3] Histamine and serotonin are presumed to play an important role in the first stage of carrageenin-induced edema and acetic acid-induced increase of vascular permeability. Compound 48/80 is well known to be a releaser of chemical mediators such as histamine and serotonin from the mast cells. Compound 48/80-induced scratching behavior is considered to be due to an itch by the released chemical mediators, and it has been reported that only histamine didn't show the behavior and the role of serotonin was seemed to be important for the behavior.[4]

We investigated the anti-inflammatory activities of the pure escins in acute inflammatory models. Escins dose-dependently exhibited inhibitory effects on the increase of vascular permeability by acetic acid in mice, and on the earlier phase of swelling by carrageenin in rats. Escins also inhibited histamine- or serotonin-induced increase of vascular permeability, excepting escin Ia on serotonin-induced one. Escin Ib (**2**) showed significant inhibition on the second phase of swelling by carrageenin, and escins IIa (**3**) and IIb (**4**) also showed tendency to inhibit it. These results indicate that escins, given orally, effect on the early exudative stage of inflammation and itch owing to these chemical mediators and suggest that these inhibitory effects are

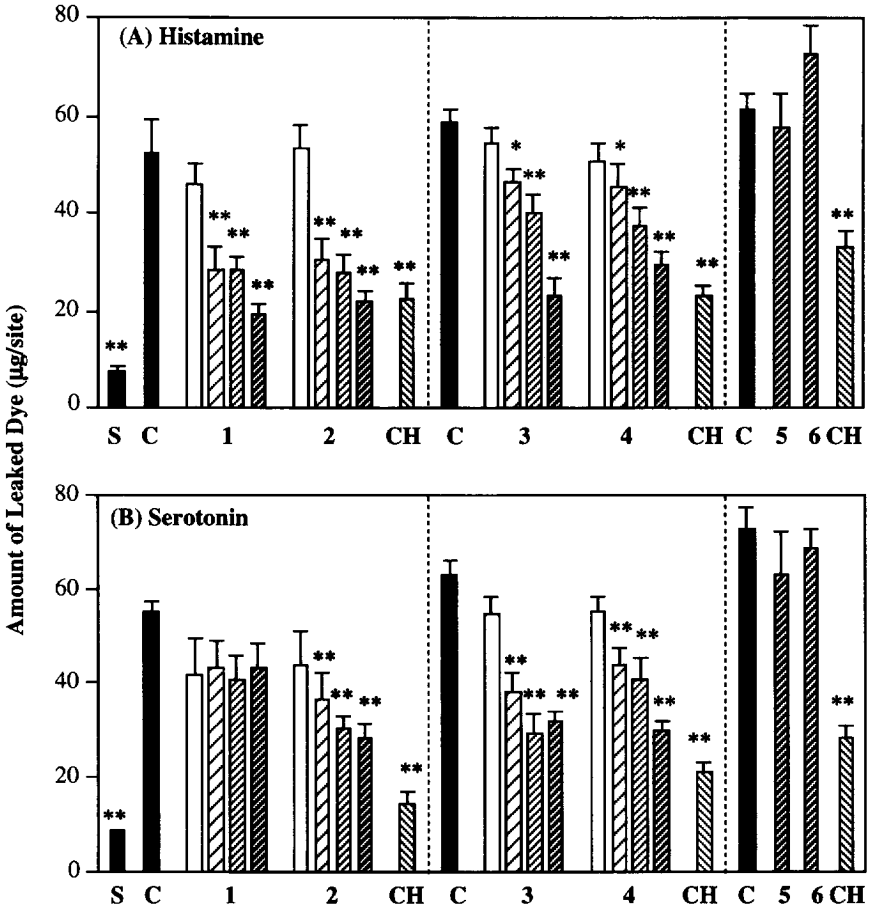

Fig. 4. Effect of Escins (1 - 4) and Desacylescins (5, 6) on Increase of Vascular Permeability Induced by Histamine (A) or Serotonin (B) in Rats

Wistar male rats weighing 140 - 150g were used. Two percent (w/v) Evans blue solution in saline was injected (10 ml/kg, *i.v.*) 55 min after the oral administration of a test compound. Five minutes later, histamine hydrochloride (100μg/site) or serotonin-creatinine sulfate monohydrate (2.5μg/site) were injected intracutaneously into the back of the rats. Thirty minutes later, the animals were sacrificed by cervical dislocation, the blue colored skin in the back was cut down and soaked in 10 ml of acetone/water (7 : 3) solution for 24 h. The absorbance of the filtered solution was measured at 620 nm. The capillary permeability was assessed by the amount of Evans blue leaked in the skin.

S : saline, 50 μl/site, **C**: control, **CH**: cyproheptadine HCl (5 mg/kg),

□: 25 mg/kg, ▨: 50mg/kg, ▨: 100 mg/kg, ▨: 200 mg/kg.

Each value represents the mean with S.E. (n=6 - 10), $*p<0.05$, $**p<0.01$.

mainly depend on their anti-histaminic and anti-serotonic activities, though the effects of escins against kinin formation and arachidonic acid cascade are not clarified.

Comparing with escins Ia (**1**) and Ib (**2**) having the 2'-*O*-glucopyranoside, **2** having the 21-angeloyl group showed more potent activities than **1** having the 21-tigloyl group on carrageenin-induced edema at second phase, compound 48/80-induced scratching behavior and serotonin-induced increase of vascular permeability. Escin IIa (**3**) having the 2'-*O*-xylopyranosyl moiety and the 21-tigloyl group also showed more potent activities than **1** having the 2'-*O*-glucopyranosyl moiety and the 21-tigloy groups. But, escins Ib (**2**), IIa (**3**), and IIb (**4**) having either of the 2'-*O*-xylopyranosyl moiety or the 21-angeloyl group exhibited similar effects on these experiments. Additionally, desacylescins I (**5**) and II (**6**) lacked the activities in these experiments. These results led us to presume the following structure-requirements of escins for the activity : 1) the 21, 22-acyl groups were essential to the activity; 2) the 21-angeloyl group instead of the 21-tigloyl group intensified the activity; 3) the 2'-*O*-xylopyranosyl moiety instead of the 2'-*O*-glucopyranosyl moiety also intensified the activity.

In this study, it has become apparent that escins have antiinflammatory activities in the acute stage, but there are differences among the activities of escins, especially anti-serotonic activity. Furthermore the differences of chemical structure in acyl groups and in oligosaccharide moiety are apparently be important to exhibit the antiinflammatory activity.

References

1. a) Vogel, G.; Marek, M. L.; Stoeckert, I., *Arzneim. Forsch.*, **1963**, 13, 59 ; b) Vogel, G.; Marek, M. L.; Oertner, R.; *ibid.*, **1970**, 20, 699; c) Rothkopf, M., Vogel, G., *ibid.*.; **1976**, 26, 225.
2. a) Yoshikawa, M.; Harada, E.; Murakami, T.; Matsuda, H.; Wariishi, N.; Yamahara, J.; Murakami, N.; Kitagawa, I.; *Chem. Pharm. Bull.*, **1994**, 42, 1357; b) Yoshikawa, M.; Murakami, T.; Matsuda, H.; Yamahara, J.; Murakami, N.; Kitagawa, I.; *ibid.*, 44, **1996**, 1454; c) Yoshikawa, M.; Murakami, T.; Murakami, N.; Matsuda, H.; Araki, N.; Ohtsuji, K.; Yamahara, J.; presented at the 43rd Annual Meeting of the Japanese Society of Pharmacognosy, Tokyo, Sep. 1996, Abstract Papers p. 62.
3. a) Winter, A. C.; Risley, A. E.; Nuss, W. G.; *Proc. Soc. Exp. Biol. Med.*, **1962**, 111, 544; b) Whittle, B. A., *Br. J. Pharmacol.*, **1964**, 22, 246.
4. a) Kuraishi, Y.; Nagasawa, T.; Hayashi, K.; Satoh, M., *Eur. J. Pharmacol.*, **1995**, 275, 229; b) Kubo, M.; Matsuda, H.; Dai, Y.; Ido, Y.; Yoshikawa, M.; *Yakugaku Zasshi*, **1997**, 117. in press.

(*Received in Japan* 21 *April* 1997; *accepted* 19 *May* 1997)