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# Antinociceptive Antiinflammatory Effect of a Diterpene Isolated from the Herb of *Siegesbeckia pubescens*

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The root of *Siegesbeckia pubescens* (Compositae) has been used to treat rheumatoid arthritis and hypertension in the Oriental medicine. This crude drug has been used without process (SP-0) or with three times-process of steaming and drying (SP-0) or the nine times of that process (SP-9). To search for the antinociceptive anti-inflammatory components from this crude drug, activity-directed fractionation was performed on this crude drug. Since the $CHCl_3$ extract was shown to have a more potent effect than other extracts, it was subjected to silica gel & ODS column chromatography to yield two diterpene compounds (**1**). Compound **1** was structurally identified as ent-16H,17-hydroxykauran-19-oic acid, which were tentatively named siegeskaurolic acid A (Fig.1). A main diterpene, siegeskaurolic acid A was tested for the antiiflammatory antinociceptive effects using both hot plate- and writhing antinociceptive assays and carrageenan-induced anti-inflammatory assays in mice and rats. Pretreatment with siegeskaurolic acid A (20 and 30 mg/kg) significantly reduced the stretching episodes, action time of mice (Fig. 2) and carrageenan-induced edema (Fig. 3). These results support that siegeskaurolic acid is a main diterpene responsible for antinociceptive and antiiflammatory action of *S. pubescens*. In addition, the assays on SP-0, SP-3 and SP-9 produced the experimental results that SP-9 had more significant effects than other two crude drugs (Table 1 and 2). These results suggest that the processing on the original plant may lead to the higher pharmacological effect

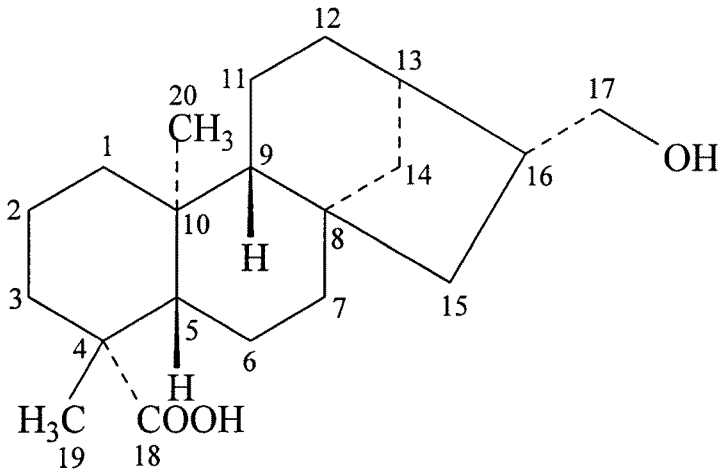
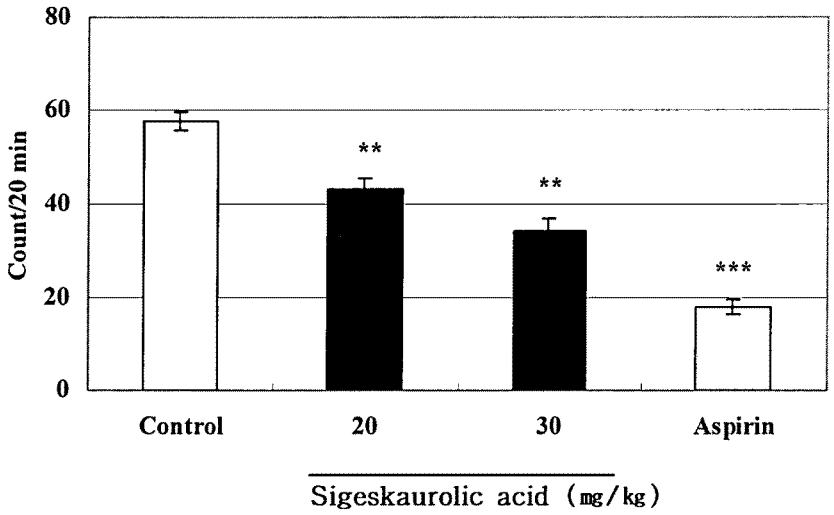



Fig. 1. Structure of compound **1** isolated from *S. pubescens*



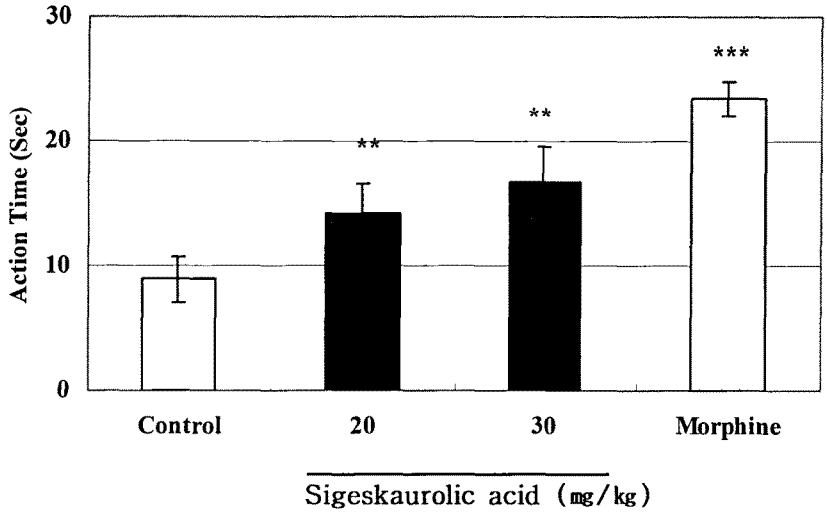



Fig. 2. Antinociceptive effect of siegeskaurolic acid (comp. **1**) isolated from the roots of *S. pubescens* by acetic acid-induced writhing and hot plate method in mice.

Methods indicate writhing- (upper), and hot plate tests (down), respectively. Values represent meansS.D. (n=10). *, **Astericks indicate the means which are significantly different ($p<0.05$ and $p<0.01$), respectively, from the control.

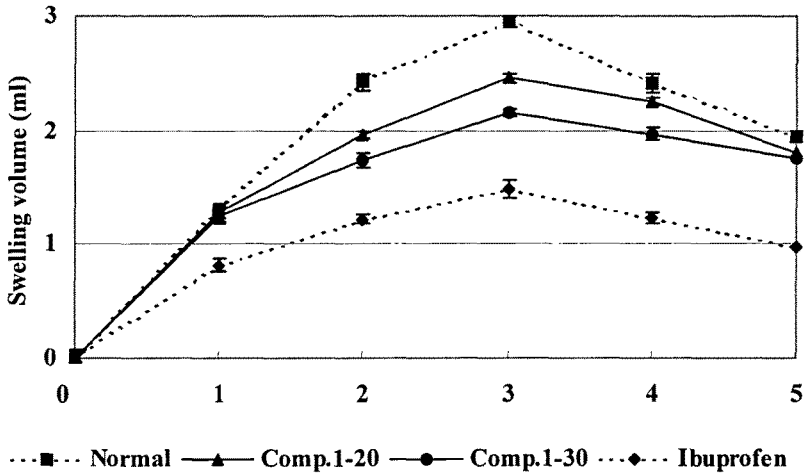



Fig. 3. Effects of siegeskaurolic acid (comp. **1**) isolated from the roots of *S. pubescens* on carrageenan-induced paw edema in rats. Values represent meansS.D. (n=10).

Table 1. Antinociceptive effect of the MeOH extracts (0-SP, 3-SP, and 9-SP) of *Siegesbeckia pubescens* herbs by acetic acid-induced writhing syndrome and hot-plate test in mice.

| Group | Dose (mg/kg, p.o.) | Stretching episodes[1] (count/20 min) | Inhibition (%) | Action time[2] (sec) | Increase (%) |
|---|---|---|---|---|---|
| Untreated | 0 | 60.6 2.07$^{a}$ | 0 | 8.9 1.33$^{e}$ | 0 |
| SP-0 | 100 | 57.4 2.41$^{b}$ | 5.3 | 11.6 2.17$^{d}$ | 30.3 |
| | 200 | 54.2 2.28$^{c}$ | 10.6 | 12.8 1.20$^{c,d}$ | 43.8 |
| SP-3 | 100 | 57.8 1.93$^{a,b}$ | 4.62 | 12.0 1.16$^{c,d}$ | 34.8 |
| | 200 | 51.0 1.58$^{d}$ | 15.8 | 13.1 1.24$^{c,d}$ | 47.2 |
| SP-9 | 100 | 48.0 1.59$^{e}$ | 20.8 | 13.0 1.09$^{c,d}$ | 46.1 |
| | 200 | 41.0 3.16$^{f}$ | 32.3 | 13.8 1.48$^{c,d}$ | 55.1 |
| Aspirin | 100 | 18.0 1.58$^{h}$ | 70.3 | NT | - |
| Morphine | 10 | NT | - | 24.5 2.33$^{a}$ | 175 |

[1], [2]Methods indicate writhing-, and hot plate tests, respectively. NT (not tested). Values represent meansS.D. (n=10). Values sharing the same superscript letter are not significantly different each other ($p<0.05$) by Duncan's multiple range test.

Table 2 Inhibitory effect of the MeOH extracts (SP-0, SP-3, and SP-9) of *Siegesbeckia pubescens*herbs on carrageenan-induced edema of the hind paw in rats

| Group | mg/kg (p.o) | 1 h | 2 h | 3 h | 4 h | 5 h |
|---|---|---|---|---|---|---|
| | | ml | | | | |
| Untreated | 0 | 1.290.08$^{t}$ | 2.420.06$^{de}$ | 2.990.07$^{a}$ | 2.410.05$^{de}$ | 1.950.08$^{lm}$ |
| SP-0 | 100 | 1.280.07$^{t}$ | 2.210.05$^{ijk}$ | 2.760.08$^{b}$ | 2.380.03$^{def}$ | 1.900.05$^{lmno}$ |
| | 200 | 1.300.09$^{t}$ | 2.170 03$^{k}$ | 2.650.04$^{c}$ | 2.310 07$^{fgh}$ | 1.870.07$^{mnop}$ |
| SP-3 | 100 | 1.250.03$^{t}$ | 2.300.07$^{fghi}$ | 2.700.05$^{bc}$ | 2 360.06$^{efg}$ | 1.920.06l$^{mn}$ |
| | 200 | 1.270.07$^{t}$ | 2.240.04$^{hijk}$ | 2.610.03$^{c}$ | 2.270.05$^{ghij}$ | 1.850.07$^{mnop}$ |
| SP-9 | 100 | 1.280.04$^{t}$ | 2.200.06$^{ijk}$ | 2.650.07$^{c}$ | 2.310.08$^{fgh}$ | 1.830.04$^{nopqr}$ |
| | 200 | 1.260.02$^{t}$ | 1.980.04$^{l}$ | 2.470.05$^{d}$ | 2.250.04$^{hijk}$ | 1.800.07$^{pqr}$ |
| Ibuprofen | 100 | 0.820.03$^{v}$ | 1.220 05$^{t}$ | 1.490.04$^{s}$ | 1.230 08$^{t}$ | 0.970 05$^{u}$ |

Values represent means S.D. (n=10). Values sharing the same superscript letter are not significantly different each other ($p<0.05$) by Duncan's multiple range test.