Acteoside as the Analgesic Principle of Cedron (*Lippia triphylla*), a Peruvian Medicinal Plant

Tomonori NAKAMURA,[a] Emi OKUYAMA,*[,a] Atsushi TSUKADA,[a] Mikio YAMAZAKI,[a] Motoyoshi SATAKE,[b] Sansei NISHIBE,[c] Takeshi DEYAMA,[d] Akira MORIYA,[d] Masao MARUNO,[e] and Hiroaki NISHIMURA[e]

Faculty of Pharmaceutical Sciences, Chiba University,[a] 1–33 Yayoicho, Inage-ku, Chiba 263, Japan, National Institute of Health Sciences,[b] 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158, Japan, Faculty of Pharmaceutical Sciences, Health Science University of Hokkaido,[c] Ishikari-Tobestu, Hokkaido 061–02, Japan, Central Research Laboratories, Yomeishu Seizo Co., Ltd.,[d] 2132–37, Nakaminowa, Minowamachi, Kamiina-gun, Nagano 399–46, Japan, and Research Institute for Biology and Chemistry, Tsumura Co., Ltd.,[e] 3586 Yoshiwara, Amimachi, Inashiki-gun, Ibaraki 300–11, Japan.
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Acteoside (verbascoside) was isolated as an analgesic principle from Cedron (leaves and stem of *Lippia triphylla* (L'HER) O. KUNTZE; Verbenaceae), a Peruvian medicinal plant, by activity-guided separation. The compound exhibited analgesia on acetic acid-induced writhing and on tail pressure pain in mice by the oral administration of 300 mg/kg and 100 mg/kg, respectively. Acteoside also caused weak sedation by its effect on the prolongation of pentobarbital-induced anesthesia and on the depression of locomotion enhanced by methamphetamine. An intravenous injection of acteoside reduced the effective dose to 2 mg/kg by the writhing method. Thirteen related compounds were tested for the activity by intravenous and oral administration to obtain information on the active structure.

Key words acteoside; *Lippia triphylla*; analgesia; phenylethanoid glycoside; sedation

During our survey of Peruvian medicinal plants for neurotropic effects, the extract of Cedron showed an analgesic effect in mice.[1] Cedron (*Lippia triphylla* (L'HER) O. KUNTZE; Verbenaceae) has long been used as a calmative and a carminative and for the treatment of stomachache *etc.*, in Peru.[2] The plant is a shrub widely distributed throughout South America, and the leaves and stem have a lemon-like fragrance.[2c] In this paper, we report the isolation and identification of the analgesic principle from this plant, as well as some pharmacological properties of the component. The active structure was also discussed by its comparison with the analgesic activities of the thirteen related compounds.

Oral administration (*p.o.*) of the methanol extract of Cedron showed an inhibitory effect of 52% ($p<0.05$) on acetic acid-induced writhing in mice at a dose of 3 g/kg. The methanol extract was separated by following the writhing inhibition as an analgesia-guide (Chart 1). The extract was partitioned with *n*-hexane, ethyl acetate and water. The ethyl acetate part exhibited inhibitory activity of 66% ($p<0.05$) at a dose of 2 g/kg, *p.o.* Separation of the ethyl acetate part by chromatography on Sephadex LH-20 gave the active fractions 1-C and 1-D, which inhibited the writhing at a dose of 1 g/kg, *p.o.* Both fractions showed a major component (**1**), indicating a $FeCl_3$-positive spot (dark blue) on TLC, and it was isolated by ODS and silica gel chromatographies from these fractions.

The isolation procedure was then modified to obtain a sufficient amount of the compound for further experimentation. The methanol extract (50.4 g) was partitioned with *n*-hexane and water, and the water part was applied to Diaion HP-20. The eluate of water–methanol (1 : 1) was separated by repeated silica gel chromatography to yield **1** (2.88 g, 5.7% from the methanol extract).

The compound (**1**) was a pale-yellow powder and demonstrated a molecular weight of 624 by fast atom bombardment mass spectrometry (FAB-MS). The structure was elucidated by ^1H- and ^{13}C-NMR spectra in DMSO-d_6, including 2D-NMRs such as pulse field gradient (FG) correlation spectroscopy (COSY), FG-

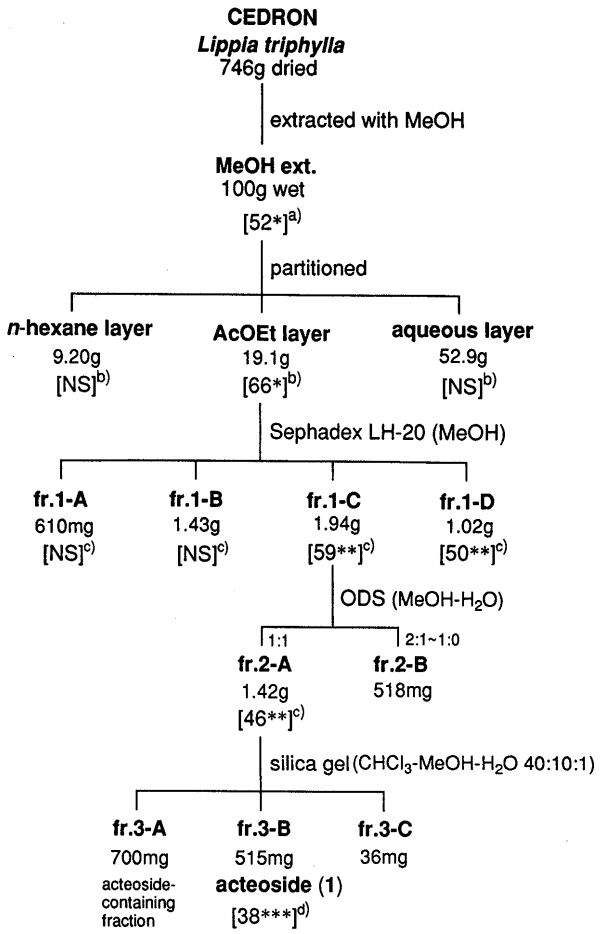

Chart 1. Isolation Procedure of Acteoside (**1**)

[ ]: inhibitory % of acetic acid-induced writhing. NS: no significance. a) 3 g/kg, *p.o.*, b) 2 g/kg, *p.o.*, c) 1 g/kg, *p.o.*, d) 500 mg/kg, *p.o.* $*p<0.05$, $**p<0.01$, $***p<0.001$.

* To whom correspondence should be addressed.

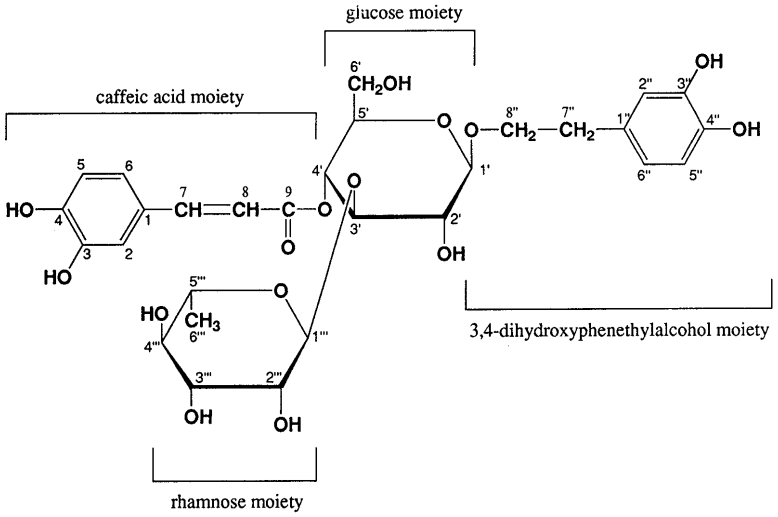

Fig. 1. Structure of Acteoside (**1**)

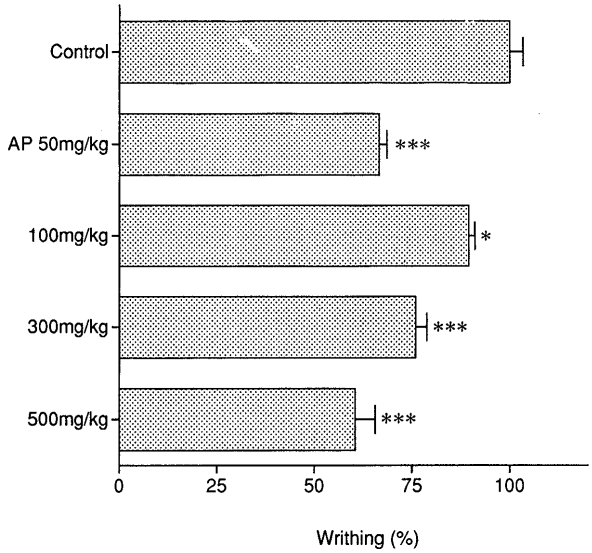

Fig. 2. Analgesic Effect of Acteoside (**1**) on Acetic Acid-Induced Writhing in Mice

Each bar represents the mean ± S.E. The number of squirms in control (26.6 ± 0.898) was taken as 100%. AP: aminopyrine. $*p<0.05$, $***p<0.001$. $n=6$.

heteronuclear multiple quantum coherence spectrum (HMQC) and FG-heteronuclear multiple-bond correlation spectrum (HMBC), and **1** was identified as acteoside (verbascoside) by direct comparison with the authentic sample (Fig. 1). Acteoside has been isolated from many other plants such as *Cistanche*, *Forsythia*, *Rehmannia* and *Orobanche* sp.[3]

The analgesic activity of **1** was examined in mice by the writhing and the tail pressure methods. Oral administration of 100, 300 and 500 mg/kg of **1** indicated analgesia dose-dependently in the writhing method, as shown in Fig. 2. The threshold in the tail pressure pain method was increased in a dose-dependent manner at doses of 100 and 300 mg/kg, *p.o.*[4] as shown in Fig. 3.

The sedative effect of **1** was also indicated in mice by the prolongation of pentobarbital-induced anesthesia and the inhibition of methamphetamine-enhanced locomotor activity. Compound **1** exhibited the prolongation of the anesthesia by oral administration of 100, 300 and 500 mg/kg (Fig. 4). The inhibition of methamphetamine-enhanced locomotion was also observed at doses of 300 and 500 mg/kg in Fig. 5. Compound **1** did not show any effects on body temperature or general behavior in mice at a dose of 1 g/kg, *p.o.* From these data, **1** seemed to have weak sedation.

The analgesic effect of **1** was tested by alternative administration such as subcutaneous (s.c.) and intravenous (i.v.) injections. The subcutaneous injection of 300 mg/kg indicated 40% ($p<0.05$) inhibition of the writhing that was almost an equivalent potency to that in the oral administration. On the other hand, intravenous injection, as shown in Fig. 6, extremely increased the activity: 23% ($p<0.001$), 41% ($p<0.001$) and 63% ($p<0.001$) at doses of 2, 10 and 50 mg/kg, respectively. The analgesic potency of **1** by i.v. was almost equal to aminopyrine (44%, 5 mg/kg) at about same dose of injection, although it was much weaker than aminopyrine (29%, 50 mg/kg) by oral administration. Caffeic acid (**2**) and 3,4-dihydroxyphenethyl alcohol (**3**), the aglycone parts of **1**, did not significantly exhibit analgesia at a dose of 500 mg/kg, *p.o.* The activity was neither observed in the mixture of **2** and **3** at doses of 144 and 124 mg/kg, *p.o.*, respectively, which were equivalent moles per kg to 500 mg/kg of **1**.

The thirteen related compounds in Fig. 7 were tested by the writhing method in order to investigate the active structure of **1** for its analgesia. The results by intravenous injection (2, 10 and 50 mg/kg) are summarized in Fig. 8, and oral administration (100, 300 and 500 mg/kg) in Fig. 9, together with the 30%-inhibition dose (ID_{30}), which were all calculated from the regression lines or curves of these compounds. Among those related compounds, **5**, **15** and **16** were not included in Fig. 8 because of their insolubility in water for intravenous injection. In Fig. 8, all of the compounds were less potent than **1** (ID_{30}: 2.1 mg/kg). The potencies of leucosceptoside A (**4**) and jionoside D (**6**) (ID_{30}: 9.3 and 13 mg/kg, respectively) in group I, in which some of the hydroxyl groups were methylated, did not exhibit extensively decreased potency. Modification of the sugar moiety varied the potency in group IIa and IIb. Activity appeared to be maintained, even if the rhamnose moiety in **1** was replaced by glucose

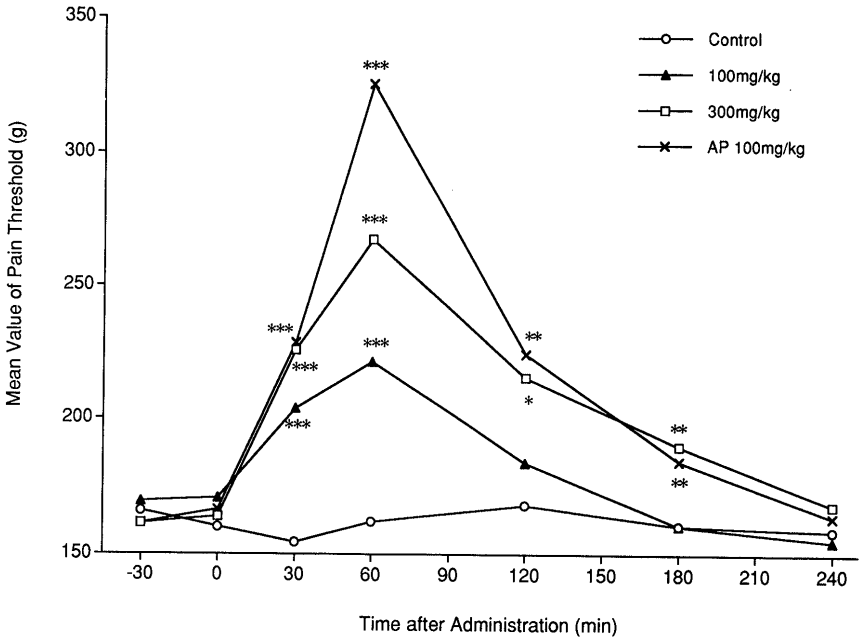

Fig. 3. Analgesic Effect of Acteoside (**1**) on Tail Pressure Pain Threshold in Mice

AP: aminopyrine. $*p<0.05$, $**p<0.01$, $***p<0.001$. $n=8$.

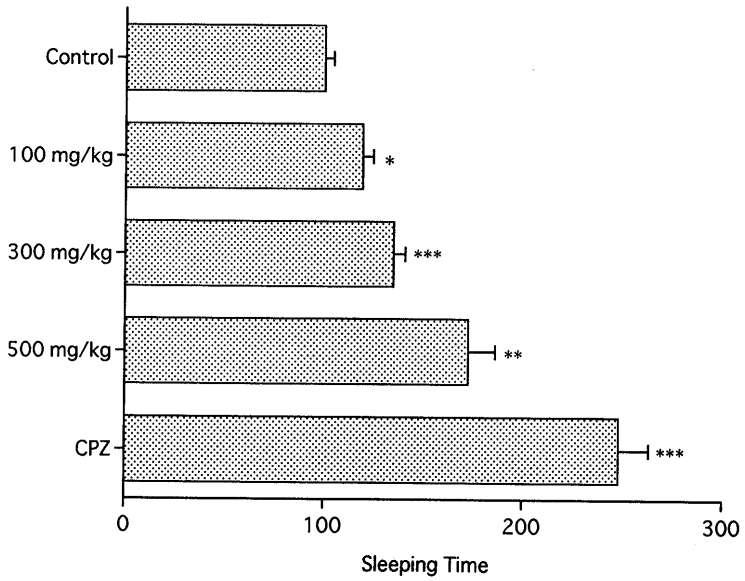

Fig. 4. Effect of Acteoside (**1**) on Pentobarbital-Induced Anesthesia in Mice

Each bar represents the mean ± S.E. The sleeping time of the control (45.0 ± 1.96 min) was taken as 100%. CPZ: chlorpromazine. $*p<0.05$, $**p<0.01$, $***p<0.001$. $n=8$.

in plantamajoside (**7**) (ID_{30}: 3.9 mg/kg). Deletion or migration of the rhamnose, however, decreased the activity, as shown in desrhamnosyl acteoside (**8**) and forsythiaside (**9**) (ID_{30}: 16 and 25 mg/kg, respectively). Echinacoside (**11**) and purpureaside C (**12**) in group IIb, which have an additional glucose or galactose substituent, showed a notable reduction in their potencies (ID_{30}: >50 and 42 mg/kg, respectively). In group III, compounds which lack some of the hydroxyl groups in the aglycone part lost their activity. Lack of caffeoyl and phenethyl moieties, such as in cistanoside F (**13**) and decaffeoyl acteoside (**14**), respectively, in group IV, completely decreased the potency (ID_{30}: >50 mg/kg).

Acteoside (**1**) was most potent, and a similar tendency of activity depending on the modification of the structures was also observed in its oral administration. In group V, isoacteoside (**15**), which has migration of the caffeoyl moiety, and salidroside (**16**), which loses caffeoyl and rhamnose moieties and one aromatic hydroxyl group, were not potent.

Considering the activities of acteoside (**1**), it seemed to have a supportive effect on the central nervous system (CNS), although **1** does not influence the general behavior or body temperature of mice at a dose of 1 g/kg, *p.o.* (the data were not shown). Sato and co-workers have reported the CNS effect of acteoside; the effect on sexual and learning behavior in hanging stress loaded mice.[5] Interesting effects such as the inhibition of arachidonate

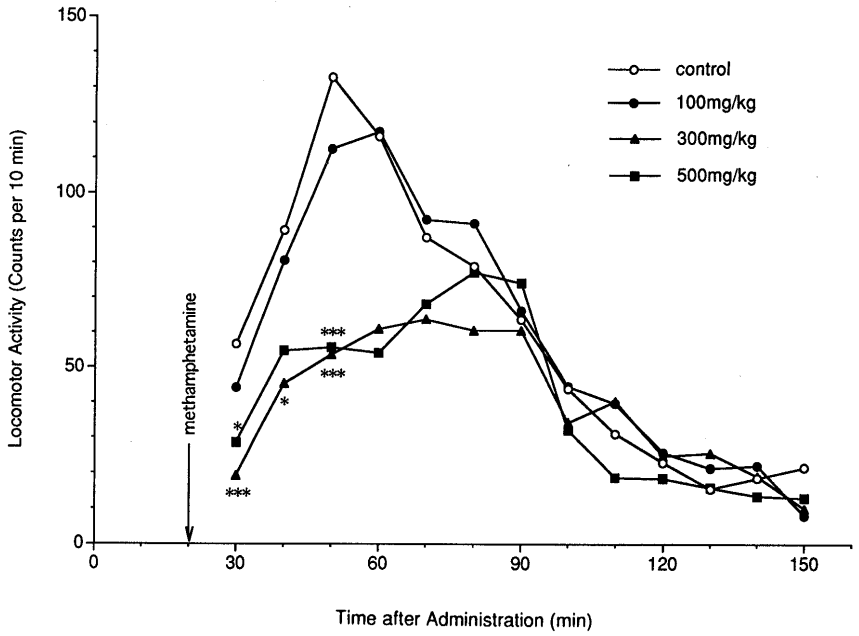

Fig. 5. Effect of Acteoside (**1**) on Methamphetamine-Enhanced Locomotor Activity in Mice

Methamphetamine (2 mg/kg, s.c.) was injected 20 min after administration of samples. $*p<0.05$, $**p<0.01$, $***p<0.001$. $n=10$.

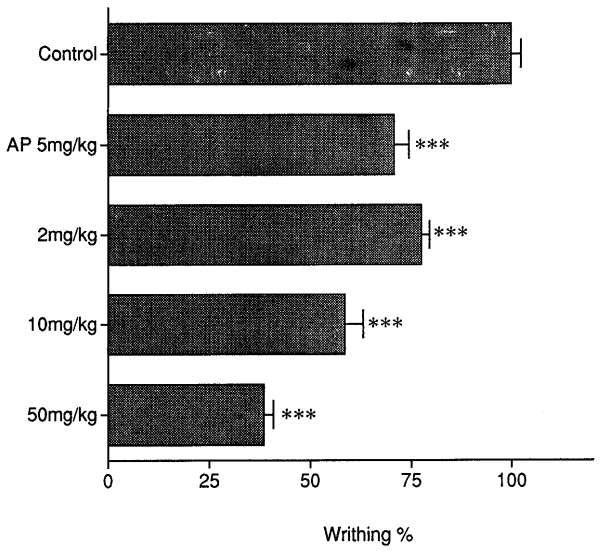

Fig. 6. Analgesic Effect of Acteoside (**1**) on Acetic Acid-Induced Writhing in Mice by Intravenous Injection

Each bar represents the mean ± S.E. The number of squirms in control (27.8 ± 0.655) was taken as 100%. AP: aminopyrine. $***p<0.001$. $n=6$.

metabolism,[6a] antinephritic effect,[6b] the inhibition effects of lens-aldose reductase[6c] and hyarulonidase,[6d] were also reported in relation to acteoside. Considering its distribution in a wide range of medicinal plants, acteoside may participate in the exhibition of their pharmacological activities.

Experimental

The melting point was determined on a Yanagimoto melting point apparatus and was uncorrected. Specific rotation was measured on a JASCO DIP-140 digital polarimeter. Infrared (IR) spectra were recorded on a Hitachi grating infrared spectrophotometer, model EPI-G3. Ultra-violet (UV) spectra were taken on a Hitachi spectrophotometer, model U-3200. FAB-MS was measured on a JEOL JMS-HX 110A spectrometer. NMR spectra were measured on JEOL GSX-400, GSX-500 and GSX-A500 spectrometers, and FG-HMQC and FG-HMBC on GSX-A500 with an FG control unit spectrometer. Thin layer chromatography was performed on Kieselgel 60 F_{254} and HPTLC RP-18 WF_{254} (Merck). Column chromatography was carried out on Diaion HP-20 (Mitsubishi Kasei Corp.), Sephadex LH-20 (Pharmacia Fine Chemicals Co., Ltd.), Silica gel C-200 (Wako Pure Chem. Ind., Ltd.), Silica gel 60 (230—400 mesh) (Nacalai Tesque, Inc.), and Chromatorex ODS (Fuji Silysia Chemical, Ltd.). Caffeic acid and 3,4-dihydroxyphenylacetic acid were purchased from Nacalai Tesque, Inc.

Material Cedron (Leaves and stem of *Lippia triphylla* (L'Her) O. Kuntze) from Peru was obtained in April, 1989 and in January, 1991. The materials were comfirmed by Mr. M. Satake and Mr. T. Shiota.

Isolation The methanol extract of Cedron was chromatographed with the guidance of the inhibitory effect on acetic acid-induced writhing in mice by oral administration. Cedron (746 g, obtained in 1989) was extracted with methanol at room temperature, and the extract (100 g) was partitioned with *n*-hexane, ethyl acetate and water. As the ethyl acetate part (19.1 g) only indicated activity, it was chromatographed on Sephadex LH-20 with methanol to provide fr. 1-A (610 mg), 1-B (1.43 g), 1-C (1.94 g) and 1-D (1.02 g). Fraction 1-C, one of the latter two fractions showing the activity, was separated by ODS column chromatography using water–methanol (1 : 1—0 : 1) into fr. 2-A (1.42 g) and fr. 2-B (518 mg). In the active fr. 2-A, a major spot was observed on TLC, which was isolated by silica gel chromatography using chloroform–methanol–water (40 : 10 : 1) as an eluent. The component (**1**, 515 mg) was obtained. The compound (**1**) was also observed in the other active fraction, fr. 1-D, which showed **1** on TLC.

The isolation of **1** proceeded from the other extract of Cedron (655 g, obtained in 1991). After partition of the methanol extract (50.4 g) with *n*-hexane and water, the aqueous part (37.6 g) was chromatographed on DIAION HP-20. The water–methanol (1 : 1) eluate, fr. 4-C, was separated by repeated column chromatographies on silica gel using chloroform–methanol–water (40 : 10 : 1) as an eluent. Compound **1** (2.88 g) was obtained (5.7% yield from the methanol extract).

Acteoside (**1**): Pale-yellow amorphous powder, mp 142—147 °C, $[\alpha]_D^{24}$ −83.0° ($c=1.0$, MeOH). FAB-MS *m/z*: 624 $[M]^+$, 647 $[M+Na]^+$. IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1690, 1630, 1610, 1520. UV λ_{max}^{MeOH} nm (log ε): 220 (4.18), 233 sh (4.02), 248sh (3.92), 292 (4.04), 333 (4.19). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 0.97 (3H, d, 6.1 Hz, H-6‴), 2.66—2.76 (2H, m, H-7‴), 3.12 (1H, td-like, 9.4, 3.4 Hz, H-4‴), 3.23 (1H, td-like, 8.4, 5.4 Hz, H-2′), 3.31—3.36 (2H, m, H-3‴, H-6′), 3.36—3.40 (1H, m, H-5‴), 3.39—3.43 (1H, m, H-6′), 3.48 (1H, ddd, 9.8, 6.1, 2.2 Hz, H-5′), 3.62 (1H, td, 9.1, 6.7 Hz, H-8″), *ca.* 3.70 (1H, overlapped with the other signal, H-2‴), 3.71 (1H, t-like, 9.3 Hz, H-3′), 3.89 (1H, td, 9.1, 6.8 Hz, H-8″), 4.35 (1H, d, 7.8 Hz, H-1′), *ca.* 4.35 (1H, overlapped with the other signal, OH-3‴), 4.44 (1H, br d, 3.7 Hz, OH-4‴), 4.48 (1H, d, 3.4 Hz, OH-2‴),

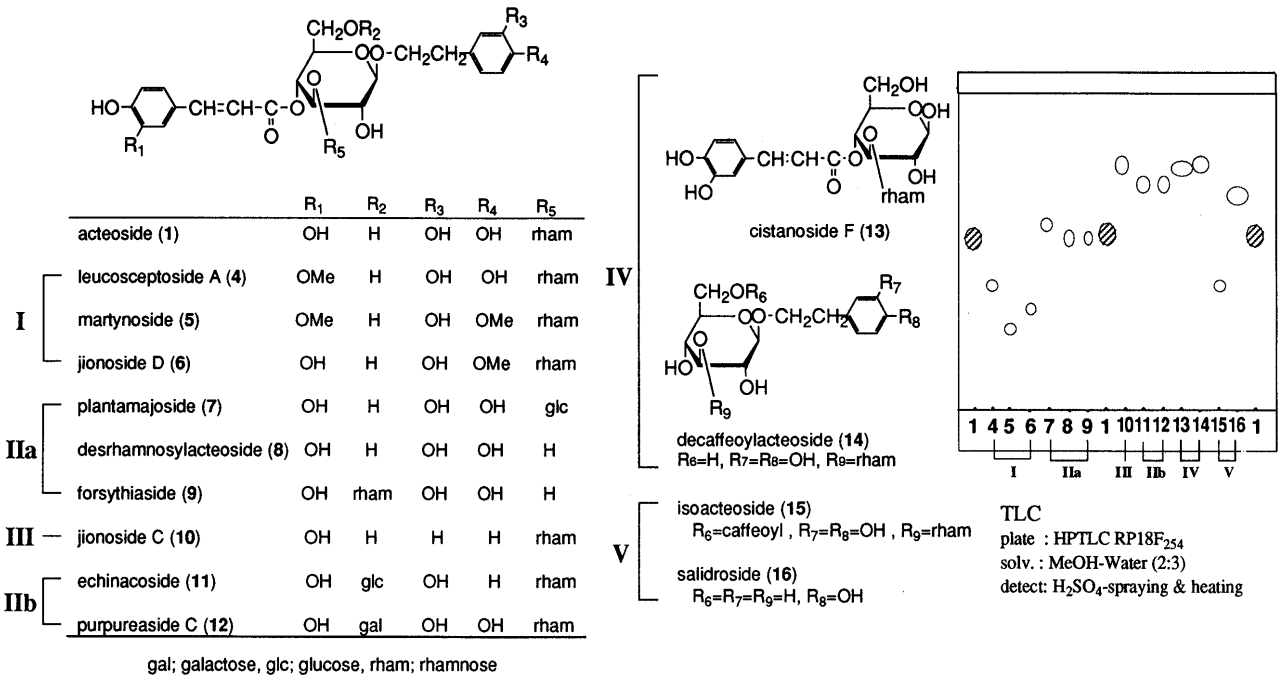

Fig. 7. Structures of the Acteoside-Related Compounds

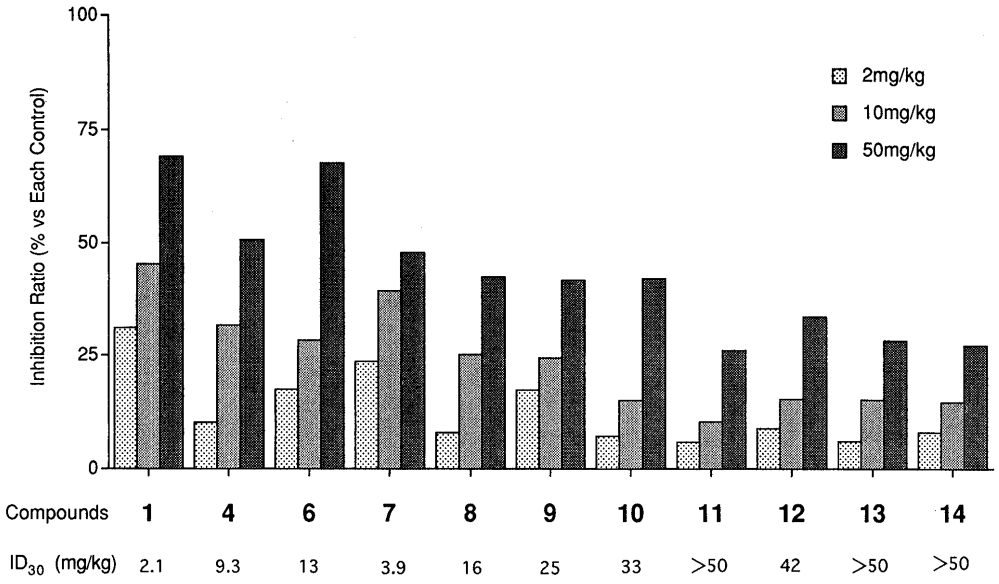

Fig. 8. Analgesic Effect of the Acteoside-Related Compounds on Acetic Acid-Induced Writhing in Mice by Intravenous Injection
ID_{30}, 30% inhibition dose. $n=6$.

4.59 (1H, brt, 5.6 Hz, OH-6′), 4.72 (1H, t-like, 9.6 Hz, H-4′), 5.05 (1H, brs, H-1‴), 5.41 (1H, brd, 5.6 Hz, OH-2′), 6.19 (1H, d, 15.9 Hz, H-8), 6.50 (1H, dd, 8.0, 2.1 Hz, H-6″), 6.64 (1H, d, 2.1 Hz, H-2″), 6.64 (1H, d, 8.0 Hz, H-5″), 6.77 (1H, d, 8.3 Hz, H-5), 6.97 (1H, dd, 8.3, 2.0 Hz, H-6), 7.02 (1H, d, 2.0 Hz, H-2), 7.46 (1H, d, 15.9 Hz, H-7), 8.55 (1H, brs, OH-4″), 8.61 (1H, brs, OH-3″), 9.07 (1H, brs, OH-3), 9.48 (1H, brs, OH-4). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 18.03 (C-6‴), 34.93 (C-7″), 60.71 (C-6′), 68.60 (C-5‴), 69.16 (C-4′), 70.12 (C-8″), 70.37 (C-3‴), 70.46 (C-2‴), 71.66 (C-4‴), 74.46 (C-2′, C-5′), 79.02 (C-3′), 101.09 (C-1‴), 102.26 (C-1′), 113.60 (C-8), 114.67 (C-2), 115.41 (C-5″), 115.72 (C-5), 116.24 (C-2″), 119.45 (C-6″), 121.27 (C-6), 125.50 (C-1), 129.13 (C-1″), 143.45 (C-4″), 144.91 (C-3″), 145.39 (C-7), 145.47 (C-3), 148.34 (C-4), 165.58 (C-9). The direct comparison with the authentic sample was done by ^{1}H-NMR (400 MHz, CD_3OD).

Synthesis of 3,4-Dihydroxyphenethylalcohol (3) The mixture of $LiAlH_4$ (34 mg, 0.90 mmol) and 3,4-dihydroxyphenylacetic acid (100 mg, 0.59 mmol) was stirred in diethyl ether (20 ml) at room temperature for 24 h. After the addition of water (10 ml) and 10%–H_2SO_4 (6 ml) into the reaction solution, the precipitates were removed by suction. The filtrate (112 mg) was purified by silica gel flash column chromatography using chloroform–methanol to afford **3** (88 mg). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.44 (1H, brs), 2.76 (2H, t, 6 Hz), 3.82 (2H, dd, 10, 6 Hz), 5.14—5.17 (1H, m), 5.32—5.39 (1H, m), 6.65—6.67 (1H, d, 8 Hz), 6.74—6.75 (1H, d, 2 Hz), 6.80 (1H, dd, 8.0, 1.7 Hz). The signals of δ 1.44, δ 5.14—5.17 and δ 5.32—5.39 disappeared with the addition of D_2O.

Pharmacological Assay Male ddY strain mice (5 weeks, 25.0—27.0 g; Nippon SLC Co., Ltd., Hamamatsu, Japan) were used after 1 week acclimation under temperature and humidity-controlled conditions (22 ± 1 °C, 55 ± 2%) and a circadian cycle of 12 h light and 12 h darkness. Food and water were made available *ad libitum*. The following drugs were used: acetic acid (Nacalai Tesque, Inc.), aminopyrine (Wako Pure Chem. Ind., Ltd.), powdered acacia (Iwaki Seiyaku Co., Ltd.), methamphetamine hydrochloride (Dainippon Pharm. Co., Ltd.), pentobarbital sodium (Tanabe Pharm. Co., Ltd.). In each of the experiments, the vehicle without samples was used as the control.

Analgesic Effect on Acetic Acid-Induced Writhing: A modified

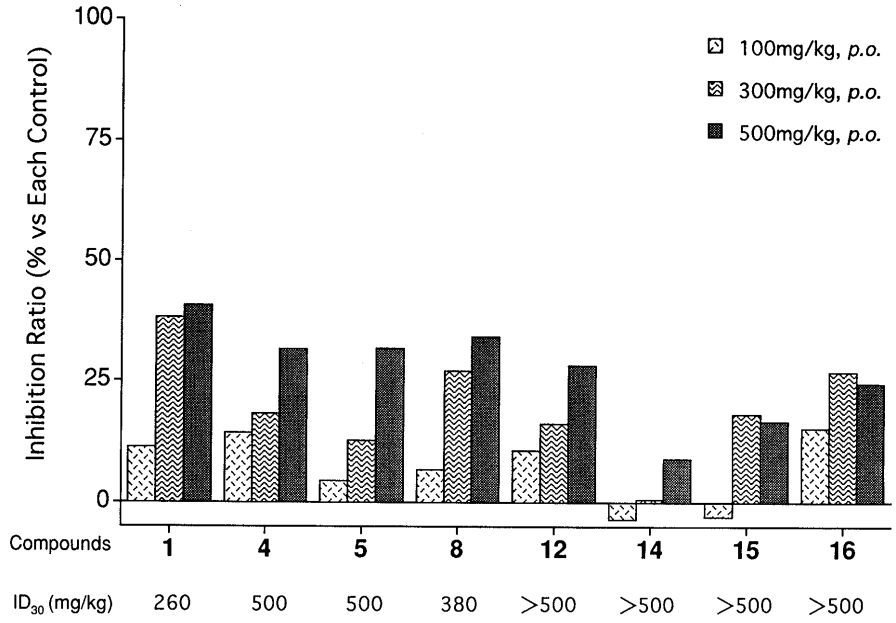

Fig. 9. Analgesic Effect of the Acteoside-Related Compounds on Acetic Acid-Induced Writhing in Mice by Oral Administration
ID_{30}, 30% inhibition dose. $n=6$.

Whittle's method was used as reported previously.[7] The samples were administered to mice 40 min (in the case of *p.o.*) or 20 min (in the case of i.v.) before the i.p. injection of 0.7%-acetic acid (0.1 ml/10 g body weight). After 5 min, the number of squirms was measured for the next 15 min. Aminopyrine was used as a positive control.

Analgesic Effect on Pressure Pain Threshold: A gradient pressure was given at the base of the mouse tail by the use of a Basile Analgesy-Meter (Ugo Basile, Italy).[7b] Mice having a pain threshold range of 100—200 g were used, after they had been tested twice in the pre-experiment. The pain threshold was measured every 30 min after sample administration. Aminopyrine (100 mg/kg, *p.o.*) was used as a positive control.

Effect on the Pentobarbital-Induced Anesthesia: The samples were administered to mice 40 min before the i.p. injection of pentobarbital sodium (50 mg/kg). The time required for the righting reflex to vanish was measured. Chlorpromazine hydrochloride (5 mg/kg, *p.o.*) was used as a positive control.

Effect on the Locomotion Enhanced by Methamphetamine: The locomotor activities were counted every 10 min by the use of Ambulometer AMB-10 (O'hara & Co., Ltd., Japan), as reported previously.[7c] The samples were orally administered 20 min prior to the subcutaneous injection of methamphetamine hydrochloride (2 mg/kg).

Comparison of Analgesic Efficacy among the Related Compounds: Each value of ID_{30} was obtained by calculation of the regression equation which was computed with the statistic software, Microsoft Excel, version 5.0 (Microsoft Corporation). High correlations were indicated with *R*-square (>0.97), in which the significance ($p<0.10$), except for **7**, was judged by the *F*-test in the case of the i.v. injection experiment. The experiment for oral administration, however, indicated a value of $p<0.25$.

Statistics: All data were statistically analyzed using the Student's *t*-test, except for ID_{30} calculation.

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References and Notes

1) Okuyama E., Gao L.-H., Nishimura S., Yamazaki M., *J. Pharmacobio-Dyn.*, **15**, s-11 (1992); Okuyama E., Nakamura T., Umeyama K., Yamazaki M., "Trends in Traditional Medicine Research," ed. by the Scientific Committee, the School of Pharm. Sci., Univ. of Sci. Malaysia, Penang, Malaysia, pp. 380—381, 1995 (Proceedings of the International Conference on the Use of Traditional Medicines and Other Natural Products in Health Care, 1993).

2) *a*) Jiménez P. A., "El Libro Verde, Guia de Recursos Terapeuticos Vegetales 1," ed. by Gustavo Dongo Aguirre, published by Ministerio de Salud, Instituto Nacional de Medicina Traditional, Lima, Peru, 1992, p. 35; *b*) Velásquez N. A. C., "La Materia Medica en el Incanato," Editorial Mejía Baca, Lima, Peru, 1977; *c*) Cheiej R., "The Macdonald Encyclopedia of Medicinal Plants," Macdonald & Co., Ltd., UK, 1980; *d*) Mr. Shiota, Fundación F. I. T.-list of 100 undeveloped useful plants in Peru.

3) Kobayashi H., Oguchi H., Takizawa N., Miyase T., Ueno A., Usmanghani K., Ahmad M., *Chem. Pharm. Bull.*, **35**, 3309—3314 (1987); Yoshizawa F., Deyama T., Takizawa N., Usmanghani K., Ahmad M., *ibid.*, **38**, 1927—1930 (1990); Nishibe S., Okabe K., Tsukamoto H., Sakushima A., Hisada S., *ibid.*, **32**, 1209—1213 (1984); Sasaki H., Nishimura H., Chin M., Mitsuhashi H., *Phytochemistry*, **28**, 875—879 (1989).
After our first report on this subject,[1] Lamaison *et al.* analyzed acteoside in *Aloysia triphylla* (syn. *Lippia triphylla*) by TLC and HPLC; Lamaison J. L., Petitjean-Freytet C., Carnat A., *Plant. Méd. Phytothér.*, **26**, 225—233 (1993).

4) Andary C., Privat G., Chevallet P., Orzalesi H., Serrano J. J., Boucard M., *Il Farmaco Ed. Sc.*, **35**, 3—30 (1980). They reported that a mixture containing acteoside showed analgesic activity by i.p. injection of 500 mg/kg and 1 g/kg in the phenylbenzoquinone-induced writhing and hot plate tests.

5) Sato T., Kojima S., Kobayashi K., Kobayashi H., *Yakugaku Zasshi*, **105**, 1131—1144 (1985).

6) *a*) Kimura Y., Okuda H., Nishibe S., Arichi S., *Plant Med.*, **53**, 148—153 (1987); *b*) Hayashi K., Nagamatsu T., Ito M., Yagita H., Suzuki Y., *Jpn. J. Pharmacol.*, **70**, 157—168 (1996); *c*) Kohda H., Tanaka S., Yamaoka Y., Yahara S., Nohara T., Tanimoto T., Tanaka T., *Chem. Pharm. Bull.*, **37**, 3153—3154 (1989); *d*) Takeda Y., Fujita T., Satoh T., Kakegawa H., *Yakugaku Zasshi*, **105**, 955—959 (1985).

7) *a*) Whittle B. A., *Br. J. Pharmacol. Chemother.*, **22**, 246—253 (1964); *b*) Okuyama E., Umeyama K., Yamazaki M., Kinoshita Y., Yamamoto Y., *Planta Med.*, **61**, 101—198 (1995); *c*) Okuyama E., Umeyama K., Saito Y., Yamazaki M., Satake M., *Chem. Pharm. Bull.*, **41**, 1309—1311 (1993).