Analgesic Principles from *Aralia cordata* THUNB.

Emi OKUYAMA, Satoshi NISHIMURA, and Mikio YAMAZAKI

Faculty of Pharmaceutical Sciences, Chiba University, 1–33 Yayoi-cho, Chiba 260, Japan. Received August 20, 1990

The analgesic principles from *Aralia cordata* THUNB. were identified with (ent)-kaur-16-en-19-oic acid (KA) and (ent)-pimara-8(14),15-dien-19-oic acid (PA), respectively. Both compounds were significantly effective regarding analgesics, hypothermia, duration of pentobarbital-induced anesthesia, and depression of locomotor activity enhanced by methamphetamine at doses of 300 mg/kg (KA) and 500 mg/kg (PA) by oral administration.

**Keywords** *Aralia cordata; Kyohkatsu; Dokukatsu; Qiang-huo; Du-huo; (ent)-kaur-16-en-19-oic acid; (ent)-pimara-8(14),15-dien-19-oic acid; pharmacologically active principle; analgesics

During our screening of neurotropic components from traditional medicinal plants, the methanol extract of *Aralia cordata* THUNB. (Araliaceae) showed hypothermic and analgesic effects and an elongation effect on pentobarbital-induced anesthesia in mice. Isolation of the pharmacologically active principles from this plant is described in this paper.

Traditional Chinese medicines named “Du-huo” and “Qiang-huo” in Chinese (“Dokukatsu” and “Kyohkatsu” in Japanese, respectively) have been used in remedies to produce analgesic effects. However, there is some confusion about their original plants. Nowadays, in Japan, *A. cordata* is used as (Wa-)kyohkatsu or (Wa-)dokukatsu (“Wa” means Japanese in Japanese) with the same expectation.

The methanol extract of the roots of *A. cordata* showed a 66% inhibitory effect (p < 0.01) on acetic acid-induced writhing at a dose of 2.0 g/kg, and 444% elongation (p < 0.001) of pentobarbital-induced sleeping time, as well as −1.0°C (p < 0.01) of ΔTmax (the maximum of body temperature differences) at a dose of 3.0 g/kg by oral administration (p.o.) in mice.

The extract was separated with the guidance of hypothermic effects in mice (Chart 1). Partition of the extract with n-butanol and water gave the hypothermic organic layer, which was then fractionated by LH-20 column chromatography. Fractions C and D, which were the most potent, were further purified by silica gel column chromatography to get frs. C-b and D-b (the total yield of combined frs. C-b and D-b: 10.8% from the extract) which appeared as a single spot on the silica gel thin-layer chromatography (TLC) plate, but as two spots on a reverse-phase plate. This fraction showed −3.1°C of ΔTmax at a dose of 500 mg/kg (p.o.) and seemed to contain the major components of *A. cordata*. The potency did not increase extensively during purification, perhaps due to the fact that purification caused less solubility and less absorption of the samples by the mice during the bioassay. The fraction also exhibited 69% inhibition (p < 0.001) on acetic acid-induced writhing symptoms (500 mg/kg, p.o.) and 250% elongation (p < 0.001) of pentobarbital-induced sleeping time (500 mg/kg, i.p.: intraperitoneal administration) in mice. By using reverse-phased medium pressure liquid chromatography (MPLC), the effective fraction was finally separated into compounds I and II (comps I and II).

Compd I was crystallized from n-hexane to give colorless needles, which have a melting point (mp) 164.5—166°C and [α]D20 −120°. Crystallization of compd II from methanol afforded colorless prisms, mp 178—180°C. [α]D20 16 −110°.

The carbon-13 nuclear magnetic resonance (13C-NMR) spectra of both compounds showed 20 carbon signals, one of which was assigned as carboxylic acid at δ 184.2 (compd I) and 184.6 (compd II) with relation to the absorptions at 1696 (compd I) and 1693 cm−1 (compd II) in the infrared (IR) spectra. In the proton nuclear magnetic resonance (1H-NMR) spectra there were no OH signals except from the carboxylic acids assigned at δ 11.74 (compd I) and ca.

**Chart 1. Isolation of Analgesic Principles from *A. cordata* THUNB.**

1) 3.6 g/kg, 2) 2.0 g/kg, 3) 500 mg/kg. a) p < 0.05, b) p < 0.01, c) p < 0.001, n = 3—6.
11.58 (compd II). Compds I and II seemed to be diterpenoic acids with no hydroxyl group, because some diterpenoic acids have been isolated from A. cordata. Compd I was shown to have three methyl signals at δ 13.8, 29.1 and 29.3 in the $^{13}$C-NMR, and δ 0.65, 1.26 and 1.00 (each 3H, s) in the $^1$H-NMR, respectively. Two types of double bond moieties were also observed at δ 137.9 and 128.0 (1H, s) in the $^1$H-NMR and δ 147.2 and 122.9 (1H, dd, J = 17.1 and 10.5 Hz), 4.91 (1H, dd, J = 17.3 and 1.9 Hz) and 4.94 (1H, dd, J = 10.7 and 1.9 Hz) in the $^1$H-NMR in the $^{13}$C-NMR. These data and the other properties of compd I were quite similar to those of (ent)-pimar-8(14),15-dien-19-oic acid (PA), a major diterpenoic acid isolated from A. cordata (Fig. 1). Compd I was directly identified with the authentic sample by TLC (Merck HPTLC RP-18, methanol; Merck Kieselgel GF$_{254}$, benzene-acetone 10:1), IR and $^1$H-NMR. The mp was not depressed by mixed fusion.

The $^1$H- and $^{13}$C-NMR of compd II revealed the presence of an exo-methylene by observing the signals at δ 155.9 and 103.0 in the $^{13}$C-NMR and δ 4.78 and 4.80 (each 1H, s) in the $^1$H-NMR, indicating a similarity of compd II to (ent)-kaur-16-en-19-oic acid (KA), another major component of A. cordata. Compd II and the authentic sample were identical by TLC (Merck HPTLC RP-18, methanol; Merck Kieselgel GF$_{254}$, benzene-acetone 10:1), IR and $^1$H-NMR. The mp was not depressed by mixed fusion.

The hypothermic effects of compds I (PA) and II (KA) in mice are shown in Fig. 2. Compd II (KA) caused hypothermia with $-1.6^\circ$C ($p < 0.001$) and $-0.7^\circ$C ($p < 0.05$) of $A_{max}$'s at doses of 500 and 300 mg/kg, respectively by oral administration. Intraperitoneal injection of 100 mg/kg of compd I (PA) indicated $-0.8^\circ$C ($p < 0.01$) of $A_{max}$, although it did not show a significant effect at 500 mg/kg of oral administration.

The analgesic effects of both compounds in mice are
indicated in Fig. 4. Compds I(PA) and II (KA) reduced 43% (p<0.01) and 40% (p<0.01) of acetic acid-induced writhing by oral administration of 500 and 300 mg/kg, respectively. Both compounds also seemed to have dose-dependent effects on the duration of sleeping time induced by pentobarbital. The results are shown in Fig. 5.

Compds I(PA) and II(KA) produced sedative effects at oral doses of 500 and 300 mg/kg, respectively, on locomotor activity enhanced by methamphetamine (Fig. 6).

These results indicate that compds I and II, which were identified with (ent)-pimara-8(14),15-dien-19-oic acid (PA) and (ent)-kauren-16-en-19-oic acid (KA), respectively are pharmacologically active principles of A. cordata; however compd I was less effective in mice than compd II on analgesics, duration of pentobarbital-induced anesthesia, hypothermic effect and depression of locomotor activity enhanced by methamphetamine. Although the chemical structure of steviol (the 13-hydroxy derivative) was similar to that of KA, it did not show a hypothermic effect at the dose of 500 mg/kg (p.o.) in mice. This is the first report that a kaurenoid acid and a pimaradienoid acid contain pharmacological activities such as analgesics and sedative effects.

Experimental
Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on a Hitachi EPI-G3 spectrometer, and optical rotations were measured with a JASCO DIP-140 polarimeter. 1H- and 13C-NMR were recorded on JEOL GXS 400 and 500 spectrometers using tetramethylsilane as an internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, m=multiplet, br=brd. Column chromatographies were performed on Sephadex LH-20, Wakogel C-200 and Narakai Silica gel 60. Two pre-packed columns (Kusano CPO-HS-212-20) connected in series were used for MPLC.

Isolation
The dried plant was purchased by Kinokunija Kan-yakkyoku Co., Ltd.

The material (900 g) was extracted with methanol at room temperature. The methanol extract (106.2 g) was partitioned with n-butanol and water to get a hypotonic n-butanol layer (49.3 g), which was chromatographed on Sephadex LH-20 (methanol). Fractions C and D containing activity were further separated independently by silica gel column chromatography eluted with benzene to give effective fractions, frs. C-b and D-b, respectively, which appeared as a single spot on silica gel TLC (Merck HPTLC, benzene–ethyl acetate 5:1). Both fractions were combined (12.7 g of the total yield calculated from the extract), and ca. 5.9 g of it was pursued by reverse-phased MPLC (methanol). Thus, the bioactive compounds, compds I (2.33 g) and II (1.43 g) were obtained.

Compd I Colorless needles from n-hexane, mp 164.5~166°C, [α]20
−120° (c=0.75, CHCl3). IR (KBr): 3452br, 2955, 1696, 1451, 1267, 920 cm−1. 1H-NMR (CDCl3) 6: 0.65 (3H, s), 1.00 (3H, s), 1.05 (2H, td, 13.5, 3.9), 1.22 (1H, td, 13.5, 3.9), 1.26 (3H, s), 1.28 (1H, dd, 12.4, 2.8), 1.31—1.39 (1H, m), 1.44—1.48 (1H, m), 1.51—1.56 (1H, m), 1.54 (1H, brd, 10.2), 1.68 (1H, dd, 10.1, 6.2), 1.73 (1H, brd, 13.8), 1.78—1.90 (3H, m), 1.99 (1H, td, 13.7, 5.2), 2.17 (1H, brd, 13.5), 2.33 (1H, dd, 13.8, 4.1, 2.3), 4.91 (1H, dd, 17.3, 1.9), 4.94 (1H, dd, 10.7, 1.9), 5.14 (1H, s), 5.71 (1H, dd, 17.1, 10.5), ca. 11.58 (1H, br). 13C-NMR (CDCl3) 6: 13.8, 19.2, 19.5, 24.1, 29.2, 29.3, 35.8, 36.4, 37.9, 38.5, 39.20, 39.23, 44.0, 50.5, 56.1, 112.9, 128.0, 137.9, 147.2, 184.2.

Compd II Colorless prisms from methanol, mp 178~180°C. [α]20
−110° (c=1.00, CHCl3). IR (KBr): 3450br, 2935, 1693, 1471, 1460, 1256, 877 cm−1. 1H-NMR (CDCl3) 6: 0.81 (1H, td, 13.2, 3.5), 0.95 (3H, s), 1.01 (1H, td, 13.9, 4.4), ca. 1.06 (1H), 1.07 (1H, t-likje, 7.4), 1.13 (1H, dd, 11.4, 4.9), 1.24 (3H, s), 1.40—1.48 (3H), 1.52 (1H, dt, 13.2, 3.3), 1.56—1.66 (3H), 1.83 (2H, td, 10.4, 3.3), 1.88—1.90 (2H), 1.99 (1H, brd, 11.1), 2.05 (2H, dd, 5.3, 2.7), 2.16 (1H, brd, 14.3), 2.64 (1H, brs), 4.74 and 4.80 (each 1H, t), 7.4 (1H, br). 13C-NMR (CDCl3) 6: 15.6, 18.4, 19.1, 21.9, 28.0, 33.1, 37.8, 39.67, 39.70, 40.7, 41.3, 45.7, 45.8, 44.2, 49.0, 51.7, 57.1, 103.0, 155.9, 184.6.

Pharmacological Assay
Male ddY mice (5 weeks old, 22—32 g), propagated at Shizuoka Agricultural Cooperative Association (Hamamatsu, Japan) were used. Test samples were suspended in normal saline with 2% Tween 80 and 20—40% olive oil. Experiments were pursued under constant conditions at 23—25°C.

Hypothermic Effect: Rectal temperatures were measured until 5 h after administration of the test samples by thermistor (Takara Instrumental Co., Ltd.).

Analgesic Activity: An acetic acid-induced writhing method was used. At 30 min after administration of the test samples, 0.75% acetic acid (0.1 ml/10 g) was injected intraperitoneally. The number of squirms was counted in each mouse for 15 min beginning from 5 min after acetic acid injection.

Effect on Pentobarbital-Induced Anaesthesia: Test samples were administered 30 min before i.p. injection of 50 mg/kg of sodium pentobarbital (Tanabe Pharmaceutical Co., Ltd.). The time required to regain the righting reflex was measured.

Effect on Locomotor Activity: Spontaneous motor activity was recorded by an Ambulatory Activity Analyzer (O’Hara & Co., Ltd.). Test samples were administered at 20 min before subcutaneous injection of methamphetamine (Dainippon Pharmaceutical Co., Ltd.) at a dose of 2 mg/kg. The movements were counted for every 10 min up until 120 min.

Statistics: Statistical significance was evaluated by the Student’s t test.

Acknowledgements
This research was supported in part by a Grant-in-Aid (No. 1771901) from the Ministry of Education, Sciences and Culture. We are grateful to Professors O. Tanaka, K. Yamasaki and Kohda of Hiroshima University and Prof. Kuroyanagi of the University of Shizuoka for providing samples, and to Mr. S. Mitake for the medicinal plants. We thank Ms. L.-H. Gao and Mr. N. Iwase of this University for their technical assistance. We also thank the Chemical Analysis Center of this University for 1H- and 13C-NMR spectra.

References and Notes
3) The authentic sample was used after crystallization from n-hexane.