Ascaridole as a Pharmacologically Active Principle of "Paico," a Medicinal Peruvian Plant

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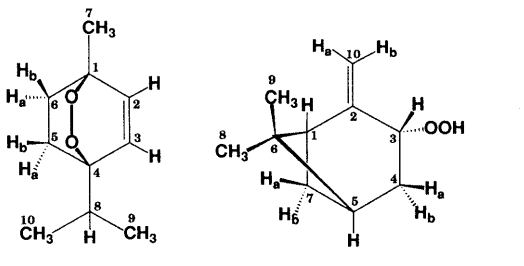
"Paico," *Chenopodium ambrosioides* L., is a traditional Peruvian medicine which is considered to be nervine, antirheumatic, anthelmintic, *etc.* An attempt was made to isolate the component having sedative and/or analgesic properties from "Paico" and "Aritasou" (the Japanese name for *C. ambrosioides*). Ascaridole was identified as the active principle in both materials.

Keywords *Chenopodium ambrosioides*; ascaridole; analgesia; sedation; Paico; *trans*-pinocarveylhydroperoxide

"Paico" is a Peruvian medicinal plant, *Chenopodium ambrosioides* L. (Chenopodiaceae), which has been used as nervine, antirheumatic, anthelmintic, emmenagogue, *etc.*[1] The methanol extract showed a hypothermic effect (ΔT_{max} $-0.6\,°C$, $p<0.05$, 2 g/kg) as well as inhibition of acetic acid-induced writhing (88%, $p<0.001$, 3 g/kg) in mice during a survey of Peruvian herbal medicines for neurotropic effects, suggesting that this plant has some sedative and/or analgesic effects. *C. ambrosioides* was also used for its anthelmintic property in Japan ("aritasou" being its Japanese name), while no sedative or analgesic effect of the plant was reported. In this paper, the isolation of the pharmacologically active components of *C. ambrosioides* from Peru and from Japan are described.

The methanol extract of "Paico" (Peruvian *C. ambrosioides*) was separated following hypothermia in mice. Successive partition of the extract with organic solvents and water gave an *n*-hexane layer which exhibited activity, as shown in Chart 1. The fraction was separated by repeated silica gel column chromatography, and then purified by passing it through Sep-Pak (ODS and silica gel). Compound **1** was obtained as the effective component. This compound indicated a positive color by the reagent for detecting organic peroxides,[2] and was identified as ascaridole.[3] (Fig. 1)

"Aritasou" (Japanese *C. ambrosioides*) was also tested for analgesic effects by the acetic acid-induced writhing method.[4] Because the methanol extract showed an inhibitory effect (56%, $p<0.01$, 2 g/kg) by oral administration, the extract was separated according to writhing inhibition. After being partitioned with *n*-hexane and water, the organic layer involved in the inhibition was separated by silica gel chromatography. Since the effective fraction showed positive spots on TLC caused by the peroxide-reagent, an attempt was made to isolate the compounds by repeated chromatography using MPLC and HPLC. Two components were obtained, one of which showed analgesic effects, and was identified with ascaridole. The other compound, compound 2, did not inhibit writhing at doses of up to 100 mg/kg. The structure of compound **2** was estimated to be *trans*-pinocarveylhydroperoxide, which was previously isolated from *Anthemis nobilis*,[5] using a



1 (ascaridole) 2 (*trans*-pinocarveylhydroperoxide)

Fig. 1. Structures of Compounds **1** and **2**

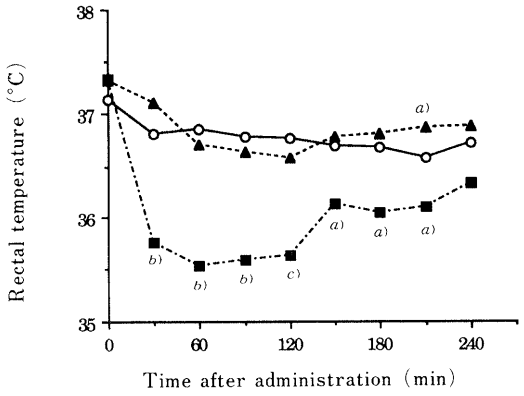


Fig. 2. Effect of Ascaridole on Rectal Temperature in Mice

—○—, control; ---▲---, 30 mg/kg; ——■——, 100 mg/kg. *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$. $n=7$.

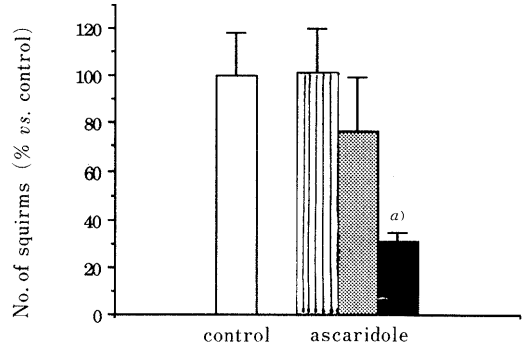


Fig. 3. Effect of Ascaridole on Acetic Acid-Induced Writhing in Mice

□, control; ▨, 10 mg/kg; ▩, 30 mg/kg; ■, 100 mg/kg. *a*) $p<0.05$. Each bar represents the mean ± S.E. $n=6$.

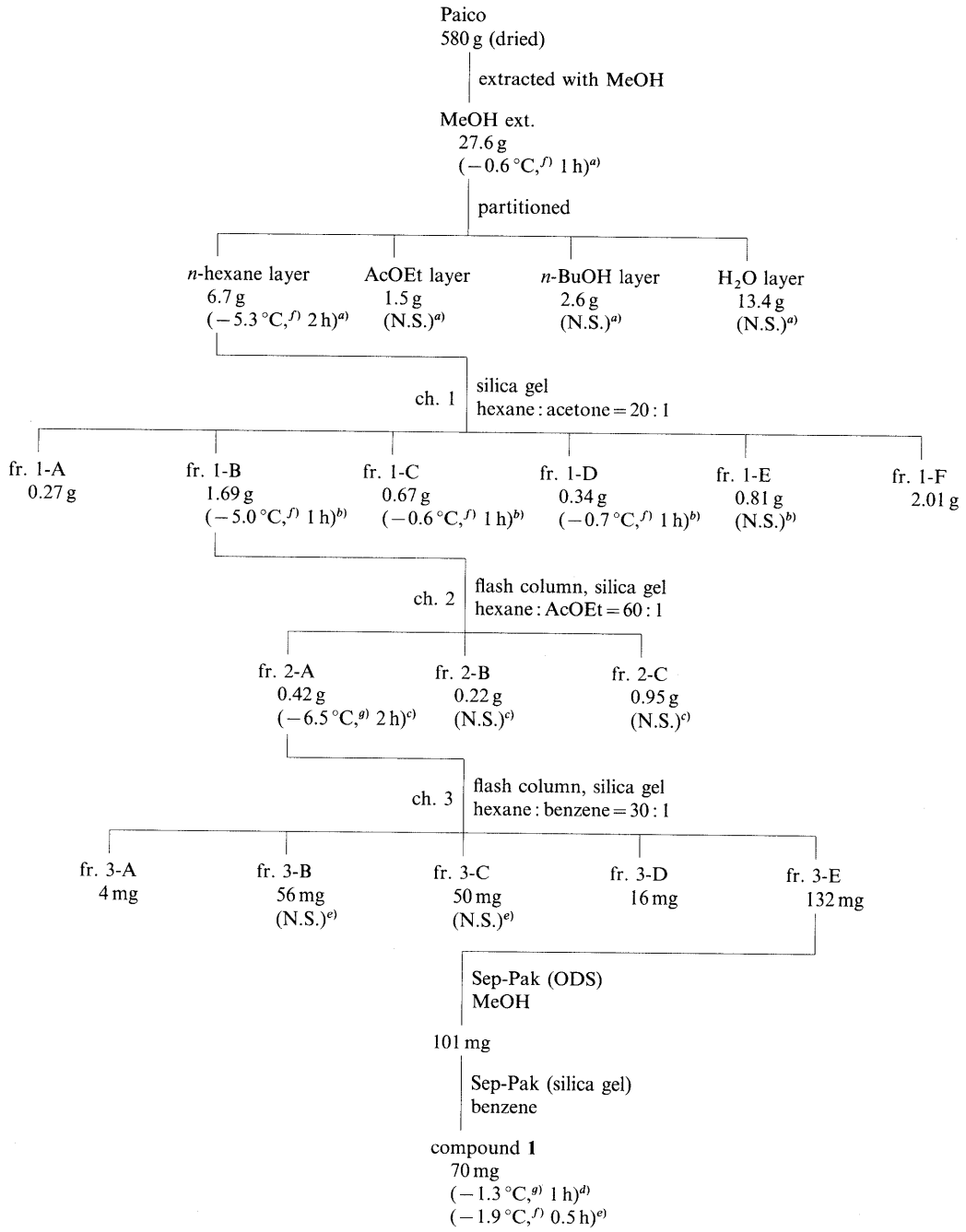


Chart 1. Isolation Procedure of "Paico"

(ΔT_{max}, h): effects on body temperature. *a*) 2 g/kg, *p.o.*, *b*) 1 g/kg, *p.o.*, *c*) 750 mg/kg, *p.o.*, *d*) 100 mg/kg, *p.o.*, *e*) 50 mg/kg, i.p. *f*) $p<0.05$, *g*) $p<0.01$. N.S.: no significance.

spectroscopic method, and it was reduced to pinocarveol[6] by treatment with methyl sulfide.

The oral administration of ascaridole at a dose of 100 mg/kg showed the hypothermic effect of ΔT_{max} −1.3 °C ($p<0.01$, 1 h) and an analgesic effect (69%, $p<0.05$) on acetic acid-induced writhing in mice (Figs. 2 and 3). Prolongation of the anesthesia induced by sodium pentobarbital was also observed at the same dose of this compound (Fig. 4). Ascaridole reduced the locomotor activity which was enhanced by methamphetamine, as shown in Fig. 5. The administration of 300 mg/kg, however, produced convulsions and lethal toxicity in mice (3/4). These facts indicate that ascaridole possibly has sedative and analgesic effects, and is the pharmacologically active principle of both of Peruvian and Japanese *C. ambrosioides*, although the effective dose range is narrow.

Experimental

Optical rotations were measured with JASCO DIP-140 and JASCO J-20 polarimeters. IR spectra were recorded on a Hitachi 260-10 spectrometer, UV spectra on a Hitachi U-3400 spectrometer, and GC-MS spectra on a Hewlett-Packard GC-MS 5995A. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL-JMN-GSX 400 and JEOL-JMN-GSX 500 spectrometers with tetramethylsilane as an internal standard. The following abbreviations are used: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet; br, broad. Column chromatographies were performed on Wakogel C-200, Chromatorex ODS (100—200 mesh), and Sephadex LH-20. Pre-packed columns (Kusano CPS-HS-221-5, Senshu Pak. Silica-5251-N and Senshu Pak. Silica-3031-N) were used for MPLC and HPLC.

Isolation "Paico" (*Chenopodium ambrosioides* L.) was provided by

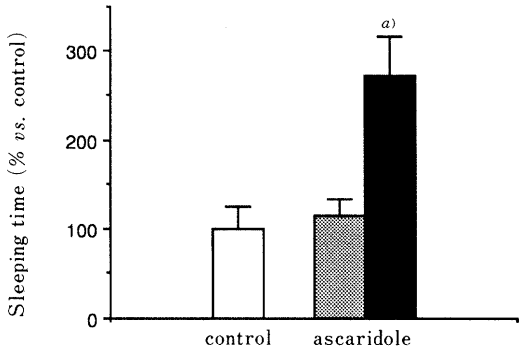


Fig. 4. Effect of Ascaridole on Prolongation of the Anesthesia Induced by Sodium Pentobarbital in Mice

□, control; ▨, 30 mg/kg; ■, 100 mg/kg. *a*) $p<0.05$. Each bar represents the mean ± S.E. The mean of the control (21.4 min) is set as 100%. $n=5$–6.

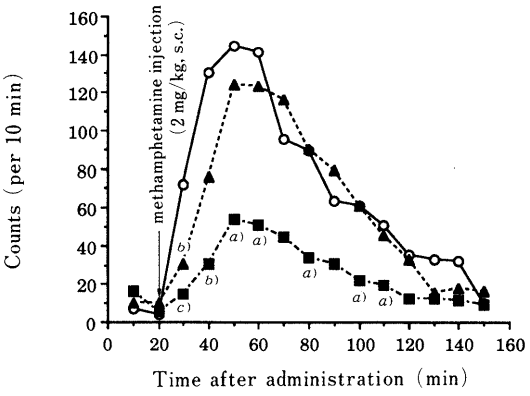


Fig. 5. Effect of Ascaridole on Locomotor Activity in Methamphetamine-Treated Mice

—○—, control; ---▲---, 50 mg/kg; —·—■—·—, 100 mg/kg. *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$, $n=10$.

Mr. Shiota, Peru, in April, 1989. "Aritasou" (*C. ambrosioides* L.) was collected in Dejima, Ibaraki Prefecture, Japan, in September, 1991. Both were botanically identified by Satake, one of the authors.

Paico (580 g; the dried aerial part) was extracted with methanol at room temperature to obtain the methanol extract (27.6 g). The extract was partitioned successively with *n*-hexane, ethyl acetate, *n*-butanol and water. The *n*-hexane fraction (6.7 g), which showed a hypothermic effect in mice, was chromatographed by silica gel eluted with *n*-hexane–acetone (20 : 1) to get the active fraction, fr. 1-B (1.69 g). The fraction was further separated by flash column chromatographies (silica gel) using with *n*-hexane–ethyl acetate (60 : 1) and *n*-hexane–benzene (30 : 1) as eluents. Fraction 3-E (132 mg) was purified by ODS Sep-Pak (methanol) and silica gel Sep-Pak (benzene) to give a colorless oil, compound **1** (70 mg).

The Aritasou extract (333 g) was obtained by extraction of the dried aerial part (2.5 kg) with methanol at room temperature. The extract (107 g) was partitioned with *n*-hexane–water. Because the *n*-hexane fraction (22.1 g) inhibited acetic acid writhing in mice, it was separated by silica gel with *n*-hexane–ethyl acetate (5 : 1). Fraction 1-B (6.06 g), showing the inhibition, was flash chromatograhed by silica gel, and fr. 2-B (2.25 g) and fr. 2-C (1.13 g) were obtained from an *n*-hexane–ethyl acetate (15 : 1) eluate. Both fractions were separated repeatedly by MPLC or HPLC using silica gel (*n*-hexane–ethyl acetate (25 : 1 or 30 : 1)) and then purified by ODS (methanol) and LH-20 (benzene) to give compounds **1** (710 mg) and **2** (134 mg).

Compound **1**: Colorless oil, $[\alpha]_D^{16}$ 0.0° ($c=2.0$, *n*-hexane). IR ν_{max}^{neat} cm^{-1}: 3050, 2970, 2925, 1465, 1450, 1375, 1110, 1010, 880. UV λ_{max}^{EtOH} nm: 229 (sh). GC-MS *m/z* (%): 168 (M^+, 3), 136 (17), 121 (100), 107 (31), 93 (41). ^{1}H-NMR (C_6D_6) δ: 0.82 (3H, d, $J=7.0$ Hz, 9-H_3), 0.90 (3H, d, $J=6.8$ Hz, 10-H_3), 1.13 (3H, s, 7-H_3), 1.14—1.19 (2H, m, 5-H_a, 6-H_a), 1.74—1.81 (1H, m, 8-H), 1.81—1.87 (2H, m, 5-H_b, 6-H_b), 6.10 (1H, d, $J=8.4$ Hz, 2-H), 6.21 (1H, d, $J=8.4$ Hz, 3-H). ^{13}C-NMR (C_6D_6) δ: 17.21 (C-9), 17.37 (C-10), 21.51 (C-7), 26.16 (C-5), 29.98 (C-6), 32.45 (C-8), 73.71 (C-1), 79.19 (C-4), 133.08 (C-3), 136.63 (C-2).

Compound **2**: Colorless oil, $[\alpha]_D^{24}$ −82.7° (Calcd by ORD, $c=0.133$, $CHCl_3$) (lit. 5, $[\alpha]_D^{20}$ −38.2° in $CHCl_3$). IR ν_{max}^{neat} cm^{-1}: 3400, 3080, 2950, 2925, 1645, 1470, 1385, 1370, 905. UV λ_{max}^{EtOH} nm: 276, 282 (sh). GC-MS *m/z* (%): 135 (M^+ − OOH, 41), 107 (8), 81 (18), 52 (70), 41 (100). ^{1}H-NMR (C_6D_6) δ: 0.54 (3H, s, 9-H_3), 1.10 (3H, s, 8-H_3), 1.65 (1H, d, $J=10.0$ Hz, 7-H_b), 1.70—1.73 (1H, m, 5-H), 1.96—1.99 (2H, m, 4-H_2), 2.19 (1H, dtd, $J=10.0$, 5.9, 1.9 Hz, 7-H_a), 2.34 (1H, dd, $J=5.9$, 5.3 Hz, 1-H), 4.49 (1H, dd, $J=6.5$, 2.7 Hz, 3-H), 4.83 (1H, brs, 10-H_a), 5.05 (1H, dd, $J=1.9$, 1.0 Hz, 10-H_b), 7.35 (1H, s, 3-OOH). ^{13}C-NMR (C_6D_6) δ: 21.90 (C-9), 25.94 (C-8), 27.86 (C-7), 30.90 (C-4), 39.70 (C-5), 41.06 (C-6), 50.76 (C-1), 80.34 (C-3), 114.98 (C-10), 148.64 (C-2).

(−)-*trans*-Pinocarveol: Compound **2** (4 mg) in benzene was treated with dimethyl sulfide at room temperature. After evaporation, the residue was separated by silica gel column chromatography eluted with benzene and *n*-hexane–ethyl acetate (15 : 1) to give (−)-*trans*-pinocarveol (2 mg). ^{1}H-NMR ($CDCl_3$) δ: 0.65 (3H, s, 9-H_3), 1.28 (3H, s, 8-H_3), 1.72 (1H, d, $J=10.0$ Hz, 7-H_b), 1.85 (1H, dd, $J=14.6$, 4.4 Hz, 4-H_b), 2.00 (1H, m, 5-H), 2.24 (1H, ddt, $J=14.6$, 7.6, 2.0 Hz; 4-H_a), 2.40 (1H, dtd, $J=10.0$, 5.5, 2.0 Hz, 7-H_a), 2.52 (1H, t, $J=5.5$ Hz, 1-H), 3.52 (1H, brs, 3-OH), 4.43 (1H, d, $J=7.6$ Hz, 3-H), 4.82 (1H, s, 10-H_a), 5.00 (1H, s, 10-H_b).

Pharmacological Assay Male ddy mice (5 weeks) weighing 22—35 g were used. The animals, propagated at Shizuoka Agricultural Cooperative Association (Hamamatsu, Japan), were housed under a 12 h light/dark cycle at 22—25 °C, and allowed free access to food and water. Food was withheld three hours before the experiments. Test samples were suspended in saline with 5% arabic gum and/or 5% Tween 80.

Effect on Body Temperature: Rectal temperatures were measured every 30 min up to 4 h following the oral administration of the samples by a thermistor (Takara Instrumental Co., Ltd.).

Analgesic Activity: Samples were given 40 min prior to the intraperitoneal injection of 0.7% acetic acid. After 5 min, the number of squirms was counted in each mouse for the next 15 min.

Effect on Pentobarbital-Induced Hypnosis: Samples were administered 40 min before intraperitoneal 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: Locomotor activity was measured by a Tilting-type ambulometer AMB-10 (O'hara & Co., Ltd.). Samples were orally administered at 20 min before the subcutaneous injection of methamphetamine hydrochloride (Dainippon Pharmaceutical Co., Ltd.) at a dose of 2 mg/kg. The movements were counted for every 10 min up to 150 min. One mouse was put in a cage at a time.

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

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