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Pharmacologically Active Components of a Peruvian Medicinal Plant, Huanarpo (*Jatropha cilliata*)

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From *Jatropha cilliata* M. ARG., a Peruvian medicinal plant, isoorientin and orientin were isolated as anxiolytic components by using the isolation guide of anti-conflict effect in mice. Several related flavonoids were also tested for this effect. Another bioassay-guided isolation using the acetic acid-induced writhing method gave a coumarin compound, fraxetin, as an analgesic principle.

Key words *Jatropha cilliata*; flavone C-glycoside; anti-anxiety; fraxetin; analgesia; Peruvian medicinal plant

During our continuous study of Peruvian medicinal plants having neurotropic effects,[1)] the extract of *Jatropha* (*J.*) *cilliata* M. ARG. (Euphorbiaceae) indicated an anti-conflict effect. An analgesic effect was also observed in the extract. *J. cilliata* is a shrub with many branches, and is one of the medicinal plants named Huanarpo in Peru which has been used by natives as a tonic or for tranquilizing purposes.[2)] In this paper, the active components having an anxiolytic or analgesic effect were reported.

Anti-conflict effects are often used for evaluating anxiolytic-like activity, and the modified Vogel-type procedure (the drinking conflict test) in water-deprived mice by Kuribara, Tadokoro *et al.* is a simple and reliable method.[3,4)] The methanol extract from the dried stem of *J. cilliata* presented anti-conflict activity by subcutaneous (s.c.) injection of 2 g/kg to mice according to this method, while neither sedative behavior nor a hypothermic effect were observed. The extract was separated by using the isolation guide of anti-conflict activity as shown in Chart 1. After being partitioned with ethyl acetate, *n*-butanol and water, the activity was moved to the *n*-butanol part, which was further separated by Sephadex LH-20 and octadecyl silica (ODS) column chromatographies. The activity was concentrated to fr. 2b (200 mg/kg, s.c., $p<0.01$) presenting major yellow spots on TLC. The corresponding substances, compounds JC-1 and JC-2, were isolated as the active components by HPLC and then by passing them through Sephadex LH-20 and/or SEP-PAC.

In addition to its anxiolytic effect, the methanol extract showed analgesia by oral administration (*p.o.*) of 1 g/kg to mice in the acetic acid-induced writhing method, although 2 g/kg administration did not indicate any significant effect. After the partition mentioned above, the activity was observed in the ethyl acetate part at a dose of 500 mg/kg, *p.o.* The fraction was also tested by the tail-pressure method, which produced an analgesic effect in a dose-dependent manner at oral doses of 250 and 500 mg/kg. The activity (writhing inhibition)-guided isolation from the ethyl acetate part was performed by Sephadex LH-20 and ODS flash chromatographies, as shown in Chart 1. Compound JC-3 was obtained as the analgesic principle.

Compounds JC-1 (**1**) and JC-2 (**2**) were flavone glucosides with the same molecular formula, $C_{21}H_{20}O_{11}$. The anomeric proton and carbon of the glucose moiety in **1** were assigned at δ 4.59 (1H, d, 9.8) in the ^{1}H-NMR and δ 73.09 in the ^{13}C-NMR, respectively, suggesting a C-glucoside structure. By 2D-NMR experiments such as HH-correlation spectroscopy (COSY), CH-COSY and heteronuclear multiple-bond connectivity (HMBC), compound JC-1 (**1**) was established to be isoorientin, and was identified directly with the authentic sample.[5)] The spectra of **2** were very similar to those of **1**, except that the aromatic proton of C-8 at δ 6.45 (1H, br s) in the latter compound was slightly shifted to δ 6.20 (1H, br s) in the former.[6a)] Compound JC-2 (**2**) was identified with orientin by comparing the spectral data.[6)]

Compound JC-3 (**3**), yellow needles, mp 228—230 °C, was a phenolic compound with a molecular formula of $C_{10}H_8O_5$, which was estimated by MS and NMR spectra. An ester carbonyl signal at δ 163.78 was observed in the ^{13}C-NMR, and *ortho*-coupled protons at δ 6.20 (1H, d, $J=9.4$) and δ 7.83 (1H, d, $J=9.4$) in the ^{1}H-NMR indicated a coumarin-skeleton. The positions of the substituents were confirmed by NOE difference and 2D-NMRs. Compound JC-3 (**3**) was identified with fraxetin by comparing spectral data.[7)]

Anti-conflict effects of compounds JC-1 (**1**) and JC-2 (**2**) were shown in Fig. 2. Diazepam was used as a positive control. The injection of 40 mg/kg of each substance significantly increased the number of punished shocks in mice. Since these active components are flavonoids, some related flavonoids were tested for anti-conflict activity (Fig. 3). None of those compounds showed such activity except for vitexin, a flavone C-glucoside. Anti-conflict activity was not observed in the case of luteolin, which is the aglycone of compounds JC-1 and JC-2. Therefore, the C-glucoside part probably plays a role in the activity. Although the hypotensive effects of flavone C-glycosides have been reported,[8)] this result may elucidate another interesting activity of flavone C-glycosides. Compound JC-3 did not contribute to any anxiolytic effect in this medicinal plant (the data was not shown).

The analgesic effect of compound JC-3 (**3**) was shown in Fig. 4. The compound exhibited about 60% inhibi-

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This paper is dedicated to Dr. Masanori Kageyama (deceased on July 30, 1994) by Emi Okuyama, one of the authors, for his warmest encouragement.

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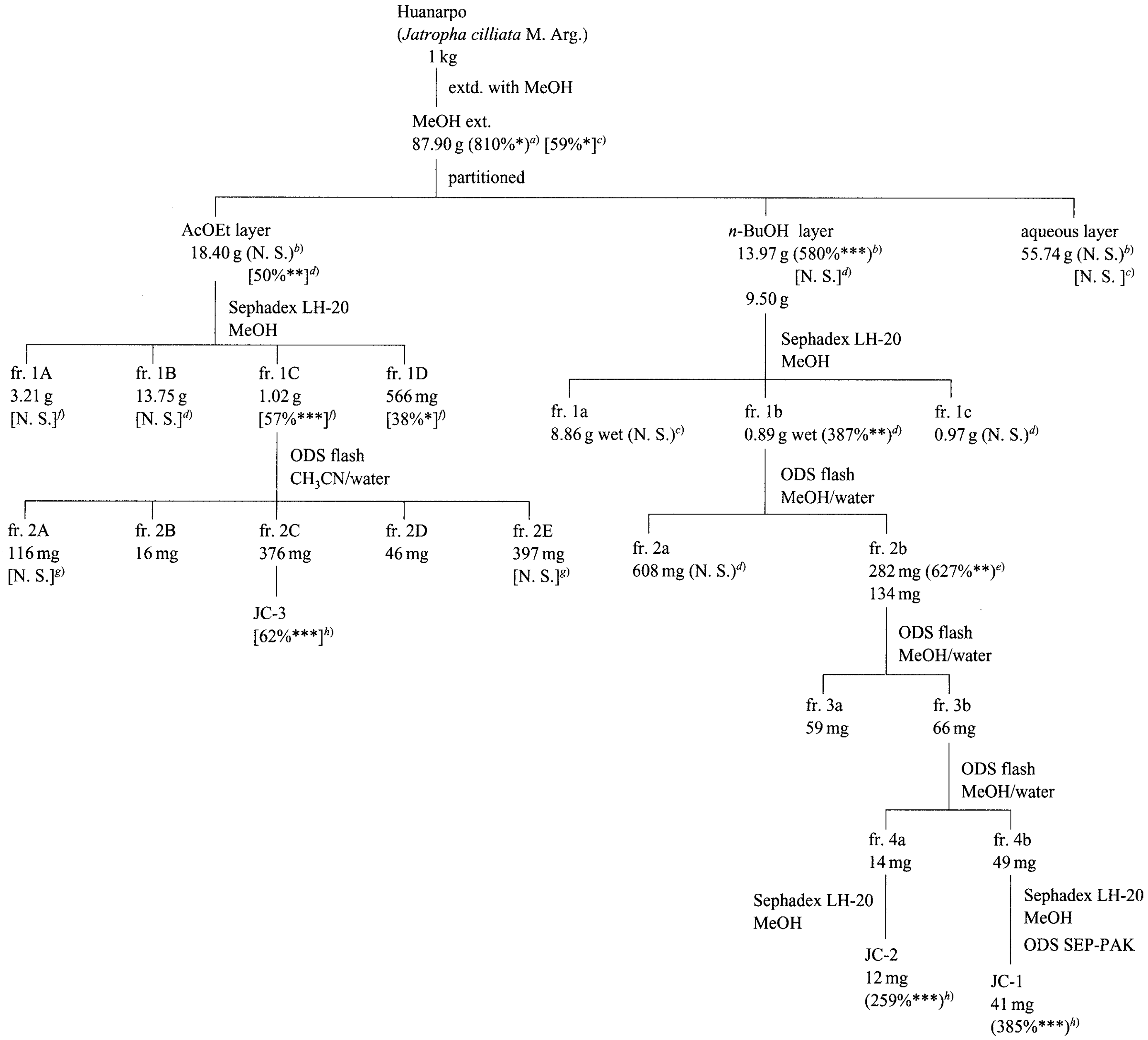
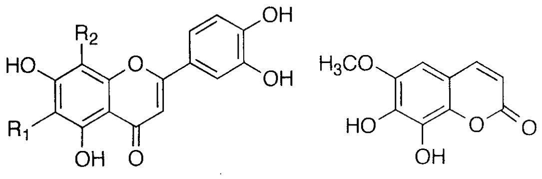


Chart 1. Separation Procedure of *Jatropha cilliata* Using Bioassay-Guides of Anti-conflict and Analgesic Activities

( ): punishment number, % *vs.* control; [ ]: writhing inhibition, % *vs.* control; *a*) 2.0 g/kg, *b*) 1.2 g/kg, *c*) 1.0 g/kg, *d*) 500 mg/kg, *e*) 200 mg/kg, *f*) 100 mg/kg, *g*) 70 mg/kg, *h*) 40 mg/kg. Samples were administered subcutaneously in the case of the conflict test, and orally in the analgesic test. $*p<0.05$, $**p<0.01$, $***p<0.001$; N.S.: no significance.



JC-1(**1**, isoorientin): R1=-βGlc, R2=H
JC-2(**2**, orientin) : R1=H, R2=-βGlc

JC-3(**3**, fraxetin)

Fig. 1. Structures of the Pharmacologically Active Components from *Jatropha cilliata*

tion of writhing in both cases at oral doses of 40 and 70 mg/kg in mice, which is the similar potency to that of aminopyrine (a positive control). Such doses, however, showed dose-dependent analgesia by the tail-pressure method in mice. These suggested that compound JC-3 (**3**) was the analgesic principle of *J. cilliata*, and that it also contributes to the plant's neuronal effect. Compound JC-3 was not effective on body temperature in normal mice, and it might have a weak effect in lipopolysaccharide (LPS)-treated mice (Fig. 5).

From the results, some flavone C-glucosides and fraxetin are responsible for the pharmacological activities of *J. cilliata*, such as its anxiolytic and analgesic effects, respectively.

Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on a Hitachi 260-10 spectrometer, UV on a Hitachi U-3400 spectrometer, high-resolution (HR)-FAB-MS on a JEOL HX-110, and electron impact (EI)-MS on a JEOL AUTO MS-20. Optical rotations were measured with a JASCO J-20 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL-JNM-GSX 400 and JEOL-JNM-GSX-500 spectrometers with tetramethylsilane or a solvent as an internal standard. Column chromatographies were performed on Sephadex LH-20, Wakogel C-200, Nacalai Silica gel 60 and Chromatorex ODS (100—200 mesh). Pre-packed columns: Kusano CPO-HS-221-20 and CPS-HS-221-05 for medium pressure liquid chromatography (MPLC) and Sensyu Pak ODS-5251-S for HPLC, were used.

Plant Material and Isolation The dried stem of *J. cilliata* M. Arg. was obtained in April, 1991 from Mr. Shiota in Peru, and was identified by himself and Mr. Satake, one of the authors.

The plant (1 kg) was extracted with methanol at room temperature, and the solvent was evaporated to leave an extract of 87.9 g which showed both anti-conflict and analgesic effects in mice. Partition of the extract with ethyl acetate, *n*-butanol and water successively gave fractions of 18.40, 13.97 and 55.74 g, respectively. The anti-conflict effect was observed only in the *n*-butanol part, while the analgesic effect was in the ethyl acetate part.

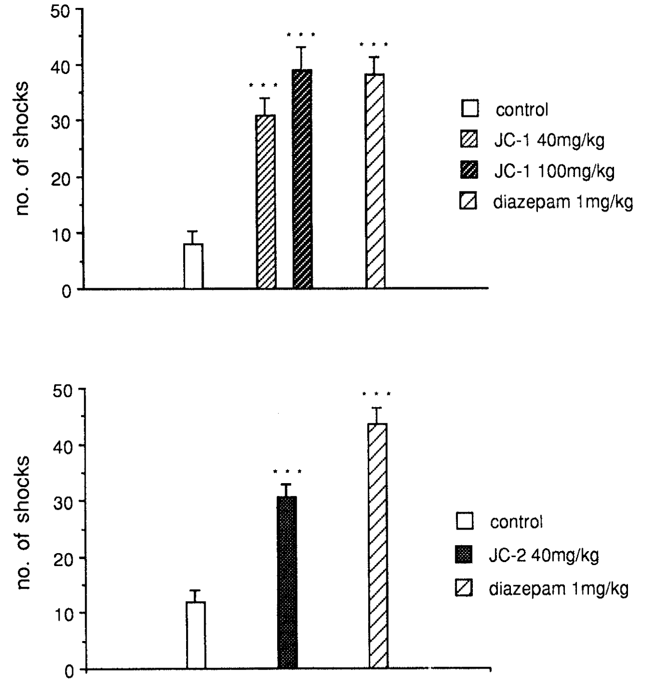


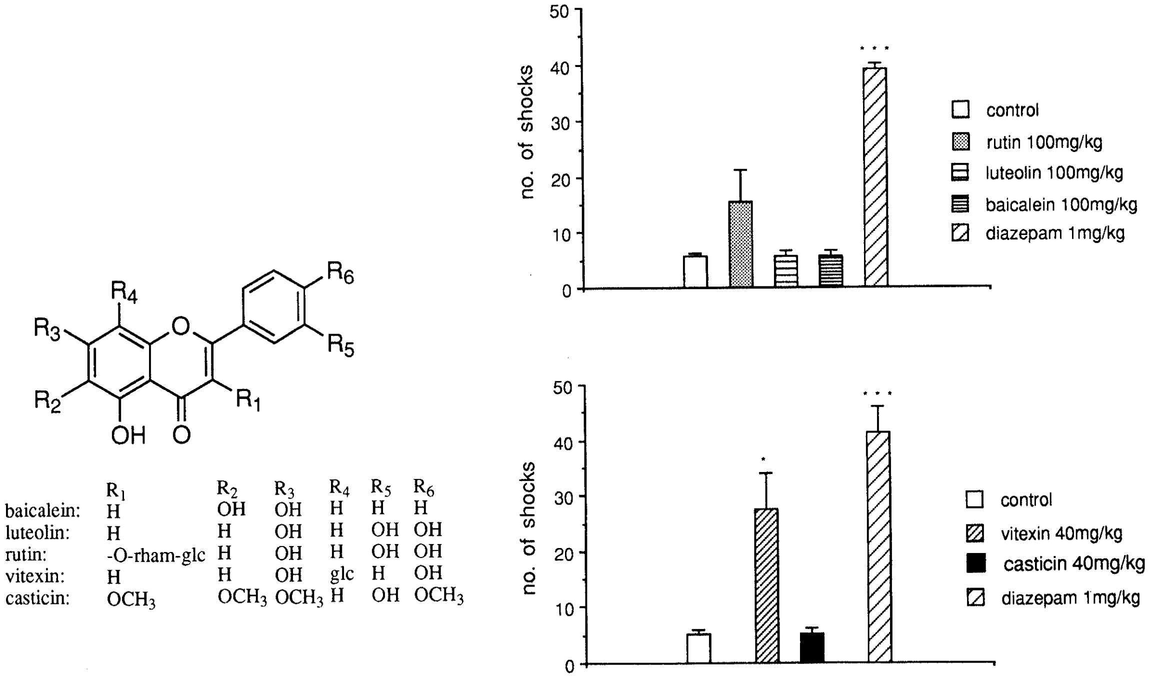
Fig. 2. Anticonflict Effects of Compounds JC-1 (above) and JC-2 (below) in Mice

$***p<0.001$. $n=6$ (above) and 7 (below).

The *n*-butanol part of 9.50 g was separated by the use of the anti-conflict effect as an isolation-guide. It was chromatographed on Sephadex LH-20 with methanol, and the active fraction of fr. 1b (0.89 g) was then applied to ODS flash chromatography. The solvent of methanol–water, 1 : 1 eluted active fr. 2b (282 mg), which showed major yellow spots on TLC. After ODS flash rechromatography of 134 mg of fr. 2b, fr. 3b (66 mg) eluted with methanol–water, 1 : 1, was separated to fr. 4a (14 mg) and 4b (49 mg) by HPLC (ODS, acetonitrile–water, 1 : 5). Compound JC-1 (41 mg) and compound JC-2 (12 mg) were obtained as active components from fr. 4b and fr. 4a, respectively, by passing them through Sephadex LH-20 (methanol) and/or ODS Sep-Pak (methanol–water, 1 : 1).

The ethyl acetate part was separated by using writhing inhibition in mice as an analgesia-guide. Chromatography on Sephadex LH-20 with methanol gave fr. 1A through fr. 1D, and the latter two fractions showed writhing inhibition. Fraction 1C (1.02 g) was flash chromatographed on ODS with a gradient elution of acetonitrile–water (1 : 5—acetonitrile only). Fraction 2C (376 mg) was a major active component, compound JC-3, which was further crystallized from methanol. No activity was observed in the other fractions, fr. 2A and 2E. Although fr. 1D showed weak activity, it was found to have some amount of compound JC-3 by TLC. Therefore, compound JC-3 was determined to be the analgesic principle.

Compound JC-1 (**1**): Yellow powder, mp 237—239 °C (lit.,[5] mp 237—239 °C), $[\alpha]_{589}^{26}$ +18° (4.7 mg/ml, methanol) [lit.[5] $[\alpha]_D$ +31.5° (methanol)]. HR-FAB-MS (PEG600) *m/z*: 449.1067 ([M+1]$^+$) (err. −1.7 mmu for $C_{21}H_{21}O_{11}$). UV λ_{max}^{MeOH} nm: 260 sh, 271, 351. ^{1}H-NMR (DMSO-d_6) δ: 3.13 (1H, dd, J=9.2, 8.3, H-4″), 3.15—3.18 (1H, m, H-5″), 3.20 (1H, dd, J=8.8, 8.3, H-3″), 3.42 (1H, dd, J=11.7, 5.7, H$_a$-6″), 3.68 (1H, dd, J=11.6, 5.5, H$_b$-6″), 4.06 (1H, dd, J=9.8, 8.8, H-2″), 4.59 (1H, d, J=9.8, H-1″), 6.45 (1H, br s, H-8), 6.62 (1H, s, H-3), 6.87 (1H, d, J=7.9, H-5′), 7.38 (1H, d, J=2.1, H-2′), 7.39 (1H, dd, J=7.9, 2.1, H-6′), 13.56 (1H, s, OH-5). ^{13}C-NMR (DMSO-d_6) δ: 61.45 (C-6″), 70.16 (C-2″), 70.59 (C-4″), 73.09 (C-1″), 78.95 (C-3″), 81.46 (C-5″), 93.59 (C-8), 102.47 (C-3), 102.94 (C-10), 108.89 (C-6), 113.04 (C-2′), 116.00 (C-5′), 118.85 (C-6′), 120.98 (C-1′), 145.89 (C-3′), 150.22 (C-4′), 156.22 (C-9), 160.63 (C-5), 163.49 (C-2), 164.20 (C-7), 181.59 (C-4). The ^{1}H- and ^{13}C-NMR of compound JC-1 were identical with published data.[6a)] It was also directly identified with the authentic sample by TLC (SiO$_2$, *n*-butanol–acetic acid–water 2 : 1 : 1; ODS, methanol–water 3 : 2 contain-



	R_1	R_2	R_3	R_4	R_5	R_6
baicalein:	H	OH	OH	H	H	H
luteolin:	H	H	OH	H	OH	OH
rutin:	-O-rham-glc	H	OH	H	OH	OH
vitexin:	H	H	OH	glc	H	OH
casticin:	OCH_3	OCH_3	OCH_3	H	OH	OCH_3

Fig. 3. Anticonflict Effects of Some Related Flavonoids in Mice

$*p<0.05$, $***p<0.001$, $n=6$—7.

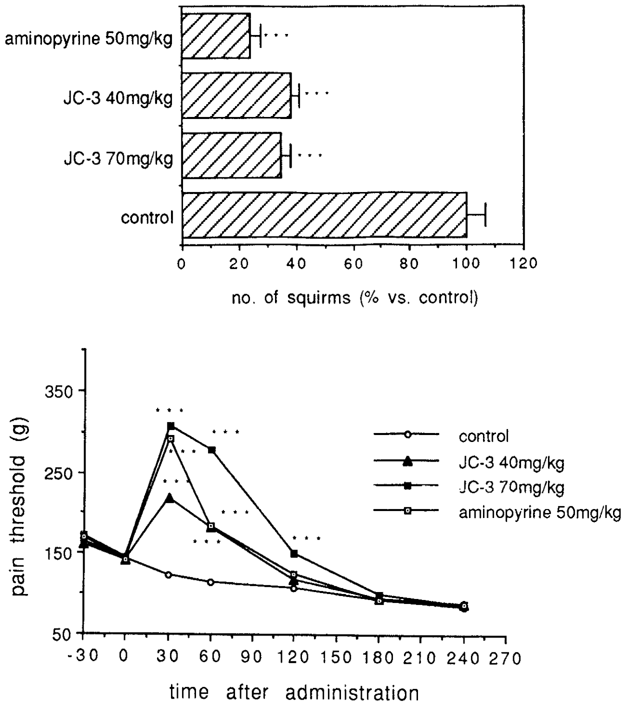


Fig. 4. Analgesic Effects of Compound JC-3 on Acetic Acid-Induced Writhing (above) and on Pressure Pain Threshold (below) in Mice

AP: aminopyrine, $***p<0.001$. $n=6$ (above) and 8 (below).

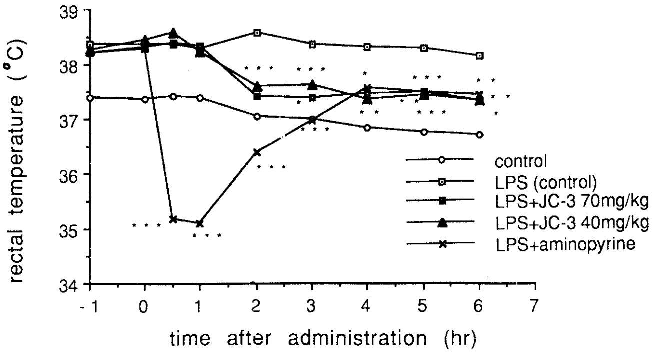


Fig. 5. Antipyretic Effect of Compound JC-3 in LPS-Treated Mice

Control means non-treated mice. The significance of each sample expresses the statistical difference from LPS (control), meaning LPS-treated mice were used as a control. Samples were administered at 0 h. $*p<0.05$, $**p<0.01$, $***p<0.001$. $n=6$.

ing one drop of acetic acid), UV and mixed mp.

Compound JC-2 (**2**): Yellow powder, mp 273—275 °C (lit.[6a] mp 275—277 °C). ORD ($c=0.85$ mg/ml, methanol) $[\alpha]^{25}$ (nm): +12° (589) [lit.,[6c] $[\alpha]_D$ +17.0° (pyridine)], +24° (500), +55° (450), +240° (420). HR-FAB-MS (NBA/PEG400) *m/z*: 449.1114 ($[M+1]^+$) (err. +3.0 mmu for $C_{21}H_{21}O_{11}$). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 259, 270 sh, 353. ^{1}H-NMR (DMSO-d_6) δ: *ca.* 3.34 (4H, overlapped with water signal, H-3″—5″, 6″a), 3.77 (1H, br d, $J=11.3$, H-6″b), 3.85 (1H, *tert*-like, $J=9.3$, H-2″), 4.71 (1H, d, $J=9.7$, H-1″), 6.20 (1H, br s, H-6), 6.59 (1H, s, H-3), 6.84 (1H, d, $J=8.3$, H-5′), 7.44 (1H, br s, H-2′), 7.49 (1H, m, H-6′), 13.17 (1H, br s, OH-5). The data were identical with published data.[6]

Compound JC-3 (**3**): Yellow needles (methanol), mp 228—230 °C (lit.,[7a] mp 228 °C). EI-MS *m/z* (%): 208 (M^+, 100). UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 210, 230 sh, 258 sh, 341. ^{1}H-NMR (CD_3OD) δ: 3.89 (3H, s, OCH_3), 6.20 (1H, d, $J=9.6$, H-3), 6.70 (1H, s, H-5), 7.83 (1H, d, $J=9.6$, H-4). ^{13}C-NMR (CD_3OD) δ: 56.78 (OCH_3), 101.03 (C-5), 112.13 (C-10), 112.61 (C-3), 134.02 (C-8), 140.60 (C-9), 140.74 (C-7), 146.77 (C-4), 147.10 (C-6), 163.78 (C-2). The NMR spectra were identical with published data.[7]

Pharmacological Assay Male mice (Std: ddY) propagated at Japan SLC, Inc. (Hamamatsu, Japan) were used. The animals were housed for around one (in analgesia and other animal tests except for the conflict test: 5 weeks, 24—28 g) or two weeks (in conflict test: 6 weeks, 29—33 g) under a 12-h light/dark cycle at 25±2 °C and were allowed free access to food and water. Samples were suspended in saline with 3% Tween 80. The following drugs were used: diazepam and aminopyrine (Wako Pure Chemical Ind., Ltd.), Tween 80 (Nacalai Tesque Co., Ltd.) and saline (Otsuka Pharmaceutical Co., Ltd.).

Anti-conflict Effect: A modified Vogel-type conflict experiment was carried out using an O'Hara Experimental Unit for Punishment for Water Consumption (PWC-5, O'Hara & Co., Japan).[4] Test animals which had been water-deprived 48 h before the experiment were individually put into the chamber of the apparatus. The mouse was punished by an electric foot shock (60 V) with every water-consumption of 0.05 ml. The voltage of the electric foot shock was altered 50 and 85 V in summer and winter time, respectively, by testing in our pre-experiment. The sum of the numbers of shocks (drinking count) for 40 min was measured 20 min after the sample was injected subcutaneously. The experiment was performed in a dark room from 19:30 to 22:30. The following substances were used: luteolin (>90% purity, Wako Pure Chemical Ind., Ltd.), baicalein (>99% purity, Wako Pure Chemical Ind., Ltd.), vitexin (chromatographycally pure standard for HPLC, Funakoshi Co., Ltd.), casticin isolated from *Vitex rotundiforia* and identified by us.

Analgesic Effect by Acetic Acid-Induced Writhing Test: Samples were orally administered 40 min prior to the intraperitoneal injection of 0.7% acetic acid (0.1 ml/10 g mouse). After 5 min, the number of squirms was counted in each mouse for the next 15 min. Aminopyrine (50 mg/kg, *p.o.*) was used as a positive control.

Analgesic Effect by Tail Pressure Test: The gradient pressure was given at the base of the mouse tail using a BASILE Analgesy-Meter (Ugo Basile, Italy). Prior to the experiment, mice were tested twice, and those having a pressure range of 100—200 g for pain-reaction were used. The reaction threshold in each animal was measured and noted at 30, 60, 90, 120 and 180 min after sample administration. Aminopyrine (50 mg/kg, *p.o.*) was used as a positive control.

Antipyretic Effect: Mice having a higher rectal temperature than the mean temperature of the control (saline) were used 12 h after the s.c. injection of LPS (50 mg/kg). Samples were administered (*p.o.*) 14 h after the LPS-treatment. Rectal temperatures in mice were measured with a thermister (Technol Seven Co., Ltd., Japan).

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

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