Antinociceptive Anti-inflammatory Effect of Monotropein Isolated from the Root of *Morinda officinalis*

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The root of *Morinda officinalis* (Rubiaceae) is used to treat rheumatoid arthritis and impotence in the traditional Oriental medicine. To identify the antinociceptive anti-inflammatory components of this crude drug, we adopted an activity-directed fractionation approach. The active fraction of the BuOH extract of *M. officinalis* root was subjected to silica gel and ODS column chromatography to yield two diterpenes, compounds 1 and 2 and these were identified as monotropein and deacetylasperulosidic acid, respectively. The iridoid glycoside, monotropein, was tested for its anti-inflammatory antinociceptive effects using hot plate- and writhing antinociceptive assays and by using carrageenan-induced anti-inflammatory assays in mice and rats. Pretreatment with monotropein (at 20, 30 mg/kg/d, *p.o.*) significantly reduced stretching episodes and prolonged action time in mice. It also significantly reduced acute paw edema by carrageenan in rats. These results indicate that monotropein contributes to the antinociceptive and anti-inflammatory action of *Morinda officinalis* root.

Key words *Morinda officinalis*; Rubiaceae; iridoid glycoside; monotropein; antinociceptive; anti-inflammatory

Morinda citrifolia (Rubiaceae) is commonly known as noni is indigenous to the Hawaiian and Tahitian islands. A number of constituents have already been isolated from this plant, and they include anthraquinones, flavonoids, iridoids and oligosaccharides.¹⁾ Moreover, it has been established that the constituents of Hawaiian noni differ from those of noni found on the Indian sub-continent. Cimanga²⁾ reported on the isolation of iridoids with complement-inhibitory activity from *Morinda* spp. Moreover, the roots of *Morinda officinalis* (Rubiaceae) continue to be traditionally used to treat rheumatoid arthritis, diabetes and hypertension in North East Asia rather than *M. citrifolia*, which is also used in traditional medicine and said to have similar pharmacologic effect. Yang *et al.*³⁾ have reported on the anthraquinones from a CHCl₃ extract of *M. officinalis*.

To isolate the active principle of *M. officinalis*, we used activity-directed fractionation. The main iridoid glycoside isolated, monotropein, was also examined by antinociceptive assay (hot plate test and writhing test) in mice and anti-inflammatory assay in rats with carrageenan-induced edema. Our investigation of the components of *M. officinalis*, resulted in the isolation of iridoid glycosides that were somewhat different from noni constituents. It is commonly known that many iridoid glycosides or other natural glycosides are easily hydrolyzed by intestinal bacteria. Therefore, we adopted oral administration approach for *in vivo* model to find the exact active compounds.

MATERIALS AND METHODS

Instruments Melting points were determined on an Electrothermal digital melting point apparatus; optical rotations on a JASCO DIP-360 digital polarimeter and IR spectra on a Bomem MB-100 FT-IR spectrometer. ¹H-NMR spectra (δ ppm, *J* in Hz) were recorded in CD₃OD using a Bruker AM-500 spectrometer (500 MHz), whereas ¹³C-NMR spectra were recorded in the same solvent using an AM-500 instru-

ment at 125.5 MHz with tetramethylsilane (TMS) as an internal standard. Sample purity was checked by TLC (Si gel, precoated plates, Merck, PF₂₅₄, 20×20 cm, 0.25 mm).

Plant Material The dried roots of *Morinda officinalis* (Rubiaceae) were purchased from the Chun-II Herb Store in Wonju, Gangwon province, Korea, and the plant was identified by Prof. S. Y. Yun, Department of Botanical Resources, Sangji University, Korea.

Extraction, Fractionation and Isolation Dried powdered roots (2.5 kg) were extracted with MeOH (2.5 l) three times under reflux. The extract was then filtered and evaporated on a rotatory evaporator under reduced pressure. The concentrated extract was freeze-dried to give a solid MeOH extract (185 g). This MeOH extract was then suspended in H₂O, and partitioned with CHCl₃. The CHCl₃ layer was then concentrated *in vacuo*, and the extract so obtained was freeze-dried to give the CHCl₃ extract (28 g). The residual H₂O layer was successively fractionated with EtOAc (8 g) and BuOH (89 g), in order, to give an EtOAc extract and a BuOH extract.

The BuOH extract (40 g) was found to contain an active fraction by activity-directed fractionation, and thus was chromatographed on a silica gel column (600 g, 8.0×30.0 cm, Merck, Art 7734, Germany) using CHCl₃–MeOH–H₂O (65:35:10, lower phase) as eluent to yield six fractions (Fr. 1–Fr. 6). On TLC documentation system (spectrophotometer CV-415.LS), it was shown that monotropein was contained in BuOH extract more than 85%. Based on the results of preliminary anti-inflammatory testing, Fr. 3 was selected for further isolation. Accordingly, Fr. 3 was chromatographed on a ODS column (octadecylsilane 30 g, 3.0×12.0 cm, YMC, AA12S75, Japan) using MeOH–H₂O (10:2) to afford compounds 1 and 2. These compounds were identified as monotropein and deacetylasperulosidic acid, respectively by spectral data.

Compound 1: Amorphous powder, mp 161–163°C, $[\alpha]_D^{25}$ –130.7° (*c*=0.05, H₂O), ¹H-NMR (500 MHz, CD₃OD) δ :

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aglycone-2.68 (1H, dd, $J=3.0, 8.6$ Hz, H-8), 3.56 (1H, m, H-5), 4.67 (1H, d, $J=7.9$ Hz, H-1'), 5.55 (1H, d, $J=7.9$ Hz, H-1), 5.61 (1H, dd, $J=1.9, 5.6$ Hz, H-7), 7.35 (1H, s, H-3), 6.21 (1H, dd, $J=2.5, 5.6$ Hz, $J=6$ Hz, H-6); ^{13}C -NMR (CD_3OD , 125.5 MHz) δ : aglycone-40.1 (C-5), 46.2 (C-9), 68.9 (C-10), 86.8 (C-8), 95.7 (C-1), 112.8 (C-4), 133.9 (C-7), 138.8 (C-6), 152.3 (C-3), 172.2 (C-11), glucose-100.6 (C-1), 73.7 (C-2), 78.8 (C-3), 71.9 (C-4), 78.4 (C-5), 62.2 (C-6).

Compound 2: Amorphous powder, $[\alpha]_{\text{D}}^{20} +26^\circ$ ($c=1.7$, MeOH), ^1H -NMR (500 MHz, CD_3OD) δ : 2.55 (1H, m, H-9), 3.00 (1H, m, H-5), 3.61 (1H, dd, $J=6.0, 12.0$ Hz, H_a-6'), 3.85 (1H, d, $J=12.0$ Hz, H_b-6'), 4.21 (1H, d, $J=15.5$ Hz, H_b-10), 4.45 (1H, d, $J=15.5$ Hz, H_b-10), 4.72 (1H, d, $J=8.0$ Hz, H-1'), 4.81 (1H, dd, $J=1.8, 5.9$ Hz, H-6), 5.05 (1H, d, $J=8.9$ Hz, H-1), 6.01 (1H, d, $J=1.8$ Hz, H-7), 7.65 (1H, s, H-3); ^{13}C -NMR (CD_3OD , 125.5 MHz) δ : aglycone-45.8 (C-1), 62.2 (C-10), 75.4 (C-5), 108.5 (C-4), 129.9 (C-7), 151.5 (C-8), 155.4 (C-3), 170.9 (C-11); glucose-101.4 (C-1), 75.0 (C-2), 78.5 (C-3), 71.6 (4), 77.8 (C-5), 61.7 (C-6).

Animals and Sample Preparation ICR male mice weighing 20–25 g and Sprague-Dawley male rats weighing 100–120 g were purchased from the Korean Experimental Animal Co. and maintained under constant conditions (temperature: $20 \pm 2^\circ\text{C}$, relative humidity: 40–60%, light/dark cycle: 12 h) for at least two weeks. Twenty-four hours before the experiment, animals were given only water. Due to diurnal enzyme activity, animals were sacrificed at a fixed time (10:00 am–12:00 am). The animal experiment was all performed without anesthesia.

The test samples (*i.e.*, MeOH extract, CHCl_3 extract, EtOAc extract, BuOH extract, and compound 1) were first dissolved in 10% Tween 80 and diluted with 0.9% saline before being orally administered. The same volume of solvent was administered to control rats and mice. Extracts were administered at 100 and 200 mg/kg once a day for a week and compound 1 was also orally administered at 20 and 30 mg/kg for the same period. Animal experiments were performed 30 min after the final administration; Aspirin (100 mg/kg, *p.o.*) and morphine (10 mg/kg, *p.o.*) were used for positive controls.

Acetic Acid (Chemical-Induced) Writhing Assay Thirty minutes after orally administering test solutions (100 or 200 mg/kg) or compound 1 (20 or 30 mg/kg) were orally

administered, 0.1 ml/10 g of 0.7% acetic acid was injected to the mice (10 mice/experimental group).⁴⁾ The numbers of abdominal contractions over the period of 20 min were counted. Acetylsalicylic acid (100 mg/kg, *p.o.*) was used as a positive control. A significant reduction in the number of abdominal contractions compared to the control was considered as a positive analgesic response.

Hot Plates (Thermal) Testing Mice (10 mice/experimental group) were administered different doses of test solutions (100 or 200 mg/kg daily) or compound 1 (20 or 30 mg/kg daily) for 1 week at 1 h after final administration they were placed on a plate (Ugo Basile, Italy) maintained at $55 \pm 1.0^\circ\text{C}$. The times taken by the animals to lick a fore or hind paw or to jump off the plate were defined as the reaction time. Morphine (10 mg/kg, *p.o.*) was used as a reference drug.

Anti-inflammatory Activity Acute inflammation was produced by injecting 0.1 ml of 1% carrageenan solution into the plantar surface of a single rat hind paw (10 mice/experimental group). Test solutions (100, 200 mg/kg) and compound 1 (20, 30 mg/kg), ibuprofen (100 mg/kg orally) as a reference agent were administered for a week before carrageenan injection. Paw volumes were monitored for 0–5 h using a plethysmometer.⁵⁾

Acute Toxicity Male ICR mice ($n=30$) were fasted 18 h prior to the toxicity test and then treated with different doses of monotropein (250, 500, 1000, 2000 mg/kg, *p.o.*). The animals were kept for a period of 48 h under observation. LD_{50} by oral administration route of compound 1 was measured at 937.5 ± 773.9 mg/kg.

RESULTS AND DISCUSSION

The MeOH extract of *M. officinalis* root was fractionated into CHCl_3 -, EtOAc- and BuOH extracts in an effort to isolate the component with antinociceptive/anti-inflammatory activity. The pretreatment of mice and rats with BuOH extract (100, 200 mg/kg, *p.o.* daily for 7 d) more significantly inhibited nociceptive or inflammatory response than the CHCl_3 and EtOAc extracts by writhing- and hot plate testing in mice and carrageenan-induced edema testing in rats (Tables 1, 2).

As shown in Table 1, the oral administration of 100,

Table 1. Antinociceptive Effect of the Methanolic Extract of *M. officinalis* Roots and of Its Fractionated Extract According to the Acetic Acid-Induced Writhing and the Hot-Plate Assay in Mice

Treatment	Dose (mg/kg, <i>p.o.</i>)	Stretching episodes ^{a)} (count/20 min)	Inhibition (%)	Action time ^{b)} (s)	Increase (%)
Control	—	60.6 ± 2.07^a	—	9.13 ± 1.27^f	—
MeOH extract	100	46.8 ± 2.28^d	19.5 ^a	14.5 ± 1.23^{cd}	58.8 ^a
	200	37.8 ± 1.30^f	37.6	15.6 ± 1.27^{bcd}	70.9
CHCl_3 extract	100	55.8 ± 2.39^b	7.9	10.4 ± 1.14^{ef}	13.9
	200	54.0 ± 1.87^{bc}	10.9	11.6 ± 1.33^{ef}	27.1
EtOAc extract	100	55.0 ± 3.53^{bc}	9.2	11.7 ± 1.39^{ef}	28.1
	200	52.6 ± 3.05^c	13.2	13.2 ± 2.10^{de}	44.6
BuOH extract	100	43.2 ± 1.48^c	36.6	14.9 ± 1.42^{cd}	63.2
	200	34.2 ± 2.77^g	47.5	16.3 ± 2.11^{bc}	78.5
Aspirin	100	18.0 ± 1.79^h	70.3	NT	—
Morphine	10	NT	—	21.4 ± 2.00^g	134.4

^a Values are % antinociceptive effect versus the control group. *a, b*) Methods indicate writhing-, and hot plate tests, respectively. NT (not tested). Values represent means $\pm$ S.D. ($n=10$). Values sharing the same superscript are not significantly different ($p<0.05$) by Duncan's multiple range test.

Table 2. Inhibitory Effect of the MeOH Extract of *M. officinalis* Roots and of Its Fractionated Extracts on Carrageenan-Induced Edema in Rats Hind Paw Model

Treatment	Dose (mg/kg)	Swelling volume (ml)				
		1	2	3	4	5 (h)
Control	—	1.33±0.08 ^a	2.39±0.06 ^a	2.90±0.05 ^a	2.38±0.03 ^a	1.90±0.05 ^a
MeOH ext.	100	1.29±0.05 ^a	1.97±0.07 ^{de}	2.21±0.06 ^d	1.77±0.02 ^c	1.87±0.07 ^{ab}
	200	1.27±0.09 ^a	1.53±0.03 ^f	1.93±0.04 ^f	1.84±0.06 ^{de}	1.58±0.08 ^{de}
CHCl ₃ ext.	100	1.30±0.05 ^a	2.28±0.05 ^b	2.86±0.07 ^{a,b}	2.26±0.05 ^b	1.85±0.06 ^{ab}
	200	1.31±0.07 ^a	2.16±0.09 ^c	2.77±0.09 ^b	2.27±0.08 ^b	1.89±0.05 ^{ab}
EtOAc ext.	100	1.28±0.09 ^a	2.18±0.08 ^{bc}	2.79±0.05 ^b	2.20±0.07 ^b	1.84±0.06 ^{ab}
	200	1.31±0.08 ^a	2.03±0.07 ^d	2.60±0.08 ^c	2.18±0.04 ^b	1.79±0.03 ^{bc}
BuOH ext.	100	1.25±0.04 ^a	1.95±0.04 ^{de}	2.17±0.03 ^d	1.73±0.04 ^{cd}	1.73±0.05 ^c
	200	1.26±0.03 ^a	1.48±0.06 ^f	1.86±0.05 ^f	1.59±0.03 ^{ef}	1.62±0.09 ^d
Ibuprofen	100	0.79±0.04 ^b	1.17±0.06 ^g	1.41±0.06 ^h	1.22±0.09 ^g	0.93±0.05 ^f

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

200 mg/kg of the BuOH extract reduced the number of writhings and stretchings induced by a 0.7% acetic acid i.p. injection by 28.7% and 43.6%, respectively, and increased hot plate latency response by 63.2% and 78.5%, respectively. Table 2 shows the inhibitory effect of MeOH extract and of its fractionated extract on carrageenan-induced edema; the MeOH extract had a significant effect at 100 and 200 mg/kg. Of the fractionated extracts, the BuOH extract exhibited most effect, thus indicating that this fraction contains active components. It was also observed that the CHCl₃ extract, which has been reported to contain anthraquinones,³⁾ was almost inactive.

We passed the BuOH extract through a silica gel column and then further purified the active fraction by ODS column chromatography to yield compounds **1** and **2**, which were identified as monotropein (**1**) and deacetylasperulosidic acid (**2**) based on its physicochemical and spectroscopic data.^{6,7)} It was shown on TLC that BuOH fraction contained much higher amount of the two compounds (**1**, **2**) than other two fractions. The structure of these two compounds is shown in Fig. 1. The NMR assignments are based on the known iridoid glycosides of asperulosidic acid, asperuloside and geniposidic acid from other plant sources, the ¹³C-NMR assignment of **2** has not been reported, and thus is reported here for the first time, as shown in Experimental section, after comparisons of the NMR data of those iridoid glycosides reported.⁸⁾ The present study is also the first to report the isolation of iridoid glycosides from *M. officinalis*. The more abundant compound **1** was examined for its antinociceptive/anti-inflammatory activity using *in vivo* assay system. Interestingly, compounds **1** and **2** were found to have the same molecular formula, and it is known that the former monotropein is chemically unstable.⁹⁾ Therefore, it is possible that deacetylasperulosidic acid is produced through nucleophilic attack by H₂O at olefinic C-6 of monotropein and the successive elimination of C₉-OH. Compound **1** was successfully purified by sequential silica gel and ODS column chromatography.

Monotropein administration significantly reduced the number of writhings and stretchings caused by 0.7% acetic acid (Fig. 2). The percentage reductions in the writhing response afforded by monotropein were 36.6% and 47.5%, respectively, at 100 mg/kg and 200 mg/kg and latencies by hot plate testing were prolonged by 64.3% and 96.1%, respectively. These results suggest that monotropein possesses cen-

Fig. 1. Structure of Monotropein (**1**) and Deacetylasperulosidic Acid (**2**) Isolated from *M. officinalis* Fig. 2. Antinociceptive Effect of Monotropein Isolated from the Roots of *M. officinalis* by Acetic Acid-Induced Writhing and Hot-Plate Assays in Mice

Methods indicate writhing- (upper), and hot plate tests (down), respectively. Values represent means ± S.D. (n = 10). **, *** indicate significantly different means ($p < 0.01$ and $p < 0.001$, respectively) versus the normal untreated control.

trally and peripherally mediated antinociceptive properties.

As shown in Fig. 3, monotropein was found to have an anti-inflammatory effect 1 h after the carrageenan injection, and maximal edema inhibition was observed at 3 h after edema induction. In particular, treatment with monotropein (30 mg/kg, *p.o.*) reduced the edema by 39.6% at 3 h, and ibuprofen (100 mg/kg, *p.o.*) treatment reduced edema rate by 62% on a volume basis. Moreover, this effect was significant

Fig. 3. Effects of Monotropein Isolated from the Roots of *M. officinalis* on Carrageenan-Induced Paw Edema in Rats

Values represent means $\pm$ S.D. ($n=10$).

for least 5 h after edema induction. When subjected to acute toxicity test using mice, any lethality was not observed up to 2000 mg/kg dose (*p.o.*). This result indicates that monotropein is very safe for clinical use.

Although a small number of reports have been issued on the biological activity of *M. citrifolia*, *i.e.* its inhibition of angiogenesis¹⁰ and LDL oxidation,¹¹ and on its nitric oxide scavenging activity,¹² few reports on the biological activity of *M. officinalis* have been reported. Soon¹³ reported that *M. officinalis* extract has hypoglycemic and anti-oxidant activities, but did not isolate active compounds. In conclusion, the present report demonstrates for the first time that monotropein a component of the root of *M. officinalis* has an-

tinociceptive/anti-inflammatory activities.

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