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In Vivo Anti-Nociceptive and Anti-Inflammatory Effect of the Two Triterpenes, Ursolic Acid and 23-Hydroxyursolic Acid, from *Cussonia bancoensis*

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Triterpenoids, ursolic acid (**1**) and 23-hydroxyursolic acid (**2**) were obtained from the hydrolysis of BuOH fraction of *Cussonia bancoensis* extract to test antinociceptive and anti-inflammatory effect of *C. bancoensis* (Araliaceae). Compound **1** and **2** exhibited anti-nociceptive effects, which were determined by acetic acid-induced writhing test and hot plate test. The effect of **2** was much more potent in acetic acid-induced writhing test than in hot plate test. Compound **1** and **2** significantly inhibited 1%-carrageenan-induced edema in the rat. These results suggest that the two triterpenes, ursolic acid and 23-hydroxyursolic acid, are responsible for the anti-nociceptive and anti-inflammatory effect of *C. bancoesnsis*.

Key words: *Cussonia bancoensis*, Triterpenes, Ursolic acid, 23-Hydroxyursolic acid, Anti-nociceptive, Anti-inflammatory

INTRODUCTION

Cussonia bancoensis Aurev. & Pellegr. (Araliaceae) is a medium size tree of 15-25m in height and mostly found in the dense humid forest and widespread from Ivory Coast to Nigeria. It also has been used in Nigeria folk medicine for the treatment of dizziness and women sterility.

We have already reported the isolation of ursolic acid (**1**), 23-hydroxyursolic acid (**2**), 3-*O*-α-L-arabinopyranosyl-23-hydroxyursolic acid, 3-*O*-β-D-glucopyranosyl-23-hydroxyursolic acid and 28-*O*-α-L-rhamnopyranosyl (1-4)-β-D-glucopyranosyl (1-6)-3-D-glucopyranosylester of **1** in LPS-activated macrophage 264.7 from *Cussonia bancoensis* Aurev. & Pellegr. (Araliaceae) and more potent NO inhibitory effect of 23-hydroxyursolic acid in LPS-induced macrophage 264.7 cells than ursolic acid (Tapondjou *et al.*, 2003).

Saponins show a broad spectrum of biological and pharmacological activities and many of them have revealed anti-tumor and anti-inflammatory activities against several *in vivo* and *in vitro* models (Lacaille-Dubois and Wagner, 1996). In the previous studies on bioactivity of natural triterpenes and triterpenoidal saponins (Park *et al.*, 1998; Choi *et al.*, 2002), we have found that sugar-linkage positions of triterpenes usually determine the cytotoxic potency against cancer cell lines or inhibition of NO production in LPS-activated macrophage cell lines (Lee *et al.*, 2000; Park *et al.*, 2001; Kim *et al.*, 2002). Ursane-type triterpenoids very rarely attach oligosaccharides at C-3 when compared with oleanane-type triterpenoids.

Since *C. bancoensis* contains mainly the triterpene glycosides than the aglycone, the isolation of hydrolysate of saponin fraction afforded high amount of ursolic acid and 23-hydroxyursolic acid to test anti-nociceptive and anti-inflammatory effect. We attempted to find any discrepancy of the pharmacological effect.

MATERIALS AND METHODS

Materials

The stem bark and leaves of *C. bancoensis* were

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collected in Bafou village (Menoua Division, Western Province of Cameroon) in April 2000. The plant material was identified by the botanists of the Department of Plant Biology of the University of Yaounde I. Specimens documenting the collection are deposited at the Cameroon National Herbarium (ref. 16896/SRF/CAM)

Preparation of ursolic acid and 23-hydroxyursolic acid from *C. bancoensis*

The dried and pulverized stem bark of *C. bancoensis* (4.0 kg) was extracted with MeOH at room temperature for three days, and the MeOH extract concentrated to dryness under reduced pressure. The residue obtained (320 g) was suspended in H_2O and successively fractionated with $CHCl_3$, and *n*-BuOH, respectively, which were dried *in vacuo* to give $CHCl_3$-soluble fraction (65 g) and *n*-BuOH fraction (142 g). n-BuOH extract was hydrolyzed in 5%-H_2SO_4 for 5 h under reflux. After cooling, it was fractionated with EtOAc and washed two times with distilled water. The EtOAc fraction was dried *in vacuo* to give 69 g of the hydrolysate. A part of hydrolysate (18 g) was subjected to column chromatography over silica gel by the eluent of $CHCl_3$-MeOH (9:1). The eluate were collected by each 80 mL and checked by spraying 50%-H_2SO_4. The fractions over retention volume 960-1280 mL (ursolic acid-containing fraction) and over 1440-1760 mL (23-hydroxyursolic acid-containing fraction) were evaporated *in vacuo*, respectively, and crystallized in MeOH to yield compounds **1** (2.3 g) and **2** (3.5 g). Compounds **1** and **2** were identified as ursolic acid and 23-hydroxyursolic acid by direct comparisons of mp, $[\alpha]_D$, TLC with authentic specimens that we have previously isolated from this plant. Compound **3** was used for the activity comparison and it was identified as tormentic acid on the basis of mp, $[\alpha]_D$ and NMR data.

Compound **1** (Ursolic acid) - Mp 279-281°C, $[\alpha]_D^{23}$ = + 70.0° (c = 0.20, MeOH) (Park *et al.*, 1993).

Compound **2** (23-hydroxyursolic acid) - Mp 280-282°C, (lit. 283-287°C), $[\alpha]_D^{25}$ = + 64° (c = 0.27, MeOH) (Srivastava *et al.*, 1989).

Compound **3** (tormentic acid) - Mp 266-268°C, $[\alpha]_D^{23}$ = +31.5° (c = 0.80, MeOH) (Yamagishi *et al.*, 1988; Takahashi *et al.*, 1974).

Preparation of test sample solutions

Test samples (MeOH extract and compounds **1-3**) were first dissolved with 10% tween 80 and diluted by saline. The same volume of solvent only was administered for the normal group. The extract and the isolated compounds (ursolic acid, 23-hydroxyursolic acid, and tormentic acid) were intraperitoneally administered at 10 mg/kg based on the preliminary experiments.

Animals

Both 4 week-old ICR male mice and Sprague-Dawley male rats were purchased from Korean Experimental Animal Co. and adapted them in a constant condition (temperature: 20±2°C, dampness: 40-60%, light/dark cycle: 12 h) for two weeks or more. Twenty-four hours before the experiment, only water was offered to the animals. Considering the variation of enzyme activity during one day, the animals were sacrificed at fixed time (10:00 A.M.-12:00 A.M.).

Acetic acid writhing and hot plate method

Test solution was orally or intraperitoneally administered 30 min before the experiment, and further 0.1 mL/10 g of 0.7% acetic acid-saline was injected intraperitoneally. 10 min after the injection, the frequency of writhing in mice was counted for 10 min (Whittle, 1949). Aminopyrine (10 mg/kg) was used as a positive control.

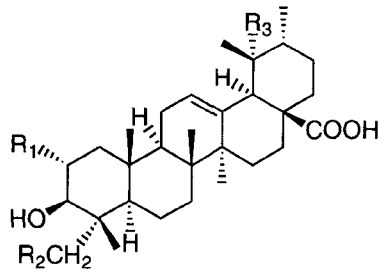
Hot plate made by Ugo Basile (Italy) was used for the measurement of antinociceptive effect by hot plate method. The response-time showing writhing syndrome was recorded.

Induction and measurement of edema

Each 0.1 mL of 1% carrageenan (Sigma Co., U.S.A.) was injected to right footprints of rats and edema was induced. The test solution (each extract: 150, 250 mg/kg p.o.; each compound: 5, 10 mg/kg) had been orally administered for 7 days. The effect was taken by plethysmometer (Ugo Basile, Italy) (Winter *et al.*, 1962). The inhibitory effect was calculated as follows: Inhibitory effect of edema (%) = (volume of control group-volume of treatment group/volume of control group)×100.

RESULTS AND DISCUSSION

MeOH extract of *C. bancoensis* exhibited significant antinociceptive effects in hot plate test and writhing test. The extract inhibited carrageenan-induced edema in the rat. Complete hydrolysis of the *n*-BuOH extract and further chromatographic isolation yielded ursolic acid (Park *et al.*, 1993) and 23-hydroxyursolic acid (Srivastava *et al.*, 1989), which were identified on the basis of mp, $[\alpha]_D$, and spectral data. The structures are shown in Fig. 1. Based on the *in vitro* anti-inflammatory effect of ursolic acid and 23-hydroxyursolic acid (data not shown) in LPS-induced macrophage 264.7, it was thought as necessary to find the *in vivo* anti-nociceptive and anti-inflammatory effect. Tormentic acid was almost inactive throughout the three assays whereas the two compounds, ursolic acid and 23-hydroxyursolic acid were active. The two triterpenes, ursolic acid and 23-hydroxyursolic acid, were more potent in writhing test assay (Table II) than in hot plate assay (Table I). In particular, 23-hydroxyursolic acid showed strong antinociceptive effect in writhing assay at 10 mg/kg dose



1 R_1 = R_2 = H (Ursolic acid)
2 R_1 = H, R_2 = OH, R_3 = H (23-Hydroxyursolic acid)
3 R_1 = OH, R_2 = H, R_3 = OH (Tormentic acid)

Fig. 1. Structure of ursolic acid, 23-hydroxyursolic acid and tormentic acid.

Table I. Effect of the extract of *C. bancoensis* and its components on action time in hot plate assay in mice at 10 mg/kg dose

Group	Action time (second)	Prolongation (%)
Control	30.2 ± 2.54	0
MeOH extract	40.7 ± 5.31	35
1 (ursolic acid)	45.8 ± 5.49*	52
2 (23-hydroxyursolic acid)	70.9 ± 7.53**	135
3 (tormentic acid)	35.7 ± 2.49	18
Aminopyrine	155.4 ± 13.5***	415

Values represent means ± S.D. (n = 10).
***p<0.001, **p<0.01, *p<0.05 compared with the control.

(i.p.), which activity was comparable with that of aminopyrine (10 mg/kg, i.p.). The active triterpenes, ursolic acid and 23-hydroxyursolic acid, significantly inhibited paw edema induced by 1%-carrageenan injection and no activity difference of potency between the two compounds was observed (Table III).

The above bioassay results suggested that the active component could be attributed to ursolic acid and 23-hydroxyursolic acid rather than to tormentic acid or their glycosides. Ryu *et al.* (2000) have reported potent *in vitro* anti-inflammatory effect of 2α-hydroxyursolic acid. Kinoshita *et al.* (1998) reported the screening assay results on *in vivo* antinociceptive action by several triterpenes isolated from cacti. It has been also reported that ursolic acid has various biological actions such as anti-inflammatory, and anti-mutagenic action in animals. In addition, some triterpenoids such as aescin, asiaticoside and glycyrrhizin, are known to have clinical significance. These bioactivities encouraged us to search for the *in vivo* anti-inflammatory activity of 23-hydroxyursolic acid. Our results suggest that this compound has especially anti-nociceptive significance in writhing assay.

Table II. Effect of the extract of *C. bancoensis* and its components on the writhing syndrome induced by acetic acid in mice at 10 mg/kg dose

Group	Frequency (Count/10 min)	Inhibition (%)
Control	41.2 ± 4.54	0
MeOH extract	34.8 ± 4.07*	15.5
1 (ursolic acid)	26.8 ± 4.96**	35.0
2 (23-hydroxyursolic acid)	16.5 ± 3.78***	60.0
3 (tormentic acid)	39.2 ± 6.11	4.9
Aminopyrine	9.83 ± 2.32***	76.1

Values represent means ± S.D. (n = 10).
***p<0.001, **p<0.01, *p<0.05 compared with the control.

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Table III. Antiinflammatory effect of *C. bancoensis* and its components, **1** (ursolic acid), **2** (23-hydroxyursolic acid), **3** (tormentic acid), on carrageenan-induced edema of the hind paw in rats at 10 mg/kg dose (i.p.).

Group	Swelling (%)			
	30 min	60 min	120 min	180 min
Control	20.2 ± 2.73	34.4 ± 4.26	53.9 ± 5.15	63.3 ± 8.58
MeOH extract	22.1 ± 4.12	34.9 ± 2.05	45.4 ± 5.430*	57.1 ± 4.56
1 (ursolic acid)	20.0 ±1.70	32.1 ± 3.41	38.7 ± 1.71*	46.5 ± 3.45*
2 (23-hydroxyursolic acid)	19.6 ± 2.03	32.3 ± 3.08	30.0 ± 2.73**	43.4 ±4.55*
3 (tormentic acid)	21.7 ± 3.36	38.4 ± 4.21	55.4 ± 5.62	57.1 ± 4.56
Indomethacin	18.2 ± 1.92	16.2 ± 1.49**	12.1 ± 1.55***	10.7 ± 1.24***

Values represent means ± S.D. (n = 10).
***p<0.001, **p<0.01, *p<0.05 compared with the control.

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