Cassiaindoline, a New Analgesic and Anti-Inflammatory Alkaloid from *Cassia alata*

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Cassiaindoline is a new dimeric indole alkaloid isolated from *Cassia alata* L. leaves whose structure was elucidated through spectroscopic analyses. It exhibited analgesic activity at a dosage of 125.0 mg/kg mouse and decreased the number of writhings induced by acetic acid by 49.4%. It also showed a 57.1% anti-inflammatory activity at a dosage of 75 mg/kg mouse.

Key words: Cassia alata L., Analgesic, Anti-Inflammatory, Cassiaindoline

Introduction

Cassia alata L. (Fabaceae), commonly known as ringworm bush or seven golden candlesticks and originated from tropical America, is now found locally abundant throughout the Philippines (Quisumbing, 1978). The known chemical constituents of *C. alata* include the anthraquinones emodin, physcion, rhein, obtusifolin, chryso-obtusin, aurantion-obtusin, aloe-emodin, chrysophanol, and alatonal (Hauptman and Nazario, 1950; Rao *et al*., 1975; Rai, 1975; Villaroya and Bernal-Santos, 1976; Smith and Sadaquat, 1979; Hemlata, 1994); the anthrones sennosides A, B and C; the bis-anthraquinones siameanine and cassiamine; cassiaxanthone, kaempferol, and β-sitosterol.

Preliminary bioactivity studies (Villaseñor *et al*., 2002) showed that the hexane extract of *C. alata* leaves is analgesic, as it reduced the number of writhings induced by acetic acid by 59.5% at a dosage of 250 mg/kg mouse. The present paper reports the isolation and structure elucidation of another analgesic constituent of *C. alata* L. through bioassay-directed fractionation, and its subsequent screening for anti-inflammatory activity.

Results and Discussion

Bioassay-directed purification of the hexane extract of *C. alata* leaves resulted in 15 fractions, FB1–FB15, of which FB8 and FB9 reduced the number of writhings induced by HOAc by 43.1% and 43.5%, respectively, at a dosage of 125 mg/kg mouse (Table I). The analgesic activities of FB8 and FB9 were statistically similar to that of mefenamic acid, a known analgesic compound, at $\alpha = 0.001$.

Subsequent purification of the combined fractions FB8 and FB9 (FB8-9) resulted in 5 subfractions with FB8-9A and FB8-9B showing analgesic activity with 43.6% and 51.4% inhibition, respectively, at the same dosage (Table I). Both contained a common constituent with $R_f = 0.30$ in EtOAc/hexane (4:6), which was eluted with EtOAc/hexane (3:7) and was detected as a brownish orange spot with vanillin/H_2SO_4. Recrystallization using Me_2CO/hexane yielded a yellowish powder, labeled as cassiaindoline, which showed analgesic activity with 49.4% inhibition (Table I) at 125 mg/kg mouse. Its variance differed from that of the solvent control at $\alpha = 0.05$.

The positive ion mode ESI-MS of cassiaindoline showed a molecular ion peak at *m/z* 568 and a base peak at *m/z* 284. Its FT-IR spectrum showed signals characteristic of O-H (3323 cm^{-1}), C=O (1662 cm^{-1}), aromatic C-C (1616 and 1507 cm^{-1}), and C-O (1130 cm^{-1}) vibrations. The ^{13}C NMR and DEPT spectra of cassiaindoline showed 12 aromatic carbon signals, four of which are -CH and the rest are quarternary, ranging from δ_C 94.5–165.0, and an acyl carbon atom at δ_C 176.6. The HMQC and COSY spectra showed that the aromatic protons at δ_H 8.16, δ_C 130.5 and δ_H 7.02, δ_C 116.4 are *ortho*-coupled ($J = 8.8$ Hz),

Table I. Analgesic activity of *Cassia alata* L. isolates using the acetic acid-induced writhing test.

Sample	Dosage [mg/kg mouse]	Average number of squirms ± SD	Inhibition (%)
HOAc	10.0 mL	51.2 ± 7.7	
Corn oil	10.0 mL	45.0 ± 8.0	
Mefenamic acid	3.5	22.4 ± 10.9	56.3
FB8	125.0	25.6 ± 9.6	43.1
FB9	125.0	25.4 ± 7.2	43.5
HOAc	10.0 mL	60.0 ± 6.0	
Corn oil	10.0 mL	51.4 ± 3.9	
Water	10.0 mL	50.0 ± 4.5	
Mefenamic acid	3.5	15.0 ± 9.1	74.1
FB8-9A	125.0	29.0 ± 5.5	43.6
FB8-9B	125.0	25.0 ± 16.4	51.4
Cassiaindoline (**1**)	125.0	26.0 ± 8.1	49.4

while the aromatic protons at δ_H 6.51, δ_C 94.5 and δ_H 6.25, δ_C 99.2 are *meta*-disposed (J = 2.0 Hz). The HMBC spectrum showed phenolic O-H cross peaks, ^2J, at δ_H 9.71 and δ_C 165.0, δ_H 9.11 and δ_C 160.2, and δ_H 12.18 and δ_C 162.3. Further cross peaks at δ_C 162.3 and δ_H 6.25 (^2J); δ_C 99.2 and δ_H 6.51 (^3J), 9.71 (^3J), 12.18 (^3J); δ_C 165.0 and δ_H 6.51 (^2J); δ_C 94.5 and δ_H 6.25 (^3J), 9.71 (^3J); δ_C 157.8 and δ_H 6.51 (^2J); and δ_C 104.2 and δ_H 6.25 (^3J), 12.18 (^3J) showed connectivities in the *meta*-substituted aromatic ring. The *para*-substituted aromatic ring showed cross peaks at δ_C 123.3 and δ_H 7.02 (^3J); δ_C 116.4 and δ_H 9.11 (^3J); and δ_C 160.2 and δ_H 7.02 (^2J).

To account for the quarternary aromatic carbon atoms at δ_C 147.1 and δ_C 136.7, an indole alkaloid is proposed with the molecular formula $C_{15}H_9NO_5$ with *m/z* 283. Inspection of the positive ion mode ESI-mass spectrum showed a base peak at *m/z* 284 $[M+H]^+$. The molecular ion peak at *m/z* 568 suggests the presence of a dimer. An indole moiety absorbed at 222.5, 262, 280, 288 nm using hexane as solvent (Kemp, 1975). The shift to longer wavelength, λ_{max} at 366.50 nm, exhibited by cassiaindoline confirmed that the indole moiety and benzene are co-planar. The structure of cassiaindoline is shown in Fig. 1.

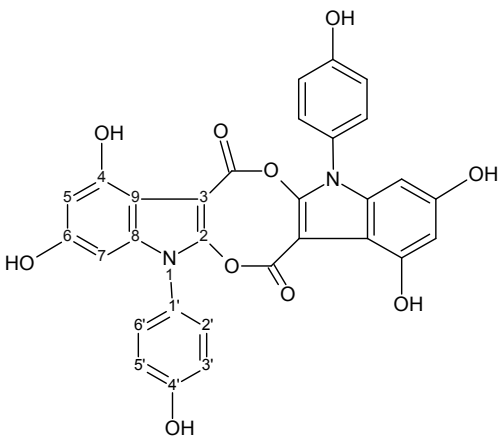

Fig. 1. Chemical structure of cassiaindoline (**1**).

When tested for anti-inflammatory activity, cassiaindoline showed a higher anti-inflammatory activity, 57.1% at a dosage of 75 mg/kg mouse (Table II), as compared to its analgesic activity, 49.4% at a dosage of 125 mg/kg mouse (Table I). Interestingly, the positive controls used in the bioassays are both *N*-containing compounds. Mefenamic acid is an anthranilic acid derivative while indomethacin is an indole derivative.

Experimental

General

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in Me_2CO-d_6 with TMS as internal standard. Normal phase liquid chromatography (NPVLC) and TLC were performed on silica gel using gradient ratios of hexane, EtOAc/hexane, and EtOH/EtOAc. Detection includes I_2, vanillin/H_2SO_4 spray followed by heating, and UV light. All bioassay data were analyzed statistically using Kruskal Wallis one-way analysis of variance by ranks (Walpole, 1997).

Table II. Anti-inflammatory activity of cassiaindoline using the carrageenan-induced mouse paw edema assay.

Sample	Dosage [mg/kg mouse]	Change in average volume [mL]	Inhibition (%)
DMSO	5.0 mL	0.047	
Cassiaindoline (**1**)	75.0	0.020	57.1
Water	5 mL	0.030	
Indomethacin	1.4285	0.016	46.7

Plant materials

Leaves of *C. alata* L. were sampled at Quezon City and dried in-doors. They were authenticated, and a voucher specimen with accession no. 13942 was deposited at the Dr. Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman.

Extraction and isolation

Air-dried leaves were homogenized in MeOH. The MeOH solution was concentrated *in vacuo* and was then partitioned between hexane and water (6:1). The analgesic hexane extract (20 g) was subjected to NPVLC using 500 mL hexane, 10% gradient ratios of EtOAc/hexane, EtOAc, and 25% gradient ratios of EtOH/EtOAc resulting in 15 fractions, labeled as FB1 to FB15, which were pooled based on their analytical TLC profiles. NPVLC of the combined fractions of FB8 and FB9 (3.9566 g) using 200 mL 5% gradient ratios of EtOAc/hexane (20–65%) resulted in 5 subfractions, labeled as FB8-9A to FB8-9E, which were combined based on their analytical TLC profiles. Both subfractions FB8-9A and FB8-9B contained a common constituent with R_f = 0.30 in EtOAc/hexane (4:6), which was eluted with EtOAc/hexane (3:7) and was detected as a brownish orange spot with vanillin/H_2SO_4. Recrystallization using Me_2CO and hexane yielded a yellowish powder.

Cassiaindoline (**1**): M.p. (uncorr.) 236 °C, with decomposition. – UV (0.001% MeOH): λ (log ε) = 222.5 (4.45), 262 (4.43), 265.5 (4.44), 280 (4.11), 288 (4.12), 317 (4.22), 366.5 (4.52) nm. – FT-IR (KBr): ν = 3323 br (O-H), 1662 (C=O), 1616 (arom. C-C), 1570, 1507 (arom. C-C), 1453 (arom. C-C), 1385, 1315, 1256, 1226, 1179, 1130 (C-O), 1090, 1009, 975 cm^{-1}. – ^1H NMR (Me_2CO-d_6): δ = 12.18 (1H, s, H-bonded 4-OH), 9.71 (1H, br s, 6-OH), 9.11 (1H, br s, 4'-OH), 8.16 (2H, br d, J = 8.8 Hz, H-2'), 7.02 (2H, br d, J = 9.2 Hz, H-3'), 6.51 (1H, d, J = 2.0 Hz, H-7), 6.25 (1H, d, J = 2.2 Hz, H-5). – 2D COSY cross peaks: H-2'/H-3'. – ^{13}C NMR (Me_2CO-d_6): δ = 176.6 (-COO-), 165.0 (C-6), 162.3 (C-4), 160.2 (C-4'), 157.8 (C-8), 147.1 (C-2), 136.7 (C-3), 130.5 (C-2'), 123.3 (C-1'), 116.4 (C-3'), 104.2 (C-9), 99.2 (C-5), 94.5 (C-7). – DEPT-CH: C-2', C-3', C-5, C-7. – 2D HMQC cross peaks: C-2'/H-2', C-3'/H-3', C-5/H-5, C-7/H-7. – 2D HMBC cross peaks: (^3J) C-1'/H-3', C-3'/4'-OH, C-9/H-5, C-9/4-OH, C-5/6-OH, C-5/H-7, C-5/4-OH, C-7/6-OH, C-7/H-5; (^2J) C-4'/4'-OH, C-4'/H-3', C-6/6-OH, C-6/H-7, C-4/H-5, C-4/4-OH, C-8/H-7. – ESI-MS: *m/z* (rel. int.) = 568 $[M]^+$ (26), 346 (15), 284 $[[M+H]^+$ (100).

Acetic acid-induced writhing test

Swiss Webster albino mice, weighing 20–25 g, were used as test animals. Five mice were used per test sample. The samples from *C. alata* leaves were dissolved in corn oil while mefenamic acid was dissolved in water. Approximately 30 min after oral administration of the test samples, 0.7% acetic acid was injected intraperitoneally (0.01 mL/g mouse). The number of writhes for each mouse was then counted for 15 min beginning 5 min after acetic acid injection (Villaseñor *et al.*, 2002).

Carrageenan-induced mouse paw edema test

The initial volume of the mouse hind paw was measured using a plethysmometer. Cassiaindoline was dissolved in DMSO. 1 h after intraperitoneal injection of cassiaindoline at a dosage of 75 mg/kg mouse, 0.01 mL 1% carrageenan solution was injected intradermally into the left hind paw. After 3 h, the volume of the mouse hind paw was measured. The difference in the initial and final volumes was then computed and compared with that of the solvent control (Villaseñor *et al.*, 2002). Five mice per test solution were used. The positive control, indomethacin, was administered orally.

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