Synthesis and Anti-inflammatory Activity of Fructigenine A Derivatives

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Abstract Several derivatives were synthesized from fructigenine A, which was isolated from *Penicillium fructigenum*. The anti-inflammatory properties of fructigenine A was evaluated *in vivo* with a 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced mouse ear edema model and a carrageenan-induced rat paw edema model. Results showed that the anti-inflammatory activity was significantly higher with fructigenine derivatives than with indomethacin, which was used as a standard. We concluded that fructigenine derivatives could exert an anti-inflammatory effect.

Keywords: *Penicillium fructigenum*, fructigenine, anti-inflammatory, edema model

Fructigenine A is an indole alkaloid isolated from *Penicillium fructigenum* by Kunizo *et al.* [1]. They proposed that fructigenine A (Fig. 1) prevents the end of a plant stem from growing, but any anti-inflammatory activity has not been reported yet. On the examination of substances with anti-inflammatory activity from fermented products of various microorganisms, we found unexpectedly a novel species of *Penicillium* producing a substance with strong anti-inflammatory activity. Our analysis surprisingly revealed that the substance produced from the novel strain was a known fructigenine A [2]. In addition, we successfully prepared some novel fructigenine derivatives by chemical synthesis methods, which exhibited the same or improved anti-inflammatory activity. Herein, we describe the synthesis of the fructigenine derivatives and their anti-inflammatory activities.

For the synthesis of fructigenine A derivatives, we started with fructigenine A (Fig. 1), which was converted to N-deacetyled compound (**3**) using a reducing agent with lithium aluminum hydride at 0°C [3]. Reaction of N-deacetylated fructigenine with acyl chlorides and triethylamine in the presence of dichloromethane at 25°C gave the each product **4a**, **4b**, and **4c** with the yield of 57~75%, respectively [4]. The structures of these products were confirmed by ^{1}H NMR spectral data as follows: (**1**) ^{1}H-NMR (400MHz, $CDCl_3$) δ 8.0 (1H, bs), 7.12-7.34 (8H, m), 6.1 (1H, bs), 5.74-5.81(1H, dd, J=17, 11 Hz), 5.09-5.14 (2H, m), 4.26(1H, dd, J=6, 2. Hz), 3.82(1H,m), 3.52(1H, dd, J=14, 3 Hz), 2.84(1H, dd, J=14, 10 Hz), 2.66(3H, s), 2.58(1H, dd, J=12.5, 3.7 Hz), 2.29(1H, t, J=12.8 Hz), 1.12(3H, s), 0.97(3H, s); ^{13}C NMR (100MHz, $CDCl_3$) δ 170.0, 168.1, 164.8, 143.4, 143.1, 135.4, 129.4, 129.2, 129.1, 127.7, 124.6, 119.2, 114.6, 79.4, 60.9, 59.2, 56.0, 40.4, 37.1, 36.2, 23.6, 23.3, 22.4; MS (FAB) *m/z* $C_{27}H_{29}N_3O_5$ $(M+H)^+$=444.55, found=444.1; (**3**) ^{1}H-NMR (400MHz, $CDCl_3$) δ 7.55 6.7(9H, m), 6.75(1H, t, J=7 Hz), 6.57(1H, d, J=7.7 Hz), 5.94(1H, m), 5.57(1H, s), 5.13(2H, m), 5.05 (1H, bs), 4.21(1H, m), 3.90(1H, m), 3.58(1H, dd, J=14.5, 7.7Hz), 2.84(1H, dd, J=14.4, 10.3 Hz), 2.50(1H, dd, J=12, 6.2 Hz), 2.40(1H, dd, J=11.3, 10 Hz), 1.14(3H, s), 1.0(3H, s); (**4a**) ^{1}H-NMR (400MHz, $CDCl_3$) δ 8.0 (1H, d, J=8.1Hz), 7.3~7.15(8H, m), 6.36(1H, bs), 5.79(1H, m), 5.68~5.64 (1H, d, J=15.4 Hz), 5.22(3H, m), 4.3(1H, m), 3.8 (1H, m), 3.49(1H, m), 2.90(1H, m), 2.56(1H, m), 2.2(1H, m), 2.2(3H, s), 1.1(3H, s), 0.98(3H, s); (**4b**) ^{1}H-NMR (400MHz, $CDCl_3$) δ 8.13 (1H, m), 7.52~7.06(14H, m), 6.36(1H, s), 5.77(1H, m), 5.04(2H, m), 4.22 (1H, m), 4.12(2H, s), 3.80(1H, m), 3.49(1H, m), 2.9(1H, m), 2.6(1H, m), 2.3(1H, m), 1.1(3H, s), 1.0(3H, s); (**4c**) ^{1}H-NMR (400MHz, $CDCl_3$) δ 8.08 (1H, d, J=8.8 Hz), 7.5-7.1(13H, m), 6.5(1H, s), 5.98(1H, m), 5.18(2H, m), 4.24(1H, m), 3.80(1H, m), 3.55 (1H, m), 2.79(1H, m), 2.57(1H, m), 2.33(1H, t, J=11.5 Hz), 1.17(3H, s), 1.09(3H, s). On the other hand, reduction of

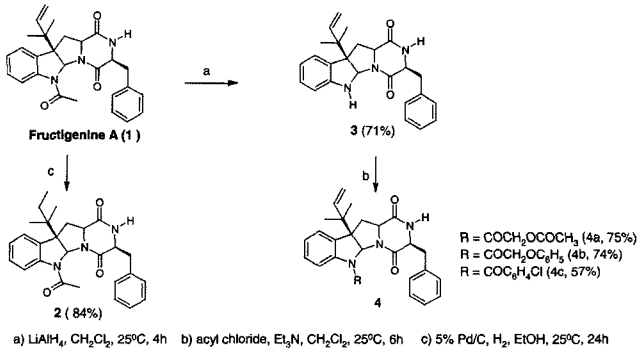

Fig. 1. Chemical synthesis of fructigenine derivatives.

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Table 1. Inhibition of edema on mouse ear* (administered topically, [a]n = 5)

Compounds	Dosage (mg/ear)	Ratio of edema occurrence (%)	Ratio of edema inhibition (%)
Control	0	101.92[a]	-
	0.175	88.75	12.92
Indomethacin	0.35	61.84	39.33
	0.7	44.89	56.45
Hydrocortisone	0.3	25.56	79.88
	0.15	65.00	36.23
Fructigenine A (**1**)	0.3	52.16	48.82
	0.6	26.91	78.82
2	0.6	29.76	76.57
3	0.6	21.33	83.21
4a	0.6	113.09	10.94
4b	0.6	92.26	27.37
4c	0.6	66.16	47.91

* The data presented are for one of two sets of separated experiments. Student's *t*-test and two-factor analysis of variance are employed (*pharmacological calculation system version* 4.1, $P < 0.05$).

the double bond was also carried out using standard hydrogenation conditions (60 psi H_2, 5% Pd/C, and EtOH) to provide **2** in near quantitative yield. Its spectral data are (**2**): ^{1}H-NMR (400MHz, $CDCl_3$) δ 8.0 (1H, bs), 7.00-7.51(8H, m), 56.18 (1H, bs), 4.26(1H, dd, J=6.7, 2.6Hz), 3.78(1H, m), 3.55(1H, dd, J=14.4, 3.5 Hz), 2.87(1H, dd, J=14.4, 4.7 Hz), 2.68(3H, s), 2.57(1H, dd, J=12.4, 5.6 Hz), 2.21 (1H, t, J=12 Hz), 1.21(2H, q, J=7 Hz), 0.88(6H, d, J=7.1 Hz), 0.83(3H, t, J=7.4 Hz).

The anti-inflammatory activities of the above compounds were determined through a mouse edema inhibition test. They were dissolved in acetone, and spotted on the right ear of the mouse (10 μL/ear) with the dosage shown in Table 1. A solution of TPA (12-O-tetradecanoylphorbol-13-acetate, Sigma Co., St. Louis, MO, USA), which was an inflammation inducible substance [5], was immediately applied to the right ear to cause edema. The TPA solution was prepared by dissolving 1 μg of TPA in 1 μL of acetone. Prior to treatment with the TPA solution, the thickness of the animal ear was calibrated. Five hours after the TPA treatment, the thickness of the mouse ear was measured again. To determine the anti-inflammatory activity of each compound, the ratio of edema occurrence and the ratio of edema inhibition were calibrated using the following equations.

Ratio of edema occurrence (%) = $(T_t - T_n) / T_n \times 100$

Ratio of edema inhibition (%) = $(E_c - E_t) / E_c \times 100$

Where

T_t = thickness of the mouse ear following TPA treatment.

T_n = thickness of the mouse ear prior to TPA treatment.

E_c = ratio of edema occurrence following topical application of the vehicle.

E_t = ratio of edema occurrence following topical application of the test compound.

As shown in Table 1, we disclosed that compounds **1**, **2**, and **3** showed stronger anti-inflammatory effect towards the mouse ear edema than indomethacin, a well-known anti-inflammatory agent in the art. Especially, *N*-deacetylfructigenine (**3**) was identified as the most potent activity. It was concluded from the above results that fructigenine A and compounds of the present study were useful for an anti-inflammatory medicament because of their 1.5 times higher anti-inflammatory activities than indomethacin.

Here, we investigated the potency of fructigenine A by measuring edema inhibition on the feet of rats (Table 2). Rat paw edema induced by carrageenan (1.0% (w/v) in distilled water), an inflammation inducible substance, was measured using a plethysmometer (Model 7140, Ugo Basile, Italy) [6]. The rats were starved for 1 day prior to the experiment and volume of rat's right foot was calibrated by the plethysmometer prior to the administration of the medicament. Purified fructigenine A (1, 10, and 100 mg/kg dissolved in 10% Tween-80) [7] and control material, indomethacin (10 mg/kg dissolved in 10% Tween-80), were independently administered separately to rat orally, after 30 min, 0.1 mL of 1% carrageenan suspension was injected into each of rat's right foot. At 1.5, 3 and 4.5 h after carrageenan injection, volume of rat's right foot was again measured. Anti-inflammatory activity was determined by the ratio of edema occurrence and the ratio of edema inhibition calculated by comparing the volume of rat's right foot before and after the injection.

Table 2. Inhibition of edema on rat foot

Compounds	Dosage (mg/kg)	Administration route	Ratio of edema inhibition (n=5)		
			Time after injection of carrageenan (h)		
			1.5	3	4.5
Fructigenine A (**1**)	1	Orally	−14.16	−7.77	−11.47
	10		10.35	35.11	18.58
	100		43.44	49.73	35.13
Indomethacin	10	Orally	18.01	53.42	40.71

In summary, the synthesis of fructigenine derivatives has been accomplished in a short step sequence. This synthesis can provide access to near class of indole alkaloid derivatives, which may be useful in the development of medications for the treatment of anti-inflammatory. Preliminary biological testings showed that *N*-deacetylated fructigenine (**2**) and a dehydrated compound (**3**) exerted potent anti-inflammatory effects in mice. As result, anti-inflammatory effects in a mouse ear edema inhibition test and a rat paw edema inhibition test could be equal and superior to indomethacin. In conclusion, the results obtained indicate that the fructigenine derivatives may have a potential anti-inflammatory activity on inhibiting the edema formation.

REFERENCES

[1] Kunizo, A., K. Kazutaka, M. Taisei, and Y. Yuzura (1989) Structure of Fructigenines A and B, new alkaloids isolated from *Penicillium fructigenum* Takeuchi. *Chem. Pharm. Bull.* 37: 2937-2939.

[2] Yoo, W. I., J. H. Chang, D. H. Kim, S. H. Lee, C. S. Lyu, H. S. Moon, K. H. Lee, D. H. Ko, S. B. Rhee, S. G. Kang, J. H. Kim, and C. H. Lee (2000) Novel fructigenine A derivatives, use of fructigenine A or its derivatives as an anti-inflammatory agent, and a novel microorganism producing fructigenine A. *WO 0020004*.

[3] Das, B. P., and D. W. Boykin (1971) Synthesis of some 10-cycloalkylaminodibenz [b,f] azepines *J. Med. Chem.* 14: 56-58.

[4] Pegurier, C., L. Morellato, E. Chahed, J.Andrieux, J. P. Nicolas, J. A. Boutin, C. Bennejean, P. Delagrange, M. Langlois, and M. Mathe-Allainmat (2003) Synthesis of New arylalkoxy amino derivatives as melatoninergic ligands *Bioorg. Med. Chem.* 11: 789-800.

[5] Young, J. M., D. A. Spires, C. J. Bedord, B. Wagner, S. J. Ballaron, and L. M. De Young (1984) The mouse ear inflammatory response to topical arachidonic acid. *J. Invest. Dermatol.* 82: 367-371.

[6]Kim, K. S., S. Y. Jung, D. K. Lee, J. K. Jung, J. K. Park, D. K. Kim, and C. H. Lee (1998) Suppression of inflammatory responses by surfactin, a selective inhibitor of platelet cytosolic phospholipase A_2. *Biochem. Pharmacol.* 55: 975-985.

[7] Osadebe, P. O. and F. B. C. Okoye (2003) Anti-inflammatory effects of crude methanolic extract and fractions of *Alchornea cordifolia* leaves. *J. Ethnopharmacol.* 89: 19-24.

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