STUDIES ON THE DIFFERENTIATION INDUCERS OF MYELOID LEUKEMIC CELLS
II. CITRININ, A NEW INDUCER OF THE DIFFERENTIATION OF M1 CELLS

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In the course of our screening program for inducers of myeloid leukemic cell differentiation[1], we have recently isolated citrinin as an active substance from the culture filtrate of a fungus, *Monosporascus cannonballus*. Citrinin induced the differentiation of mouse myeloid leukemic cells (M1)[2].

The fungus was cultivated in 500-ml Erlenmeyer flasks containing a Raulin-Thom medium (100 ml) at 27°C for 7 days.

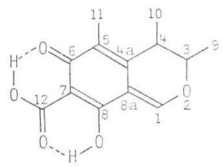
The filtrate of the fermentation broth (1 liter) was extracted twice with 500 ml of ethyl acetate at pH 2. The organic layer was back extracted twice with 5% sodium bicarbonate (500 ml). The aqueous layer was subsequently adjusted to pH 2 and extracted with 500 ml of ethyl acetate. The solvent layer was concentrated *in vacuo* to a small volume and the active material was obtained as yellow needle crystals (**1**).

The ^1H and ^{13}C NMR spectra (Fig. 1 and Table 1) indicated the presence of $=C-CH(CH_3)-CH(CH_3)-O-$ (δ_H in $CDCl_3$; 1.23 (3H, d, J=6.5 Hz), 1.35 (3H, d, J=6.5 Hz), 2.98 (1H, dq, J=6.5, <1 Hz), 4.78 (1H, dq, J=6.5, <1 Hz)), $CH_3-\underset{|}{C}=C-$ (δ_H; 2.02 (3H, s)), one olefinic proton (δ_H; 8.24 (1H, s)), chelated phenolic (δ_H; 15.88 (1H, s)) and carboxylic protons (δ_H; 15.12 (1H, br. s)). UV_{max} 238 and 340 nm in 0.1 N HCl - methanol. Based on these data, **1** was strongly suggested to be citrinin. This was proved by the ^{13}C NMR spectral data which were identical with literature values[3] and finally confirmed by direct comparison with authentic citrinin.

The effect of citrinin on the differentiation of

Table 1. ^{13}C NMR spectral data of **1** in $CDCl_3$.

Carbon	Chemical shift (ppm)
1	162.6
3	81.6
4	34.5
4a	139.0
5	122.9
6	183.6
7	100.2
8	177.1
8a	107.3
9	18.1
10	9.3
11	18.4
12	174.4



1 Citrinin

Table 2. Effect of citrinin on the growth and induction of phagocytic activity of M1 cells.

Citrinin (μg/ml)	Phagocytic cells (%)	Number of cells (cells/ml)
0	0.8	1.7×10^6
5.0	0.9	1.3×10^6
10.0	9.0	1.2×10^6
20.0	37.8	5.8×10^5
25.0	0	0

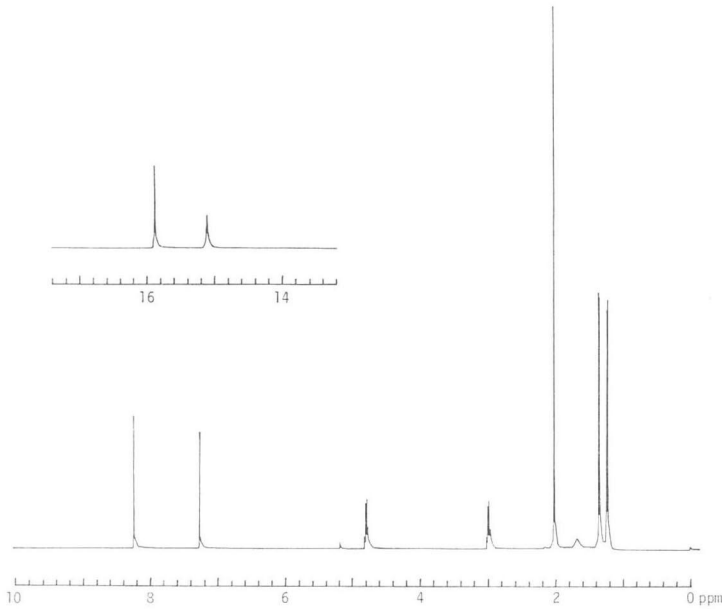
M1 cells at 1.5×10^5 cells/ml were incubated with various concentrations of citrinin for 72 hours and then their phagocytic activities were examined.

Table 3. Effect of citrinin on the growth and the rate of benzidine positive cells of B8 cells.

Citrinin (μg/ml)	Benzidine positive cells (%)	Number of cells (cells/ml)
0	0.1	2.8×10^6
5.0	0.4	2.7×10^6
10.0	19.1	1.6×10^6
15.0	39.5	4.0×10^5
20.0	40.0	4.0×10^5
30.0	0	0

B8 cells at 3.0×10^5 cells/ml were incubated with various concentrations of citrinin for 4 days.

Fig. 1. ^{1}H NMR spectrum of **1** (400 MHz in $CDCl_3$).



M1 cells were examined. On treatment with 1~30 μg/ml of citrinin for 1~3 days, the cells were induced to phagocytize latex beads and to synthesize lysozyme. Table 2 shows the phagocytic activity induced by various concentrations of citrinin for 3 days. The morphology of the cells changed into myelocyte during treatment with 20 μg/ml of citrinin for 3 days. Citrinin also induced the cell differentiation of mouse erythroleukemic cells (B8) into benzidine positive cells. Table 3 shows the rate of benzidine positive cells induced by various concentrations of citrinin for 4 days. On the other hand citrinin showed no effect to the differentiation of human promyelocytic cells (HL-60).

Citrinin is known to be active against Gram-positive bacteria and to possess carcinogenic activity[4]. Further studies on the biological activity of citrinin are in progress.

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