COMMUNICATIONS TO THE EDITOR

Cytotrienin A, a Novel Apoptosis Inducer in Human Leukemia HL-60 Cells

Sir:

Cell death occurs *via* two distinct mechanisms: necrosis and apoptosis[1]. While necrosis is a degenerative phenomenon produced by severe environmental disturbances, apoptosis is regarded as an active suicidal response to various physiological or pathological stimuli. Apoptosis is involved in embryonic development, tissue remodeling, and tumor regression. Autoreactive T cells are selectively depleted by apoptosis during clonal selection in the immune system.[2~4] Recently, it has been shown that the efficacy of various anticancer agents is dependent on the intrinsic propensity of the target cells whether if the cells respond to the anticancer agents by apoptosis or not. In fact, apoptosis is induced by various anticancer agents such as cisplatin[5], camptothecin[6], etoposide[7], and taxol[8]. The compounds which induced apoptosis in tumor cells might be a good candidate for anticancer drugs. From this point of view, we started to explore new apoptosis inducers derived from microorganisms by utilizing a human promyelocytic leukaemia cell line, HL-60 as an indicator of apoptosis. In the course of our screening, we found that an actinomycete strain RK95-74 isolated from a soil sample collected in Shiki City, Saitama Prefecture, Japan, produced a new potent apoptosis inducer, cytotrienin A (**1**) containing a triene-ansamycin, a 1-aminocyclopropane carboxylic acid and a 1-cyclohexene-1-carboxylic acid moieties. (Fig. 1).

The strain RK95-74 was identified to be *Streptomyces* sp. and deposited in the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan, with the accession No. FERM P-15904.

Cytotrienin A was obtained by the general purification procedures as a yellowish powder with mp 132~135°C and optical rotation $[\alpha]_D^{25}$ +271° (*c* 1.0, MeOH). It is soluble in MeOH, $CHCl_3$ and DMSO, and substantially insoluble in H_2O and *n*-hexane. The molecular formula of **1** was determined to be $C_{37}H_{48}N_2O_8$ from the result of positive HR-EI-MS spectrometry (Found: *m/z* 648.3355, Calcd: *m/z* 648.3407), which was supported by ^{13}C and ^1H NMR data. ^1H NMR and ^{13}C NMR are shown in Figs. 2 and 3. The UV spectrum showed absorption maxima at 262 nm (ε 45500), 273 nm (ε 46600) 283 nm (ε 45400) in MeOH, which suggested the presence of triene moiety. Its IR spectrum (KBr) revealed the absorption at 3400, 1720, 1660, 1000 cm^{-1}. Cytotrienin A has a novel structure consisting of a triene-ansamycin and a unique 1-aminocyclopropane carboxylic acid moieties. The structure determination will be a subject

Fig. 1. Structure of cytotrienin A (**1**).

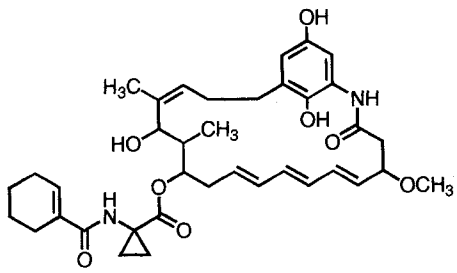


Fig. 2. ^1H NMR spectrum of cytotrienin A (400 MHz, in DMSO-d_6).

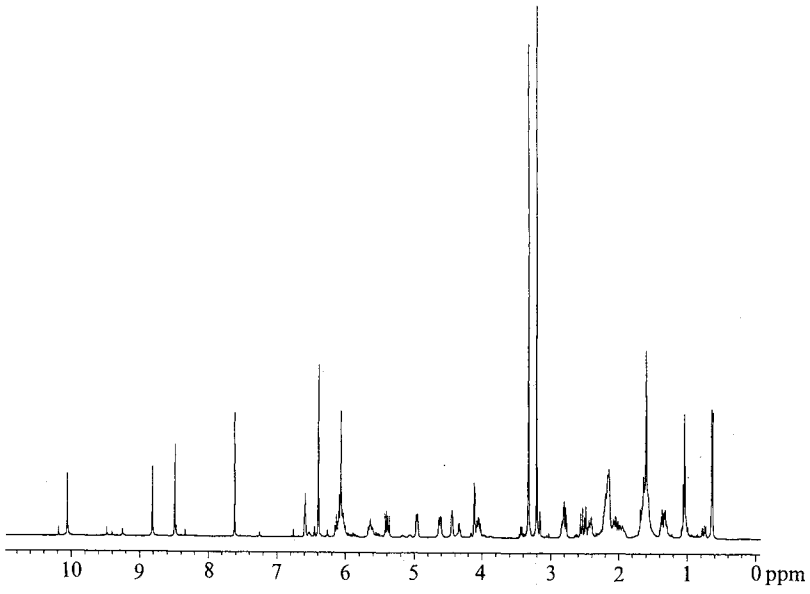


Fig. 3. ^{13}C NMR spectrum of cytotrienin A (100 MHz, in DMSO-d_6).

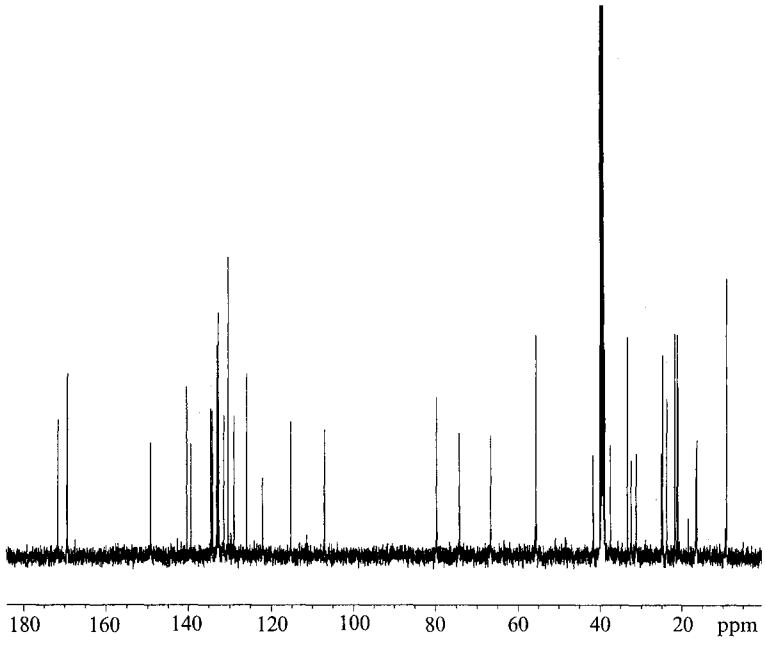
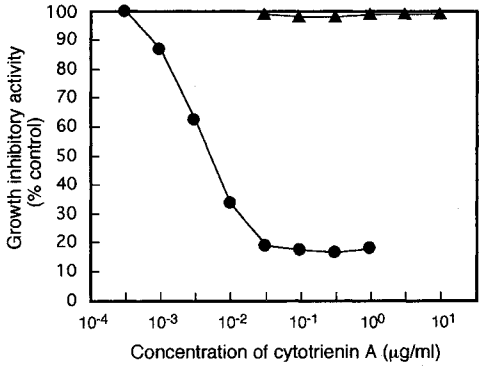
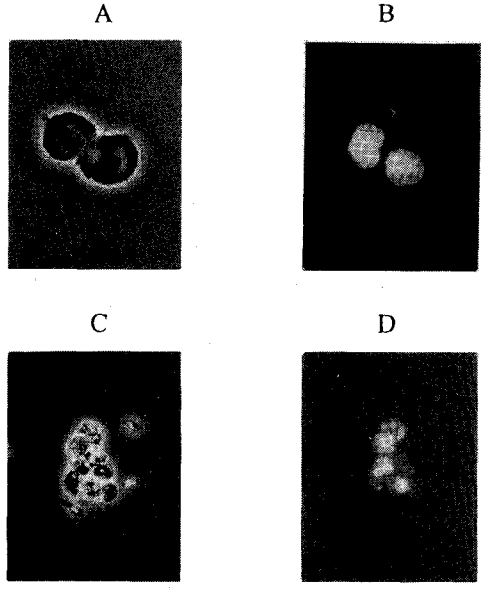


Fig. 4. Effect of cytotrienin A on the cell growth in HL-60 and WI-38 cells.



HL-60 (—●—) and WI-38 (—▲—) cells were cultured in RPMI-1640 containing 10% fetal bovine serum and DMEM containing 10% fetal bovine serum, respectively, for 1 day with various concentarations of cytotrienin A at 37°C in 5% CO_2 humidified atmosphere. The growth was measured by MTT method.

Fig. 5. Phase contrast and fluorescence micrographs of HL-60 cells treated with cytotrienin A.



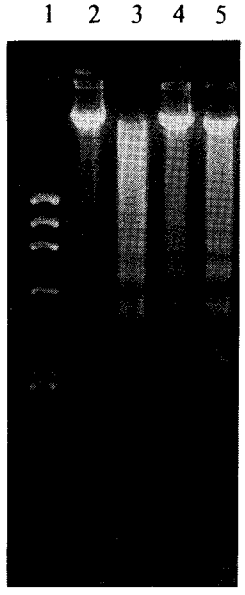
HL-60 cells were incubated for 16 hours without (A, B) or with 0.01 μg/ml of cytotrienin A (C, D). Then, the cells were fixed and stained with Hoechst Dye 33258, which observed the cells and nuclei under the phase contrast (A, C) and fluorescence microscopes (B, D).

of a separate publication[9]. Cytotrienin A is closely related to mycotrienin II[10,11] in its structure, however, different from the the ansamycin group compounds reported in the respect of the 1-aminocyclopropane carboxylic acid moiety which was found in plant metabolites but not in this group.

The antibacterial activity *in vitro* was determined by the standard agar dilution techniques. Cytotrienin A showed antimicrobial activity against *Pyricularia orizae* with a MIC value of 12.5 μg/ml. However, cytotrienin A showed no activity against *Staphylococcus aureus* FDA 209P, *Escherichia coli* BE1186, *Pseudomonas aeruginosa* N-10 *L*-form, *Candida albicans*, and *Chlorella vulgaris* at the dose of 100 μg/ml.

The cytotoxic effect of cytotrienin A on mammalian cell lines, HL-60 and human lung fibroblast WI-38 cells were shown in Fig. 4. The growth inhibitory effects of

Fig. 6. DNA fragmentaion caused by cytotrienin A in HL-60 cells.



HL-60 cells were incubated for 16 hours with 0 (lane 2), 0.1 μg/ml of actinomycin D (lane 3), 0.001 (lane 4), and 0.01 (lane 5) of cytotrienin A, respectively. Cellular DNA was extracted and electrophoresed on agarose gel as described in the previous report[8]. Lane 1: HaeIII-digested ϕX174 DNA.

cytotrienin A on these cells with the IC_{50}'s values of 0.005 and $>10\,\mu$g/ml in HL-60 and WI-38 cells, respectively. The HL-60 cells treated with 0.01 μg/ml of cytotrienin A for 16 hours contained condensed chromatin and fragmentated nuclei as visualized by staining with Hoechst Dye 33258 (Fig. 5). When HL-60 cells were treated with 0.01 μg/ml of cytotrienin A, agarose gel electrophoresis of DNA revealed a "ladder" pattern as shown in Fig. 6, indicating preferential DNA degradation at the internucleosomal cleavage of genomic DNA. These data suggest that cell death induced by cytotrienin A resulted a programmed cellular response, apoptosis[3]. Further studies on the biological studies of cytotrienin A are in progress.

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