STUDIES ON DIFFERENTIATION INDUCING SUBSTANCES OF ANIMAL CELLS. I DIFFERENOL A, A DIFFERENTIATION INDUCING SUBSTANCE AGAINST MOUSE LEUKEMIA CELLS

Sir:

We have established a screening system for differentiation inducing substances of animal cells[1]. Using this system, we have recently isolated a new substance from culture filtrate of a streptomycete, which induces differentiation of mouse leukemia cells. The active substance was named differenol A.

Streptomyces sp. ANS-127, the differenol A-producing organism was isolated from a soil sample collected on our campus in Wako-shi, Saitama and the taxonomic study indicated that it resembles *Streptomyces antibioticus*. This strain was cultivated in jar fermentors for 90 hours at 28°C with a medium containing 2% glucose, 1% starch, 0.1% meat extract, 0.4% dried yeast, 0.2% NaCl, 0.005% K_2HPO_4 and 2.5% soy bean meal in tap water.

Differenol A was extracted from the culture filtrate (28 liters, pH 8.2) with ethyl acetate at pH 7. The ethyl acetate layer was concentrated under reduced pressure and subjected to silica gel column chromatography with methanol in chloroform (0→100%). Further purification was carried out with HPLC (Nucleosil, $5C_{18}$), which was developed with acetonitrile - water (55: 45). One of the active substances, differenol A, was recrystallized from methanol - water to give colorless needles (50 mg).

Differenol A is a weakly acidic compound with melting point of 305~310°C. It has two titrable groups with pKa' of 8.6 and 10.7 in 66.7% dioxane. Elementary analysis and FD-mass spectroscopic determination of molecular weight (M^+ 270) gave the formula, $C_{15}H_{10}O_5$.

Fig. 1. UV spectrum of differenol A.

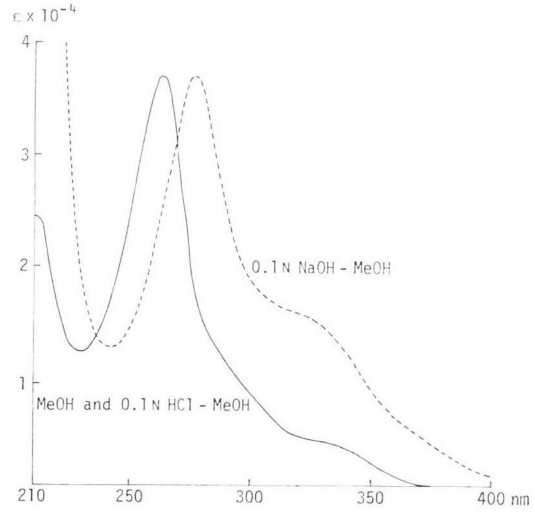
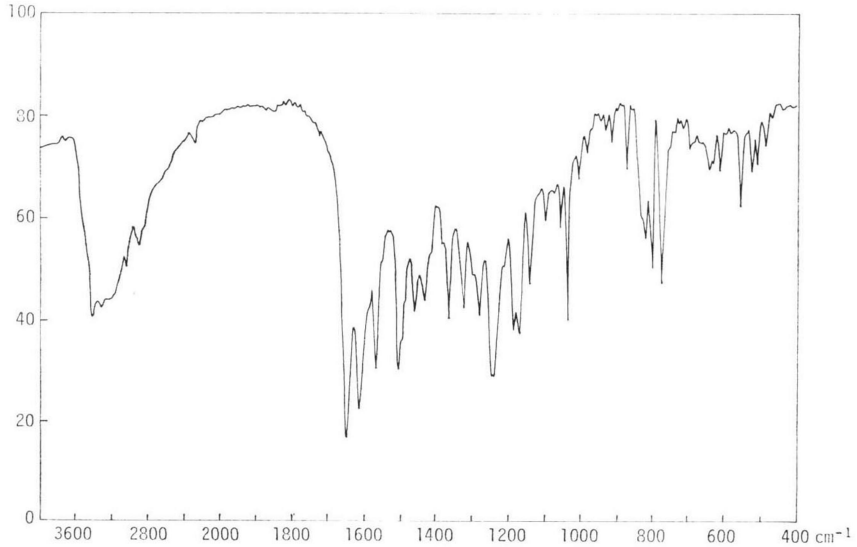


Fig. 2. IR spectrum of differenol A (KBr).



Anal. Calcd. for $C_{15}H_{10}O_5$: C 66.67, H 3.73, O 29.60
Found : C 66.67, H 3.72, O 29.58

It is optically inactive and has characteristic UV absorption maxima (Fig. 1): λ_{max} (in MeOH and 0.1 N HCl-MeOH), nm (ε): 262 (37,000), 337 (sh. 3,600); λ_{max} (in 0.1 N NaOH-MeOH): 274 (37,000), 326 (sh. 15,200). IR absorption spectrum is shown in Fig. 2. It gave the following main absorption maxima at 3430, 2920, 1650, 1617, 1571, 1510, 1465, 1436, 1240, 1188, and 1170 cm^{-1}. Differenol A is soluble in common organic solvents, *e.g.* acetone, ethyl acetate, and methanol but hardly soluble in water. It gives positive $FeCl_3$ and $KMnO_4$ reactions but negative FEHLING and 2,4-dinitrophenylhydrazine reactions.

Differenol A induces the cell differentiation of mouse erythroleukemia cells (B8) into benzidine-positive cells at concentrations of 20~400 mcg/ml (higher concentrations were not examined). Maximum % of differentiated cells was 45%, which is comparable to the value induced by 1.5% DMSO. Differenol A also induces lysozyme activity in mouse myeloid leukemia cells (M1) at 10~60 mcg/ml. Maximum lysozyme induced was 1,100 U/ml. Differenol A shows weak antimicrobial activity against *Escherichia coli* BE1186 and *Xanthomonas oryzae* (Table 1). Other microorganisms tested were not sensitive. No acute toxicity was observed after intraperitoneal injection of 100 mg per kg of body weight of mice.

Table 1. Antimicrobial activity of differenol A by paper-disc method.

Microorganism	Inhibitory diameter (mm)	
	1,000	5,000 (mcg/ml)
Xanthomonas oryzae	+	12
Escherichia coli BE1186	+	13

Added in Proof

X-Ray analysis of differenol A has shown that it is identical with genistein (4′,5,7-trihydroxyisoflavone)[2,3].

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