Communications to the Editor

DIFFERANISOLE A, A NEW DIFFERENTIATION INDUCING SUBSTANCE[1]

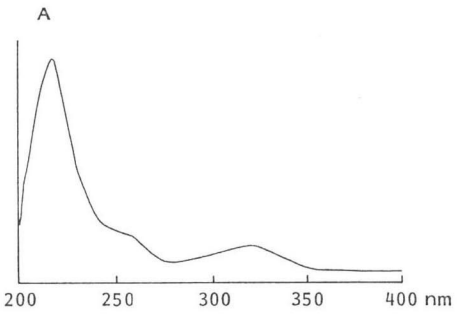
Sir:

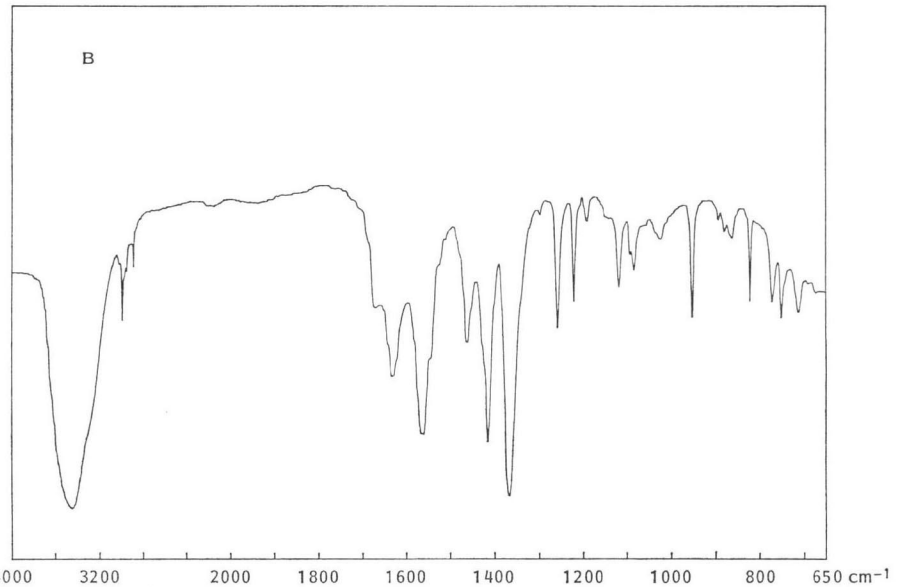
A new differentiation inducing substance against mouse leukemia cells was found in the cultured broth of a *Chaetomium* strain RB-001 isolated from a soil sample. In this communication, the isolation, characterization, and structural elucidation are reported.

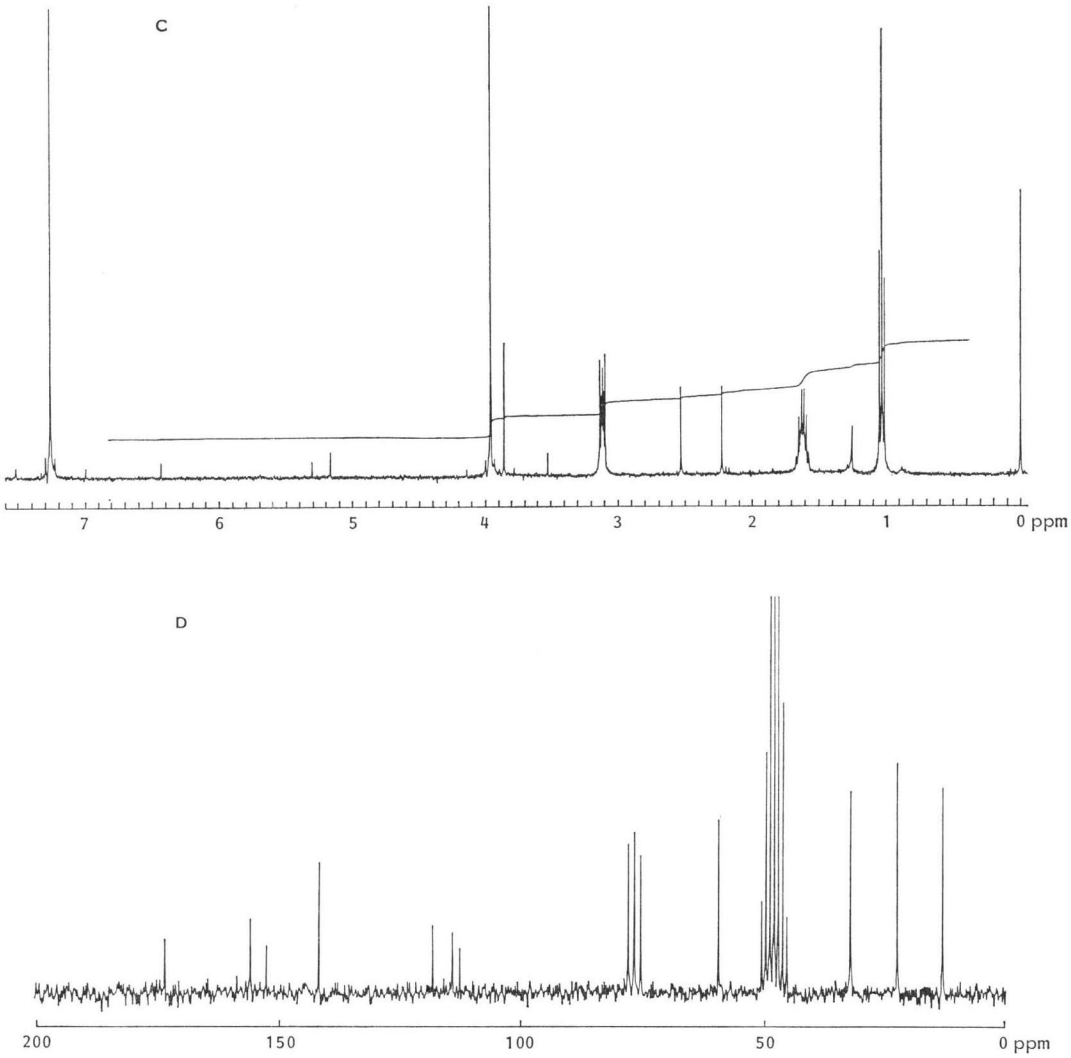
The strain RB-001 was cultivated for 7 days at 28°C with a medium containing sucrose 2%, corn steep liquor 1%, peptone 0.2%, dried yeast 0.05%, NaCl 0.1%, K_2HPO_4 0.02%, and $MgSO_4 \cdot 7H_2O$ 0.05%. The active substance was extracted from the broth filtrate with EtOAc at pH 2.5, and then transferred to 0.05 M Tris-buffer (pH 8.5). After reextraction using EtOAc under the acidic condition, the organic solvent was concd under the reduced pressure. The residue was subjected to silica gel column chromatography, which was developed with $CHCl_3$ - MeOH (9: 1). The fractions which had the differentiation inducing activities[2] were further purified by using Sephadex LH-20 column chromatography developed with 0.05 M acetate buffer (pH 4.5) - MeOH (3: 7). One of

Fig. 1.

(A) UV spectrum of differanisole A in MeOH.
(B) IR spectrum of differanisole A. Sample was run in KBr.
(C) 400 MHz ^1H NMR spectrum of differanisole A in $CDCl_3$. Peak at 0 ppm is due to Me_4Si.
(D) ^{13}C NMR of differanisole A in CD_3OD (100 MHz, proton spin decoupled).







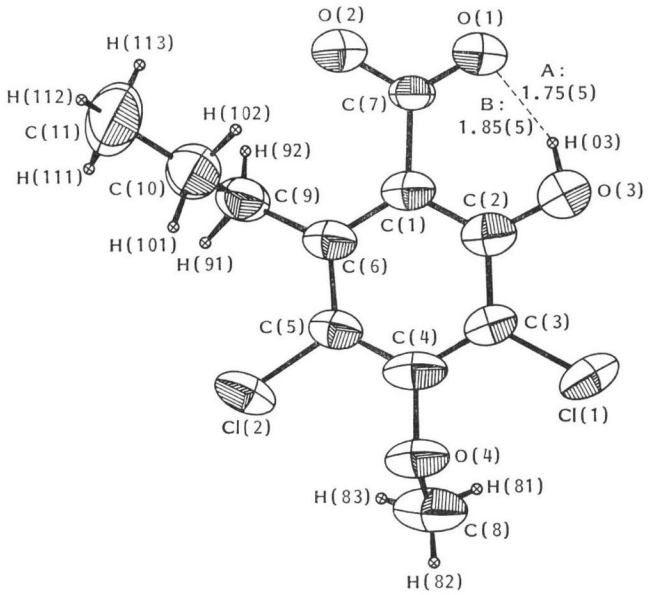
the active substances thus purified was recrystallized from MeOH - H_2O to give colorless needles, and was named differanisole A.

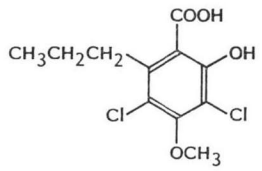
Differanisole A is an acidic compound with the melting point of 128°C. High resolution mass spectroscopic analysis showed the molecular formula of $C_{11}H_{12}O_4Cl_2$ (MW: 279.1193). It is optically inactive and has characteristic UV absorption maxima (Fig. 1A): λ_{max} in MeOH; 318 nm (ε 2,700), 257 nm (ε 4,060) and 220 nm (ε 23,000). IR, ^1H NMR, and ^{13}C NMR spectra are shown in Figs. 1B, 1C, and 1D, respectively. Differanisole A is soluble in H_2O, MeOH, acetone, DMF and DMSO, but insoluble in *n*-hexane, or petroleum ether. It gives positive iodine and $KMnO_4$ reactions, but negative Fehling and 2,4-dinitrophenylhydrazine reactions. Six aromatic carbons (δ ppm: 113.4 (1C), 115.0 (1C), 119.1 (1C), 142.7 (1C), 153.8 (1C), and 157.1 (1C) in ^{13}C NMR spectrum) suggest that the molecule is a highly substituted benzene. It is also suggested from the ^1H NMR and ^{13}C NMR spectra that differanisole A possesses *n*-propyl, methoxy, and carboxyl groups as the substituted groups.

The molecular structure was solved by single crystal X-ray analysis,[3] and was determined as 3,5-dichloro-2-hydroxy-4-methoxy-6-*n*-propylbenzoic acid as illustrated in Fig. 2.

Differanisole A induces cell differentiation of mouse erythroleukemia (B8) cells[4] to hemoglobin-producing erythrocyte-like cells at concentra-

Fig. 2. Molecular structure and atomic numbering of differanisole A.
The thermal ellipsoids are 50% probability for non-H atoms (H atoms are arbitrarily scaled).





tions above 5 μg/ml. Further studies on cell differentiation induction by this substance, its cytotoxicity, antitumor activities *in vivo etc.* are in progress (differanisole A has no antimicrobial activity against the usual test organisms). These results will be published elsewhere in the near future.

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References

1) This is the third paper of this series. The previous paper: OHNO, Y.; T. TAKUMA, K. ASAHI & K. ISONO: Differentiation induction of murine erythroleukemia cells by butylated hydroxytoluene. FEBS Lett. 165: 277~279, 1984
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