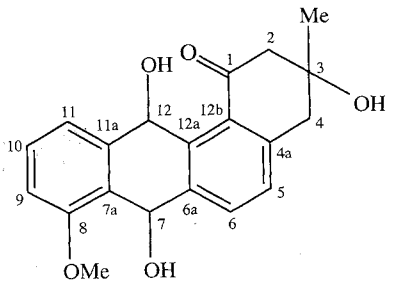
Hydranthomycin, a New Agroactive Antibiotic Produced by *Streptomyces* sp. K93-5305

Sir:

In the course of screening for new herbicidal antibiotics using *Euglena gracilis*, *Streptomyces* sp. K93-5305 was found to produce a new antibiotic, which was named hydranthomycin. This communication describes the fermentation, isolation, physico-chemical properties and biological activity of hydranthomycin (Fig. 1).

Strain K93-5305 was isolated from a soil sample collected at Shirokane, Minato-ku, Tokyo, Japan. The spores and mycelia of this strain were inoculated into test tubes (i.d. 2.0 × 20 cm) containing 10 ml of a seed medium composed of glucose 2.0%, peptone 0.5%, dry yeast 0.3%, meat extract 0.5%, NaCl 0.5%, $CaCO_3$ 3.0%, pH 7.0 before sterilization, and incubated at 27°C for 2 days on a reciprocal shaker. A 2 ml aliquot of the culture thus obtained was transferred into 500 ml Erlenmeyer flasks containing 100 ml of the same seed medium, and the flasks were incubated on a rotary shaker. For production of the antibiotics 2% vol of the second seed culture was transferred into a 50-liter jar fermenter containing 35 liters of a medium composed of starch 2.4%, glucose 0.1%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5%, $CaCO_3$ 0.4%, trace metal solution 5 ml/liter, allophane 0.5%, pH 7.0 before sterilization, and incubated at 27°C for 4 days with aeration (17 liter/minute) and agitation (250 rpm). The trace metal solution contained (each at 1 g/liter): $FeSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$ and $CoCl_2 \cdot 6H_2O$, pH 7.0.

Fig. 1. Structure of hydranthomycin.

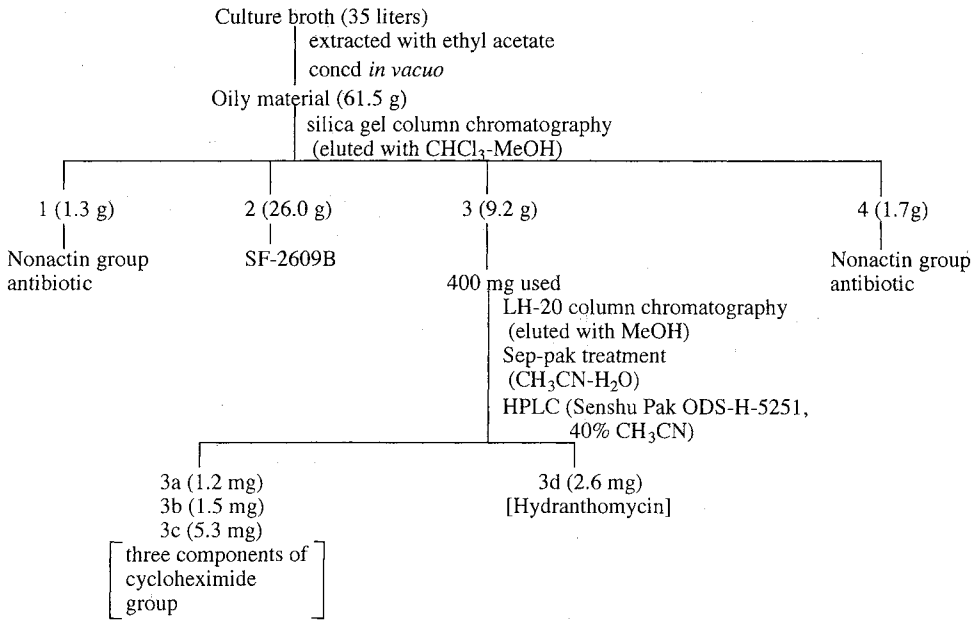


The isolation procedure for active compounds from the culture broth of strain K93-5305 is shown in Scheme 1. From the ethyl acetate extract of the fermentation broth (35 liters), followed by column chromatography on silica gel, fractions 1 (1.3 g), 2 (26.0 g), 3 (9.2 g), and 4 (1.7 g) were obtained. Fractions 1 and 4 contained nonactin group antibiotics. Fraction 2 contained SF-2609B[1]. Whereas, fraction 3 was found, after separation by HPLC, to contain three components (3a, 3b and 3c) of the cycloheximide group, and a new antibiotic hydranthomycin (3d).

The physico-chemical properties of hydranthomicin were summarized in Table 1. Its UV spectrum is presented in Fig. 2. The molecular formula of $C_{20}H_{20}O_5$ was revealed by HR FAB-MS. The analysis of these data led to the deduced structure of hydranthomycin as shown in Fig. 1.

It showed moderate antifungal activity against *Pyricularia oryzae* (MIC of 25 μg/ml). It inhibited the

Scheme 1. Isolation procedures for hydranthomycin.



Condition for HPLC: Column: Senshu Pak ODS-H-5251 (i.d. 20 × 250 mm), Mobile phase: 40% CH_3CN, flow rate: 6 ml/minute, detection by UV at 210 nm.

Fig. 2. UV spectrum of hydranthomycin (in MeOH).

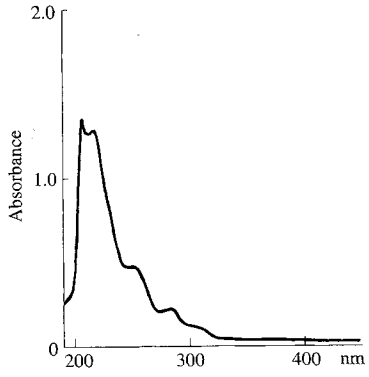


Table 1. Physico-chemical properties of hydranthomycin.

Appearance	Pale yellow powder
Melting point	117~118°C
$[\alpha]_D^{21}$	-139° (*c* 0.6, MeOH)
Molecular formura	$C_{20}H_{20}O_5$
HR-FAB-MS *m/z*, $(M+Na)^+$	Found : 363.1237 Calcd : 363.1208 ($C_{20}H_{20}O_5Na$)
UV λ_{max}^{MeOH} nm (ε)	215 (20410), 250 (6800), 283 (2890), 305 (1360)
IR ν_{max}^{KBr} cm^{-1}	3419, 1672, 1603, 1475, 1377, 1265, 1074, 987, 800

Table 2. Growth inhibitory activities of hydranthomycin.

Test organism	Amount of hydranthomycin (mg/ml)	Growth inhibition (% of control)
Euglena gracilis	1.0	96
	0.1	72
Caenorhabditis elegans	0.1	<20
Sorghum bicolor (Sorghum)	1.0	97
	0.2	41
Rhaphanus sativus (Radish)	1.0	85
	0.2	54

growth of *E. gracilis* and showed herbicidal activity (Table 2).

Several compounds of the benz[*a*]anthraquinone group were reported to date. These are tetrangomycin[2], 6-deoxy-8-*O*-methylrabelomycin[3], 8-*O*-methylrabelomycin[3], MM47755[4], SF-2609 A, B and C[1], and fujianmycin A and B[5]. The quinone moieties of them are changed to alcohol in hydranthomicin. Hydranthomycin was produced in a culture containing the phosphate-trapping agent allophane. The effects of allophane on phosphate-depression and enhancement of antibiotic production were described previously by the present authors[6]. Inspite of its weak antimicrobial activity, the cytotoxic activity of this compound could be detected by using *E. gracilis* as test organism. It is suggested that the use of *E. gracilis* led to the discovery of a new antibiotic, hydranthomycin. Detailed accounts on the taxonomy of the producing microorganism, fermentation and structure elucidation will be forthcoming.

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