NOTES

NEW ANTITUMOR ANTIBIOTIC DC-14

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In the course of our screening for new antitumor antibiotics, an actinomycete strain DO-14 isolated from a soil sample collected in Ube-shi, Yamaguchi, Japan, was found to produce a new antibiotic named DC-14.

Taxonomic studies according to the method used in the International Streptomyces Project (ISP)[1] showed that the strain DO-14 can be identified as *Streptomyces olivaceus*.

A 30-liter jar fermentor containing 18 liters of the fermentation medium was inoculated with 0.9 liters of the seed culture grown in 2-liter Erlenmeyer flasks containing 300 ml of the seed medium on a rotary shaker for 72 hours. The seed medium consisted of 10 g tryptone, 5 g yeast extract, 5 g NaCl and 1 g glucose per liter of tap water (pH 7.2) and the fermentation medium consisted of 70 g glycerol, 3 g ammonium sulfate, 20 g polypeptone, 60 mg $ZnSO_4 \cdot 7H_2O$, 30 mg $CaCl_2 \cdot 2H_2O$, 10 mg $FeSO_4 \cdot 7H_2O$, 4 mg $MnSO_4 \cdot H_2O$ and 2 mg $CuSO_4 \cdot 5H_2O$ per liter of tap water (pH 7.0). Fermentation was carried out at 30°C for 72 hours under aeration (18 liters per minute) and agitation (300 r.p.m.). The production of DC-14 was followed by an agar diffusion assay using *Bacillus subtilis* No. 10707 as the test organism.

A flow diagram for the isolation of DC-14 is given in Fig. 1. Pure DC-14 was obtained by repeated recrystallization from ethyl ether.

The physico-chemical properties of the purified DC-14 are as follows: orange needles; orange or yellow appearance in acidic solution and purple in alkaline solution; melting point, 214~215°C (dec.); UV absorption, λ_{max}^{MeOH} (nm) 243, 265~270 (sh.), 290~295 (sh.), 420; IR spectrum, characteristic absorptions attributed to OH, C=O and aromatic ring (Fig. 2); elemental analysis, PMR and CMR spectroscopic studies suggested the molecular formula $C_{43}H_{50}N_2O_{13}$; elemental analysis, Found C 65.11, H 6.24, N 3.49%, Calcd. for $C_{43}H_{50}N_2O_{13}$ C 64.34, H 6.22, N 3.49%.

These data indicate that DC-14 belongs to the anthraquinone class of antibiotics. But, DC-14

Fig. 1. Isolation procedure for DC-14.

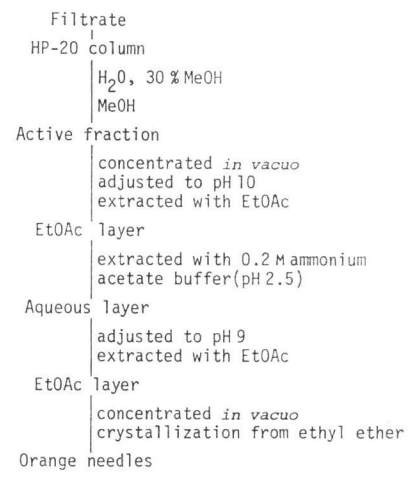

Fig. 2. IR absorption spectrum of DC-14 in KBr pellet.

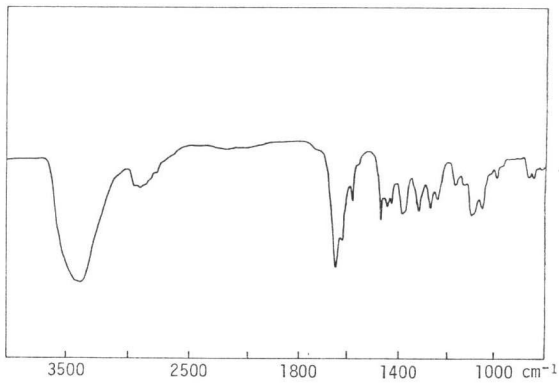

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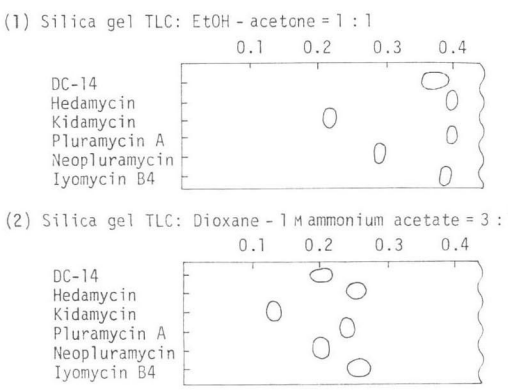
Table 1. Comparison of DC-14 with known anthraquinone antibiotics.

Compounds	Molecular formulae
DC-14	$C_{43}H_{50}N_2O_{13}$
Hedamycin	$C_{41}H_{50}N_2O_{11}$
Kidamycin	$C_{38}H_{48}N_2O_9$
Indomycin A	$C_{40}H_{50}N_2O_{10}$
Indomycin B	$C_{38}H_{48}N_2O_{10}$
Indomycin C	$C_{41}H_{52}N_2O_{11}$
Pluramycin A	$C_{43}H_{52}N_2O_{11}$
Neopluramycin	$C_{41}H_{50}N_2O_{10}$
Rubiflavin	$C_{23}H_{29\sim31}NO_5$

Fig. 3. Comparison of Rf values of DC-14 with known anthraquinone antibiotics.



is clearly different from known anthraquinone antibiotics[2~7] as shown in Table 1. The behavior of DC-14 and other anthraquinone antibiotics on silica gel TLC plates (Fig. 3) also provided support for the uniqueness of DC-14.

The antibacterial activities of DC-14 are shown in Table 2. This test was carried out by an agar dilution method using the medium consisting of 3 g tryptone, 3 g meat extract, 1 g yeast extract, 1 g glucose and 16 g agar per liter of water (pH 7.0). DC-14 inhibited the growth of Gram-positive bacteria and weakly Gram-negative bacteria. As shown in Tables 3 and 4, DC-14 showed antitumor activities against murine tumors. At a single injection of 6 mg/kg, it inhibited growth of sarcoma 180 solid-type tumor with T/C of 0.22 and it is also effective against leukemia P388 with 94% ILS (increase of life span) at a single injection of 6.2 mg/kg.

The acute toxicity (LD_{50}) of DC-14 by the intraperitoneal route in mice was about 8 mg/kg.

Table 2. Antibacterial activity of DC-14 by the agar dilution method.

Test organisms	MIC (μg/ml)
Staphylococcus aureus	1.0
Bacillus subtilis	1.0
Klebsiella pneumoniae	8.0
Escherichia coli	32
Shigella sonnei	60
Salmonella typhosa	120

Table 3. Antitumor activity of DC-14 against murine sarcoma 180.

	Dose (mg/kg)	Tumor volume (mm^3)	T/C
DC-14	0	1403	—
	1.5	1123	0.80
	3.0	944	0.67
	6.0	306	0.22
Mitomycin C	4.2	454	0.32

Drugs were injected intraperitoneally after 24 hours of tumor implantation. T/C represents the ratio of the median tumor volume of the treated group divided by that of the control group.

Table 4. Antitumor activity of DC-14 against murine leukemia P388.

	Dose (mg/kg)	Survival (day)	ILS (%)
DC-14	0	13	—
	3.1	17.5	35
	6.2	25.2	94
Mitomycin C	4.2	28.6	120

Drugs were injected intraperitoneally after 24 hours of tumor implantation. ILS % represents the increase of life span in the treated group to that of the control.

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