Notes

A NEW ANTIBIOTIC, STRUCTURALLY RELATED TO LEPTOMYCIN A, FLATTENS THE MORPHOLOGY OF v-*ras*^{ts} NRK CELLS

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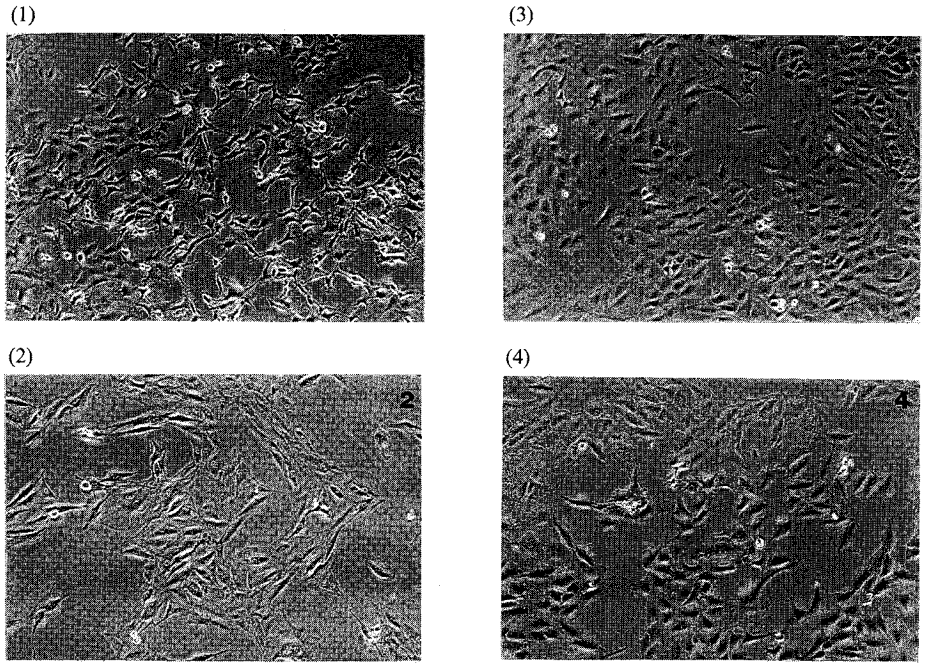
We have been screening *in vitro* microbial products which could be preferentially active against *ras* oncogene-expressed cells. For this purpose, we are using a rat kidney cell line integrating temperature-sensitive v-K-*ras* (*ras*^{ts} NRK), grown either at 33°C (a permissive temperature) or at 39°C (a nonpermissive temperature)[1]. The cells grown under the former and latter conditions are referred to as *ras*$^{+}$ and *ras*$^{-}$ cells, respectively. We found that a strain of streptomyces produced a metabolite which altered the morphology of *ras*$^{+}$ cells into something like that of *ras*$^{-}$ cells (Fig. 1). The active component was isolated and the structure was determined. The studies revealed that the active component was a new antibiotic, structurally related to leptomycin A[2]. We report here the production, isolation, physico-chemical and some biological properties of this antibiotic.

The producing microorganism, isolated from a soil sample collected at Shinagawa-ku, Tokyo, Japan was classified as *Streptomyces* sp. MJ132-NF5. From an agar slant culture of this strain, mycelial specimens were taken and inoculated into two 500-ml Erlenmeyer flasks each containing 110 ml of a medium composed of meat extract

Fig. 1. Effects of reductoleptomycin A on the morphology of *ras*^{ts} NRK cells.



Phase-contrast microscopies, *ras*^{ts} NRK cells were grown as reported (1) at 33°C (No. 1 and No. 2) and at 39°C (No. 3 and No. 4). No. 2 and No. 4 received 0.2 μl/culture of 5-day-fermented broth of *Streptomyces* sp. MJ132-NF5, while No. 1 and No. 3 received equal volumes of water.

0.3%, Tryptose 0.5%, yeast extract 0.5%, glucose 0.1%, soluble starch 2.4% and $CaCO_3$ 0.2% (pH 7.4 before sterilization). After 2 days of fermentation at 27°C on a rotary shaker, about 2 ml portions of the seed culture were transferred to 90 flasks of the same type each containing 110 ml of the same medium. Fermentation was continued for 4 days under the same conditions as for the seed culture. Throughout the purification procedures described below, the active material was chased by monitoring the activity converting the cell morphology of *ras*^{ts} NRK. The mycelia (390 g, wet) were collected on a filter and extracted with MeOH (1.5 liters × 2). The extract was concentrated *in vacuo* and the resulting solution (100 ml) was stirred with 2 liters of EtOAc. The EtOAc layer was taken and evaporated *in vacuo*, leaving an oily residue (3.2 g) which was applied to a silica gel column (5 × 30 cm in $CHCl_3$). Active fractions were eluted with $CHCl_3$-MeOH (50:1), collected and concentrated *in vacuo*. The dried residue (190 mg) was added on a Sephadex LH-20 column (3 × 60 cm) equilibrated with MeOH, and eluted with MeOH. Active fractions were combined and concentrated to yield yellow sticky oil (10 mg). The oily substance was further purified by reverse-phase HPLC (YMC-pack C_{18}, 10 × 250 mm, 80% MeOH) yielding pure material (1.3 mg).

Physico-chemical properties of the purified mate-

Table 1. Physico-chemical properties of reductoleptomycin A.

Appearance	Yellow sticky oil
$[\alpha]_D^{24}$ (*c* 0.59, EtOH)	−56.7°
Molecular formula	$C_{32}H_{48}O_5$
FAB-MS (negative, *m/z*)	511 (M^-)
HRFAB-MS (negative, *m/z*)	
Calcd. for $C_{32}H_{48}O_5Na$:	535.3399
Found:	535.3372 [$(M+Na)^+$]
UV λ_{max} nm (ε)	204 (25,000), 240 (27,000), 295 (3,700)
IR ν_{max} ($CHCl_3$) cm^{-1}	3450, 2970, 2940, 1720, 1610, 1460, 1380, 1295, 1255, 1100, 1000, 970, 830
Rf value[a]	0.50
HPLC (minutes)[b]	8.0

[a] Silica gel TLC (Merck Art, No. 5715): Toluene-EtOH-EtOAc (5:1:2).

[b] YMC-pack 5 μ C_{18}-100 Å (6 × 150 mm), mobile phase; 80% MeOH (pH 5.0), detection; 245 nm, flow rate; 1.5 ml/minute.

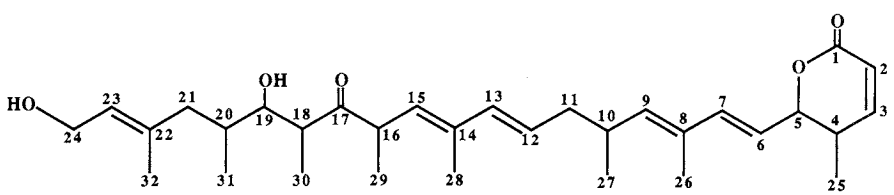
Table 2. ^{13}C (100 MHz) and ^1H (400 MHz) NMR data of reductoleptomycin A in acetone-d_6.

Position	δ_C	δ_H
1	164.0 s	—
2	120.3 d	5.90 (dd, J=1.0, 9.8)
3	152.8 d	7.09 (dd, J=5.6, 9.8)
4	34.0 d	2.63 (m)
5	81.5 d	5.09 (m)
6	125.5 d	5.79 (dd, J=6.4, 15.6)
7	130.9 d	6.85 (br d, J=15.6)
8	130.7 d	—
9	138.9 d	5.29 (br d, J=9.8)
10	33.0 d	2.77 (m)[a]
11	41.5 t	2.10 (m)[b]
12	128.0 d	5.65 (dt, J=7.3, 15.6)
13	136.5 d	6.07 (d, J=15.6)
14	135.5 s	—
15	130.0 d	5.14 (br d, J=10.3)
16	46.3 d	3.78 (dq, J=6.6, 10.3)
17	214.4 s	—
18	49.1 d	2.85 (m)[c]
19	74.2 d	3.59 (m)
19-OH		3.55
20	34.4 d	1.55 (m)
21	45.6 t	1.85 (dd, J=8.1, 12.0)[d], 2.08[e]
22	135.9 s	—
23	127.9 d	5.33 (br t, J=6.6)
24	59.2 t	4.06 (br d, J=6.6)
24-OH		3.37
25	12.5 q	1.02 (d, J=7.3)
26	20.6 q	1.84 (d, J=0.7)
27	21.0 q	0.96 (d, J=6.6)
28	13.2 q	1.85 (d, J=1.2)
29	16.7 q	1.06 (d, J=6.6)
30	14.5 q	1.11 (d, J=6.8)
31	13.4 q	0.72 (d, J=6.6)
32	15.9 q	1.54 (br s)

δ: ppm from internal TMS.

[a] Overlapped H_2O signal, [b] overlapped 21-H, [c] overlapped 10-H and H_2O signal, [d] overlapped 26-H_3 and 28-H_3, [e] overlapped 11-H_2.

Fig. 2. The structure of reductoleptomycin A.



rial are summarized in Table 1. The molecular formula was determined to be $C_{32}H_{48}O_5$ by HRFAB-MS. UV absorption maxima in neutral ethanol are at 204 nm (ε 25,000), 240 nm (27,000) and 295 nm (3,700). ^1H NMR and ^{13}C NMR data are summarized in Table 2. The assignments are in accord with those for leptomycins[2], except an alcohol group at the carbon position number 24, which is replaced by a carboxyl group in leptomycin A (Fig. 2). Because of the structural similarity, we tentatively name this compound reductoleptomycin A (RLA).

Various microorganisms were insensitive to RLA at a concentration as high as 200 μg/ml except *Schizosaccharomyces pombe* (3.2 μg/ml) by the paper disc method. Acute toxicity of RLA to mice by i.p. injection was between 6.2 mg/kg (killed) and 3.1 mg/kg (survived). Vulval formation of *Caenorhabditis elegans*, a function of the *ras*-related protein of the organism[3], was also inhibited by RLA. Studies are in progress on the molecular basis for the biological activities of RLA. Leptomycin B, a member of this family antibiotics, has been reported to act on progression of both G1 and G2 in the eukaryotic cell cycle[4].

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