DECILORUBICIN, A NEW ANTHRACYCLINE ANTIBIOTIC

Sir:

A new antitumor antibiotic, decilorubicin has been found in the culture broth of *Streptomyces virginiae* MF266-g4 (FERM P-5401, ATCC 31910) which was isolated from a soil sample collected at Nishi-Shinagawa, Tokyo. The antibiotic exhibits an antibacterial activity against Gram-positive bacteria and a prolongation effect on experimental mouse leukemia L-1210. Decilorubicin consists of an anthracyclinone chromophore, rhodosamine[1] and four other sugars.

The strain was cultured at 27°C for 48 hours on a rotatory shaker (180 r.p.m.) in a 500-ml Erlenmeyer flask containing 110 ml of a medium (2.0% glycerol, 2.0% dextrin, 1.0% soy peptone, 0.3% yeast extract, 0.2% ammonium sulfate and 0.2% calcium carbonate, pH 7.4). This culture (220 ml) in 2 flasks was used to inoculate 15 liters of a medium containing 2.0% starch, 2.0% soybean meal, 1.0% glucose, 1.0% corn steep liquor, 0.6% calcium carbonate, 0.3% sodium chloride and 0.25% ammonium chloride (pH (6.2~6.4) in a 30-liter jar fermenter and the fermentation was carried out at 27°C for 90 hours with stirring at 300 r.p.m. and aeration at 15 liters per minute. This strain produced several antibiotics, and the total antibiotic activity was measured by a cylinder-plate method using *Bacillus subtilis* PCI219 as the test organism and pure decilorubicin as the assay standard. The amount of decilorubicin was determined by HPLC with a Waters 204 Compact System with a μBondapack C18 column (6.3×305 mm) developed with a mobile phase of methanol and 10% aqueous ammonium acetate (3:1) at a flow rate of 1.0 ml/minute. Decilorubicin which was detected by UV absorption at 254 nm produced a single peak at 8.2 minutes of retention time.

Methanol was added to the culture broth (24 liters) in two fermenters (12 liters). After stirring for 1 hour, the suspension was filtered and the mycelium was washed with methanol (5 liters) and water (10 liters). The filtrate (34 liters, 430 μg/ml) and washing (15 liters, 120 μg/ml) were combined and passed through a column of Amberlite XAD-2 (3 liters) to adsorb the antibiotics. After washing the column with water (6 liters), antibiotics were eluted with a mixture of acetone and 0.001 M hydrochloric acid (1:1). The active eluate (5 liters, 2,790 μg/ml) was adjusted to pH 6.0 (NaOH), and the antibiotics in the eluate were adsorbed on a column of Amberlite CG-50 (H^+, 400 ml). After washing with a mixture (1 liter) of methanol and water (3:1), the column was eluted with a mixture of methanol and 0.1 M hydrochloric acid (4:1). The active eluate (850 ml) was adjusted to pH 5.0 with Amberlite IR-45 (OH^-) and concentrated to yield a crude powder (6,300 mg, 2,020 μg/mg). The powder was dissolved in water (500 ml). The antibiotics were extracted from the solution with a mixture (1 liter×3) of chloroform and methanol (9:1) and the extract was concentrated to yield a reddish powder (1,386 mg, 2,920 μg/mg).

The reddish powder (1,380 mg) was purified by countercurrent distribution technique using a solvent system of chloroform, methanol and 0.1 M sodium acetate buffer of pH 4.0 (2:2:1, 10 ml upper, 10 ml lower). After 79 transfers, the upper and lower phases of tube Nos. 9~26 were combined and concentrated to 110 ml. The antibiotics in the concentrate was extracted with a mixture (500 ml) of chloroform and methanol (9:1) at pH 7.4. The lower layer was concentrated to give a red powder (588 mg, 2,140 μg/mg). The red powder (580 mg) was further purified by repeated countercurrent distributions at pH 3.5 to yield a red powder (85.5 mg, 1,337 μg/mg), which contained 65% of decilorubicin (HPLC analysis).

The red powder was purified by HPLC (0.5 ml aliquots of a 10 mg/ml solution were injected) using a Waters 201 Compact System with a μ-Bondapack C18 column (9.5×305 mm) developed with a mixture of methanol and 10% aqueous ammonium acetate solution (3:1) as the mobile phase at a flow rate of 2.0 ml/minute (detected by refractive index). The eluates (220 ml) containing decilorubicin (retention time: 22~29 minutes) were combined and diluted with water (500 ml). Decilorubicin in the solution was extracted with chloroform (900 ml) at pH 7.4. The chloroform layer was washed with water (500 ml×2), dehydrated over anhydrous sodium sulfate and concentrated to dryness, yielding a red powder of pure decilorubicin, mp 170~174°C (decomp.): $[\alpha]_D^{25}+460°$ (*c* 0.05, $CHCl_3$ - MeOH, 1:1), UV maximum in methanol: 220 (ε 28000), 235 (35000), 254 (27400),

290 (6800), 380 (3200), 476 (9400), 496 (10200), 535 (7600), 586 (4300) nm, in 0.1 M HCl - 90% methanol: 220 (ε 28000), 235 (37100), 255 (32200), 292 (8300), 383 (3400), 475 (11800), 498 (12500), 535 (7000) nm, in 0.1 M NaOH-90% methanol: 253 (ε 35400), 295 sh (6400), 360 (5000), 560 (13600), 597 (13500) nm; IR (KBr): 3480, 2920, 1735, 1620, 1570, 1545, 1430, 1360, 1290, 1255, 1200, 1160, 1120, 1080, 1060, 1020, 980, 955, 915, 880, 850, 810, 760 cm^{-1}. *Anal.* Calcd. for $C_{60}H_{82}N_4O_{26}$: C 56.51, H 6.48, N 4.39. Found: C 56.42, H 6.42, N 4.59. FD-MS: *m/z* 1,275 (MH^+).

TLC on Silica gel 60 plates (E. Merck) showed Rf 0.27 (chloroform - methanol - 10% aqueous ammonium acetate, 20: 15: 1), Rf 0.06 (chloroform - methanol, 1: 1) and Rf 0.10 (chloroform - methanol - acetic acid, 20: 5: 1). Under high-voltage paper electrophoresis at 3,500 V for 10 minutes in formic acid - acetic acid - water (1: 3: 36) as an electrolyte solution, it moved to cathode with an Rm (relative mobility to alanine) of 0.74. The antibiotic is soluble in dimethyl sulfoxide, *N,N*-dimethylformamide, pyridine and a mixture of chloroform and methanol. It is almost insoluble in water, but its dihydrochloride is slightly soluble in water.

Decilorubicin (79.0 mg) in a mixture of chloroform and methanol (9: 1) was treated with an excess of diazomethane in ethyl ether to yield its methyl ester (76.0 mg), mp 178~182°C (decomp.): $[\alpha]_D^{25}$ +462° (*c* 0.05, $CHCl_3$); UV maximum in methanol: 220 (ε 29000), 235 (36700), 254 (27000), 290 (7700), 380 (3600), 476 (11300), 495 (11600), 533 (7100), 580 (600) nm, in 0.1 M HCl - 90% methanol: 221 (ε 28300), 235 (37400), 254 (32500), 292 (8400), 380 (3900), 476 (12000), 498 (12600), 535 (7300) nm, in 0.1 M NaOH - 90% methanol: 253 (ε 33900), 295 sh (6700), 360 (5200), 561 (13400), 598 (13100) nm; IR (KBr): 3400, 2920, 1735, 1620, 1570, 1545, 1425, 1380, 1360, 1270, 1200, 1160, 1120, 1080, 1060, 1020, 980, 955, 915, 885, 855, 810 cm^{-1}. *Anal.* Calcd. for $C_{61}H_{84}N_4O_{26}$: C 56.83, H 6.57, N 4.35. Found: C 56.15, H 6.70, N 4.23. FD-MS: *m/z* 1,289 (MH^+).

Hydrogenolysis of the methyl ester (76.0 mg) with palladium on barium sulfate in methanol in a Parr apparatus at 3.5 kg/cm^2 for 3 hours followed by silica gel column chromatography (chloroform - methanol, 3: 1) gave L-rhodosamine[1] (7.0 mg). Its hydrochloride showed

Table 1. Minimum inhibitory concentrations of decilorubicin dihydrochloride on nutrient agar plates.

Test organism	MIC (μg/ml)
Staphylococcus aureus FDA209P	1.56
Staphylococcus aureus Smith	6.25
Micrococcus flavus FDA16	3.13
Micrococcus lysodeikticus	3.13
Micrococcus luteus PCI1001	1.56
Bacillus anthracis	<0.78
Bacillus subtilis NRRL B-558	1.56
Bacillus subtilis PCI219	1.56
Bacillus cereus ATCC10702	1.56
Corynebacterium bovis 1810	6.25
Mycobacterium smegmatis ATCC607	25
Escherichia coli NIHJ	>100
Escherichia coli K-12	>100
Shigella dysenteriae JS11910	>100
Shigella flexneri 4b JS11811	50
Shigella sonnei JS11746	>100
Salmonella typhi T-63	>100
Salmonella enteritidis 1891	>100
Proteus vulgaris OX19	>100
Proteus mirabilis IFM OM-9	>100
Proteus rettgeri GN311	>100
Proteus rettgeri GN466	>100
Serratia marcescens	>100
Pseudomonas aeruginosa A3	>100
Klebsiella pneumoniae PCI602	100

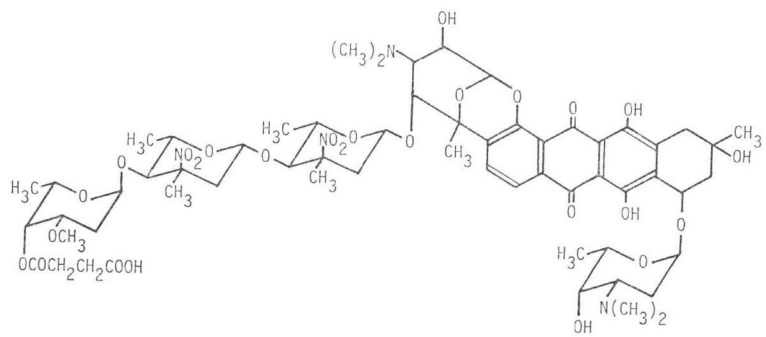

Table 2. Antitumor effect of decilorubicin dihydrochloride on mouse leukemia L-1210.

Dose (mg/kg/day)	T/C (%)
2.50	177
1.25	139
0.625	127
0.313	120
0.156	114

Inoculation: 10^5 cells/CDF_1 mouse i p.,
Administration: i.p. for 10 days (day 0~9),
Prolongationrate (T/C, %)=mean survival period of treated/mean survival period of controls.

$[\alpha]_D^{24}$ −46° (*c* 1, H_2O) (Reference 2: $[\alpha]_D^{20}$ −48.2°).

A tentative structure for decilorubicin derived from spectral data and degradation studies is as shown in the preceding page. The stereochemistry of the aglycone remains undefined. Structural studies will be reported in due course.

Minimum inhibitory concentrations of decilorubicin dihydrochloride against a selection of bacteria on nutrient agar plates were determined. Decilorubicin dihydrochloride inhibited the growth of Gram-positive bacteria as shown in Table 1. A prolongation effect in the survival period of mice implanted with the mouse leukemia L-1210 cells was observed after intraperitoneal injection of decilorubicin dihydrochloride, as shown in Table 2. Acute LD_{50} of decilorubicin dihydrochloride in mice was 50~100 mg/kg by the intravenous or intraperitoneal injection.

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