Communications to the editor

ARUGOMYCIN, A NEW ANTHRACYCLINE ANTIBIOTIC

Sir:

During the course of screening for new antitumor antibiotics, the cultured broth of an organism 1098-AV_2 showed antitumor activity and was found to contain a new anthracycline antibiotic which we named arugomycin. In this communication, the isolation and characterization of arugomycin are reported.

Strain 1098-AV_2 was isolated from a soil sample collected at Motoyama, Saga, Japan. The taxonomic studies were carried out in accordance with the method adopted by the International Streptomyces Project (ISP). The microscopical and cultural studies and cell wall analysis of 1098-AV_2 indicated that this strain belongs to the genus *Streptomyces*. Based on the cultural characteristics and physiological properties, it was identified as a strain of *Streptomyces violochromogenes*. A detailed description of the classification will be reported elsewhere. This organism was cultured at 27°C for 6 days in 500 ml Erlenmeyer flasks containing 100 ml of a medium, composed of 2% starch, 1% soybean meal, 0.2% yeast extract, and 0.4% $CaCO_3$ (pH 7.0).

The cultured broth filtrate (2 liters) was adjusted to pH 2.0 and applied to a column of Diaion HP-20. The column was developed successively with water, 50% methanol, and then eluted with methanol. The eluate was concentrated to a small volume *in vacuo* and extracted with chloroform. The solvent layer was evaporated *in vacuo* to give crude arugomycin (0.4 g). The crude material was chromatographed on a silicic acid column with chloroform - methanol (20: 1). The active fraction collected was then applied to a Sephadex LH-20 column and developed with chloroform - methanol (1: 1). The active eluate was concentrated *in vacuo* to give an orange

Fig. 1. Structure of arugomycin.

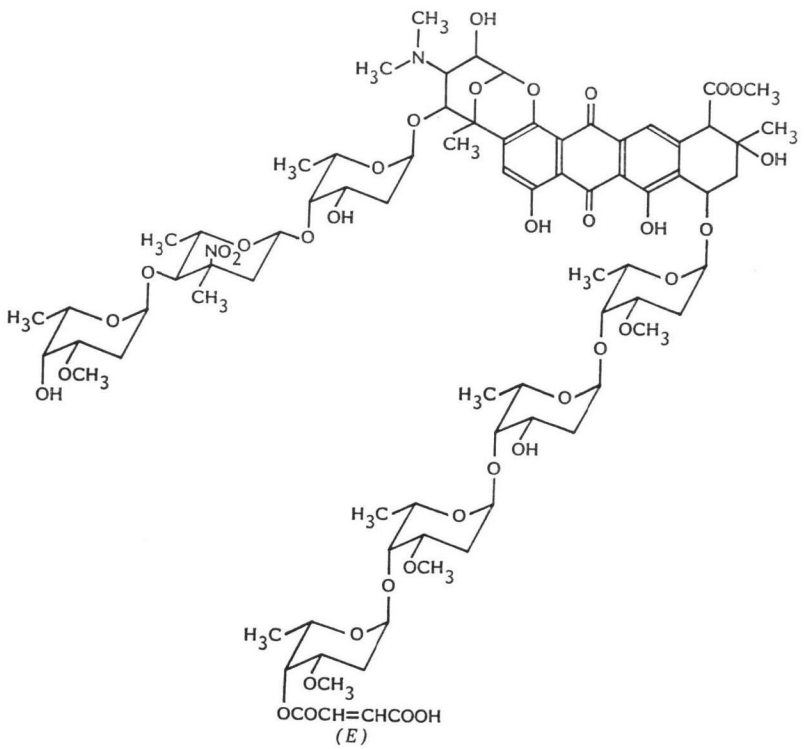

Table 1. Antimicrobial activity of arugomycin.

Organisms	MIC (μg/ml)
Bacillus subtilis PCI 219	12.5
Staphylococcus aureus FDA 209 P	12.5
Micrococcus luteus ATCC 9341	12.5
Pseudomonas aeruginosa NCTC 10490	>100
Salmonella typhimurium IFO 12529	>100
Escherichia coli NIHJ JC-2	>100
Saccharomyces cerevisiae No. Yu 1200	>100
Candida albicans IFO 0396	>100
Aspergillus fumigatus IFO 4400	>100
Penicillium chrysogenum ATCC 10002	>100

Table 2. Antitumor activity of arugomycin against Ehrlich carcinoma.

Dose (mg/kg)	Effect T/C(%)	Survivors on day 40
0.063	96	0 / 6
0.125	107	0 / 6
0.250	154	0 / 6
0.5	189	2 / 6
1.0	119	0 / 6

Treatment: 1, 3, 5 days, i. p.
Tumor inoculum: 10^6 ascites cells implanted i.p.
Host: *dd*Y♂ mice.

powder (0.2 g) of pure arugomycin; mp 208~212°C (dec.): $[\alpha]_D^{25}$ +112° (*c* 0.1, $CHCl_3$ - MeOH, 9:1): UV maximum in methanol λ_{max} ($E_{1cm}^{1\%}$) 235 (363), 258 (167), 292 (61), 476 (104) nm, in acidic methanol 235 (387), 258 (159), 292 (61), 468 (110) nm, in alkaline methanol 239 (302), 294 (41), 543 (88) nm: IR (KBr) 3430, 2970, 2930, 2830, 1740, 1660, 1640, 1570, 1545, 1450, 1410, 1380, 1300, 1255, 1235, 1207, 1185, 1165, 1105, 1000, 975, 930, 880, 830, 805 and 770 cm^{-1}: mass spectrum $(MH)^+$ *m/z* 1,694 (SIMS). The ultraviolet and visible spectra of arugomycin are similar to those of nogalarol[1]. IR spectrum (KBr) indicates the presence of hydroxyl groups (3430 cm^{-1}), ester carbonyl (1740 cm^{-1}), and an α-hydroxyanthraquinone (1640, 1660 cm^{-1}). The ^1H NMR spectrum (400 MHz, in DMSO-d_6) indicated the presence of carbomethoxy (3.8 ppm), four methoxy (3.3~3.5 ppm), dimethylamino group (2.5 ppm), and deoxysugars (1.8~2.1 ppm).

Treatment of arugomycin with 0.4 N hydrochloric acid (100°C, 30 minutes) gave a mixture of the aglycone and sugars. By TLC comparison with an authentic sample, the aglycone was identified as nogalarol[1]; mass spectrum $(MH)^+$ *m/z* 586 (SIMS): ^1H NMR (400 MHz, in CD_3OD) 1.50 (s, 3H), 1.68 (s, 3H), 2.55 (dd, *J*=5.1, 14.0 Hz, 1H), 2.75 (s, 6H), 3.15 (dd, *J*=2.6, 11.0 Hz, 1H), 3.70 (s, 3H), 3.80 (s, 1H), 4.18 (d, *J*=2.6 Hz, 1H), 4.42 (dd, *J*=3.0, 11.0 Hz, 1H), 5.21 (dd, *J*=5.1 Hz, 1H), 5.88 (d, *J*=3.0 Hz, 1H), 6.72 (s, 1H), and 7.25 (s, 1H). The carbohydrate fraction contains 2-deoxyfucose, diginose, and decilonitrose[2], but nogalose could not be detected. Thus, arugomycin is a new anthracycline antibiotic and clearly distinguished from decilorubicin[3] recently reported by UMEZAWA *et al.* A tentative structure for arugomycin derived from both spectral data and degradation studies is as shown in Fig. 1. The structural studies will be reported in due course.

The antimicrobial activity of arugomycin is summarized in Table 1, it inhibited the growth of Gram-positive bacteria. The LD_{50} for arugomycin in mice was 1.75 mg/kg by intraperitoneal injection. Table 2 shows the effect of arugomycin on Ehrlich ascites carcinoma. The intraperitoneal injection of arugomycin on day 1, 3, 5 caused prolongation of the life span of the treated mice.

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