AKROBOMYCIN, A NEW ANTHRACYCLINE ANTIBIOTIC

Sir:

During the course of screening for new antitumor antibiotics, the cultured broth of a strain of microorganism 1029-AV1 showed a marked antitumor activity and was found to contain a new anthracycline antibiotic which we named akrobomycin. In this communication, the isolation and characterization of akrobomycin are reported.

Strain 1029-AV1 was isolated from a soil sample collected at Kaho, Fukuoka, Japan. On the basis of taxonomic studies, it was identified as a strain of *Actinomadura roseoviolacea* and was designated *Actinomadura roseoviolacea* 1029-AV1. A detailed description of this strain will be reported in the following paper.

This organism was cultured at 27°C for 7 days in 500-ml Erlenmeyer flasks containing 100 ml of a medium, composed of 2.5% glucose, 1.5% soybean meal, 0.2% dry yeast and 0.4% $CaCO_3$ (pH 7.4).

The culture filtrate (10 liters) was adjusted to pH 2.0 and applied to a column of Diaion HP-20. The column was washed successively with water, 80% methanol, and then the active material was eluted with methanol. The eluate was concentrated to dryness *in vacuo*. The dried residue was dissolved in a small amount of chloroform - methanol (10: 1) and subjected to a silica gel column chromatography. After washing with chloroform, the active fraction was eluted with chloroform - methanol (10: 1), concentrated to a small volume *in vacuo*, and then applied to a Sephadex LH-20 column with methanol - acetic acid (100: 0.5). The active fractions were collected and concentrated *in vacuo* to yield an oily solid which was dissolved in chloroform - methanol (10: 1). The mixture was washed twice with water to remove acetic acid and the organic layer was concentrated *in vacuo* to yield a reddish purple powder of akrobomycin (8 mg) in pure form.

Physicochemical properties of akrobomycin are: mp 143~148°C; λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 255 (472), 268 (548), 490 (238), 513 (262), 550 (162); IR (KBr) 1593 cm^{-1} (quinone and aromatic C=C); FAB-MS *m/z* 482 $(M+H)^+$; *Anal.* Calcd. for $C_{26}H_{27}NO_8$: C 64.86, H 5.65, N 2.91; Found: C 64.79, H 5.69, N 2.87.

The ^1H NMR spectrum of akrobomycin in $CDCl_3$ showed the signals assigned to a 9,10-anhydroanthracyclinone: δ 7.83 (H-1, d, J=7.6 Hz), 7.64 (H-2, t, J=7.6), 7.27 (H-3, d, J=7.6), 5.22 (H-7, d, J=4.8), 2.55 (H-8a, dd, J=4.8, 18.4), 2.72 (H-8b, d, J=18.4), 6.85 (H-10, s), 2.38 (CH_2-13, q, J=7.8) and 1.22 (CH_3-14, t, J=7.8), with additional daunosamine as a sugar residue linked at C-7: δ 5.27 (H-1′, d, J=4.0 Hz), 1.54 (H-2′a, dd, J=4.8, 12.0), 1.67 (H-2′b, ddd, J=4.0, 11.2, 12.0), 3.16 (H-3′, dd, J=4.8, 11.2), 3.44 (H-4′, s), 3.97 (H-5′, q, J=6.8) and 1.33 (CH_3-6′, d, J=6.8).

Fig. 1. The structure of akrobomycin.

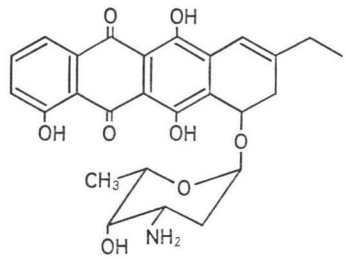

Table 1. Antimicrobial activity of akrobomycin.

Organisms	MIC (μg/ml)
Staphylococcus aureus IFO 12732	6.25
Bacillus subtilis IFO 3134	25
Micrococcus luteus ATCC 9341 (MS-1)	3.13
Pseudomonas aeruginosa IFO 12582	100
Salmonella typhimurium IID 971 (MS-1)	>100
Escherichia coli IFO 12734	>100
Saccharomyces cerevisiae ATCC 9763	>100
Candida albicans No. Yu 1200	>100
Penicillium chrysogenum ATCC 10002	>100
Trichophyton mentagrophytes	25

Table 2. Antitumor activity of akrobomycin against P388 leukemia.

Dose (mg/kg/day)	Effect T/C(%)
16	153
8	143
4	141
2	138
1	128

Injection: day 1, 5, ip.

Tumor inoculum: P388 cells, 10^6 cells/mouse, ip.

Prolongation rate (T/C, %)=mean survival period of mice treated/mean survival period of the control.

Acid hydrolysis (0.1 N HCl, 100°C, 30 minutes) of akrobomycin gave an amino sugar, identified as daunosamine[1] by direct comparison with an authentic sample obtained by hydrolysis of daunomycin, and a dehydrated aglycone which was identified as decarbomethoxybisanhydro-ε-rhodomycinone[2]: mass spectrum m/z 334 (M^+); ^1H NMR (in $CDCl_3$) δ 7.76 (H-1, d, J=7.6 Hz), 7.71 (H-2, t, J=7.6), 7.29 (H-3, d, J=7.6), 8.41 (H-7, d, J=8.4), 7.67 (H-8, dd, J=2.0, 8.4), 8.32 (H-10, d, J=2.0), 2.91 (CH_2-13, q, J=8.0) and 1.38 (CH_3-14, t, J=8.0).

These results indicate that the structure of akrobomycin is 9,10-anhydro-13-deoxocarminomycin[3] as shown in Fig. 1.

Table 1 shows the antimicrobial activity of akrobomycin as determined by the agar dilution method. Akrobomycin inhibited the growth of Gram-positive bacteria and *Trichophyton mentagrophytes*.

As shown in Table 2, akrobomycin prolonged the survival period of CDF_1 mice to which P388 leukemia cells were intraperitoneally inoculated. The LD_{50} of akrobomycin by intraperitoneal injection in mice was more than 20 mg/kg.

Further studies on the biological activity of akrobomycin are under progress and will be reported in a subsequent paper.

KANJI IMAMURA
ATSUO ODAGAWA
KOZO TANABE

Applied Bioscience Laboratory
Kirin Brewery Co. Ltd.
1-2-2 Souja, Maebashi, Gunma, 371, Japan

YOICHI HAYAKAWA
NOBORU ŌTAKE

Institute of Applied Microbiology,
University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan

(Received September 17, 1983)

References

1) ARCAMONE, F.; G. CASSINELLI, P. OREZZI, G. FRANCESCHI & R. MONDELLI: Daunomycin. II. The structure and stereochemistry of daunosamine. J. Am. Chem. Soc. 86: 5335~5336, 1964
2) BROCKMANN, H.; R. ZUNKER & H. BROCKMANN, Jr.: Antibiotics from actinomycetes. LV. Rhodomycines. 11. Synthesis of decarbomethoxybisanhydro-ε-rhodomycinone. Ann. Chem. 696: 145~159, 1966
3) BRAZHNIKOVA, M. G.; V. B. ZBARSKY, V. I. PONOMARENKO & N. P. POTAPOVA: Physical and chemical characteristics and structure of carminomycin, a new antitumor antibiotic. J. Antibiotics 27: 254~259, 1974