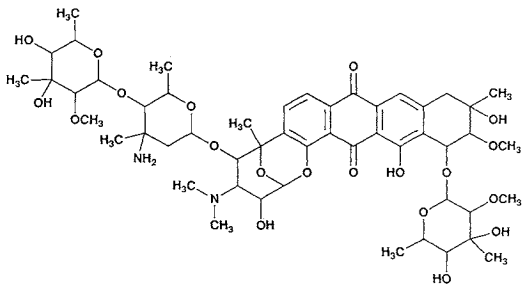
RESPINOMYCIN A1, A NEW ANTHRACYCLINE ANTIBIOTIC

Sir:

In the course of our screening for new antibiotics, we found that a strain of *Streptomyces* sp. RK-483 (FERM P-11622) isolated from a soil sample collected in Suwa-shi, Nagano Prefecture, Japan, produces a series of new anthracycline antibiotics designated respinomycins. The antibiotics induce the terminal differentiation of a human leukemia cell, K-562. In this communication, we wish to describe isolation and characterization of a major component respinomycin A1 (Fig. 1) and the biological activities.

Fig. 1. The structure of respinomycin A1.



Fermentation was carried out at 28°C for 72 hours in jar fermenters containing a medium consisting of glucose 2%, soluble starch 1%, meat extract 0.1%, dry yeast 0.4%, soybean flour 2.5%, NaCl 0.2% and K_2HPO_4 0.005%. The culture broth (32 liters) was filtered and the mycelial cake was extracted with 80% aq acetone. After removal of acetone, the aqueous extract was combined with the filtrate and the mixed solution was adjusted to pH 2 with HCl and extracted with EtOAc, which was discarded.

Fig. 2. UV spectra of respinomycin A1.

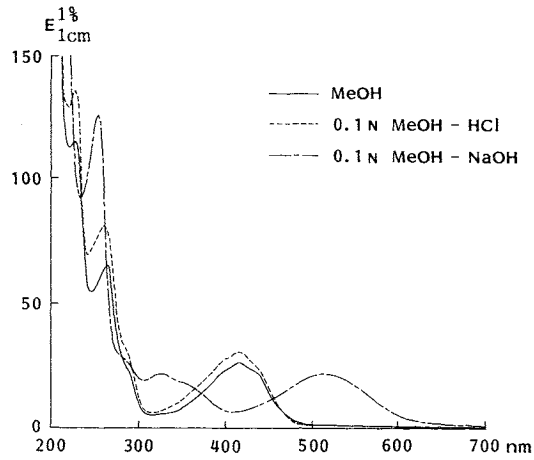


Fig. 3. ^1H NMR spectrum of respinomycin A1 (400 MHz, $CDCl_3$).

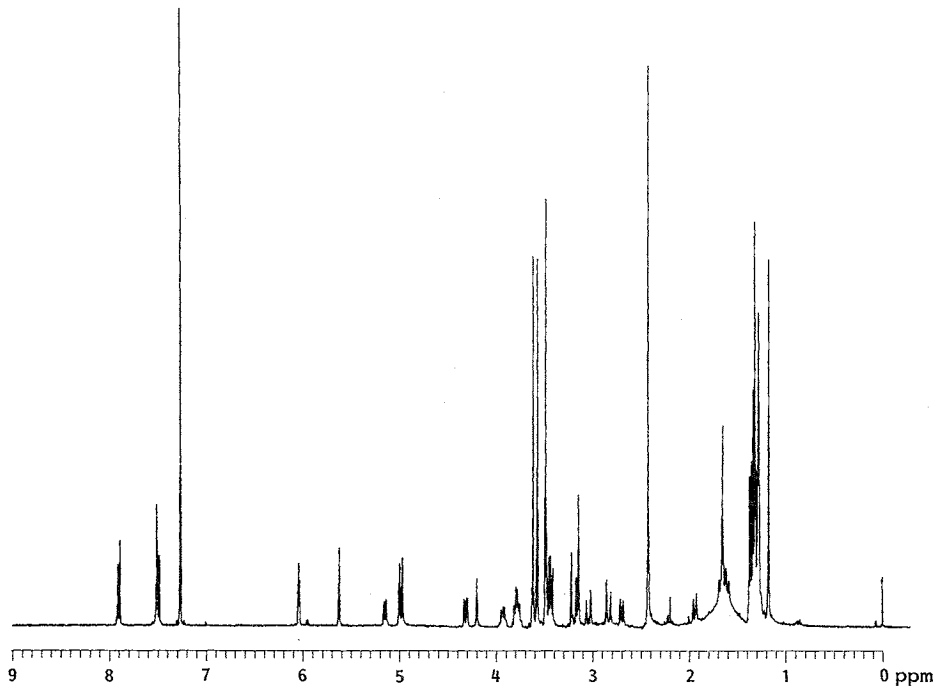
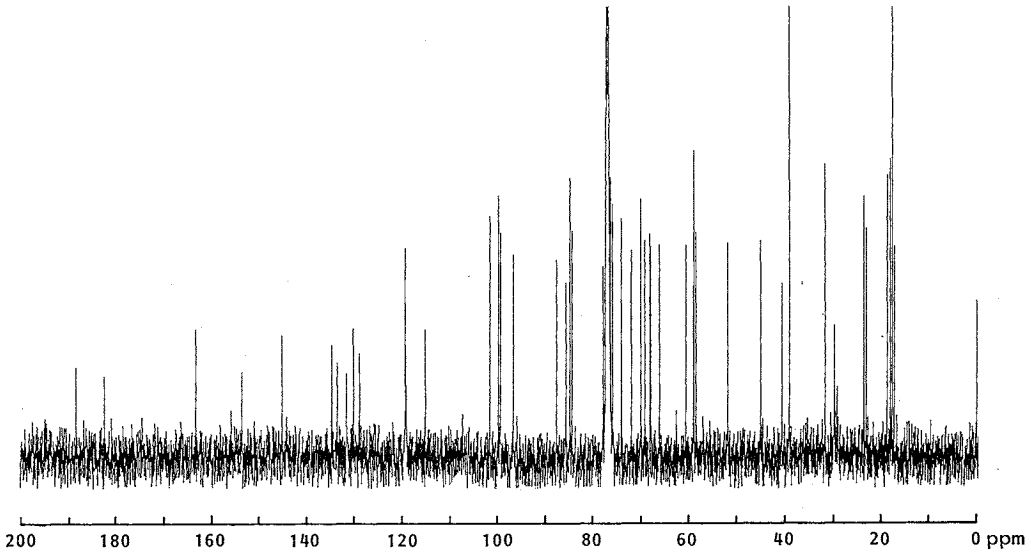


Fig. 4. ^{13}C NMR spectrum of respinomycin A1 (100 MHz, $CDCl_3$).



The aqueous layer was adjusted to pH 7.5 and extracted with $CHCl_3$. The organic layer was evaporated *in vacuo* giving 1.57 g of a yellow powder. The crude material (680 mg) was partitioned on a centrifugal partition chromatograph (Sanki Model CPC-LLN) employing following condition: BuOH-MeOH-0.01 M NH_4OAc (upper phase stationary, 4:1:5), 4 ml/minute in the descending mode at 900 rpm. After further purification by preparative silica gel TLC (Rf 0.35, $CHCl_3$-MeOH-H_2O-AcOH (80:20:6:14); Silica gel $60F_{254}$, Merck), 41.25 mg of respinomycin A1 was isolated. The remaining crude powder (872 mg) was chromatographed on a silica gel column (Silica gel 60, 70~230 mesh, Merck) with $CHCl_3$-MeOH-H_2O-AcOH (80:20:6:14) to give 39 mg of respinomycin A1.

Respinomycin A1 is a yellow powder with mp>250°C (dec) and is optically active, $[\alpha]_D^{18}$ +173° (*c* 0.3, $CHCl_3$-MeOH, 2:1). The molecular formula was established to be $C_{51}H_{72}N_2O_{20}$ on the basis of HRFAB-MS (*m/z* 1,033.47610 $(M+H)^+$, Δ0.4) and the total number of carbons detected by ^{13}C-^1H COSY. The UV spectrum (Fig. 2) showed a maxima at 220 ($E^{1\%}_{1cm}$ 114.4), 265 ($E^{1\%}_{1cm}$ 64.8), 290 ($E^{1\%}_{1cm}$ 24.0, sh), 395 ($E^{1\%}_{1cm}$ 23.2, sh), 415 ($E^{1\%}_{1cm}$ 26.4), and 440 nm ($E^{1\%}_{1cm}$ 20.8, sh) in MeOH. Main absorption bands of IR spectra (KBr) occur at the following approximate wavelengths: 3410, 2910, 1675, 1630, 1580, 1410, 1255, 1095 cm^{-1}. Respinomycin A1 is soluble in MeOH, DMSO, $CHCl_3$, EtOAc and acidic H_2O, but insoluble in neutral or alkaline H_2O and hexane. It gives positive reactions with anisaldehyde-H_2SO_4 and I_2 but is negative to a ninhydrin test.

Table 1. Antimicrobial activity of respinomycin A1.

Test organism	MIC (μg/ml)[a]
Escherichia coli JE1011	25
E. coli BE1186	12.5
Pseudomonas aeruginosa L-mutant N-10	6.3
Staphylococcus aureus FDA 209P	25
Xanthomonas campestris pv citri IFO 3781	25
Bacteroides fragelis ATCC 25285	>500
Pyricularia oryzae IFO 5994	>500
Botryotinia fuckeliana IFO 5365	>500
Alternaria mali IFO 8984	>500
Candida albicans IFO 1594	>500
Chlorella vulgaris	500

[a] The conventional agar dilution method was used. Medium: Potato-sucrose agar for fungi and *X. campestris pv citri*, glucose-peptone-yeast extract for chlorella, nutrient agar-yeast extract for bacteria.

^1H and ^{13}C NMR in $CDCl_3$ are shown in Figs. 3 and 4, respectively. The structure of respinomycin A1 was deduced from spectroscopic evidences including ^1H-^1H COSY, ^1H-^{13}C COSY and heteronuclear multiple-bond correlation (HMBC) spectra data. Details of the structure elucidation of respinomycin A1 and other components will be reported elsewhere. The aglycone of respinomycin A1 is a new type of anthracycline and distinguished from those of nogalamycin[1] and the nogalamycin related antibiotics, arugomycin[2], decilorubicin[3]

and viriplanins[4].

Respinomycin A1 induced differentiation on 50% of K-562 cells at the concentration of *ca.* 1 μg/ml, and it showed cytotoxicity to the same cells at the concentration of *ca.* 10 μg/ml.

The antimicrobial activity of respinomycin A1 is summarized in Table 1, it is inhibitory to the Gram-negative and Gram-positive bacteria. It is toxic to mice: LD_{50} is approximately 37.5 mg/kg by intraperitoneal administration.

Acknowledgments

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