Communications to the Editor

ISOLATION AND STRUCTURE OF A NEW ANSAMYCIN ANTIBIOTIC KANGLEMYCIN A FROM A *NOCARDIA*

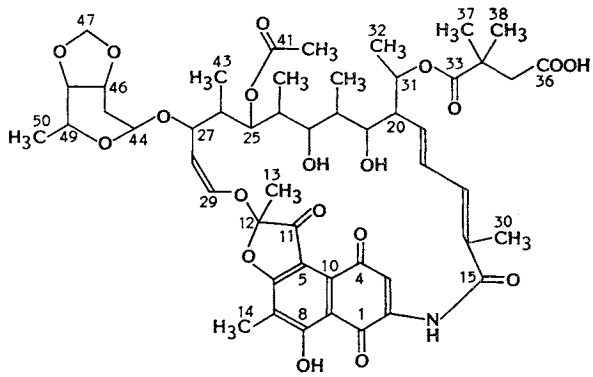
Sir:

During the course of a screening for new antibiotics from *Nocardia*, *Nocardia mediterranei* var. *kanglensis* 1747-64 was found to produce a new ansamycin antibiotic, kanglemycin A which is active against Gram-positive bacteria. In this paper, we wish to report the isolation and structure of the antibiotic.

The stock culture of a strain, *Nocardia mediterranei* var. *kanglensis* 1747-64[1] was inoculated into a seed medium (glucose 2.5%, yeast meal 0.5%, KCl 0.25%, soybean meal 0.5%, K_2HPO_4 0.02%, $(NH_4)_2SO_4$ 0.5%, $MgSO_4$ 0.02% and $CaCO_3$ 0.5%) in a 20-liter jar fermentor and incubated at 28°C for 48 hours. The seed culture (10 liters) was transferred to 200 liters of a production medium (glucose 3%, yeast meal 1%, peanut meal 0.5%, peptone 0.2%, $CaCO_3$ 0.1%, pH 7.0) in a 300-liter fermentor and the fermentation was carried out at 28°C for 96 hours.

The fermentation broth (200 liters) of kanglemycin producing strain was cooled at 5°C and filtered. The filtrate was extracted with ethyl acetate (70 liters) at pH 2. The extract was concentrated to a small volume under reduced pressure. After washing the concentrate with a phosphate buffer (pH 7.3), it was evaporated in an atmosphere of N_2 gas under reduced pressure to give a syrup. This was then chromatographed on silica gel and developed with benzene - methanol (100 : 4). The active fractions were collected and washed with acidic water (pH 2~3). Kanglemycin A in the benzene layer was extracted with a solution of 5% $NaHCO_3$ and then re-extracted with ethyl acetate from the aqueous solution at pH 7. After evaporation of the extract, the resulting crude material was dissolved in methanol. After standing for several hours, kanglemycin A (1 g) was obtained as orange rod crystals, mp 156°C (dec), $[\alpha]_D^{25}$ + 315.4° (*c* 0.2, MeOH). The molecular formula, $C_{50}H_{63}NO_{19}$ for kanglemycin A was deduced from its elemental analysis (C 60.63, H 6.53, N 1.46), electron impact (EI)- and field desorption (FD)-MS data (*m/z* 981 (M^+)) and ^{13}C NMR spectral data. The IR spectrum (KBr) exhibited characteristic bands at 3450 cm^{-1} (NH/OH), 1783 cm^{-1} (ester CO), 1640 cm^{-1} (NHCO) and 1700 and 1618 cm^{-1} (naphthoquinone CO), as shown in Fig. 1. The UV and visual absorption maxima (λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$), 232 (225), 275 (178), 304 (105) and 397 (38)) of kanglemycin A showed a close similarity absorption to that of rifamycin S,[2] indicating that it possesses the same chromophore.

Kanglemycin A is an acidic lipophilic compound. It is soluble in organic solvents such as acetone, ethyl acetate and chloroform, slightly



Kanglemycin A

Fig. 1. IR spectrum of kanglemycin A (KBr).

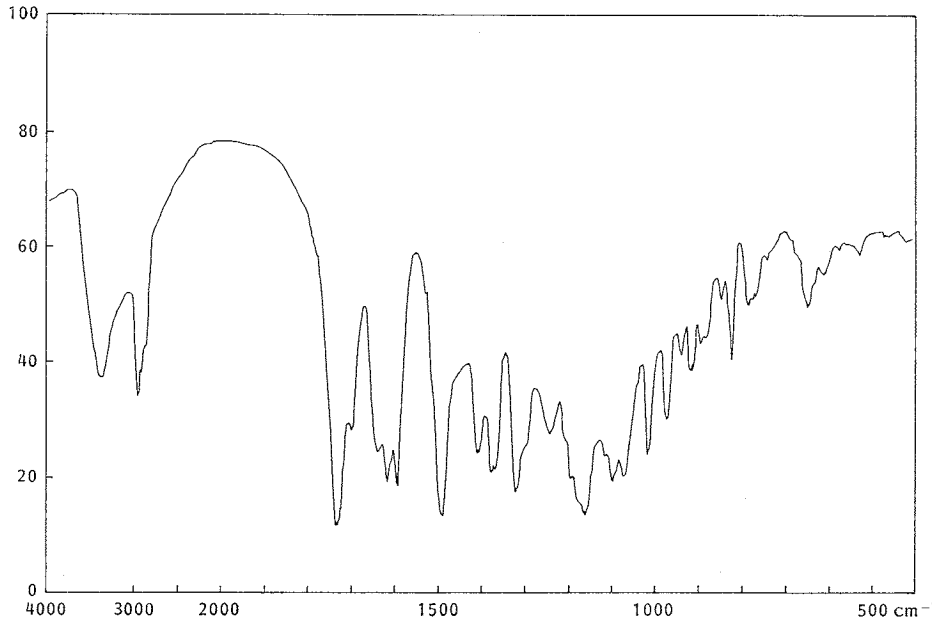
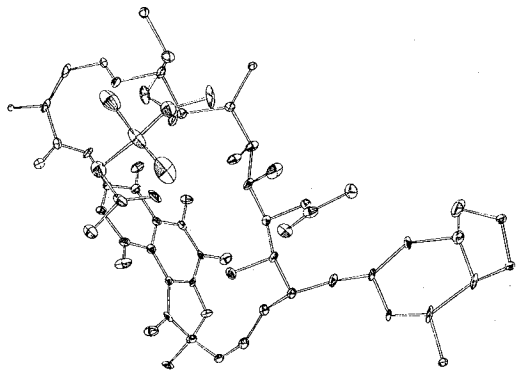


Fig. 2. The molecular structure of kanglemycin A.



soluble in methanol and ethanol, and insoluble in petroleum ether. The Rf values on silica gel TLC are follows: 0.58 ($CHCl_3$ - MeOH, 9 : 1), 0.37 (MeOH - petroleum ether - benzene, 4 : 6.5 : 10).

The structure of kanglemycin A was determined by X-ray crystallographic analysis[3] of its single crystal. The crystal of kanglemycin A ($C_{50}H_{83}NO_{19}\cdot MeOH$) belongs to triclinic, space group P1, unit cell: $a=12.760(3)$, $b=10.287(2)$, $c=9.926(2)$ Å, $\alpha=88.39(2)°$, $\beta=78.64(2)°$, $\gamma=89.14(2)°$. RANTAN direct method[4] was used for solving the structure. The final discrepancy factor is $R=0.0689$, after coordinates and temperature factors were refined with full matrix least squares. The molecular structure of kanglemycin A is shown in Fig. 2. The structural skeleton of kanglemycin A consists of four parts: A naphthalenoid nucleus containing a 5-membered ring, a 17-membered ansa chain, monoethyl ester of 2,2-dimethyl succinic acid and *β*-*O*-3,4-*O*,*O*′-methylene digitoxose on the ansa chain. The ^{13}C NMR spectrum (25 MHz) of the antibiotic shows well separated signals and supports the structure deduced from the X-ray analysis, as shown in Fig. 3. As shown in Table 1, the ^{13}C chemical shift assignments of kanglemycin A were made essentially by ^{13}C NMR spectral comparison with those of rifamycin S,[5] digitoxose and 2,2-dimethyl succinic acid. It should be noted that kanglemycin A is a novel ansamycin that possesses two structural moieties, dimethyl succinic acid and digitoxose at C-20 and C-27, respectively, on the ansa chain differing from other ansamycin antibiotics. The antibiotic shows a potent antimicrobial activity against Gram-positive bacteria, especially *Streptococcus sanguis* No. 10 (MIC 0.1～0.2 μg/ml), *Streptococcus pyogenes* A 12 (0.003～0.006) and *Streptococcus pneumoniae* (0.05). Details of the taxonomy of the producing strain, the fermentation and the

Fig. 3. The ^{13}C NMR spectrum of kanglemycin A (25 MHz, $CDCl_3$).

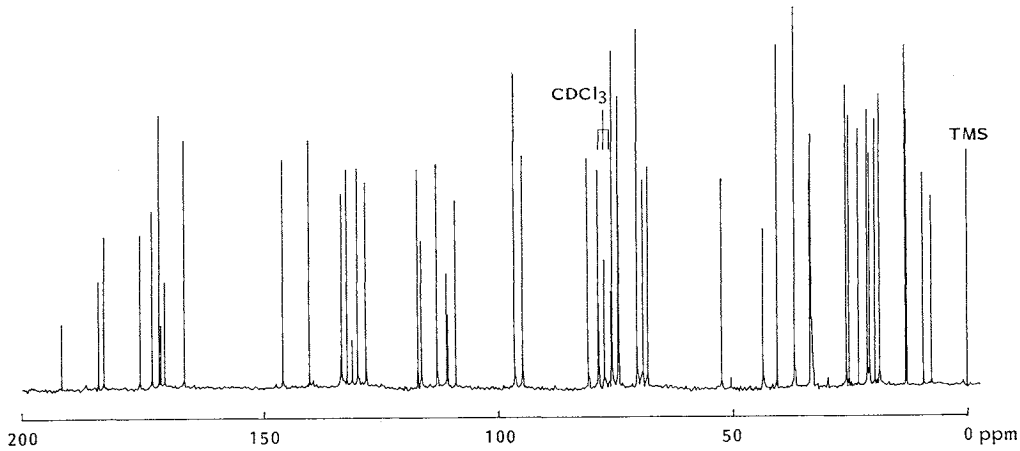


Table 1. ^{13}C Chemical shift assignments of kanglemycin A (25 MHz, $CDCl_3$).

Carbon No.	δ_C[a]	Multiplicity	Carbon No.	δ_C[a]	Multiplicity
C-1	184.7	s	C-26	36.5	d
C-2	110.5	s	C-27	80.4	d
C-3	116.9	d	C-28	112.7	d
C-4	183.5	s	C-29	145.5	d
C-5	110.8	s	C-30	20.6	q
C-6	171.9	s	C-31	75.3	d
C-7	116.1	s	C-32	18.4	q
C-8	166.5	s	C-33	175.6	s
C-9	131.9	s	C-34	40.4	s
C-10	139.6	s	C-35	43.1	t
C-11	192.5	s	C-36	171.1	s
C-12	108.8	s	C-37	24.9	q
C-13	7.5	q	C-38	25.6	q
C-14	23.0	q	C-39	9.1	q
C-15	170.4	s	C-40	12.8	q
C-16	130.8	s	C-41	172.9	s
C-17	133.0	d	C-42	21.2	q
C-18	127.8	d	C-43	12.6	q
C-19	129.6	d	C-44	96.4	d
C-20	51.8	d	C-45	32.8	t
C-21	73.9	d	C-46	69.8	d
C-22	33.5	d	C-47	94.5	t
C-23	73.7	d	C-48	61.4	d
C-24	36.5	d	C-49	68.5	d
C-25	78.3	d	C-50	19.3	q

[a] Chemical shifts in values (ppm down-field from internal TMS in $CDCl_3$).

biological property of kanglemycin A will be reported elsewhere.

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