TWO NEW ANTIBIOTICS, A-218 AND K-41 ISOLATION AND CHARACTERIZATION

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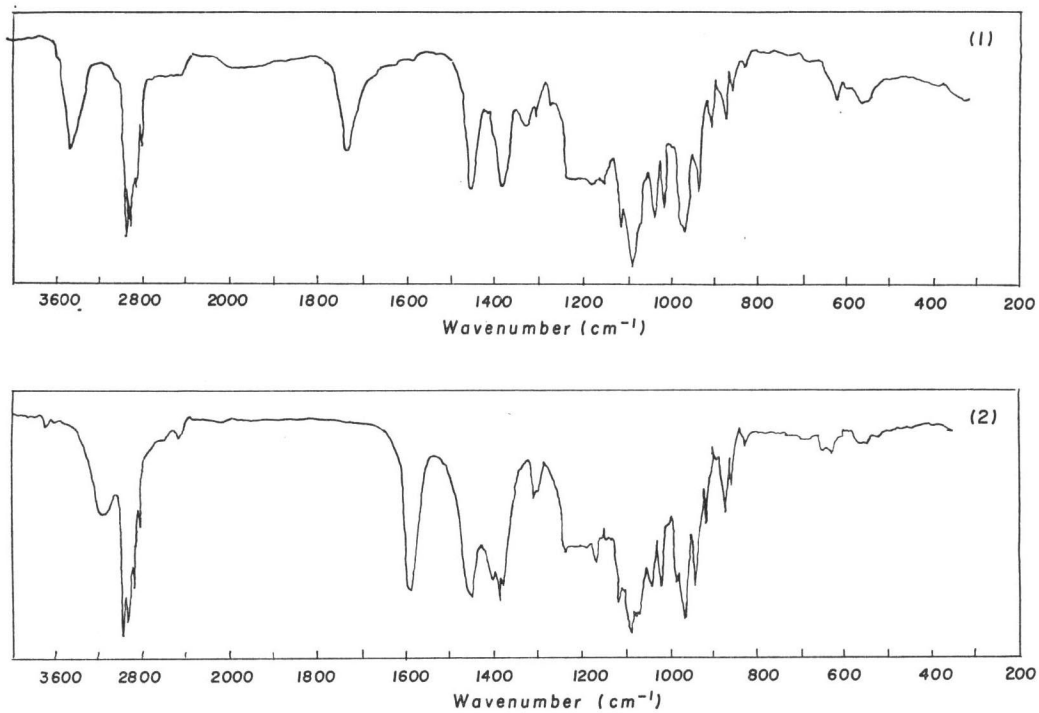
Two new antibiotics, A-218 and K-41, were isolated from strains identified as *Streptomyces hygroscopicus*. The antibiotics are characterized as polycyclic polyether compounds and are active against gram-positive bacteria.

Two strains of *Streptomyces hygroscopicus*, A-218 (FERM-P 928*) and K-41 (FERM-P 1342**), produce similar antibiotics active against gram-positive bacteria. Both antibiotics were extracted from fermentation broths with organic solvents, isolated by chromatography on silica gel, and finally purified as sodium salts, respectively.

The two antibiotics, A-218 and K-41, are monocarboxylic acids, and easily soluble in most organic solvents and sparingly soluble in water even in the form of sodium salts. The antibiotics are positive to DRAGENDORFF reagent though they do not contain nitrogen atoms in their

Fig. 1a. IR spectra of A-218 (in $CHCl_3$). (1) Free acid. (2) Sodium salt



* Japan Kokai 48-80793 (1973). ** Japan Kokai 49-14692 (1974).

Fig. 1b. IR spectra of K-41 (in $CHCl_3$). (1) Free acid. (2) Sodium salt

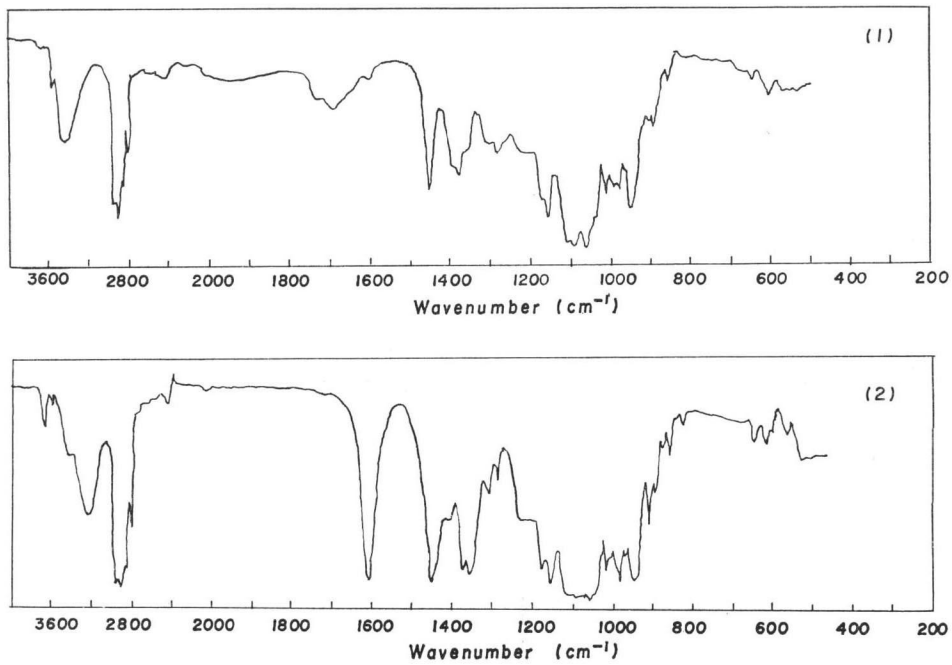


Table 1. Properties of sodium salts of A-218 and K-41

	A-218	K-41
M. P.	187~188°C	196~198°C (dec.)
$[\alpha]_D^{23}$	52.3°	1.9°
M. W.*	921	1039
MeO	4	5
M. Formula**	$C_{46}H_{79}O_{15}Na$	$C_{48}H_{81}O_{19}Na$
UV max	no max	no max

* Osmometry in $CHCl_3$.
** Assumed from elementary analysis and ^{13}C NMR spectra.

molecules. These are characteristic of the polycyclic polyether antibiotics such as nigericin.

The IR spectra, shown in Fig. 1, exhibit the presence of hydroxyl groups, carboxylic bands (COO^- bands near 1590 cm^{-1} in sodium salts) and the strong absorption bands attributable to ethereal groups (1060~1120 cm^{-1} region). These evidences support the above assumption that antibiotics A-218 and K-41 belong to the nigericin group.

The other properties of the sodium salts are summarized in Table 1. The number of the methoxyl groups is corroborated by the elementary analyses and NMR spectra (Fig. 2).

Several polycyclic polyether antibiotics which have no absorption maximum in UV have been reported. Nigericin[1] (identical with X-464 and polyetherin A), monensins[2] and grisorixin[3] have one methoxyl group in their respective molecules, while antibiotic X-206[4], salinomycin[5] lysocellin[6] laidlomycin[7] and alborixin[8] have no methoxyl group. Therefore, A-218 and K-41 should differ from these antibiotics. Duamycin[9], presumably one of this family, lacks the description concerning the methoxyl group, but is distinguished from A-218 and K-41 by direct comparison of IR spectra. Antibiotics A-204[10], A-28695[11] and septamycin[12], which have four to five methoxyl groups, closely resemble A-218 and K-41. The direct comparison studies, however, establish that A-218 and K-41 are not identical with these antibiotics.

The antimicrobial activity of both antibiotics A-218 and K-41 is shown in Table 2. The

Fig. 2. NMR spectra of A-218 and K-41 (sodium salts) in $CDCl_3$ at 60 MHz. (1) A-218 (2) K-41

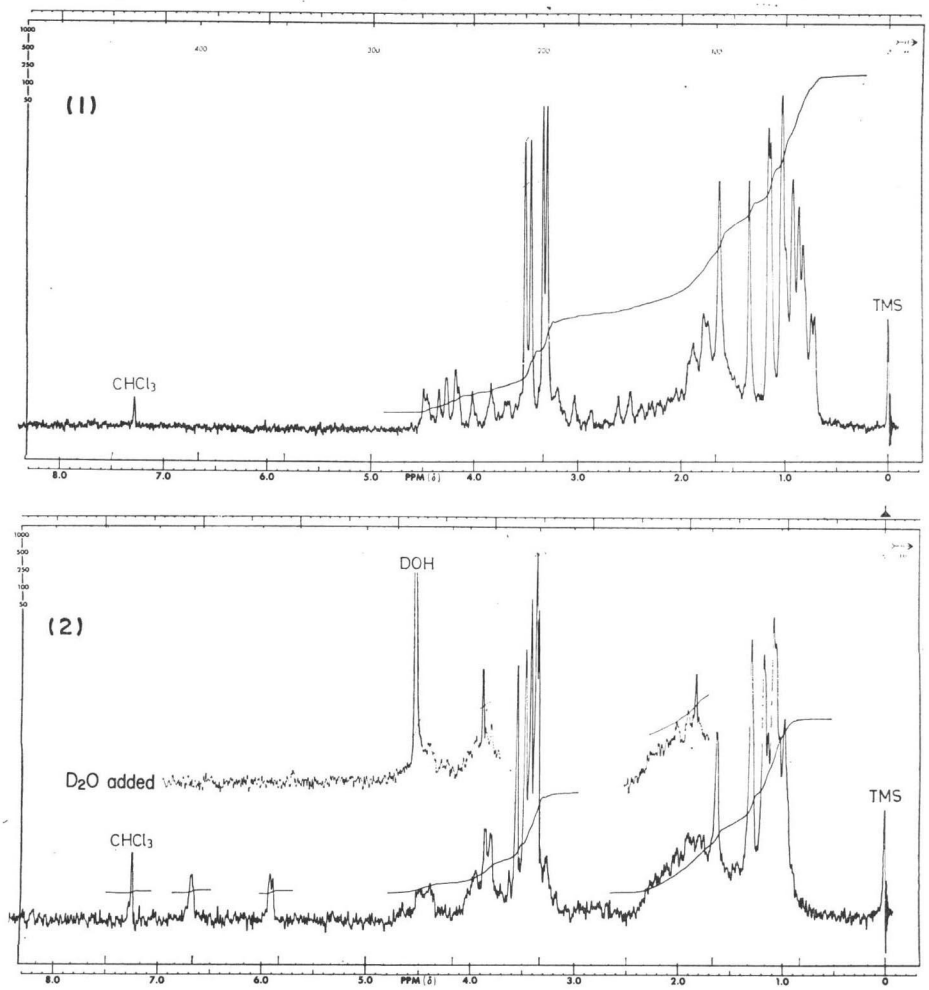


Table 2. Antimicrobial spectra of A-218 and K-41 (agar dilution method)

Test organisms	MIC (mcg/ml)	
	A-218 (Na)	K-41 (Na)
Bacillus subtilis PCI 219	1.56	3.13
Bacillus anthracis	0.78	1.56
Staphylococcus aureus 209P JC-1	1.56	1.56
Staphylococcus aureus 80257*	1.56	1.56
Streptococcus pyogenes C 203**	0.78	0.78
Streptococcus pneumoniae type I**	0.78	0.39
Corynebacterium diphtheriae Tront**	1.56	0.78
Mycobacterium tuberculosis 37H RV	50	3.13
Escherichia coli NIHJ JC-2	>50	>50
Klebsiella pneumoniae	>50	>50
Pseudomonas aeruginosa	>50	>50

* Resistant to sulfonamides and antibiotics
** Medium fortified with 3% human plasms.

antibiotics showed no therapeutic effect in mice infected with *Streptococcus pyogenes*. The LD_{50} (i.p. in mice) values of A-218 and K-41 were 10~20 mg/kg and 48 mg/kg, respectively.

Experimental

A-218

Production The streptomyces strain A-218 was inoculated into 100 ml of a medium composed of Bacto-Soytone (DIFCO) 1.0 %, soluble starch 2.0 %, glycerin 0.5 %, corn steep liquor 0.5 %, NaCl 0.35 %, glucose 0.3 % (pH adjusted to 6.6), and $CaCO_3$ 0.5 %, in a 500-ml SAKAGUCHI flask and cultured at 28°C for 4 days on a reciprocal shaker.

Isolation and purification About one liter of the culture broth was filtered using filter-aid. The wet mycelial cake was stirred in 500 ml of ethyl acetate for 30 minutes, and the mixture was filtered. The ethyl acetate layer was evaporated *in vacuo* to give 390 mg of a dark brown oil. The culture filtrate was extracted with ethyl acetate (500 ml×2), and the evaporation of the solvent gave 140 mg of a pale brown oil.

The active principle in the crude oils was separated by preparative TLC on silica gel with $CHCl_3$-MeOH (95:5).

By this procedure the mycelial cake and the filtrate gave 33 mg and 69 mg of crude antibiotics respectively as an oil which was crystallized from acetone-water as colorless prisms, m.p. 183~188°C. The crystals should be a mixture of the salts originating from the cations in the adsorbent. The mixed salts were dissolved in ether and shaken twice with 1 % oxalic acid solution, and the ether layer was shaken twice with 1 % Na_2CO_3 solution and successively with saturated NaCl solution, dried over Na_2SO_4, and evaporated. The residue was recrystallized from petroleum ether to give pure sodium salt as colorless prisms, m.p. 187~188°C, $[\alpha]_D^{23}+52.3\pm0.8°$ (*c* 0.664, EtOH).

Anal. Calcd. for $C_{48}H_{79}O_{15}Na$: C, 61.72; H, 8.90; Na, 2.57; 4MeO*, 13.87 %.
Found: C, 61.85; H, 8.95; Na, 2.66; MeO, 14.74 %.

As mentioned above, the treatment of the ether solution of the sodium salt with oxalic acid gave free acid as an amorphous powder.

K-41

Production The fermentation of *Streptomyces* K-41 was carried out the same as A-218.

Isolation and purification About 2.5 liters of the cultured broth was treated the same as A-218. On extraction with ethyl acetate, the mycelial cake and the filtrate gave 1.0 g and 0.8 g of crude products, respectively.

The crude products were combined and fractionated on a silica gel column (40 g) with a solvent system of $CHCl_3$-CH_3OH (CH_3OH 0~2 %) to give 750 mg of active fraction, which was further purified by preparative TLC on silica gel with $CHCl_3$-CH_3OH (95:5). The mixed salts (520 mg) were dissolved in $CHCl_3$ and shaken with 2 % tartaric acid, and the $CHCl_3$-layer was treated with 5 % Na_2CO_3, dried over Na_2SO_4 and evaporated. The residue was crystallized with petroleum ether to afford 248 mg of pure sodium salt in colorless prisms, m.p. 196~198°C (decomp.), $[\alpha]_D^{23}+1.9\pm0.4°$ (*c* 1.017, MeOH).

Anal. Calcd. for $C_{48}H_{81}O_{10}Na$: C, 58.52; H, 8.29; Na, 2.33; 5MeO**, 15.75 %.
Found: C, 58.62; H, 8.33; Na, 2.28; MeO, 16.61 %.

The sodium salt readily gave the free acid as a colorless amorphous powder, but K-41, as well as A-218, is less stable in acid form.

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* The presence of four methoxyl groups is confirmed by PMR and ^{13}C NMR spectra.
** The presence of five methoxyl groups is confirmed by PMR and ^{13}C NMR spectra.

Added in Proof

Two antibiotics (lonomycin and DE-3936) similar to A-218 were recently presented (September 1975). From the direct comparison of IR spectra lonomycin is presumably identical with A-218.

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