ANTIBIOTIC A-130, ISOLATION AND CHARACTERIZATION

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An antibiotic, A-130, was isolated from a strain identified as *Streptomyces hygroscopicus*, strain A-130. The antibiotic belongs to the nigericin group and, like dianemycin, has an α, β-unsaturated ketone chromophore in its molecule. A-130 is active against gram-positive organisms.

A *Streptomyces*, strain A-130, found in a soil sample collected in Ikeda city, Osaka prefecture, Japan, produces an antibiotic mixture active against gram-positive bacteria and fungi. From the morphological, cultural and physiological characteristics, the strain was identified as *Streptomyces hygroscopicus*, strain A-130.

The active components were mainly extracted from the mycelial cake with acetone. The antifungal component, present in the acetone extract, was purified and was identified as venturicidin A[1] from its chemical and physical properties.

The antibacterial component was isolated by column chromatography and purified as the sodium salt. Treating the sodium salt with dilute hydrochloric acid in organic solvents gave the antibiotic in acidic form.

The antibiotic A-130 is a colorless amorphous powder, and its molecular formula was indicated as $C_{47}H_{78}O_{13}$ by analytical and mass spectrometry data. The antibiotic is positive to Dragendorff reagent and decolorizes potassium permanganate solution.

The following groups are present; hydroxyl groups (3525 cm^{-1}, 3450 cm^{-1}), a carboxyl group (1720 cm^{-1}), an α, β-unsaturated carbonyl group (1661 cm^{-1}, 1638 cm^{-1}), a methoxyl group (*vide* NMR), ether groups (1000~1120 cm^{-1}, signals at 5.5~7 τ) and many C-CH_3 groups (*vide* NMR) are suggested by the IR and NMR spectra. The existence of an α, β-unsaturated carbonyl group is supported by the UV spectrum which shows an absorption maximum at 234.5 nm (ε=14200). The carboxyl group was confirmed by the IR spectrum of the sodium salt. In this spectrum, as shown in Fig. 1, the carboxyl band of free acid at 1720 cm^{-1} has disappeared and a new band corresponding to COO^- is evident (1556 cm^{-1}).

Both the sodium salt, $C_{47}H_{77}O_{13}Na$, and the free acid are soluble in most organic solvents and almost insoluble in water. This, together with the above-mentioned spectroscopic evidence, indicates that antibiotic A-130 is a polycyclic polyether compound like nigericin, X-206, monensin *etc.*

Among the members of this group, dianemycin[2] is the only antibiotic which has an α, β-unsaturated carbonyl group.

By direct comparison with dianemycin, kindly supplied by Dr. R. L. Hamill of the Lilly

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Fig. 1. IR spectra of A-130.
a: free acid in $CHCl_3$, b: Na salt in $CHCl_3$, c: Na salt (KBr tablet)

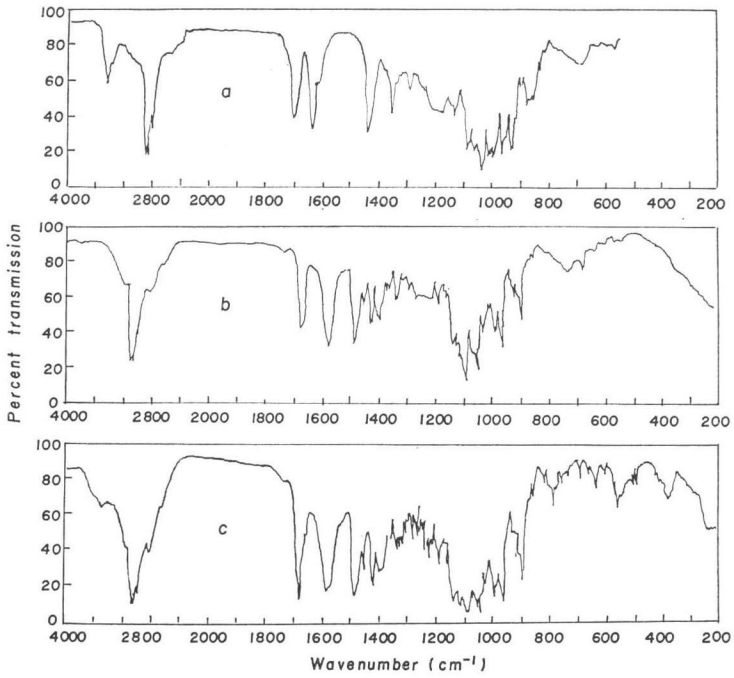
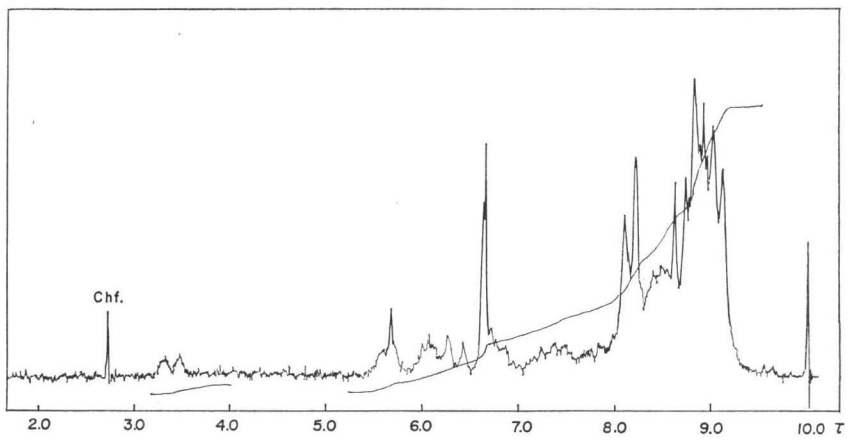


Fig. 2. NMR spectrum of A-130 (free acid) in $CDCl_3$ at 60 Mc



Laboratories, antibiotic A-130 and dianemycin were distinguishable by TLC, IR, and NMR. Antibiotic A-130 is thus established as a new antibiotic having a carboxyl group, an α,β-unsaturated ketone group, and presumably six rings in its molecule. The X-ray analysis of the silver salt of A-130 is in progress.

The *in vitro* antimicrobial activities (agar dilution method) of A-130 and its sodium salt are summarized in Table 1. The compounds showed no chemotherapeutic effect in mice. The LD_{50} of the sodium salt in mice is 2.5 mg/kg (ip) and 34.3 mg/kg (sc) using 5 % gum arabic vehicle.

Table. 1. Antimicrobial spectrum of A-130 and its sodium salt (MIC mcg/ml)

Test organism	A-130	A-130 Na
Bacillus subtilis PCI 219	1.25	1.25
Bacillus anthracis	1.25	1.25
Staphylococcus aureus FDA 209P JC-1	2.5	2.5
Streptococcus pyogenes C-203	0.625	0.625
Streptococcus faecalis	1.25	1.25
Streptococcus viridans	0.625	0.625
Diplococcus pneumoniae type I	0.625	0.625
Corynebacterium diphtheriae Tront	0.625	0.625
Mycobacterium tuberculosis H 37 Rv	2.5	2.5
Escherichia coli NIHJ JC-2	>20	
Pseudomonas aeruginosa Denken	>20	
Klebsiella pneumoniae	>20	
Trichophyton rubrum	>20	
Trichomonas vaginalis 4F	10	

Experimental

Production:

The vegetative medium was composed of starch 1%, peptone 0.5%, beaf extract 0.5%, yeast extract 0.25%, and NaCl 0.5%. The *Streptomyces*, strain A-130, was inoculated into 500 ml of the medium in a 2-liter Erlenmeyer flask, and the medium was incubated at 28°C for 48 hours on a rotary shaker. The vegetative culture was then transferred to a 30-liter jar fermentor containing 15 liters of a medium composed of starch 1.2%, soy bean meal 1.2%, corn steep liquor 0.5%, glycerin 0.6%, NaCl 0.36%, and $CaCO_3$ 0.42% (pH 7.0). The fermentation was performed at 29°C for 65 hours under aeration of 15 liters/min and agitation of 350 rpm.

Isolation and purification:

About 95 liters of the cultured broth was filtered at pH 4.0 using filter-aid. The mycelial cake was extracted with acetone. The acetone solution was evaporated *in vacuo* and the remaining water phase was extracted three times with ethyl acetate (1, 0.5 and 0.5 liter). The ethyl acetate solution was washed successively with 2% NaOH and H_2O, dried over Na_2SO_4, and evaporated to give 105 g of a crude oil. The oil was dissolved in 500 ml of ether-*n*-hexane (2:1) and allowed to stand overnight. The precipitate which formed was filtered off and recrystallized from acetone-benzene to give colorless needles, mp 144°, $[\alpha]_D$+118° (*c* 1, $CHCl_3$). This compound was identified spectroscopically as the antifungal antibiotic venturicidin.

The filtrate, which retained antibacterial activity, was concentrated to an oil (66 g), which was dissolved in $CHCl_3$ and submitted to column chromatography on 600 g of silica gel. The active fractions, eluted with chloroform-methanol (49:1~30:1) were combined and solvent was removed. The crude oily substance (40 g) was dissolved in ethyl acetate and the solution was washed with 2% HCl. After evaporation of the solvent, the residue was extracted with *n*-hexane and inactive precipitate was removed. The material from the evaporation of the *n*-hexane extract was dissolved in ethyl acetate and this solution was shaken with 2% NaOH and then with 2% NaCl, dried over Na_2SO_4, and evaporated to yield 25.4 g of crude sodium salt of A-130. Recrystallization from aceton-H_2O gave 10.0 g of the pure sodium salt as colorless needles, mp 227~231°C, $[\alpha]_D$+97.9° (*c* 1.0, $CHCl_3$), UV λ_{max}^{EtOH} 235 nm (ε=14700). The highest peak in the mass spectrum was *m/e* 872.

Anal. Found: C 64.71, H 8.90, MW* 854, 889
Calcd. for $C_{47}H_{77}O_{13}Na$: C 64.66, H 8.89, MW 872.98

The sodium salt was dissolved in ether and the solution was shaken with 3% HCl and then with 2% NaCl solution. The ether solution was dried over Na_2SO_4 and evaporated to give the free acid as a colorless amorphous powder, $[\alpha]_D+64.5°$ (*c* 1.0, $CHCl_3$), UV λ_{max}^{EtOH} 234.5 nm ($\varepsilon=14200$). The highest peak in the mass spectrum was *m/e* 832 (M^+-H_2O).

Anal. Found: C 66.47, H 9.15, O 24.07
Calcd. for $C_{47}H_{78}O_{13}$: C 66.32, H 9.24, O 24.44

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* Osmometry in $CHCl_3$.