AA-57, A NEW ANTIBIOTIC RELATED TO PENTALENOLACTONE

Sir:

As a result of screening cultures of actinomycetes for metabolites having antibacterial properties against Gram-positive and Gram-negative bacteria, including acid-fast bacteria, we have isolated an acidic lipophilic antibiotic AA-57 from the fermentation broth of *Streptomyces* sp. strain AA-57.

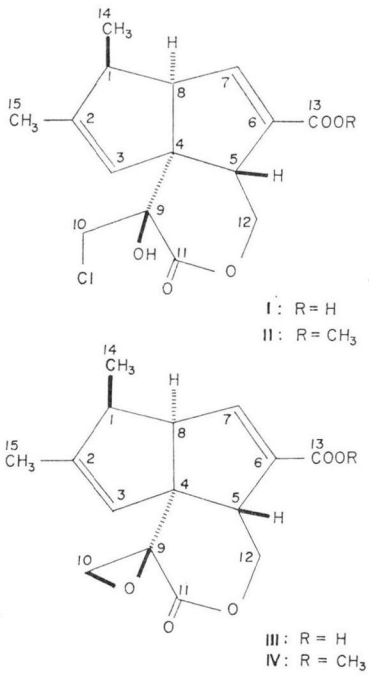
Strain AA-57, which was isolated from a soil sample collected at Koriyama, Fukushima-ken, Japan, was grown at 27°C on ISP starch agar medium slants. The seed culture was incubated at 27°C for 72 hours on a rotary shaker in a medium containing 5% glycerol, 1% glucose, 1% corn steep liquor, 2% soy-bean flour, and 0.2% $CaCO_3$ (pH 7.5). Tank fermentation was carried out using the same production medium after inoculation with the seed culture; fermentors were operated at 27°C with stirring at 250 rpm under an aeration of 100 liters/min. Antibiotic production reached a maximum after 120 hours, as determined by a paper disc-agar diffusion assay using *Staphylococcus aureus* FDA 209P.

The broth filtrate adjusted to pH 6.8 was applied to an Amberlite XAD-2 column, and the column eluted with 50% aqueous methanol. The active fraction was concentrated *in vacuo* to remove methanol, and lyophilized, providing a crude powder which was designated AA-57. The aqueous solution of the crude product was adjusted to pH 3.0 and extracted with benzene; the extract was evaporated *in vacuo* to a syrup which was purified by silica gel chromatography, using chloroform as eluting solvent; fractions were assayed for antibiotic activity. The active substance was further purified by chromatography on a Sephadex LH-20 column using methanol. Recrystallization of the residue obtained from the appropriate fractions gave a colorless prismatic crystal of antibiotic AA-57 from ether - benzene (1: 5). AA-57 showed positive REMIEUX reaction and positive BEILSTEIN's flame test. Rf values on silica gel TLC (Kieselgel GF254) were 0.56 (ethyl acetate - methanol, 1: 1), and 0.60 (chloroform - methanol, 2: 1); the active spot was detected either by autobiography or by 10% H_2SO_4.

We have now established that AA-57 is a novel antibiotic (Chart 1, structure **I**) which belongs to the class of pentalenolactones. It has the following physical properties: $C_{15}H_{17}O_5Cl$* (7-degree of unsaturation); mass spectrum M^+ 312.0782 (calcd., 312.0763); mp 123°C; $[\alpha]_D^{20}$ −143° (*c* 1.0, $CHCl_3$); pKa′ 4.70; uv (H_2O) 210 nm (inflection); and ir (nujol) 3470 (OH), ~2500 (COOH), 1732 (δ-lactone), 1685 (α,β-unsaturated COOH), and 1638 (double bond) cm^{-1}. Upon treatment with diazomethane in ether, AA-57 methyl ester (**II**) was obtained: $C_{16}H_{19}O_5Cl$*; mass spectrum M^+ 326.0923 (calcd., 326.0920); mp 130°C; uv (EtOH) λ_{max} 220 nm (ε 7,200); ir (KBr) 1735 (δ-lactone), 1715 (α,β-unsaturated ester), and 1640 (double bond) cm^{-1}. **I** and **II** could not be acetylated with acetic anhydride-pyridine, which suggested the presence of a tertiary hydroxyl group. The uv and ir data along with the acidity determination indicated the presence of an α,β-unsaturated carboxylic acid moiety in the structure.

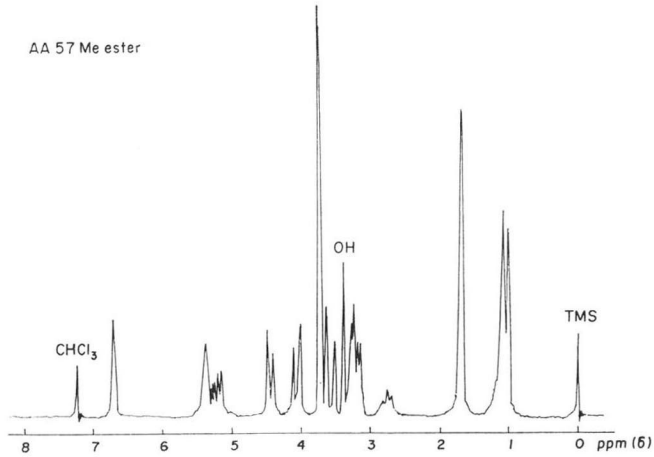
The ^{1}H-nmr spectrum**(Fig. 1) of the ester **II** showed a closed similarity to that of pentalenolactone methyl ester (**IV**)[4], except for one

Chart 1. Structures of pentalenolactones



* Microanalyses are in agreement with the molecular formula assigned.

Fig. 1. ^{1}H-Nmr spectrum of AA-57 methyl ester (**II**) at 100 MHz in $CDCl_3$



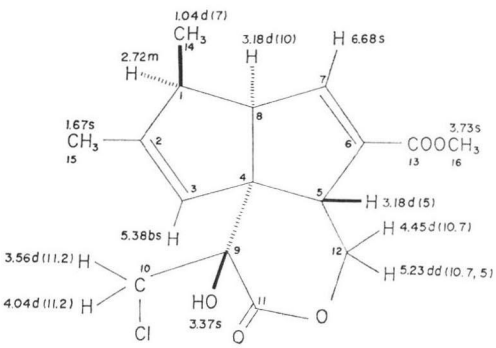
methylene signal. In the latter the exocyclic epoxide methylene protons (H10) are observed at 2.76 (d, 5.5) ppm and 3.24 (d, 5.5) ppm[1,2], whereas the methylene protons (H10) of the former are observed at 3.56 (d, 11.2) ppm and 4.04 (d, 11.2) ppm as an AB system pattern, suggesting that the exocyclic epoxide methylene group in **IV** is replaced by the chloromethyl group in **II**. The geminal tertiary hydroxyl proton (exchangeable with D_2O) was observed at 3.37 (s) ppm. The oxymethylene protons (H12) in **IV** appeared at 4.64 (d, 3) ppm[2,3], and the corresponding oxymethylene protons (H12) in **II** were at 4.45 (d, 10.7) ppm and 5.23 (dd, 10.7 and 5) ppm, the center of these signals being at 4.84 ppm. These facts suggest that the oxymethylene protons of **IV** are in bisectional position against H5 proton. On the other hand, from the coupling pattern the H12 methylene protons of **II** must be in an unequivalent position against the vicinal H5 proton. The pertinent ^{1}H-nmr data of ester **II** are shown in Chart 2.

An exhaustive ^{13}C-nmr analysis of the unique structure of **I** was carried out on its methyl ester **II**, using (A) proton noise decoupled (natural abundance), (B) off-resonance ^{1}H-decoupled (gated–1) and (C) ^{1}H weak noise decoupled ^{13}C-nmr spectra.

In the ^{13}C-nmr spectra, the difference between **II** and **IV**[2,3] was observed at one quaternary carbon signal; the 76.9(s)-ppm peak was assigned to the C9 carbon bearing tertiary hydroxyl group in **II**, as compared with the assignment of the 59.1(s)-ppm peak to the epoxide carbon of **IV**[2]. The 46.8-ppm signal (t, $^1J_{C\text{-}H}$ 152.6 Hz) was assigned to the C10 chloromethyl carbon of **II***, whereas in **IV** the exocyclic epoxide methylene carbon was assigned to the peak at 47.1 (t, $^1J_{C\text{-}H}$ 178 Hz) ppm[2,4].

The other carbon signals observed in **II** were in good agreement with those in **IV**; the multiplicity of signals in off-resonance decoupling spectrum and quaternary carbon detection by ^{1}H weak noise decoupled spectrum were also

Chart 2. ^{1}H-Nmr data of AA-57 methyl ester (**II**)



** ^{1}H-Nmr (at 100 MHz) and ^{13}C-nmr (at 25.03 MHz) spectra were taken on a JEOL PFT-100/EC-100 spectrometer operating in $CDCl_3$. The chemical shifts are expressed in ppm from internal TMS.

* The assignment of chloromethyl carbon is based on the chemical shift of 3-chloro-1,2-propanediol at 46.8 ppm: JOHNSON, L. F. & W. C. JANKOWSKI; "Carbon-13 NMR Spectra," Index No. 32, A Wiley-Interscience Publication, John Wiley & Sons, Inc., New York, 1972.

comparable. The pertinent assignment of ^{13}C-nmr data of **II** are shown in Chart 3.

The absolute configuration depicted in structure **I** was deduced from the evidence that specific rotation of $-143°$ is comparable to that of pentalenolactone (**III**)[1]. Therefore, the authors propose structure **I** for the new antibiotic AA-57 from the evidences described above.

Chart 3. ^{13}C-Nmr data of AA-57 methyl ester (**II**)

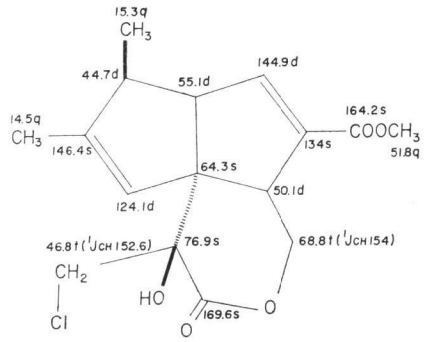


Table 1. Antibacterial spectrum of AA-57

Test organism[a]	MIC (μg/ml)
Staphylococcus aureus FDA 209P-JC1	2
Staphylococcus aureus IFO 3060	2
Staphylococcus aureus IFO 3183	2
Bacillus cereus IAM 1729P	250
Bacillus subtilis PCI 219	125
Micrococcus flavus IFO 3242	125
Sarcina lutea NIHJ	62
Escherichia coli NIHJ-JC2	2
Escherichia coli CCM 1707	62
Proteus rettgeri CCEB 137	16
Proteus mirabilis IFO 3849	31
Proteus vulgaris OX 19	16
Serratia marcescens IAM 1106	>500
Mycobacterium smegmatis ATCC 607[b]	31
Xanthomonas oryzae NIAS[c]	>500
Xanthomonas citri[c]	>500

a) Determined at 37°C for 48 hours on a medium consisting of 0.5% peptone, 0.3% meat extract bouillon, 0.2% NaCl and 1.2% agar.

b) Determined at 37°C for 48 hours on a glycerol medium consisting of 1% polypeptone, 1% meat extract bouillon, 0.2% NaCl, 4% glycerol, 0.025% Tween 80, 0.006% malachite green and 1.2% agar.

c) Determined at 27°C for 24 hours on potato-sucrose medium consisting of 0.3% potato extract, 0.02% sucrose, 0.005% polypeptone, 0.002% Na_2HPO_4, 0.0005% $NaNO_3$ and 0.01% agar (pH 7.0).

The minimum inhibitory concentration (MIC) of AA-57 was determined by the serial two-fold agar dilution method against Gram-positive and Gram-negative bacteria including acid-fast bacteria. The results are shown in Table 1. The acute intraperitoneal LD_{50} of AA-57 was 75 mg/kg in ICR/JCL female mice. The *in vitro* antitumor activity of AA-57 on SV40-transformed mouse fibroblast cells will be reported elsewhere.

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