

STRUCTURE DETERMINATION OF PYRACRIMYCIN A,
A NEW ANTIBIOTIC SUBSTANCE

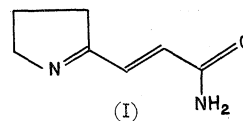
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Pyracrimycin A, a new antibiotic isolated from cultures of *Streptomyces eridani* n. sp. (ATCC 21619), was shown to be *trans* 3-(1-pyrrolin-2-yl)acrylamide (I). The structure determination was based on chemico-physical properties of I and of its dihydroderivative II. Structure I was confirmed by synthesis of the tetrahydro derivative III.

Pyracrimycin A is a metabolic product isolated from *Streptomyces eridani* n. sp. (ATCC 21619) (BERETTA, 1970), possessing an *in vitro* activity against Gram-positive and Gram-negative bacteria¹⁾. The product was recovered from the fermentation broth by extraction with *n*-butanol and obtained in a pure state by crystallization from methanol.



The structure of *trans* 3-(1-pyrrolin-2-yl)acrylamide (I) was assigned to pyracrimycin A on the basis of the chemical and physical properties and confirmed by the synthesis of the tetrahydro derivative III.

Pyracrimycin A is a white crystalline substance, m. p. 215~216°C. The elemental analysis accounts for C₇H₁₀N₂O (M. W. 138.17) confirmed by the M⁺ peak in the mass spectrum (Fig. 1). The IR spectrum (Fig. 2) is consistent with the presence of a primary amide group, as indicated by the bands at 3250 and 3050 cm⁻¹ (ν_{N-H} solid state) and 1670 cm⁻¹ broad (amide I and amide II), and of C=C or C=N groups, as indicated by the bands at 1620 and 1590 cm⁻¹. The presence of a strong band at 972 cm⁻¹ indicates that the C=C double bond bears two hydrogens in position *trans*. The PMR spectrum in DMSO-d₆ (Fig. 3) shows the presence of the sequence -CH₂-CH₂-CH₂- carrying at both sides heteroatoms or unsaturated carbon atoms, in any case not bearing hydrogen atoms (two triplets at δ 2.67 ppm and 3.90 ppm and a multiplet at 1.87 ppm; attribution confirmed by spin decoupling), of two *trans* hydrogens on a conjugated C=C group (two doublets at δ 6.44 and 7.16 ppm, J=16.0 Hz), whose two adjacent atoms do not bear hydrogens, and of a primary amide group (two broad signals at δ 7.2 and 7.6 ppm; slowly exchangeable with D₂O).

The UV spectrum in methanol solution shows a maximum at 235 m μ (ϵ 24,000) suggesting a conjugated chromophore. The maximum undergoes a small red shift in acidic solution; the ionizable function is basic, as shown by potentiometric titration

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Fig. 1. Mass spectrum of pyracrimycin A.

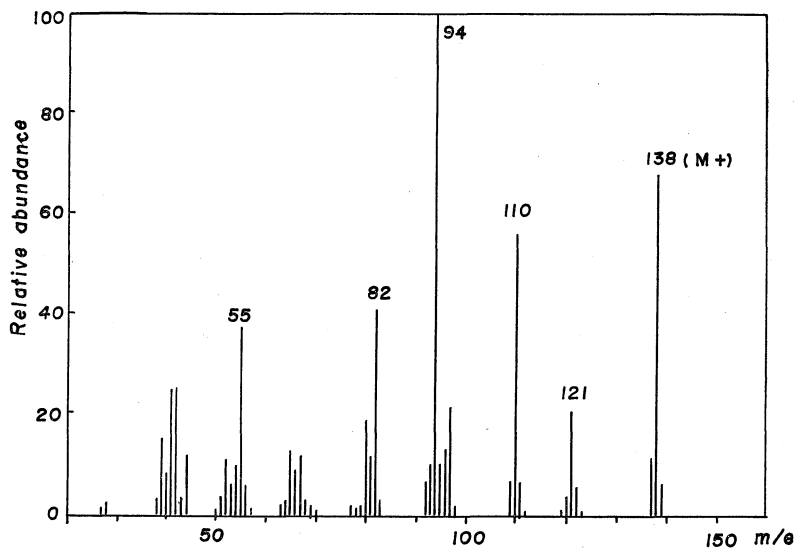
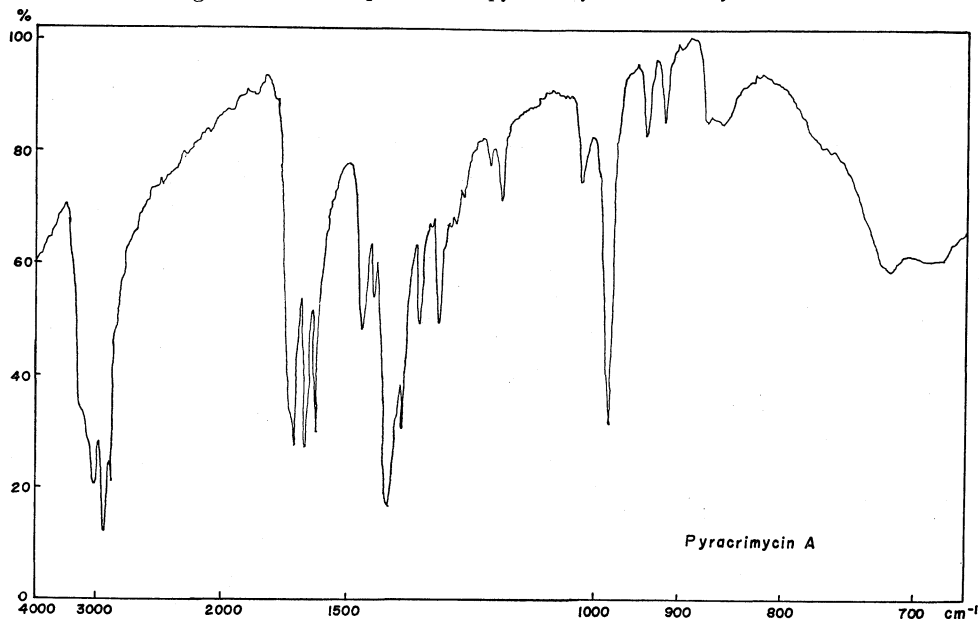
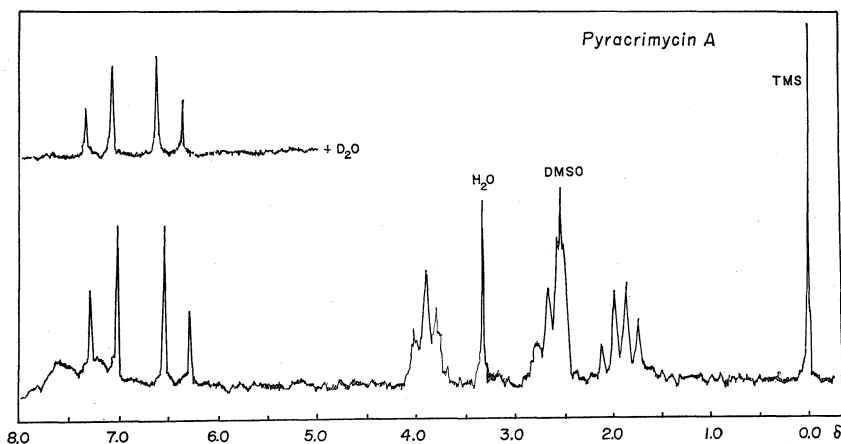
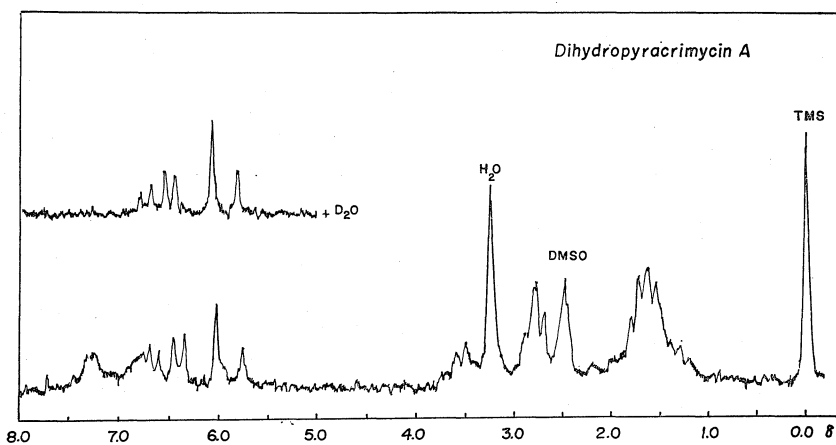


Fig. 2. Infrared spectrum of pyracrimycin A in Nujol mull.



in $\text{H}_2\text{O}/\text{MCS}$ 1/4 with 0.1 N HCl (pK_{MCS} 5.4). These properties were considered indicative of an unsaturated nitrogen function^{2,9}). To confirm the presence of this function pyracrimycin A was reduced with NaBH_4 ⁴) in ethanol to a dihydro-derivative (II), with elemental analysis in accordance for $\text{C}_7\text{H}_{12}\text{N}_2\text{O}$ (M. W. 140.19) confirmed by the M^+ peak in the mass spectrum. A pK_a value of 9.4 was obtained by potentiometric titration in H_2O , indicating that the postulated unsaturated nitrogen function was reduced. The PMR spectrum in DMSO-d_6 (Fig. 4) shows again two hydrogens on a C=C group, one as a doublet at δ 5.92 ppm and the other as a doublet of doublets at

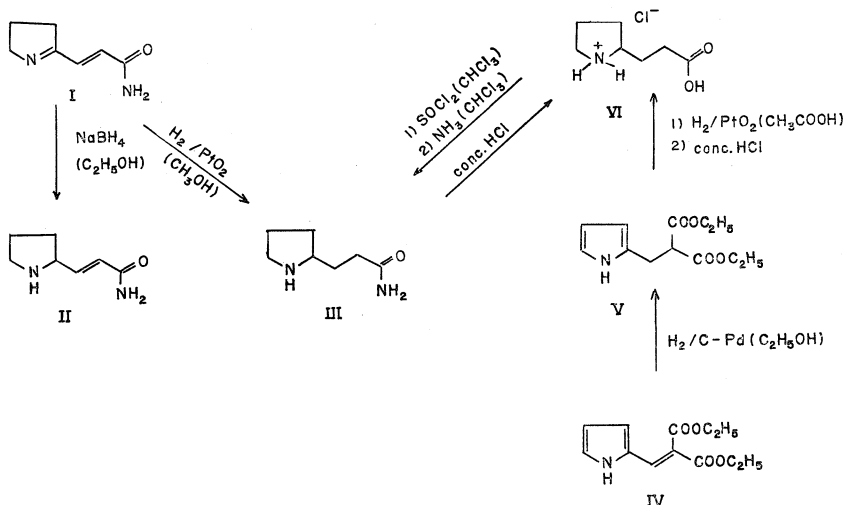
Fig. 3. PMR spectrum of pyracticimycin A in DMSO-d₆ solution.Fig. 4. PMR spectrum of dihydropyracticimycin A in DMSO-d₆ solution.

6.55 ppm, clearly indicating that in the reduced product a CH group is adjacent to the latter hydrogen. This can derive only from the selective reduction of the N=C group of the moiety $-\text{N}=\text{C}-\text{CH}=\text{CH}-$. All the data reported account for structure I, which at this point can be assigned to pyracticimycin A.

In order to confirm structure I pyracticimycin A was reduced to the tetrahydro-derivative III (Scheme 1) for comparison with the 3-(pyrrolidin-2-yl)propionamide obtained by synthesis. Diethyl 2-pyrrolylmethylenemalonate (IV)⁶ was reduced on Pd/C to diethyl 2-pyrrolylmethylmalonate (V), which by reduction on PtO₂ in acetic acid followed by hydrolysis in concentrated hydrochloric acid yielded 3-(pyrrolidin-2-yl)propionic acid hydrochloride (VI)⁶. Finally, VI was treated in chloroform solution with thionyl chloride and then with ammonia to give compound III which was identical (m. p., IR, PMR) to the one obtained from the natural product. Also compound III obtained from pyracticimycin was converted to VI by acid hydrolysis.

The Δ_1 pyrroline nucleus is generally considered in tautomeric equilibrium with the Δ_2 form but physical measurements proved that the equilibrium lies almost com-

Scheme 1



pletely towards the Δ_1 imino form⁸). In pyracrimycin A the presence of the Δ_2 tautomer is not detectable.

Concerning the products obtained from microorganisms, acrylamide moiety was found to be present in different metabolites, whereas the pyrrole nucleus was found almost exclusively in the Δ_2 or Δ_3 form^{9,10}. Anyhow, no relationship could be established between pyracrimycin A and other antibiotics so far described.

Experimental

Melting points were determined in capillary tubes and are uncorrected. IR spectra were taken on a Perkin-Elmer Model 157 spectrophotometer as nujol mulls or CHCl₃ solutions. UV spectra were recorded with a Unicam S. P. 800 spectrophotometer. PMR spectra were taken with a Varian A-60 (60 MHz) or with a Varian HA-100 (100 MHz) spectrometer in the solvents indicated (5~10% w/v), with TMS as internal reference (δ 0.00 ppm). The mass spectra were recorded with a Hitachi Perkin-Elmer RMU-6L spectrometer (single focus) at electron ionizing voltage 70 V. Analytical thin-layer chromatographic separations (TLC) were performed on Silicagel HF/UV 254 to a distance of 10.0 cm. The spots were detected after spraying with 0.1 N I₂ solution followed by conc. H₂SO₄.

Isolation of pyracrimycin A (I). The product was isolated from cultures of *S. eridani* as described¹¹.

Dihydropyracrimycin A (II). To a solution of I (0.5 g) in dry ethanol (300 ml) cooled in ice-bath, NaBH₄ (0.25 g) was added, the solution was kept at 4°C for 2 hours and at room temperature for 12 hours under gentle stirring. The excess of NaBH₄ was decomposed with 10% HCl, the ethanol was evaporated under reduced pressure after dilution with water. The residual aqueous solution, after alkalization with 10% Na₂CO₃, was repeatedly extracted with ethyl acetate. The extracts were dried and concentrated to dryness obtaining 0.1 g of a highly hygroscopic product; TLC (ethyl acetate - acetic acid - water, 3:1:1): R_f 0.6. Found: C 59.45, H 8.80, N 19.60, C₇H₁₂N₂O requires: C 59.98, H 8.63, N 19.98. IR (nujol): 3300 and 3130 (ν NH and ν NH₂), 1680 (ν C=O), 1620 (ν C=C, δ NH and δ NH₂), 990 cm⁻¹ (γ CH=CH *trans*).

Tetrahydropyracrimycin A (III). A solution of I (0.5 g) in methanol (110 ml) was reduced at normal pressure and room temperature with H₂ over PtO₂ catalyst (0.15 g) with an H₂ consumption of 2 moles/mole. The solution, filtered from the catalyst, was evaporated to

dryness; the oily residue by treatment with ethyl ether yielded a white solid (0.3 g), m.p. 99~100°C; TLC (ethyl acetate - acetic acid - water, 3:1:1): Rf 0.2. Found: C 58.80, H 10.02, N 19.88; $C_7H_{14}N_2O$: requires C 59.13, H 9.92, N 19.70. Potentiometric titration with 0.1 N HCl in water: pKa 9.85. IR (nujol): 3250 and 3130 (νNH and νNH_2), 1670 ($\nu C=O$), 1635 cm^{-1} (δNH and δNH_2).

Hydrolysis of tetrahydropyracrimycin A. Compound III (0.3 g) was refluxed with conc. HCl for 8 hours and the solution evaporated several times to dryness from water; by treating the oily residue with anhydrous acetone a solid product was obtained which by crystallization from isopropyl alcohol yielded 0.05 g of 3-(pyrrolidin-2-yl)propionic acid, hydrochloride (VI), m.p. 112~118°C. IR and PMR spectra, TLC and melting points are in accordance with those obtained for the synthetic compounds prepared as described below.

3-(Pyrrolidin-2-yl)propionic acid hydrochloride (VI). Diethyl 2-pyrrolidylmethylmalonate (V) (b. p. 120°C/0.2 mm, reported⁷⁾ 140~145°C/1 mm) was obtained by reduction of diethyl 2-pyrrolidylmethylene malonate (IV)⁵⁾ at normal pressure and temperature in ethanol over 10% Pd/C catalyst. A solution of V (8 g) in glacial acetic acid (50 ml) was hydrogenated in the presence of PtO_2 (0.7 g) for 6 hours at 4 atmospheres and room temperature. The solution filtered from the catalyst was evaporated under reduced pressure at 40°C, the residue was dissolved in water and treated with solid potassium carbonate. The solution was extracted with ethyl ether and the extract dried on Na_2SO_4 , filtered and concentrated. The residue was distilled *in vacuo* at 135~142°C/0.8 mm obtaining an oily product (5.7 g) which was heated at reflux for 12 hours with conc. HCl (110 ml). The solution was treated with charcoal and concentrated *in vacuo*. The residue after treating with acetone gave VI (4.07 g, 67.1%), hygroscopic product, m.p. 115~118°C (lit.⁶⁾ m.p. ca. 130°C); TLC (methanol): Rf 0.20. Found: C 46.68, H 7.77, N 7.78, Cl 20.01; $C_7H_{14}ClNO_2$ requires: C 46.80, H 7.85, N 7.79, Cl 19.73. IR (nujol) 3070~2200 (νOH and NH_2^+), 1700 ($\nu C=O$), 1600 (δNH_2^+), 1200 ($\nu C-O$), 880 cm^{-1} (νOH).

3-(Pyrrolidin-2-yl)propionamide (III). To a suspension of 3-(pyrrolidin-2-yl)propionic acid hydrochloride (1 g) in anhydrous chloroform (20 ml) containing one drop of pyridine, thionyl chloride (5 ml) was added, maintaining the temperature at 5°C. The mixture was stirred for 2 hours at room temperature and then concentrated *in vacuo*. The residue was treated at 0°C with a saturated solution of gaseous ammonia in anhydrous chloroform (40 ml). After stirring for 30 minutes the precipitate was filtered off, and the filtrate was evaporated *in vacuo* at room temperature. The oily product was washed with light petroleum and then with ethyl ether. A solid compound was obtained, m.p. 98~100°C (0.14 g, 17.6%), which was found to be identical (TLC, IR and PMR spectra) with the product obtained as described above. The picrolonate has m.p. 150°C (from ethanol). Found: C 50.54, H 5.26, N 20.41; $C_{17}H_{22}N_6O_6$ requires: C 50.24, H 5.46, N 20.68.

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