

FR-900148, A NEW ANTIBIOTIC

II. STRUCTURE DETERMINATION OF FR-900148

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The structure of FR-900148, a new antibiotic produced by a strain of *Streptomyces xanthocidicus*, has been established as 1-N-valyl-3-chloro-2,5-dihydro-5-oxo-1H-pyrrole-2-carboxylic acid on the basis of spectroscopic and chemical evidence.

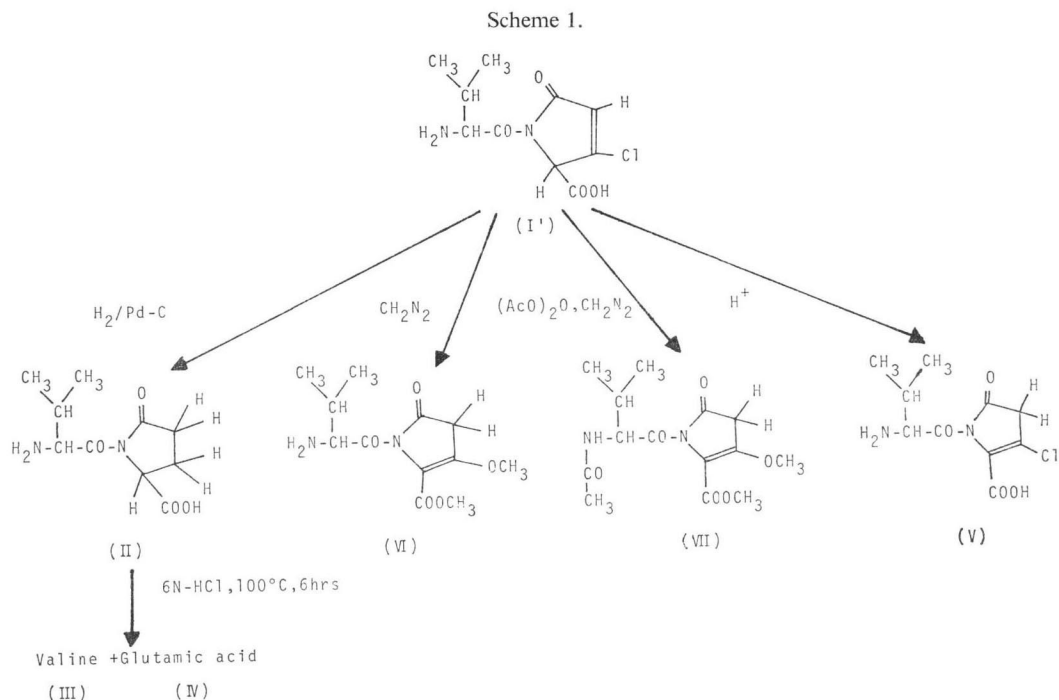
In the course of screening for cell wall inhibitors, we found that a strain of *Streptomyces xanthocidicus* produces an antibiotic. The product, designated FR-900148, shows activity against Gram-positive as well as Gram-negative bacteria, although *Pseudomonas aeruginosa* is not inhibited. The taxonomy of the producing organism, and procedures for isolation and characterization of FR-900148 have been reported in a preceding paper¹⁾. This paper describes elucidation of the structure of FR-900148.

Results and Discussion

FR-900148 (**I**) was isolated as an amphoteric white powder [sodium salt: m.p. 143~147°C (decomp.)]. The antibiotic shows pKa' values of 3.25 and 7.90, and is soluble in water and methanol. Its Rf value is 0.65 on silica-gel thin-layer plates developed with *n*-propanol - water (6:4). The antibiotic was detected by its positive reaction with ninhydrin. Elemental analysis and titration of **I** established the molecular formula as C₁₀H₁₂N₂O₄Cl Na·2H₂O. The IR spectrum exhibited absorption bands attributable to an amide group and double bond at 1500~1700 cm⁻¹ and to C-Cl at 670 cm⁻¹. The pmr spectrum showed the following signals; six methyl protons, three methine protons and one olefinic proton (see the preceding paper¹⁾). The signals at δ 1.04 (3 H × 2, d, J = 7 Hz), 2.25 (1 H, m), and 3.92 (1 H, d, J = 7 Hz) are assigned to a valyl residue. A decoupling experiment supported the presence of valine.

The reactions described below are outlined in Scheme 1. Hydrogenation of **I** consumed two moles of hydrogen and the reaction solution had a pH of 2.0. This is attributed to the hydrochloric acid liberated in the reaction. The hydrogenated product (**II**) was subjected to acid hydrolysis which afforded valine (**III**) and glutamic acid (**IV**). The pmr of **II** also indicated that **II** is valyl glutamic acid (Fig. 1). Because **I** has only one pKa' at 3.25 in the acidic region, it is believed to contain a cyclic glutamic acid structure (pyro-glutamic acid).

Treatment of **I** with acetic anhydride and subsequent methylation with diazomethane gave an N-acetyl methyl derivative (**VII**). Considering that the fragment left in **I** after accounting for valine is C₅H₂NO₃Cl Na and that glutamic acid was obtained by hydrogenation, the partial structure of **I** should be one of the three shown in Fig. 2 (**VIII**~**X**). The pmr spectrum of **I** shows signals at δ 4.98



(1 H, s) and δ 6.46 (1 H, s) in addition to those attributed to valine. Structures IX and X are excluded since signals corresponding to their two protons should be observed as doublets and as an AB system or singlet, respectively. Thus, VIII is the most probable structure. Theoretical calculations of δ values for C₄-H of VIII gave δ 6.54²³, which is in good agreement with the observed value (δ 6.46).

Treatment of I with mild acid gave the biologically inactive compound (V), which showed a molecular ion peak at 260 (M^+) and 262 ($M+2$) in the mass spectrum (peak height ratio, 3:1). Elemental analysis established the molecular formula as C₁₀H₁₃N₂O₄Cl. The pmr spectrum indicated that isomerization had occurred as shown in Scheme 1. The free acid of I (I') was methylated with diazomethane to give compound VI, for which the molecular formula C₁₂H₁₈N₂O₅ was indicated by elemental analysis and mass spectrometry (M^+ 270). Compound VI contains no

Fig. 1. Pmr spectrum of compound II (D₂O).

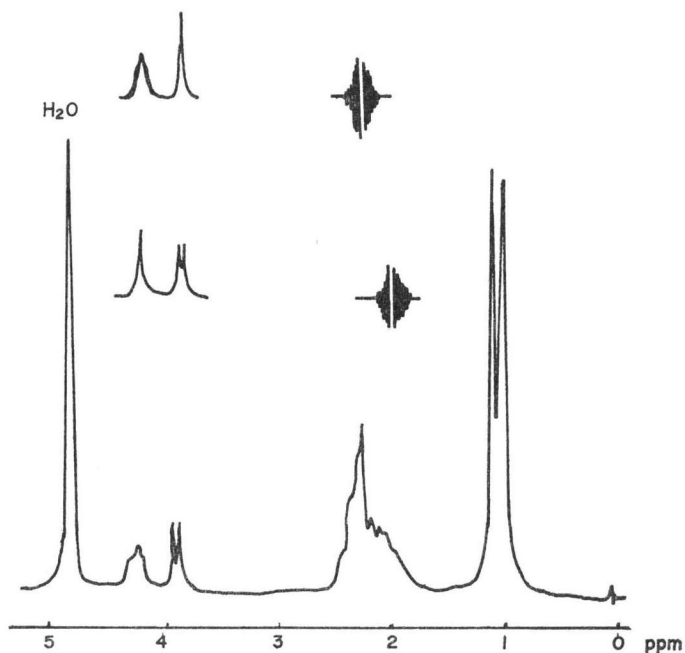


Fig. 2. Partial structure of FR-900148.

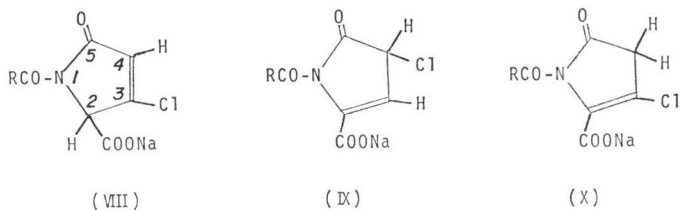
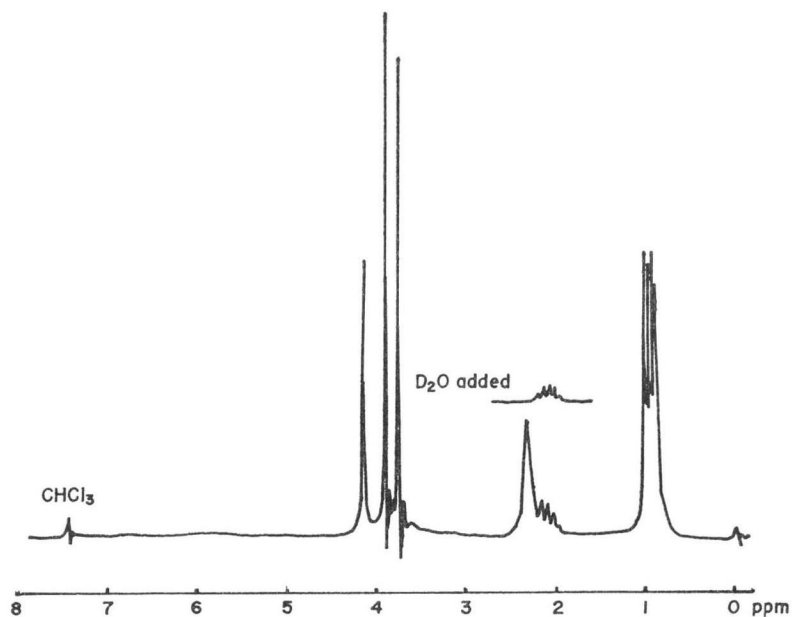
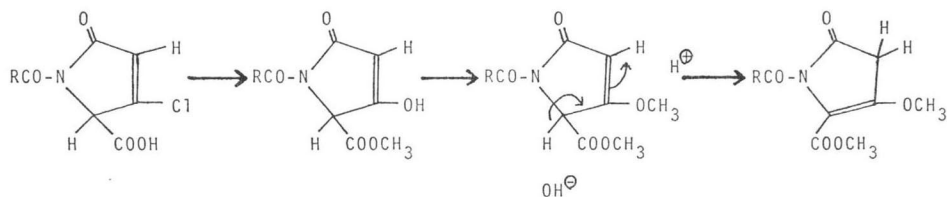
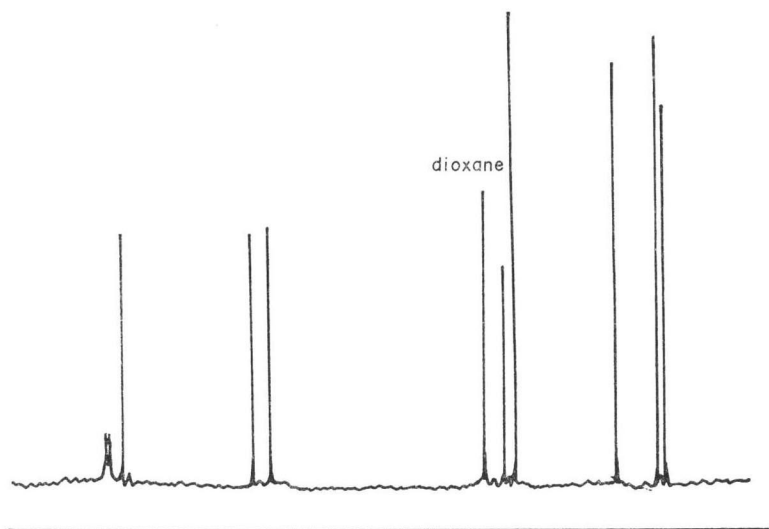
Fig. 3. Pmr spectrum of compound VI ($\text{CDCl}_3/\text{D}_2\text{O}$).

Fig. 4. Methylation of FR-900148-free acid with diazomethane.



chlorine and its pmr spectrum showed two methoxy groups (Fig. 3). The signals at δ 4.98 and δ 6.46 in I have coalesced to a two-proton singlet at δ 4.10. The same phenomenon was observed upon mild acid treatment of I. The mechanism proposed for this methylation (Fig. 4) involves hydrolysis of the vinyl chloride to give an enol. The enol is then methylated and the product isomerizes.

The ^{13}C -nmr spectrum of I (Fig. 5) is consistent with the proposed structure. Chemical shifts are assigned (Table 1) on the basis of predicted values in conjunction with the results of ^1H off-resonance experiments. Consequently, the structure deduced for FR-900148 is 1-N-valyl-3-chloro-2,5-dihydro-5-oxo-1H-pyrrole-2-carboxylic acid. Synthetic studies are now in progress.

Fig. 5. ^{13}C -Nmr spectrum of FR-900148 (D_2O).

Experimental

(1) Material

FR-900148 (I) was obtained from the fermentation broth of *Streptomyces xanthocidicus* by the methods described in the preceding paper which also reports spectral and chemical characteristics¹².

(2) Hydrogenation

A 400 mg sample of I in 40 ml of 50% aqueous ethanol was hydrogenated over palladium-carbon at 20°C under one atmosphere of hydrogen. Two moles of hydrogen were absorbed within 2 hours. The pH of the reaction solution fell to 2.0 and was adjusted to 6.5 with 6 N NaOH. After concentration, the hydrogenation reaction mixture was desalted by the use of Sephadex G-15 (1.5 liters). Concentration and freeze-drying of the ninhydrin-positive fraction gave 300 mg of white powder (II). Compound II showed an R_f value of 0.6 when developed on a silica gel G plate with *n*-propanol - water (6: 4).

Analysis Calcd. for $\text{C}_{10}\text{H}_{15}\text{N}_2\text{O}_4\text{Na} \cdot \text{H}_2\text{O}$:

C 44.78, H 6.34, N 10.45, Cl 0.00, Na 8.58

Found: C 44.22, H 6.94, N 9.97, Cl 0.03, Na 7.90

Pmr (D_2O): δ 1.04 (3H \times 2, d, J=7 Hz), 1.80~2.40 (5H, m), 3.84 (1H, d, J=7 Hz), and 4.20 (1H, m).

(3) Hydrolysis of compound II

A solution of 50 mg of II in 6 N HCl was refluxed at 100°C for 6 hours, and concentrated to dryness *in vacuo*. Two ninhydrin-positive substances were detected by TLC on cellulose with *n*-butanol - acetic acid - water (4: 1: 1) as developing solvent. They were identified as valine (III) and glutamic acid (IV) by comparison of their R_f values with those of authentic samples.

Table 1. ^{13}C -Nmr data for FR-900148.

Chemical shift ¹⁾	Off-resonance ²⁾	Assignment ³⁾
17.7	q	} $(\text{CH}_3)_2\text{-CH-}$
18.6	q	
30.8	d	$(\text{CH}_3)_2\text{-CH-}$
59.3	d	$-\text{CH}(\text{NH}_2)\text{-CO-}$
62.2	d	$-\text{N-CH}(\text{COONa})\text{-C=}$
127.9	d	$=\text{CH-CO-}$
133.2	s	$=\text{C}(\text{Cl})\text{-}$
169.3	s	$-\text{CO-}$
173.2	s	$-\text{CO-}$
173.3	s	$-\text{CO-}$

1) δ value in ppm from TMS.

2) Multiplicity: q=quartet, d=doublet, s=singlet.

3) Refer to structure I in Scheme 1.

(4) Treatment of I with mild acid

A solution of 500 mg of I in acidic water (pH 2.0, 50 ml) was kept at 20°C for 3 days. Concentration of this solution yielded 450 mg of colorless crystal (V).

Analysis Calcd. for $C_{10}H_{13}N_2O_4Cl \cdot H_2O$:

C 43.16, H 5.40, N 10.07, Cl 12.59

Found: C 42.82, H 5.50, N 10.05, Cl 12.62

Mass spectrum: m/e 260 (M), m/e 262 (M+2)

NMR ($D_2O/NaHCO_3$): δ 1.03 (3H \times 2, d, J=7 Hz), 2.15 (1H, m), 3.65 (2H, s), and 3.95 (1H, m)

(5) Methylation

A solution of 600 mg of I in 30 ml water was passed through a CM-Sephadex column (H^+ cycle) in order to prepare the free acid (I'). To this solution was added 100 ml of methanol and then ethereal diazomethane at 20°C until the yellow color of diazomethane did not disappear. The reaction mixture was concentrated and purified by chromatography on a silica gel column with ethyl acetate-methanol (10:1) as eluant. Fractions giving positive ninhydrin reactions were collected and concentrated to give 300 mg of colorless oil (VI).

Analysis Calcd. for $C_{12}H_{18}N_2O_5$: C 53.33, H 6.67, N 10.37, Cl 0.00

Found: C 51.22, H 6.50, N 9.80, Cl 0.67

Mass spectrum: m/e 270 (M), m/e 255 (M-15)

NMR ($CDCl_3/D_2O$): δ 0.90 (3H, d, J=6 Hz), 0.95 (3H, d, J=6 Hz), 2.20 (1H, m), 2.30 (2H, m exchangeable), 3.70 (3H, s), 3.85 (3H, s), and 4.10 (2H, s)

(6) Acetylation and methylation

To a solution of 400 mg of I in 10 ml of 50% aqueous methanol was added 0.7 ml of acetic anhydride. The mixture was stirred vigorously at 20°C for 2 hours at pH 8.0, then concentrated and diluted with water to give 20 ml of aqueous solution. After adjustment to pH 2.0, the acetylated compound was extracted with an equal portion of *n*-butanol. Methylation was carried out by the addition of ethereal diazomethane. The methyl ester was purified by silica gel chromatography using ethyl acetate as eluant and iodine vapor for detection. Concentration of the positive fractions gave 400 mg of colorless oil (VII).

Analysis Calcd. for $C_{14}H_{20}N_2O_6$: C 53.85, H 6.41, N 8.97, Cl 0.00

Found: C 53.08, H 6.77, N 8.47, Cl 0.58

Mass spectrum: m/e 312 (M)

NMR ($CDCl_3/D_2O$): δ 0.87 (3H, d, J=6 Hz), 0.92 (3H, d, J=6 Hz), 2.00 (3H, s), 2.25 (1H, m), 3.70 (3H, s), 3.85 (3H, s), 4.09 (2H, s), 5.08 (1H, dd, J=6, 9 Hz) dd changed to d (J=6 Hz), when D_2O was added and 6.50 (1H, d, J=9 Hz, exchangeable)

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References

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