FR-900137, A NEW ANTIBIOTIC

II. STRUCTURE DETERMINATION OF FR-900137

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The structure of FR-900137, a new antibiotic produced by a new strain of *Streptomyces* has been established as methyl hydrogen N^1-methyl N^2-(L)-leucyl phosphorohydrazidate on the basis of spectroscopic and chemical evidence.

In the course of screening for new cell wall inhibitors, we found that *Streptomyces unzenensis* sp. nov. produces a novel antibiotic designated as FR-900137, which shows antibacterial activity particularly against *Escherichia coli*. Taxonomy of the producing organism, isolation and characterization of FR-900137 have been reported in the preceding paper[1]. This paper describes the structure elucidation of this antibiotic.

Results

FR-900137 (**I**) was isolated as an amphoteric white powder [mp 134°C (decomp.)], which is soluble in water and methanol. Compound **I** showed positive reactions to ninhydrin and ammonium molybdate reagents. Elemental analysis established the molecular formula of **I** as $C_8H_{20}N_3O_4P$. Its IR spectrum exhibited absorption bands attributable to amide at 1690 cm^{-1} and P=O at 1230 cm^{-1}. Pmr spectrum analysis revealed that **I** contains CH_3–N–P, CH_3–O–P and leucine moiety. The signals at δ 0.95 (3 H×2, d, J=6 Hz), 1.60 (1 H, m), 1.70 (2 H, m), 3.90 (1 H, m) are assigned to the leucyl moiety. Decoupling experiments confirmed the presence of this segment. The signals at δ 2.80 (3 H, d, J=8 Hz), and 3.56 (3 H, d, J=11 Hz) are assigned to N-methyl and O-methyl, respectively, both of which are coupled to phosphorus.

Scheme 1.

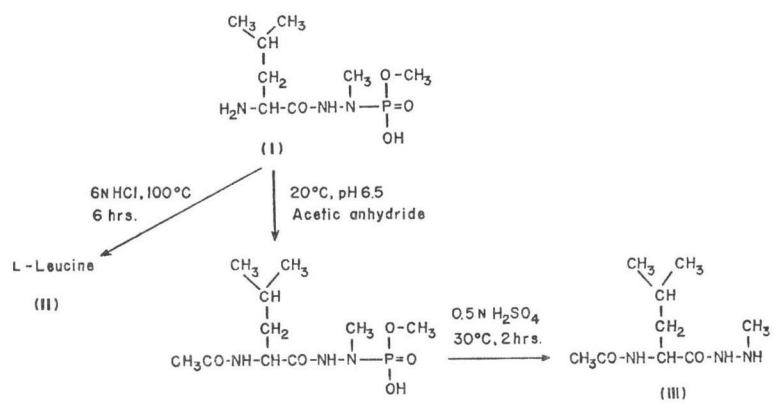
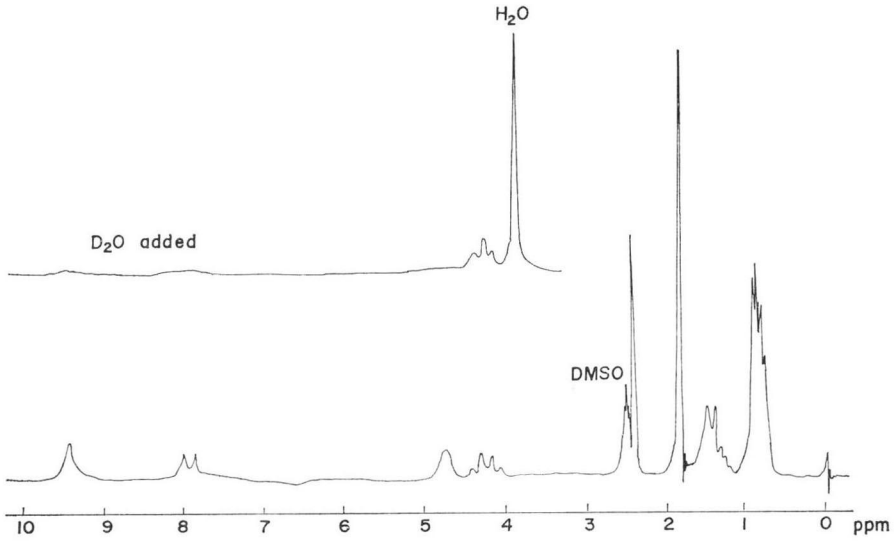


Fig. 1. Pmr spectrum of compound **III** ($DMSO/D_2O$).



The reactions described below are outlined in Scheme 1. Hydrolysis of **I** with 6 N HCl at 100°C for 6 hours gave a ninhydrin-positive compound (**II**), which was determined to be L-leucine by inspection of its IR spectrum and its optical rotation.

After treatment of **I** with acetic anhydride, the resulting acetyl derivative was subjected to hydrolysis with 0.5 N H_2SO_4 at 30°C for 2 hours to give colorless crystals (**III**). Their molecular formula was established as $C_9H_{19}N_3O_2$ by elemental analysis and mass spectrometry (M^+ 201). The pmr spectrum of **III** (Fig. 1) revealed acetyl, leucyl and N-methyl groups. The signal corresponding to the α-methine proton of leucine at δ 3.90 in the pmr spectrum of **I** shifted to a lower field (δ 4.25) as a double triplet, which implies that the α-methine proton of leucine is coupled to the amide proton. Thus, **III** contains acetyl leucine, and the remaining CH_5N_2 fragment was deduced to be N-methyl hydrazine from the fact that N-methyl function is present in **III**. The N-methyl signal appeared at δ 2.40 as a singlet in the pmr spectrum of **III** instead of δ 2.80 (doublet, J = 8 Hz) in the spectrum of **I**. This indicates that the CH_3–N–P function is present in **I**, but not in **III**. That is, N^1-methyl-N^2-leucyl hydrazine is bonded to phosphorus by a phosphoramide bond, which is easily cleaved by the mild acid treatment.

In the pmr spectrum of **I**, the doublet signal at δ 3.56 (J = 11 Hz) suggested the presence of CH_3–O–P in **I**. The coupling constant of the methyl phosphate is in good agreement with that of phosphonic acid esters of FR-33289[2] and phosphoramidon[4]. Thus, **I** is composed of methylphosphate and N^1-methyl-N^2-

Table 1. ^{13}C NMR data of FR-900137.

Chemical shift[1]	Off-resonance[2]	Assignment[3]
21.9	q	$(\underline{C}H_3)_2$–CH–
22.1	q	
24.4	d	$(CH_3)_2$–$\underline{C}$H–
37.9	q	$\underline{C}H_3$–N–P–
38.2	q	
40.9	t	–CH–$\underline{C}H_2$–CH–
51.4	d	–$\underline{C}$H(NH_2)–CO–
53.0	q	$\underline{C}H_3$–O–P–
53.2	q	
171.8	s	–CH(NH_2)–$\underline{C}$O–

1) δ value in ppm from TMS.
2) Multiplicity: q = quartet, t = triplet, d = doublet, s = singlet.
3) Refer to the structure in Scheme 1.

leucyl hydrazine (phosphorohydrazidate skeleton).

The proposed structure was confirmed by the cmr spectrum of **I**. The chemical shifts are assigned as shown in Table 1. It was observed that the O-methyl and N-methyl carbons are coupled with phosphorus ($J_{\underline{C}H_3\text{-}O\text{-}\underline{P}}=6.1$ Hz, $J_{\underline{C}H_3\text{-}N\text{-}\underline{P}}=7.6$ Hz). These assignments were made on the basis of their chemical shifts, along with a ^{1}H-off resonance experiment.

Chemical synthesis corroborated the proposed structure for FR-900137, thus, establishing the structure as methyl hydrogen N^1-methyl-N^2-(L)-leucyl phosphorohydrazidate.

Experimental

1. Material

FR-900137 (**I**) was obtained from fermentation broth of *Streptomyces unzenensis* sp. nov. by the method described in the preceding paper. The spectral and chemical characteristics have been reported in that paper.

2. Strong acid hydrolysis

A solution of 1 g of **I** in 30 ml of 6 N HCl was refluxed at 100°C for 6 hours. The degradation product was purified on a cellulose column using *n*-butanol saturated with water as an eluant. Fractions positive to ninhydrin reagent were, collected and concentrated to give 410 mg of white powder (**II**). The IR and pmr spectra of this compound were identical with those of L-leucine. $[\alpha]_D^{25}$ +13.8 (*c* 1, 1 N HCl); L-leucine showed optical rotation +13.7 in the same condition.

Analysis Calcd. for $C_6H_{13}NO_2$: C 54.96, H 9.92, N 10.69
Found : C 54.69, H 10.11, N 10.54

3. Acetylation and mild hydrolysis

To a solution of 1 g of FR-900137 (**I**) in 10 ml water was added 5 ml of acetic anhydride and the solution was vigorously stirred at 20°C at pH 6.5 until ninhydrin reaction was negative (for 2 hours). Then the reaction solution was kept at 30°C for 2 hours in 0.5 N H_2SO_4. The hydrolysate was detected on tlc by iodine vapor, and was purified by silica gel column chromatography using a solvent mixture of chloroform - methanol (20: 1). Concentration of the eluate gave 320 mg of colorless crystals (**III**).

Analysis Calcd. for $C_9H_{19}N_3O_2$: C 53.73, H 9.45, N 20.90
Found : C 53.78, H 9.49, N 20.75

Mass spectrum: *m/e* 201 (M^+)

IR (nujol): 3300, 2900, 2850, 1635, 1540, 1500, 1460, 1440, 1385, 1370, 1320, 1285, 1275, 1260, 1200, 1150, 1120, 1100, 1050, 1000, 965, 925, 880, 780, and 700 cm^{-1}

NMR (DMSO/D_2O): δ 0.82 (3H, d, J=6 Hz), 0.90 (3H, d, J=6 Hz), 1.20~1.50 (1H, m), 1.30~1.60 (2H, m), 1.87 (3H, s), 2.40 (3H, s), 4.25 (1H, q, J=8 Hz D_2O addition changed q to t), 4.74 (1H, br s, exchangeable), 7.95 (1H, d, J=8 Hz, exchangeable) and 9.40 (1H, br s, exchangeable).

Discussion

FR-900137, a new antibiotic is a unique natural product containing a phosphorohydrazidate function. Negamycin has been reported as an antibiotic effective against Gram-negative bacteria, which is composed of N^1-methyl hydrazino acetic acid[3]. Phosphoramidon[4] and L-(N^5-phosphono)methionine-S-sulfoxyminyl-L-alanyl-L-alanine[5] are known as metabolites from *Streptomyces* with phosphoramide bond in the molecule.

FR-900137 is very labile in acidic solution. This is due to the cleavage of phosphoramide bond as has been reported by UMEZAWA (phosphoramidon: room temperature, 1 N HCl, for one day) and PRUESS (L-(N^5-phosphono)methionine-S-sulfoxyminyl-L-alanyl-L-alanine: 100°C, pH 4, for 1 hour).

Treatment of FR-900137 with acetone formed unelucidated products which were detected on thin-layer chromatography (probably acetone adducts). Therefore, acid and acetone were not used

during the isolation process and structure elucidation studies.

Acknowledgement

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