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Studies on Phosphonic Acid Antibiotics. III. Structure and Synthesis of 3-(N-Acetyl-N-hydroxyamino)propylphosphonic Acid (FR-900098) and 3-(N-Acetyl-N-hydroxyamino)-2(*R*)-hydroxypropylphosphonic Acid (FR-33289)

KEIJI HEMMI, HIDEKAZU TAKENO, MASASHI HASHIMOTO, and TAKASHI KAMIYA*

Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-1-6, Kashima, Yodogawa-ku, Osaka, 532, Japan

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The structures of the antibiotics FR-900098 (II) and FR-33289 (III), isolated from *Streptomyces rubellomurius*, have been established as 3-(N-acetyl-N-hydroxyamino)-propylphosphonic acid and 3-(N-acetyl-N-hydroxyamino)-2(*R*)-hydroxypropylphosphonic acid, respectively, on the basis of spectroscopic and chemical evidence and, finally, by synthesis.

Keywords——antibiotic; inhibitor of bacterial cell wall synthesis; structure determination; phosphonic acid; hydroxamic acid; Michaelis-Becker reaction; 3-(N-acetyl-N-hydroxyamino)propylphosphonic acid; 3-(N-acetyl-N-hydroxyamino-2(*R*)-hydroxypropylphosphonic acid

Phosphonic acid-containing antibiotics, such as fosfomycin (I), have attracted considerable interest in recent years.[1] As part of an antibiotic screening program in our laboratories, a search has been undertaken for inhibitors of bacterial cell wall synthesis, and this has recently led to the isolation of novel phosphonic acid antibiotics, FR-900098 (II) and FR-33289 (III), from the culture broth of *Streptomyces rubellomurius*.[2a,b] A preliminary bioassay indicated that II is active against a variety of Gram-negative bacteria including *Pseudomonas* species, while III shows somewhat lower activity.[2c] We have determined the structures of these two antibiotics on the basis of spectroscopic studies and confirmed them by synthesis, as reported previously.[3] The present paper is devoted to a more detailed account of the structure determination and synthesis of these antibiotics.

The first antibiotic, FR-900098 (II), $C_5H_{11}O_5NPNa$ (monosodium salt), mp 193—194° (dec.), contains phosphonic acid and hydroxamic acid functions as revealed by potentiometric titration and positive color reaction to phosphomolybdate and $FeCl_3$ reagents.[4]

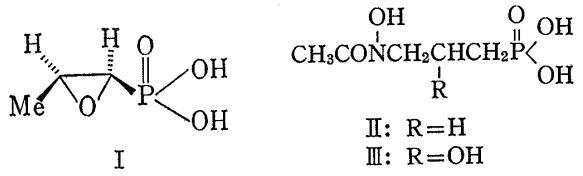

Chart 1

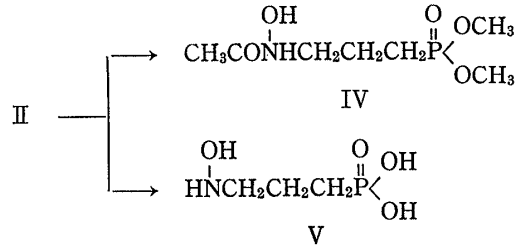

Chart 2

On treatment with diazomethane, II was converted into the dimethyl ester (IV), which was still positive in the $FeCl_3$ test. The mass spectrum of IV showed the molecular ion peak at *m/e* 225.0760 (calcd. 225.0766), thus confirming the molecular formula of II. The presence of the phosphonic acid function in II was corroborated by the following characteristic nuclear magnetic resonance (NMR) coupling data.[5] The ^{13}C NMR spectrum of II (D_2O) showed a doublet with an extremely large coupling constant (J=207.5 Hz) at δ 24.32, attributable

to the C-1 carbon, and two doublets (J=3.1 and 18.3 Hz) at δ 21.47 and 54.44, which were assigned to the C-2 and C-3 carbons, respectively. In the ^{1}H NMR spectrum ($CDCl_3$) of IV, the two-methyl signal (6H) appeared as a doublet (J=10 Hz) at δ 3.70, indicating that IV is the phosphonic acid dimethyl ester. Moreover, the ^{1}H NMR spectrum (D_2O) of II showed an acetyl signal at δ 2.16, which appeared at δ 2.13 in the spectrum of IV. On treatment with 1 N hydrochloric acid under reflux, this acetyl group was readily hydrolyzed to give the hydroxylamine (V), which was positive to the triphenyltetrazolium color reaction. These data clearly indicated the presence of the acetyl hydroxamic acid function in II. The structure of FR-900098 was thus deduced to be 3-(N-acetyl-N-hydroxyamino)propylphosphonic acid (II). Having thus established the structure of FR-900098, we turned to the synthesis of II for the decisive structural confirmation and also for the acquisition of a large quantity of the compound for biological testing.

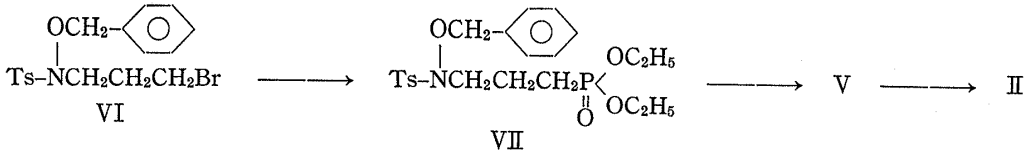

Chart 3

The starting material was 3-(N-tosyl-N-benzyloxyamino)propyl bromide (VI),[6] easily derivable by condensation of N-tosyl-N-benzyloxyamine and 1,3-dibromopropane. Michaelis-Becker reaction of VI with sodium diethyl phosphinate in benzene gave the condensation product (VII). Then, VII was subjected to hydrolysis with concentrated hydrochloric acid-acetic acid under reflux to provide in 63% overall yield the hydroxylamine (V), which was proved to be identical with a sample derived from the natural product. Final acetylation of V with acetic anhydride in water at room temperature gave 83% yield of II as the monosodium salt. This synthetic product was identical with the naturally occurring material as judged by comparison of mp, and infrared (IR) and NMR spectra.

The structure of the second antibiotic FR-33289 (III), isolated as a minor component, was deduced to be 3-(N-acetyl-N-hydroxyamino)-2-hydroxypropylphosphonic acid on the basis of spectroscopic evidence.[2b]

For confirmation of the structure and determination of the configuration at C-2, III was synthesized as follows. With regard to the determination of the C-2 configuration, we decided to synthesize one of the two possible diastereoisomers and chose, as the starting material, diethyl 2(R), 3-dihydroxypropylphosphonate (VIII), which is readily accessible from D-mannitol.[7]

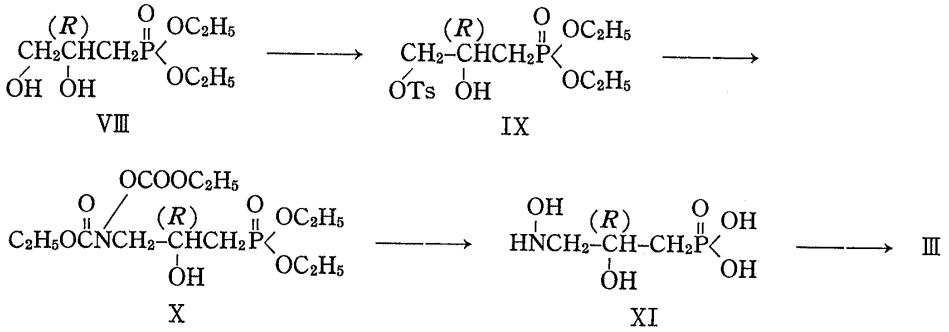

Chart 4

The primary alcohol function of VIII was selectively tosylated by using 1.0 molar equivalent of p-toluenesulfonyl chloride in pyridine to give the monotosylate (IX) in 83% yield. The secondary alcohol function of IX was then protected by silylation using bis (trimethyl-

silyl)acetamide in N,N-dimethylformamide and the silylated product was treated *in situ* with potassium N,O-dicarboethoxyhydroxamide[8] at 70° to give 41% yield of the condensation product (X). In an attempt to remove the protective groups and obtain the hydroxylamine (XI), X was heated in 6 N hydrochloric acid. This resulted in the formation of a complex mixture from which the desired product could not be isolated. Therefore, stepwise removal of the protecting groups was carried out. The phosphonic acid protective group was first removed by treatment with trimethylsilyl bromide[9] in methylene chloride at room temperature and, without purification, the product was subjected to hydrolysis with 1 N hydrochloric acid under reflux to give the hydroxylamine (XI) in 47% yield. This synthetic compound was identical with the sample derived from the natural product as judged by mp, optical rotation, and IR and NMR spectral comparison, establishing the C-2 configuration to be *R*.[10] Acetylation of XI in a manner similar to that used for preparing II yielded III as the monosodium salt, which was identical with the naturally occurring material as judged by comparison of the physical and biological properties.

The structural features of these antibiotics II and III are new among naturally occurring products bearing the phosphonic acid or hydroxamic acid function. These antibiotics showed an unique but relatively low activity.[2c,d] Structural modification directed toward the enhancement of their biological activity will be the theme of the following paper in this series.

Experimental

Melting points were determined on a Thomas–Hoover capillary melting point apparatus. Infrared spectra were recorded on a Hitachi 260-10 spectrophotometer. NMR spectra were determined on a JEOL JNM-PMX 60 NMR spectrometer and a JEOL JNM-MH 100 NMR spectrometer. Mass spectra (MS) were measured with a Hitachi PMV-6M mass spectrometer. Optical rotations were measured on a JASCO DIP-140 polarimeter.

Materials——Isolation procedures for FR-900098 (II) and FR-33289 (III) were reported by Imanaka *et al.*[2a,b]

FR-900098 (II) (Na Salt)[11]——^{13}C NMR (in D_2O) δ: 21.47 (d, J=3.1 Hz), 23.11 (s), 24.32 (d, J=207.5 Hz), 54.44 (d, J=18 Hz), 171.76 (s).

Methylation of FR-900098 (II)——A solution of II (500 mg, 3.05 mmol) in methanol (20 ml) was treated with an excess of diazomethane in ether under ice-bath cooling, and the mixture was stirred for 1 hr at the same temperature. The solvent was removed under reduced pressure, and the residue was chromatographed on a silica gel column (25 g) with chloroform–methanol (19: 1) to give an oil (350 mg). This purification procedure was repeated using 15 g of silica gel to yield 250 mg (37.0%) of dimethyl 3-(N-acetyl-N-hydroxyamino)propylphosphonate (IV) as an oil. MS *m/e*: 225.0760 (Calcd for $C_7H_{16}O_5NP$ 225.0766). IR ν_{max}^{film} cm^{-1}: 1740, 1640, 1230, 1030. ^{1}H-NMR (in $CDCl_3$) δ: 1.6—2.2 (4H, m), 2.13 (3H, s), 3.66 (2H, t, J=6 Hz), 3.70 (6H, d, J=10 Hz), 9.65 (1H, broad s).

Acid Hydrolysis of FR-900098 (II)——A solution of II (Na salt, 500 mg, 2.3 mmol) in 1 N hydrochloric acid (10 ml) was refluxed for 1 hr. The reaction mixture was concentrated under reduced pressure, and the residue was dissolved in ethanol (5 ml) and neutralized with pyridine. The precipitated crystals were collected by filtration and washed with ethanol. Recrystallization from a mixture of water and ethanol (1: 4) gave 270 mg (75.6%) of 3-(N-hydroxyamino)propylphosphonic acid (V). mp 160—164° (dec.). IR ν_{max}^{Nujol} cm^{-1}: 1640, 1595, 1240, 1220, 1190. ^{1}H-NMR (in D_2O) δ: 1.3—2.4 (4H, m), 3.36 (2H, t, J=7 Hz). *Anal.* Calcd for $C_3H_{10}N_1O_4P\cdot 1/2H_2O$: C, 21.96; H, 6.76; N, 8.54. Found: C, 22.18; H, 6.57; N, 8.55.

Diethyl 3-(N-Tosyl-N-benzyloxyamino)propylphosphonate (VII)——Sodium hydride dispersion (50%) in mineral oil (10.2 g, 0.21 mol) was washed with dry petroleum ether (100 ml) and suspended in dry benzene (500 ml). To this suspension, diethyl phosphite (24.3 g, 0.15 mol) was added dropwise during 30 min with vigorous stirring. The mixture was refluxed for 3.5 hr until a clear solution was obtained, then a solution of 3-(N-tosyl-N-benzyloxyamino)propyl bromide (VI)[6] (64.1 g, 0.16 mol) in dry benzene (250 ml) was added and the whole was refluxed with stirring for an additional 5 hr. The reaction mixture was washed with water (400 ml), dried over magnesium sulfate and evaporated to leave an oil (75 g). This was purified by chromatography on silica gel with chloroform to yield 64.4 g (90%) of VII as an oil. IR ν_{max}^{film} cm^{-1}: 1590, 1350, 1240. ^{1}H-NMR (in $CDCl_3$) δ: 1.28 (6H, t, J=8 Hz), 1.6—2.0 (4H, m), 2.35 (3H, s), 2.89 (2H, m), 4.05 (4H, q, J=7 Hz), 5.07 (2H, s), 7.2—7.4 (7H, m), 7.71 (2H, d, J=9 Hz).

3-(N-Hydroxyamino)propylphosphonic Acid (V)——A mixture of VII (13.2 g, 0.029 mol), concentrated hydrochloric acid (130 ml) and acetic acid (130 ml) was refluxed for 50 hr. The reaction mixture was concentrated under reduced pressure, and the residue was dissolved in water (50 ml). The solution was treated

with activated charcoal, which was then removed by filtration. The filtrate was concentrated to leave an oil, which was dissolved in ethanol (25 ml) and neutralized with pyridine. The precipitated crystals were collected by filtration and recrystallized from water–ethanlo to give 3.15 g (70.0%) of V, mp 160—166° (dec.); mixed mp with a sample derived from the nautral product, mp 159—165° (dec.).

3-(N-Acetyl-N-hydroxyamino)propylphosphonic Acid (FR-900098) (II)——Acetic anhydride (820 mg, 8 mmol) was added dropwise to a suspension of V (620 mg, 4 mmol) in water (3 ml) at room temperature. The reaction mixture was stirred for 1 hr at the same temperature and concentrated under reduced pressure to give an oily residue. The residue was dissolved in water (15 ml) and concentrated under reduced pressure. This operation was repeated twice more. The resulting oil was dissolved in 1 N sodium hydroxide (4 ml) and concentrated under reduced pressure to give a white powder. This powder was taken up in methan ol (5ml) and heated at a bath temperature of 70—80° for 1 hr. Acetone was added to the hot solution until crystals separated. After cooling, the crystals were collected by filtration and washed with acetone. Recrystallization from a mixture of methanol–acetone (1: 1) gave colorless prisms (730 mg, 83.3%) of II as the monosodium salt, mp 193—195° (dec.): mixed mp with the natural product, 193—195° (dec.).

Diethyl 3-Tosyl-2(*R*), 3-Dihydroxypropylphosphonate (IX)——*p*-Toluenesulfonyl chloride (7.7 g, 0.04 ml) was added portionwise to a solution of diethyl 2(*R*), 3-dihydroxypropylphosphonate (VIII)[7] (7.8 g, 0.036 mol) in dry pyridine (40 ml) under ice-bath cooling. After being stirred for 30 min at the same temperature, the mixture was allowed to stand in a refrigerator for 60 hr. The reaction mixture was poured into a mixture of 5% hydrochloric acid (400 ml) and ethyl acetate (200 ml). The ethyl acetate layer was separated and the aqueous layer was extracted with ethyl acetate (100 ml). The combined organic layer was washed with water (200 ml), dried over magnesium sulfate and concentrated under reduced pressure to leave an oil (13.5 g), which was chromatographed on silica gel with ethyl acetate as the eluting solvent to yield 10.9 g (83%) of IX as an oil. IR ν_{max}^{film} cm^{-1}: 3300 (broad), 1360, 1220, 1185, 1095, 1020, 970. ^{1}H-NMR (in $CDCl_3$) δ: 1.51 (6H, t, J=7 Hz), 1.85—2.15 (2H, m), 2.24 (3H, s), 3.00 (1H, s, disappeared with D_2O), 3.9—4.3 (7H, m), 7.34 (2H, d, J=10 Hz), 7.78 (2H, d, J=10 Hz). MS m/e: 366 (M^+), 211, 195.

Diethyl 3-(N,O-Diethyloxycarbonylhydroxyamino)-2(*R*)-hydroxypropylphosphonate (X)——Bis(trimethylsilyl)acetamide (12.2 g, 0.06 mol) was added to a solution of IX (7.3 g, 0.02 mol) in dry N,N-dimethylformamide (100 ml) under ice-bath cooling, and the mixture was stirred for 30 min at the same temperature. Potassium N,O-dicarboethoxyhydroxamide[8] (5.16 g, 0.024 mol) was then added and the mixture was stirred at 70° for 10 hr. After removal of the solvent by evaporation, the residue was poured into a mixture of ethyl acetate (200 ml) and 2% hydrochloric acid (200 ml). The organic layer was separated and washed with water (100 ml), dried over magnesium sulfate and concentrated under reduced pressure to give an oily residue, which was chromatographed on a silica gel column. Elution with chloroform–ethyl acetate (3: 1) gave 3.04 g (41%) of X. IR ν_{max}^{film} cm^{-1}: 3600—3100 (broad), 1780, 1720, 1220, 1020. ^{1}H-NMR (in $CDCl_3$) δ: 1.2—1.5 (12H, m), 1.8—2.2 (2H, m), 3.6—3.85 (1H, m), 4.0—4.5 (10H, m). MS m/e: 371 (M^+), 354, 326.

3-(N-Hydroxyamino)-2(*R*)-hydroxyaminopropylphosphonic Acid (XI)——Trimethylsilyl bromide[9] (25 g, 0.16 mol) was added to a solution of X (13.8 g, 0.037 mol) in dry methylene chloride (25 ml) under ice-bath cooling. The mixture was stirred at room temperature for 1.5 hr and then concentrated under reduced pressure to leave an oil. This oil was dissolved in water (50 ml) and stirred at room temperature for 1 hr. The solution was washed with chloroform (20 ml×3), and 1 N hydrochloric acid (140 ml) was added. The mixture was refluxed for 15 hr and concentrated under reduced pressure. The residual oil was dissolved in a mixture of water (10 ml) and methanol (20 ml) and adjusted to pH 4 with propylene oxide. Ethanol (200 ml) was then added to give precipitates, which were collected by filtration and washed with ethanol. Recrystallization from methanol-ethanol gave 2.97 g (47%) of XI, mp 138—140° (dec.). $[\alpha]_D$=+34° (c=0.19, H_2O). IR ν_{max}^{Nujol} cm^{-1}: 3170, 3500—2200, 1240, 1130, 1035. ^{1}H-NMR (in D_2O) δ: 2.00 (2H, dd, J=6 Hz and 18 Hz), 3.3—3.7 (2H, m), 4.3 (1H, m). *Anal.* Calcd for $C_3H_{10}N_1O_5P$: C, 21.06; H, 5.89; N, 8.19. Found: C, 19.78; H, 5.83; N, 7.64.

3-(N-Acetyl-N-hydroxyamino)-2(*R*)-hydroxypropylphosphonate (FR-33289) (III)——Acetic anhydride (1.02 g, 10 mmol) was added to a suspension of XI (855 mg, 5 mmol) in water (10 ml), and the mixture was stirred for 15 min under ice-bath cooling. After concentration of the reaction mixture, the residual oil was dissolved in water, adjusted to pH 10 with 5% ammonium hydroxide and stirred for 3 hr at room temperature. The solution was adjusted to pH 2 with 1 N hydrochloric acid and passed through a column of activated charcoal. The column was washed with water and then eluted with 80% aqueous acetone. The eluate was concentrated under reduced pressure to give the free acid (400 mg) of III as an oil. This was dissolved in methanol (5 ml) and a solution of sodium hydroxide (80 mg) in methanol (3 ml) was added. Addition of ethanol (30 ml) gave a crude powder (370 mg), which was reprecipitated from a mixture of methanol and ethanol to give III as the monosodium salt, an amorphous solid (230 mg, 20%). This synthetic sample was identical with the natural product on the basis of IR and ^{1}H-NMR spectral data. $[\alpha]_D$=−18° (c=0.2, H_2O). *Anal.* Calcd for $C_5H_{11}O_6NPNa$: C, 25.54; H, 4.72; N, 5.96. Found: C, 25.42; H, 4.95; N, 6.03.

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