[Chem. Pharm. Bull. 30(1) 111—118 (1982)]

Studies on Phosphonic Acid Antibiotics. IV.[1] Synthesis and Antibacterial Activity of Analogs of 3-(*N*-Acetyl-*N*-hydroxyamino)-propylphosphonic Acid (FR-900098)

Keiji Hemmi, Hidekazu Takeno, Masashi Hashimoto, and Takashi Kamiya*

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

(Received May 25, 1981)

The synthesis of analogs of 3-(*N*-acetyl-*N*-hydroxyamino)propylphosphonic acid (FR-900098, Ia) and an analysis of their structure–activity relationship are described. Among them, compounds Ib (FR-31564) and XIIb (FR-32863) have recently been found to be produced naturally by *Streptomyces lavendulae*. The antibacterial activity of Ib against *Pseudomonas* species is especially significant.

Keywords——antibiotic; 3-(*N*-acetyl-*N*-hydroxyamino)propylphosphonic acid; 3-(*N*-formyl-*N*-hydroxyamino)propylphosphonic acid; 3-(*N*-formyl-*N*-hydroxyamino)-*trans*-1-propenylphosphonic acid; inhibitor of bacterial cell wall synthesis; Michaelis-Arubuzov reaction

In the preceding paper of this series,[1] we reported the structures of new antibiotics, 3-(*N*-acetyl-*N*-hydroxyamino)-propylphosphonic acid (FR-900098, Ia) and its 2(*R*)-hydroxypropyl homolog (FR-33289, IIa), which were isolated from *Streptomyces rubellomurinus* as cell wall biosynthesis inhibitors. The unique structure of these antibiotics, when considered in terms of biogenesis, suggested that further congeners might occur in nature. This assumption prompted us to investigate the synthesis of the congeners and their structure–activity relationship. This report describes the synthesis of the semisynthetic antibiotic, 3-(*N*-formyl-*N*-hydroxyamino)propylphosphonic acid (FR-31564, Ib).[2] It is noteworthy that Ib was found to be a superior and clinically useful antibiotic[3c,d] and was later detected as a minor product in the culture broth of *Streptomyces lavendulae*.[3a,b]

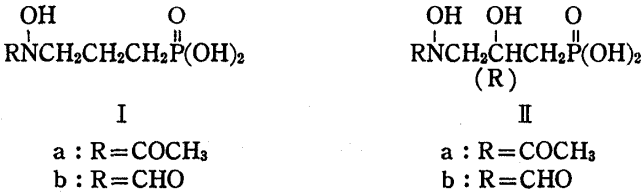

Chart 1

The biogenesis of microbial metabolites containing a phosphonic acid moiety has not been well established. However, ciliatine (III)[4] and fosfomycin (IV)[5] have been suggested to be derived from phosphoenolpyruvic acid (V) *via* 3-phosphonopyruvic acid (VI). Although the biogenesis of Ia and IIa has not yet been investigated, it seems to be a reasonable assumption that these antibiotics might be derived from the common intermediate (VI). It is also assumed that VI might be converted to 2-keto-4-phosphonobutyric acid (VII) by a pathway analogous to the normal TCA cycle.[6a] This keto acid (VII) could be subsequently converted to 3-amino-propylphosphonic acid (VIII) by decarboxylation-transamination.[6b] This hypothetical aminophosphonic acid (VIII) might be further oxidized and acylated to yield Ia and IIa, by analogy with the biogenesis of hadacidin.[7a]

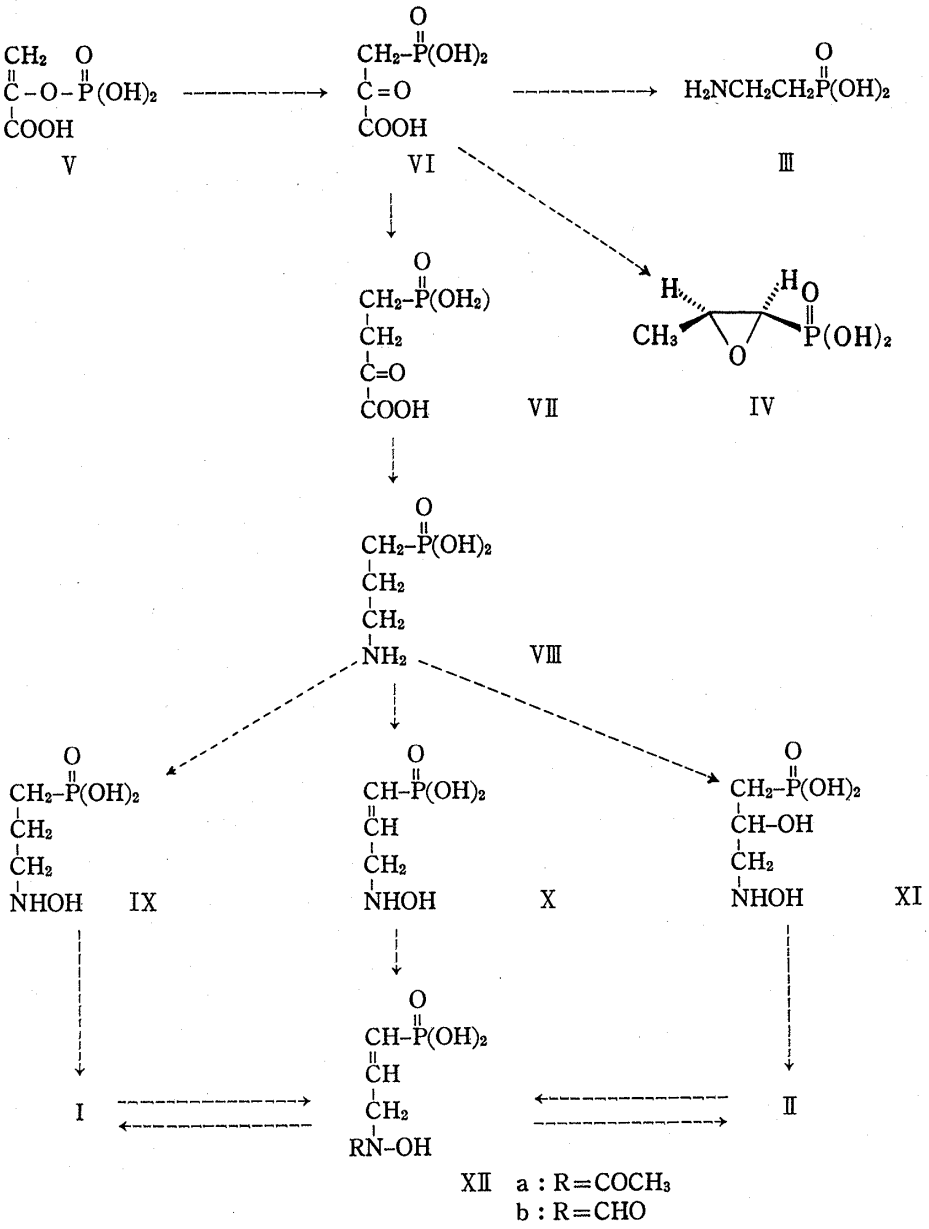

Chart 2

Based on these considerations, we supposed that there might be the following groups of compounds in nature: (1) propenyl analogs (XII) which might be further metabolites of II or intermediates in the transformation to I; (2) *N*-acyl analogs which might be derived by acylation of the hydroxylamine (IX), (X), or (XI).

These compounds were thus prepared as described below. The propenyl analog (XII) was synthesized by starting from diethyl 1-*trans*-propenylphosphonate (XIII).[8] Allylic bromination of XIII was carried out with *N*-bromosuccinimide in carbon tetrachloride under reflux in the presence of benzoyl peroxide as a catalyst to give the bromide (XIV). This was then treated with potassium *N*,*O*-dicarboethoxyhydroxamide (XV)[1] at −25°C in *N*,*N*-dimethylformamide to give the condensation product (XVI). It was presumed that the hydroxylamine (X) might be unstable under severe hydrolysis conditions. A stepwise cleavage of the protecting groups of XVI was carried out in a manner similar to that used for the preparation of XI, which was described in the preceding paper.[1] Removal of the protecting groups of the phosphonic function with trimethylsilyl bromide at 0°C, followed by hydrolysis of the

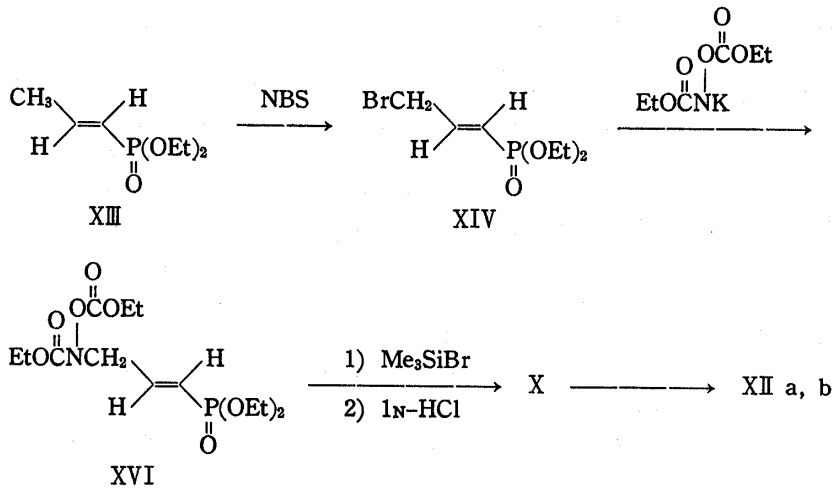

Chart 3

carboethoxy groups with 1 N hydrochloric acid under reflux gave X in 50% yield. Acetylation of X with acetic anhydride in water gave 3-(*N*-acetyl-*N*-hydroxyamino)-1-*trans*-propenylphosphonic acid (XIIa), which was isolated as the monosodium salt.

In connection with the unique biological activity of hadacidin,[7b] we were further interested in the preparation of the *N*-formyl derivatives of the hydroxylamines (IX), (X), and (XI). This was also an important objective in order to clarify the effect of the acyl groups on the biological activities. Thus, the *N*-formyl derivatives of the hydroxyamine compounds were prepared. Formylation of IX with acetic-formic anhydride at room temperature gave 70% yield of Ib as the monosodium salt. Similarly, X and XI were also formylated to give XIIb and IIb, respectively.

Among those derivatives, FR-32863 (XIIb) and FR-31564 (Ib) were later found to be present as minor products in the culture broth of *Streptomyces lavendulae*.[3a,b] This result

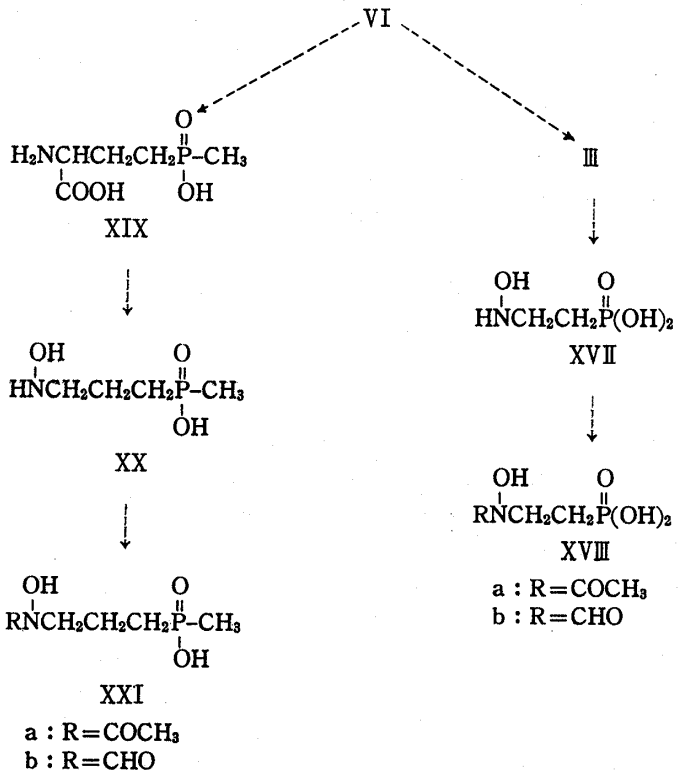

Chart 4

was consistent with our expectation based on the possible biogenesis of these antibiotics.

It is also speculated that the ethylene analogs (XVIII) and the methylphosphinic acid analogs (XXI) could be derived *via* pathways similar to those of the biosynthesis of ciliatine (III) and phosphinothricin (XIX),[9] respectively, as shown in chart 4.

For the synthesis of the ethylene analog (XVIII), we first examined the introduction of a hydroxyamino functionality into diethyl 2-bromoethylphosphonate (XXII) by using XV. Treatment of XXII with XV in *N*,*N*-dimethylformamide resulted in preferential dehydrobromination of XXII to give diethyl vinylphosphonate, and the desired product could not be obtained. Therefore, the condensation of triethyl phosphite and 2-(*N*-tosyl-*N*-benzyloxyamino)ethyl bromide (XXIV) was next examined. The bromide (XXIV) was prepared by reaction of *N*-tosyl-*N*-benzyloxyamine (XXIII)[10] with ethylene dibromide in the presence of sodium methoxide in methanol. Michaelis-Arbuzov reaction of XXIV with triethyl phosphite at 160°C gave the desired condensation product (XXV). The protecting groups of XXV were then removed by hydrolysis with concentrated hydrochloric acid-acetic acid under reflux to give the hydroxylamine (XVII). Acylation of XVII with acetic anhydride and acetic-formic anhydride gave XVIIIa and XVIIIb, respectively.

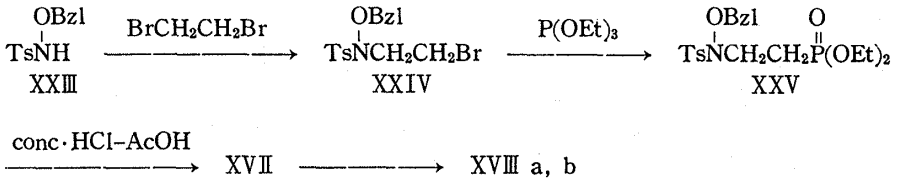

Chart 5

The synthesis of the methylphosphinic acid analogs (XXI) was carried out by starting from *n*-butyl methylphosphinate (XXVI).[11] The lithium salt of XXVI, prepared by treatment with *n*-butyl lithium in tetrahydrofuran at −35——40°C under a nitrogen atmosphere, was treated with 3-(*N*-tosyl-*N*-benzyloxyamino)propyl bromide[10] at the same temperature to give the condensation product (XXVII). This product was then subjected to hydrolysis with concentrated hydrochloric acid-acetic acid under reflux to provide the hydroxylamine (XX). Final acylation of XX in a way similar to the procedure described above for preparing XIIa and XIIb gave XXIa and XXIb.

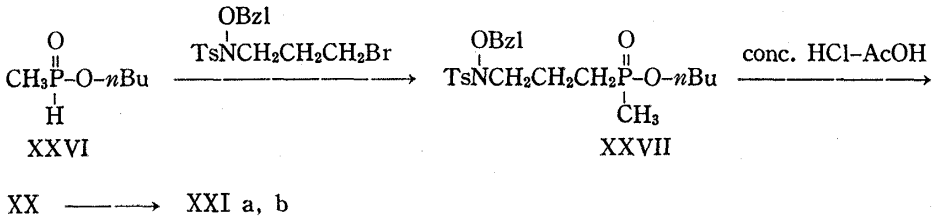

Chart 6

The antimicrobial spectra of these derivatives are shown in Table I. The ethylene analogs (XVIIIa), (XVIIIb) and the methylphosphinic acid analogs (XXIa), (XXIb) did not show any activity against the tested bacteria. 2-(*R*)-Hydroxypropyl analogs (IIa), (IIb) exhibited less activity, whereas the activity of the propenyl analogs (XIIa), (XIIb) was comparable to that of the propyl analogs (Ia), (Ib). The *N*-formyl derivatives showed greatly enhanced activity as compared with the *N*-acetyl derivatives. A number of *N*-acyl derivatives common in penicillins and cephalosporins were synthesized. However, none of the *N*-acyl derivatives proved to have significant activity, except for *N*-formyl derivatives. FR-31564 (Ib) was found to be most potent and active against a variety of gram-negative bacteria including *Pseudomonas* species.[3c] It is interesting to note that the activity of Ib was greater *in vivo* than *in vitro*, and Ib was effective orally.[3d]

Table I. Antimicrobial Spectrum

Compound	MIC (μg/ml)[a]				
	Staphylococcus aureus 209 P	*Bacillus subtilis* ATCC 6633	*Proteus vulgaris* IAM 1025	*Escherichia coli* NIHJ JC 2	*Pseudomonas aeruginosa* IAM 1095
Ia	>1000	200	125	400	250
Ib	>1000	6.25	3.13	12.5	0.78
IIa	>400	400	400	50	400
IIb	>100	100	50	25	25
XIIa	>1000	2.5	600	10	600
XIIb	>100	6.25	3.13	12.5	1.56
XVIIIa	>1000	>1000	>1000	>1000	>1000
XVIIIb	>1000	>1000	150	>1000	>1000
XXIa	—[b]	>1000	—	>1000	>1000
XXIb	—	40	—	150	>1000

a) Nutrient agar (Difco), 1000-fold dilution, stamp method, 37°C, 20 h.
b) Not tested.

In recent years, considerable efforts have been made to prepare chemotherapeutically useful agents by chemical modification of naturally occurring antibiotics. A synthetic approach on the basis of biogenetic considerations as described above seems to be one of the efficient methodologies for seeking new, biologically active substances.

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.

Diethyl 3-Bromo-1-*trans*-propenylphosphonate (XIV)——N-Bromosuccinimide (41.65 g, 0.234 mol) was added to a solution of diethyl 1-*trans*-propenylphosphonate (XIII)[8] (32.04 g, 0.18 mol) in carbon tetrachloride (320 ml), and the mixture was refluxed for 1.5 h in the presence of benzoyl peroxide (2.8 g, 0.01 mol) as a catalyst, then cooled to 0°C. The resulting precipitates were filtered off. The filtrate was concentrated under reduced pressure to leave an oil (63.1 g), which was chromatographed on silica gel using chloroform as the eluting solvent to yield 29.8 g (64.3%) of XIV as an oil. IR ν_{max}^{Film} cm^{-1}: 1630, 1240, 1160. ^{1}H-NMR (in $CDCl_3$) δ: 1.32 (6H, t, J=7 Hz), 3.9—4.3 (6H, m), 5.93 (1H, d t, J=17 Hz, 1 Hz), 6.81 (1H, dd t, J=21 Hz, 17 Hz, 1 Hz). MS m/e: 258, 256 (M^+).

Diethyl 3-(*N*,*O*-Diethoxycarbonylhydroxyamino)-1-*trans*-propenylphosphonate (XVI)——A solution of XIV (23.83 g, 0.093 mol) in *N*,*N*-dimethylformamide (50 ml) was added to a suspension of potassium dicarboethoxyhydroxamide (XV) (19.94 g, 0.093 mol) in the same solvent (100 ml) at −25——30°C. The mixture was stirred for 1 h at the same temperature and for an additional 1 h at −5——10°C. The reaction mixture was then poured into a mixture of ethyl acetate (700 ml) and water (1 l). The organic layer was separated and washed with water. Drying over magnesium sulfate and evaporation gave an oil, which was chromatographed on silica gel with chloroform–ethyl acetate to give 27.2 g (83.0%) of XVI as an oil. IR ν_{max}^{Film} cm^{-1}: 1795, 1730, 1640, 1210, 1170. ^{1}H-NMR (in $CDCl_3$) δ: 1.1—1.45 (12H, m), 3.8—4.5 (10H, m), 5.95 (1H, dd t, J=19 Hz, 17 Hz, 2 Hz), 6.74 (1H, dd t, J=22 Hz, 17 Hz, 5 Hz). MS m/e: 353 (M^+).

3-(*N*-Hydroxyamino)-1-*trans*-propenylphosphonic Acid (X)——Trimethylsilyl bromide (4.70 g, 31 mmol) was added to a solution of XVI (2.17 g, 6.1 mmol) in dry methylene chloride (20 ml) under ice-bath cooling. The mixture was stirred at room temperature for 1.5 h and then concentrated under reduced pressure to leave an oil. This was dissolved in water (15 ml) and stirred at room temperature for 1 h. The solution was washed with chloroform (10 ml×3), and 1 N hydrochloric acid (90 ml) was then added to the aqueous phase. The mixture was refluxed for 12 h and concentrated under reduced pressure. The residual oil was dissolved in water (20 ml) and the solution was washed with ethyl acetate (10 ml) and treated with activated charcoal. After removal of the charcoal by filtration, the filtrate was concentrated under reduced pressure. The residue was dissolved in ethanol (10 ml) and adjusted to pH 4.5 with pyridine to give precipitates, which were collected and washed with ethanol. Recrystallization from water–ethanol gave 467 mg (50.0%) of X. mp

127—129°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 1630, 1260. ^{1}H-NMR (in D_2O) δ: 3.99 (2H, dd, J=5 Hz, 1 Hz), 6.05—6.55 (2H, m). *Anal.* Calcd for $C_3H_8NO_4P$: C, 23.54; H, 5.27; N, 9.15. Found: C, 23.35; H, 5.49; N, 9.02.

3-(*N*-Acetyl-*N*-hydroxyamino)-1-*trans*-propenylphosphonic Acid (XIIa)——Acetic anhydride (2.04 g, 20 mmol) was added to a suspension of X (1.53 g, 10 mmol) in water (7 ml), and the mixture was stirred for 30 min at room temperature. After evaporation of the solvent, the residual oil was dissolved in 1 N potassium hydroxide (10 ml), and the mixture was stirred for 1 h at 80°C and concentrated to dryness. The residue was chromatographed on cellulose powder with isopropyl alcohol–water (8: 2) to give a powder. This was reprecipitated with methanol–ethanol and dried over phosphorus pentoxide to give the monopotassium salt of XIIa (1.79 g, 76.9%) as an amorphous solid. IR ν_{max}^{Nujol} cm^{-1}: 1650, 1620 (shoulder), 1140. ^{1}H-NMR (in D_2O) δ: 2.13 (3H, s), 4.35 (2H, m), 5.7—6.6 (2H, m). *Anal.* Calcd for $C_5H_9KNO_5P$: C, 25.75; H, 3.89; N, 6.01. Found: C, 25.72; H, 3.86; N, 5.75.

3-(*N*-Formyl-*N*-hydroxyamino)propylphosphonic Acid (Ib)——A sample (1.64 g, 10 mmol) of IX[1] was added to acetic-formic anhydride, prepared from acetic anhydride (1.33 g, 13 mmol) and formic acid (1.20 g, 26 mmol). The mixture was stirred for 1 h at room temperature and concentrated under reduced pressure to leave an oil. This was dissolved in 1 N sodium hydroxide (10 ml) and the solution was evaporated to dryness. The residue was dissolved in methanol (40 ml), and ethanol (50 ml) was added to the solution. The whole was stirred overnight at room temperature, then the precipitated crystalline solides were collected and recrystallized from methanol–ethanol to give 1.44 g (70.1%) of Ib as the monosodium salt. mp 189—191°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3600—2200, 1675, 1510, 1270, 1230, 1165, 1015, 985, 920. ^{1}H-NMR (in D_2O) δ: 1.2—2.2 (4H, m), 3.62 (2H, t, J=6 Hz), 8.00 (s), 8.35 (s)....total 1H.[12] *Anal.* Calcd for $C_4H_9NNaO_5P$: C, 23.43; H, 4.42; N, 6.83. Found: C, 23.24; H, 4.36; N, 6.75.

3-(*N*-Formyl-*N*-hydroxyamino)-2(*R*)-hydroxypropylphosphonic Acid (IIb)——A sample (1.40 g, 8.2 mmol) of XI[1] was added to acetic-formic anhydride, prepared from acetic anhydride (1.67 g, 16 mmol) and formic acid (1.51 g, 32 mmol). The mixture was stirred for 1.5 h at room temperature and concentrated under reduced pressure. The residue was dissolved in 1 N potassium hydroxide (8.2 ml) and the solution was evaporated to dryness. The residue was chromatographed on cellulose powder with isopropyl alcohol–water (8: 2) to give a powder. This was reprecipitated with methanol–ethanol and dried over phosphorus pentoxide to give the monopotassium salt of IIb (723 mg, 37.3%) as an amorphous solid. $[\alpha]_D$=+30° (c=0.12, H_2O). IR ν_{max}^{Nujol} cm^{-1}: 3130, 1660, 1140, 1040, 890. ^{1}H-NMR (in D_2O) δ: 1.85 (2H, dd, J=6 Hz, 8 Hz), 3.2—3.9 (2H, m), 4.0—4.4 (1H, m), 7.95 (s), 8.37 (s)....total 1H. *Anal.* Calcd for $C_4H_9KNO_6P$: C, 20.25; H, 3.83; N, 5.91. Found: C, 19.91; H, 4.05; N, 5.73.

3-(*N*-Formyl-*N*-hydroxyamino)-1-*trans*-propenylphosphonic Acid (XIIb)——A solution of X (306 mg, 2 mmol) in formic acid (1 ml) was added to acetic-formic anhydride, prepared from acetic anhydride (265 mg, 2.3 mmol) and formic acid (210 mg, 4.6 mmol). The mixture was stirred for 30 min at room temperature and concentrated under reduced pressure. The residue was dissolved in methanol (2.5 ml) and a solution of potassium hydroxide (120 mg) in methanol (0.5 ml) was added. The resulting crystals were collected by filtration to give 258 mg (59.0%) of XIIb as the monopotassium salt. mp 178—180°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3600—2200, 1665, 1250. ^{1}H-NMR (in D_2O) δ: 4.30 (2H, m), 6.00 (1H, m), 6.38 (1H, m), 8.02 (s), 8.35 (s).... total 1H. *Anal.* Calcd for $C_4H_7KNO_5P$: C, 21.92; H, 3.22; N, 6.39. Found: C, 21.65; H, 3.12; N, 6.25.

2-(*N*-Tosyl-*N*-benzyloxyamino)ethyl Bromide (XXIV)——*N*-Tosyl-*N*-benzyloxyamine (XXIII)[10] (100 g, 0.346 mol) was added to a solution of sodium (8.0 g, 0.346 g-atom) in methanol (450 ml) with vigorous stirring at 60°C. When the solution had become clear, ethylene dibromide (406 g, 2.16 mol) was added. The mixture was refluxed for 4 h and concentrated under reduced pressure to leave an oil, which was extracted with ethyl acetate. The extract was washed with water and dried over magnesium sulfate. After evaporation of the solvent, the residue was recrystallized from ethyl acetate–ether to give 112.4 g (82.0%) of XXIV. mp 120—122°C. ^{1}H-NMR (in $CDCl_3$) δ: 2.37 (3H, s), 3.22 (4H, s), 5.10 (2H, s), 7.35 (5H, s), 7.29 (2H, d, J= 8 Hz), 7.75 (2H, d, J=8 Hz). *Anal.* Calcd for $C_{16}H_{18}BrNO_3S$: C, 50.00; H, 4.72; N, 3.65. Found: C, 49.95; H, 4.58; N, 3.64.

Diethyl 2-(*N*-tosyl-*N*-benzyloxyamino)ethylphosphonate (XXV)——A mixture of XXIV (16.2 g, 0.04 mol) and triethyl phosphite (19.7 g, 0.12 mol) was heated with stirring for 10 h at 160°C. The reaction mixture was diluted with ethyl acetate (100 ml), washed with water and dried over magnesium sulfate. Evaporation of the solvent gave an oil, which was crystallized from isopropyl ether to give 10.6 g (60.2%) of XXV. The mother liquid was concentrated and chromatographed on silica gel using chloroform as the eluting solvent. A further 2.1 g (11.7%) of XXV was obtained. An analytically pure sample was prepared by recrystallization from isopropyl ether, mp 78—80°C. ^{1}H-NMR (in $CDCl_3$) δ: 1.25 (6H, t, J=7 Hz), 1.85 (2H, m), 2.36 (3H, s), 3.14 (2H, m), 4.01 (4H, quintet, J=7 Hz), 5.06 (2H, s), 7.30 (2H, d, J=8 Hz), 7.74 (2H, d, J=8 Hz). *Anal.* Calcd for $C_{21}H_{30}NO_6PS$: C, 54.41; H, 6.39; N, 3.17. Found: C, 54.37; H, 6.50; N, 3.10.

2-(*N*-Hydroxyamino)ethylphosphonic Acid (XVII)——A solution of XXV (12.5 g, 0.028 mol) in a mixture of acetic acid (65 ml) and concentrated hydrochloric acid (130 ml) was heated under reflux for 42 h. The solution was treated with activated charcoal and concentrated under reduced pressure. The residue was dissolved in ethanol (50 ml) and the solution was neutralized to pH 5 with pyridine. The resulting solid was collected by filtration, washed with ethanol and recrystallized from water–ethanol to give 3.50 g (87.5%) of XVII. mp 173—173.5°C. ^{1}H-NMR (in D_2O) δ: 2.04 (2H, m), 3.60 (2H, m). *Anal.* Calcd for $C_2H_8NO_4P$:

C, 17.03; H, 5.71; N, 9.93. Found: C, 17.01; H, 5.80; N, 9.86.

2-(*N*-Acetyl-*N*-hydroxyamino)ethylphosphonic Acid (XVIIIa)——Acetic anhydride (820 mg, 8 mmol) was added to a solution of IX (564 mg, 4 mmol) in water (10 ml). After being stirred for 1 h at room temperature, the reaction mixture was concentrated under reduced pressure. The syrupy residue was dissolved in 1 N sodium hydroxide (4 ml) and stirred for 1 h at 80°C. The solvent was evaporated off. The residue was pulverized with ethanol to give 640 mg (78.0%) of XVIIIa as the monosodium salt. An analytically pure sample was prepared by recrystallization from ethanol–water, mp 185—192°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3600—2200, 1620, 1230, 1160, 1040, 940, 890. ^{1}H-NMR (in D_2O) δ: 1.6—2.3 (2H, m), 2.12 (3H, s), 3.5—4.1 (2H, m). *Anal.* Calcd for $C_4H_9NPO_5Na$: C, 23.43; H, 4.42; N, 6.83. Found: C, 23.47; H, 4.35; N, 6.60.

2-(*N*-Formyl-*N*-hydroxyamino)ethylphosphonic Acid (XVIIIb)——A sample (563 mg, 4 mmol) of XVII was added to acetic-formic anhydride, prepared from acetic anhydride (530 mg, 5.2 mmol) and formic acid (480 mg, 10.4 mmol). The mixture was stirred for 2 h at room temperature and concentrated under reduced pressure. The residue was dissolved in methanol (10 ml) and a solution of potassium hydroxide (250 mg) in methanol (2 ml) was added. The whole was stirred for 30 min at room temperature, then the precipitated crystalline solids were collected and recrystallized from water–methanol to give 630 mg (76.1%) of XVIIIb as the monopotassium salt. mp 201—203°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3600—2200, 1650, 1280, 1250, 1230, 1160, 1100, 1020, 920. ^{1}H-NMR (in D_2O) δ: 1.7—2.4 (2H, m), 3.6—4.2 (2H, m), 8.00 (s), 8.31 (s)....total 1H. *Anal.* Calcd for $C_3H_7KNO_6P$: C, 17.40; H, 3.41; N, 6.76. Found: C, 17.38; H, 3.49; N, 6.73.

***n*-Butyl 3-(*N*-Tosyl-*N*-benzyloxyamino)propylmethylphosphinate (XXVII)**——A solution of *n*-butyl lithium (3.8 g, 0.059 mol) in *n*-hexane (38 ml) was added to a solution of *n*-butyl methylphosphinate (XXVI)[11] (6.7 g, 0.049 mol) in tetrahydrofuran (70 ml) at −40——50°C under a nitrogen atmosphere, and the mixture was stirred for 30 min at the same temperature. A solution of 3-(*N*-tosyl-*N*-benzyloxyamino)propyl was stirred for 30 min at the same temperature. A solution of 3-(*N*-tosyl-*N*-benzyloxyamino)propyl bromide[10] (19.1 g, 0.049 mol) in tetrahydrofuran (71 ml) was then added to the mixture at the same temperature. The mixture was stirred for 1.5 h, during which time the temperature was gradually raised to room temperature, and stirred for an additional 4 h at the same temperature. The reaction mixture was concentrated and diluted with ethyl acetate (200 ml). The solution was washed with 2% hydrochloric acid and brine, dried over magnesium sulfate and evaporated to leave an oil, which was chromatographed on silica gel with chloroform–ethyl acetate to give 9.55 g (43.0%) of XXVII as an oil. IR ν_{max}^{Film} cm^{-1}: 1595, 1350, 1305, 1200, 1165, 1120, 1080, 900. ^{1}H-NMR (in $CDCl_3$) δ: 0.90 (3H, t, J=6 Hz), 1.55 (3H, d, J=13 Hz), 1.4—2.0 (8H, m), 2.40 (3H, s), 2.93 (2H, t, J=6 Hz), 3.7—4.1 (2H, m), 5.10 (2H, s), 7.1—7.8 (9H, m). MS *m/e*: 453 (M^+).

3-(*N*-Hydroxyamino)propylmethylphosphinic Acid (XX)——A solution of XXVII (9.06 g, 0.02 mol) in a mixture of acetic acid (100 ml) and concentrated hydrochloric acid (100 ml) was heated under reflux for 35 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethanol (30 ml) and the solution was neutralized to pH 4.5 with concentrated ammonium hydroxide. The resulting solid was collected by filtration, washed with ethanol and recrystallized from 95% aqueous ethanol to give 1.54 g (49.7%) of XX. mp 176—178°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3200—2200, 1125, 1100, 1030, 1020, 900. ^{1}H-NMR (in D_2O) δ: 1.28 (3H, d, J=14 Hz), 1.3—2.3 (4H, m), 3.35 (2H, t, J=7 Hz). *Anal.* Calcd for $C_4H_{12}NO_3P$: C, 31.37; H, 7.90; N, 9.15. Found: C, 31.35; H, 7.86; N, 9.23.

3-(*N*-Acetyl-N-hydroxyamino)propylmethylphosphinic Acid (XXIa)——Acetic anhydride (404 mg, 4 mmol) was added to a solution of XX (306 mg, 2 mmol) in water (5 ml) and the mixture was stirred for 1.5 h at room temperature. After removal of the solvent, the residue was dissolved in 1 N sodium hydroxide (2 ml), and the mixture was stirred for 1 h at 80°C then evaporated to dryness. The residue was chromatographed on cellulose powder with isopropyl alcohol–water (8: 2) to give a powder. This was reprecipitated with methanol–ethanol and dried over phosphorus pentoxide to give the monosodium salt of XXIa (250 mg, 57.6%) as an amorphous solid. IR ν_{max}^{Nujol} cm^{-1}: 3500—2200, 1650, 1130, 1030. ^{1}H-NMR (in D_2O) δ: 1.28 (3H, d, J=14 Hz), 2.10 (3H, s), 1.3—2.1 (4H, m), 3.65 (2H, t, J=6 Hz). *Anal.* Calcd for $C_6H_{13}NNaO_4P$: C, 33.19; H, 6.03; N, 6.45. Found: C, 33.25; H, 6.02; N, 6.15.

3-(*N*-Formyl-*N*-hydroxyamino)propylmethylphosphinic Acid (XXIb)——A sample (306 mg, 2 mmol) of XX was added to acetic-formic anhydride, prepared from acetic anhydride (266 mg, 2.6 mmol) and formic acid (240 mg, 5.2 mmol). After being stirred for 2 h at room temperature, the mixture was concentrated under reduced pressure to leave an oil. This was dissolved in 1 N sodium hydroxide (2 ml) and the solution was evaporated to dryness. The residue was reprecipitated with ethanol–acetone to give the sodium salt of XXIb (350 mg, 86.3%) as an amorphous solid. IR ν_{max}^{Nujol} cm^{-1}: 3500—2200, 1660, 1130, 1030, 890. ^{1}H-NMR (in D_2O) δ: 1.27 (3H, d, J=14 Hz), 1.3—2.1 (4H, m), 3.63 (2H, t, J=6 Hz), 8.00 (s), 8.35 (s)....total 1H. *Anal.* Calcd for $C_5H_{11}NNaO_4P$: C, 29.57; H, 5.46; N, 6.90. Found: C, 29.45; H, 5.47; N, 6.68.

Acknowledgement The authors wish to thank Mr. Miyazaki for his technical assistance during the course of this work.

References and Notes

1) Part III: K. Hemmi, H. Takeno, M. Hashimoto, and T. Kamiya, *Chem. Pharm. Bull.*, **29**, 645 (1981).

2) *a*) A preliminary report dealing with certain aspects of this work has appeared: T. Kamiya, K. Hemmi, H. Takeno, and M. Hashimoto, *Tetrahedron Lett.*, **21**, 95 (1980); *b*) M. Hashimoto, K. Hemmi, H. Takeno, and T. Kamiya, *ibid.*, **21**, 99 (1980); *c*) T. Kamiya, K. Hemmi, H. Takeno, and M. Hashimoto; Presentation at the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy, Boston, 1979, Abstract, p. 235.
3) *a*) M. Okuhara, Y. Kuroda, T. Goto, M. Okamoto, H. Terano, M. Kohsaka, H. Aoki, and H. Imanaka, J. Antibiot., **33**, 24 (1980); *b*) Y. Kuroda, M. Okuhara, T. Goto, M. Okamoto, H. Terano, M. Kohsaka, H. Aoki, and H. Imanaka, *ibid.*, **33**, 29 (1980; *c*) Y. Mine, T. Kamimura, S. Nonoyama, and M. Nishida, *ibid.*, **33**, 36 (1980); *d*) H. Kojo, Y. Shigi, and M. Nishida, *ibid.*, **33**, 44 (1980).
4) *a*) W.A. Warren, *Biochim. Biophys. Acta*, **156**, 340 (1968); *b*) M. Horiguchi, *ibid.*, **261**, 102 (1972).
5) T.O. Rogers and J. Birnbaum, *Antimicrob. Ag. Chemother.*, **5**, 121 (1974).
6) *a*) For metabolites formed from intermediates of the TCA cycle in microorganisms, see *e.g.* W.B. Turner, 'Fungal Metabolites," Academic Press, 1971, pp. 280—290; *b*) For the amino-acid biosynthesis in microorganisms, see *e.g. ibid.*, pp. 297—300.
7) *a*) R.L. Stevens and F.E. Thomas, *Biochemistry*, **5**, 74 (1966); *b*) E.A. Kaczka, C.O. Gitterman, E.L. Dulaney, and K. Folkers, *ibid.*, **1**, 340 (1962).
8) L. Horner, J. Ertel, H.D. Ruprecht, and O. Belovsky, *Chem. Ber.*, **103**, 1582 (1970).
9) E. Bayer, K.H. Gugel, K. Hagele, H. Hagenmaier, S. Jessipow, W.A. Konig, and H. Zahner, *Helv. Chim. Acta*, **55**, 224 (1972).
10) Y. Isowa, T. Takashima, M. Ohmori, H. Kurita, M. Sato, and K. Mori, *Bull. Chem. Soc. Japan*, **45**, 1461 (1972).
11) M.I. Kabachinik, E.N. Tsvetkov, and C. Chang, *Doklady Acad. Nauk S.S.S.R.*, **125**, 1260 (1959) [C.A., **53**, 2172f (1959)].
12) The formyl proton signal was a double one in a ratio of 4: 1, presumably due to a rotational restriction of the formyl group. At 90°C, this pair of signals coalesced to a singlet at δ 8.16.