STRUCTURE AND STEREOCHEMISTRY OF ANTIBIOTIC PS-5

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The structure and stereochemistry of a new β-lactam antibiotic PS-5 were determined as shown in Fig. 3.

PS-5[1] is a new β-lactam antibiotic isolated from the fermentation broth of a soil microorganism, *Streptomyces cremeus* subsp. *auratilis* A271 (ATCC 31358)[2] or *Streptomyces fulvoviridis* A933[3]. It shows a broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria including β-lactamase-producing organisms resistant to the known β-lactam antibiotics[4]. From its biological and physico-chemical properties, PS-5 was considered to resemble a new type of β-lactam antibiotics such as thienamycin[5~8], N-acetylthienamycin[9], epithienamycins[10] and olivanic acids[11~16].

This paper describes the full structure of antibiotic PS-5 based on spectroscopic and chemical degradative studies.

Results and Discussion

Antibiotic PS-5 was isolated as freeze-dried sodium salt. Its specific rotation* is $[\alpha]_D^{22}+77.3°$ (*c* 1.59). It migrates to the anode on high voltage paper electrophoresis (pH 8.6 Veronal buffer, 28 mm for 30 minutes at 42 V/cm). The infrared spectrum of PS-5 sodium salt shows the characteristic carboxylate anion band at 1600 cm^{-1} and the strong β-lactam carbonyl absorption at 1760 cm^{-1}. The ultraviolet absorption in neutral buffer has a maximum at 301 nm[1]. These physical properties strongly suggested that PS-5 is a member of the family of antibiotics having the 7-oxo-1-azabicyclo [3.2.0]hept-2-ene ring system such as thienamycin, epithienamycin and olivanic acid derivatives.

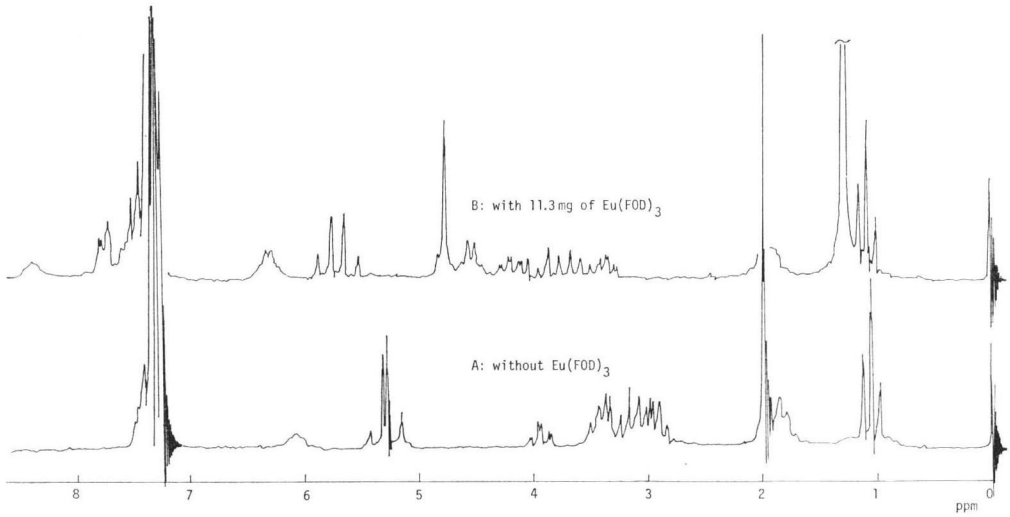
The molecular formula of PS-5 was deduced from the methyl ester of PS-5, because PS-5 sodium salt was hygroscopic; inappropriate for elemental analysis and did not give the molecular ion peak in the field desorption mass spectrum.

The methyl ester of PS-5 was prepared by treating PS-5 sodium salt with methyl iodide in dimethylformamide. The infrared spectrum of the methyl ester showed a new band attributable to ester carbonyl at 1710 cm^{-1} in addition to bands at 1780 and 1680 cm^{-1} assigned to β-lactam and amide carbonyls, respectively. The esterification resulted in a bathochromic shift of 15 nm for the UV absorption maximum. Similar shifts were found in the esterification of thienamycin and olivanic acid derivatives[6,13]. The ^1H-NMR spectrum of the methyl ester corresponded well to that of PS-5 sodium salt except for the signal due to the ester methyl group.

The high resolution mass spectrum of PS-5 methyl ester gave the following elemental composition:

* The value of +1.23° previously reported[1] was not a specific rotation, but an observation of rotation (*c* 1.59). It must be corrected to $[\alpha]_D^{22}+77.3°$ for the specific rotation.

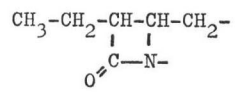
Fig. 1. ^{1}H-NMR spectra of PS-5 benzyl ester at 100 MHz.



$C_{14}H_{20}N_2O_4S$(calcd. 312.1143, found 312.1131). Thus the elemental composition of PS-5 itself was calculated to be $C_{13}H_{18}N_2O_4S$.

The ^{1}H-NMR spectrum of the methyl ester displayed signals at δ 1.04 (3H, t, J=7 Hz) and δ 1.87 (2 H, m) due to an ethyl group; signals at δ 1.95 (3 H, s) and δ 6.20 (1 H, exchangeable in D_2O) due to an acetamide group; peaks at δ 3.94 (1 H) attributable to a proton on the carbon atom attached to the β-lactam nitrogen atom; a signal at δ 3.81 (3 H, s) due to the methyl ester, and unassignable peaks in the region of δ 2.80~3.56. The proton corresponding to the signal at δ 3.94 in the ^{1}H-NMR spectrum of PS-5 methyl ester appeared at δ 4.04 as a clear doublet of triplets (1 H) in the spectrum of PS-5 sodium salt and at δ 3.96 (1 H, dt) in that of PS-5 benzyl ester.

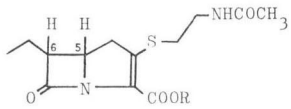
To clarify interproton relationships, spin-decoupling experiments were carried out with the benzyl ester in the presence of $Eu(FOD)_3$ as a shift reagent. The signals were effectively separated from each other when 11.3 mg (0.37 mol. ratio) of $Eu(FOD)_3$ was added (Fig. 1). Irradiation at δ 1.05 (3 H, t, J=7 Hz) converted the multiplet at δ 1.98(2 H) to a doublet of J=7 Hz. When the center of δ 1.98 was irradiated, the doublet of triplets at δ3.36 (1 H) turned to be a doublet of J=3 Hz with simultaneous transformation of the triplet at δ 1.05 to a singlet. On irradiation at δ 4.21 (corresponding to the signal at δ 3.96 in the spectrum without the shift reagent) the doublet of triplets at δ 3.36 was converted to a triplet of J=7 Hz while the multiplet (2 H) at δ 3.48~4.28 changed to an AB type quartet of J=18 Hz. These results indicated that PS-5 had the following partial structure:



Furthermore, irradiation at δ 6.30 (2 H, corresponding to δ 3.43 in the spectrum without the shift reagent) transformed the peaks attributable to an amide proton at δ 8.37 (1 H, corresponding to δ 6.02 in the spectrum without the shift reagent) to a broad singlet with concurrent change of the peaks at δ 4.53 (2 H, corresponding to δ 2.95 in the spectrum without the shift reagent). These findings suggested the existence of the following partial structure:

$-CH_2-CH_2-NH-COCH_3$

Based on these spectral data, the structure of PS-5 and its esters was concluded to have a 7-oxo-1-azabicyclo[3.2.0]hept-2-ene ring system as follows:



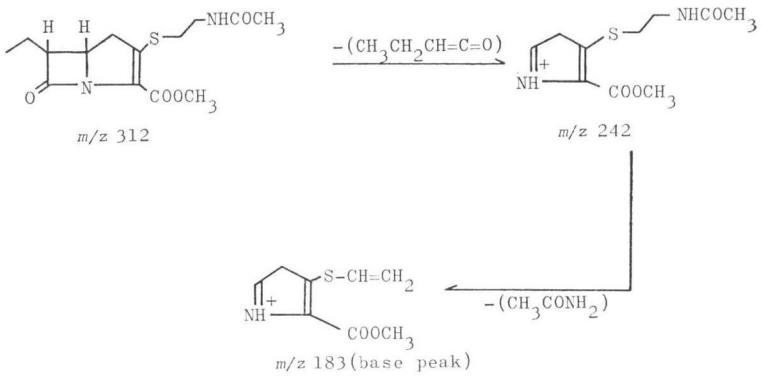
R : H
: Na
: CH_3
: CH_2-⬡

Table 1. ^{13}C-NMR data of PS-5 sodium salt.

Assignment	ppm
–CO–	184.0 (s)
	175.0 (s)
	169.3 (s)
>C=	141.1 (s)
	130.4 (s)
–CH–	60.2 (d)
	55.6 (d)
$-CH_2-$	40.0 (t)
	39.9 (t)
	31.5 (t)
	22.5 (t)
$-CH_3$	22.6 (q)
	11.4 (q)

The ^{13}C-NMR spectrum of PS-5 sodium salt also supported the structure; thirteen signals observed were unequivocally assignable to individual carbon atoms on the basis of off-resonance measurements and their chemical shifts (Table 1). Three carbonyl carbon signals could be attributed to the carboxylate (δ 184.0), acetyl group (δ 175.0) and β-lactam (δ 169.3) and the other two trigonal carbon signals (δ 141.1 and δ 130.4) indicated the presence of a tetrasubstituted carbon-carbon double bond.

Fig. 2. Mass fragmentation of PS-5 methyl ester.

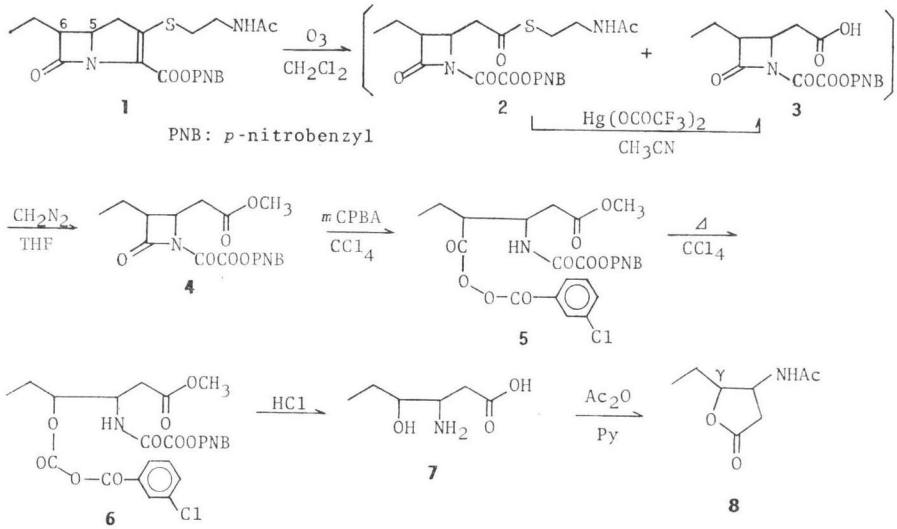


The mass fragmentation pattern of PS-5 methyl ester (Fig. 2) also validated the structure of 3-(2-acetamidoethyl)thio-6-ethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid for PS-5.

The vicinal coupling constant observed in PS-5 sodium salt and its esters ($J_{5,6}$=3 Hz) allowed the assignment of the *trans* β-lactam configuration to PS-5, because the *cis* relation is known to be always associated with a larger coupling constant ($J \geq 5 \sim 6$ Hz) than the corresponding *trans* relation ($J \leq 2 \sim 3$ Hz)[17,18,19].

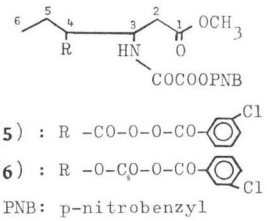
The absolute configurations of C-5 and C-6 were determined by chemical degradation as shown in Scheme 1. The *p*-nitrobenzyl ester **1** was treated with ozone in methylene chloride at −70°C, affording **2** and **3**. Without isolation, the mixture of **2** and **3** was treated with mercuric trifluoroacetate in acetonitrile at room temperature followed by methylation with diazomethane in tetrahydrofuran at 5°C, yield-

Scheme 1.



ing azetidinone **4**. In the IR spectrum of **4**, the characteristic bands at 1810 and 1760 cm^{-1} indicated that **4** had a structure similar to N-*p*-nitrobenzoxalyl-2-azetidinone derivatives[20]. Compound **5** was obtained by treatment of **4** with *m*-chloroperbenzoic acid in carbon tetrachloride at 75°C. On monitoring by IR, the conversion of **4** to **5** was proved by appearance of new bands at 3400 cm^{-1} (NH) and 1800 and 1770 cm^{-1} (carbonyl) and disappearance of the band assignable to the β-lactam carbonyl. Heating **5** in carbon tetrachloride afforded **6**[21,22]. In the IR spectrum of **6** a new strong band appeared at 1815 cm^{-1}, whereas the bands at 1800 and 1770 cm^{-1} seen in the IR spectrum of **5** disappeared. The NMR data of **5** and **6** are shown in Table 2. The structure of **6** was supported by a decoupling experiment. Irradiation at the center of δ 1.72 (methylene multiplet) caused the triplet at δ 1.04 to collapse to a singlet, while the doublet of triplets at δ 5.07 became a doublet of J=3 Hz. Hydrolysis with 6 N hydrochloric acid at 110°C for 16 hours converted **6** to γ-hydroxy-β-amino acid **7**[23]. Lactone **8** was obtained by treating **7** with acetic anhydride in pyridine at room temperature. It showed a strong infrared absorption at 1780 cm^{-1} and a molecular ion peak at m/z 171 in the field desorption mass spectrum.

Table 2. ^{1}H- and ^{13}C-NMR data of compounds **5** and **6**.



Assignment	^{1}H-NMR		^{13}C-NMR
	5	6	6
CH_3-6	1.06	1.04	9.4 (q)
CH_2-5	1.72	1.72	24.4 (t)
CH-4	3.00	5.07	82.6 (d)
CH-3	4.72	4.67	48.4 (d)
CH_2-2	2.76	2.74	36.0 (t)
OCH_3	3.72	3.71	52.2 (q)
OCH_2Ar	5.35	5.37	67.0 (t)

Other signals in ^{13}C-NMR

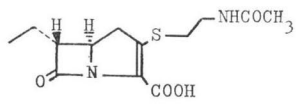
77.2 (d), 123.8 (d), 128.6 (d), 128.8 (d), 130.4 (d), 134.8 (d), 130.1 (s), 135.0 (s), 141.1 (s), 147.9 (s), 148.8 (s), 157.7 (s), 159.8 (s), 160.2 (s), 170.4 (s).

Determination of the absolute configuration of the γ-position of lactone **8** was based on HUDSON's

lactone rule[23,24]. According to HUDSON's lactone rule, when the absolute configuration at the γ-position of lactone **8** is *R*, the optical rotation must be dextrorotatory, while, when it is *S*, the rotation must be levorotatory.

As the optical rotation of lactone **8** was $[\alpha]_D^{22}+63.3°$ (*c* 0.3, water), the absolute configuration of the γ-position of lactone **8** was established to be *R*. Since the γ-position of lactone **8** corresponded to position C-6 of the starting material (PS-5 *p*-nitrobenzyl ester **1**), the absolute configuration at position C-6 of PS-5 was determined to be *R*. Furthermore, since the relative configuration of the β-lactam hydrogens of PS-5 was *trans*, the absolute configuration at C-5 was concluded to be *R*. Thus the full structure of PS-5 is shown in Fig. 3.

Fig. 3.



Experimental

Ultraviolet absorption spectra were taken with a Hitachi 200–20 spectrophotometer. Infrared absorption spectra were obtained with a Hitachi 260–30 spectrophotometer. ^{1}H-NMR spectra were recorded in D_2O or $CDCl_3$ with TSP (2,2,3,3-tetradeutero-3-trimethylsilyl-propionic acid sodium salt) or Me_4Si as internal standard on a JEOL PS-100 and ^{13}C-NMR spectra in D_2O with dioxane as internal standard on a Varian CFT-20 and a JEOL FX-100 instruments. The high resolution mass spectrum of PS-5 methyl ester was obtained on a Hitachi RMU-7 mass spectrometer. Specific rotations were measured with a JASCO DIP-181 digital polarimeter.

General procedure for preparation of PS-5 esters

Triethylamine (3 eq.) and alkyl halide (3 eq.) corresponding to the desired ester were added to a solution of PS-5 sodium salt (1 eq.) in dried dimethylformamide. The solution was stirred for 3 hours at room temperature and diluted with 100 ml of benzene. The reaction mixture was washed with sodium phosphate buffer (0.1 M, pH 6.8) and dried over sodium sulfate. The evaporation residue was chromatographed on silica gel and the ester of PS-5 was eluted with benzene - acetone (3: 1).

Methyl ester

IR ($CHCl_3$) 1780, 1710, 1680, 1560, 1505, 1445, 1350 cm^{-1}; UV λ_{max}^{MeOH} 316 nm (ε 10,100); ^{1}H-NMR ($CDCl_3$) δ 1.04 (3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.87 (2H, m, $\underline{CH_2}CH_3$), 1.95 (3H, s, $COC\underline{H_3}$), 2.80~3.56 (7H, m, $-SC\underline{H_2}C\underline{H_2}N-$, C-4H, C-6H), 3.81 (3H, s, $OC\underline{H_3}$), 3.94 (1H, dt, J=3.0, 9.2 Hz, C-5H), 6.20 (1H, m, CONH); mass *m/z* 312 (M^+), 242 ($M^+-EtCH=C=O$), 183; $[\alpha]_D^{22}+72.9°$ (*c* 1.0, THF).

Benzyl ester

IR($CHCl_3$) 1770, 1700, 1660, 1550, 1510 cm^{-1}; UV λ_{max}^{MeOH} 317 nm (ε 10,700); ^{1}H-NMR ($CDCl_3$) δ 1.06 (3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.88 (2H, m, $\underline{CH_2}CH_3$), 1.97 (3H, s, $COC\underline{H_3}$), 2.80~3.60 (7H, m, $-SC\underline{H_2}C\underline{H_2}N-$, C-4H, C-6H), 3.96 (1H, dt, J=3.0, 9.2 Hz, C-5H), 5.20 (1H, d, J=14 Hz, C$\underline{H}$HAr), 5.38 (1H, d, J=14 Hz, CH$\underline{H}$Ar), 6.10 (1H, m, CONH), 7.30~7.50 (5H, m, ArH); mass *m/z* 388 (M^+), 318 ($M^+-EtCH=C=O$), 259; $[\alpha]_D^{22}+38.6°$ (*c* 1.0, THF).

p-Nitrobenzyl ester

IR ($CHCl_3$) 1780, 1710, 1675, 1530, 1350 cm^{-1}; UV $\lambda_{max}^{CHCl_3}$ 322 nm (ε 12,000); ^{1}H-NMR ($CDCl_3$) δ 1.04 (3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.84 (2H, m, $\underline{CH_2}CH_3$), 1.96 (3H, s, $COC\underline{H_3}$), 2.80~3.60 (7H, m, $-SC\underline{H_2}C\underline{H_2}N-$, C-4H, C-6H), 3.98 (1H, dt, J=3.0, 9.2 Hz, C-5H), 5.19 (1H, d, J=14 Hz, C$\underline{H}$HAr), 5.49 (1H, d, J=14 Hz, CH$\underline{H}$Ar), 5.95 (1H, m, CONH), 7.61 (2H, d, J=9 Hz, ArH), 8.18 (2H, d, J=9 Hz, ArH); mass *m/z* 433 (M^+), 363 ($M^+-EtCH=C=O$); $[\alpha]_D^{22}+70.7°$ (*c* 1.0, $CHCl_3$).

Anal. Calcd. for $C_{20}H_{23}O_6N_3S$: C, 55.43; H, 5.35; N, 9.70.
Found: C, 55.29; H, 5.24; N, 9.40.

N-*p*-Nitrobenzoxalyl-3-ethyl-4-methoxycarbonylmethyl-2-azetidinone (**4**)

Into a solution of 100 mg (0.231 mmol) of PS-5 *p*-nitrobenzyl ester **1** in 20 ml of methylene chloride, an excess of ozone was introduced at −70°C for 5 minutes. After the ozone remaining was removed in a stream of N_2, the solvent was evaporated *in vacuo*. The residue was treated with 200 mg of mercuric trifluoroacetate in 20 ml of acetonitrile at room temperature for one hour. The reaction mixture was diluted with 60 ml of ethyl acetate, washed with brine (60 ml×3), and dried over sodium sulfate. After evaporation of the solvent *in vacuo*, the viscous oil obtained was treated with an excess of diazomethane in 20 ml of tetrahydrofuran at 5°C for 30 minutes. The evaporation residue was dissolved in benzene and chromatographed on Bio-Beads S×3 (Bio-Rad Laboratories) using benzene to give 70 mg (80.2% from **1**) of **4**.

IR ($CHCl_3$) 1810, 1760, 1740, 1705, 1610, 1530, 1355 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.06 (3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.85 (2H, m, $\underline{CH_2}CH_3$), 2.72 (1H, dd, J=9, 17 Hz, CHC$\underline{H}$HCO), 3.27 (1H, dd, J=4, 17 Hz, CHC$\underline{H}$HCO), 3.16 (1H, dt, J=3, 7 Hz, C-4H), 3.72 (3H, s, OC$\underline{H_3}$), 4.19 (1H, m, C-3H), 5.43 (2H, s, OC$\underline{H_2}$Ar), 7.60 (2H, d, J=8 Hz, ArH), 8.26 (2H, d, J=8 Hz, ArH).

Methyl-3-N-*p*-nitrobenzoxalylamino-4-(*m*-chlorobenzoyldioxycarbonyl)hexanoate (**5**)

Compound **4** (154.6 mg, 0.409 mmol) was heated in 15 ml of carbon tetrachloride at 75°C and 490 mg (2.463 mmol) of *m*-chloroperbenzoic acid was added in small portions. After heating at 75°C for 45 minutes, the reaction mixture was diluted with 60 ml of benzene, washed with 4% aqueous sodium bicarbonate (30 ml×3) and brine (30 ml×3) and dried over sodium sulfate. The evaporation residue was subjected to chromatography on Bio-Beads S×3 with benzene to provide 55.6 mg of **4** and 49.5 mg (34.3%) of **5**.

IR ($CHCl_3$) 3400, 1800, 1770, 1745, 1715, 1610, 1580, 1530, 1355 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.06 (3H, t, J=7 Hz, C–6H), 1.72 (2H, m, C–5H), 2.76 (2H, d, J=7 Hz, C–2H), 3.00 (1H, dt, J=3, 7 Hz, C–4H), 3.72 (3H, s, OC$\underline{H_3}$), 4.72 (1H, m, C-3H), 5.35 (2H, s, OC$\underline{H_2}$Ar), 7.30~8.36 (9H, m, ArH, N$\underline{H}$).

Methyl-3-N-*p*-nitrobenzoxalylamino-4-(*m*-chlorobenzoyloxycarbonyloxy)hexanoate (**6**)

Compound **5** (49 mg, 0.089 mmol) was dissolved in 15 ml of carbon tetrachloride and kept at 75°C for 2 hours. After the solvent was removed by evaporation *in vacuo*, the residue was purified by chromatography on Bio-Beads S×3 with benzene to give 20 mg (40.8%) of **6**.

IR ($CHCl_3$) 3400, 1815, 1750, 1715, 1615, 1580, 1530, 1355 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.04 (3H, t, J=7 Hz, C-6H), 1.72 (2H, m, C-5H), 2.74 (2H, d, J=7 Hz, C-2H), 3.71 (3H, s, OC$\underline{H_3}$), 4.67 (1H, m, C-3H), 5.07 (1H, dt, J=3, 7 Hz, C-4H), 5.37 (2H, s, OC$\underline{H_2}$Ar), 7.30~8.29 (9H, m, ArH, N$\underline{H}$).

3-Acetamido-4-ethyl-butyrolactone (**8**)

Compound **6** (50 mg, 0.091 mmol) was heated in 10 ml of 6 N hydrochloric acid at 110°C for 15 hours in a sealed tube. The concentrated reaction mixture was purified by column chromatography on Sephadex G-10 (Pharmacia Fine Chemicals AB, eluted with distilled water) and by ion-exchanger column chromatography on Dowex 50W×4 (H-form, Dow Chemical Co., eluted with 1.6 N aqueous ammonia) to yield 7.5 mg (43.6%) of 3-amino-4-hydroxyhexanoic acid **7**. Compound **7** was treated with 0.1 ml of acetic anhydride in 0.2 ml of pyridine at room temperature for 2 hours. The reaction mixture was poured in ice-water and stirred for 30 minutes. The aqueous layer was extracted with ethyl acetate, and the organic layer was dried over sodium sulfate and then chromatographed on Bio-Beads S×3 (eluted with benzene) to yield 6.5 mg (95.0%) of **8**.

IR ($CHCl_3$) 1780, 1745, 1680 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.03 (3H, t, J=7 Hz, $CH_2\underline{CH_3}$), 1.66 (2H, m, $\underline{CH_2}CH_3$), 2.04 (3H, s, CO$\underline{CH_3}$), 2.42 (1H, dd, J=2, 18 Hz, C-2H), 2.92 (1H, dd, J=8, 18 Hz, C-2H), 4.43 (1H, dt, J=6, 7 Hz, C-4H), 4.80 (1H, m, C-3H), 6.50 (1H, m, N$\underline{H}$); FD mass m/z 171 (M^+); $[\alpha]_D^{22}$ +63.3° (c 0.3, water).

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