Synthesis and Activity of 3-*epi*-Actinobolin

HAYAMITSU ADACHI, YOSHIO NISHIMURA*, SHINICHI KONDO and TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

(Received for publication October 2, 1997)

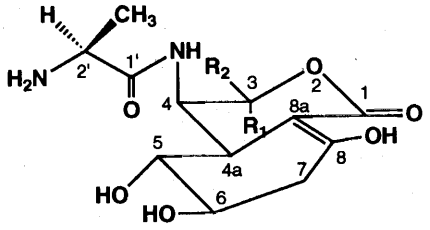
3-*epi*-Actinobolin was synthesized by the chemical transformation of actinobolin involving a key step of the reconstruction of fused δ-lacton skelton *via* intramolecular acylation reaction. The analogue with low toxicity weakly inhibits Gram-positive and Gram-negative bacteria.

Actinobolin (**1**) and its structurally related bactobolin (**2**) were isolated from the culture broths of *Streptomyces*[1~3] and *Pseudomonas*,[4~6] respectively. They demonstrated various kinds of biological effects including antimicrobial and antitumor activities,[1,4,6~8] suppressing effect on antibody production[9] and therapeutic effect on autoimmune encephalomyelitis.[10] Due to the unique structures and potent activities, these antibiotics have attracted intensive synthetic interest in the total synthesis[11~22] and in the chemical modification.[23~25] While **1** bears a structural resemblance to **2** except for the different substitution of C-3 (Fig. 1), its biological activity and cytotoxicity are considerably distinct from those of **2**. The biological diversity stimulated our interest in the alteration of functionality at C-3 of **1**. We considered the epimerization of the stereochemistry at C-3 of **1** as an initial probe of the structure-activity profile of this class of antibiotic. We here report the synthesis of 3-*epi*-actinobolin (**17**) by the chemical conversion of **1**.

Synthesis

The synthetic route to **17** is outlined in Scheme 1. The synthesis of **17** began with the known carbamate **3**.[20] Stereoselective reduction of **3** with $NaBH_4$ under WARD's conditions[20] (50% MeOH in CH_2Cl_2, $-78°C$) gave the alcohol **4** in 98% yield. The large coupling constants (12 and 11.2 Hz) between H-5′ and Hax-6′ and between H-5′ and Hax-4′ in ^1H NMR spectrum of **4** are indicative of the equatorial hydroxyl group. Protection of the hydroxyl of **4** afforded the *tert*-butyldimethylsilyl (TBDMS) ether **5** (96% yield), which was converted into the N-PMS carbamate **6** (54% yield) by treatment with (4-methylphenyl)methanesulfonyl chloride (PMS-Cl).[26] Alkaline hydrolysis of **6** gave **7** in 96% yield. Epimerization of methyl group at C-2′ was successfully carried out by two step sequences of oxidation and stereoselective reduction. Reduction of **8** with $NaBH_4$ under WARD's conditions[20] gave the desired stereoisomer **9** as a sole product in 84% yield. The compound **9** has the different specific rotation and ^1H NMR spectrum from those of the starting **7**, clearly indicating an epimerization at C-2′. This was also supported by the distinct physical data of the final 3-*epi*-actinobolin (**17**) from those of the natural actinobolin (**1**). Upon acetylation, **9** afforded the acetate **10**. Removal of TBDMS group of **10** resulted in the alcohol **11** (92% yield), which was oxidized to the ketone **12** (94% yield). Hydrolysis of **12** gave the key intermediate, keto alcohol **13** in 93% yield. The critical reconstruction of the fused δ-lactone skelton was best achieved by treatment of **13** with 1,1′-carbonyldiimidazole and with sodium hy-

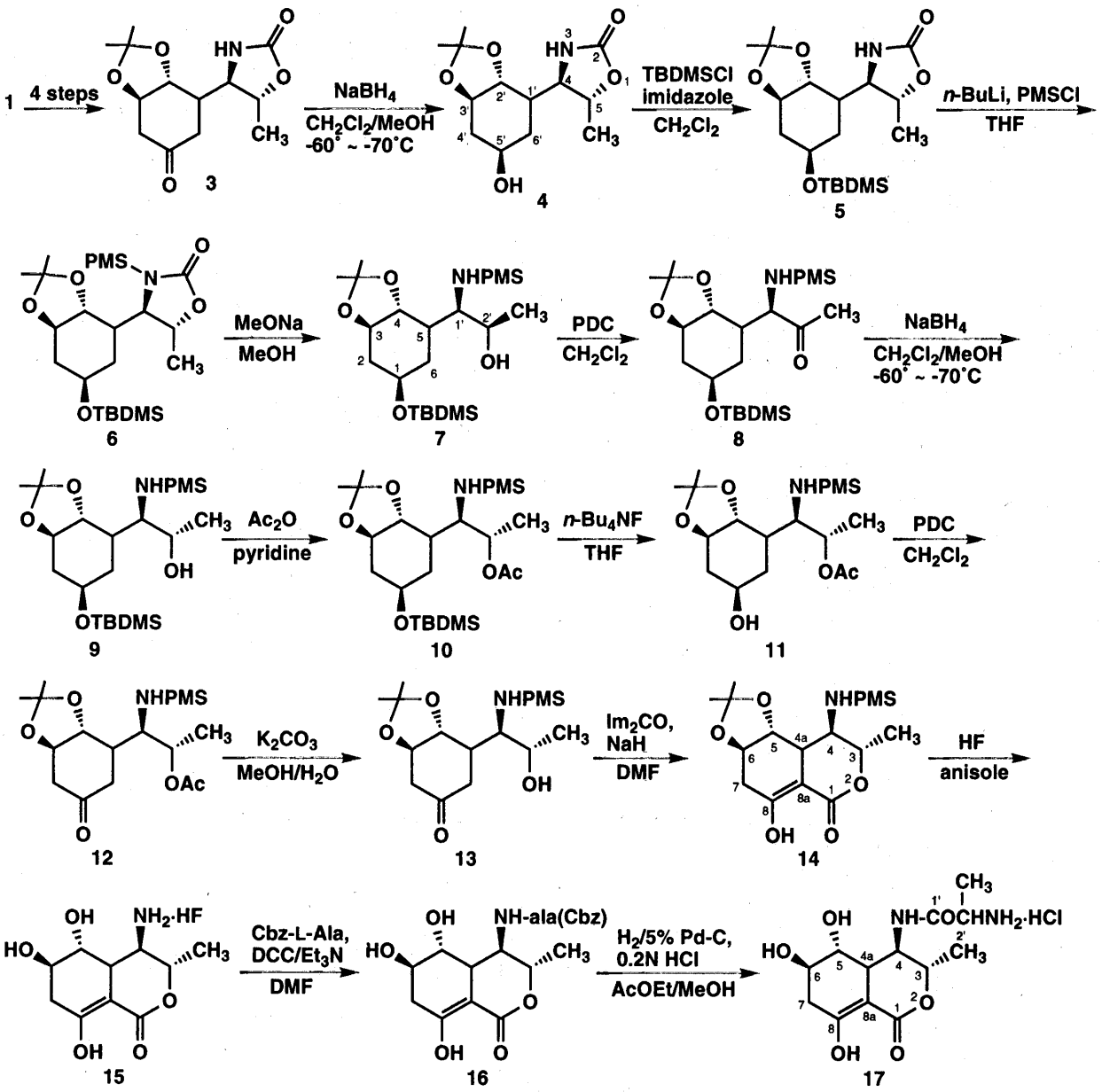
Fig. 1. The structures of actinobolin and bactobolin.



Actinobolin (1): R_1=H, R_2=CH_3

Bactobolin (2): R_1=CH_3, R_2=$CHCl_2$

Scheme 1. Synthesis of 3-*epi*-actinobolin.



dride[14] to afford the enol lactone **14** in 98% yield. Simultaneous removal of both the PMS and the acetonide groups of **14** smoothly proceeded with HF to afford the 3-*epi*-actinobolamine as its HF salt **15**. The synthesis of 3-*epi*-actinobolin (**17**) was completed by condensation of **15** with *N*-benzyloxycarbonylalanine followed by hydrogenolysis with H_2/Pd-C.

Biological Activities

As shown in Table 1, 3-*epi*-actinobolin (**17**) showed less inhibitory activity than actinobolin (**1**) against several organisms. The analogue **17** was also found to have less cytotoxicity than **1** (Table 2). These results indicate that the stereochemistry at C-3, which would cause the conformational change, critically participates in the biological activity. Further chemical modification of **1** based on the alteration of functionality at C-3 are now in progress.

Experimental

General Methods

Melting points were determined with a Yanagimoto apparatus and were uncollected. IR spectra were determined on a Hitachi Model 260-10 spectrometer. Optical rotations were measured with a Perkin-Elmer

Table 1. Antibacterial activities of actinobolin (**1**), bactobolin (**2**) and 3-*epi*-actinobolin (**17**) by broth dilution method.

Test organism	MIC (μg/ml)		
	1	**2**	**17**
Staphylococcus aureus FDA209P	3.13	0.20	25
Staphylococcus aureus Smith	6.25	0.20	25
Staphylococcus aureus MRSA No. 5	12.5	0.39	50
Staphylococcus aureus MS16526 (MRSA)	6.25	0.39	50
Staphylococcus epidermidis 109	6.25	0.20	50
Micrococcus luteus FDA16	1.56	0.10	12.5
Micrococcus luteus PCI1001	1.56	0.10	12.5
Bacillus anthracis	50	6.25	>50
Bacillus subtilis PCI219	25	0.39	>50
Corynebacterium bovis 1810	1.56	0.10	12.5
Escherichia coli NIHJ	1.56	0.20	25
Escherichia coli K-12 ML1629	25	6.25	>50
Shigella dysenteriae JS11910	1.56	0.05	12.5
Salmonela typhi T-63	25	6.25	>50
Proteus vulgaris OX19	3.13	0.39	25
Serratia marcescens	>50	25	>50
Pseudomonas aeruginosa A3	>50	25	>50
Pseudomonas aeruginosa GN315	>50	50	>50
Klebsiella pneumoniae PCI602	25	3.13	>50
Mycobacterium smegmatis ATCC607*	12.5	3.13	>50

MICs were determined by 2-fold agar dilution streak method at 37°C for 18 and 42 hours.*

Table 2. Cytotoxicity of actinobolin (**1**), bactobolin (**2**) and 3-*epi*-actinobolin (**17**).

Cell	IC_{50} (μg/ml)		
	1	**2**	**17**
L1210	48.1	0.11	>100
EL4	42.1	0.087	>100
P388	39.6	0.068	>100
IMC ca.	40.8	0.078	>100
Colon 26	30.6	0.035	>100
HeLa	>100	0.34	>100
FS-3	99.6	0.28	>100
LB32T	37.2	0.11	>100
Methyl green FS-3	>100	0.71	>100

The rate of survival cells was measured by MTT assay and IC_{50} value was calculated.

Model 241 polarimeter. ^{1}H NMR spectra were recorded with Jeol GX-400 spectrometer. Chemical shifts are expressed in δ values (ppm) with tetramethylsilane as an internal standard. The MS spectra were taken by Jeol SX102 in the FAB mode using 3-nitrobenzyl alcohol as a matrix.

(1′*R*,2′*R*,3′*R*,4*R*,5′*R*,5*R*)-4-[5′-Hydroxy-2′,3′-(isopropylidenedioxy)cyclohexyl]-5-methyl-2-oxazolidinone (**4**)

To a solution of **3** (1.20 g, 4.5 mmol) in a mixture of dichloromethane (6 ml) and methanol (6 ml) was added sodium borohydride (0.510 g, 13.5 mmol) at $-60\sim70$°C, and the reaction mixture was stirred for 30 minutes. After dilution with ethyl acetate, the solution was washed with water, dried over $MgSO_4$ and filtered. The filtrate was evaporated to give an oil, which was subjected to column chromatograpy on silica gel. Elution with chloroform-methanol (10 : 1) gave a colorless solid of **4** (1.18 g, 98% yield), which was crystallized from a mixture of dichloromethane and hexane (10 : 1) to give colorless crystals; mp 106~108°C; $[\alpha]_D^{24}$ +53.5° (*c* 0.72, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3450 (br), 2990, 2940, 2875, 1745, 1465, 1455, 1385, 1375, 1235, 1100, 1060, 1050 (sh), 1005, 840; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.18 (1H, q, $J=\sim12$ Hz, 6′-Hax), 1.40 and 1.42 (each 3H, s, isopropylidene), 1.45 (3H, d, $J=5.9$ Hz, 5-CH_3), 1.51 (1H, q, $J=11.2$ Hz, 4′-Hax), 1.77 (1H, m, 1′-H), 2.10 (1H, d with small couplings, $J=\sim12$ Hz, 6′-Heq), 2.44 (1H, d with small couplings, $J=11.2$ Hz, 4′-Heq), 3.17 (1H, dd, $J=8.8$ and 10.3 Hz, 2′-H), 3.35~3.45 (3H, m, 3′-H, 4-H and 5′-OH), 3.89 (1H, m, 5′-H), 4.66 (1H, dq, $J=6.1$ and 7.6 Hz, 5-H), 7.01 (1H, br s, NH); MS (FAB positive) *m/z* 272 $(M+H)^+$.

(1′*R*,2′*R*,3′*R*,4*R*,5′*R*,5*R*)-4-[5′-(*tert*-Butyldimethylsilyl)-oxy-2′,3′-(isopropylidenedioxy)cyclohexyl]-5-methyl-2-oxazolidinone (**5**)

To a solution of **4** (0.685 g, 2.5 mmol) in dichloromethane (7 ml) were added *tert*-butyldimethylsilyl chloride (0.753 g, 5.0 mmol) and imidazole (0.510 g, 7.5 mmol) at room temperature, and the reaction mixture was stirred for 2 hours. After dilution with chloroform, the solution was washed with satd $NaHCO_3$ aq solution and water, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give an oil, which was subjected to preparative TLC on silica gel developed with chloroform-methanol (30 : 1) to give **5** (0.866 g, 90% yield). $[\alpha]_D^{24}$ +33.2° (*c* 0.85, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3475, 3250, 2990 (sh), 2960, 2940, 2870, 1755, 1465, 1410 (sh), 1385, 1375, 1260 (sh), 1250 (sh), 1235, 1100, 1060, 840; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.065 and 0.071 (each 3H, s, –$(CH_3)_3$–Si–), 0.88 (9H, s, $(CH_3)_3$C–), 1.21 (1H, q, $J=\sim12$ Hz, 6′-Hax), 1.39 and 1.40 (each 3H, s, isopropylidene), 1.44 (3H, d, $J=5.9$ Hz, 5-CH_3), 1.54 (1H, q, $J=\sim11$ Hz, 4′-Hax), 1.74 (1H, m, 1′-H), 1.93 (1H, d with small couplings, $J=12.7$ Hz, 6′-Heq), 2.32 (1H, d with small couplings, $J=11.7$ Hz, 4′-Heq), 3.13 (1H, dd, $J=8.8$ and 10.3 Hz, 2′-H), 3.30~3.40 (2H, m, 3′-H and 4-H), 3.83 (1H, m, 5′-H), 4.67 (1H, dq, $J=6.1$ and 7.1 Hz, 5-H), 6.08 (1H, br s, 4-NH); MS (FAB positive) *m/z* 386 $(M+H)^+$.

(1′*R*,2′*R*,3′*R*,4*R*,5′*R*,5*R*)-4-[5′-(*tert*-Butyldimethylsilyl)-oxy-2′,3′-(isopropylidenedioxy)cyclohexyl]-5-methyl-3-*N*-[(4-methylphenyl)methanesulfonyl]-2-oxazolidinone (**6**)

BuLi (1.0 M solution in hexane; 1.88 ml, 3 mmol) was added to a solution of **5** (0.388 g, 1 mmol) and 5-nitro-1,10-phenanthroline (trace) in tetrahydrofuran (40 ml) at 0°C until a red color persisted. To a mixture was added a solution of PMS-Cl (0.409 g, 2.0 mmol) in tetrahydrofurane (5 ml), and the mixture was stirred at 0°C for 1 hour. After dilution with chloroform, the solution was successively washed with satd $NaHCO_3$ aq solution, satd NH_4Cl aq solution and water, dried over $MgSO_4$, and filtered. Evaporation of the solvent gave an oil, which was subjected to preparative TLC on silica gel developed with ethyl acetate-hexane (1 : 2) to give **6** (0.300 g, 54% yield). $[\alpha]_D^{24}$ −35.4° (*c* 0.72, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 2995, 2960, 2940, 2870, 1780, 1475, 1390 (sh), 1380, 1375 (sh), 1175, 1150, 1105, 845; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.06 (6H, s, –$(CH_3)_2$Si–), 0.88 (9H, s, $(CH_3)_3$C–), 1.13 (1H, dt, $J=10.7$ and 12.7 Hz, 6-Hax), 1.23 (3H, d, $J=6.3$ Hz, 5-CH_3), 1.37 and 1.40 (each 3H, s, isopropylidene), 1.45 (1H, q, $J=\sim11$ Hz, 4′-Hax), 1.80 (1H, d with small couplings, $J=\sim13$ Hz, 6′-Heq), 2.19 (1H, m, 1′-H), 2.30 (1H, d with small couplings, $J=11.2$ Hz, 4′-Heq), 2.36 (3H, s, CH_3–Ph–), 3.14 (1H, dd, $J=8.8$ and 10.7 Hz, 2′-H), 3.36 (1H, ddd, $J=3.4$, 8.3 and 11.7 Hz, 3′-H), 3.78~3.90 (2H, m, 4-H and 5′-H), 4.57~4.64 (1H, 5-H overlapped with aromatic proton), 4.64 and 5.00 (2H, ABq, $J=14.2$ Hz, –CH_2–Ph–), 7.21 (2H, d, $J=7.8$ Hz, aromatic protons), 7.36 (2H, d, $J=8.3$ Hz, aromatic protons); MS (FAB positive) *m/z* 554 $(M+H)^+$.

(1*R*,1′*R*,2′*R*,3*R*,4*R*,5*R*)-1-[(*tert*-Butyldimethylsilyl)-oxy]-5-[2′-hydroxy-1′-[(4-methylphenyl)methanesulfonamide]propyl]-3,4-(isopropylidenedioxy)cyclohexane (**7**)

To a solution of **6** (0.313 g, 0.57 mmol) in methanol (6

ml) was added sodium methoxide (0.305 g, 5.7 mmol) at 0°C, and the reaction mixture was stirred for 3 hours. After dilution with ethyl acetate, the solution was washed with sat. NH_4Cl aq. solution and water, dried over $MgSO_4$, and filtered. Evaporation of the solvent gave an oil, which was subjected to preparative TLC on silica gel developed with ethyl acetate - hexane (1 : 2) to give **7** (0.287 g, 96% yield) which was identical to the known compound reported by WEINREB *et al.*[17]

(1*R*,1′*R*,3*R*,4*R*,5*R*)-1-[(*tert*-Butyldimethylsilyl)oxy]-3,4-(isopropylidenedioxy)-5-[1′-(4-methylphenyl)methanesulfonamide-2′-oxopropyl]cyclohexane (**8**)

To a solution of **7** (0.170 g, 0.32 mmol) in dichloromethane (4 ml) was added pyridinium dichromate (1.2 g, 3.2 mmol) at room temperature, and the reaction mixture was stirred overnight. Filtration and evaporation of the filtrate gave an oil, which was subjected to preparative TLC on silica gel developed with ethyl acetate - hexane (1 : 2) to give **8** (0.106 g) and the starting material **5** (0.034 g) (conversion yield 79%). $[\alpha]_D^{24}$ −53.7° (*c* 0.91, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3010 (sh), 2975, 2950, 2880, 1730, 1395, 1385, 1350, 1270, 1240, 1165, 1140, 1120 (sh), 1095, 1070, 845; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.034 and 0.027 (each 3H, s, $-(CH_3)_2-Si-$), 0.87 (9H, s, $(CH_3)_3C-$), 1.21 (1H, dt, J=10.8 and 13.2 Hz, 6-Hax), 1.41 and 1.42 (each 3H, s, isopropylidene), 1.44∼1.55 (2H, 6-Heq and 2-Hax), 1.91 (1H, m, H-5), 2.20 (3H, s, CH_3CO-), 2.29 (1H, d with small couplings, J=11.7 Hz, 2-Heq), 2.36 (3H, s, CH_3-Ph-), 3.26 (1H, dd, J=8.8 and 10.7 Hz, 4-H), 3.35 (1H, ddd, J=3.4, 8.8 and 11.7 Hz, 3-H), 3.75 (1H, m, 1-H), 4.06 (1H, dd, J=3.4 and 9.3 Hz, 1′-H), 4.19 and 4.28 (2H, ABq, J=13.7 Hz, $-CH_2-Ph-$), 5.27 (1H, d, J=9.3 Hz, 1′-NH), 7.18 (2H, d, J=7.8 Hz, aromatic protons), 7.28 (2H, d, J=8.3 Hz, aromatic protons); MS (FAB positive) *m/z* 526 $(M+H)^+$.

(1*R*,1′*R*,2′*S*,3*R*,4*R*,5*R*)-1-[(*tert*-Butyldimethylsilyl)oxy]-5-[2′-hydroxy-1′-[(4-methylphenyl)methanesulfonamide]propyl]-3,4-(isopropylidenedioxy)cyclohexane (**9**)

To a solution of **8** (0.556 g, 11 mmol) in a mixture of methanol (13.2 ml) and dichloromethane (13.2 ml) was added sodium borohydride (0.119 g, 3.2 mmol) at −70°C. The reaction mixture was stirred at −70°C for 1 hour and was further stirred at room temperature for 2 hours. Dilution with ethyl acetate and evaporation of solvent gave a crude oil, which was dissolved in water. The aqueous layer was extracted with ethyl acetate. The extract was dried over $MgSO_4$ and filtered. Evaporation of the filtrate gave an oil, which was subjected to preparative TLC on silica gel developed with ethyl acetate - hexane (1 : 2) to give **9** (0.465 g, 84% yield). $[\alpha]_D^{24}$ +16.9° (*c* 0.51, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3500 (sh), 3350, 2990 (sh), 2960, 2930, 2870, 1515, 1465, 1385, 1375, 1335, 1260, 1150 (sh), 1130 (sh), 1095, 845; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.06 (6H, s, $-(CH_3)_2Si-$), 0.87 (9H, s, $(CH_3)_3C-$), 1.24 (3H, d, J=6.4 Hz, 2′-CH_3), 1.31 (1H, dt, J=∼2 and 12.9 Hz, 6-Hax), 1.37 and 1.39 (each 3H, s, isopropylidene), 1.51 (1H, q, J=∼11 Hz, 2-Hax), 1.75 (1H, m, 5-H), 1.93 (1H, d with small couplings, J=∼13 Hz, 6-Heq), 2.17 (1H, d, J=6.8 Hz, 2′-OH), 2.31 (1H, d with small couplings, J=11.2 Hz, 2-Heq), 2.36 (3H, s, CH_3-Ph-), 3.24 (1H, dd, J=8.8 and 10.3 Hz, 4-H), 3.31 (1H, ddd, J=3.4, 8.8 and 11.4 Hz, 3-H), 3.44 (1H, dt, J=3.7 and 10.3 Hz, 1′-H), 3.77 (1H, m, 1-H), 3.97 (1H, m, 2′-H), 4.29 and 4.39 (2H, ABq, J=13.7 Hz, $-CH_2-Ph-$), 4.72 (1H, d, J=9.8 Hz, 1′-NH), 7.19 (2H, d, J=8.3 Hz, aromatic protons), 7.34 (2H, d, J=7.8 Hz, aromatic protons); MS (FAB positive) *m/z* 528 $(M+H)^+$.

(1*R*,1′*R*,2′*S*,3*R*,4*R*,5*R*)-5-[2′-Acetoxy-1′-[(4-methylphenyl)methanesulfonamide]propyl]-1-(*tert*-butyldimethylsilyloxy)-3,4-(isopropylidenedioxy)cyclohexane (**10**)

A solution of **9** (0.465 g, 0.88 mmol) in pyridine (4.5 ml) was added acetic anhydride (1.1 ml), and the mixture was stirred at room temperature overnight. Evaporation of the solvent gave an oil, which was subjected to preparative TLC on silica gel developed with ethyl acetate - hexane (1 : 2) to give **10** (0.491 g, 98% yield). $[\alpha]_D^{24}$ −34.1° (*c* 0.36, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 2990 (sh), 2960, 2930, 2870, 1735, 1435, 1385 (sh), 1375, 1335, 1260 (sh), 1235, 1095, 1060, 845; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.050 and 0.054 (each 3H, s, $-(CH_3)_2Si-$), 0.87 (9H, s, $(CH_3)_3C-$), 1.27 (3H, d, J=6.3 Hz, 2′-CH_3), 1.25∼1.40 (1H, m, 6-Hax), 1.38 (6H, s, isopropylidene), 1.51 (1H, q, J=11.5 Hz, 2-Hax), 1.72 (1H, m, 5-H), 1.96 (1H, d with small couplings, J=13.2 Hz, 6-Heq), 2.09 (3H, s, CH_3CO_2-), 2.30 (1H, d with small couplings, J=11.2 Hz, 2-Heq), 2.36 (3H, s, CH_3-Ph-), 3.24 (1H, dd, J=8.5 and 10.5 Hz, H-4), 3.31 (1H, ddd, J=3.4, 8.3 and 11.7 Hz, 3-H), 3.70∼3.80 (2H, m, 1-H and 1′-H), 4.30 (2H, s, $-CH_2-Ph-$), 4.61 (1H, d, J=10.3 Hz, 1′-NH), 5.12 (1H, dq, J=3.4 and 6.4 Hz, 2′-H), 7.19 (2H, d, J=7.8 Hz, aromatic protons), 7.33 (2H, d, J=8.3 Hz, aromatic protons); MS (FAB positive) *m/z* 570 $(M+H)^+$.

(1*R*,1′*R*,2′*S*,3*R*,4*R*,5*R*)-5-[2′-Acetoxy-1′-[(4-methylphenyl)methanesulfonamide]propyl]-1-hydroxy-3,4-(isopropylidenedioxy)cyclohexane (**11**)

To a solution of **10** (0.491 g, 0.86 mmol) in tetrahydrofuran (10 ml) was added 1 M solution of tetrabutylammonium fluoride in THF (1.05 ml, 1.1 mmol), and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with satd NH_4Cl aq solution and extracted with ethyl acetate. The extract was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure. The residue was subjected to preparative TLC on silica gel developed with ethyl acetate-hexane (1:1) to give **11** (0.369 g, 94% yield). $[\alpha]_D^{24}$ −36.3° (*c* 0.72, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3375, 2990, 2940, 1735, 1390 (sh), 1385, 1340, 1245, 1230 (sh), 1165, 1140, 1095, 1065; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, J=6.4 Hz, 2′-CH_3), 1.20~1.33 (1H, 6-Hax overlapped with 2′-CH_3), 1.38 and 1.41 (each 3H, s, isopropylidene), 1.47 (1H, q, J=~11 Hz, 2-Hax), 1.65~1.75 (1H, m, 5-H), 2.09 (3H, s, $-OCOCH_3$), 2.14 (1H, d with small couplings, J=13.2 Hz, 6-Heq), 2.33 (3H, s, $-CH_2-Ph-$), 2.40 (1H, d with small couplings, J=~11 Hz, 2-Heq), 2.62 (1H, br s, 1-OH), 3.26 (1H, t, J=~9 Hz, 4-H), 3.32 (1H, ddd, J=3.4, 8.3 and 11.2 Hz, 3-H), 3.70 (1H, ddd, J=3.9, 6.4 and 9.8 Hz, 1′-H), 3.77 (1H, m, 1-H), 4.16 and 4.27 (2H, ABq, J=13.7 Hz, $-CH_2-Ph-$), 5.05 (1H, d, J=9.8 Hz, 1′-NH), 5.26 (1H, dq, J=3.4 and 6.4 Hz, 2′-H), 7.19 (2H, d, J=7.8 Hz, aromatic protons), 7.30 (2H, d, J=8.3 Hz, aromatic protons); MS (FAB positive) m/z 456 $(M+H)^+$.

(1′*R*,2′*S*,3*R*,4*R*,5*R*)-5-[2′-Acetoxy-1′-[(4-methylphenyl)methanesulfonamide]propyl]-3,4-(isopropylidenedioxy)cyclohexanone (**12**)

To a solution of **11** (0.344 g, 0.76 mmol) in dichloromethane (4 ml) was added pyridinium dichromate (0.568 g, 1.5 mmol) at room temperature, and the reaction mixture was stirred at room temperature for 1 hour. After filtration, the filtrate was evaporated to give an oil, which was subjected to preparative TLC on silica gel developed with ethyl acetate-hexane (1:1) to give **12** (0.322 g, 94% yield). $[\alpha]_D^{24}$ −53.5° (*c* 0.98, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3025, 2990, 2940, 2875, 1740 (sh), 1725, 1395 (sh), 1385, 1340, 1240, 1160, 1135; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.26 (3H, d, J=6.4 Hz, 2′-CH_3), 1.44 and 1.47 (each 3H, s, isopropylidene), 2.01 (1H, dddd, J=4.9, 5.1, 10.0 and 12.2 Hz, 5-H), 2.08 (3H, s, $-OCOCH_3$), 2.26 (1H, dd, J=12.0 and 16.4 Hz, 6-Hax), 2.34 (3H, s, CH_3-Ph-), 2.54 (1H, t, J=13.9 Hz, 2-Hax), 2.54 (1H, ddd, J=2.0, 4.9 and 16.1 Hz, 6-Heq), 2.88 (1H, ddd, J=2.0, 4.9 and 13.9 Hz, 2-Heq), 3.61 (1H, ddd, J=4.9, 8.8 and 13.9 Hz, H-3), 3.69 (1H, dd, J=8.8 and 10.0 Hz, H-4), 3.76 (1H, dt, J=~4.1 and 9.8 Hz, 1′-H), 4.26 and 4.30 (2H, ABq, J=13.9 Hz, $-CH_2-Ph-$), 4.71 (1H, d, J=9.8 Hz, 1′-NH), 5.16 (1H, dq, J=3.4 and ~6.5 Hz, 2′-H), 7.19 (2H, d, J=7.8 Hz, aromatic protons), 7.30 (2H, d, aromatic protons); MS (FAB positive) m/z 454 $(M+H)^+$.

(1′*R*,2′*S*,3*R*,4*R*,5*R*)-5-[2′-Hydroxy-1′-[(4-methylphenyl)methanesulfonamido]propyl]-3,4-(isopropylidenedioxy)cyclohexanone (**13**)

To a solution of **12** (0.282 g, 0.62 mmol) in a mixture of methanol (4.8 ml) and water (3.8 ml) was added potassium carbonate (0.342 g, 2.5 mmol), and the reaction mixture was stirred at room temperature for 1 hour. After dilution with ethyl acetate, the solution was washed with water, dried over $MgSO_4$ and filtered. Evaporation of the filtrate gave an oil, which was subjected to preparative TLC on silica gel developed with ethyl acetate-hexane (1:1) to yield a colorless solid of **13** (0.236 g, 93% yield), which was crystallized from a mixture of dichloromethane and hexane (10:1) to give colorless crystalls; mp 145~146°C; $[\alpha]_D^{24}$ −34.3° (*c* 0.85, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3525 (br), 3350 (br), 3025 (sh), 2980, 2930, 2875, 1721, 1390, 1335, 1230, 1215 (sh), 1115, 1135, 915; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.27 (3H, d, J=6.4 Hz, 2′-CH_3), 1.43 and 1.45 (each 3H, s, isopropylidene), 1.88 (1H, d, J=5.4 Hz, 2′-OH), 2.03 (1H, m, 5-H), 2.31 (1H, dd, J=12.5 and 16.9 Hz, 6-Hax), 2.36 (3H, s, CH_3-Ph-), 2.53 (1H, t, J=~14 Hz, 2-Hax), 2.50~2.60 (1H, m, 6-Heq), 2.88 (1H, ddd, J=2.0, 4.9 and 13.9 Hz, 2-Heq), 3.54 (1H, dt, J=~4.1 and 9.8 Hz, 1′-H), 3.62 (1H, ddd, J=4.9, 8.8 and 13.9 Hz, 3-H), 3.73 (1H, dd, J=8.8 and 10.7 Hz, 4-H), 4.05 (1H, dq, J=6.3 and ~10 Hz, 2′-H), 4.31 and 4.40 (2H, ABq, J=13.9 Hz, $-CH_2-Ph-$), 4.69 (1H, d, J=9.8 Hz, 1′-NH), 7.19 (2H, d, J=7.8 Hz, aromatic protons), 7.33 (2H, d, J=7.8 Hz, aromatic protons); MS (FAB positive) m/z 412 $(M+H)^+$.

De-*N*-alanyl-5,6-*O*-(isopropylidene)-*N*-[(4-methylphenyl)methanesulfonyl]-3-*epi*-actinobolin (**14**)

A solution of **13** (0.013 g, 0.032 mmol) and 1,1′-carbonyldiimidazole (0.025 g, 0.16 mmol) in anhydrous DMF (1.3 ml) was stirred at 70°C overnight, and to the mixture sodium hydride (7.6 mg, 60% oil dispersion) was added at 0°C. The reaction mixture was stirred at 20°C for 1 hour. After being quenched with satd NH_4Cl aq solution, the resulting suspension was diluted with ethyl acetate. The solution was washed with water, dried over

$MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to preparative TLC on silica gel developed with ethyl acetate-hexane (2:1) to give **14** (0.014 g, 98% yield). $[\alpha]_D^{24}$ −4.1° (*c* 0.46, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3370, 3010, 2980, 2925, 1650, 1590, 1425, 1390, 1335, 1225, 1155, 1135, 1105; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.36 (3H, d, J=6.8 Hz, 3-CH_3), 1.47 and 1.48 (each 3H, s, isopropylidene), 2.36 (3H, s, CH_3–Ph–), 2.65 (1H, ddd, J=2.9, 11.2 and 17.6 Hz, 7-Hax), 2.90~3.0 (2H, m, 7-Heq and 4a-H), 3.56 (1H, t, J=9.3 Hz, H-5), 3.81 (2H, m, 4-H and 6-H), 4.21 and 4.39 (2H, ABq, J=13.7 Hz, –CH_2–Ph), 4.49 (1H, dq, J=1.5 and 6.8 Hz, 3-H), 4.63 (1H, d, J=9.8 Hz, 4-NH), 7.20 (2H, d, J=7.8 Hz, aromatic protons), 7.31 (2H, d, J=8.3 Hz, aromatic protons), 13.7 (1H, br s, 8-OH); MS (FAB positive) m/z 438 $(M+H)^+$.

2′-*N*-(Benzyloxycarbonyl)-3-*epi*-actinobolin (**16**)

Anhydrous hydrogen fluoride (HF) (20 ml) was condensed into a Teflon round-bottomed flask containing a solution of **14** (0.031 g, 0.07 mmol) in anisole (4 ml) at −200°C. The reaction mixture was then stirred at 0°C overnight. Hydrogen fluoride and anisole were removed under reduced pressure to give the crude **15**, which was subjected to the next step without purification. A solution of **15**, *N*-benzyloxycarbonyl-L-alanine (0.045 g, 0.20 mmol), dicyclohexylcarbodiimide (0.049 g, 0.24 mmol) and triethylamine (57 μl) in dry dimethylformamide (0.4 ml) was stirred overnight. Evaporation of the solvent gave an oil, which was subjected to preparative TLC on silica gel developed with chloroform-methanol (5:1) to give **16** (0.015 g, 69% yield). $[\alpha]_D^{24}$ −66.5° (*c* 0.22, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3430, 3010, 2960 (sh), 1725, 1660, 1615, 1515, 1230, 1150; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.39 (3H, d, J=6.8 Hz, 3-CH_3), 1.44 (3H, d, J=6.8 Hz, 2′-CH_3), 2.48 (1H, br d, J=9.6 and 18.7 Hz, 7-Hax), 2.72 (1H, br d, J=9.3 Hz, 4a-H), 2.93 (1H, dd, J=7.1 and 18.7 Hz, 7-Heq), 3.05~3.25 (2H, m, 5-H and 6-OH), 3.92 (1H, dt, J=7.1 and 9.6 Hz, 6-H), 4.20~4.30 (2H, m, 4-H and 2′-H), 4.57 (1H, br d, J=6.8 Hz, 3-H), 4.70 (1H, br s, 5-OH), 5.04 and 5.10 (2H, ABq, J=12.2 Hz, –CH_2–Ph–), 5.25 (1H, br d, J=~5 Hz, 2′-NH), 7.04 (1H, br d, J=~5 Hz, 4-NH), 7.30~7.40 (5H, m, aromatic protons), 13.2 (1H, br s, 8-OH); MS (FAB positive) m/z 435 $(M+H)^+$.

3-*epi*-Actinobolin (**17**)

Compound **16** (0.0117 g, 0.027 mmol) in a mixture of ethyl acetate (0.3 ml), methanol (2 ml) and 0.2 N HCl (0.098 ml) was stirred with 5% Pd/C (48 mg) under atmosphere of hydrogen at room temperature for 1 hour. After filtration, evaporation of the filtrate gave a hydrochloride of 3-*epi*-actinobolin **17** (0.008 mg, 88% yield). $[\alpha]_D^{24}$ +32.5° (*c* 0.55, MeOH); IR (KBr) cm^{-1} 3350, 2990 (sh), 1690, 1665 (sh), 1605, 1570, 1500, 1420, 1225, 1205, 1145, 1080; ^1H NMR (CD_3OD, 400 MHz) δ 1.43 (3H, d, J=6.8 Hz, 3-CH_3), 1.49 (3H, d, J=6.8 Hz, 2′-CH_3), 2.40 (1H, ddd, J=2.4, 9.8 and 18.6 Hz, 7-Hax), 2.82 (1H, dd, J=6.6 and 18.6 Hz, 7-Heq), 2.89 (1H, d with small couplings, J=9.8 Hz, 4a-H), 3.25 (1H, t, J=9.6 Hz, 5-H), 3.81 (1H, dt, J=6.6 and 9.8 Hz, 6-H), 3.98 (1H, q, J=6.8 Hz, 2′-H), 4.34 (1H, dd, J=1.5 and 3.4 Hz, 4-H), 4.69 (1H, dq, J=1.5 and 6.8 Hz, 3-H); MS (FAB positive) m/z 301 $(M+H)^+$.

Acknowledgments

The authors would like to express their appreciation to Dr. MASA HAMADA and Mr. KATSUHISA YAMAZAKI for evaluation of biological activity. The authors are grateful to members of Pharmaceutical Technology Laboratories, Meiji Seika Kaisha, Ltd. for the large scale preparation of actinobolin. We gratefully acknowledges Prof. SHOSUKE SOFUKU, Rikkyo University for his technical advice of removal of PMS protecting groups.

References

1) HASKELL, T. H. & Q. R. BARTZ: Actinobolin, a new-broad spectrum antibiotic. Isolation and characterization. Antibiot. Ann. 1958–1959: 505~509, 1959
2) STRUCK, R. F.; M. C. THORPE, W. C. COBURN & Y. F. SHEALY: Actinobolin. I. Basic degradation studies and the structure of a major degradation product (1). Tetrahedron Lett.: 1589~1595, 1967
3) ANTOSZ, F. J.; D. B. NELSON, D. L. HERALD, Jr. & M. E. MUNK: The structure and chemistry of actinobolin. J. Am. Chem. Soc. 92: 4933~4942, 1970
4) KONDO, S.; Y. HORIUCHI, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: A new antitumor antibiotic, bactobolin, produced by *Pseudomonas*. J. Antibiotics 32: 1069~1071, 1979
5) UEDA, I.; T. MUNAKATA & J. SAKAI: Structure of 2-amino-*N*-(3-dichloromethyl-3,4,4a,5,6,7-hexahydro-5,6,8-trihydroxy-3-methyl-7-oxo-1*H*-2-benzopyran-4-yl)propanamide hydrobromide dihydrate. Acta Crystallogr. B36: 3128~3131, 1980
6) MUNAKATA, T.; Y. IKEDA & H. MATSUKI: Cultural conditions and strain improvement for production of bactobolin. Agric. Biol. Chem. 47: 929~934, 1983
7) OKUMOTO, T.; M. KOTANI, H. HOSHINO & M. NAKANISHI: Antitumor activity of newly isolated antibiotics, 3-dichloromethylactinobolins. J. Pharm. Dyn. 3: 177~182, 1980
8) HORI, M.; K. SUZUKAKE, C. ISHIKAWA, H. ASAKURA & H. UMEZAWA: Biochemical studies on bactobolin in relation to actinobolin. J. Antibiotics 34: 465~468, 1981
9) ISHIZUKA, M.; S. FUKASAWA, T. MASUDA, J. SATO, N. KANBAYASHI, T. TAKEUCHI & H. UMEZAWA: Antitumor effect of bactobolin and its influence on mouse immune system and hematopoietic cells. J. Antibiotics 33:

1054～1062, 1980

10) TABIRA, T.; Y. DA-LIN, T. YAMAMURA & T. AOYAGI: Prophylactic and therapeutic effect of bactobolin on autoimmune encephalomyelitis. Proc. Jpn. Acad. 63, Ser. B: 127～130, 1987

11) YOSHIOKA, M.; H. NAKAI & M. OHNO: Stereocontrolled total synthesis of (+)-actinobolin by an intramolecular Diels-Alder reaction of a chiral Z diene derived from L-threonine. J. Am. Chem. Soc. 106: 1133～1135, 1984

12) ASKIN, D.; C. ANGST & S. DANISHEFSKY: A total synthesis of *N*-acetylactinobolamine. J. Org. Chem. 50: 5005～5007, 1985

13) KOZIKOWSKI, A. P.; T. R. NIEDUZAK, T. KONOIKE & J. P. SPRINGER: π-Facial selection in intermolecular Diels-Alder reactions: total syntheses of (+)-actinobolin and (+)-5,6,10-triepi-actinobolin. J. Am. Chem. Soc. 109: 5167～5175, 1987

14) GARIGIPATI, R. S.; D. M. TSCHAEN & S. M. WEINREB: Total synthesis of (+)-actinobolin. J. Am. Chem. Soc. 107: 7790～7792, 1985

15) RAHMAN, M. A. & B. FRASER-REID: Actinobolin *via* the anomeric effect. J. Am. Chem. Soc. 107: 5576～5578, 1985

16) GARIGIPATI, R. S. & S. M. WEINREB: Stereoselective total synthesis of the antitumor antibiotic (−)-bactobolin. J. Org. Chem. 53: 4143～4145, 1988

17) GARIGIPATI, R. S.; D. M. TSCHAEN & S. M. WEINREB: Stereoselective total syntheses of the antitumor antibiotics (+)-actinobolin and (−)-bactobolin from a common bridged lactone intermediate. J. Am. Chem. Soc. 112: 3475～3482, 1990

18) WARD, D. E. & B. F. KALLER: The diastereoselective synthesis of (+)-actinobolin from D-glucose. Tetrahedron Lett. 34: 407～410, 1993

19) WARD, D. E. & B. F. KALLER: Diastereoselective synthesis of actinobolin from D-glucose by application of a novel [3+3] annulation. J. Org. Chem. 59: 4230～4238, 1994

20) WARD, D. E.; Y. GAI & B. F. KALLER: Synthesis of (−)-bactobolin from D-glucose and from (+)-actinobolin. Tetrahedron Lett. 35: 3485～3488, 1994

21) WARD, D. E.; Y. GAI & B. F. KALLER: [3+3] annulation based on 6-endo-trig radical cyclization: regioselectivity and diastereoselectivity. J. Org. Chem. 60: 7830～7836, 1995

22) WARD, D. E.; Y. GAI & B. F. KALLER: Synthetic studies on actinobolin and bactobolin: synthesis of *N*-desalanyl-*N*-[2-(trimethylsilyl)ethanesulfonyl] derivatives from a common intermediate and attempted removal of the SES protecting groups. J. Org. Chem. 61: 5498～5505, 1996

23) MUNAKATA, T.: Bactobolins, antitumor antibiotics from *Pseudomonase*. II. Synthesis and biological activities of related compounds. Yakugaku Zasshi 101: 138～147, 1981

24) MUNAKATA, T. & T. OKUMOTO: Some structure-activity relationships for bactobolin analogs in the treatment of mouse leukemia P388. Chem. Pharm. Bull. 29: 891～894, 1981

25) NISHIMURA, Y.; S. KONDO & T. TAKEUCHI: Syntheses and activities of some bactobolin derivatives. J. Antibiotics 45: 735～741, 1992

26) FUKUDA, T.; C. KITADA & M. FUJINO: *p*-Tolylmethylsulphonyl: a new amino-protecting group in peptide synthesis. J. Chem. Soc., Chem. Commun.: 220, 1978