Resormycin, a Novel Herbicidal and Antifungal Antibiotic Produced by a Strain of *Streptomyces platensis*

II. Structure Elucidation of Resormycin

MASAYUKI IGARASHI, HIKARU NAKAMURA, HIROSHI NAGANAWA and TOMIO TAKEUCHI

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

(Received for publication July 25, 1997)

A novel herbicidal antibiotic, resormycin, was isolated from the culture broth of *Streptomyces platensis* MJ953-SF5.

The structure of resormycin is determined to be (2*Z*)-2-*N*-[2*N*-[(3*S*)-3,6-diaminoheptanoyl]-(2*S*)-3-hydroxyvalyl]amino-3-(4-chloro-3,5-dihydroxy)phenylpropenoic acid by spectroscopic analyses, degradation studies and X-ray crystallography.

Resormycin (**1**) is a novel tripeptide antibiotic, produced by *Streptomyces platensis* MJ953-SF5, which showed strong herbicidal activity against weeds and antifungal activity against some phytopathogenic fungi. The taxonomic study of the producing strain, fermentation, isolation and biological activity of **1** are reported in the preceding paper[1].

In this paper, we describe the physico-chemical properties, degradation studies and structural elucidation of **1**.

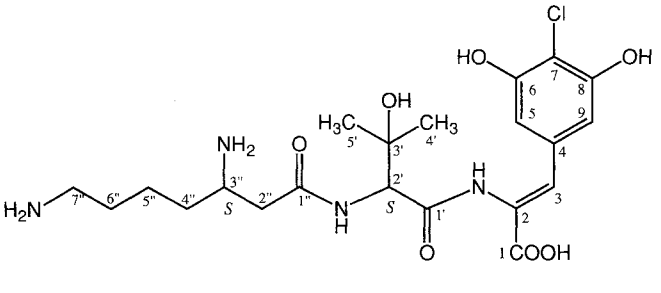
Results and Discussion

Structure Determination

The molecular formula of resormycin was established as $C_{21}H_{32}ClN_4O_9$ on the basis of HRFAB-MS and NMR spectral analysis. The IR (1649 cm^{-1} and 1585 cm^{-1}) and ^{13}C NMR spectral data (169.8 and 170.0 ppm) of **1** HCl salt suggested that **1** was a peptide antibiotic. In the UV spectrum, **1** HCl salt showed absorption maxima at 220 (16,500) and 286 nm (12,500) in MeOH and at 243 (16,300) and 310 nm (9,800) in MeOH-0.1 M NaOH. The UV spectra and positive color reaction with $FeCl_3$ suggested the presence of a phenolic group in the molecule. The physico-chemical properties of **1** HCl salt are summarized in Table 1.

The ^{13}C NMR spectral data of **1** HCl salt showed 19 signals including two overlapping signals at 108.7 and 154.1 ppm. All bond connections between ^1H and ^{13}C signals were interpreted by DEPT and heteronuclear multiple quantum coherence (HMQC) experiments. The DEPT and HMQC experiments revealed the presence of two methyl carbons, five methylene carbons, two methine

Fig. 1. Structure of resormycin (**1**).



(**1**)

Table 1. Physico-chemical properties of resormycin (**1**) HCl salt.

Molecular formula	$C_{21}H_{31}N_4O_7Cl$
FAB-MS (*m/z*)	487 $(M+H)^+$, 485 $(M-H)^-$
HRFAB Calcd:	487.1960
Found:	487.1954 $(M+H)^+$
$[\alpha]_D^{23}$	+146.8° (*c* 1, MeOH)
UV λ_{max}^{MeOH} nm (ε):	286 (12,500), 220 (16,500, sh)
MeOH-0.1 N HaOH:	310 (9,800), 243 (16,300)
IR ν_{max} (KBr) cm^{-1}	3400~3100, 1695, 1649, 1585, 1059, 1429, 1390, 1259, 1203, 1005
TLC[a]	0.63
HVPE (Rm)[b]	0.86
Color reaction	Ninhydrin, $FeCl_3$
positive	Rydon-Smith reagent

[a] The Rf values of **1** on silica gel TLC (Kieselgel 60 F_{254}, art 5715, Merck) developed with $CHCl_3$: MeOH : conc. NH_3OH : H_2O = 1 : 4 : 2 : 1).

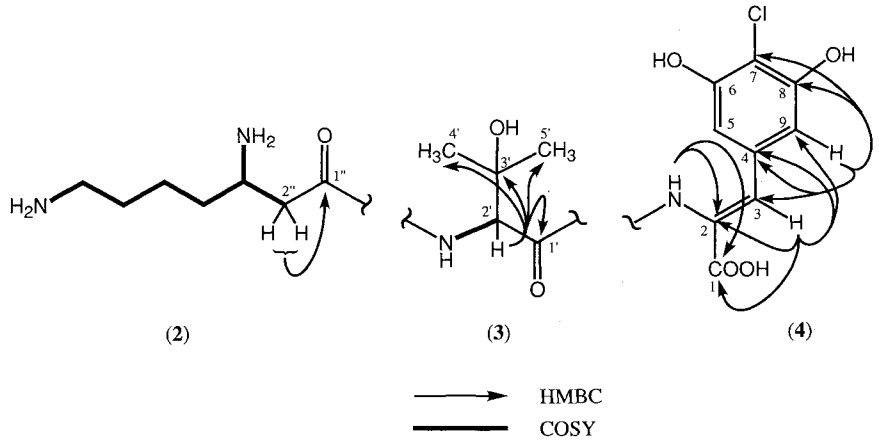
[b] High Voltage Paper Electrophoresis (HVPE), buffer (HCOOH : AcOH : H_2O = 1 : 3 : 36) Ref. alanine (Rm = 1.0).

Table 2. ^{13}C and ^{1}H NMR data of resormycin (**1**) HCl salt and **1** free base in DMSO-d_6.

Position	**1** HCl salt		**1** free base	
	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]
1	166.2 s		168.3 s	
2	126.8 s		134.3 s	
2-NH		9.42 (1H, br s)		
3	130.9 d	6.95 (1H, s)	124.7 d	6.83 (1H, s)
4	132.0 s		132.5 s	
5, 9	108.7 d	6.80 (2H, s)	107.7 d	6.55 (2H, s)
6, 8	154.1 s		153.9 s	
7	108.6 s		106.7 s	
1′	169.8 s		169.2 s	
2′	60.5 d	4.52 (1H, d, 9.0)	61.6 d	4.26 (1H, s)
2′-NH		8.54 (1H, br d, 9.0)		
3′	71.6 s		70.8 s	
4′	26.1 q	1.21 (3H, s)	26.1 q	1.19 (3H, s)
5′	27.2 q	1.20 (3H, s)	27.5 q	1.20 (3H, s)
1″	170.0 s		171.2 s	
2″	37.5 t	2.63 (1H, dd, 15.0, 3.0)	38.8 t	2.71 (2H, br t, 7.0)
		2.72 (1H, m)		
3″	48.0 t	3.38 (1H, m)	48.0 d	3.11 (1H, br dd, 5.0, 7.0)
3″-NH_3		8.16 (3H, br d, 2.5)		
4″	31.4 t	1.53 (1H, m)	34.8 t	1.48 (2H, m)
		1.58 (1H, m)		
5″	21.4 t	1.41 (2H, m)	21.8 t	1.37 (2H, m)
6″	26.4 t	1.53 (2H, m)	27.6 t	1.37 (2H, m)
7″	38.4 t	2.72 (1H, m)	41.1 t	2.39 (2H, br d, 5.0)
7″-NH_3		8.04 (3H, br t, 3.0)		

[a] 125 MHz, chemical shifts in ppm (multipulicity).
[b] 500 MHz, chemical shifts in ppm (integration, multipulicity, coupling constant in Hz).

Fig. 2. Partial structures in resormycin.



carbons, three olefinic methine carbons, one quaternary carbon, five olefinic quaternary carbons and three carbonyl carbons. The ^{1}H and ^{13}C NMR spectral data of **1** HCl salt and free base are shown in Table 2.

The ^{1}H-^{1}H COSY and HMBC spectra of **1** HCl salt suggested **1** consisted of three partial structures (**2**, **3** and **4**) as shown in Fig. 2. The partial structure for 3,6-diaminoheptanoyl residue (**2**) was depicted as follows: $NH_2CH_2CH_2CH_2CH_2CH(NH_2)CH_2$– by analysis of the ^{1}H-^{1}H COSY spectrum. In the HMBC spectrum, a methylene signal at δ_H 2.63 and 2.72 (H-2″) was coupled to a carbonyl carbon at δ_C 170.0 (C-1″).

The long-range ^{13}C-^{1}H couplings in 3-hydroxyvalyl residue (**3**) showed that a methine proton at δ_H 4.52 (2′-H) was coupled to a carbonyl carbon at δ_C 169.8 (C-1′), an oxygen bearing sp^3 quaternary carbon at δ_C 71.6 (C-3′)

Fig. 3. HMBC experiments of resormycin.

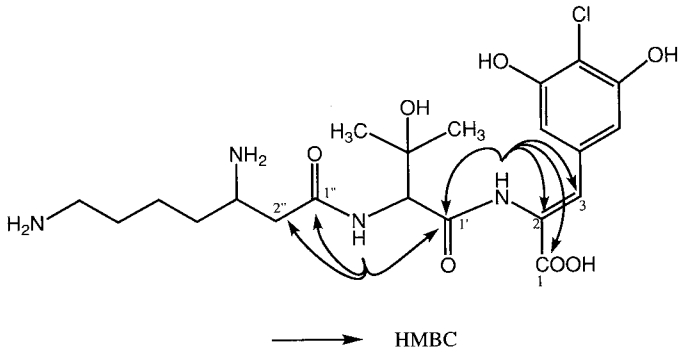


Fig. 4. NOE experiments of diacetyl-trimethyl-resormycin (7).

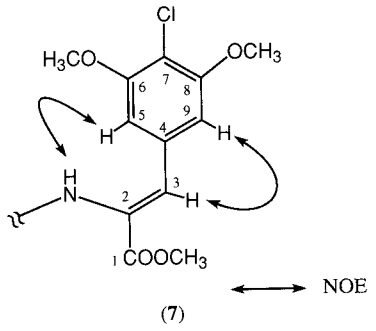
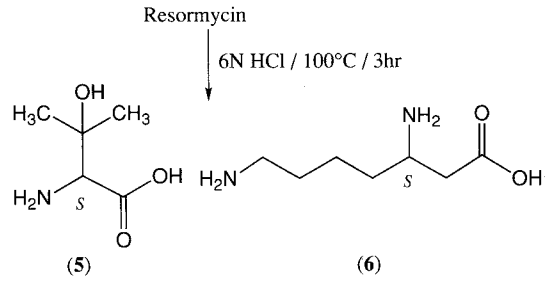


Fig. 5. Acid hydrolysis of resormycin.



and two methyl carbons at δ_C 26.1 (C-4′) and δ_C 27.2 (C-5′).

The long-range cross peaks in 4-chloro-3,5-dihydroxy phenylpropenoic acid residue (**4**) showed that an olefinic proton at δ_H 6.95 (3-H) was coupled to a carbonyl carbons at δ_C 166.2 (C-1), an olefinic carbon at δ_C 126.8 (C-2), an aromatic quaternary carbon at δ_C 132.0 (C-4) and two aromatic carbons at δ_C 108.7 (C-5 and C-9). The two overlapping singlet aromatic protons at δ_H 6.80 (5 and 9-H) were coupled to three quaternary aromatic carbons at δ_C 132.0 (C-4) and δ_C 154.1 (C-6 and 8).

An aromatic quarternary carbon (C-7) at δ_C 108.6 ppm was very close to the signals of C-5 and C-9 (δ_C 108.7) in the HCl salt form of **1**. Therefore, the HMBC spectrum was observed using the free base form. The HMBC spectrum of the free base form showed that the singlet signal at δ_H 6.55 (5 and 9-H) were coupled to the aromatic carbon at δ_C 106.7 ppm (C-7) bearing a chlorine atom.

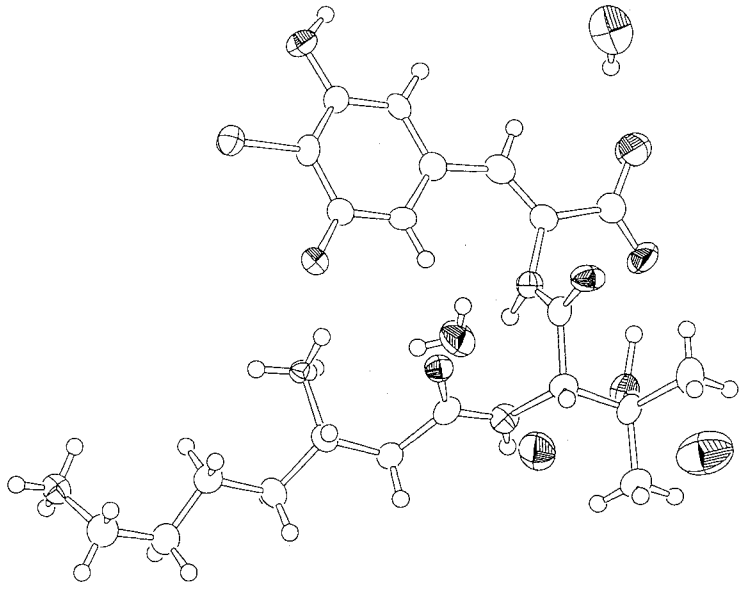
The connectivities among the three partial structures in **1** were done by HMBC experiment. An amide proton at δ_H 9.42 (2-NH) was coupled to two carbonyl carbons at δ_C 166.2 (C-1) and δ_C 169.8 (C-1′) and two olefinic carbons at δ_C 126.8 (C-2) and δ_C 130.9 (C-3). An amide proton at δ_H 8.54 (2′-NH) was coupled to two carbonyl carbons at δ_C 170.0 (C-1″) and δ_C 169.8 (C-1′). The result of HMBC experiment of **1** is summarized in Fig. 3. Thus, the planar structure of resormycin was elucidated to be 2-*N*-[2*N*-(3,6-diaminoheptanoyl)-3-hydroxyvalyl]amino-3-(4-chloro-3,5-dihydroxy) phenylpropenoic acid.

Stereochemistry of Resormycin

Geometry of phenylpropenoic acid residue of **1** was elucidated by the NOE experiment for resormycin derivative (**7**). As shown in Fig. 4, the NOE between the singlet amide proton at δ_H 9.79 (2-NH) and the singlet aromatic protons at δ_H 7.08 (5 and 9-H) indicated that the geometry of the phenylpropenoic acid residue was *Z*.

Acid hydrolysis of **1** with 6 M HCl at 100°C for 3 hours gave two amino acid components, **5** and **6** (Fig. 5). The structures of **5** and **6** were elucidated by the MS and NMR spectra to be 3-hydroxyvaline and 3,7-diaminoheptanoic acid, respectively. The optical rotations of **5** and **6** were dextro-rotatory: $[\alpha]_D^{23}$ +7.2° (*c* 0.98, 5 M HCl) and +26.2° (*c* 1.22, pH 2.0 H_2O). Therefore, both configurations of **5** and **6** were determined to be *S* by the comparisons with the optical rotation values of reference compounds in the literatures $[\alpha]_D^{22}$ +10.0° (*c* 2.0, 5 M HCl)[2] and $[\alpha]_D^{22}$ +29° (*c* 1, H_2O)[3].

Fig. 6. ORTEP drawing of resormycin.



Finally the absolute structure of **1** was confirmed by X-ray crystallographic analysis of the free base form. The ORTEP drawing of **1** is shown in Fig. 6.

From all the results described above, the structure of **1** was determined to be (2*Z*)-2-*N*-[2*N*-[(3*S*)-3,6-diaminoheptanoyl]-(2*S*)-3-hydroxyvalyl]amino-3-(4-chloro-3,5-dihydroxy)phenylpropenoic acid.

Experimental

General

Optical rotation was measured with a Perkin-Elmer model 241 polarimeter. UV spectra were recorded with a Hitachi 557 spectrophotometer. IR spectra were recorded with a Horiba FT-210 fourier transform infrared spectrometer. The ^1H and ^{13}C NMR spectra were measured with a JEOL JNM-A500 spectrometer at 24°C, using TMS ($\delta_H = 0$ ppm) in DMSO-d_6 solution, HOD ($\delta_H = 4.8$ ppm) as an internal reference and dioxane ($\delta_C = 67.4$) was used as an external reference in the case of D_2O solutions. The mass spectrum was recorded with a JEOL JMS-SX102 mass spectrometer.

Acid Hydrolysis of Resormycin

Acid hydrolysis of resormycin (140 mg, 0.29 mmol) was carried out with 6 M HCl at 100°C for 3 hours. The products in the reaction mixture showed the presence of an UV (254 nm)-quenched spot and two ninhydrin-positive spots attributed to **5** and **6** on a silica gel TLC (Kieselgel 60 F_{254}, art 5715, Merck) developing with $CHCl_3$ - MeOH - conc. NH_4OH - H_2O (1 : 4 : 2 : 1) as a solvent system. The Rf values of the three components, **5**, **6** and the UV-quenched spot were 0.81, 0.35 and 0.75, respectively. The reaction mixture of the acid hydrolysate was concentrated *in vacuo* to give a brownish oil. The residue was separated to a fraction of the quenched spot and a mixture of **5** and **6** by a Sephadex LH-20 column chromatography developing with 50% aqueous MeOH. The mixture of **5** and **6** were further purified by an Amberlite CG-50 (H^+, 5 ml) column, developing with distilled water and 1 M NH_4OH. Compound **5** was eluted with distilled water (20 ml). The fractions containing **5** were collected and concentrated *in vacuo* yielding **5** (23.3 mg, 61%) as an HCl salt. Compound **6** was eluted with 1 M NH_4OH. The fractions containing of **6** were collected, neutralized and concentrated *in vacuo*. The residue was neutralized with 1 M HCl and concentrated *in vacuo* yielding pure **6** (38.2 mg, 67%) as a HCl salt.

(*S*)-3-Hydroxyvaline (**5**)

FABMS *m/z* 134 $(M+H)^+$; $C_5H_{10}NO_3$; $[\alpha]_D^{23}$ +7.2 (*c* 0.98, 5 M HCl); ^1H NMR (500 MHz, D_2O): δ 1.25 (3H, s), 1.47 (3H, s), 3.62 (1H, s); ^{13}C NMR (125 MHz, D_2O): δ 24.0 d, 28.0 d, 64.1 d, 70.6 s, 172.9 s.

(*S*)-3,7-Diaminoheptanoic Acid (**6**)

FABMS *m/z* 161 $(M+H)^+$; $C_7H_{16}N_2O_2$; $[\alpha]_D^{23}$ +26.2° (*c* 1.22, pH 2.0·H_2O); ^1H NMR (500 MHz, D_2O): 3.48 (1H, m), 3.00 (2H, t), 2.70 (2H, m), 2.67 (2H, m), 2.54 (1H, dd, 16.0, 5.0), 2.43 (1H, dd, 16.0, 8.0), 1.47

(2H, m); ^{13}C NMR (125 MHz, D_2O): 22.5 t, 27.3 t, 32.7 t, 49.8 d, 39.8 s, 39.9 t, 179.1 s.

Preparation of Resormycin *N*-Diacetyl-dimethoxy-monomethyl Ester (7)

To a solution of 43.1 mg of **1** free base (0.089 mmol) in 50% aq dioxane (3 ml), 46 mg of di-*t*-butyl dicarbonate (0.2 mmol) and 25 mg triethylamine was added at room temperature, and the reaction mixture was stirred for 3 hours. The product in the reaction mixture was extracted with 50 ml of ethyl acetate. The ethyl acetate layer was separated and washed with 10 ml of 0.2 M citric acid. The organic layer was separated and concentrated to give 58.7 mg of *N*-diBoc-resormycin (yield 96 %). A solution of 51.7 mg of *N*-diBoc-resormycin (0.074 mmol) in methanol was treated with a large excess of diazomethane in diethyl ether at room temperature. The solution was concentrated and purified by silica gel column chromatography ($CHCl_3$: MeOH = 9 : 1) to give 46 mg of *N*-diBoc-trimethyl-resormycin (yield 62.5%). A 41.5 mg sample of *N*-diBoc-trimethyl-resormycin (0.056 mmol) was dissolved in 2 M HCl - 50% aq THF at room temperature and allowed to stand for 3 hours. The reaction mixture was adjusted to pH 7.0 with 1 M NaOH and concentrated *in vacuo*. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH : conc. NH_4OH : H_2O = 10 : 5 : 0.5 : 1). The fractions containing the product were collected and concentrated to give 19.9 mg of trimethylresormycin (yield 68%). To a solution of 10 mg of trimethylresormycin (0.019 mmol) in methanol (0.5 ml), 40 mg of acetic anhydride and 40 mg of triethylamine was added, and stirred for 22 hours at room temperature. The reaction mixture was extracted with 10 ml of ethyl acetate and the extract was washed with 2 ml of 0.2 M citric acid. The organic layer was collected and concentrated *in vacuo*, which was purified by preparative TLC (Kieselgel 60 F_{254}, art 5715, Merck) developing with $CHCl_3$ - MeOH (9 : 1, Rf 0.37) to give 7.0 mg of *N*-diacetyl-dimethoxy-monomethyl ester of resormycin (**7**, yield 61%). **7**; FABMS *m/z*: 613 $(M+H)^+$; $C_{28}H_{41}ClN_4O_9$; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.1 ~ 1.41 (6H, m), 1.18 (3H, s), 1.2 (3H, s), 1.75 (3H, s), 1.77 (3H, s), 2.24 (1H, dd, 14.0, 6.4), 2.34 (1H, dd, 14.0, 7.2), 2.97 (2H, dt, 6.0, 6.8), 3.70 (3H, s), 3.85 (6H, s), 4.02 (1H, br m), 4.67 (s, OH), 7.08 (2H, s), 7.12 (1H, s), 7.60 (1H, d, 9.0), 7.76 (1H, t, 6.0), 7.86 (1H, d, 9.0), 9.79 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 22.5 q, 22.6 q, 22.8 t, 26.2 q, 26.6 q, 29.6 t, 33.6 t, 38.4 t, 40.8 t, 46.0 d, 52.0 q, 57.9 q, 57.9 q, 60.2 d, 73.5 s, 106.5 (×2) d, 110.0 s, 126.6 s, 129.7 d, 132.6 s, 155.3 s, 155.3 s, 162.9 (×2) s, 165.3 s, 170.0 s, 170.1 s.

Free Base Form of **1**

A 105 mg sample of resormycin HCl salt was dissolved in H_2O (1 ml) and adjusted with 1 M NH_4OH to pH 9.0. The solution was concentrated *in vacuo* to give a 111.2 mg residue of the free base form of **1**. An aqueous solution (6 ml) of the above sample was allowed to stand for 24 hours at 5°C to give 34.5 mg of colorless prism crystals. MP: 183 ~ 190 (dec.)

X-Ray Crystallographic Analysis of **1**

A crystal of **1** with approximate dimensions of 0.03 × 0.03 × 0.25 mm was chosen for X-ray crystallography. All measurements were made on Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα radiation and a rotation anode generator. The crystal data are as follows: Empirical formula $C_{21}H_{31}N_4O_7Cl \cdot 4H_2O$; F.W. 559.01; Crystal system Monoclinic; Space group $P2_1$; Lattice parameters $a = 11.087(2)$ Å, $b = 7.687(4)$ Å, $c = 15.652(2)$ Å, $\beta = 95.09(1)°$, $V = 1328.7(7)$ Å^3; Z value 2. Dcalc 1.397 g/cm^3; μ(Cu-Kα) 18.37 cm^{-1}. Of the 3701 reflections which were collected, 2158 were unique. No decay correction was applied. An empirical absorption correction using the program DIFABS[4] was applied which resulted in transmission factors ranging from 0.67 to 1.00. The structure was solved by a direct method (SIR92)[5] and expanded using a Fourier technique (DIRDIF94)[6]. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 1607 observed reflections ($I > \sigma(I)$) and 333 variable parameters and converged with unweighted agreement factors of $R = 0.059$ and $Rw = 0.059$. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.40 and $-0.29e^-/Å^3$, respectively. Comparing $|Fo(hkl)|/|Fo(\bar{h}\bar{k}\bar{l})|$ and $|Fc(hkl)|/|Fc(\bar{h}\bar{k}\bar{l})|$ for 201 Bijvoetmates for which the difference $||Fc(hkl)| - |Fc(\bar{h}\bar{k}\bar{l})||/\sqrt{\delta^2 Fo(hkl) + \delta^2 Fo(\bar{h}\bar{k}\bar{l})}$ are larger than 0.5, 162 pairs showed consistently the absolute configuration in Fig. 6. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation.

References

1) IGARASHI, M.; N. KINOSHITA, T. IKEDA, M. KAMEDA, M. HAMADA & T. TAKEUCHI: Resormycin, a novel herbicidal and antifungal antibiotic produced by a strain of *Streptomyces platensis*. I. Taxonomy, production, isola-

tion and biological properties. J. Antibiotics 50: 1020～1025, 1997

2) ITO, Y.; Y. OHASHI, S. KAWABE, H. ABE & T. OKUDA: β-Hydroxy-L-valine, a constitutional amino acid of antibiotics YA-56 X and Y. J. Antibiotics 25: 360～361, 1972

3) KONDO, S.; H. IWASAWA, D. IKEDA, Y. UMEDA, Y. IKEDA, H. IINUMA & H. UMEZAWA: The total synthesis of spergualin, an antitumor antibiotic. J. Antibiotics 34: 1625～1627, 1981

4) WALKER, N. & D. STUART: An empirical absorption correction program. Acta Cryst. A39: 158～166, 1983

5) ALTOMARE, A.; M. C. BULARA, M. CAMALLI, M. CASCARANO, C. GIACOVAZZO, A. GUAGLIARDI & G. POLIDORI: SIR92- a program for automatic solution of crystal structures by direct methods. J. Appl. Crryst. 27: 435, 1994

6) BEURSKENS, P. T.; G. ADMIRAAL, G. BEURSKENS, W. P. BOSMAN, R. DE GELDER, R. ISRAEL & J. M. M. SMITS: The DIRDF-94 program system, Technical Report of the Crystallography Laboratory, University of Nijmegen, The Netherlands. 1994