VOL. 55 NO. 8, AUG. 2002 THE JOURNAL OF ANTIBIOTICS pp. 715 — 721

Argyrins, Immunosuppressive Cyclic Peptides from Myxobacteria

II. Structure Elucidation and Stereochemistry†

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(Received for publication March 11, 2002)

The structures of argyrins A~H were elucidated by NMR spectroscopy, chemical degradation and X-ray analysis as cyclic octapeptides. Argyrins A and B, in addition to the common amino acids tryptophan, glycine, dehydroalanine and alanine or α-aminobutyric acid, sarcosine, contain 2-(1-aminoethyl)thiazol-4-caboxylic acid and the novel amino acid 4′-methoxytryptophan. In argyrins C and D the latter is replaced by 4′-methoxy 2′-methyl-tryptophan. According to NMR analysis the solution and crystal conformations of argyrins A and B are identical in $CDCl_3$ and slightly different in acetone-d_6. Argyrins A and B are identical with the antibiotics A21459 A and B, whose structures are revised with respect to 4′-methoxytryptophan.

Screening for cytostatic compounds from myxobacteria led to the isolation of the argyrins, a group of cyclic peptides which were isolated from the culture broth of strains of the myxobacterium *Archangium gephyra*. The production, isolation and physico-chemical as well as biological characterization have been published elsewhere[1].

In this paper we describe the structure elucidation and absolute stereochemistry of the argyrins as obtained from NMR spectroscopy, degradation and X-ray analysis.

Methods

NMR Spectroscopy

The ^1H, ^{13}C and 2D NMR spectra of the argyrins A and B in $CDCl_3$ were recorded on a BRUKER Avance DMX-500 spectrometer, using an inverse triple resonance probe for ^1H and 2D spectra and a dual probe for the ^{13}C measurements.

^1H NMR spectra in acetone-d_6 were recorded on a Bruker AM 600 spectrometer. The H/D exchange rates of NH protons were determined with a 5 mg sample of argyrin A in 0.7 ml of acetone-d_6/D_2O 9 : 1 using the integral of the thiazole 3-H as internal reference. ^1H- and ^{13}C shifts are listed in Table 1.

X-Ray Crystallography

a) Structure Determination and Refinement

Crystals of argyrin B were grown from acetone solution by slow evaporation of the solvent. A Nonius CAD4 automatic diffractometer was used for data collection with Cu-Kα radiation and a graphite monochromator. The structure was solved by direct methods[2]. The parameters were refined by full-matrix least squares calculations[3] with anisotropic displacement parameters for all non-H atoms. A subsequent difference Fourier map showed 38 of 46 hydrogen atoms of the molecule. The positions of the remaining ones (all of side chains) were calculated

† Dedicated to Professor EKKEHARD WINTERFELDT on the occasion of his 70th birthday.

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Table 1. NMR data of argyrin A and B.

		Argyrin A [a]				Argyrin B [b]			
Res	Group	δ ^{13}C [c]	Δ ^{1}H [c]	Mult.[d]	J [Hz]	δ ^{13}C [c]	δ ^{1}H [c]	Mult.[d]	J [Hz]
Thiaz	NH		8.83	d	8.4		8.83	d	8.7
1	CO	159.8				159.8			
2	Cq	150.4				150.4			
3	CH	122.9	8.06	s		122.8	8.06	s	
4	Cq	170.8				170.8			
5	CH	45.3	5.49	m		45.2	5.48	m	
6	CH_3	20.3	1.75	d	7.1	21.1	1.72	d	7.1
Trp[1]	NH		8.58	d	7.2		8.60	d	7.1
1	CO	172.9				172.9			
2	CH	52.1	5.07	m		52.1	5.06	m	
3	CH_2	26.6	3.56	dd	3.2/15.2	26.6	3.55	dd	3.2/15.4
			2.85	dd	3.2/15.2		2.84	dd	3.2/15.4
1'	NH		10.68	s	broad		10.65	s	broad
2'	CH	125.5	6.97	d	2.6	125.5	6.96	d	2.2
3'	Cq	105.7				105.7			
4'	CH	115.9	5.40	d	7.9	115.9	5.40	d	7.9
5'	CH	119.2	6.34	t	7.3/7.9	119.2	6.34	t	7.6/7.9
6'	CH	121.2	6.89	t	8.1/7.3	121.2	6.90	t	8.1/7.6
7'	CH	111.3	7.06	d	8.1	111.4	7.05	d	8.1
8'	Cq	134.7				134.7			
9'	Cq	126.5				126.5			
Trp[2]	NH		8.77	d	1.8		8.81	s	
1	CO	170.0				170.0			
2	CH	57.7	4.22	m		57.8	4.20	m	
3	CH_2	26.9	3.50	dd	3.8/14.9	26.9	3.50	dd	overlap
			3.33	dd	3.9/14.9		3.32	dd	3.8/14.9
1'	NH		8.37	d	2.3		8.39	d	1.5
2'	CH	123.7	6.83	d	2.3	123.7	6.83	d	2.2
3'	Cq	108.3				108.3			
4'	Cq	152.3				152.3			
5'	CH	101.3	6.92	m		101.3	6.92	m	
6'	CH	123.6	7.34	m		123.6	7.34	m	
7'	CH	106.6	7.34	m		106.6	7.34	m	
8'	Cq	138.3				138.4			
9'	Cq	117.4				117.3			
4'-OCH_3		56.1	4.37	s		56.1	4.34	s	
Gly	NH		4.53	dd	5.3/7.8		4.54	dd	5.8/7.2
1	CO	171.0				171.4			
2	CH_2	40.0	3.50	dd	7.8/17.3	40.5	3.49	dd	overlap
			1.07	dd	5.2/17.3		1.03	dd	5.2/17.3
Ala	NH		6.86	d	6.5				
1	CO	169.8							
2	CH	48.4	4.24	m					
3	CH_3	13.9	1.44	d	7.0				
Abu	NH						6.81	d	6.6
1	CO					169.2			
2	CH					54.3	3.98	m	
3	CH_2					20.5	2.00	m	
							1.88	m	
4	CH_3					10.5	0.88	t	7.3
DeAla	NH		9.49	s			9.36	s	
1	CO	168.3				168.2			
2	Cq	136.8				136.9			
3	CH_2	99.7	5.02	d	1.5	99.4	5.00	d	1.3
			4.72	d	1.5		4.71	d	1.3
Sarc	N-CH_3	37.4	3.11	s		37.2	3.10	s	
1	CO	166.8				166.8			
2	CH_2	51.0	4.96	d	16.9	51.0	4.97	d	16.9
			3.40	d	16.9		3.40	d	16.9

a ^{1}H and ^{13}C: 3 mg/0.5 ml $CDCl_3$

b ^{1}H and ^{13}C: 4.8 mg/0.5 ml CDCl3

c ^{13}C shifts relative to $CDCl_3$: 77.0 ppm, ^{1}H shifts relative to $CHCl_3$: 7.26 ppm

d s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet

assuming normal geometry. Hydrogen atom parameters were idealized and not refined.

b) Determination of the Absolute Configuration

On the basis of the anomalous scattering of the S and O atoms the absolute configuration was determined using two different tests:

1. Hamilton R-factor[4]: Both enantiomers were refined separately. The enantiomer shown in the figures gave a final R-value of 0.066, whereas the parameters of the other enantiomer converged to an R-value of 0.070.

2. Flack parameter x[5]: The Flack parameter x was calculated for 1816 reflection pairs. It's value of 0.01_2 for the enantiomer shown supported the result of the Hamilton R-factor. The theoretical values are x=0 for the correct enantiomer and x=1 for the wrong one.

Chemical Degradation and Fragment Analysis

For the determination of amino acid building blocks 1 mg amounts of argyrins were dissolved in 0.1 ml 6 M hydrochloric acid and heated to 100°C for 12 hours. After evaporation to dryness a freshly prepared 4 : 1 mixture of ethanol and acetyl chloride was added, heated to 100°C for 30 minutes and evaporated to dryness. Then 100 μl of a 2 : 1 mixture of dichloromethane and trifluoracetic anhydride was added, heated to 100°C for 2 minutes and evaporated to dryness. The residue was dissolved in 100 μl of dichloromethane and analysed by GLC on a Shimadzu GC-17 A with FID detection. Columns: L-Chirasil-Val temperature gradient: 10 minutes 85°C, 6°C/minute to 190°C and Lipodex E gradient: 15 minutes 115°C, 15°C/minute to 190°C. (both 25 m×0.4 mm, Machery+Nagel); Injection port 230°C.

For ozonolysis, 1~5 mg amounts of argyrins were dissolved in 1 ml methanol and a stream of ozone was bubbled through the solution at either −70°C or room temperature for 10 minutes. After purging the system with oxygen for 5 minutes 0.2 ml 1 N NaOH and 20 μl 30% H_2O_2 were added and heated to 100°C for 5 minutes. Excess H_2O_2 was destroyed with Pt/C, the solvents were evaporated and the residue analysed by GC after hydrolysis and derivatisation as described above.

Results

NMR Spectra

$^1H,^1H$-COSY[6], $^1H,^1H$-ROESY[7,8], $^1H,^{13}C$-COSY[9] and long range $^1H,^{13}C$-COSY[10] spectra were used to elucidate the chemical constitutions of argyrins A and B. Argyrin A consists of tryptophan, 4′-methoxytryptophan, glycine, sarcosine, alanine, dehydro-alanine and 2-(1-aminoethyl)-thiazole-4-carboxylic acid ("alanine-thiazole"). In argyrin B alanine is replaced by α-aminobutyric acid. The amino acid sequence is based on sequential backbone NOE's like α_i–N_{i+1} and N_i–N_{i+1}, and on sequential HMBC correlations, as summarized in Table 2.

Table 2. Sequential NMR correlations in argyrin A and B.

NOE's		HMBC correlations	
Trp^1-H2	Trp^2-NH	Trp^1-NH	Thiaz-1, Trp^1-1
Trp^2-H2	Gly-NH	Trp^1-H2	Trp^1-1
Trp^2-NH	Gly-NH	Trp^2-NH	Trp^1-1, Trp^2-1
Gly-NH	Ala/Abu-NH	Trp^2-H2	Trp^2-1
Gly-H2	Ala/Abu-NH	Gly-H2	Trp^2-1, Gly-1
Ala/Abu-H2	DeAla-NH	Ala/Abu-NH	Gly-1
DeAla-NH	DeAla-H3	Ala/Abu-H2	Ala/Abu-1
DeAla-H3	Sarc-N-CH_3	Ala/Abu-H3	Ala/Abu-1
Sarc-N-CH_3	Sarc-H2	DeAla-NH	Ala/Abu-1, DeAla-1
Sarc-H2	Thiaz-NH	DeAla-H3	DeAla-1
		Sarc-N-CH_3	DeAla-1
		Sarc-H2	DeAla-1, Sarc-1
		Thiaz-NH	Sarc-1
		Thiaz-H5	Thiaz-4
		Thiaz-H3	Thiaz-2, Thiaz-4
		Thiaz-H6	Thiaz-4

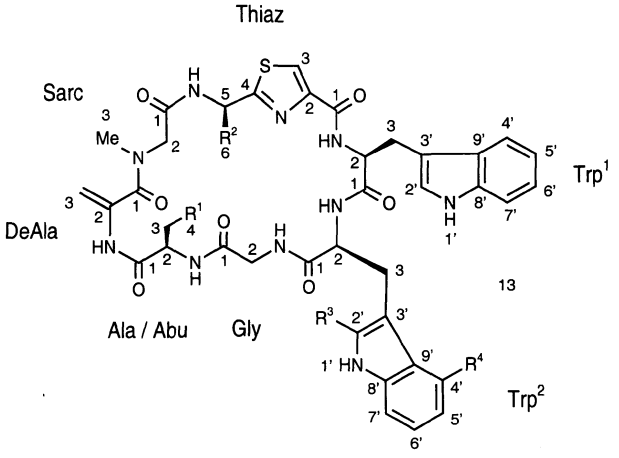
Chemical Degradation

Independently from NMR spectral data the amino acid building blocks of argyrins A and B were determined by total hydrolysis in combination with ozonolysis and gas chromatographic comparison with authentic samples. Both argyrins A and B gave glycine and sarcosine. In addition, argyrin A gave D-alanine, argyrin B D-α-aminobutyric acid. A late eluting double peak observed in both argyrins was tentatively assigned to racemic 2-(1-aminoethyl)-thiazole-4-carboxylic acid. Expectedly, dehydroalanine and the tryptophans were destroyed during hydrolysis.

Selective ozonolysis of argyrin A at −70°C followed by total hydrolysis yielded in addition to the amino acids observed before L-aspartic acid formed by degradation of tryptophan[11].

This proves that both tryptophans occur in the L-form. Similarly, ozonolysis of argyrin B at room temperature destroyed also the thiazole ring liberating the aminoethyl

Fig. 1. Chemical structure of the argyrins A~H.



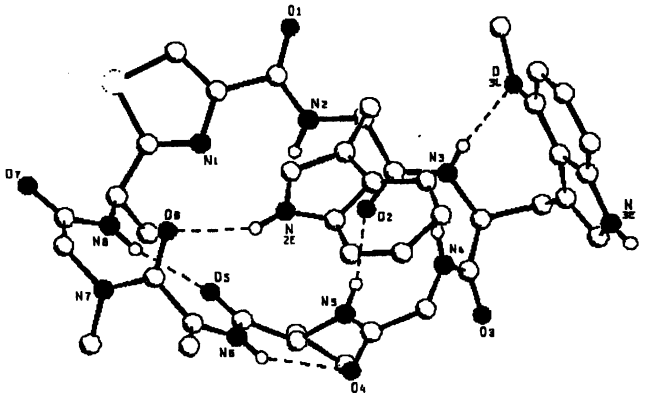
	R^1	R^2	R^3	R^4
Argyrin A	H	CH_3	H	OCH_3
B	CH_3	CH_3	H	OCH_3
C	H	CH_3	CH_3	OCH_3
D	CH_3	CH_3	CH_3	OCH_3
E	H	CH_3	H	H
F	H	CH_2OH	H	OCH_3
G	CH_3	CH_2OH	H	OCH_3
H	H	H	H	OCH_3

side chain as alanine. GC analysis gave additional signals for D- and L-alanine in the ratio of 3 : 1, whereas the late eluting double peak was absent. Thus, the partially racemised aminoethyl thiazole residue belongs to the D-series.

These results and the analysis of NMR spectra provide structure and absolute stereochemistry of argyrins A and B shown in Figure 1, which is in full agreement with the X-ray structure discussed below.

On the basis of their elemental composition and NMR spectra the argyrins C–H are close relatives of argyrins A and B. Thus, argyrins C and D are higher homologues with an additional methyl group (δ_H=2.12, δ_C=11.4) on C-2′ (δ_C 133.5) of Trp^2 whereas argyrin H is a lower homologue of argyrin A lacking the methyl group on C-5 of the thiazole amino acid. In the latter compound C-4 and C-5 are shifted to δ 167.6 and 40.2, the geminal protons attached to C-5 are observed at δ 4.27 (m) and 5.15 (dd, J=15.5, 8.5 Hz). Argyrin E lacks the Trp^2 methoxy group which causes the expected upfield shift of C-4′ (δ 119.2) and downfield shifts of C-7′, C-5′ and C-9′ (δ 112.4, 120.0 and 128.5). The corresponding protons are found at δ 7.51 (7′-H), 7.16 (6′-H), 7.03 (5′-H) and 7.48 (4′-H) showing the expected coupling patterns. Argyrins F and G are relatives of argyrins A and B containing a hydroxy group on C-6 of the thiazole amino acid. This shifts C-5 and C-6 to δ 53.1 and 64.4, the C-6 methylene protons are found as multiplets at δ 4.10 and 4.12.

Fig. 2. Intramolecular hydrogen bonds of argyrin B in the crystal.



Residues are numbered starting from thiazole-alanine as residue 1.

Crystal Structure of Argyrin B

The two tryptophans are found to be of L-configuration, the alanine and the α-aminobutyric acid of D configuration. Bond lengths agree within the limits of accuracy with expected values[12].

The overall conformation is stabilized by five intramolecular hydrogen bonds (Fig. 2), three of them within the macro-cyclic ring and two to the tryptophan side chains. Particularly remarkable is the hydrogen bond from the dehydroalanine N6H group to the carbonyl-oxygen atom 04 of the α-aminobutyric acid. A very similar H-bond could be observed in the solid states of cyclosporin A[13] and PSC833[14].

From the α-aminobutyric acid residue to the tryptophan amino acid a classical β-turn of type I is formed. The relevant torsion angles are listed in Table 4 together with their theoretical values[15].

The dihedral angle between the C1=O1 amide group and the thiazole ring is 2.5°. The hydrogen atoms of N2 and N4 have no hydrogen bond partners. The NH group of the 4′-methoxytryptophan (N3H) forms an intermolecular

Table 3. NOE's with distance ranges in comparison with the distances in the crystal structure.

NOE from / to [a]	Intensity	Range (Å)	Distance in crystal (Å)
Thiaz-NH / Trp^1-H5	w	3.5-4.5	4.3
Thiaz-NH / Ala-H3	w	3.5-4.5	4.3
Trp^1-NH / Ala-NH	m-w	2.5-4.5	3.7
Trp^1-NH / Trp^1-H2'	s	1.5-2.5	2.6
Trp^1-H2 / Trp^2-4'-OCH_3	m	2.5-3.5	2.94
Trp^1-H3b / Trp^2-4'-OCH_3	m-s	1.5-3.5	2.9
Trp^2-NH / Trp^1-H4'	m	2.5-3.5	3.4
Trp^2-NH / Gly-NH	s	1.5-2.5	2.6
Trp^2-NH / Trp^2-4'-OCH_3	s	1.5-2.5	3.1
Trp^2-NH / Trp^1-H3[b]	s	1.5-2.5	2.25
Gly-NH / Trp^1-H4'	s	1.5-2.5	2.7
Gly-NH / Ala/Abu-NH	s	1.5-2.5	2.7
Gly-H2b / Trp^1-H6'	m	2.5-3.5	3.1
Gly-H2b / Trp^1-H5'	w	3.5-4.5	3.3

[a] The upfield shifted protons of methylene groups are marked as 'b'.

Table 4. Observed dihedral angles in the β-I turn of argyrin B together with the theoretical values.[a]

	Observed angles	theoretical values
N5-C4-C4α-N4	3	0
C4-C4α-N4-C3	-81	-90
N4-C3-C3α-N3	-20	-30
C3-C3α-N3-C2	-67	-60

[a] Residue numbering is 2, tryptophan; 3, 4'-methoxy-tryptophan; 4, glycine; 5, α-amino-butyric acid.

hydrogen bond to O4 of a neighboring argyrin molecule.

Solution Conformation of the Argyrins

In $CDCl_3$ solution, the aromatic protons H-4′ and H-5′ of Trp^1 as well as the NH and one of the glycine C-2 protons (1.07 and 1.03 ppm in argyrin A and B, resp.) are strongly upfield shifted. While the latter experience shielding from the indole ring of Trp^1 situated above them, the former are shielded by the 4′-methoxyindole ring of Trp^2, which is clearly visible in the crystal structure (Figure 2).

A selection of long range NOE's extracted from the ROESY spectrum with a mixing time of 200 ms were classified by level counting and assigned to distances. Strong (s) NOE's with 5~8 levels correspond to the distance range of 1.5~2.5 Å, medium (m) NOE's with 3~5 levels correspond to the distance range of 2.5~3.5 Å and weak (w) NOE's with 1~3 levels correspond to the distance range of 3.5~4.5 Å. These distances correlate well with the distances in the crystal and are listed in Table 3.

A similar situation is observed in acetone-d_6 solution where the majority of signals in the ^1H NMR spectra of argyrins A and B show essentially the same or very similar chemical shifts as in $CDCl_3$. Only a few signals summarised in Table 5 are shifted significantly. In particular the reduced up-field shifts of glycine and Trp^1 protons indicate that in acetone Trp^1 and Trp^2 are slightly moved away from each other and the peptide backbone. According to the H/D exchange rate of amide protons also rearrangement of hydrogen bonds has occurred. We now find three

Table 5. Selected ^{1}H chemical shifts of argyrin A in $CDCl_3$ and acetone-d_6 ($\Delta\delta = \delta_{acetone}\ d_6 - \delta_{CDCl_3}$), and H/D exchange rates (half-life $\tau/2$)[a].

Residue	Proton δ (ppm)	$CDCl_3$ δ (ppm)	Aceton-d_6 (ppm)	Δδ (ppm)	τ/2 (min)
Thiaz	NH	8.71	8.33	-0.38	60
Trp[1]	NH	8.58	8.66	0.08	180
	3-Ha	2.85	3.07	0.22	-
	1'-NH	10.68	10.73	0.05	< 1
	5'-H	6.34	6.60	0.26	-
	4'-H	5.40	6.12	0.72	-
Trp[2]	NH	8.77	8.43	-0.34	< 1
	1'-NH	8.37	10.37	2.00	< 1
	6'-H	7.34	7.20	-0.14	-
Gly	2-Ha	1.07	1.70	0.63	-
	2-Hb	3.50	3.36	-0.14	-
	NH	4.53	5.39	0.86	< 1
Ala	NH	6.86	7.09	0.23	10
	3-H_3	1.44	1.27	-0.17	-
DeAla	NH	9.49	9.34	-0.15	< 1
Sarc	2-Ha	3.40	3.49	0.09	-
	2-Hb	4.96	4.66	-0.30	-

[a] in acetone-d_6/D_2O 9:1

slowly exchanging protons assigned to Ala-NH, Trp[1]-NH and Thiaz-NH (Table 5).

Whereas Ala-NH and Trp[1]-NH form hydrogen bonds to the Trp[1] and Ala carbonyls thus extending the β-turn to a β-hair-pin, the DeAla carbonyl is left to form a hydrogen bond to the Thiaz-NH. This moves the Sarc methyl group in an axial position flanked by the Ala and Thiaz methyl groups which is in perfect agreement with strong NOE's between the Sarc methyl and the neighbouring methyl groups.

Discussion

The experimental NMR data obtained from the argyrins A and B are identical to those reported for the two antibiotics A21459 A and B by FERRARI *et al.*[16]. We could show, however, that the methoxy group of Trp[2] residue is located in position 4', while it was originally suggested to contain a 5'-substituted indole moiety. Instead, substitution in position 4' could be unambiguously confirmed by correlations in the ROESY and HMBC spectra. Additionally, the ^{1}H spectrum in C_6D_6 solution reveals a first order spin system of H-5' (doublet), H-6' (triplet) and H-7' (doublet) whereas in $CDCl_3$ these aromatic protons form a higher order spectrum. We conclude that our structure of argyrins A and B with the 4'-methoxy-tryptophan moiety revise the antibiotics A21459A and B of FERRARI and coworkers.

The close similarity of the distances estimated from intensities of ROESY cross-peaks in $CDCl_3$ solution and those observed in the crystal structure indicates that both conformations in crystal and in $CDCl_3$ solution are identical. In acetone-d_6 solution the gross structure is retained, however, two hydrogen bonds are rearranged and the tryptophan side-chains are less densely packed.

From a biosynthetic point of view the argyrins are cyclic non-ribosomally derived octapeptides containing two

unusual tryptophan and thiazole derived amino acids. Of these 4′-methoxy-L-tryptophan found in argyrins A and B and in the minor components F, G and H is a novel natural amino acid. It is presumably formed from tryptophan by hydroxylation and *O*-methylation. An additional *C*-methylation in the electron-rich pyrrol ring leads to 4′-methoxy-2′-methyltryptophan found in argyrins C and D, the sole products of strain Ar 315. Most remarkably, zelkovamycin[17], a related cyclic peptide from a *Streptomyces* species, contains instead of 4′-methoxy a 7′-methoxy substituted tryptophan. In addition, both argyrin and zelkovamycin contain 2-(1-aminoethyl)-thiazole-4-carboxylic acid formed from a dipeptide fragment alanyl-cysteine by cyclisation and dehydrogenation[18]. This type of cyclisation of cysteine containing peptides is frequently observed in microbial and marine natural products, *e.g.* thiostrepton[19] and dolastatin 3[20]. According to this scheme the thiazoles found in argyrins F, G and H seem to be derived from seryl-cysteine and glycyl-cysteine. More probably, however, argyrins F and G are formed from argyrin A and B by hydroxylation at C-6. Argyrin F, by a retro-aldol like fragmentation, yields argyrin H and formaldehyde.

Based on the structures described above a total synthesis of argyrin B has been achieved recently[21].

References

1) SASSE, F.; H. STEINMETZ, T. SCHUPP, F. PETERSEN, K. MEMMERT, H. HOFMANN, C. HEUSSER, V. BRINKMANN, P. VON MATT, G. HÖFLE & H. REICHENBACH: Argyrins, immunosuppressive cyclic peptides from myxobacteria. I. Production, isolation, physico-chemical and biological properties. J. Antibiotics 55: 543~551, 2002
2) SHELDRICK, G. M.: SHELXS86. Program for the Solution of Crystal Structures, University of Göttingen, Germany, 1986
3) SHELDRICK, G. M.: SHELXL93. Program for the Refinement of Crystal Structures, University of Göttingen, Germany, 1993
4) HAMILTON, W. C.: Acta Cryst. 18: 502, 1965
5) FLACK, H. D.: On enantiomorph-polarity estimation. Acta Cryst. A39: 876, 1983
6) DEROME, A. & M. WILLIAMSON: Rapid pulsing artifacts in double-quantum-filtered COSY. J. Magn. Reson. 88: 177~185, 1990
7) BAX, A. & D. G. DAVIS: Practical aspects of two-dimensional transverse NOE spectroscopy. J. Magn. Reson. 63: 207~213, 1985
8) HWANG, T.-L. & A. J. SHAKA: Cross relaxation without TOCSY: Transverse rotating-frame overhauser effect spectroscopy. J. Am. Chem. Soc. 114: 3157~3159, 1992
9) BAX, A.; R. H. GRIFFEY & B. L. HAWKINS: Correlation of proton and nitrogen-15 chemical shifts by multiple quantum NMR. J. Magn. Reson. 55: 301~315, 1983
10) BAX, A. & M. F. SUMMERS: 1H and 13C assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. J. Am. Chem. Soc. 108: 2093, 1986
11) BAILEY, P. S., *In*, Ozonation in Organic chemistry, Vol. 2, p. 139, Academic Press, New York, 1982
12) ALLEN, F. H.; O. KENNARD, D. G. WATSON, L. BRAMMER & A. G. ORPAN: Tables of bond lengths. J. Chem. Soc. Perkin Trans. II: 1~19, 1987
13) LOOSLI, H. R.; H. KESSLER, H. OSCHKINAT, H. P. WEBER, T. J. PETCHER & A. WIDMER: The conformation of cyclosporin A in the crystal and in solution. Helv. Chim. Acta 68: 682~704, 1985
14) POHL, E.; R. HERBST-SCHIRMER, G. M. SHELDRICK, Z. DAUTER, K. S. WILSON, J. J. BÖLSTERLI, P. BOLLINGER, J. KALLEN & M. D. WALKINSHAW: Crystal structures of two modifications of (3,O-didehydro-MeBmt,1, Val2)-cyclosporin and comparison of three different X-ray data sets. Helv. Chim. Acta 78: 355~366, 1995
15) RICHARDSON, J. S.: The anatomy and taxonomy of protein structure. Adv. Protein Chem. 34: 167~339, 1981
16) FERRARI, P.; K. VEKEY, M. GALIMBERTI, G. G. GALLO, E. SELVA & L. F. ZERILLI: Antibiotics A21459 A and B, new inhibitors of bacterial protein synthesis, II. structure elucidation. J. Antibiotics 49: 150~154, 1996
17) TABATA, N.; H. TOMODA, H. ZHANG, R. UCCIDA & S. OMURA: Zelkovamycin, a new cyclic peptide antibiotic from *Streptomyces* sp. K96-0670. II. Structure elucidation. J. Antibiotics 52: 34~39, 1999
18) KONZ, D.; A. KLENS, K. SCHÖRGENDORFER & M. A. MARAHIEL: The bacitracin biosynthesis operon in *Bacillus lichemiformis* ATCC 10716: Molecular characterisation of the three muli-modular peptide syntheses. Chem. Biol. 4: 927~937, 1997
19) MOCEK, U.; Z. ZENG, D. O'HAGAN, P. ZHOU, L.-D.G. FAN, J. M. BEALE & H. G. FLOSS: Biosynthesis of the modified peptide antibiotic thiostrepton in *Streptomyces azureus* and *Streptomyces laurentii*. J. Am. Chem. Soc. 115: 7992~8001, 1993
20) PETTIT, G. R.; Y. KAMANO, C. W. HOLZAPFEL, W. J. VAN ZYL, A. A. TUINMAN, C. L. HERALD, L. BACZYNSKYJ & J. M. SCHMIDT: The structure and synthesis of dolastatin 3. J. Am. Chem. Soc. 109: 7581~7582, 1987
21) LEY, S. V.; A. PRIOUR & C. HEUSSER: Total synthesis of the cyclic heptapeptide argyrin B: A new potent inhibitor of T-cell independent antibody formation. Org. Lett. 4: 711~714, 2002