Communications to the Editor

NEW GLYCOPEPTIDE ANTIBIOTICS: II. THE ISOLATION AND STRUCTURES OF CHLOROORIENTICINS

Sir:

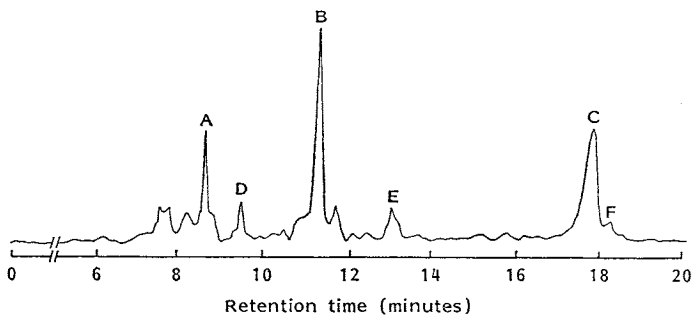
During screening studies to find new glycopeptide antibiotics, we elucidated the structure of orienticins[1] which have excellent antibacterial activity against methicillin-resistant *Staphylococcus aureus* equivalent to vancomycin. We isolated also the new vancomycin-type antibiotics, chloroorienticins, from the fermentation broth of *Amycolatopsis orientalis* (*Nocardia orientalis*)[2] PA-45052 which had been identified by Y. KAWAMURA and studied preliminarily by E. KONDO and his co-workers in Shionogi Research Laboratories. Some of them possessed antibacterial activity more excellent than that of vancomycin. In this communication paper, we report the isolation of chloroorienticins and their structures. The screening, fermentation and biological properties will be reported elsewhere.

The chloroorienticin complex including A, B, C, D and E were separated by MCI gel CHP20P (Mitsubishi Chemical Industries Limited) column and Packed column RQ-2 (C_{18}, Fuji gel) chromatography according to Scheme 1. The structures of these molecules were elucidated by ^1H, ^{13}C NMR and mass spectroscopies and were confirmed by the chemical transformations or degradations. Physico-chemical properties of the molecules are listed in Table 1†.

Chloroorienticin A (**1**) is very similar to orienticin A (**6**)[1] on comparison of the ^1H and ^{13}C NMR spectra involving asparagine, *N*-methylleucine, glucose and two 4-*epi*-vancosamine units. With regard to Cl substitution to the aromatic ring, chloroorienticin A (**1**) has two positions, A-3 and C-5 (A-3; δ_C 126.7 (s), C-5; δ_C 127.2 (s)), like vancomycin (**8**) (A-3; δ_C 126.3 (s), C-5; δ_C 127.2 (s)), but orienticin A (**6**) does not have the Cl at C-5 (C-5; δ_C 122.9 (d), δ_H 7.12 (dd, J=8.4 and 2.2 Hz))[1]. Based on the data, the structure **1** shown in Fig. 1 was deduced. To confirm the structure, including the absolute structure, chloroorienticin A (**1**) was transformed to orienticin A (**6**) by selective hydrogenolysis at C-5[1,3] and the aglycone of chloroorienticin A (**1**) was identified with that of vancomycin (**9**) by hydrolysis, and the sugar parts, D-glucose and L-4-*epi*-vancosamine[1], were also identified. Thus, the structure of chloroorienticin A (**1**) was elucidated.

Chloroorienticin B (**2**), one of the major components, lacks one of the 4-*epi*-vancosamine untis

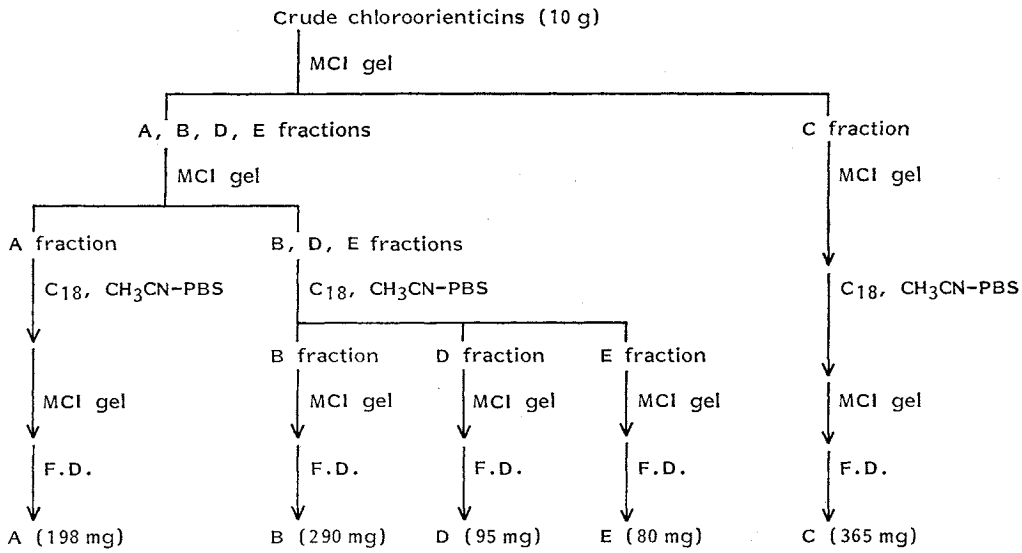
Chart 1. HPLC analysis of chloroorienticins.



Sample: Crude chloroorienticins. Column: Cosmosil 5Ph (4.6×150 mm). Mobile phase: (a) 7% CH_3CN - 0.05 M phosphate buffer solution (pH 3.5), (b) 40% CH_3CN - 0.05 M phosphate buffer solution (pH 3.5), gradient from 100% (a) to 100% (b) over 30 minutes, 1 ml/minute. Detection: 220 nm.

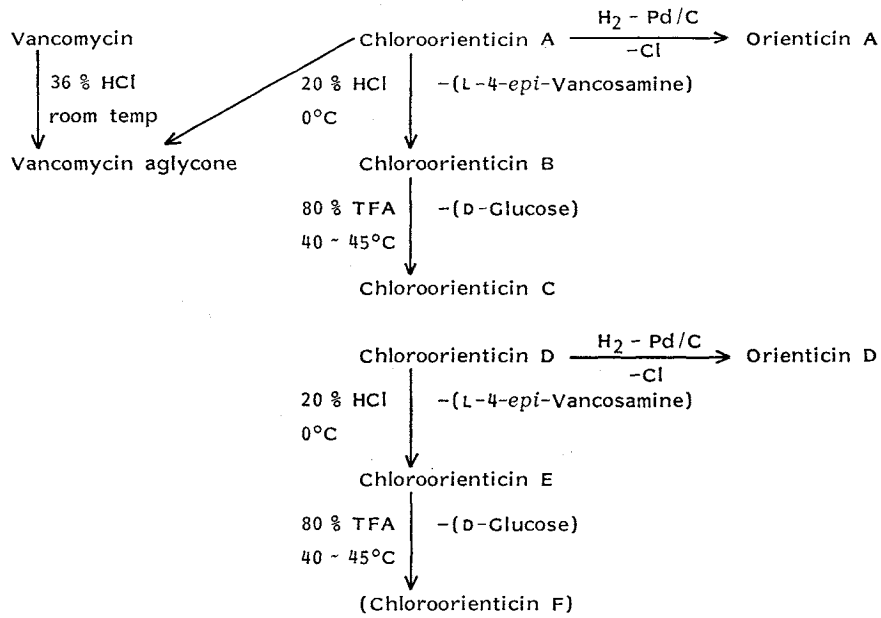
† According to the referee's opinion on the previous paper[1], tables listing the NMR signals and assignments of analogues were omitted from the paper on account of space consideration. In this report also, the related key NMR signals are selectively shown in text.

Scheme 1. Isolation of chloroorienticins.



PBS: Phosphate buffer solution.
F.D.: Freeze-drying.
MCI gel: High porous polymer (Mitsubishi Chemical Industries Limited).

Scheme 2. Chemical transformation of chloroorienticins.



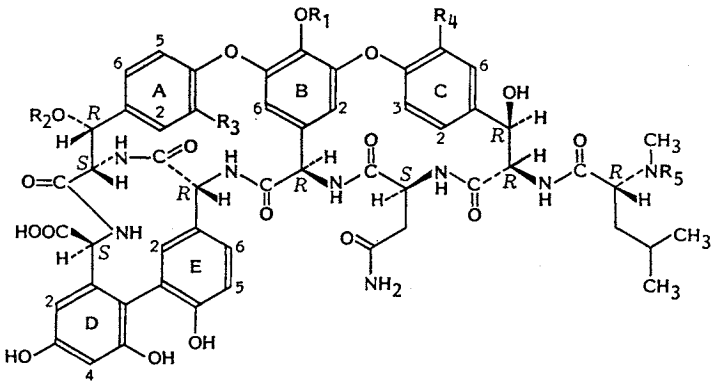
from chloroorienticin A (**1**) according to comparison of its ^{1}H, ^{13}C NMR and mass spectra, but **2** has another one connecting to A-1′ (anomeric; δ_C 93.9 (d), δ_H 4.67 (d like, J=4.2 Hz), A-1′; δ_e 74.2 (d))[1]. Chloroorienticin B (**2**) and L-4-*epi*-vancosamine were identified from the products of the partial hydrolysis of chloroorienticin A (**1**) by 20% HCl at 0°C.

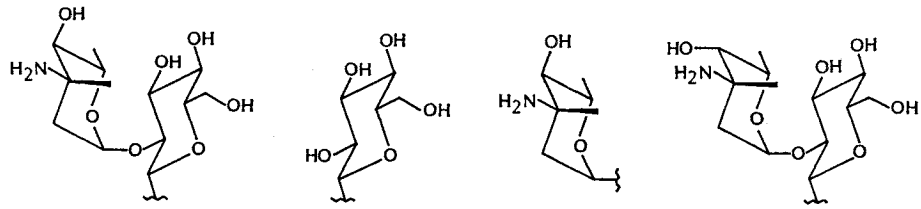
Chloroorienticin C (**3**), a major component, lacks D-glucose from chloroorienticin B (**2**) and

Table 1. Physico-chemical data on chloroorienticins.

	A (1)		B (2)		C (3)		D (4)		E (5)	
$[\alpha]_D$ (H_2O)	−87.2±2.5°		−67.3±2.1°		−59.9±1.9°		−86.1±2.5°		−71.2±2.1°	
	(*c* 0.52)		(*c* 0.51)		(*c* 0.52)		(*c* 0.50)		(*c* 0.52)	
Elemental analysis	$C_{73}H_{88}N_{10}O_{26}Cl_2 \cdot 1\frac{1}{2}HCl \cdot 8H_2O$:		$C_{66}H_{75}N_9O_{24}Cl_2 \cdot HCl \cdot 5H_2O$:		$C_{60}H_{85}N_9O_{19}Cl_2 \cdot 2HCl \cdot 6H_2O$:		$C_{74}H_{90}N_{10}O_{26}Cl_2 \cdot 2HCl \cdot 10H_2O$:		$C_{67}H_{77}N_9O_{24}Cl_2 \cdot 2HCl \cdot 8H_2O$:	
	Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found
C	48.95,	49.01	50.21,	50.38	49.09,	49.14	47.80,	47.62	47.89,	47.79
H	5.94,	5.82	5.68,	5.60	5.42,	5.49	6.07,	6.00	5.70,	5.46
N	7.82,	7.98	7.98,	8.13	8.59,	8.76	7.53,	7.50	7.50,	7.80
Cl	6.93,	7.29	6.74,	6.75	9.66,	9.88	7.63,	7.53	8.44,	8.47
SI-MS (*m/z*, $(M+H)^+$)	1,591		1,448		1,286		1,605		1,462	
UV λ_{max}^{HCl} nm (ε)	281.0 (5,800)		281.2 (5,900)		279.6 (6,000)		280.7 (5,500)		289.0 (5,700)	
λ_{max}^{NaOH} nm (ε)	301.8 (6,400)		302.0 (6,800)		296.4 (11,000)		301.8 (6,300)		302.5 (6,900)	

Fig. 1. Structures of chloroorienticins and related analogues.





X1 X2 X3 X4

	R_1	R_2	R_3	R_4	R_5
Chloroorienticin A (**1**)	X1	X3	Cl	Cl	H
Chloroorienticin B (**2**)	X2	X3	Cl	Cl	H
Chloroorienticin C (**3**)	H	X3	Cl	Cl	H
Chloroorienticin D (**4**)	X1	X3	Cl	Cl	CH_3
Chloroorienticin E (**5**)	X2	X3	Cl	Cl	CH_3
Orienticin A (**6**)	X1	X3	Cl	H	H
Orienticin D (**7**)	X1	X3	Cl	H	CH_3
Vancomycin (**8**)	X4	H	Cl	Cl	H
Vancomycin aglycone (**9**)	H	H	Cl	Cl	H

Table 2. *In vitro* antibacterial activities of chloroorienticins (MIC, μg/ml).

	Chloroorienticin					Orienticin A	Vancomycin
	A	B	C	D	E		
Staphylococcus aureus JC-1	0.2	0.39	0.2	0.2	0.39	0.39	0.78
S. aureus 3131 (methicillin-resistant)	0.39	0.39	0.39	0.39	0.78	0.78	1.58

was converted from chloroorienticin B (**2**) by hydrolysis.

Chloroorienticins D (**4**) and E (**5**), isolated as minor components, were similar to chloroorienticins A (**1**) and B (**2**), respectively, but ^1H and ^{13}C NMR spectra showed that the *N*-methylleucine part (NCH_3 of **1** and **2**; δ_C 33.9 (q) and 33.9 (q), δ_H 2.31 (3H, s) and 2.32 (3H, s)) was replaced by *N*-dimethylleucine ($N(CH_3)_2$ of **4** and **5**; δ_C 41.7 (q) and 41.5 (q), δ_H 2.30 (6H, s) and 2.33 (6H, s)). Confirmation came from hydrogenolysis[1,3] of chloroorienticin D (**4**) to orienticin D (**7**) and the partial hydrolysis product of chloroorienticin D (**4**) being transformed to chloroorienticin E (**5**). In addition, the hydrolysis of chloroorienticin E (**5**) gave chloro-

orienticin F according to HPLC but could not be isolated because of its small amount. The conditions used in the chemical reactions are shown in Scheme 2.

The NMR data offered information on the glycoside bond also. When δ value of glucose-C2 (δ_C 77.0, δ_H 3.67) of chloroorienticin A (**1**) is compared with that (δ_C 74.6, δ_H 3.445) of the hydrolysate (*i.e.* **2**), we can recognize the clear shift ascribable to glycosylation or deglycosylation. The fact indicated one of the 4-*epi*-vancosamine units was connected to the glucose-C2. The $^1J_{C,H}$ values (170~172 Hz) of anomeric carbons indicated that the 4-*epi*-vancosamine units had an α-glycoside bond. The H-H coupling feature (d like, J=4.0~4.2 Hz) of anomeric protons also supported the α-bond of the units. While, a β-glycoside bond at anomeric position of the glucose unit was conclusive from the ^1H data of anomeric proton (*e.g.* **1**; δ_H 5.67 (d, J=7.5 Hz)). From the comparison of δ value of B-4 carbon of the molecules having glucose unit **1**, **2**, **4** or **5**) δ_C 132.4~132.9) with that of the molecule losing the glucose unit **3** (δ_C 128.9) it was concluded that the glucose was connected to the B-4. Thus, the type and position of glycoside bond were clarified.

We isolated the new vancomycin-type glycopeptide chloroorienticins A (**1**), B (**2**), C (**3**), D (**4**) and E (**5**) by elucidating the structures by ^1H ^{13}C and secondary ion (SI)-MS. Moreover, the correlation of chloroorienticin A (**1**) with orienticin A (**6**) and vancomycin (**8**) and of chloroorienticin D (**4**) with orienticin D (**7**) established their structures completely. Their antibacterial activities† are equal to or stronger than those of orienticin A and vancomycin (Table 2).

Acknowledgment

The authors are indebted to Mr. K. MATSUMOTO (Shionogi & Co., Ltd.) for the supply of the crude chloroorienticins and to Dr. Y. NAKAGAWA (Shionogi & Co., Ltd.) and his co-workers for measurement of the mass spectra. Thanks are also due to Dr. E. KONDO (Shionogi & Co., Ltd.) for the information about the preliminary studies.

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(Received April 5, 1988)

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† Dr. Y. KOMATSU of this laboratory provided this information.