NEW GLYCOPEPTIDE ANTIBIOTICS I. THE STRUCTURES OF ORIENTICINS†

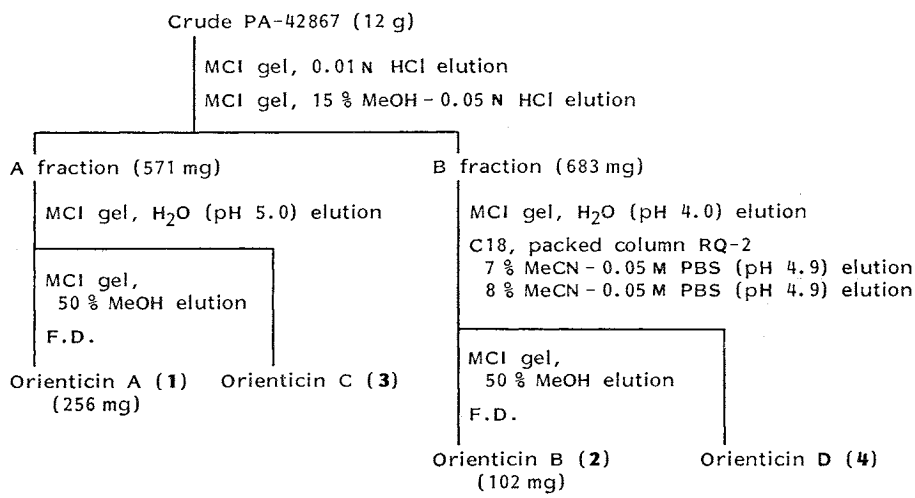
Sir:

Glycopeptide antibiotics have been attracting much interest owing to their activity against methicillin-resistant *Staphylococcus aureus*. Many new glycopeptides have been reported[1], but only vancomycin (**5**)[2] has been used clinically. It has excellent antibacterial activity but some problems remain with its intravenous administration, the most important being the side-effect of the so-called red man's syndrome and the presence of undesirable minor components. Here we report the structures of newly isolated vancomycin-type antibiotics and their derivatives.

The glycopeptides, orienticins, were found by Y. KAWAMURA in the metabolites of a microorganism identified as *Nocardia orientalis* PA-42867 and suggested to be new glycopeptides by E. KONDO and his co-workers from preliminary chromatographic experiments. The details, including the fermentation procedure, will be published elsewhere.

Orienticins are composed of mainly two components and can be easily separated into orienticins A and B according to Scheme 1. Comparison of the ^{1}H NMR†† (in DMSO-d_6, DMSO-d_6+D_2O; at 60°C, 80°C, 100°C) with that of vancomycin (**5**) gave valuable information; orienticin A (**1**) has an aglycone composed of *N*-methylleucine and asparagine as vancomycin (**5**) but has two amino sugars (anomeric (C-1): δ_H 4.675 (d like, J=4.3 Hz) and 5.305 (d, J=4.3 Hz), δ_C 93.7 (d) and 96.4 (d)) which should be epimeric to vancosamine at C-4 (C-4; δ_H 2.89 (d, J=9.4 Hz) and 2.95 (d, J=9.6 Hz), δ_C 78.1 (d) and 78.1 (d)). Furthermore, orienticin A (**1**) lacks Cl substitution at the *C*-aromatic ring (C-5; δ_H 7.12 (dd, J=8.4 and 2.2 Hz), δ_C 122.9 (d)). ^{13}C NMR supported the above assumption, and the location of the second amino sugar at the A-1′ carbon (A-1′; δ_C 74.1 (d)) was deduced from the fact that the A-1′ carbon proved by the long-range coupling with the A-2 and A-6 protons (A-2; δ_H 7.86 (d, J=2.0 Hz), A-6; δ_H 7.33 (dd, J=8.4 and 2.0 Hz)) exhibits the chemical shift considerably lower field than that of vancomycin (A-1′; δ_C 71.5 (d)). The gross structure (**1**) was established by precise examina-

Scheme 1. Isolation of orienticins.

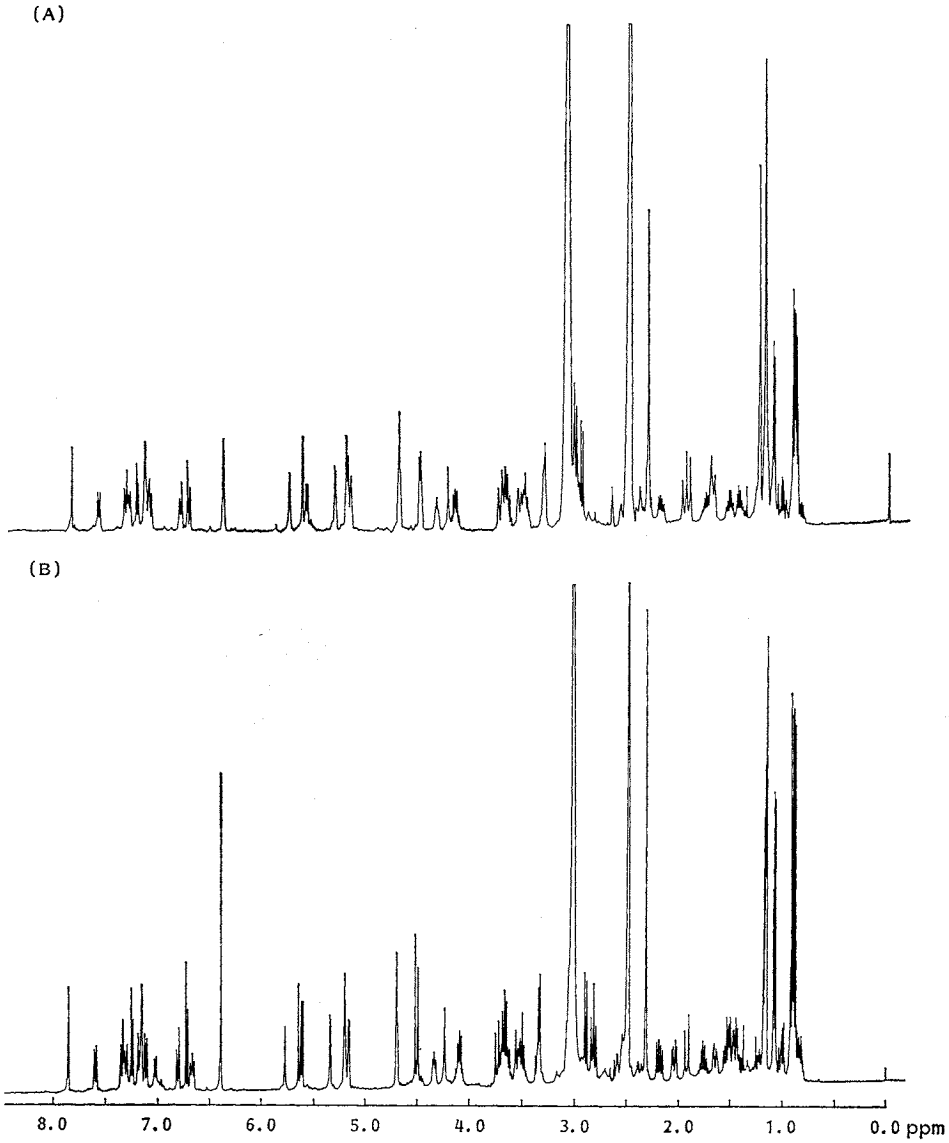


MCI gel: High porous polymer
Mitsubishi Chemical Industries Limited.
PBS: Phosphate buffer solution.
F.D.: Freeze-drying.

† Orienticins are originally designated as PA-42867 A, B, C and D (Jpn. Kokai 174099 ('87), July 30, 1987).

†† NMR spectra were taken on a Varian XL-400 at 400 MHz for ^{1}H and at 100 MHz for ^{13}C.

Fig. 1. ^1H NMR spectra of orienticins A (A) and B (B) (DMSO-d_6+D_2O at 100°C).



tion of the decoupled NMR spectra.

Orienticin B (**2**) has the same amino sugar (anomeric; δ_C 93.5 (d)) at the A-1′ carbon (A-1′; δ_C 74.1 (d)) but has a neutral sugar linked to glucose. The structure was concluded to be (**2**) based on NMR. Secondary ion mass spectroscopy (SI-MS) in accordance with the above structures gave satisfactory (M+H): A; ($C_{73}H_{89}N_{10}O_{26}Cl$+H) 1,557, B; ($C_{72}H_{86}N_9O_{27}Cl$+H) 1,544.

It has been reported[3] that acid hydrolysis of vancomycin (**5**) is accompanied by rearrangement of the aglycone at the asparagine moiety to isoaspartate. Preliminary experiments using HPLC showed that lower temperatures allowed the acid hydrolysis of sugar moieties to proceed without this rearrangement. The selective cleavage of sugars is shown in Scheme 2.

The two amino sugars, which proved to be identical, were isolated as a syrup and purified as methyl 4-*epi*-vancosamine diacetate (α-anomer and β-anomer, respectively). The structure was determined by NMR (including nuclear Overhauser effect (NOE)) and comparison of

Scheme 2. Acid treatment of orienticins A and B.

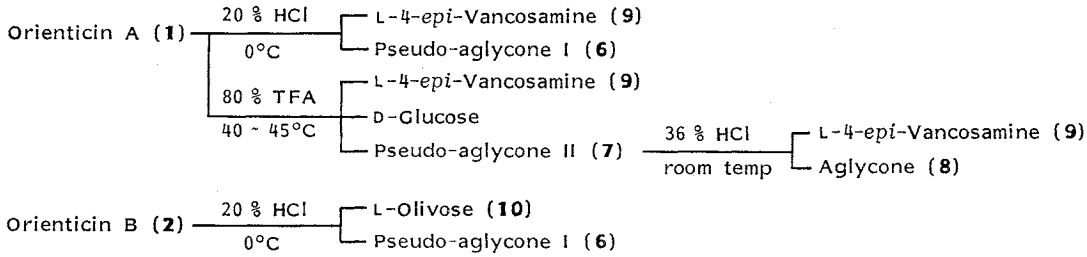
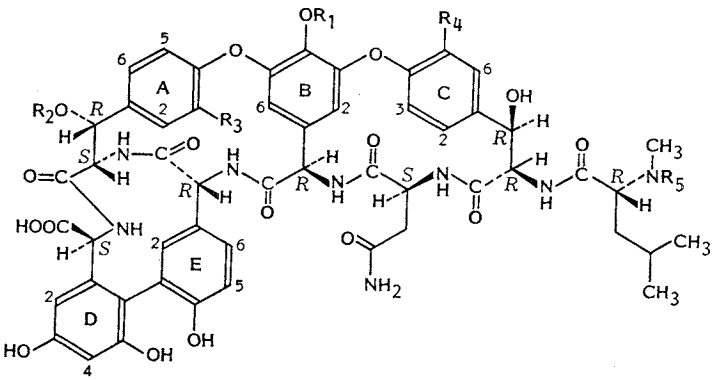
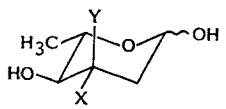


Fig. 2. Structures of orienticins and vancomycin.



	R_1	R_2	R_3	R_4	R_5
Orienticin A (1)	(Glc)-(4-*epi*-VCN)	(4-*epi*-VCN)	Cl	H	H
Orienticin B (2)	(Glc)-(olivose)	(4-*epi*-VCN)	Cl	H	H
Orienticin C (3)	(Glc)-(4-*epi*-VCN)	(4-*epi*-VCN)	H	H	H
Orienticin D (4)	(Glc)-(4-*epi*-VCN)	(4-*epi*-VCN)	Cl	H	CH_3
Vancomycin (5)	(Glc)-(VCN)	H	Cl	Cl	H
Pseudo-aglycone I (6)	(Glc)	(4-*epi*-VCN)	Cl	H	H
Pseudo-aglycone II (7)	H	(4-*epi*-VCN)	Cl	H	H
Aglycone (8)	H	H	Cl	H	H

Glc: Glucose, VCN: vancosamine.



(4-*epi*-VCN) (9) X=NH_2 Y=CH_3
(Olivose) (10) X=OH Y=H

Table 1. *In vitro* antibacterial activities of orienticins and vancomycin (MIC, μg/ml).

	Orienticin A (1)	Orienticin B (2)	Orienticin C (3)	Orienticin D (4)	Pseudo-aglycone I (6)	Pseudo-aglycone II (7)	Vancomycin (5)
Staphylococcus aureus JC-1	0.39	1.56	0.39	0.78	0.78	0.39	0.78
S. aureus 3131 (methicillin-resistant)	0.78	1.56	1.56	1.56	1.56	0.78	1.56

$[\alpha]_D^{24}$ values with literature data[4]. Glucose was also isolated and determined to be D-glucose by optical rotation. The neutral sugar from orienticin B (2) proved to be L-olivose which has the opposite configuration to D-olivose obtained from olivomycin B†.

Since orienticin A (1) gave a CD curve similar to that of vancomycin (5), the configurations of

† Olivomycin B was kindly supplied by Dr. M. KOENUMA of this laboratory.

both aglycones were thought to be the same. This was confirmed by hydrogenation of the aglycone of vancomycin on Pd-C[5] in the presence of acetic acid. The mono dehalogenated product was identical with the aglycone of orienticin A in all respects. The additional minor components C and D were isolated by K. MATSUMOTO and his colleague. The structures of these compounds were easily deduced to be (**3**) (A-3; δ_H 6.96 (dd, J=8.4 and 2.5 Hz), δ_C 122.4 (d)) and (**4**) (Leu-N(CH_3)$_2$; δ_H 2.32 (s, 6 protons), δ_C 41.6 (q)), respectively, by NMR and SI-MS.

The catalytic hydrogenolysis of orienticin A (**1**), we found, gave orienticin C (**3**) as was expected. The antibacterial activity† of orienticins and their derivatives are summarized in Table 1. Other details, including information on the *in vivo* activity will be published elsewhere.

After completion of this work, eremomycin[6] having properties very similar to those of orienticin A was reported without disclosing the chemical structure.

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