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

Sir:
During screening studies to find new glyco-
peptide antibiotics, we elucidated the structure
of orienticins\(^1\) which have excellent antibacterial
activity against methicillin-resistant \textit{Staphylococ-
cus aureus} equivalent to vancomycin. We iso-
lated also the new vancomycin-type antibiotics,
chloroorienticins, from the fermentation broth
of \textit{Amycolatopsis orientalis} (\textit{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 anti-
bacterial 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) col-
umn and Packed column RQ-2 (C\(_18\), Fuji gel)
chromatography according to Scheme 1. The
structures of these molecules were elucidated by
\(^1\)H, \(^13\)C NMR and mass spectroscopies and were
confirmed by the chemical transformations or
degradations. Physico-chemical properties of
the molecules are listed in Table I.

Chloroorienticin A (1) is very similar to
orienticin A (6)\(^1\) on comparison of the \(^1\)H and
\(^13\)C NMR spectra involving asparagine, N-
methyleucine, glucose and two 4-\textit{epi}-vanco-
samine units. With regard to Cl substitution
to the aromatic ring, chloroorienticin A (1) has
two positions, A-3 and C-5 (A-3; \(\delta_0\) 126.7 (s),
C-5; \(\delta_0\) 127.2 (s)), like vancomycin (8) (A-3;
\(\delta_0\) 126.3 (s), C-5; \(\delta_0\) 127.2 (s)), but orienticin A
(6) does not have the Cl at C-5 (C-5; \(\delta_0\) 122.9
(d), \(\delta_H\) 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\(^1\) 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-\textit{epi}-vancosamine\(^1\), were
also identified. Thus, the structure of chloro-
orienticin A (1) was elucidated.

Chloroorienticin B (2), one of the major com-
ponents, lacks one of the 4-\textit{epi}-vancosamine units

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\(^1\) 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.
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; $\delta_0$ 93.9 (d), $\delta_6$ 4.67 (d like, $J=4.2$ Hz), A-1'; $\delta_6$ 74.2 (d))$^3$. 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.

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<tr>
<th></th>
<th>A (1)</th>
<th>B (2)</th>
<th>C (3)</th>
<th>D (4)</th>
<th>E (5)</th>
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<tr>
<td>([\alpha]_D) (H2O)</td>
<td>-87.2±2.5° (c 0.52)</td>
<td>-67.3±2.1° (c 0.51)</td>
<td>-59.9±1.9° (c 0.52)</td>
<td>-86.1±2.5° (c 0.50)</td>
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<td>Elemental analysis</td>
<td>C73H88N10O26Cl2 *</td>
<td>C66H75N9O24Cl2 *</td>
<td>C60H65N9O19Cl2 *</td>
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<tr>
<td></td>
<td>1/2HCl·8H2O:</td>
<td>HCl·5H2O:</td>
<td>2HCl·6H2O:</td>
<td>2HCl·10H2O:</td>
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<tr>
<td>C</td>
<td>48.95, 49.01</td>
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<td>49.09, 49.14</td>
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<td>5.94, 5.82</td>
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<td>6.07, 6.00</td>
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<td>H</td>
<td>7.82, 7.98</td>
<td>7.98, 8.13</td>
<td>8.59, 8.76</td>
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<tr>
<td>N</td>
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<td>9.66, 9.88</td>
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<td>Cl</td>
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<td>UV (\lambda_{max}) nm ((\varepsilon))</td>
<td>281.0 (5,800)</td>
<td>281.2 (5,900)</td>
<td>279.6 (6,000)</td>
<td>280.7 (5,500)</td>
<td>289.0 (5,700)</td>
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<td></td>
<td>301.8 (6,400)</td>
<td>302.0 (6,800)</td>
<td>296.4 (11,000)</td>
<td>301.8 (6,300)</td>
<td>302.5 (6,900)</td>
</tr>
</tbody>
</table>
Fig. 1. Structures of chloroorienticins and related analogues.

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Table 2. In vitro antibacterial activities of chloroorienticins (MIC, \(\mu g/ml\)).

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<td>H</td>
<td>H</td>
<td>H</td>
<td>Cl</td>
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</tr>
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</table>

was converted from chloroorientin 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 \(^1\)H and \(^{13}\)C NMR spectra showed that the N-methylleucine part (NCH\(_3\) of 1 and 2; \(\delta_H 33.9 \text{ (q)}\) and 33.9 \(\text{ (q)}\), \(\delta_N 2.31 \text{ (3H, s)}\) and 2.32 \(\text{ (3H, s)}\)) was replaced by N-dimethylleucine (N(CH\(_2\))\(_2\)) of 4 and 5; \(\delta_H 41.7 \text{ (q)}\) and 41.5 \(\text{ (q)}\), \(\delta_N 2.30 \text{ (6H, s)}\) and 2.33 \(\text{ (6H, s)}\)). Confirmation came from hydrogenolysis\(^1\) of chloroorientin D (4) to orienticin D (7) and the partial hydrolysis product of chloroorientin 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 (δC 77.0, δH 3.67) of chloroorienticin A (1) is compared with that (δC 74.6, δH 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 1JCH values (170 ÷ 172 Hz) of anomeric carbons indicated that the 4-epi-vancosamine units had an α-glycoside bond. The H-H coupling feature (δ 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; δH 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 δC 132.4 ÷ 132.9) with that of the molecule losing the glucose unit 3 (δC 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 13C 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 activities1 are equal to or stronger than those of orienticin A and vancomycin (Table 2).

Acknowledgment

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Naoki Tsuji
Toshiyuki Kamigauchi
Masaaki Kobayashi
Yoshhiro Terui
Shionogi Research Laboratories,
Shionogi & Co., Ltd.,
Fukushima-ku, Osaka 553, Japan

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References


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