# CHLOROPOLYSPORINS A, B AND C, NOVEL GLYCOPEPTIDE ANTIBIOTICS FROM *FAENIA INTERJECTA* SP. NOV.

## III. STRUCTURE ELUCIDATION OF CHLOROPOLYSPORINS

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Structure elucidations of chloropolysporins A, B and C were achieved mainly by chemical degradation studies. These components possessed the same pseudoaglycone in common and their structures were closely related to that of $\beta$-avoparcin. The structures of chloropolysporins differ from that of $\beta$-avoparcin in the presence of vancomycinic acid moiety instead of monodechlorovancomycinic acid moiety and glucose, not ristosaminylglucose, in its side chain. Chloropolysporin C was identified as derhamnosylchloropolysporin B based on its $^1$H NMR and mass spectral analysis and degradation studies. Two minor components, chloropolysporins D and E, were identified as the epimers of each of chloropolysporins B and C, respectively, based on their Cotton effects and retention times on reverse phase HPLC.

Chloropolysporins A, B and C are new members of the glycopeptide antibiotics produced by *Faenia interjecta* sp. nov. SANK 60983. Taxonomy of the producing organism[1], fermentation, isolation and physico-chemical characterization[2] of chloropolysporins were reported in the preceding papers. It was also found that each component possessed the same constituents of the amino acids[2]. Partial deglycosylation[3] and biological properties[4] of chloropolysporins will be reported in the succeeding papers.

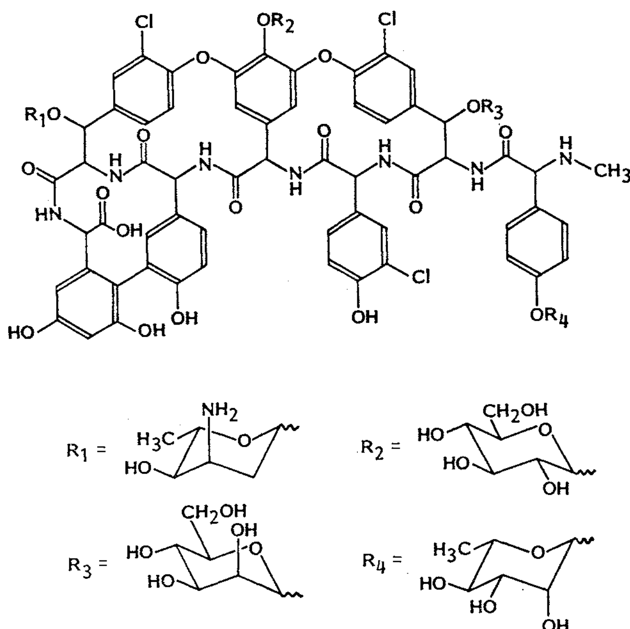
This report deals with the structural elucidations of chloropolysporins, mainly A, B and C.

### Structural Elucidation of Chloropolysporin B

Chloropolysporin B (**1**) is an amphoteric, slightly brownish powder possessing the following physico-chemical properties: Molecular formula $C_{83}H_{89}O_{34}N_8Cl_3$ (MW 1,846), UV $\lambda_{max}^{0.01N\text{-}HCl}$ ($E_{1cm}^{1\%}$) 280 nm (51), $[\alpha]_D^{25}-64.5°$ (*c* 1.04, 0.1 N HCl) and IR $\nu_{max}^{KBr}$ cm$^{-1}$ 3300 (OH, NH), 1650 and 1560 (NHCO). The $^1$H NMR spectrum of **1** taken in DMSO-$d_6$ exhibited highly complicated signals but suggested the presence of an *N*-methyl group ($\delta$ 2.18, s) and two methyl groups ($\delta$ 1.08, br d and 1.22, br d). These properties are characteristic for glycopeptide antibiotics. The structural studies of **1** were achieved mainly by the following chemical degradation studies.

Acid hydrolysis of **1** (concentrate HCl - AcOH, 1 : 1, 105°C, 20 hours) followed by separation on reverse phase HPLC gave three unusual amino acids, actinoidic acid (**2**), 3-chloro-4-hydroxyphenylglycine (**3**) and *N*-methyl-*p*-hydroxyphenylglycine (**4**), which were previously reported as the constituents of $\beta$-avoparcin by McGahren *et al.*[5], and reductive acid hydrolysis of **1** (HI/P, 105°C, 20 hours[6]), fol-

Fig. 1. Structure of chloropolysporin B (**1**).
Chloropolysporin C $R_4$=H.

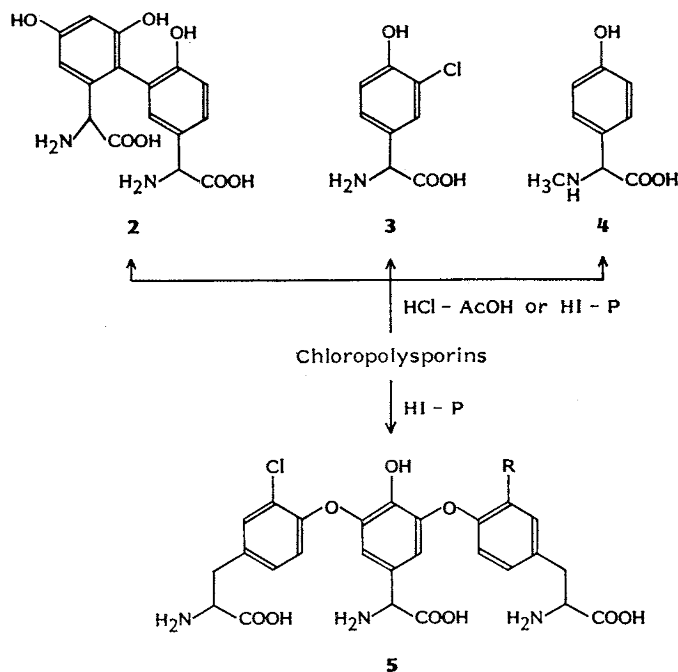



lowed by isolation on the HPLC system as described in the experimental section, yielded four amino acids. Three of them were identified as compounds **2**, **3** and **4**, respectively, and the remaining one was identified as dideoxyvancomycinic acid (**5**)[7]. These results were summarized in Fig. 2. It is well-known that allylic positions of the vancomycinic acid moiety, which is one of the characteristic components in glycopeptide antibiotics, are easily deoxygenated under reductive condition[5,6]. Therefore, it could be deduced that the compound **5** was the reductive hydrolysis product from vancomycinic acid moiety. In addition, actinoidic acid is also one of the typical amino acids in this family of antibiotics[8]. These results suggested that the aglycone of chloropolysporins was closely related to that of $\beta$-avoparcin[5], but the former contained vancomycinic acid and the latter monodechlorovancomycinic acid and thus the structure of aglycone of **1** was elucidated as shown in Fig. 1.

In order to determine the sugar moieties in **1**, degradation of **1** was carried out with 5% (w/w) HCl in absolute MeOH at 105°C for 16 hours followed by trimethylsilylation with *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) in pyridine. Gas chromatographic (GC) analysis of the hydrolysate indicated the presence of glucose, mannose and rhamnose as an equimolar mixture, and their configurations were determined to be D, D and L, respectively, by the measurements of optical rotation of each sugar derivative as described in the experimental section. An amino sugar, ristosamine, was isolated as methylristosaminide from mild acid hydrolysate of **1** (5% HCl in absolute MeOH, 105°C, 5 hours). Its acetylated ($Ac_2O$ - pyridine) compound, methyl-*N*,*O*-diacetylristosaminide, was found to have L configuration[9].

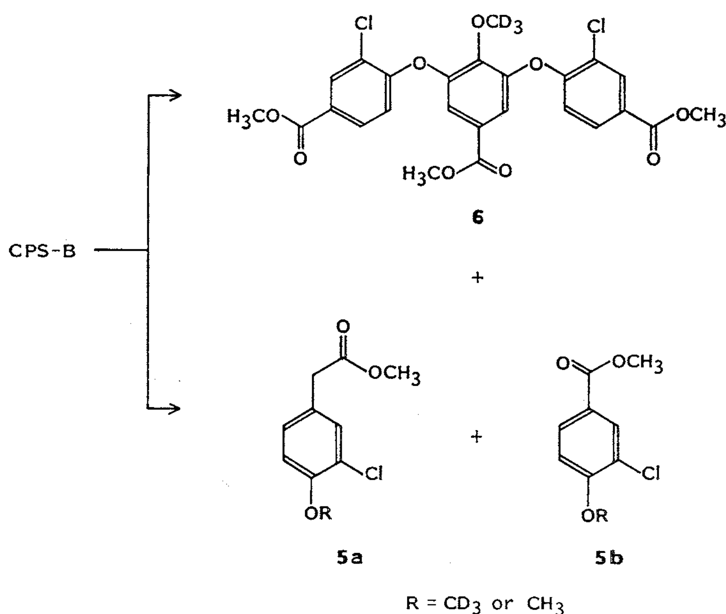
To decide the linkage position of these sugars to the aglycone moiety, Hakomori permethylation of **1** was carried out[5,10]. Permethylated glucose, mannose and rhamnose were isolated from the acid hydrolysate (5% HCl in absolute MeOH) of permethylchloropolysporin B. This result clearly indicated that these neutral sugars attached as monosaccharide at their C-1 position. On the contrary, in the case of $\beta$-avoparcin, one more ristosamine bound at the 2′-position of glucose forms the disac-

Fig. 2. Degradation of chloropolysporins.
**2**: Actinoidic acid, **3**: 3-chloro-4-hydroxyphenylglycine, **4**: *N*-methyl-*p*-hydroxyphenylglycine, **5**: dideoxyvancomycinic acid R=Cl; monodechlorodideoxyvancomycinic acid R=H.




charide, 2′-ristosaminylglucose side chain[5]. It is well-known that the sugars which bind to aliphatic hydroxyl residues are slowly hydrolyzed with acid, whereas the sugars which bind to phenolic group are easily hydrolyzed[11]. In the case of chloropolysporin B, glucose and rhamnose were easily released, but mannose and ristosamine were not. These phenomena were very similar to the observation in the structural studies on $\beta$-avoparcin by McGahren *et al.*[5]. Ristosamine and mannose, therefore, might attach to the positions at $R_1$ and $R_3$ in the aglycone, respectively. Determination of the attachment sites of the remaining rhamnose and glucose to the aglycone was done as follows: First, Edman degradation of **1** was carried out with $\beta$-avoparcin as a control[5]. The only one common product obtained was the compound containing rhamnose. In the case of $\beta$-avoparcin, the binding site of rhamnose in this compound was reported to be at the phenolic position of the *N*-terminal amino acid residue, *N*-methyl-*p*-hydroxyphenylglycine[5]. Therefore, the rhamnose moiety in **1** was determined to be also located at the same position as $\beta$-avoparcin, $R_4$, as shown in Fig. 1. Second, elucidation of the binding site of glucose in **1** was performed by the differential methylation technique which was used in the structural studies on avoparcins by McGahren *et al.*[5] and ristocetin A by Sztaricskai *et al.*[10]. The first methylation step of **1** was done with $CH_3I$ - $K_2CO_3$ in absolute MeOH. After removal of the sugars by mild acid hydrolysis (1 N HCl, 95°C, 1 hour), the second methylation was carried out under the same conditions except $CD_3I$ - $K_2CO_3$ was used instead of $CH_3I$ - $K_2CO_3$. The reaction mixture was hydrolyzed with KOH - $NaBH_4$ in water to yield differentially methylated amino acids. The products were oxidized in sequence with NaOCl and $KMnO_4$ under acidic condition. They were finally methylated with $CH_2N_2$ and separated by a preparative TLC on silica gel to afford three compounds as shown in Fig. 3. Both compounds **5a** and **5b** were the mixture of $CD_3$ and $CH_3$ derivatives (in each approx-

Fig. 3. Differential methylation of chloropolysporin B (CPS-B).




imate 6 : 4), which might be derived from the 3-chloro-4-hydroxyphenylglycine moiety. But the remaining compound **6** was the completely deutero-methylated material derived from the vancomycinic acid moiety of **1**. This result suggested that the phenolic position of the vancomycinic acid moiety took part in the glycosyl bond linkage, *i.e.* glucose was attached to this $R_2$ position. Thus the structure of chloropolysporin B was elucidated as shown in Fig. 1.

## Structural Elucidations of Chloropolysporins A, C, D and E

As described in the preceding paper[2], acid hydrolysates of chloropolysporins A, B and C gave the same amino acids, **2**, **3**, **4** and **5**, and chloropolysporins A and B had glucose, mannose, rhamnose and ristosamine but chloropolysporin C gave the same sugar components except for the absence of rhamnose. Moreover chloropolysporin C and enzymatically prepared derhamnosylchloropolysporin B[3] showed the same retention time on analytical reverse phase HPLC. Chloropolysporins A, B and C gave the identical pseudoaglycone on mild acid hydrolysis as described in the preceding paper[3]. $^1$H NMR and fast atom bombardment mass spectral (FAB-MS) analyses of chloropolysporin C as well as its physicochemical properties straightforwardly revealed its identity with derhamnosylchloropolysporin B. Although the structure of chloropolysporin A is not decided yet, it is clear that it has the same aglycone as that of the other two components, B and C, but possesses one mol of galactose in addition to the sugar moieties found in chloropolysporin B.

Two minor components, chloropolysporins D and E were identified as the epimers of components B and C, respectively, as follows: ELLESTAD *et al.*[12] reported that avoparcin components were easily epimerized by heating under neutral or alkaline condition, and these epimers gave the opposite sign of the peak at 232 nm in the CD spectrum. The epimers of chloropolysporins B and C prepared by heating at 70°C for 17 hours in water exhibited the same phenomenon, and these epimers showed negative Cotton effects at 237 nm while the parent compounds B and C had positive effects at 230 nm. The

retention times of these epimers in a reverse phase HPLC were identical with those of chloropolysporins D and E, respectively.

## Experimental

### General

$^1$H and $^{13}$C NMR spectra were measured with Jeol FX-90Q or GX-270 spectrometer. 1,4-Dioxane was used as an internal standard for $^{13}$C NMR spectrum taken in $D_2O$. Tetramethylsilane was used as an internal standard for $^1$H and $^{13}$C NMR in $CDCl_3$ and DMSO-$d_6$ and as external standard for $^1$H NMR in $D_2O$. IR spectra were determined on Jasco IRA-302 spectrometer employing KBr pellets. UV spectra were recorded with Hitachi 124 spectrophotometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. CD spectra were recorded with Jasco J-500 spectrometer. Low resolution, high resolution and GC electron impact mass spectra (EI-MS) were obtained on Jeol JMS D-300 mass spectrometer (70 eV). FAB-MS were obtained on Jeol JMS HX-100 mass spectrometer. HPLC were performed with Hitachi 655A-11 system. GC were done by a Shimadzu GC7AG system with 2% OV17 column.

### Drastic Acid Hydrolysis of **1** for Preparing Amino Acids as Constituents

A 200 mg sample of **1** was hydrolyzed with 5 ml of conc HCl - AcOH (1 : 1) in five sealed ampoules for 20 hours at 105°C. Reductive acid hydrolysis of **1** (60 mg) was done with 5 ml of 55% HI containing red P (60 mg) in five sealed ampoules for 20 hours at 105°C. After the hydrolysates were concentrated to dryness *in vacuo*, the residue were separated by a preparative HPLC system (YMC-Pack S-343 I15 ODS) with 5% aq acetonitrile containing 0.4% TFA. Their TMS derivatives with BSTFA in pyridine were used for the analyses by GC-MS. Their recoveries, $^1$H NMR signals in $D_2O$ and the characteristic fragment ion peaks in MS were as follows. Actinoidic acid (**2**; 26 mg, a mixture of two diastereoisomers): $^1$H NMR $\delta$ 5.00 (1H, s), 5.04 (1H, s), 6.48 (1H, d, $J$=2.0 Hz), 6.50 (1H, br s), 6.51 (2H, d, $J$=2.0 Hz), 7.00 (1H, d, $J$=8.3 Hz), 7.02 (1H, d, $J$=8.3 Hz), 7.15 (1H, d, $J$=2.0 Hz), 7.22 (1H, d, $J$=2.0 Hz), 7.31 (1H, br d, $J$=8.3 Hz) and 7.34 (1H, br d, $J$=8.3 Hz). The remaining two signals assignable to $\alpha$-methin protons are expected to be hidden in HDO signal. MS (hepta-trimethylsilylate) $m/z$ 852 $(M)^+$, 837 $(M-CH_3)^+$, 735 $(M-TMSCO_2)^+$ and 646 $(735-TMSNH_2)^+$. 3-Chloro-4-hydroxyphenylglycine (**3**; 15 mg): $^1$H NMR $\delta$ 4.74 (1H, s), 6.90 (1H, d, $J$=8.3 Hz), 7.08 (1H, dd, $J$= 8.3 and 2.4 Hz) and 7.31 (1H, d, $J$=2.4 Hz). MS (tris-trimethylsilylate) $m/z$ 402 $(M-CH_3)^+$ and 300 $(M-TMSCO_2)^+$. *N*-Methyl-*p*-hydroxyphenylglycine (**4**; 12 mg): $^1$H NMR $\delta$ 2.42 (3H, s), 4.56 (1H, s), 6.79 (2H, d, $J$=8.3 Hz) and 7.16 (2H, d, $J$=8.3 Hz). MS (tris-trimethylsilylate) $m/z$ 382 $(M-CH_3)^+$ and 280 $(M-TMSCO_2)^+$. Dideoxyvancomycinic acid (**5**; 14 mg): $^1$H NMR $\delta$ 3.01 (2H, dd, $J$=14.7 and 7.0 Hz), 3.11 (2H, dd, $J$=14.7 and 6.2 Hz), 3.99 (2H, dd, $J$=6.2 and 7.0 Hz), 4.48 (1H, s), 6.62 (2H, s), 6.87 (2H, d, $J$=8.4 Hz), 7.06 (2H, dd, $J$=8.4 and 1.8 Hz) and 7.32 (2H, d, $J$=1.8 Hz). MS (hepta-trimethylsilylate) $m/z$ 1,082 $(M-CH_3)^+$ and 964 $(M-CH_3 \text{ and } TMSCO_2H)^+$. FAB-MS $m/z$ 594 $(M+H)^+$ and 592 $(M-H)^-$.

### Acid Hydrolysis of **1** for Preparing of Sugar Components

Two g of **1** was hydrolyzed with 1 N HCl (200 ml) at 95°C for 2 hours. After the reaction mixture was neutralized with Amberlite IRA 45 $(OH^-)$ (Rohm & Haas Co.), the filtered mixture was passed through a Diaion HP-20 (Mitsubishi Chemical Ind. Ltd., 50 ml) and a Dowex 50W-X4 $(H^+$, Dow Chemical Co., 10 ml) columns and neutralized again with Amberlite IRA 45 $(OH^-)$. The hydrolysate was concentrated to 2 ml *in vacuo* and chromatographed on a silica gel (Type 60A Special, Mallinckrodt Co.) column (100 ml) with a solvent composed of EtOAc - 2-PrOH - $H_2O$ (13 : 7 : 2). Fractions containing rhamnose were combined, concentrated, passed through a SEP-PAK $C_{18}$ cartridge (Waters Associates, Inc.) for decolorization and lyophilized to yield 43 mg of rhamnose. Fractions containing glucose were also collected and subjected to the same procedure as described in the preparation method of rhamnose to obtain 35 mg of glucose. Methylmannoside was obtained as follows: After removal of glucose and rhamnose from **1** (2 g) in 1 N HCl (200 ml) at 95°C for 2 hours, the hydrolysate was neutralized with 6 N NaOH to obtain precipitate of pseudoaglycone, which was collected, lyophilized,

suspended in MeOH (25 ml), added Amberlist 15 (5 ml) as a catalyst and refluxed for 16 hours, successively. After filtration, the reaction mixture was concentrated to dryness, and the aqueous solution was passed through a Diaion HP-20 (50 ml) and a Dowex 50W-X4 ($H^+$, 10 ml) columns to remove aglycone or amino acid derivatives. The succeeding purification was done by a column chromatography on silica gel developed with a solvent mixture of EtOAc - 2-PrOH - $H_2O$ (50 : 20 : 1). Fractions containing methylmannoside were combined and concentrated to 2 ml *in vacuo* and lyophilized to yield 13 mg in pure form. The methylmannoside thus obtained was identified as $\alpha$ configuration by comparing with the $^{13}C$ NMR spectrum of the authentic sample. Methylristosaminide was prepared from 1.6 g of **1** with 5% HCl in abs MeOH (10 ml) at 105°C for 5 hours. The hydrolysate was concentrated to dryness *in vacuo*, redissolved in water, neutralized with Amberlite IRA 45 ($OH^-$), and removed the precipitate together with the ion exchange resin by centrifugation 10,000×$g$, 10 minutes. The supernatant (40 ml) was adsorbed on a Dowex 50W-X4 ($NH_4^+$) column (15 ml) followed by washing with water (50 ml) and elution of methylristosaminide with 0.5 N $NH_4OH$ (100 ml). The eluate was concentrated to 2 ml *in vacuo* and lyophilized to yield 37 mg of crude material. Purification by a Toyopearl HW40-F (Toyo Soda Co.) column chromatography (60 ml) was achieved using methanol to afford methylristosaminide (23 mg). It was identified with methyl-$\alpha$-ristosaminide by comparative studies of its $^1H$ and $^{13}C$ NMR spectra with those of the values reported by SZTARICSKAI *et al.*[10]. In order to determine the configuration, this methyl-$\alpha$-ristosaminide was acetylated with $Ac_2O$ - pyridine followed by separation on a silica gel column chromatography ($CH_2Cl_2$ - MeOH, 10 : 1) to get 6.5 mg of methyl-*N*,*O*-diacetyl-$\alpha$-ristosaminide. The optical rotations of these sugars were as follows: D-Glucose; $[\alpha]_D^{23}$ +41.4° (*c* 1.77, $H_2O$), methyl-$\alpha$-D-mannoside; $[\alpha]_D^{23}$ +57.5° (*c* 1.33, $H_2O$), L-rhamnose; $[\alpha]_D^{23}$ +6.1° (*c* 4.14, $H_2O$) and methyl-*N*,*O*-diacetyl-$\alpha$-L-ristosaminide; $[\alpha]_D^{23}$ −120° (*c* 0.65, $CHCl_3$).

Hakomori Permethylation of 1 and The Permethylated Sugars

A suspension of 2.5 g of NaH in 15 ml of dry DMSO was allowed to stand at 60°C for 1 hour. After cooling, dried compound **1** (1.2 g) in 15 ml of dry DMSO was added. After careful addition of $CH_3I$ (30 ml) at 4°C, the reaction mixture was allowed to stand at room temp for 4 hours. All these reactions described above were performed in a stream of nitrogen. The reaction was stopped by addition of water (75 ml) and the reaction product was extracted with $CH_2Cl_2$ (200 ml). The $CH_2Cl_2$ layer was washed with 2 N $Na_2S_2O_3$ (100 ml), dried over $CaCl_2$ and concentrated *in vacuo* to a crude syrup. The crude material was triturated with ether, washed and decanted with the same solvent for three times, and the residue was dried to yield the permethylation product of **1** (1.6 g). The product was refluxed with 5% HCl in abs MeOH (20 ml) for 16 hours and the hydrolysate was concentrated to dryness *in vacuo*. The yielded material was extracted with 100 ml of $CH_2Cl_2$ - ether (1 : 1) to remove an insoluble oily material. The concentrated extract of the mixture which contained permethylated glucose and rhamnose was separated by silica gel column (200 ml) chromatography ($CH_2Cl_2$ - EtOAc, 4 : 1). Each of the pure fraction was concentrated *in vacuo* to yield 72 mg of permethylated glucose and 54 mg of permethylated rhamnose, respectively. Permethylated mannose (24 mg) was prepared by further reflux of the oily material with 10% HCl in abs MeOH (20 ml) for 16 hours followed by the same purification procedure as described above. Permethylated mannose and rhamnose were in good agreement with the previously reported $^{13}C$ NMR spectra[5] and permethylated glucose was also identified by comparing with the $^{13}C$ NMR spectrum of the authentic sample.

Edman Degradation of 1

The compound **1** (100 $\mu$g) was coupled with 2 $\mu$g of phenylisothiocyanate (PITC) in dimethylallylamine buffer at pH 9.5 (200 $\mu$l) and the reaction mixture was kept at 40°C for 1 hour, washed three times with 1 ml portions of benzene and lyophilized. It was further dried by heating at 50°C for 30 minutes *in vacuo*. The phenylthiocarbamate thus obtained was dissolved in 20 $\mu$l of TFA and allowed to stand at 40°C for 10 minutes. Then the mixture was extracted two times with 1 ml portions of dichloroethane and the extract was concentrated to dryness. The material was converted to a phenylthiohydantoin derivative with 1 N HCl (200 $\mu$l) at 80°C for 10 minutes. The derivative was extracted three times with 1 ml portions of EtOAc and the extract was concentrated to dryness *in vacuo*. All of these experiments were done in a stream of nitrogen. The Edman degradation product was detected

by the HPLC method (UV at 269 nm) on a column of Senshu Pak SEQ-4 (4.6 ×300 nm) developed with a solvent mixture of $CH_3CN$ (33%), 2-PrOH (2%), butylchloride (0.4%) and 6 mM of sodium acetate in water.

Differential Methylation of **1**

A mixture of **1** (2 g), $CH_3I$ (6 ml) and $K_2CO_3$ (2 g) in MeOH (20 ml) was refluxed for 16 hours and concentrated to dryness. After confirming that the shift of UV absorption max at 280 nm was not observed in alkaline solution, the reaction product was dissolved in water (20 ml), adjusted to pH 9.0 and adsorbed on a Diaion HP-20 column (50 ml) which was washed with water (100 ml) and eluted with 50% aq acetone (100 ml). The concentrated eluate was hydrolyzed with 1 N HCl (25 ml) at 95°C for 1 hour, and the hydrolysate was neutralized with 6 N NaOH, centrifuged 10,000×$g$, 10 minutes to collect the precipitate and lyophilized to afford 740 mg of the methylated material. This methylated material was re-methylated as described above, but by the use of $CD_3I$ (5 ml) instead of $CH_3I$. The differentially methylated derivative was refluxed with 4 N KOH (30 ml) - $NaBH_4$ (1 g) in water in a stream of nitrogen for 24 hours. After neutralization, NaOCl solution (8 ml) was added to the reaction mixture which was allowed to stand at room temp for 4 hours. After addition of $Na_2SO_3$, further oxidation was done with acidic 5% $KMnO_4$ (100 ml) at ambient temp for 16 hours followed by exhaustion of the oxidant with $Na_2SO_3$. The reaction mixture was extracted with a mixture of EtOAc - MeOH (9 : 1, 200 ml), dried over $Na_2SO_4$ and concentrated *in vacuo* to 20 ml. The mixture was finally esterified with $CH_2N_2$ and concentrated *in vacuo* to afford 170 mg of the esterified mixture of the derivatives. Separation on a preparative silica gel TLC plate (*n*-hexane - EtOAc, 2 : 1) yielded three derivatives possessing UV absorption, **5a** (Rf 0.56, 2.5 mg), **5b** (Rf 0.48, 4.0 mg) and **6** (Rf 0.37, 1.3 mg). Their $^1H$ NMR (90 MHz, $CDCl_3$) and EI-MS data were as follows: **5a**; $^1H$ NMR $\delta$ 3.58 (1H, d, $J$=13.5 Hz), 3.78 (1H, d, $J$=13.5 Hz), 3.88 (3H, s), 3.96 (~1H, s), 6.95 (1H, d, $J$=8.7 Hz), 7.96 (1H, dd, $J$=8.7 and 2.6 Hz) and 8.06 (1H, d, $J$=2.6 Hz). MS $m/z$ 217 ($M^+$, $C_{10}D_3H_8O_3Cl$) and 214 ($M^+$, $C_{10}H_{11}O_3Cl$). The intensities of these signals were 100% and 68%, respectively. **5b**; $^1H$ NMR $\delta$ 3.87 (3H, s), 3.96 (~1H, s), 6.92 (1H, d, $J$=8.2 Hz), 8.00 (1H, dd, $J$=8.2 and 2.6 Hz) and 8.06 (1H, d, $J$=2.6 Hz). MS $m/z$ 203 ($M^+$, $C_9D_3H_6O_3Cl$) and 200 ($M^+$, $C_9H_9O_3Cl$). Their intensities were 61% and 41% of the base peak at $m/z$ 172, respectively. **6**; $^1H$ NMR $\delta$ 3.86 (3H, s), 3.92 (6H, s), 6.85 (2H, d, $J$=8.8 Hz), 7.56 (2H, s), 7.89 (2H, dd, $J$=8.8 and 1.9 Hz) and 8.17 (2H, d, $J$=1.9 Hz). MS $m/z$ 537 ($M^+$). Its molecular formula was established to be $C_{25}D_3H_{17}O_9Cl_2$ (found 537.0657; calcd 537.0673).

Preparation of Epimers of Chloropolysporins B and C

An aqueous solution (2 ml) of **1** (10 mg) was sealed in an ampoule and heated at 70°C for 17 hours. The epimer was separated by preparative HPLC system (YMC-Pack S-343 I15 ODS) with 20% aq acetonitrile containing 0.4% of TFA to afford 9 mg. *epi*-Chloropolysporin C was also prepared from chloropolysporin C by the same method described in the preparation of *epi*-chloropolysporin B. Identification of these compounds were achieved by an HPLC method using reverse phase column (YMC-Pack A-312 ODS) developed with 14% aq $CH_3CN$ containing 0.4% of TFA.

## References


1) OKAZAKI, T.; R. ENOKITA, H. MIYAOKA, T. TAKATSU & A. TORIKATA: Chloropolysporins A, B and C, novel glycopeptide antibiotics from *Faenia interjecta* sp. nov. I. Taxonomy of producing organism. J. Antibiotics 40: 917~923, 1987
2) TAKATSU, T.; M. NAKAJIMA, S. OYAJIMA, Y. ITOH, Y. SAKAIDA, S. TAKAHASHI & T. HANEISHI: Chloropolysporins A, B and C, novel glycopeptide antibiotics from *Faenia interjecta* sp. nov. II. Fermentation, isolation and physico-chemical characterization. J. Antibiotics 40: 924~932, 1987
3) TAKATSU, T.; S. TAKAHASHI, Y. TAKAMATSU, T. SHIOIRI, S. IWADO & T. HANEISHI: Chloropolysporins A, B and C, novel glycopeptide antibiotics from *Faenia interjecta* sp. nov. IV. Partially deglycosylated derivatives. J. Antibiotics 40: 941~945, 1987
4) TAKATSU, T.; T. KATAYAMA, M. NAKAJIMA, S. TAKAHASHI, T. HANEISHI, T. MAGARIBUCHI & M. TAJIMA: Chloropolysporins A, B and C, novel glycopeptide antibiotics from *Faenia interjecta* sp. nov. V. Comparative studies of the biological properties. J. Antibiotics 40: 946~952, 1987

5) McGahren, W. J.; J. H. Martin, G. O. Morton, K. T. Hargreaves, R. A. Leese, F. M. Lovell, G. A. Ellestad, E. O'Brien & J. S. E. Holker: Structure of avoparcin components. J. Am. Chem. Soc. 102: 1671~1684, 1980
6) Katrukha, G. S.; B. Diarra, A. B. Silaev, Z. P. Trifonova, B. V. Rozynov & O. S. Reshetova: New amino acid from ristomycin A. Antibiotiki 24: 179~181, 1979
7) Harris, C.; H. Kopecka & T. Harris: Vancomycin: Structure and transformation to CDP-1. J. Am. Chem. Soc. 105: 6915~6922, 1983
8) Barna, J. C. J. & D. H. Williams: The structure and mode of action of glycopeptide antibiotics of the vancomycin group. Annu. Rev. Microbiol. 38: 339~357, 1984
9) Bogner, R.; F. Sztaricskai, M. E. Munk & J. Tamas: Structure and stereochemistry of ristosamine. J. Org. Chem. 39: 2971~2974, 1974
10) Sztaricskai, F.; C. M. Harris, A. Neszmelyi & T. M. Harris: Structural studies of ristocetin A. (Ristomycin A): Carbohydrate-aglycone linkages. J. Am. Chem. Soc. 102: 7093~7099, 1980
11) Lomakina, N. N.; L. I. Murav'eva & M. Pushkash: Ristomycins A and B. Structure of the carbohydrate moiety of the molecules. Antibiotiki 13: 867~871, 1968
12) Ellestad, G. A.; W. Swenson & W. J. McGahren: Epimerization and stereochemistry of avoparcin. J. Antibiotics 36: 1683~1690, 1983