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

IV. PARTIALLY DEGLYCOSYLATED DERIVATIVES

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Chloropolysporins A, B and C, new members of the glycopeptide antibiotic family, were enzymatically and chemically converted to their partially deglycosylated derivatives. The α- and β-avoparcins were also deglycosylated by the same method. The conversions were achieved by treatments with rhamnosidase (Naringinase) and α-mannosidase, and by mild acid hydrolysis.

In the previous papers[1~3], we described the taxonomy of the producing organism, isolation, physico-chemical characterization and comparative studies of chloropolysporins A, B and C, which were revealed to be novel compounds in the glycopeptide family of antibiotics. It is well-known that most of the antibiotics belonging to this family are produced as complexes in culture broth, except for vancomycin[4]. The variety of components in the complexes is mainly caused by differences in the sugar components.

In this paper we report the preparations of some derivatives of chloropolysporins B and C, as well as α- and β-avoparcins[5,6], by enzymatic or mild acid hydrolysis.

Enzymatic Conversions of Chloropolysporins B, C and α- and β-Avoparcins

Naringinase (Sankyo Co., Ltd.) was used for derhamnosylation and α-mannosidase (Boehringer Mannheim, West Germany) for demannosylation. The progress of reactions and purifications were monitored by the analytical HPLC method reported in the previous paper[2].

Chloropolysporin B was converted enzymatically to its derhamnosyl derivative with Naringinase and the derivative was purified as follows: Chloropolysporin B (1 g) was dissolved in 1 liter of 0.067 M phosphate buffer at pH 5.8 containing 150 mg of Naringinase. After incubation for 16 hours at 37°C, the reaction mixture was applied to a Diaion HP-20 column (Mitsubishi Chemical Ind. Ltd., Japan, 200 ml). After washing with deionized water (400 ml), the active compound was eluted with 50% aqueous acetone (200 ml), adjusted to pH 4.0 with 1 N HCl, concentrated to 20 ml *in vacuo* and charged on a Polyamide column (Woelm Co., West Germany, 200 ml) with deionized water. The fractions containing the reaction product were combined, concentrated to 5 ml *in vacuo*, adjusted to pH 4.0 with 0.1 N HCl, and lyophilized to yield 400 mg of derhamnosylchloropolysporin B hydrochloride in pure form (yield 40%).

The structural studies of chloropolysporin C revealed that it was the derhamnosyl derivative of chloropolysporin B. The details of the identification of chloropolysporin C were described in the preceding paper[3].

In the same manner, α- and β-avoparcins, prepared from Avotan (American Cyanamid Co.), were

also converted to their corresponding derhamnosyl derivatives.

Thirteen g of crude avoparcin complex was dissolved in 1.5 liters of 0.067 M phosphate buffer containing 2 g of Naringinase. After incubation for 16 hours, the reaction mixture was applied to a Diaion HP-20 column (1 liter), followed by washing with deionized water. The active components were eluted with 50% aqueous acetone, concentrated to 50 ml *in vacuo* and adjusted to pH 5.9. The concentrated eluate was applied to a Polyamide column (500 ml) and the column was developed with deionized water. The two main fractions which contain derhamnosyl-α- and β-avoparcins, respectively, were collected, concentrated *in vacuo* and lyophilized to obtain 1.7 g of the former (purity 36%) and 2.5 g of the latter (purity 67%). Final purification of these compounds was performed by preparative HPLC (YMC-Pack S-343 I15 ODS, 20×150 mm) using 14% aqueous acetonitrile containing 0.3% of trifluoroacetic acid. Each purified compound was converted to its hydrochloride salt form as reported in the preceding paper[2] to yield 606 mg of derhamnosyl-α-avoparcin hydrochloride and 850 mg of derhamnosyl-β-avoparcin hydrochloride (yield 80% in each), respectively.

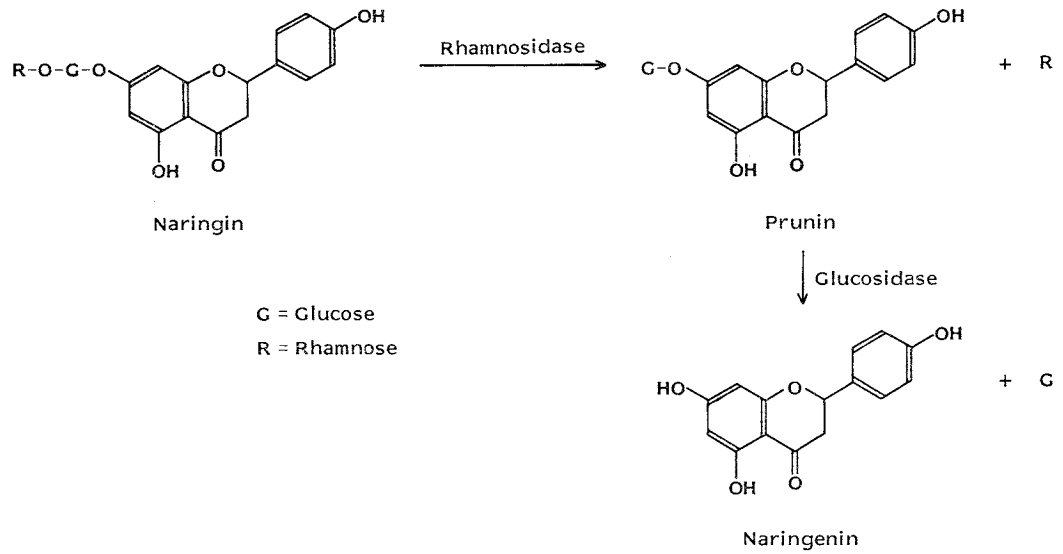
Table 1. Physico-chemical properties of the deglycosylated compounds.

Derhamnosyl-α-avoparcin hydrochloride	
Elementary analysis	
Calcd for $C_{83}H_{92}N_9O_{32}Cl\cdot 2HCl\cdot 9H_2O$ (1,998):	C 49.89, H 5.65, N 6.30, Cl 5.32.
Found:	C 49.98, H 5.45, N 6.38, Cl 5.06.
UV λ_{max} nm ($E^{1\%}_{1cm}$)	280 (48) in 0.01 N HCl, 298 (62) in 0.01 N NaOH
$[\alpha]_D^{23}$	−69.7° (*c* 1.00, 0.1 N HCl)
Derhamnosyl-β-avoparcin hydrochloride	
Elementary analysis	
Calcd for $C_{83}H_{91}N_9O_{32}Cl_2\cdot 2HCl\cdot 9H_2O$ (2,033):	C 49.05, H 5.50, N 6.20, Cl 6.98.
Found:	C 49.07, H 4.98, N 6.31, Cl 6.97.
UV λ_{max} nm ($E^{1\%}_{1cm}$)	280 (49) in 0.01 N HCl, 298 (63) in 0.01 N NaOH
$[\alpha]_D^{23}$	−74.3° (*c* 1.00, 0.1 N HCl)
Demannosylchloropolysporin B	
Elementary analysis	
Calcd for $C_{77}H_{79}O_{29}N_8Cl_3\cdot 9H_2O$ (1,849):	C 50.01, H 5.29, N 6.06, Cl 5.75.
Found:	C 50.07, H 5.31, N 5.81, Cl 5.66.
UV λ_{max} nm ($E^{1\%}_{1cm}$)	280 (47) in 0.01 N HCl, 300 (54) in 0.01 N NaOH
$[\alpha]_D^{23}$	−110.4° (*c* 1.09, 0.1 N HCl)
Demannosylchloropolysporin C hydrochloride	
Elementary analysis	
Calcd for $C_{71}H_{69}O_{25}N_8Cl_3\cdot HCl\cdot 5H_2O$ (1,667):	C 51.46, H 4.87, N 6.16, Cl 8.56.
Found:	C 51.57, H 4.96, N 6.77, Cl 8.27.
UV λ_{max} nm ($E^{1\%}_{1cm}$)	280 (53) in 0.01 N HCl, 297 (89) in 0.01 N NaOH
$[\alpha]_D^{23}$	−81.8° (*c* 1.13, 0.1 N HCl)
Chloropolysporin pseudoaglycone	
Elementary analysis	
Calcd for $C_{71}H_{69}O_{25}N_8Cl_3\cdot HCl\cdot 5H_2O$ (1,667):	C 51.46, H 4.87, N 6.16, Cl 8.56.
Found:	C 51.24, H 5.07, N 6.89, Cl 8.30.
UV λ_{max} nm ($E^{1\%}_{1cm}$)	278 (52) in 0.01 N HCl, 297 (102) in 0.01 N NaOH
$[\alpha]_D^{25}$	−33.8° (*c* 1.17, 0.1 N HCl)

α-Mannosidase was used for conversion of chloropolysporins B and C to the demannosyl derivatives as follows: The reaction mixture containing 280 mg of chloropolysporin B and 3 ml of α-mannosidase (*ca.* 50 units/ml) in 100 ml of 0.1 M phosphate buffer at pH 6.8 was incubated on a reciprocal shaker for 16 hours at 37°C. The reaction mixture was applied to a Diaion HP-20 column (10 ml). After washing with deionized water (20 ml) and 30% aqueous acetone (20 ml), successively, the eluate with 50% aqueous acetone (20 ml) was concentrated and lyophilized to yield demannosylchloropolysporin B (180 mg) in free base form (yield 71%). In the same manner, chloropolysporin C (505 mg) was demannosylated with 4 ml of α-mannosidase (*ca.* 50 units/ml) in the phosphate buffer at pH 6.8 (160 ml). As the reaction product had very low solubility, it precipitated in the reaction mixture. After addition of excess deionized water (200 ml) to the reaction mixture to dissolve the precipitate, the resultant solution was charged on a Diaion HP-20 column (100 ml). The column was washed with deionized water (200 ml), subsequently with 10% aqueous acetone (200 ml). Active compound was finally eluted with 80% aqueous acetone acidified with 1 N HCl to pH 2.0 (200 ml). The eluate was neutralized with 1 N NaOH, concentrated *in vacuo* and lyophilized to yield crude material (400 mg). The final purification was carried out on a Polyamide column (20 ml) developed successively with deionized water and 20% aqueous acetone. The fractions containing the reaction product eluted with 20% aqueous acetone were combined, concentrated to 10 ml *in vacuo*, adjusted to pH 4.0 with 0.1 N HCl and lyophilized to afford demannosylchloropolysporin C hydrochloride (230 mg, yield 50%). It was also possible to obtain the demannosyl derivative of β-avoparcin, which was identical with the known component, ε-avoparcin[6].

It is well-known that the most of glycopeptide antibiotics can be converted to various partially deglycosylated pseudoaglycones by mild acid hydrolysis[7~9]. Reflux of chloropolysporins A, B and C with 5% (w/w) HCl in absolute MeOH for 1 hour resulted in removal of the sugars linked to the phenolic groups of the aglycone and all components of chloropolysporins gave the same pseudoaglycone. In order to prepare the pseudoaglycone of chloropolysporines, chloropolysporin B (2 g) was suspended in absolute MeOH (160 ml) containing 5% HCl and refluxed for 1 hour. After chilling, chloropolysporin

Fig. 1. Enzymatic degradation of naringin by Naringinase.



pseudoaglycone formed in the reaction mixture was precipitated by adjusting to pH 7.0 with 5 N NaOH. The precipitate was obtained by centrifugation. It was redissolved in 50 ml of 40% aqueous methanol, adjusted to pH 4.0, applied to a Polyamide column (400 ml), and developed with 40% aqueous methanol. Active fractions were collected, concentrated and lyophilized to afford 810 mg of off-white powder of the pseudoaglycone (yield 75%, purity 90%). The final purification of this powder was carried out on a Toyopearl HW40-F column (Toyo Soda Co., Japan, 400 ml) equilibrated with 60% aqueous methanol. The pure fractions were pooled, concentrated to 10 ml *in vacuo* and lyophilized to obtain 560 mg of chloropolysporin pseudoaglycone (yield 77%).

Physico-chemical properties of these compounds are summarized in Table 1.

Discussion

Almost all members of glycopeptide antibiotic family exist as complexes in the culture broth except for vancomycin. The components of the complex are characterized mainly by variations of the glycosylating sugars. We have established a useful mild deglycosylation method using rhamnosidase and mannosidase. In addition to Naringinase, rhamnosidase preparation of other brands, such as Sclase (Sankyo Co., Ltd.) and Kumitanase (Tanabe Seiyaku Co., Ltd.), were also successfully used for derhamnosylation of chloropolysporin B and avoparcins (data not shown). Naringinase has been used commercially for hydrolysis of naringin to remove its bitter taste. This enzyme preparation contains not only α-rhamnosidase but also β-glucosidase[10]. As shown in Fig. 1, the enzymatic conversion of naringin to naringenin in which rhamnose and glucose moieties were successively released by the action of two enzymes described above. When chloropolysporins or avoparcins were used as the substrate, however, only rhamnose but no glucose was released by Naringinase treatment, probably because of steric hindrance caused by the substitutions at neighboring carbon or chlorine atoms of glycosidic linkage at phenolic hydroxyl of vancomycinic acid moiety in these antibiotic structures. Difference in hydrolytic activity of Naringinase was also shown between naringin or chloropolysporins and A35512 B or ristocetin as the substrate. It is assumed that rhamnose moiety was not released from ristocetin (data not shown) in which that moiety linked to the primary alcohol of glucose moiety (1→6 linkage), in contrast to secondary alcohol of glucose moiety (1→2) or phenolic hydroxy moiety is responsible for the linkage to rhamnose in naringin or chloropolysporins, respectively.

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