# THE STRUCTURAL RELATIONSHIPS OF A82846B AND ITS HYDROLYSIS PRODUCTS WITH CHLOROORIENTICINS A, B AND C

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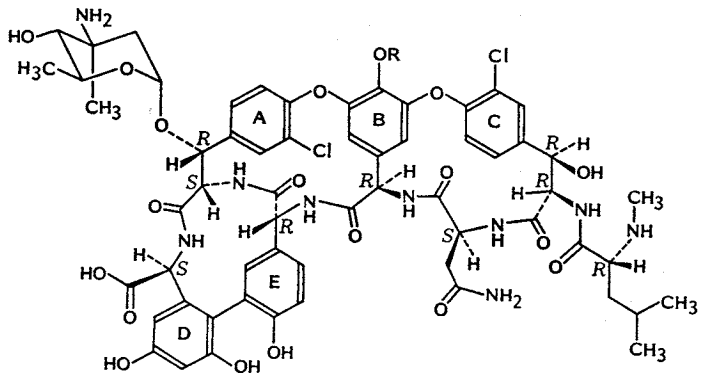
Several glycopeptide antibiotics, structurally related to the clinically important antibiotic vancomycin, containing modified amino sugars have been reported recently.[1~3] Amongst these antibiotics, we had established that the two pairs, monodechloro A82846B and orienticin A, and A82846A and eremomycin, respectively, were identical.[3] Here we report the HPLC comparison and the identity of A82846B with chloroorienticin A,[4] and the relationship of the acid hydrolysis products of A82846B with chloroorienticins B and C.

The antibiotics A82846B and chloroorienticin A have been assigned the same structure **1** (Fig. 1).[4,5] The relationship of chloroorienticins B and C to chloroorienticin A were shown by the removal, first of L-4-*epi*-vancosamine, and then of D-glucose, from the disaccharide moiety attached to the phenolic hydroxyl group of the aromatic ring B in chloroorienticin A.[4]

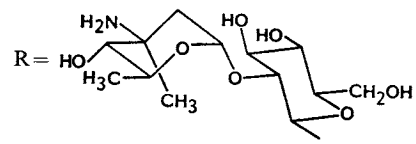
Recently, we developed a method for the selective and sequential removal, of the amino sugar vancosamine, and then the D-glucose from vancomycin, by TFA hydrolysis at carefully controlled conditions.[6] This reaction was applied to A82846B, and des-*epi*-vancosamine A82846B (**2**) and des-*epi*-vancosaminylglucose A82846B (**3**) were obtained after chromatographic separation.[7]

The three pairs of compounds, A82846B and chloroorienticin A, des-*epi*-vancosamine A82846B and chloroorienticin B, and des-*epi*-vancosaminylglucose A82846B and chloroorienticin C, respectively, were compared by HPLC.†

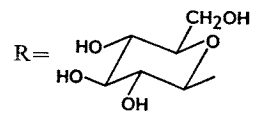
Fig. 1. Structures of A82846B and chloroorienticins.



A82846B (chloroorienticin A) (**1**)



Des-*epi*-vancosamine A82846B (chloroorienticin B) (**2**)



Des-*epi*-vancosaminylglucose A82846B (chloroorienticin C) (**3**) R=H

† Chloroorienticins A, B and C were kindly provided by Dr. TAICHIRO KOMENO, Director, Shionogi Research Laboratories, Fukushima-ku, Osaka 553, Japan for chromatographic comparison.

Table 1. Comparison of HPLC retention times of A82846B and its acid hydrolysis products with chloroorienticins A, B and C.

| | Retention time[a] (minutes) |
|---|---|
| A82846B | 7.79 |
| Chloroorienticin A | 7.71 |
| Synthetic mixture of A82846B and chloroorienticin A | 7.81 |
| Des-*epi*-vancosamine A82846B (**2**) | 4.50 |
| Chloroorienticin B | 4.47 |
| Synthetic mixture of **2** and chloroorienticin B | 4.46 |
| Des-*epi*-vancosaminylglucose A82846B (**3**) | 3.91 |
| Chloroorienticin C | 3.93 |
| Synthetic mixture of **3** and chloroorienticin C | 3.91 |

[a] The conditions used for HPLC were as follows:

1) For A82846B, **2**, chloroorienticins A and B. Column: Dupont Zorbax SCX, 4.6×150 mm. Mobile phase: Solution A=10% MeOH, 0.1 M $NaH_2PO_4$; solution B=10% MeOH, 0.9 M $NaH_2PO_4$. Gradient: 20% B to 90% B over 5 minutes period, hold at 90% B for 2 minutes. Flow rate: 1.5 ml/minute. Detection: UV 225 nm. Injection volume: 20 $\mu$l of a 1-mg/ml concentration.

2) For **3** and chloroorienticin C. Column: Beckman Ultrasphere ODS, 4.6×250 mm. Mobile phase: 23% acetonitrile-$H_2O$, 0.5% $NH_4H_2PO_4$ (w/v). Flow rate: 1 ml/minute. Detection: UV 225 nm.

The antibiotics A82846B and chloroorienticin A had retention times of 7.79 and 7.71 minutes, respectively, which are identical within experimental error. When these two antibiotics were mixed together, they eluted as a single peak with a retention time of 7.81 minutes. Furthermore, the UV profiles of both antibiotics measured at their peak maxima were superimposable.

Similarly, des-*epi*-vancosamine A82846B (**2**) and chloroorienticin B had retention times of 4.50 and 4.47 minutes, respectively, and the mixed antibiotics coeluted at 4.46 minutes as a single peak. Finally, des-*epi*-vancosaminylglucose A82846B (**3**) and chloroorienticin C had retention times of 3.91 and 3.93 minutes, respectively, and a synthetic mixture of these two compounds eluted as a single peak with a retention time of 3.91 minutes. The UV profiles at the peak maxima of the above two pairs were also superimposable.

The above data confirm our earlier observation[3] that A82846B and chloroorienticin A are identical, and their corresponding acid hydrolysis products des-*epi*-vancosamine A82846B and chloroorienticin B, and des-*epi*-vancosaminylglucose A82846B and chloroorienticin C, respectively, are also identical.

It is interesting to note that chloroorienticin D is the di-*N*-methyl leucine analog of chloroorienticin A.[4] The di-*N*-methyl leucine analog of vancomycin, designated as M43D, was also recently reported.[8]

## Experimental

### TFA Hydrolysis of A82846B

A solution of 130 mg of A82846B in 3 ml TFA was stirred at room temperature for 50 minutes. The TFA from the reaction mixture was then evaporated, and the residue dissolved in water. The aqueous solution was lyophilized. The recovered material was purified by preparative HPLC using a Waters micro Bondapak $C_{18}$ column, 19×150 cm, and eluted with acetonitrile - water gradient buffered with 1% pyridinium acetate and monitored by UV at 280 nm. The appropriate pools yielded 45.6 mg (38.5%) of **2**, and 13.3 mg (12.6%) of **3**.

In another experiment, 250 mg of A82846B was stirred in 5 ml TFA for 4~5 hours at room temperature. Using the same work-up procedure as above, the reaction yielded 45.6 mg (20%) of **2** and 63.1 mg (31.2%) of **3**.

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