

COMMUNICATIONS TO THE EDITOR

Synthesis, Isolation, and Antibacterial Activities of Monodechlorovancomycins

Sir:

Vancomycin, a glycopeptide antibiotic, is often prescribed for the treatment of staphylococcal infections, particularly infections caused by methicillin-resistant strains of staphylococcus.^{1,2)} Vancomycin was first isolated in 1956 from *Streptomyces orientalis*³⁾ (currently known as *Amycolatopsis orientalis*), and the structure was fully characterized 25 years later.⁴⁻⁶⁾ Vancomycin B (vancomycin, Figure 1) is isolated from fermentation broth and formulated for pharmaceutical use as the hydrochloride salt, vancomycin hydrochloride.⁷⁾ One of the related substances formed during the vancomycin fermentation is consistent with a monodechlorovancomycin structure. The monodechlorovancomycin has a similar retention time to its parent, vancomycin B, in USP⁸⁾ and EP⁹⁾ HPLC assays. We were interested in isolating this compound for use as an HPLC standard as well as testing the antibacterial activity versus the parent vancomycin B.

There are two chlorine atoms in the vancomycin structure; therefore there are two possible "monodechloro" products (Figure 1). Monodechlorovancomycin 1 was synthesized chemically by reduction of vancomycin B.¹⁰⁾ Monodechlorovancomycin 2 was isolated from fermentation broth. HPLC assays showed that the synthetic monodechlorovancomycin 1 was different from the fermentation product. This finding was later confirmed by NMR assignments of the two monodechlorovancomycins. This led to the determination that monodechlorovancomycin 2 was the concomitant produced during fermentation. Monodechlorovancomycin 1 has not been detected in fermentation broth, whereas vancomycin missing both chlorine atoms (didechlorovancomycin) has been found in vancomycin fermentations. This finding has implications regarding the biosynthesis of the vancomycin complex and remains to be explained. Herein, we report the synthesis and isolation of the two monodechlorovancomycins and their antibacterial activities.

Monodechlorovancomycin 1

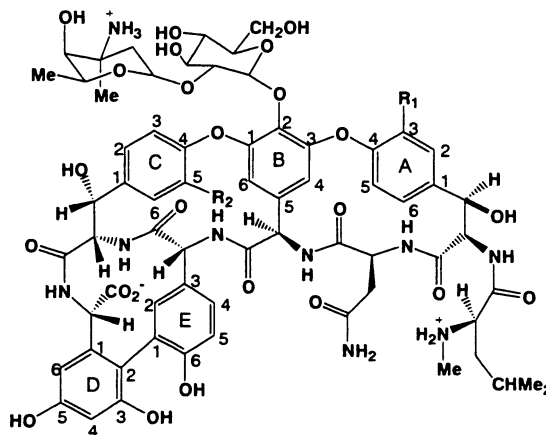
Treatment of a 10% aqueous solution of vancomycin B with 5% Pd/C under 40 psi of hydrogen gas gave crude

monodechlorovancomycin 1. The compound was then purified by reverse phase column chromatography on an ODS-A column by elution with 4% aqueous acidic acetonitrile (0.25% acetic acid) to afford a 90% pure monodechlorovancomycin 1. Purity was determined using a modification of the assay in USP 24 and is measured as peak area percent. MS (ESI, C8 column, 5~85% acetonitrile gradient screening) m/z called for $C_{66}H_{77}ClN_9O_{24}^+$: 1414.48, found: 1414.29.

Monodechlorovancomycin 2

The pH of fermentation broth containing vancomycin B and related substances was adjusted to 9.4 with aqueous sodium hydroxide followed by treatment with acidic ion-exchange resin. The spent beer was removed by decanting and the resin was washed with water followed by a dilute aqueous NH_4OH to give an aqueous vancomycin solution. The pH of the solution was adjusted to 3 with 6N aqueous hydrochloric acid to give a vancomycin hydrochloride solution. The resulting solution was desalted and concentrated by reverse osmosis. Vancomycin with the related substances was purified by crystallization after adjusting the pH to 9 with 28% NH_4OH . The final product

Fig. 1. Vancomycin and monodechlorovancomycins 1 and 2.



Vancomycin: $R_1=Cl, R_2=Cl$

Monodechlorovancomycin 1: $R_1=H, R_2=Cl$

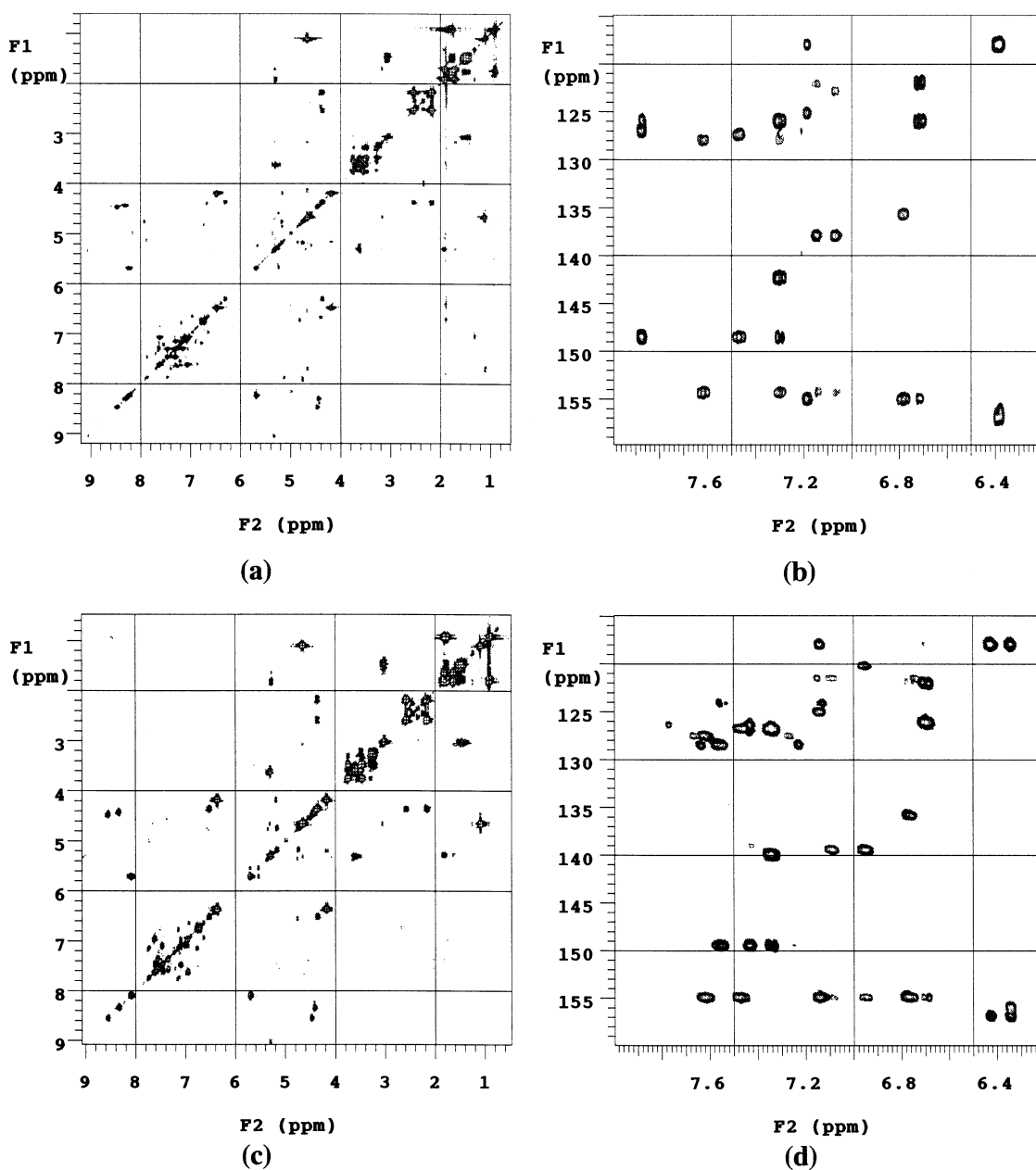
Monodechlorovancomycin 2: $R_1=Cl, R_2=H$

of vancomycin and related substances was isolated as the hydrochloride salt. Monodechlorovancomycin 2 was enriched by reverse recrystallization from ethanol/acetone/water (1.5/1/1.5) at 15°C for 18~24 hours. Monodechlorovancomycin 2 was enriched two-fold in the collected solid, leaving 82~90% of vancomycin B in the mother liquors. Monodechlorovancomycin 2 was purified by low-pressure column chromatography on Amberchrom resin (CG161-m). The compound was eluted using a gradient method. Mobile phase A was 0.2%

NH₄OH buffer. Mobile phase B was 0.2% NH₄OH buffer with 10% isopropyl alcohol. The elution solvent became 100% B after 6 bed volumes. The monodechlorovancomycin 2 was further purified on an ODS-A column with elution by 4% aqueous acidic acetonitrile (0.25% acetic acid) to afford greater than 98% pure monodechlorovancomycin 2. MS (ESI, C8 column, 5~85% acetonitrile gradient screening) *m/z* called for C₆₆H₇₇ClN₉O₂₄⁺: 1414.48, found: 1414.3.

UV spectra of monodechlorovancomycin 1 and 2 were

Fig. 2. Two-dimensional spectra of monodechlorovancomycins 1 and 2 in DMSO-*d*₆ at 60°C.



(a) gDQCOSY of monodechlorovancomycin 1; (b) aromatic region gHMBC of monodechlorovancomycin 1; (c) gDQCOSY of monodechlorovancomycin 2; (d) aromatic region gHMBC of monodechlorovancomycin 2.

nearly identical with a characteristic secondary absorption maximum at approximately 290 nm. Both compounds monodechlorovancomycin 1 and 2 degraded prior to melting. Vancomycin B demonstrated similar behavior. Mass spectra of monodechlorovancomycin 1 and 2 indicated that there was only one chlorine atom in each molecule. As shown in Figure 2a, a spectrum of gDQCOSY of 1 (ppm), there are correlations between H2 (7.29) and H3 (7.14), H5 (7.06) and H6 (7.61) on the A-ring; H2 (7.46) and H3 (7.29) on the C-ring. As depicted in a spectrum of gHMBC of monodechlorovancomycin (ppm), Figure 2b, there are long-range correlations between proton H3 (7.14) and carbons C1 (137.8), C4 (154.2), and C5 (122.0) on the A-ring; proton H3 (7.29) and carbons C5 (125.1), C4 (148.4) and C1 (142.2) on the C-ring. As shown in a spectrum of gDQCOSY of monodechlorovancomycin 2 (ppm), Figure 2c, there are correlations between H2 (7.61) and H3 (6.95), H5 (7.09) and H6 (7.46) on the C-ring and H5 (7.34) and H6 (7.55) on the A-ring. As depicted in a spectrum of gHMBC of monodechloro-

vancomycin 2 (ppm), Figure 2d, there are long range correlations between proton H5 (7.34) and carbons C1 (140.1), C3 (127.0) and C4 (149.6) on the A-ring; proton H5 (7.09) and carbons C1 (139.6), C3 (121.6), and C4 (155.1) on the C-ring. The ^1H - and ^{13}C -NMR spectra were consistent with the proposed structures.

The antibacterial activity of vancomycin B and the two monodechlorovancomycins was determined and compared with another glycopeptide antibiotic, teicoplanin (Table 1). Antibacterial activities were determined by the broth microdilution method in cation-adjusted Mueller Hinton broth (Becton Dickinson and Co., Cockeysville, Md.) as described by the National Committee for Clinical Laboratory Standards (NCCLS, M7-A4. 1997). Results are reported as the minimum inhibitory concentration (MIC), defined as the lowest concentration of compound required to inhibit visible bacterial growth. All strains tested were from the culture collection of Abbott Laboratories and were either clinical isolates or reference strains obtained from the American Type Culture Collection (Manassas, Va). The two

Table 1. Antimicrobial activity of vancomycin B and derivatives.

Organism	Vancomycin B	MIC (mcg/mL)		Teicoplanin
		Monodechloro-vancomycin 1	Monodechloro-vancomycin 2	
<i>Staphylococcus aureus</i> NCTC 10649M	0.5	4	4	0.5
<i>Staphylococcus epidermidis</i> 3519	1	4	2	4
<i>Streptococcus pneumoniae</i> ATCC 6303	0.25	1	1	0.015
<i>Enterococcus faecalis</i> ATCC 29212	2	8	4	0.12
<i>Enterococcus faecium</i> 1632	2	8	4	0.12
<i>Moraxella catarrhalis</i> 2604	64	64	64	16
<i>Haemophilus influenzae</i> 1435	>128	>128	128	32
<i>Escherichia coli</i> Juhl	>128	>128	>128	>128
<i>Pseudomonas aeruginosa</i> 5007	>128	>128	>128	>128
<i>Candida albicans</i> CCH442	>128	>128	>128	>128
<i>Enterococcus faecalis</i> 7065 VanB inducible	>128	>128	>128	0.12
<i>Enterococcus faecalis</i> 7070 VanA	>128	>128	>128	64
<i>Enterococcus faecium</i> 5205 VanA	>128	>128	>128	128
<i>Enterococcus faecium</i> 6253 VanB constitutive	>128	>128	>128	32
<i>Enterococcus faecium</i> 6973 VanB inducible	>128	>128	>128	0.25

monodechlorovancomycins demonstrated the same spectrum of activity as vancomycin B, inhibiting the Gram-positive staphylococci, vancomycin susceptible enterococci, and *Streptococcus pneumoniae*. However the activity of the monodechlorovancomycins was slightly less than vancomycin B. This correlates with the results of an autoturbidimetric microbial assay for vancomycin potency, it was found that the two monodechlorovancomycins had approximately 70% the activity of the parent vancomycin B. With the exception of very weak activity against *Moraxella catarrhalis*, none of the vancomycins demonstrated antibacterial activity against the Gram-negative pathogens. Likewise, none of the vancomycin demonstrated antifungal activity against *Candida albicans*. The compounds were also evaluated for antibacterial activity against representative strains of vancomycin resistant enterococci.¹¹⁾ Neither monodechlorovancomycin demonstrated antibacterial activity against these strains at the highest concentration tested. Teicoplanin was active against the inducible VanB strains.

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LI-SUN
ALINE C. LINDBECK[†]
ANGELA M. NILIUS
TIMOTHY B. TOWNE
CASEY CHUN ZHOU
THOMAS J. PAULUS*

Abbott Laboratories,
Global Pharmaceutical Operations &
Global Research and Development
1400 N. Sheridan Road
North Chicago, IL 60064 USA

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* Corresponding author: thomas.paulus@abbott.com

[†] Present address: Hospira, 275 N. Field Drive, Lake Forest, IL 60064.