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Chloroperoxidase-catalyzed chlorination of didechloroaglucovancomycin and vancomycin

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Abstract

The chloroperoxidase (CPO) from *Caldariomyces fumago* catalyzed the chlorination of didechloroaglucovancomycin and vancomycin in the presence of hydrogen peroxide and chloride ion. Chlorination of didechloroaglucovancomycin has afforded new derivatives, with one and two chlorine atoms attached onto the aromatic ring of residue 7 of didechloroaglucovancomycin. Vancomycin was similarly chlorinated under the same conditions to furnish a new dichloro derivative.

Keywords: Chloroperoxidase; Vancomycin; Enzymatic chlorination; Antibiotics; Biotransformations

1. Introduction

Haloperoxidases are enzymes that are capable of halogenating a large variety of organic compounds using hydrogen peroxide and either chloride, bromide or iodide ions, depending on the enzyme under study. Chloroperoxidase (CPO; chloride peroxidase: hydrogen peroxide oxidoreductase; EC 1.11.1.10) is such an enzyme that can utilize chloride, bromide and iodide ions as donors for enzymatic halogenation reactions, although it can also catalyze the classical peroxidation reactions that are characteristic of peroxidases [1].

In nature, there is a wide variety of halogenated compounds, broadly differing in their chemical structures and properties [1]. Vancomycin **(1)**, (Fig. 1)) is one among them, bearing two chlorine atoms. It is a clinically important glycopeptide antibiotic [2]. The structural complexity of vancomycin [3–6], its unique mode of action [7], and the emergence of resistant strains [8–10] have renewed interest in the study of this class of compounds. The thermal atropisomerism of aglucovancomycin **(2)**, (Fig. 1) derivatives [11], its total chemical synthesis [12,13], and the reconstruction of vancomycin by chemical glycosylation of the pseudoaglycon and aglycon [14] have been recently reported, paving the way for the search of analogs with improved antimicrobial activities than vancomycin.

At present, little is known about the mechanistic details of its biosynthesis. It is not clear at which step and how the chlorine atoms are introduced into the vancomycin molecule. Our

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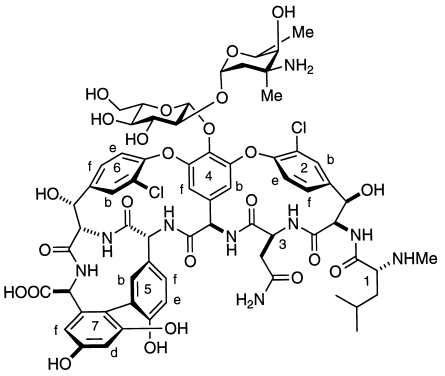


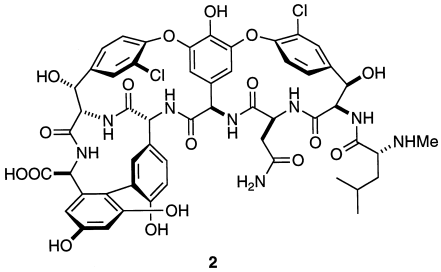


Fig. 1. Structures of vancomycin **(1)** and aglucovancomycin **(2)**.

goal was to determine the role of chloroperoxidase, if any, in such a transformation. Herein, we report our results on the enzymatic chlorination of vancomycin **(1)** and didechloroaglucovancomycin **(3)**, (see Scheme 1)

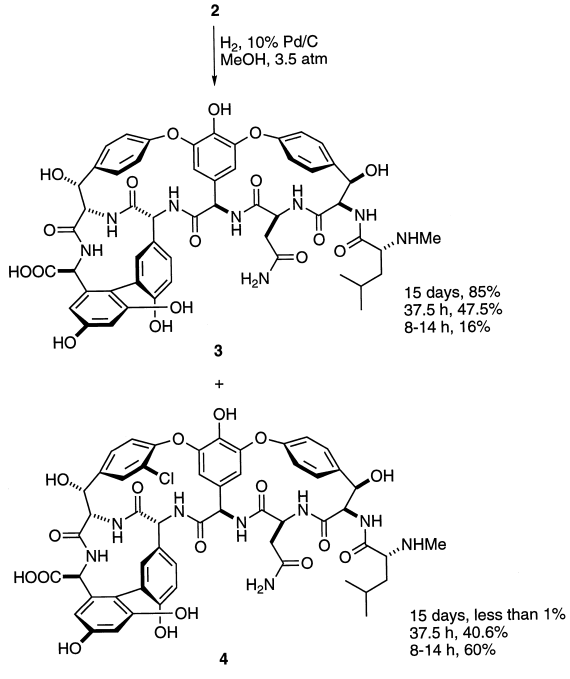
2. Experimental

2.1. General

Vancomycin and CPO (from *Caldariomyces fumago*) were purchased from Sigma. Proton NMR spectra were obtained at 24°C on a Bruker AM-300 spectrometer operating at 300 MHz using Me_2SO-d_6 as a solvent (10 mM solutions were used). MALDI-TOF mass spectra were obtained on a Bruker REFLEX II spectrometer using α-cyano-4-hydroxycinnamic acid as a matrix and neurotensin as a calibrant. Reported values are monoisotopic masses. Reverse-phase high performance liquid chromatography (RP-HPLC) was carried out on a Waters Nova-Pak C18 cartridge (8 × 100 mm) or a Prep Nova-Pak HR C18 column (19 × 300 mm) using an elution gradient from solvent A (0.1% TFA in water) to solvent B (0.01% TFA in 90% aqueous acetonitrile), and the UV absorption was monitored at 254 nm.

*2.2. Preparation of didechloroaglucovancomycin **(3)***

Didechloroaglucovancomycin **(3)** was prepared from vancomycin **(1)** following published procedures. In the first step, **(1)** was treated with TFA to give aglucovancomycin **(2)**, (Fig. 1) [11,15]. In the next step, catalytic hydrogenolysis of **(2)** was carried out in the same manner as published for **(1)** [16], except methanol was used as a solvent instead of water. It is noteworthy that the rate of dehalogenation occurred faster than that reported for **(1)**. It required 8–14 h to remove the chlorine atom from the aromatic ring of residue 2 (60% yield) and 15 days to remove the other chlorine atom from residue 6 (85% yield). Scheme 1 illustrates the catalytic hydrogenolysis of aglucovancomycin **(2)**. With both analytical and preparative HPLC, didechloro derivative **(3)** was the fastest moving



Scheme 1. Catalytic hydrogenolysis of glucovancomycin **(2)**

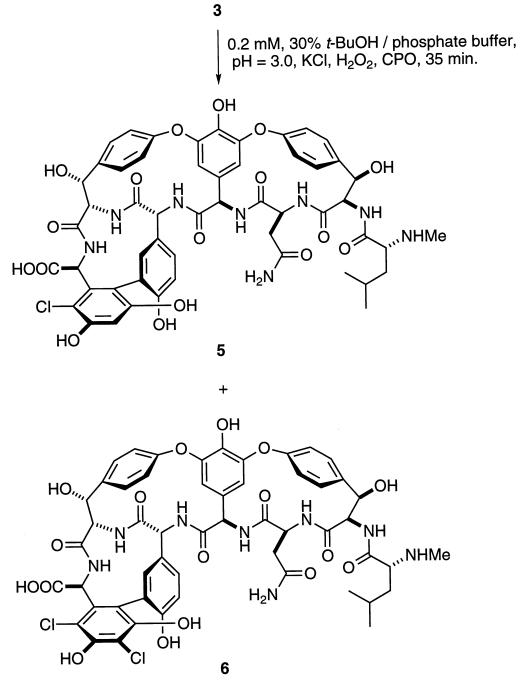
component whereas the monodechloro derivative **(4)** was in between **(2)** and **(3)**. Proton NMR spectra of compounds were in accord with the data reported in the literature [3–5,16]. MALDI-TOF-MS were as follows: aglucovancomycin **(2)**: 1142.9 (calcd. exact MH^+ 1143.3); didechloroaglucovancomycin **(3)** 1075.1 (calcd. exact MH^+ 1075.4); monodechloroaglucovancomycin **(4)** 1109.2 (calcd. exact MH^+ 1109.3).

2.3. CPO-catalyzed chlorination of didechloroaglucovancomycin *(3)*

The reaction mixture contained: 10 mg (9.33 μmol, 0.2 mM) of **(3)**, 28 μmol of hydrogen peroxide, 93 μmol of potassium chloride, 31.5 ml of 0.1 M potassium phosphate buffer, pH = 3.0, and 13.5 ml (30%) of *tert*-butanol. CPO was added to the stirring mixture at 24°C at 5-min intervals in 25-unit aliquote during the first 20 min of incubation (125 units in total) [17]. The reaction was quenched after 35 min by the addition of 100 μmol of sodium bisulfite. After work up, mono and dichlorinated products were purified by preparative HPLC (10–35% B, 9 ml/min during 35 min). Monochlorinated product **(5)**(Scheme 2) was eluted at 19.7 min (15% yield). Proton NMR spectrum showed the absence of the signal corresponding to the 7f proton (signals of the 7f and 7d protons in **(3)** appeared at δ 6.26 and δ 6.38, respectively, whereas in monochlorinated product **(5)**, only 7d proton was found at δ 6.63); MALDI-TOF-MS: 1109.4 (calcd. M H^+1109.3). Dichlorinated product **(6)**(Scheme 2) was eluted at 21.0 min (45% yield). Proton NMR spectrum showed the absence of the signals for both, 7f and 7d protons; MALDI-TOF-MS: 1143.7 (calcd. MH^+1143.3)

2.4. CPO-catalyzed chlorination of vancomycin *(1)*

When vancomycin **(1)** was used as the substrate, similar conditions were used. Typically, the reaction mixture contained: 25 mg (17.3 μmol, 0.2 mM) of **(1)**, dissolved in 59.5 ml of

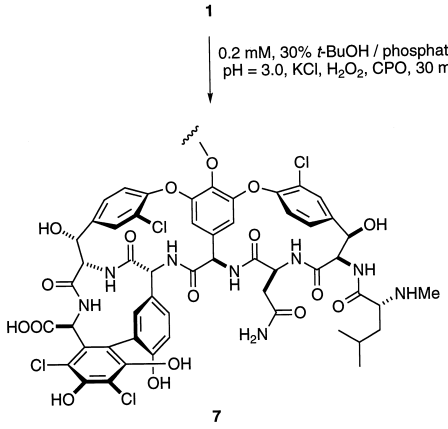


Scheme 2. CPO-catalyzed chlorination of didechloroaglucovancomycin **(3)**.

0.1 M potassium phosphate buffer, pH = 3.0, and 25.5 ml (30%) of *tert*-butanol. Then, 173 μmol of potassium chloride and 50 μmol of hydrogen peroxide were added, along with 30 units of CPO added every 5 min for the first 20 min of incubation (150 units in total). The reaction mixture was stirred at 24°C and quenched after 30 min by the addition of 150 μmol of sodium bisulfite. The isolated product **(7)** (preparative HPLC, 10–35% B, 9 ml/min during 35 min) had the retention time of 17 min(40% yield). Scheme 3 illustrates the CPO-catalyzed chlorination of vancomycin **(1)**. Proton NMR spectrum showed the absence of the signals corresponding to the 7f proton (in vancomycin at δ 6.27) and 7d proton (in vancomycin at δ 6.43); MALDI-TOF-MS: 1538.2 (calcd. MNa^+ 1538.3).

2.5. Chemical chlorination of vancomycin *(1)*

The incubation conditions for the chlorination of vancomycin with hypochlorous acid were identical to those used in the enzyme reaction



Scheme. 3. CPO-catalyzed chlorination of vancomycin **(1)**.

except enzyme, hydrogen peroxide, and chloride were replaced with 1–5 equivalents of sodium hypochlorite (1 equivalent at 5-min intervals) [17]. Results are presented in the Section 3.

3. Results and discussion

In an effort to provide some new information regarding vancomycin biosynthesis, we initiated this study with a view to determining if it was possible to yield aglucovancomycin **(2)**, (Fig. 1) by introduction of the two chlorine atoms onto residues 2 and 6 of didechloroaglucovancomycin **(3)**, (Scheme 1). The commercially available CPO from *Caldariomyces fumago* was chosen for this experiment. To simplify matters, the disaccharide moiety was first removed from the vancomycin **(1)**, (Fig. 1) molecule [11,15]. This was followed by the removal of one or both chlorine atoms from the aglycone **(2)**[16] to provide the substrates for our enzyme-catalyzed chlorination studies (see Scheme 1). Our initial experiments showed that when monodechloro derivative **(4)** was chlorinated, the resulting product did not coincide with **(2)** by HPLC coinjection. Therefore, our attention was turned to didechloro derivative **(3)**. In this case, two products were obtained, and one of them coincided with **(4)** by coinjection, and showed an ion in the MALDI-TOF mass spectrum consistent with the introduction of one chlorine. Another product showed an ion consistent with the introduction of two chlorines, but the proton NMR spectra were intriguing. The compound, we thought was **(4)**, showed the absence of the signal corresponding to the 7f proton and another compound with the same molecular weight as **(2)** showed the absence of signals corresponding to 7f and 7d protons. The signals of these two protons were very distinct in the proton NMR spectrum of **(3)**, with δ 6.26 for 7f, and δ 6.38 for 7d proton. Therefore, the new compounds were assigned as structures **(5)** and **(6)**, respectively (see Scheme 2).

While the structures **(2)** and **(4)** were not obtained, our results showed that it was possible to introduce one or two chlorine atoms into aglucovancomycin and vancomycin. These additional chlorine atoms could result in a significant change in the conformation of these molecules, which in turn could result in improved antimicrobial properties of these new derivatives. With this in mind, vancomycin **(1)** was used as a substrate for CPO-catalyzed chlorination. Although, chlorination was performed under essentially the same conditions, only one product was isolated that showed an ion in the MALDITOF mass spectrum consistent with the substitution of two protons with two chlorines. The proton NMR spectrum showed no signals corresponding to the 7f and 7d protons (in the proton NMR spectrum of vancomycin **(1)** the signals of 7f and 7d protons appeared at δ 6.27 and δ 6.43, respectively). Therefore, the structure **(7)**(Scheme 3) was proposed. In an agar diffusion assay using *E. coli* as the test organism, this new derivative has the same level of antibiotic activity as vancomycin itself.

Finally, chemical chlorination of vancomycin **(1)** was carried out to determine if either, hypochlorous acid (HOCl) or enzyme-bound intermediate (EOCl) was the halogenating species[1]. The substrate was consumed in chemical chlorination reaction, but depending on the

[1] For a review on the reaction mechanisms and the halogenating intermediate, see ref. [1], pp. 102–112

amount of HOCl used (1–5 equivalents) the reaction was very slow if 1 equivalent of HOCl was used, giving a small amount of mono and dichlorinated products. If 5 equivalents of HOCl were used, the reaction profile was very complicated with the formation of less polar, undetermined products. On the basis of these results, it seems that CPO catalyzed the oxidation of chloride ion to hypochlorous acid, which is probably bound to the enzyme during the halogenation process.

4. Conclusion

In summary, an attempt was made to chlorinate vancomycin **(1)** and didechloroaglucovancomycin **(3)**, using chloroperoxidase (CPO) from *Caldariomyces fumago*. Although, we were unsuccessful in introducing chlorine atoms onto the *ortho* positions of aromatic rings of residues 2 and 6, one and two chlorine atoms were introduced onto the benzene ring of residue 7 of didechloroaglucovancomycin **(3)** giving the products **(5)** and **(6)**, respectively. Two chlorine atoms were also introduced into vancomycin **(1)**, furnishing a new derivative **(7)** with the same level of antimicrobial activity as vancomycin **(1)**.

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