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Complestatin to Chloropeptin I via a Quantitative Acid Catalyzed Rearrangement. Absolute Stereochemical Determination of Complestatin.

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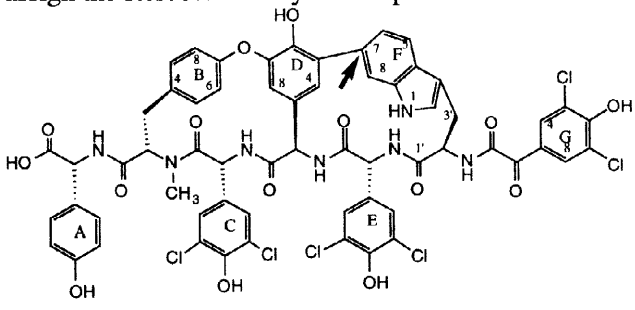
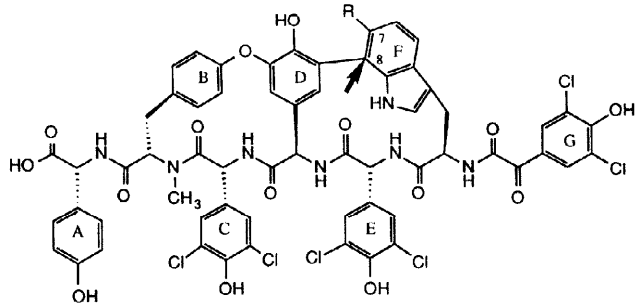
Abstract: Complestatin and isomeric chloropeptin I are bicyclo hexapeptides isolated from a *Streptomyces* sp. Both of these compounds are inhibitors of gp120-CD4 HIV fusion activity. In this paper, we describe an efficient acid catalyzed conversion of complestatin to chloropeptin I, provide a plausible mechanism for this transformation, and unambiguously assign the stereochemistry of complestatin.

Complestatin (**1**), a bicyclo hexapeptide comprised entirely of aromatic amino acids, was isolated from a *Streptomyces* sp. as an anticomplement agent.[1] Recently, complestatin and its isomeric congener, chloropeptin I (**2a**), were characterized as equipotent inhibitors of HIV-1 gp120 glycoprotein CD4 binding (IC_{50} = 2.0 μM).[2] The structure and stereochemistry of chloropeptin I were firmly established in late 1996 by Hirono, Omura and Smith.[3] Structurally, these peptides have a common 16-membered biaryl ether ring, and differ only in the position of the carbon-carbon bond linkage from the central hydroxyphenyl glycine to the indole unit of tryptophan. This bond transposition does not formally change the ring size of the cyclic peptide but has a profound effect on the conformation of this ring and surprisingly has no impact on the glycoprotein binding. The physical properties of the two compounds are also remarkably different. For example, chloropeptin I is highly soluble in methanol while complestatin is virtually insoluble.

The biological activity of chloropeptin I and its superior solubility relative to complestatin made it an attractive target for further evaluations. In this paper, we describe an efficient acid catalyzed conversion of complestatin to chloropeptin I, provide a plausible mechanism for this transformation, and unambiguously assign the stereochemistry of complestatin.



1: Complestatin



2a: R = H, Chloropeptin I
2b: R = D

A study to measure the solubility of complestatin in a number of organic solvents was performed. When complestatin was dissolved/suspended under acidic conditions, we observed the formation of a new compound. The new material was first evident by the appearance of an additional peak (with similar UV spectrum in the diode array) in the HPLC chromatogram.[4] The ^1H NMR spectrum of the mixture contained additional

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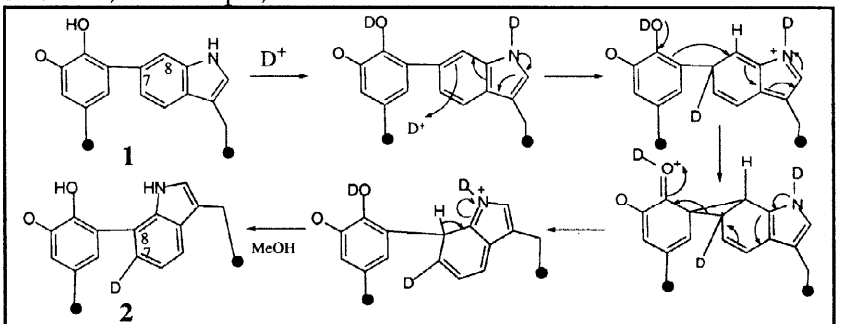
resonances. Therefore, more controlled studies of the formation of the new component were designed. The transformation of complestatin to the new component was observed under all acidic conditions examined, including, dil HCl and dil H_2SO_4. However, the rates of the conversions varied.

The most efficient conversion of complestatin to the new material was accomplished when complestatin was suspended in neat trifluoroacetic acid (TFA) and heated at 50 °C to form a homogeneous solution (~ 5 min). HPLC analysis[4a] of the resulting solution indicated complete conversion of complestatin to the new peak. This new compound was identified as chloropeptin I (**2a**) by ^1H and ^{13}C NMR methods.[2,4b] The conversion was found to be slower in the presence of a co-solvent, for example, water.

Mechanistically, the conversion of complestatin to chloropeptin I is very intriguing and may be envisioned to proceed through a cyclopropyl intermediate as illustrated in Scheme 1. The proposed mechanism of the reaction is supported by the outcome of the treatment of complestatin with neat TFA-d_1. The product, 7-deuterio-chloropeptin I (**2b**), resulted from regiospecific incorporation of deuterium at the site of complestatin bond cleavage. Examination of the ^1H NMR spectrum of **2b** indicated the disappearance of the doublet of amino acid residue F-H7 (δ = 7.08) and the appearance of a doublet at δ 6.90 instead of a triplet (*vide infra*) for F-H6.



Scheme 1: Proposed Mechanism of Conversion

NMR Comparisons: The ^1H and ^{13}C NMR spectra of **1** and **2a**, including exchangeable amide NH protons, were identical to the reported spectra of complestatin[1d,5] and chloropeptin I[2a,5] respectively. The comparison of the ^1H NMR spectra of **1** and **2a** (aside from the presence of a triplet for F-H6 at δ 6.90 and two doublet of doublets for F-H5 and F-H7 in chloropeptin I) indicated shifts of several protons in the Trp containing macrocyclic ring. The most significant of them were downfield shifts of D-H4 (Δ =+ 0.9 ppm), D-H8 (Δ= +0.24 ppm), F-αH (Δ = + 0.93 ppm), E-NH (Δ =+ 0.32 ppm), and upfield shifts of E-αH (Δ =- 0.15 ppm), and F-H1 (Δ =- 0.3 ppm) in chloropeptin I. These changes are due to diminished aromatic ring-induced anisotropy in chloropeptin I compared to complestatin.

Stereochemistry of Complestatin: Prior to our work, the stereochemistry of complestatin had not been fully established.[1] We have elucidated the stereochemistry of complestatin by extensive NMR experiments and structural calculations. Based on these results, the stereochemistry of complestatin was affirmed to be identical to that of chloropeptin I, i.e. A-*R*, B-*S*, C-*R*, D-*R*, E-*R*, F-*R*. The absolute stereochemical assignments for complestatin are further confirmed by the TFA induced conversion of complestatin to chloropeptin I under reaction/isolation conditions that are not expected to produce epimerization.

The isolation of complestatin and chloropeptin I as described in the literature[2a] involved extractions with ethyl acetate at pH 2.0. The acid catalyzed conversion of complestatin to chloropeptin I described in this paper suggests that the original acidic extraction conditions may lead to the formation of chloropeptin I as an artifact during isolation. Therefore, chloropeptin I may not be a natural product.

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4. (a) HPLC condition, Zorbax RX C-8 (4.6 X 250 mm), CH_3CN +0.1% TFA, 1mL/min, tR = 7.9 min (complestatin), 9.6 min (chloropeptin I). (b) The ^1H NMR spectrum of the reaction product showed the exclusive presence of chloropeptin I.
5. Authentic samples of complestatin and chloropeptin I were unavailable to us.