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COMPLESTATIN AND CHLOROPEPTIN I , CONDENSED AROMATIC PEPTIDES FROM TWO STRAINS OF STREPTOMYCETES

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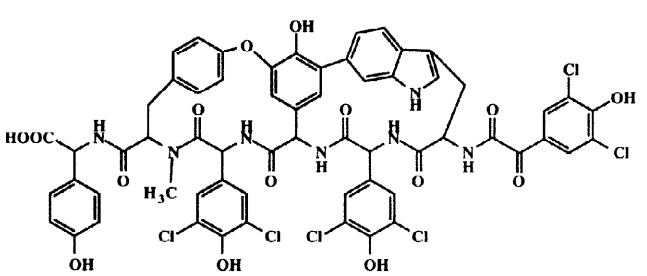
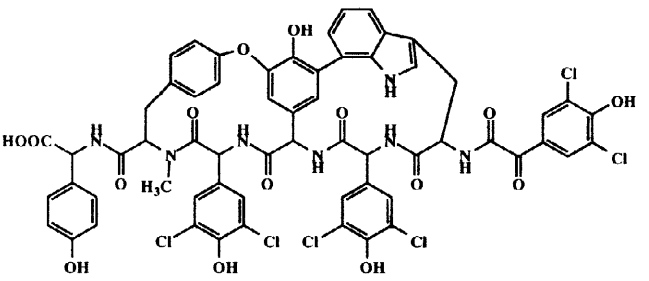
Abstract: The mycelial extract of the fermentation broths from two strains of streptomycetes were found to contain complestatin and a mixture of complestatin and chloropeptin I, respectively. Extraction and detection of these compounds using identical procedures is evidence that complestatin and chloropeptin I are indeed natural products.

Complestatin (**1**) and chloropeptin I (**2**) belong to a family of condensed aromatic hexapeptides. Complestatin was first reported by Kaneko et.al as an anticomplement agent.[1] In another report Matsuzaki et al,[2] reported the identification of chloropeptin I[3] & complestatin (chloropeptin II) as inhibitors of the binding of HIV gp-120 to CD-4 protein. These compounds also enhanced the binding of plasminogen and fibrin to U937 cells, thus potentiating fibrinolysis.[4] Complestatin and chloropeptin I are structural isomers and differ only in the position of the carbon-carbon linkage of the hydroxy phenyl (ring D) ring to the indole unit of the tryptophan as shown below. A recent report from Jayasuriya et. al,[5] concerning the conversion of complestatin to chloropeptin I in acidic conditions and the presumption that the chloropeptin I (**2**) may not be a natural product, prompted us to report our findings about these microbial metabolites. As part of our continuing investigation of bio-active natural products, we screened ethyl acetate extracts of microbial fermentation broths and identified two streptomycetes, producing these compounds. One streptomycete strain (SCC 2467) was found to produce complestatin (**1**), (yield: 145 mg/liter) while the other (SCC 2465) produced complestatin and its structural isomer chloropeptin I (**2**), (yield: 245 mg/liter; ratio: 45: 55). This paper provides evidence that both **1** and **2** are natural products.



1. Complestatin (Chloropeptin II)



2. Chloropeptin I

In the fermentation broth the inhibitors were found to be tightly bound to the mycelia and were efficiently extracted using acetone and aqueous base. The two strains were fermented in the same medium, under identical conditions.[6] After 120 hrs fermentation, the broths (5 ml) were centrifuged and the supernatants were discarded. The mycelia were washed with water and then extracted with a mixture of acetone and 1N NH_4OH (1:1). The extracts were dried, dissolved in methanol, filtered and analyzed for the presence of **1** & **2**

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using HPLC. The HPLC[7a] profiles of the extracts clearly show that one strain produces only complestatin while the other strain produces both chloropeptin I and complestatin. Both compounds were completely characterized using various spectroscopic techniques. Though we have used 0.025 % trifluoroacetic acid (TFA) (pH 2-2.2) for HPLC (Jayasuriya et. al, used 0.1%TFA) we do not see considerable decomposition under these conditions. However we have seen considerable decomposition of complestation to chloropeptin I during large scale isolation using reverse phase chromatography on XAD-16 resin and eluting with a mixture of acetonitrile and 0.05% TFA (1:1). To further validate our observation we have developed another HPLC condition[7b] and observed similar results. Under basic conditions for the isolation of these compounds, we detect complestatin from one microorganism and a mixture of complestation and chloropeptin I from the other.

Conversion of complestatin to chloropeptin I in acidic medium has been previously observed by Tachikawa et al.[4], consistent with the recent observation by Jayasuriya et. al. Our findings clearly indicate that chloropeptin I is indeed a natural product.

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6. Both were fermented using the same fermentation medium under identical fermentation conditions for 120 hours. The pH during the fermentation ranged from 5.5 to 6.5 for both strains.
7. HPLC (system): (a) Polymeric Lab. PLRPS, 5μ, (0.39 X 15 cm), CH_3CN : 0.025% TFA (45:55), 1 ml/min, Rt 14.1 min. for **1** and Rt 20.05 min. for **2**. (b) Water's Deltapak C-18 (0.39 X 15 cm) CH_3CN: 0.02M NH_4Ac (2:8) for 5 min, followed by a gradiant to 30% CH_3CN for 25 min. 1ml/min, Rt 28.4 min. for **1** and 31.8 min. for **2**.