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Structural and synthetic studies of pithomycolide

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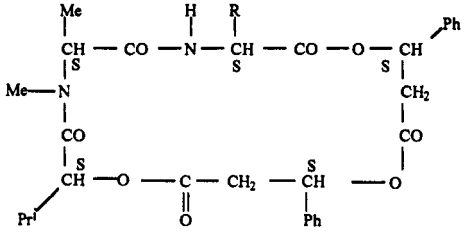
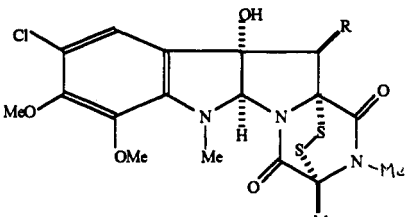
Abstract: Although the primary structure of the depsipeptide pithomycolide is known, the secondary structure has been inaccessible because of the inapplicability of x-ray techniques. We describe approaches to a secondary structure via NMR and IR data and molecular mechanics calculations, followed by a synthetic sequence designed to lead to pithomycolide and analogues.

Introduction

Pithomycolide is a depsipeptide metabolite of the fungus Pithomyces chartarum (Berk. and Curt.) M.B. Ellis. This fungus attracted attention in the early 1960's because of its implication in the etiology of the "facial eczema" disease of sheep in New Zealand pastures. The main toxic metabolites were shown to be the sporidesmins 2 and 3. Accompanying these toxins is a series of cyclodepsipeptides,

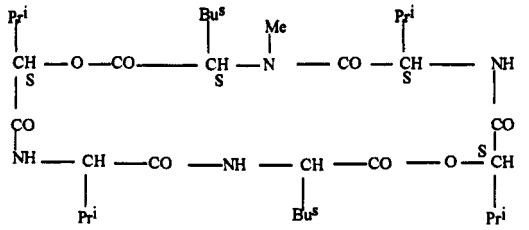


1; R=Me
5; R=Pr^i



2; R=H
3; R=OH

of which sporidesmolide I 4 is a representative major constituent. Pithomycolide



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was separated from a mixture of sporidesmin and depsipeptide constituents by chromatography and fractional crystallization and was shown to have structure 1 by chemical degradation.[1]

Structure 1 is unusual in several respects. First, the 17-membered ring containing three adjacent hydroxyacid residues including two β-hydroxy-β-phenyl propionic acid units was unprecedented in 1964; secondly, the sequence - NMeAla-Ala - appears unique. An N-MeAla residue is present in the toxins 2 and 3 but is otherwise rare.

Interest attaching to the conformation of pithomycolide prompted x-ray crystallographic investigations but attempts to solve the structure in this way have been unexpectedly unfruitful.[2] Mass spectrometric investigation[3] fully corroborated structure 1 for pithomycolide; this work also revealed the pithomycolide analogue 5 as a congeneric metabolite of P. chartarum.

Pithomycolide is labile to base hydrolysis and reduction reactions. The ester group between the Ala and the first β-hydroxy-β-phenyl propionyl residue is the first attacked chemically[1,3] and is probably the first site of ring fission on mass spectrometric electron impact.[3] It has previously been pointed out that this site is also the point of variation between 1 and 5.[3] But prior to the present work no detailed structural information on pithomycolide was available, no synthetic work had been attempted, and the biogenesis and biological function of the molecule were unknown. We now report some new structural and synthetic studies.

Structural Studies

The five acid residues of pithomycolide are self-contained with respect to proton couplings. The only conformational datum available from the early ^1H NMR work on 1 was the $^3J_{(NH\text{-}C\alpha H)}$ coupling constant for the alanyl residue of 9.0 Hz, measured in $CDCl_3$ solution. This was the basis of a calculated preferred conformation[4] shown in Figure 1. A notable implication of this conformation is the absence of an intramolecular hydrogen bond from the NH of Ala. Another implication of the conformation in Figure 1 would be two quite different ^1H-^1H coupling patterns for the β-hydroxy-β-phenylpropionic acid units. In the propionic acid unit next to alanine, the methine C_β-H is anti to one C_α-H and gauche to the other C_α-H. In the second propionic acid unit, the methine C_β-H is gauche to both C_α-H's.

Newly determined IR and NMR data rule out the predicted conformation shown in Figure 1. The NH stretch in pithomycolide, at 3322 cm^{-1} (CCl_4) suggests that this hydrogen atom is involved in a strong intramolecular hydrogen bond. The β-hydroxy-β-phenylpropionic acid units show very similar pairs of $J(HC_\beta\text{-}C_\alpha H)$ values in a 300 MHz ^1H spectrum: 11.5 and 3.1 Hz for one unit and 11.7 and 2.9 Hz for the other. These vicinal couplings imply that the correct conformation has the methine C_β-H anti to one C_α-H and gauche to the other in both propionic acid units. No significant variation of chemical shifts or couplings was observed, suggesting the possibility of a single conformation. One-dimensional steady-state nOe measurements showed no cross-ring interactions. Significant nOe's were seen from the alanine NH to the alanine CH and methyl, and to the CH and N-CH_3 of the N-methylalanine residue.

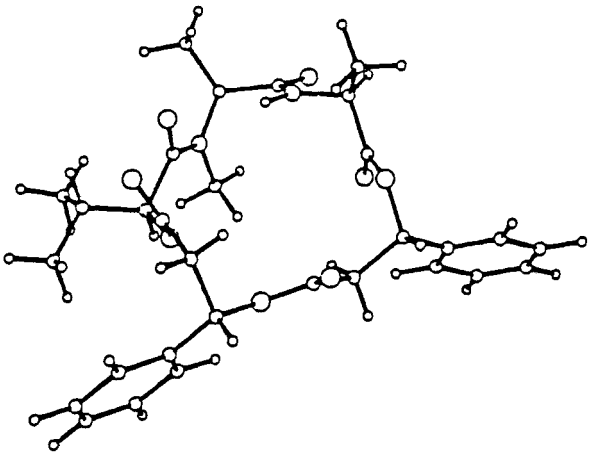


Figure 1.

1982 calculated conformation

J Compt. Chem.,1982, 3, 38.

A global conformational search was carried out in the MMX forcefield (an offshoot of the MM2 forcefield) on pithomycolide with the two phenyls replaced by methyls. More than 800 distinct minima were found. Twenty-four conformations were found within 4.0 kcal/mol of the lowest energy structure. However, the most stable conformation found was lower in energy than the second most stable by more than 1.5 kcal/mol, and the only two additional structures were found within 2.0 kcal/mol of the minimum. Furthermore, the lowest energy structure gave the best predicted agreement with the vicinal coupling constants out of all 24 structures (predicted J(HC_β-$C_\alpha H$) values of 11.6 and 3.5, and 11.7 and 3.1 Hz).

After replacing the methyls by phenyls to restore the pithomycolide formula, the lowest five structures were reminimized with conformational searching of the phenyl torsion angles. The phenyl positioning is poorly defined in regard to the torsion angles, i.e., the energy surface is rather shallow for Cβ-phenyl rotation. However, an even larger separation of 2.5 kcal/mol is found between the lowest energy structure with the most stable ring conformation and any pithomycolide structure derived from the next four most stable ring conformations.

The lowest energy structure found for pithomycolide in the MMX forcefield is shown in Figure 2. The predicted J(HC_β-$C_\alpha H$) pairs of 11.7 and 3.2 Hz, and 11.7 and 2.9 Hz are in good agreement with experiment. The 135° HN-CH dihedral

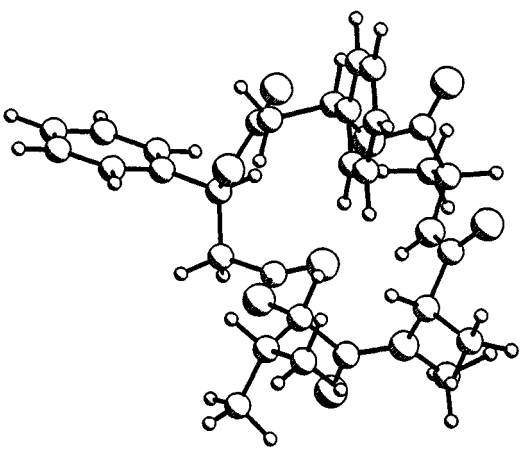


Figure 2.

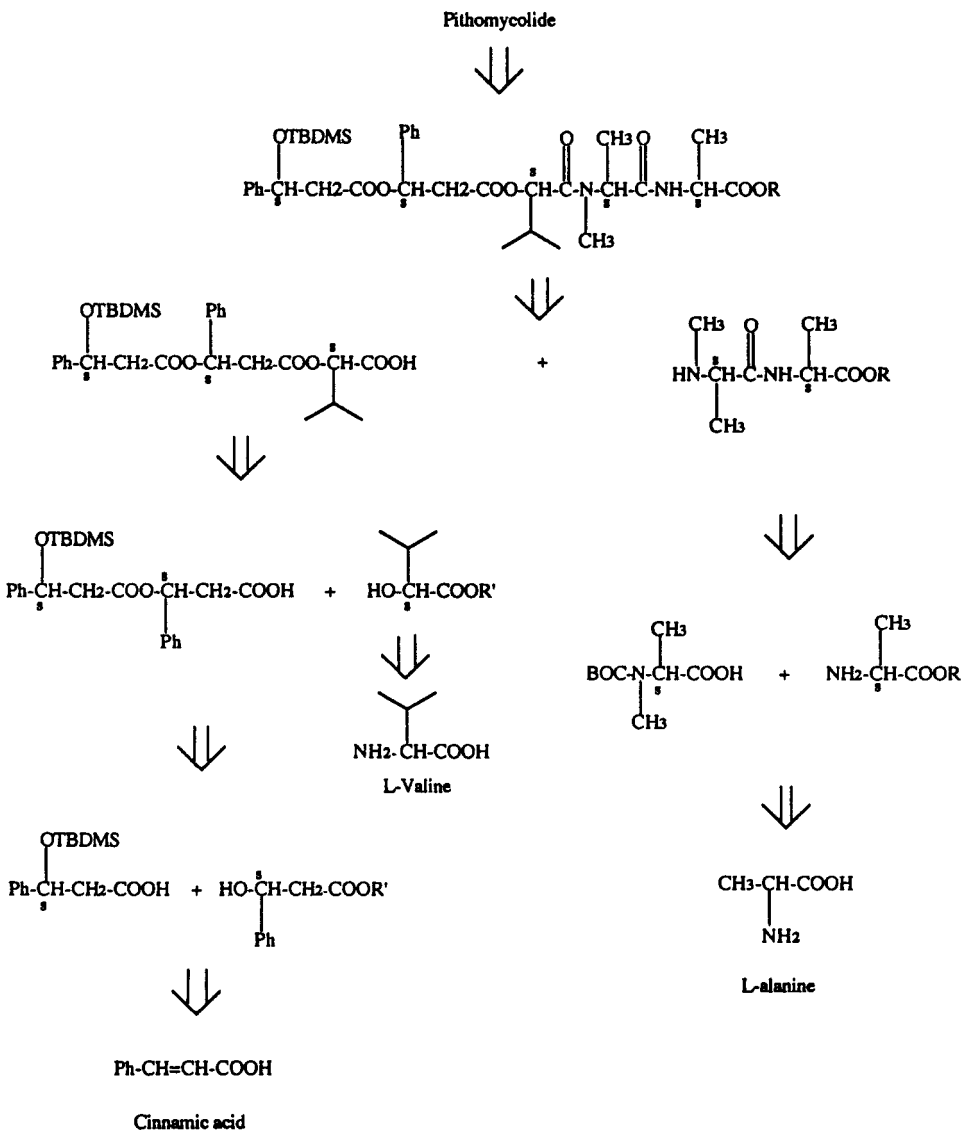
1994 Calculated Conformation of Pithomycolide (MMX)

angle is also a reasonable match for the corresponding coupling of 9.1 Hz, assuming a Karplus-type relation. The N-H is H-bonded (2.08 Å) to the carbonyl of the one of the propionic acid units. No obvious cross-ring nOe's are expected.

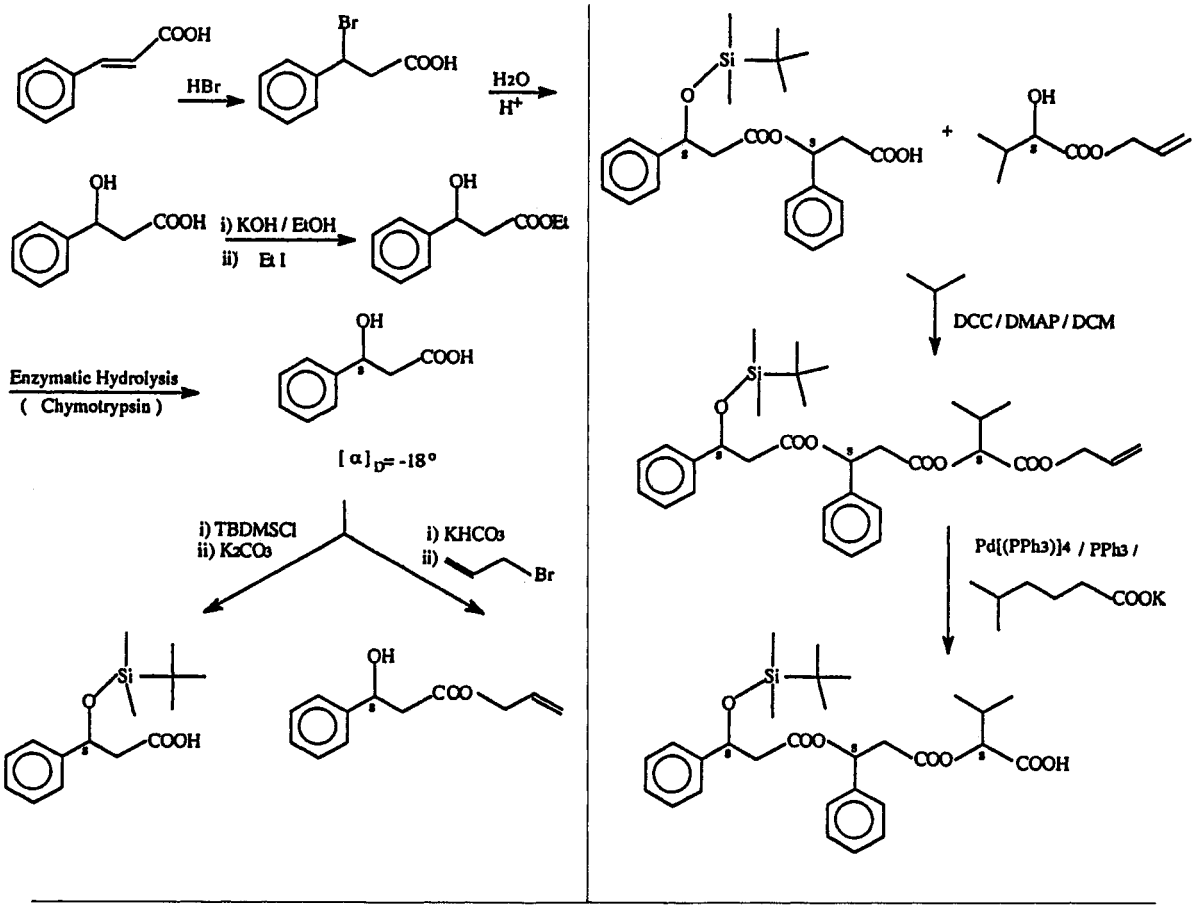
Synthetic Studies

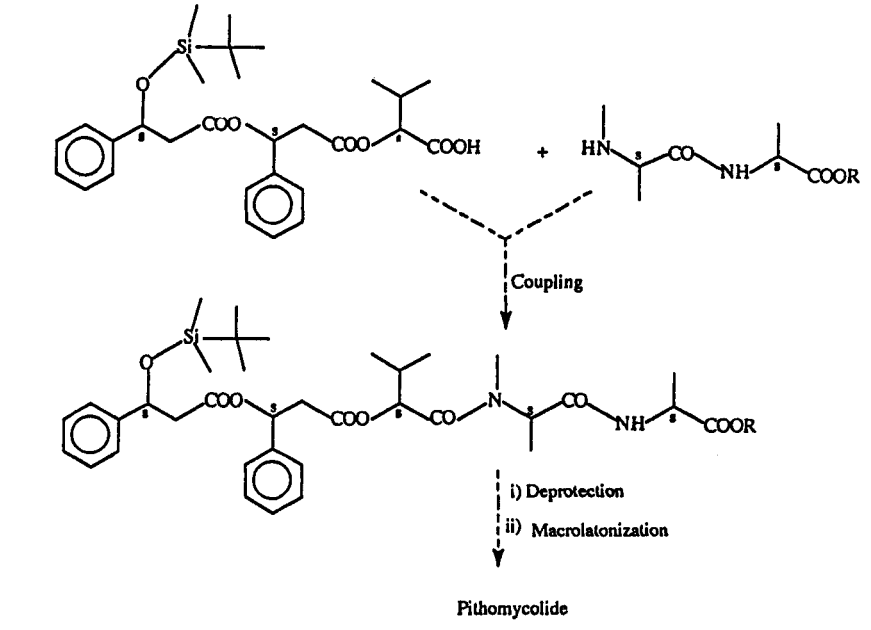
A retrosynthetic analysis followed by a summary of our synthetic studies to December 1993, is contained in the attached schemes.

RETROSYNTHESIS OF PITHOMYCOLIDE



SYNTHESIS

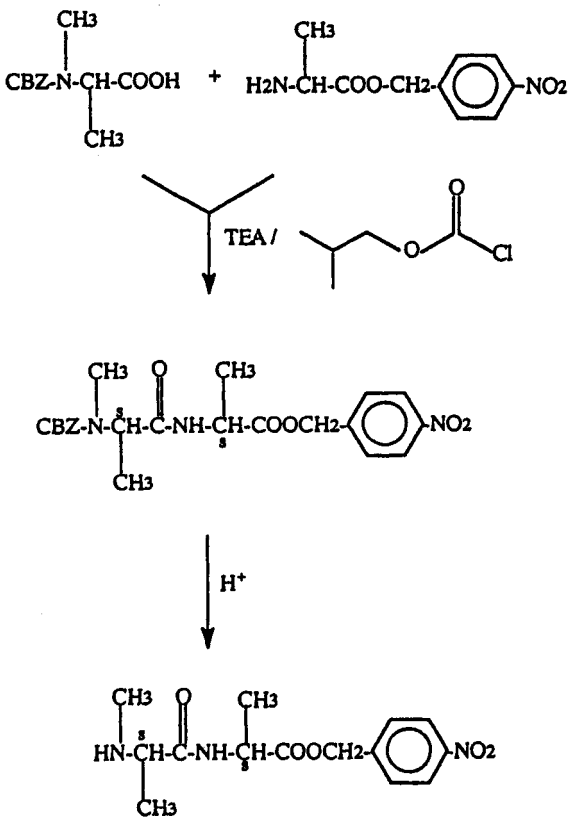




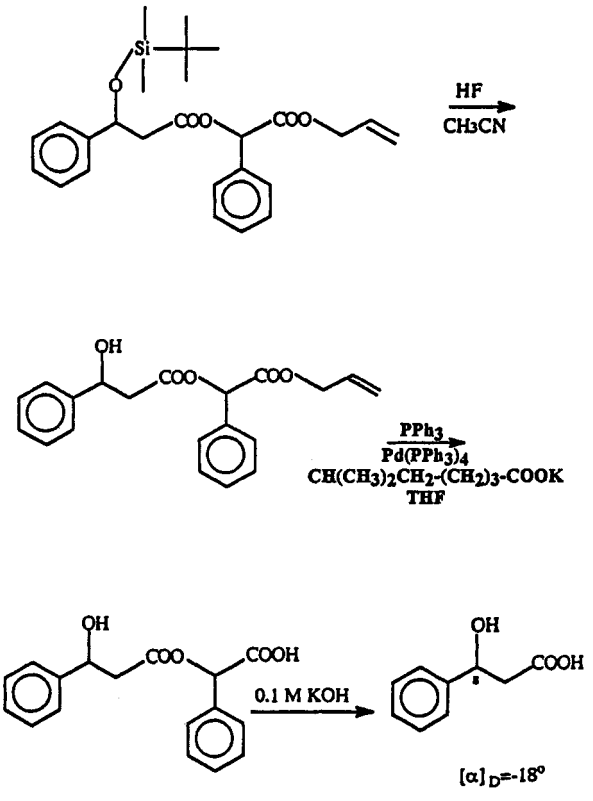
Pithomycolide

R=polymer OR -CH2- -NO2

SOLUTION PHASE SYNTHESIS OF THE DIPEPTIDE



PROOF OF ABSENCE OF RACEMIZATION DURING ESTER BOND FORMATION



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