Tetrahedron Letters,Vol.28,No.48,pp 6001-6004,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

STEREOCHEMICAL STUDIES OF THE SKIPPED-POLYOL POLYENE MACROLIDE CLASS: DEGRADATION AND PARTIAL STRUCTURE DETERMINATION OF MYCOTICIN A AND B.

Stuart L. Schreiber and Mark T. Goulet*
Sterling Chemistry Laboratory
Yale University, New Haven, Connecticut 06511

Abstract The degradation of prototype members of the skipped-polyol polyene macrolide class, mycoticins A and B, is reported. The characterization of the resultant fragments allows a partial structure determination to be achieved.

The polyene macrolides comprise a large class of natural products which display potent antiviral and antifungal activity and have been successfully employed in antifungal therapy.[1] These compounds selectively alter the permeability of membranes that contain sterols; accordingly, they show activity against yeast, fungi, and eukaryotic cells, but not bacteria.[2] The design and synthesis of analogues with increased selectivity towards ergosterol vs. cholesterol, characteristic of fungal and mammalian cells, respectively, should result in fungicides with diminished toxicity and represents a worthy and challenging goal.[3] Structural leads are available from selectivity studies that have demonstrated, for example, that phosphatidylcholine vesicles containing ergosterol were markedly more and less sensitive to amphotericin and filipin, respectively, than corresponding preparations containing cholesterol.[4] Unfortunately, the interpretation of such structure-function studies is complicated by the paucity of stereochemical information within the polyene macrolide class. Since the discovery of the first polyene macrolide nystatin (now used in antifungal therapy) in 1950[5], over 200 members have been reported. Although the constitution of over 40 members have been determined, the claim of a full stereochemical elucidation of structure is reserved for a single member of this class, the antiviral, antifungal agent amphotericin B.[6,7,8]

A striking feature of a large subclass within this family is the presence of a long chain equipped with alternating -CH(OH)- and -CH_2- groups: the skipped-polyol polyene macrolides.[9] We have previously reported that speculation of stereochemistry based on structural homology of members of this class, represented by mycoticin A and B **1**[10], to the distant relative amphotericin B is not fruitful.[11] In particular, the conjecture that these members contain the all syn-skipped-polyol configuration was demonstrated to be incorrect in the case of mycoticin A and B. Recently, we have been successful in achieving the full assignment of stereochemistry of these prototype members of the skipped-polyol polyene class.[12] Central to these investigations was the degradation of the natural products and spectroscopic analysis of the resultant fragments. The results of these studies are described in this Letter.

Mycoticin A and B were obtained from shake cultures of *Streptomyces ruber* (ATCC #3348) according to the procedure developed by Wasserman and McCaustland[10d] during their studies of mycoticin constitution and biosynthesis. Extraction of the mycelia and purification by flash chromatography[13] resulted in the acquisition of a 1:1 mixture of mycoticin A and B that exhibited the same properties as those reported by Wasserman. Refinement of the procedure led to an optimized yield of 0.8-1.0 g./liter of broth. For the purpose of stereochemical analysis (vide infra) the formation of dioxane derivatives of the mycoticins was investigated. Results of experiments performed on the tetraacetonide **2** will be described herein; the related tetraformylal derivative is described in the accompanying Letter.

Tetraacetonide **2** was obtained by treatment of an acetone suspension of the macrolide with p-toluenesulphonic acid (Scheme 1). Mycoticin A and B tetraacetonide could be separated at this stage by HPLC (μ porasil, 4:1 Hexane/EtOAc). Ozonolysis of the mixture, however, followed by

Scheme 1

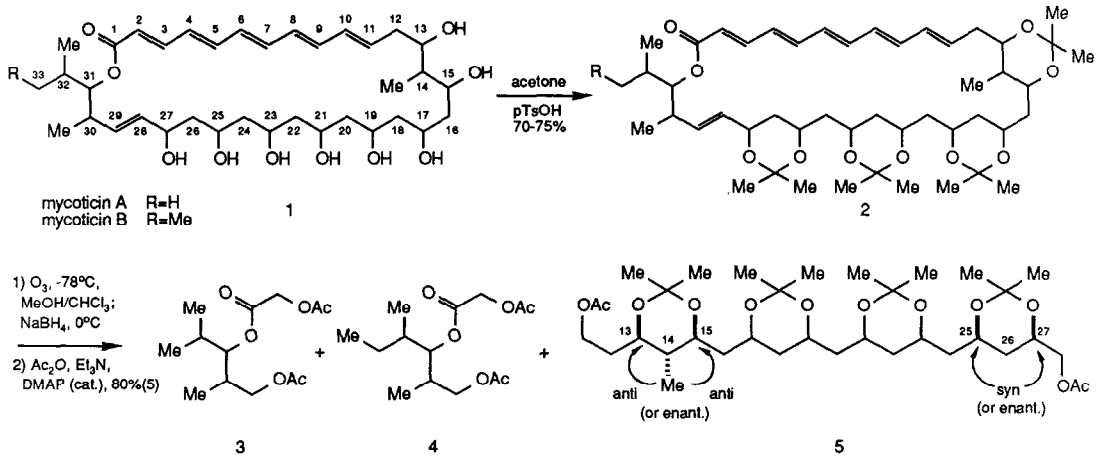
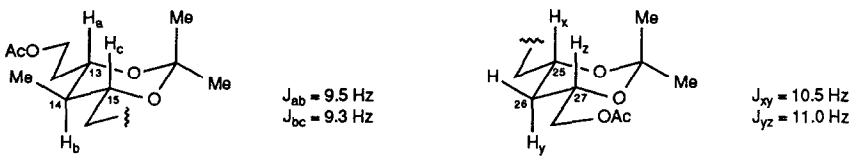


Figure 1

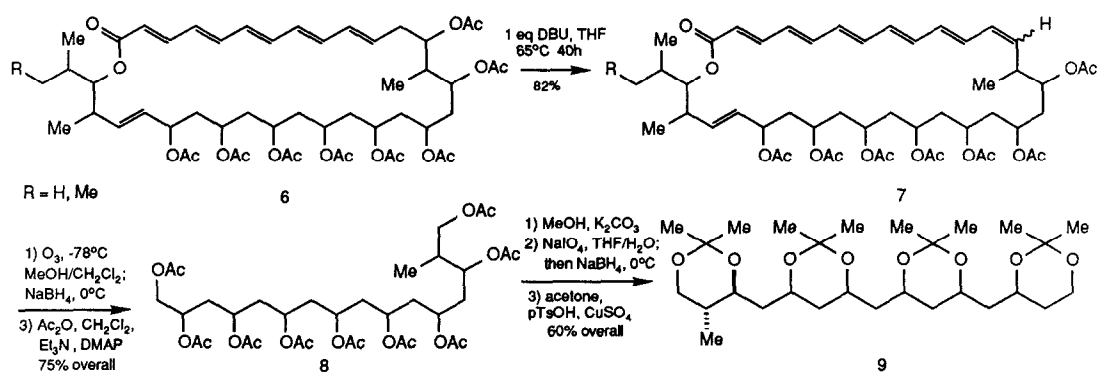


reduction with sodium borohydride and acetylation provided the readily separable products **3**, **4** and **5**, indicating a stereochemical homology along the skipped-polyol chains of mycoticin A and B.

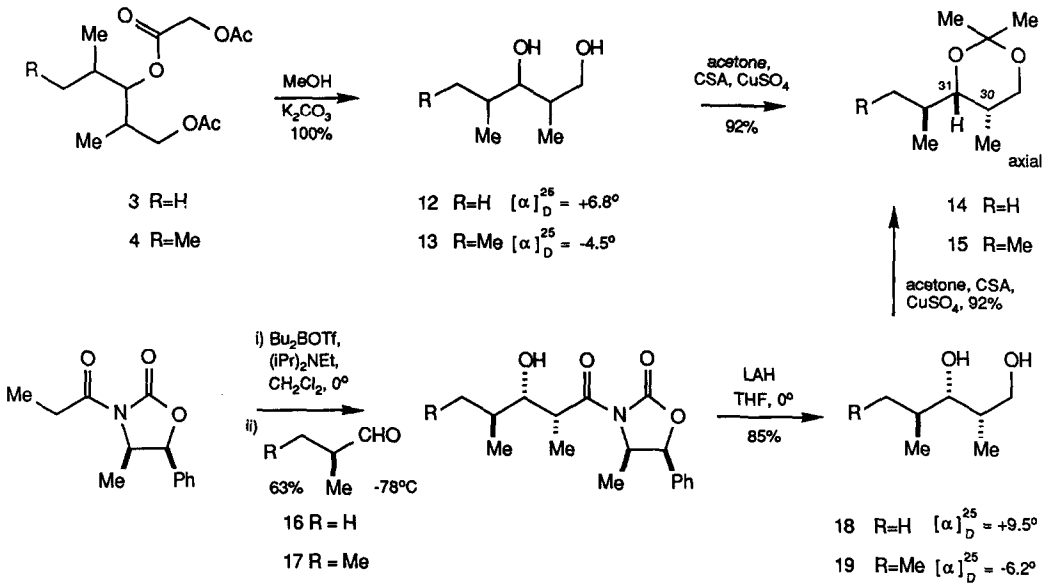
We were able to assign proton signals at several carbon centers, including C_{13}, C_{15}, C_{25}, C_{27} and C_{28}, of the bisacetate **5**.[14] The salient features of the spin multiplicities are indicated in Figure 1 and provide support for the indicated local conformations and relative stereochemical assignments illustrated in structure **5**.

With the relative stereochemistry of the outer stereocenters in **5** resolved, further degradation of **1** was undertaken in order to simplify a potential skipped-polyol chain structure as a target for synthesis. Despite reported difficulties in the attempted dehydration of flavofungin (= mycoticin),[10e]

Scheme 2



Scheme 3



we found the mono-elimination of acetic acid from the octaacetate **6** (Scheme 2) could be achieved in high yield by the action of 1 eq DBU in refluxing THF. The oxohexaene **7**, as a mixture of two isomers, was subjected to an ozonolysis, reduction ($NaBH_4$) and acetylation sequence to deliver the nonaacetate **8** as a single isomer. Further degradation of the nonaol with sodium periodate and subsequent reduction ($NaBH_4$) and ketonization produced the tetraacetonide **9**. Although the NMR analysis of **9** provided no further stereochemical information, **9** served as a key target molecule throughout these studies for comparison to related materials derived from synthesis. A recent synthesis of the all-syn (polyol) isomer of **9** is illustrative.[11,15]

The absolute stereochemistry of stereocenters along the C_{30}-C_{33} fragment of mycoticin A and B was determined by independent synthesis of derivatives of fragments **3** and **4** (Scheme 3). The naturally derived diols **12** and **13** are readily obtained by saponification of **3** and **4**, respectively. NMR analysis of the acetonides **14** and **15** resulted in the assignment of the indicated syn-stereochemistry at C_{30} and C_{31} of both isomers. The absolute stereochemistry of diols **12** and **13** was determined by employment of the Evans' asymmetric aldol method[16a] with aldehydes **16** and **17**. The resultant diols[16b] **18** and **19** were shown to be identical to the naturally derived diols **12** and **13**, respectively, by the criteria of chromatographic mobility (TLC) and NMR spectroscopy. A similar comparison was made with the derived acetonides. The sign of optical rotations of the diols, in combination with the known stereodirecting properties of the Evans' oxazolidinone auxiliary, was supportive of the indicated absolute stereochemistry.[17]

The studies described herein were successful in reducing the number of stereoisomeric permutations of mycoticin B from 4096 to 64. The concurrent synthesis and demonstrated nonequivalence of the syn-isomer of **9** to the naturally derived material demanded further spectroscopic studies of more illuminating mycoticin derivatives. One such compound is described in the following Letter.

Acknowledgements These investigations were supported by the NIGMS, NSF (Presidential Investigator Award), Alfred P. Sloan Foundation (1985-87), and The Camille and Henry Dreyfus Foundation, Inc. (Teacher/Scholar Award 1984-89) to whom we are grateful. Matching funds for the NSF-PYI Award were generously provided by Pfizer, Inc. We thank Gayle Schulte for her efforts with several crystallographic analyses and Professor Harry H. Wasserman for his advice and encouragement.

References and Footnotes

1. Reviews: (a) Gale, E.F.; Curdliffe, E.; Reynolds, P.E.; Richmond, M.H.; Waring, M.J. In *The Molecular Basis of Antibiotic Action*, John Wiley: New York, 1981, p. 201-219. (b) Omura, S.; Tanaka, H. In *Macrolide Antibiotics: Biology and Practice*, Omura, S., Ed.; Academic: New York, 1984, pp. 351-404.
2. (a) Kinsky, S.C. *Ann. Rev. Pharmacology* **1970**, *10*, 119. (b) Hamilton-Miller, J.M.T. *Adv. Appl. Microbiology* **1974**, *17*, 109.
3. Floyd, D.M.; Fritz, A.W. *Tetrahedron Lett.* **1981**, *22*, 2847.
4. (a) De Kruilff, B.; Gerritsen, W.J.; Oerlemans, F.; Van Dijck, P.W.M.; Demel, R.A.; VanDeenan, L.L.M. *Biochim. Biophys. Acta* **1974**, *339*, 44. (b) Archer, D.B. *Biochim. Biophys. Acta* **1976**, *436*, 68.
5. Hazen, E.L.; Brown, R. *Science* **1950**, *112*, 423.
6. *Structure determination*: Mechlinski, W.; Schaffner, C.P.; Ganis, P.; Avitabile, G. *Tetrahedron Lett.* **1970**, 3873. *Synthesis*: Nicolaou, K.C.; Daines, R.A.; Uenishi, J.; Li, W.S.; Papahatjis, D.P.; Chakraborty, T.K. *J. Am. Chem. Soc.* **1987**, *109*, 2205; Nicolaou, K.C.; Daines, R.A. Chakraborty, T.K. *J. Am. Chem. Soc.* **1987**, *109*, 2208.
7. Despite several attempts, we have not been successful in gaining structural information on mycoticin A and B or derivatives via X-ray crystallographic methods.
8. Stereochemical studies of the polyene macrolide lienomycin have been reported: Pawlak, J.; Nakanishi, K.; Iwashita, T.; Borowski, E. *J. Org. Chem.* **1987**, *52*, 2896.
9. An arbitrary distinction has been made between compounds such as amphotericin B, which contains two consecutive skipped hydroxyls, and the mycoticins, which contain eight consecutive skipped-hydroxyls.
10. Mycoticin A and B are the first members of this class whose constitutions were determined and are apparently identical to the flavofungins: (i) *mycoticin:* (a) Burke, R.C.; Swartz, J.H.; Chapman, S.S.; Huang, W.Y. *J. Invest. Dermatol.* **1954**, *23* , 163. (b) Wasserman, H.H.; VanVerth, J.E.; McCaustland, D.J.; Barowitz, I.J.; Kamber, B. *J. Am. Chem. Soc.* **1967**, *89*, 1535. (c) Wasserman, H.H.; Zoretic, P.A.; Mariano, P.S. *J. Chem. Soc., Chem. Commun.* **1970**, 1634. (d) McCaustland, D.J. Ph.D. Thesis, Yale University, 1960. (ii) *flavofungin:* Bognar, R.; Makleit, S.; Zsupan, K.; Brown, B.O.; Lockley, W.J.S.; Toube, T.P.; Weedon, B.C.L. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1848.
11. Schreiber, S.L.; Goulet, M.T.; Schulte, G. *J. Am. Chem. Soc.* **1987**, *109*, 4718.
12. Unpublished results of M.T. Goulet; manuscript in preparation.
13. Still, W.C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
14. C_{13}-H (δ 3.53) $J_{(13\text{-}14)}$9.5Hz, $J_{(13,12a)}$9.5Hz, $J_{(13,12b)}$2.3Hz; C_{14}-H (δ 1.31) $J_{(14,13)}$9.5Hz, $J_{(14,15)}$9.3Hz; C_{15}-H (δ 3.51) $J_{(15,14)}$9.3Hz; C_{25}-H (δ 4.18) $J_{(25,26a)}$10.5Hz; C_{27}-H (δ 4.02) $J_{(27,26a)}$11.5Hz, $J_{(27,28a)}$9.2Hz, $J_{(27,28b)}$3.8Hz.
15. The synthesis of other isomers, including one that corresponds to the natural configuration, will be reported shortly.
16. (a) Evans, D.A.; Bartoli, J.; Shih, T.L. *J. Am. Chem. Soc.* **1981**, *103*, 2127. (b) Evans, D.A.; Ennis, M.D., Mathre, D.J. *J. Am. Chem. Soc.* **1982**, *104*, 1737.
17. The reasons for the discrepancy in optical rotations have not been determined; however, our conclusions are corroborated CD comparisons of the naturally and synthetically derived dibenzoates of **18** and **19**.

(Received in USA 4 August 1987)