ORGANIC LETTERS

2006
Vol. 8, No. 21
4751−4754

Stereochemical Determination of Archazolid A and B, Highly Potent Vacuolar-Type ATPase Inhibitors from the Myxobacterium *Archangium gephyra*

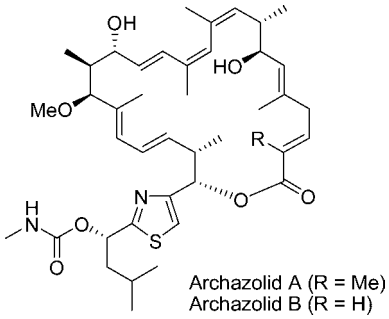
Jorma Hassfeld,[†] Christophe Farès,[§] Heinrich Steinmetz,[‡] Teresa Carlomagno,[§] and Dirk Menche*,[†]

Gesellschaft für Biotechnologische Forschung mbH, Medizinische Chemie and Umweltmikrobiologie, Mascheroder Weg 1, D-38124 Braunschweig, Germany, and Max Planck Institute for Biophysical Chemistry, Department of NMR based Structural Biology, Am Fassberg, 11, D-37077 Göttingen, Germany

dme05@gbf.de

Received July 25, 2006

ABSTRACT



The relative and absolute stereochemistry of the structurally unique 24-membered myxobacterial macrolides archazolid A and B, highly potent vacuolar-type ATPase (V-ATPase) inhibitors in vitro and in vivo, was determined on the basis of a combination of extensive high-field NMR studies, including *J*-based configuration analysis, molecular modeling, and chemical methods.

Vacuolar-type ATPases (V-ATPases) are heteromultimeric, proton translocating proteins, which are localized in a multitude of eukaryotic membranes and energize many different transport processes.[1] As their malfunction is correlated with various diseases such as renal acidosis,[2] osteoporosis,[3] or cancer,[4] the development and molecular understanding of selective inhibitors present important research goals. The polyketide natural products archazolid A and B, originally isolated at GBF in the early nineties,[5] constitute a structurally novel type of particularly efficient (IC_{50} in the low nanomolar range) and specific inhibitors of V-ATPases, both in vitro[6] and in vivo.[7] The planar structure of the archazolids (Figure 1), featuring a 24-membered

[†] Gesellschaft für Biotechnologische Forschung, Medizinische Chemie.
[§] Max Planck Institute for Biophysical Chemistry.
[‡] Gesellschaft für Biotechnologische Forschung, Umweltmikrobiologie.

(1) (a) Wieczorek, H.; Brown, D.; Grinstein, S.; Ehrenfeld, J.; Harvey, W. R. *Bioessays* **1999**, *21*, 637. (b) Nishi, T.; Forgac, M. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 94.

(2) Karet, F. E.; Finberg, K. E.; Nelson, R. D.; Nayir, A.; Mocan, H.; Sanjad, S. A.; Rodriguez-Soriano, J.; Santos, F.; Cremers, C.; Di Pietro, A.; Hoffbrand, B. I.; Winiarski, J.; Bakkaloglu, A.; Ozen, S.; Dusunsel, R.; Goodyer, P.; Hulton, S. A.; Wu, D. K.; Skvorak, A. B.; Morton, C. C.; Cunningham, M. J.; Jha, V.; Lifton, R. P. *Nat. Genet.* **1999**, *21*, 84.

(3) Schlesinger, P. H.; Blair, H. C.; Teitelbaum, S. L.; Edwards, J. C. *J. Biol. Chem.* **1997**, *272*, 18636.

(4) Sennoune, S. R.; Luo, D.; Martinez-Zaguilan, R. *Cell. Biochem. Biophys.* **2004**, *40*, 185.

(5) Höfle, G.; Reichenbach, H.; Sasse, F.; Steinmetz, H. German Patent DE 41 42 951 C1, 1993.

(6) Huss, M.; Sasse, F.; Kunze, B.; Jansen, R.; Steinmetz, H.; Ingenhorst, G.; Zeeck, A.; Wieczorek, H. *BMC Biochem.* **2005**, *6*, 13.

(7) Sasse, F.; Steinmetz, H.; Höfle, G.; Reichenbach, H. *J. Antibiot.* **2003**, *56*, 520.

10.1021/ol061831y CCC: $33.50
Published on Web 09/20/2006

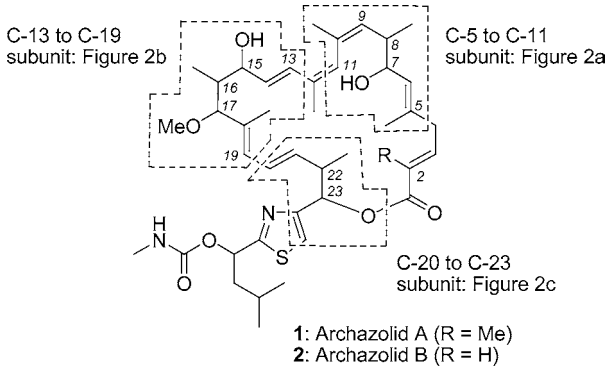


Figure 1. Planar structure of the archazolids.

macrolactone ring with seven alkenes (2*E*,5*E*,9*Z*,11*Z*,13*E*,-18*E*,20*E*), a thiazole side chain, and a characteristic sequence of eight methyl- and hydroxyl-bearing stereocenters, was elucidated by Höfle and Steinmetz on the basis of spectroscopic data, in particular ^{1}H and ^{13}C NMR, COSY, NOE, and HMQC data.[5,7,8] The contradictory UV maximum at 229 nm was explained by distortion of the conjugated triene system.[8] Eventually, the structure was proven by the analysis of a ^{13}C-labeled sample of archazolid A obtained by the feeding of ^{13}C-enriched acetate and methionine to *Archangium gephyra*.[8,9]

As a prelude to initiating a synthetic campaign to enhance the supply of the archazolids for biological evaluation, we now report the determination of the full stereostructure through the application of *J*-based configuration analysis[10] combined with extensive NOESY and ROESY experiments, molecular modeling, and synthetic derivatization.

Optimum ^{1}H signal dispersion was realized in CD_3OD and DMSO-d_6 at the highest available field strengths (600 and 900 MHz) allowing complete assignment of all resonances.[11] The $^{3}J_{H,H}$ coupling constants were extracted from a combination of 2D *J*-resolved spectra and homonuclear decoupling experiments, and measurement of heteronuclear coupling constants ($^{2,3}J_{C,H}$) relied on analysis of HSQC−TOCSY and HSQC−HECADE spectra.[12] Notably, the coupling constants and NOESY correlations suggested the C-6−C-9 and C-15−C-19 regions to be relatively rigid, whereas at least two rapidly interconverting conformations had to be considered for the C-20−C-23 region (vide infra).[13]

(8) Steinmetz, H.; Höfle, G. personal communication.

(9) Archazolid A: With the exception of C-23, the macrocyclic backbone is exclusively constructed from acetate, and the *C*-, *O*-, and *N*-methyl groups originate from methionine.

(10) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866.

(11) The observed coupling constants and NOE data in CD_3OD and DMSO-d_6 are very similar, which suggests that archazolid adopts a similar conformation in these solvents.

(12) (a) Kozminski, W.; Nanz, D. *J. Magn. Reson.* **2000**, *142*, 294. (b) Marquez, B. L.; Gerwick, W. H.; Williamson, R. T. *Magn. Reson. Chem.* **2001**, *39*, 499.

(13) Broadening of the ^{1}H NMR signals for H-15 and H-16 and the respective $^{2,3}J_{C,H}$ signals also suggest a certain degree of flexibility around the (15,16)-bond.

Establishing a relationship between the substituents at C-7 and C-8 of archazolid A (Figure 2a) relied on *J*-based

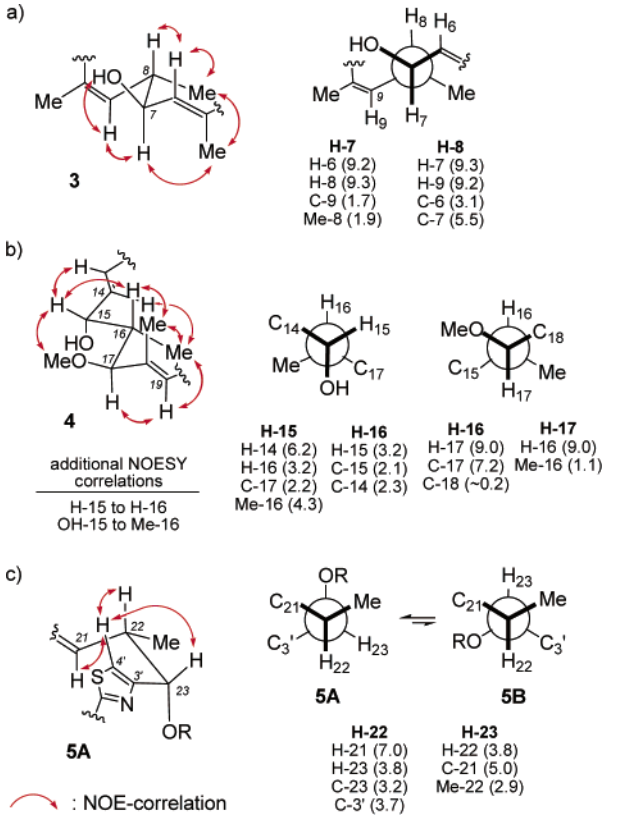


Figure 2. Rotamers determined for the C-5−C-11 subunit **3** (a), the C-13−C-19 subunit **4** (b), and the C-20−C-23 subunit **5** (c) of archazolid A; coupling constants, $^{3}J_{H,H}$ and $^{2,3}J_{H,C}$ (Hz), are in parentheses.

configuration analysis and relevant NOESY correlations. A large homonuclear coupling between H-7 and H-8, small couplings from H-7 to C-9 and Me-8 and from H-8 to C-6, and a large scalar coupling from H-8 to C-7 indicated an antiperiplanar relationship. In a similar fashion, antiperiplanar relationships between H-6 and H-7 as well as between H-8 and H-9 may be deduced, suggesting that the C-5−C-11 subunit resides in the depicted conformation **3**. Three key NOESY correlations, from Me-8 to H-6 and to Me-5 and from OH-7 to H-9, supported the relative assignment as shown.

The homo- and heteronuclear coupling constants observed for the C-13−C-19 subunit of archazolid A (**1**) are listed in Figure 2b. The available data for the C-14−C-16 region suggested a syn relationship between the adjacent methyl and hydroxyl substituents at C-15 and C-16, by small couplings observed from H-15 to H-16, from H-15 to C-17, and from H-16 to C-14. This assignment was supported by strong NOE correlations from H-15 to H-16 and from OH-15 to Me-16. A large coupling between H-16 and H-17 suggested an antiperiplanar relationship between these protons, supported by small couplings from H-16 to C-18

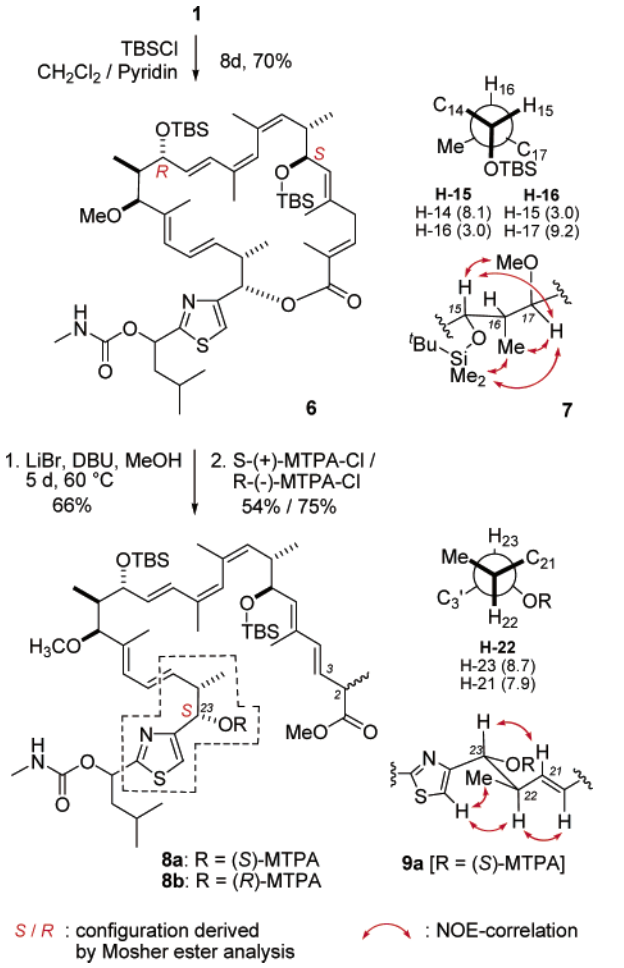
and from H-17 to Me-16 and by a large coupling from H-16 to C-17. A series of NOESY correlations (from H-15 to H-13 and OMe-17, Me-16 to H-14, H-19 and Me-18) confirmed the relative orientation depicted (16,17-anti).

As illustrated in Figure 2c, both the homo- and heteronuclear couplings observed for the C-21−C-23 segment of archazolid A suggested the contribution of two or more rapidly interconverting conformations. In particular, medium couplings from H-22 to H-23, from H-22 to Me-23, from H-22 to C-3′, and from H-23 to C-21 supported a degree of conformational flexibility. A relatively small heteronuclear coupling between H-23 and Me-22 and medium couplings, H-22−H-23, H-22−C-23, and H-23−C-21, indicated two interconverting conformers with gauche relationships of H-23 and Me-22. NOESY correlations, from H-4′ to H-21 and H-22, suggested a vicinity between the thiazol C-4′ and C-21, which is explicable by the relative assignment, as shown (**5A**, **5B**).[14] This assignment was confirmed by chemical methods (see below).

Because determination of the relationship between these stereoclusters, as well as a correlation to the stereocenter in the side chain, were not possible through *J*-based configuration analysis, molecular modeling was carried out on the possible stereochemical permutations. Using Macromodel (version 8.5),[15] 20 000-step Monte Carlo searches were performed. With the MMFFS and the generalized Born/surface area (CB/SA) water solvent model,[16] a series of discrete families of low-energy conformations were found for the various stereoisomers within 10.00 kcal mol^{-1} of the global minimum. These structures were further refined by AM1. The lowest-energy conformation for **1a** (see Supporting Information) accounted for a number of key transannular ROESY correlations (i.e., H-3−H-21, Me-8−H-13, H-17 and H-19). Examination of the calculated dihedral angles and correlation to a corresponding series of $^{3}J_{H,H}$ coupling constants resulted in an acceptable match with the experimental NMR data for the northeastern part (C-3−C-13) and the central western fragment (C-16−C-21) of the macrocycle. However, the calculated conformation for the C-15−C-16 area deviated from the observed dihedral angles and the conformational flexibility in the C-22−C-23 region was not correctly predicted. This picture was refined using conformational restraints based on dihedral angles and an approximation of selected H−H distances by NOESY experiments at different mixing times (see Supporting Information).[17] Nonetheless, defining a convincing relationship between the stereoclusters in the northern part and the southern part of the macrocycle and a correlation to the side chain called for more rigorous means for further stereochemical proof.

The 15,16-syn assignment was corroborated by *J*-based configurational analysis of the bis-TBS ether **6** (Scheme 1),

Scheme 1. Configurational Assignment of the Macrocyclic Core of Archazolid A



which adopts a conformation (**7**) that is closely related to that of the parent compound in the respective region (**4**, see Figure 2). A series of ROESY correlations (from H-15 to H-17 and OMe-17, and from TBS-15 to Me-16 and H-17) confirm the relative orientation depicted. The absolute configuration at C-7 and C-15 was deduced by Mosher ester analysis of the bis-Mosher esters of archazolid A (see Supporting Information). In a similar fashion, the configuration at C-23 was delineated on the opened derivatives **8a**/**8b**.[18,19] As presented in conformation **9a**, these Mosher ester derivatives allowed us to confirm the 22,23-anti relationship by large couplings between H-21 and H-22 and between

(14) It should be noted that the observed coupling constants can also be rationalized by related conformers suggesting a 22,23-syn relationship: see ref 10.

(15) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

(16) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127.

(17) Two families of conformers were predicted, which are closely related to structures **5A** and **5B**. These were found by AM1 to be 5.2/2.0 kcal mol^{-1} higher in energy than the global minimum as obtained from a nonrestrained conformational search under the same conditions (see Supporting Information). These structures show a very high degree of agreement with the observed dihedral angles and transannular NOEs.

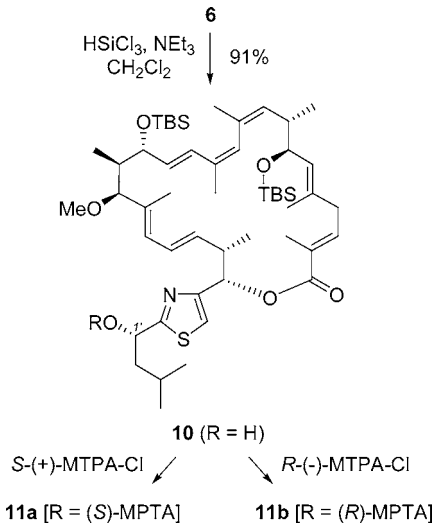
(18) (a) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543. (b) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.

(19) Ring cleavage under these conditions gives a 3:1 mixture of the shown isomer and the one without isomerization of the (2,3)-double bond. The spectral data of these isomers are identical in the crucial western region of the molecule: see Supporting Information.

H-22 and H-23 in combination with key ROESY interactions from H-4′ to H-22 and Me-22, from H-20 to H-22, and from H-21 to H-23.

For assigning the configuration at C1′, a selective cleavage of the carbamate was accomplished by a method of Pirkle and Hauske using trichlorosilane in the presence of triethylamine to give the truncated archazolid derivative **10** (Scheme 2).[20] Mosher ester analysis allowed unambiguous designation

Scheme 2. Selective Cleavage of the Side-Chain Carbamate and Configurational Assignment at C-1′.



of the C1′ configuration as shown.

Structure **12** (Figure 3) summarizes the assignment of the full relative and absolute configuration for archazolid A. On the basis of a common biogenesis, the stereochemistry of archazolid B was assigned as **13**. This assignment is in full agreement with the close similarity of the spectral data of archazolid A and B (see Supporting Information).

In conclusion, a full stereochemical assignment for the antimitotic macrolide archazolids A and B is proposed as

(20) Pirkle, W. H.; Hauske, J. R. *J. Org. Chem.* **1977**, *42*, 2781.

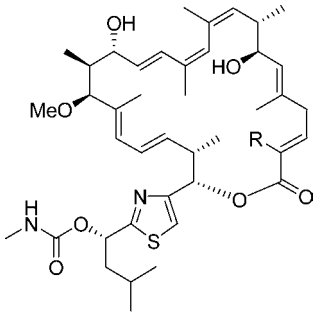


Figure 3. Stereostructures for archazolid A and B.

12 and **13** (2*E*,5*E*,7*S*,8*S*,9*Z*,11*Z*,13*E*,15*R*,16*S*,17*S*,18*E*, 20*E*,-22*S*,23*S*,1′*S*) on the basis of the results of extensive high-field NMR studies, including *J*-based configurational analysis, molecular modeling, and chemical derivatization. Confirmation of this proposal will rely on the stereocontrolled total synthesis of the archazolids.

Acknowledgment. Generous financial support from the Fonds der Chemischen Industrie (“Liebig-Stipendium” to D.M.) and the Volkswagenstiftung (Funding Initiative: “Interplay between Molecular Conformations and Biological Function”) is gratefully acknowleged. The work was supported by the MPG. We thank Gerhard Höfle (GBF, Braunschweig) for fruitful discussions, Florenz Sasse and Bettina Hinkelmann (GBF, Braunschweig) for fermentation of *Archangium gephyra*, and Tatjana Arnold (GBF, Braunschweig) for technical support.

Supporting Information Available: Tables of spectral and calculated data, experimental details and copies of 1D and 2D NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

OL061831Y