Natural Products

# Abyssomicin C—A Polycyclic Antibiotic from a Marine *Verrucosispora* Strain as an Inhibitor of the *p*-Aminobenzoic Acid/Tetrahydrofolate Biosynthesis Pathway**

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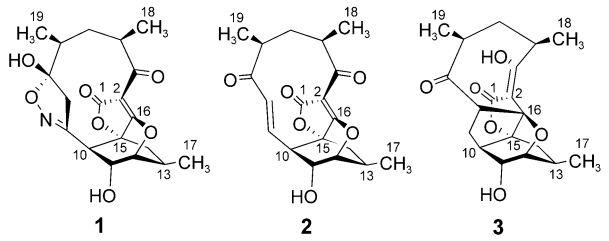
*Dedicated to Günther Jung and Louis Moroder*

For many multiresistant Gram-positive bacteria, for example, methicillin-resistant *Staphylococcus aureus* strains (MRSA), vancomycin- or teicoplanin-type glycopeptide antibiotics are the sole remaining means of treatment. As a consequence, efforts of researchers from various disciplines have been directed towards the discovery and development of novel antibiotics. In this process of drug finding, nature has always been an important source, and the cultivation of microorganisms collected from previously unexplored habitats may possibly reveal lead structures for new antibiotics. Similarly, the targeting of biosynthetic processes in the bacterial cell other than those previously investigated might also lead to the discovery of new antibiotics. In this context we have directed our attention to rare actinomycetes from the deep-sea plain and screened them for inhibitors of *p*-aminobenzoate (*p*ABA) biosynthesis.

The biosynthesis of *p*ABA is part of the biosynthesis of tetrahydrofolate (THF).[1] Among the few but prominent inhibitors of THF biosynthesis are sulfonamides and trimethoprim. The biosynthesis of *p*ABA is a very attractive target since it is found in many microorganisms but not in humans. In the present contribution we describe the structure elucidation of new polycyclic polyketide-type antibiotics, which we have named abyssomicins. Interestingly, the abyssomicins fulfill the criteria mentioned above: they are produced by the rare actinomycete *Verrucosispora* and are inhibitors of the *p*ABA biosynthetic pathway. To our knowledge, the abyssomicins are the first known substances derived from a bacterial source that inhibit the biosynthesis of *p*ABA.

The actinomycete *Verrucosispora* strain AB 18-032 was isolated from a sediment sample collected in the Japanese Sea at a depth of 289 m. By using an agar-plate diffusion assay,[2] we found that the inhibitory effects of abyssomicins, which could be depleted upon addition of *p*ABA, were localized in the bacterial biosynthesis pathway between chorismate and *p*ABA. In the subsequent HPLC/diode array analysis three compounds were detected which could not be assigned to known metabolites by means of our HPLC-UV/Vis Database.[15] Compounds **1**–**3** (Scheme 1) were purified by subsequent size-exclusion and adsorption chromatography and preparative reversed-phase HPLC, and further analyzed by mass spectrometry, NMR spectroscopy, and X-ray structure determination.




***Scheme 1.*** Structural formulae of abyssomicins B (**1**), C (**2**), and D (**3**) showing the *R* configuration at C(11).

The high-resolution ESI-FTICR mass spectra of the Na adducts of **1**, **2**, and **3** showed masses of 400.13654, 369.13079, and 371.14663 Da, respectively, corresponding to the molecular formulas $C_{19}H_{23}NO_7$ (**1**) [$(M+Na)^+_{theor} = 400.13667$, $\Delta m = 0.34$ ppm], $C_{19}H_{22}O_6$ (**2**) [$(M+Na)^+_{theor} = 369.13085$, $\Delta m = 0.20$ ppm], and $C_{19}H_{24}O_6$ (**3**) [$(M+Na)^+_{theor} = 371.14650$, $\Delta m = 0.32$ ppm]. From 1D and 2D NMR spectra the partial structural motif shown in Figure 1 was determined for all three compounds, indicating that compounds **1**–**3** indeed belong to the same family. A second partial fragment of **2** was identified as bearing a Michael system: that is, a *trans* double bond ($J = 13.5$ Hz) in conjugation with a ketone. Database searches of the molecular formulae of **1**–**3** as derived from FTICR-MS combined with the structural motif determined from NMR experiments (Figure 1) did not result

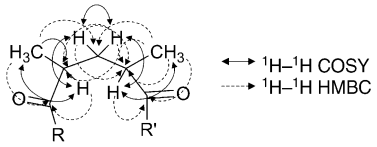



***Figure 1.*** Characteristic structural motif of abyssomicins **1**–**3** determined by NMR experiments

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[**] The work of R.D.S. has been supported by an Emmy Noether Fellowship for young investigators of the Deutsche Forschungsgemeinschaft (DFG) (SU 239/2-1) and the DFG-Forschergruppe (SU 239/1-1 and 1-2). We thank Prof. H.-J. Meyer for support in X-ray analyses and Prof. M. E. Maier for helpful discussions.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

  DOI: 10.1002/anie.200353160 

in matches with any known compound from the DNP database[3] or from the CAS-online database.[4]

For further structure determination we focused on both the evaluation of NMR data and crystallization experiments. We obtained single crystals of all three compounds (**1**–**3**), and X-ray structure determination revealed the relative configurations shown in Figure 2. NMR analyses performed on

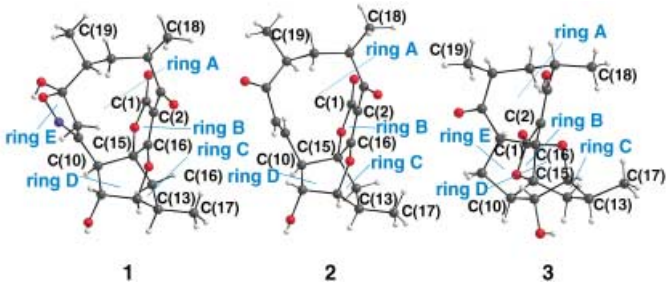



***Figure 2.*** X-ray structures of abyssomicins B (**1**), C (**2**), and D (**3**) showing the relative configuration. The ring systems are assigned alphabetically.

compound **2** (Table 1) confirmed the X-ray data. The absolute stereochemistry of compound **3** was determined by both the Mosher method[5,6] and the Helmchen method.[7,8] Both methods consistently suggest an *R* configuration at C(11) of ring D. On the basis of structural analogy, the same configurations were assumed for the analogous positions in compounds **1** and **2.** Thus the absolute configurations were deduced for **1**–**3**.

***Table 1:*** $^{1}$H and $^{13}$C NMR shifts of abyssomicin C.[a]

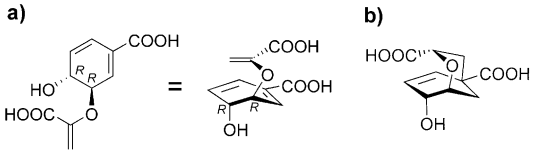
| No. | $\delta(^{1}H)$ [ppm] | Multiplicity and coupling constants [Hz] | $\delta(^{13}C)$ [ppm] |
|---|---|---|---|
| 1 | – | – | 173.8 |
| 2 | – | – | 106.7 |
| 3 | – | – | 202.8 |
| 4 | | | 45.3 |
| 5 | 2.01 (a) | m, $^{2}J_{5a,5b}=14.1$; $^{3}J_{5a,4}=11.2$; $^{3}J_{5a,6}=10.1$ | 42.3 |
| | 1.44 (b) | m, $^{2}J_{5b,5a}=14.1$; $^{3}J_{5b,4}=2.7$; $^{3}J_{5b,6}=1.6$ | |
| 6 | | | 50.3 |
| 7 | – | – | 208.4 |
| 8 | 6.55 | d, $^{3}J_{8,9}=13.5$ | 137.1 |
| 9 | 5.98 | dd, $^{3}J_{9,8}=13.5$; $^{3}J_{9,10}=9.5$ | 137.3 |
| 10 | 2.99 | dd, $^{3}J_{10,9}=9.5$; $^{3}J_{10,11}=6.1$ | 51.5 |
| 11 | 5.06 | dd, $^{3}J_{11,10}=6.1$; $^{3}J_{11,12}=3.3$ | 76.0 |
| | 4.59 (OH) | – | – |
| 12 | 4.57 | d, $^{3}J_{12,11}=3.3$; $^{3}J_{12,13}=$ n.d.[b] | 88.9 |
| 13 | 2.73 | n.d.[b] | 28.1 |
| 14 | 1.26 (a) | dd, $^{2}J_{14a,14b}=12.4$; $^{3}J_{14a,13}=4.8$ | 39.6 |
| | 2.69 (b) | dd, $^{2}J_{14b,14a}=12.4$; $^{3}J_{14b,13}=$ n.d.[b] | |
| 15 | – | – | 81.1 |
| 16 | – | – | 189.8 |
| 17 | 1.17 | d, $^{3}J_{17,13}=7.0$ | 21.5 |
| 18 | 1.09 | d, $^{3}J_{18,4}=6.7$ | 19.3 |
| 19 | 1.11 | d, $^{3}J_{19,6}=7.2$ | 23.0 |

[a] $c=20.5$ mg mL$^{-1}$, [D$_4$]methanol, 298 K. [b] n.d. = not determined.

Compounds **1**–**3** are previously unknown natural products and were named abyssomicins B, C (**1**, **2**), and D (**3**) (from "abyss", referring to the site of strain habitat). In antibacterial testing, however, only abyssomicin C (**2**) showed antibiotic activity against Gram-positive bacteria including pathogenic *Staphylococcus aureus* strains.[2] The minimal inhibition concentrations against methicillin-resistant *S. aureus* (MRSA) and a multiresistant including vancomycin-resistant *S. aureus* strain were 4 μg mL$^{-1}$ and 13 μg mL$^{-1}$, respectively.[2]

Considering their low molecular masses, abyssomicins contain an unusually large number of ring systems (four in the case of **2** and five for **1** and **3**), quarternary carbons, and stereocenters. The tetronic acid motif of ring B indicates a likely relationship to tetrocarcin-type antibiotics.[9] Further structural features characteristic of all three compounds are two methyl groups (C(18), C(19)) of ring A, and the oxabicyclooctane ring system of rings C and D.

The structures of the abyssomicins could be simply catalogued with many other newly discovered antibiotics. However, from the described structures we see several interesting details that suggest a possible mode of action and cast a different light on these compounds. A closer consideration of the oxabicyclooctane system (C and D rings) in **1**–**3** revealed striking similarities to the solution conformation of chorismate[10,11] as well as to synthetic transition-state analogues of chorismate mutase inhibitors (Scheme 2).[12,13]




***Scheme 2.*** a) Diaxial conformation of chorismate in aqueous solution.[10] b) Synthetic transition-state analogue for chorismate mutase inhibition.[13]

Furthermore, the fact that the Michael system (C(7)–C(9)) adjacent to the oxabicyclooctane system of abyssomicin C (**2**) is missing in the antibiotically inactive abyssomicins B (**1**) and D (**3**) is a strong indication for its significance for antibiotic activity. Currently, our hypothesis is that in bacterial metabolism of the abyssomicins, abyssomicin D is derived from abyssomicin C by Michael addition of a hydride equivalent (with NADH as a putative hydrogen source). Abyssomicin B might be the result of a Michael addition of hydroxylamine (as yet of unknown origin) followed by oxidation, leading to an unusual five-membered heterocycle. We therefore speculate that the Michael system is directly involved in the mechanism of action of abyssomicin C, and that nucleophilic amino acid side chains from the targeted enzyme are covalently trapped. Thus, subsequent biosynthesis steps from the branch-point metabolite chorismate especially to *p*ABA are inhibited. Viewed in this way, abyssomicin C would represent a substrate mimetic that traps the enzyme by reaction with a Michael system.

We assume that abyssomicins are formed by the polyketide biosynthesis pathway in analogy to other tetrocarcin-related antibiotics.[14] Further investigations on the abyssomi-

cins with regard to the biosynthesis, the exact determination of the targeted enzyme(s), and the mode of action are currently in progress.

## Experimental Section

LC-MS experiments were performed on a Bruker Esquire 3000plus (Bruker-Daltonics, Bremen, Germany) coupled to an Agilent 1100 HPLC system (Agilent, Waldbronn, Germany). FTICR-ESI mass spectra were recorded on an APEX II FTICR mass spectrometer (4.7 T, Bruker-Daltonics, Bremen, Germany). NMR experiments were recorded on an AMX600 NMR spectrometer (Bruker, Karlsruhe, Germany) equipped with a 5-mm triple-resonance probehead with *z* gradients. Single-crystal X-ray data were collected on an STOE IPDS 1 system with monochromated $Mo_{K\alpha}$ radiation. CCDC 226556–226558 (**1**–**3**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk). The Mosher method[5,6] and the Helmchen method[7,8] for the determination of the absolute stereochemistry were performed in each case with 10 mg of abyssomicin D. Further physico-chemical data are given in the Supporting Information.

Received: October 27, 2003
Revised: January 12, 2004 [Z53160]
Published Online: April 26, 2004

**Keywords:** antibiotics · metabolism · Michael addition · natural products

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