Configurational assignment of rhizopodin, an actin-binding macrolide from the myxobacterium *Myxococcus stipitatus*†

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The relative and absolute stereochemistry of the 38-membered myxobacterial polyketide rhizopodin, a potent actin-binding macrolide, was determined by *J*-based configurational analysis in combination with molecular modeling and chemical derivatization.

Rhizopodin (**1**, Fig. 1) is a structurally unique polyketide isolated from the myxobacterium *Myxococcus stipitatus* by the groups of Höfle and Reichenbach in the early nineties.[1] It displays impressive biological properties including antifungal activity and potent cytotoxicity against a range of tumor cell lines in the low nanomolar range.[1] Its cytotoxicity has been attributed to its ability to interact with actin and disrupt the actin cytoskeleton by binding specifically to few critical sites of G-actin.[2,3] Recently, it has also been shown to reduce phagocytosis efficiency for yeast cells.[4] The planar structure of rhizopodin (**1**) was elucidated by Höfle and Steinmetz and was originally considered to be monomeric. Being originally considered to be monomeric,[1–4] it became apparent during the course of this study to be a C_2-symmetric dimer.[5] It is distinguished by a 38-membered macrolide ring, two conjugated diene systems in combination with two disubstituted oxazole systems and two enamide side chains. In addition, a total of 18 stereogenic centers are present in the carbon backbone of **1**. The important biological properties of rhizopodin and its natural scarcity, coupled with its intriguing molecular architecture, renders it an attractive compound for further development. Herein, we report determination of the full stereostructure of rhizopodin by application of *J*-based configuration analysis in combination with extensive ROESY experiments, molecular modelling, and synthetic derivatization.

Optimal resolution of ^{1}H signals was obtained in CD_3OD and $CDCl_3$ at 600 MHz allowing complete assignment of all resonances.[7,8] The $^3J_{H,H}$ coupling constants were determined from a combination of homonuclear decoupling experiments, 1D TOCSY and 2D *J*-resolved spectra. The coupling constants and ROESY data suggested the C-15 to C-21 subunit and the C-24 to C-29 region to be relatively rigid, while a certain degree of conformational flexibility has to be considered within the C-2 to C-6 fragment.

As illustrated in Fig. 2(a), a large homonuclear coupling between H-25 and H-26 and between H-26 and H-27b indicated antiperiplanar relationships between these protons, supported by a small coupling from H-26 to H-27a. These data suggested the C-24–C-28 subunit resides in the depicted conformation **2**. Three key ROESY correlations, from H-27a to OMe-26, from H-28 to Me-25 and from H-28 to H-25 supported the relative assignment as shown, which was further confirmed by the close spectroscopic similarity with that of structurally related polyketides (see below).

Establishing a relationship between the two hydroxyl-bearing stereocenters at C-3 and C-5 (Fig. 2(b)) relied on confident assignment of the diastereotopic methylene protons at C-2 and C-4. A sequence of small and large $^3J_{HH}$ coupling constants within the C-2 to C-4 region suggested the presence of one major conformation, which was further supported by specific NOE correlations (H-2a–H-3 and H-3–H-4b). In a similar fashion, the respective data for the C-4 to C-6 subunit likewise indicated the presence of one major conformer by strong NOE correlations from H-4a to H-5, H-4b to–H-6 and H-5 to H-7, together with a much weaker NOE contact from H-4b to H-5.[9] In combinations, all these data indicated a 1,3-*syn* relationship between the substitutents at C-3 and C-5. This was supported by a series of further NOE correlations (H-2b–H-4a, H-3–H-5, H-3–H-6, H-3–H-7).

The coupling constants and ROESY data for the C-15 to C-21 subunit are shown in Fig. 2(c). These data suggested an

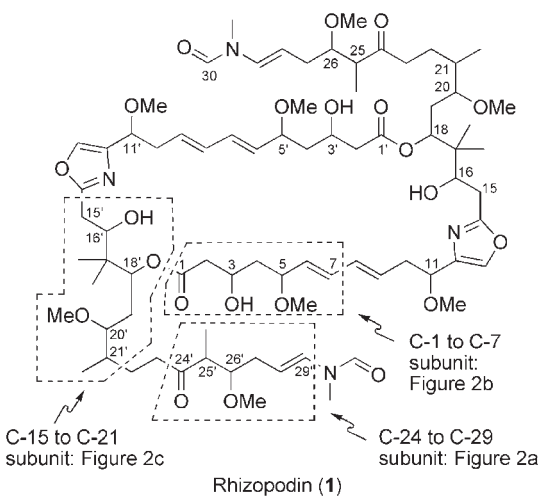

Fig. 1 Planar structure of rhizopodin.[6]

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† Electronic supplementary information (ESI) available: Tables of spectral and calculated data, copies of 1D and 2D NMR spectra for rhizopodin and comparison of its side chain NMR data with those of **6**, **7**, **8** and **9**. See DOI: 10.1039/b810405k

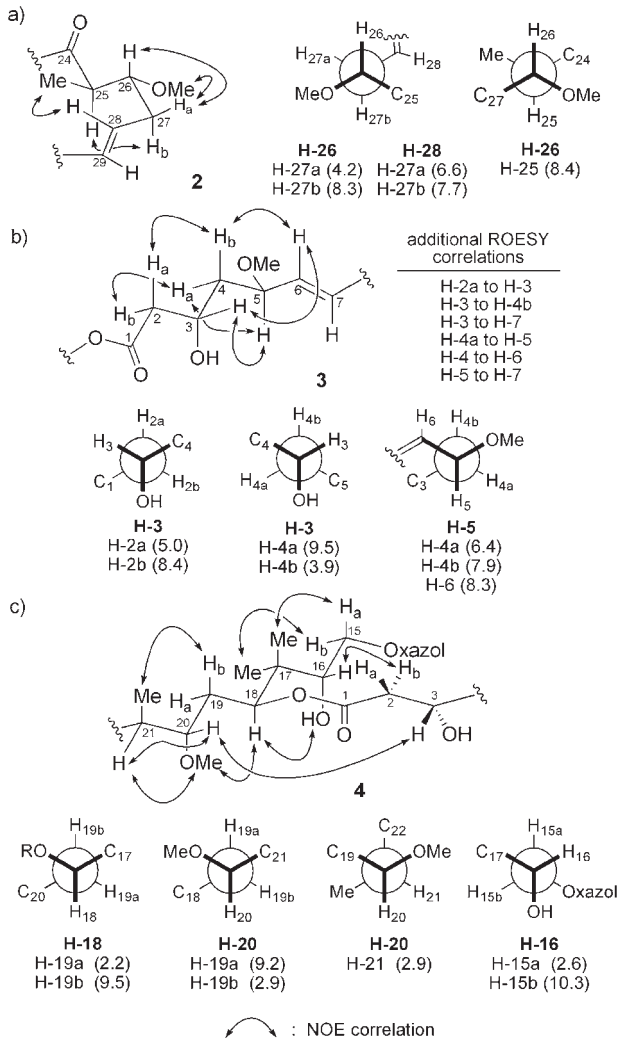

Fig. 2 Rotamers determined for subunits **2** (a), **3** (b) and **4** (c) of rhizopodin; coupling constants, $^{3}J_{H,H}$ (Hz), in parentheses.

anti-relationship between the hydroxyl-bearing stereogenic centers at Cs-16 and C-18, by strong NOE correlations between H-18 and OH-16 and from H-16 and H-2b, as well as equally strong NOE-correlations from H-15a and H-15b to both Me-17a and Me-17b, in combination with the observed coupling constants from H-16 to H-15a and H-15b.

Large homonuclear coupling constants from H-18 to H-19b and from H-19a to H-20 suggested antiperiplanar relationships between these protons, supported by small couplings H-18–H-19a and H-19b–H-20. A 18,20-*anti* relationship was deduced by strong NOE correlations between H-18 and OMe-20. Assignment of a 20,21 *syn*-relationship, in turn, relied on a small coupling between H-20 and H-21, in combination with strong NOE correlations between H-21 and OMe-20 and between Me-21 and H-19. The 18,20-*anti*, 20,21-*syn* assignment was further corroborated by a long-range NOE correlation from H-20 to H-3. These interactions suggest this fragment to reside in the conformation **4**, as depicted in Fig. 2(c). The relative assignment was further supported by an NOE correlation from H-16 to H-2b. Ultimately, this was confirmed by Mosher ester analysis of the tetra-Mosher ester of rhizopodin, which also enabled determination of the absolute configuration at C-3 (═C-3′) and C-16 (═C-16′) as *S*.

As determination of the relationship between fragment **4** and subunit **2** as well as correlation to the isolated stereogenic center at C-11 was not possible by *J*-based configurational analysis, molecular modelling was carried out on the possible stereochemical permutations, using Macromodel (Version 8.5), the MMFFS force field and the generalized Born/surface area (CB/SA) solvent model, favouring assignment of structure **5** (Fig. 3). In detail, 10 000 step Monte Carlo searches using conformational restraints based on dihedral angles resulted in a series of discrete families of low-energy conformations for **5**, which accounted for a number of key long-range ROESY correlations (*i.e.*, H-5–H-10, H-16, H-16–H-2b) and gave an acceptable match between the calculated dihedral angles and corresponding series of $^{3}J_{H,H}$ coupling constants.†

While a stereochemical correlation between the macrocyclic C-1–C-21 region and the side-chain C-25–C-26 fragment could not be deduced by this approach, the remarkable homology between the relative stereochemistry for C-3, C-16, C-18, C-20, C-21, C-25 and C-26, as assigned by these studies, and that of the structurally related polyketides jaspisamide A (**6**),[10] halichondramide (**7**),[11] mycalolide (**8**)[12] and ulapualide A (**9**),[13,14] suggests the full absolute and relative configuration of rhizopodin, as depicted in Fig. 3.[15] This assignment is in

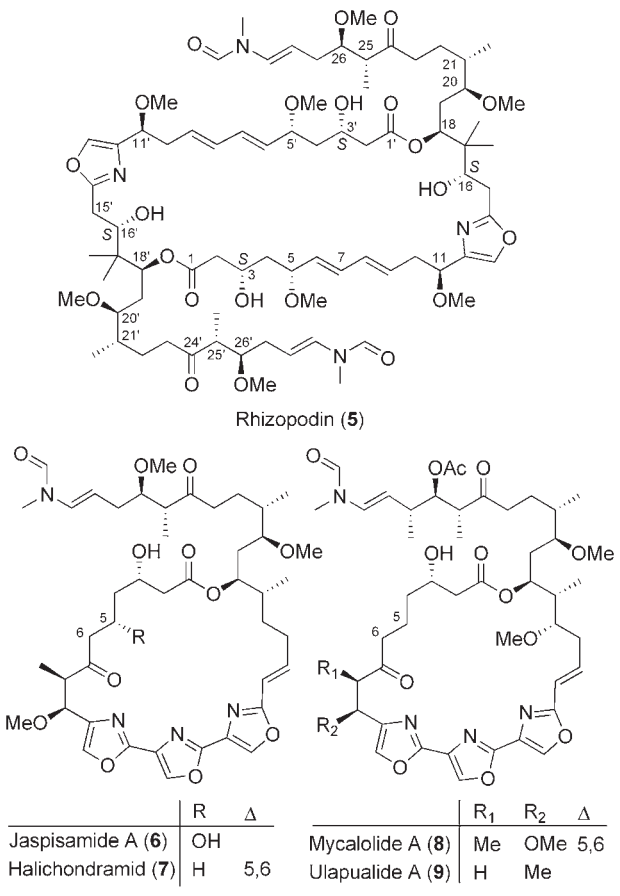

Fig. 3 Stereostructures for rhizopodin (**5**) and related macrolides (**6–8**).

complete agreement with the close similarity of relevant ^{13}C NMR and ^{1}H data for the side chain fragments of these macrolides (see ESI†). Notably, these polyketides likewise interact with the actin-network which further corroborates the viability of this homology consideration.

In conclusion, a full stereochemical assignment of the antimitotic polyketide rhizopodin is proposed as **5** (3*S*,5*R*,11*S*,15*S*,18*S*,20*S*,21*S*,25*R*,26*R*,3′*S*,5′*R*,11′*S*,15′*S*,18′*S*,20′*S*,21′*S*,25′*R*,26′*R*) on the basis of extensive NMR studies, chemical derivatization, molecular modelling and homology considerations. Confirmation of this proposal will rely on the stereocontrolled total synthesis of rhizopodin.

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Notes and references

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6 Notably, due to the C_2 symmetry of rhizopodin, the two monomeric halves (*i.e*. the C-1–C-29 and the C-1′–C-29′ fragment) are stereochemically identical.
7 All spectroscopic data were in agreement to those previously published: see refs. 1*a* and 5.
8 The coupling constants and NOE data in these solvents were very similar which suggests that rhizopodin adopts a similar conformation in these media.
9 The observed medium ^{3}J-coupling constants from H-5 to both H-4a and H-4b may be attributed to a certain degree of thermal flexibility. This is in agreement with NMR measurements at lower temperatures showing an increased H-4b–H-5 coupling constant together with a decreased value for H-4a to H-5.
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15 Notably, this stereochemical consensus holds also true for the hydroxyl bearing stereogenic center next to the oxazole-ring (C-11), which further supports our configurational assignment of this center.