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The Dynamic Structure of Jadomycin B and the Amino Acid Incorporation Step of Its Biosynthesis

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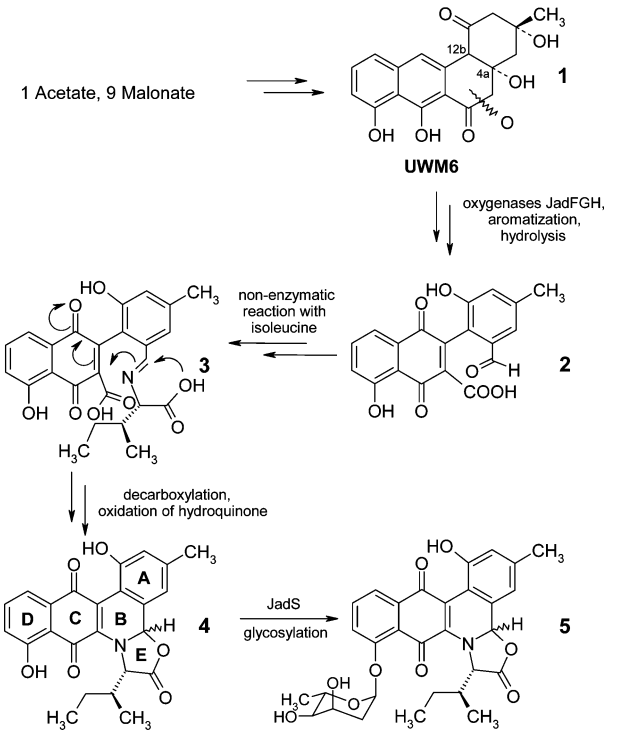
The jadomycins are a unique family of angucycline-derived antibiotics because of their pentacyclic 8*H*-benz[*b*]oxazolo[3,2-*f*]-phenanthridine backbone, which includes a dihydropyridine and an oxazolone ring. This unusual ring assembly is distinctive for the jadomycins, but its biosynthetic formation is unclear. However, it is obvious that the nitrogen heteroatom is derived from the amino acid isoleucine, a major constituent of the jadomycin production medium.[1] Jadomycin B (**5**) is the principal product of *Streptomyces venezuelae* ISP5230 when this strain is fermented under stress conditions, such as heat shock, ethanol treatment, or phage infection,[1] while chloramphenicol is the only antibiotic produced under normal growth conditions.[2] The jadomycins show biological activity against gram-positive and gram-negative bacteria, and against yeast. An interesting structural feature in this context is the deoxysugar L-digitoxose found in jadomycin B (**5**),[4] which distinguishes its structure from the minor congener jadomycin A (**4**). Because **5** displays anti-yeast activity,[1c] but **4** does not, this sugar moiety is of great importance for the bioactivity of the jadomycins.[3]

The key step of the jadomycin biosynthesis is the oxidative opening of the 5,6-bond of an angucyclinone intermediate, which likely leads to the formation of an aldehyde, possibly **2**, a compound that can easily react with the amino acid isoleucine, thereby forming aldimine **3**. As the starting point of this oxidative cleavage cascade, we favor UWM 6 (**1**)[4,5] and not rabelomycin,[6] the latter being readily formed spontaneously by 4a,12b-dehydration and oxidation of **1**.[4] The quinone structure of **3** allows a cascade of cyclization steps, initiated by the Michael addition of the imine nitrogen to the quinone moiety, through which the N-containing pentacyclic jadomycin skeleton is formed (Scheme 1). In contrast, Vining et al. postulated an initial Michael addition of isoleucine at the quinone moiety, followed by an intramolecular attack of the resulting secondary amine at the aldehyde and simultaneous lactonization.[6]

This amino acid incorporation can be either an enzyme-catalyzed or a spontaneous reaction. To date, no responsible enzyme candidate has been identified in the jadomycin gene cluster. Considering also the reactivity of the proposed intermediate **2**, as well as the excess of isoleucine in the production medium (the only significant nucleophile present), we favor this reaction to occur spontaneously, which in turn should lead to a mixture of **5**-diastereomers. To prove this hypothesis,[5] we (i) reinvestigated the structure of jadomycin B (**5**) and the production profile of *S. venezuelae* ISP5230 to find evidence for such a diastereomeric mixture, and (ii) replaced isoleucine in the production medium by other, structurally quite different amino acids to generate novel analogues of jadomycin B (**5**).

Scheme 1. Key Steps of the Jadomycin Biosynthesis: Proposed Nonenzymatic Incorporation of Isoleucine after the Oxidative 5,6-Bond Cleavage of an Angucyclinone Intermediate



A careful NMR analysis of jadomycin B (**5**) surprisingly revealed that it appears as an inseparable diastereomeric mixture with 67% 3a-*S* and 33% 3a-*R* configuration. This suggests that both forms exist in a dynamic equilibrium via, for example, an ion bond-stabilized zwitterionic intermediate, which itself is not observed due to a short lifespan (Scheme 2). Most significant for the assignment of these two diastereomers are the observed NOESY correlations between 1-H and 3a-H of the 3a-*S* isomer and between 3a-H and 1′-CH_3 of the 3a-*R* isomer, respectively, as confirmed by molecular modeling (Figure 1).

It is also apparent from Figure 1 that the ABC ring system of **5** is not in the same plane for each separate diastereomer. Therefore, the conformational restraints presented by the 8-C═O and the 7-OH groups might account for the driving force for this intriguing opening and closing mechanism of the oxazolone ring; that is, the apparently weak bond between C-3a and 2-O needs to be broken before the molecule can overcome the significant barrier for ring A to flip to the opposite face of ring C.

Replacing isoleucine by other amino acids in the culture medium of *S. venezuelae* led to the production of several novel jadomycins,[1c]

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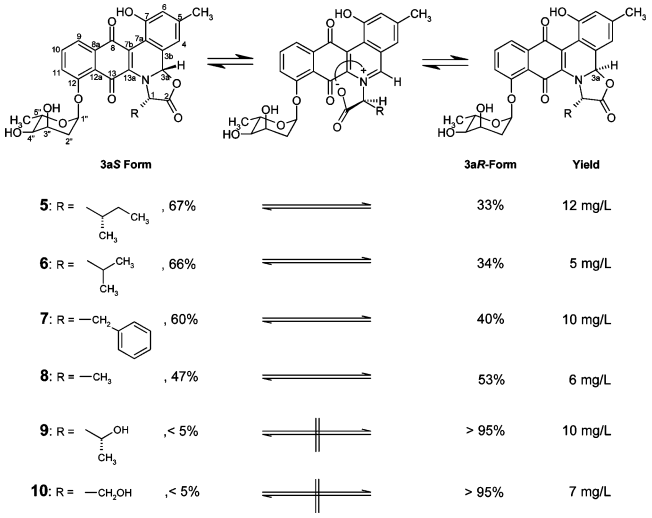
Scheme 2. Yields, and Dynamic Equilibrium Found for Jadomycins B (**5**), V (**6**), F (**7**), and Ala (**8**)[a]



[a] The percentages of the two diastereomeric forms were deduced from the integrals of the ^{1}H NMR spectra (see Supporting Information) or, for jadomycins T (**9**) and S (**10**), estimated from the HPLC chromatograms.

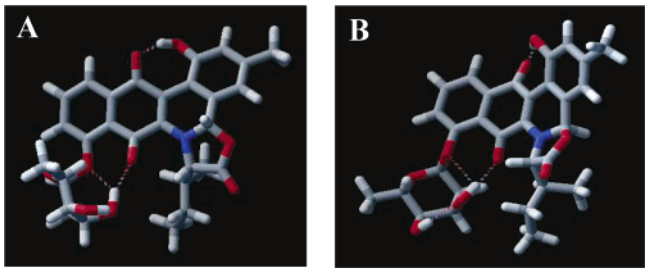


Figure 1. Molecular modeling of jadomycin B (**5**): (A) 3a-*S* diastereomer; (B) 3a-*R* diastereomer. Dashed lines represent hydrogen bonds.

some of which were characterized in detail (see Supporting Information), jadomycins V (**6**, valine), F (**7**, phenylalanine), Ala (**8**, alanine), T (**9**, threonine), and S (**10**, serine). Scheme 2 shows the structures of these new jadomycins and the distribution between the two 3a-diastereomers, which depends on the nature of the amino acid moiety. For instance, the short methyl residue in the alanine-derivative **8** does only interact minimally with the rest of the molecule, so that an almost 1:1 mixture was found, while the bulkier hydrophobic residues of **5**, **6**, and **7** force the molecule preferably into the 3a-*S* configuration.

This is due to less steric hindrance in this form between the amino acid-derived side chain and the deoxysugar. Consistently, the side chain of jadomycin V (**6**) causes essentially the same distribution pattern of diastereomers as observed for **5**. Jadomycins T (**9**) and S (**10**), however, which both contain hydroxyl groups in their amino acid side chains, do not show the dynamic equilibrium displayed by all of the other analogues. This is due to stabilization through a hydrogen bond between the side chain hydroxyl and 13-C═O, the presence of which has been confirmed by molecular modeling experiments (Figure 2). This additional H-bond locks the oxazolone ring into place and prevents it from breaking open. The two diastereomers are therefore chromatographically separable. Although we could only characterize the much more abundant 3a-*R* diastereomers of jadomycins S (**10**) and T (**9**) in detail, we also observed the minor metabolites with similar HPLC retention times and identical UV and HRMS spectra, which therefore likely

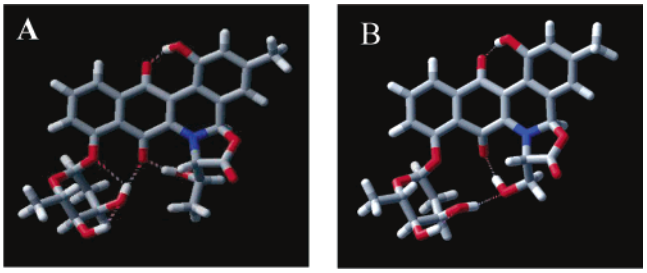


Figure 2. Molecular modeling of the predominant 3a-*R* diastereomers of jadomycin T (**9**) (A) and S (**10**) (B) each displaying an additional hydrogen bond (dashed lines) between 1′-OH and 13-C═O.

correspond to the 3a-*S* diastereomers of **9** and **10**. Most crucial for the identification of the 3a-*R* diastereomer of jadomycin T (**9**) was the observed NOESY correlation between 3a-H and 1′-H, which is confirmed by molecular modeling (Figure 2). Further jadomycins were identified from small-scale fermentations, supplemented with Leu, Tyr, Trp, Met, Asn, and His, respectively (only characterized by HPLC/MS, see Supporting Information).

In summary, the diastereomeric mixture found for jadomycin B (**5**) and other jadomycins along with the observed dynamic equilibrium (found for jadomycins B, V, F, and Ala) and the fact that various amino acids with different side chains can be incorporated into the jadomycin skeleton strongly support our hypothesis that the steps from **2** to **4** of the jadomycin biosynthesis outlined in Scheme 1 occur most likely nonenzymatically. A similar nonenzymatic aldimine formation as a biosynthetic key step embedded in the biosynthetic pathway of the alkaloid betalain was recently reported.[7] Nonenzymatic reactions also play essential roles for the formations of the urdamycins C, D, E, and H.[8]

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Supporting Information Available: Physicochemical data for jadomycin B and 11 of its analogues, including NMR data for compounds **5**−**10** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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