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Ring-Opening Dynamics of Jadomycin A and B and Dalomycin T

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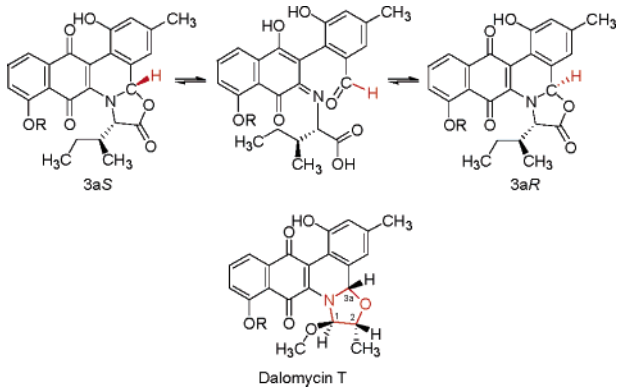
ABSTRACT



A novel oxazolone ring-opening and interconversion process between the two jadomycin diastereomeric forms has been characterized by NMR spectroscopy. An analogue, dalomycin T, has been isolated for the first time and does not undergo interconversion.

Novel atropisomeric intermediates have been identified that are involved in the interconversion of the diastereomeric 3a*R* and 3a*S* forms of both jadomycin A and B. This has enabled us to present a new mechanism for this interconversion process. Further evidence for the structural requirements of the interconversion was obtained from the first isolation of dalomycin T aglycone and dalomycin T, novel jadomycin-derived derivatives, which exist in only one isomeric form and do not undergo interconversion.

The jadomycins are a group of antibiotics active against both Gram-positive and Gram-negative bacteria, as well as drug-resistant cancer cell lines.[1,2] They are produced by *Streptomyces venezuelae* ISP5230 when the bacteria are subjected to environmental stress.[3,4] A unique distinguishing structural feature of the jadomycins is the five-membered oxazolone ring formed by incorporation of an amino acid (Figure 1).[3] The addition of the amino acid is most likely nonenzymatic,[5] and thus, analogues have been produced using various natural[5] and nonnatural[6,7] amino acids, extending the reservoir of potentially active therapeutic agents. Within the jadomycin family, substitution at C-12 with the 2,6-dideoxysugar, L-digitoxose, distinguishes jadomycin B from jadomycin A (Figure 1) and imparts improved bioactivity. During the biosynthesis of jadomycin, it has been proposed that incorporation of the amino acid occurs prior to the addition of the dideoxysugar.[8]

Two different diasteromeric forms of jadomycin B were recently reported (Figure 1),[5] and it was suggested that the

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(1) Doull, J. L.; Singh, A. K.; Hoare, M.; Ayer, S. W. *J. Ind. Microbiol.* **1994**, *13*, 120.
(2) Zheng, J. T.; Rix, U.; Zhao, L.; Mattingly, C.; Adams, V.; Quan, C.; Rohr, J.; Yang, K. Q. *J. Antibiot.* **2005**, *58*, 405.
(3) Ayer, S. W.; McInnes, A. G.; Thibault, P.; Walter, J. A.; Doull, J. L.; Parnell, T.; Vining, L. C. *Tetrahedron Lett.* **1991**, *32*, 6301.
(4) Doull, J. L.; Ayer, S. W.; Singh, A. K.; Thibault, P. *J. Antibiot.* **1993**, *46*, 849.
(5) Rix, U.; Zheng, J.; Remsing Rix, L. L.; Greenwell, L.; Yang, K.; Rohr, J. *J. Am. Chem. Soc.* **2004**, *126*, 4496.
(6) Jakeman, D. L.; Farrell, S.; Young, W.; Doucet, R. J.; Timmons, S. C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1449.
(7) Jakeman, D. L.; Graham, C. L.; Reid, T. R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5280.

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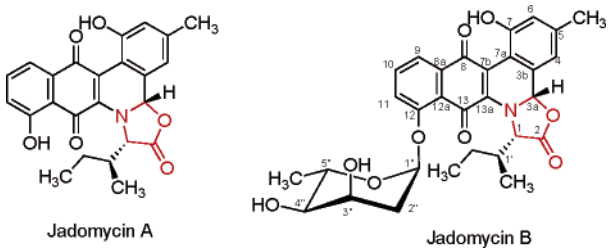
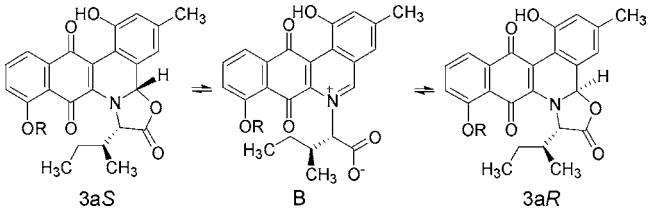


Figure 1. Jadomycin A lacks the 2,6-dideoxysugar, L-digitoxose, attached to C-12 of jadomycin B. The distinctive oxazolone ring of the jadomycins is in red. Only the major stereoisomer (3a*S*) is shown.

jadomycin B diastereomeric mixture could be the result of a dynamic equilibrium, via an ion bond-stabilized zwitterionic intermediate (B), which itself is not observed due to a short life span (Scheme 1); however, no spectroscopic

Scheme 1. Proposed Interconversion by Rohr and Co-workers[5]
R = H or L-digitoxosyl



evidence was provided for this species.[5] In an attempt to structurally characterize the interconversion between the jadomycin diastereomers, we have investigated the effects of acidic and basic conditions on the jadomycins and dalomycin T. We have clearly observed a novel aldehyde intermediate, through which interconversion of the diastereomers proceeds, that is in contrast to the intermediate species proposed by Rohr and co-workers.[5]

We have observed differences between the ratio of diastereomers at C3a for jadomycin B (≈3:2 *S*/*R*) in comparison to jadomycin A (≈10:1 *S*/*R*). Jadomycin A and B are not readily soluble in buffered water at pH < 6.8. Therefore, to investigate the effects of acidic and basic conditions, ^{1}H NMR spectra (Figure 2 and Supporting Information) of aliquots of jadomycin A and B dissolved in acetone-d_6 and D_2O buffered with 50 mM phosphate (pH 1.4−11.0) were analyzed. As the pH was raised, the intensity of two new signals at ≈9.8 ppm (Figure 2B,C) increased and a color change occurred, indicating a change in conjugation of the benzoxazolophenanthridine skeleton.

The two new signals arise from non-water-exchangeable protons characteristic of either aldehyde or pyridinium protons (potentially H-3a of the proposed intermediate B in

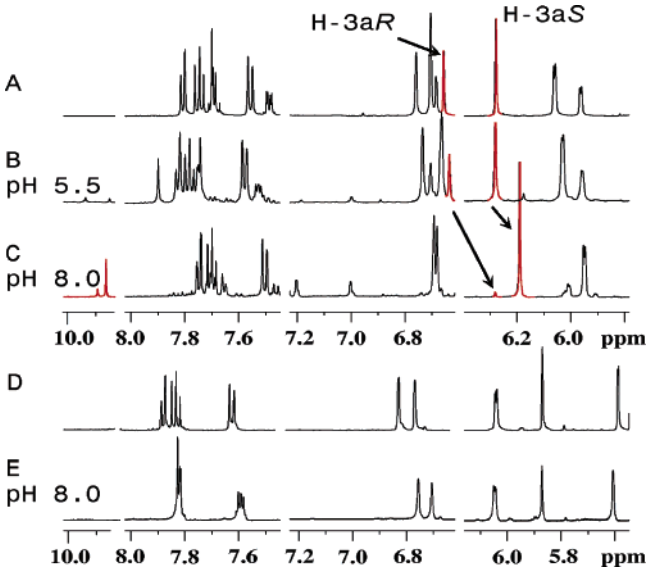


Figure 2. Jadomycin B dissolved in acetone-d_6 exists in two diastereomeric forms as observed in the ^{1}H NMR spectrum (A, only downfield region is shown). The intensity of two new peaks at ≈9.8 ppm increases as the alkalinity of added D_2O is increased (B and C). For dalomycin T (D), addition of buffered D_2O does not significantly affect the spectrum or produce signals at 9.8 ppm (E). Signals outlined in red are from H-3a.

Scheme 1). From subsequent ^{1}H−^{13}C HSQC and selective inversion exchange experiments, these signals were determined to correspond to aldehyde protons that are both in slow chemical exchange with H-3a*S* and H-3a*R* (Figure 3

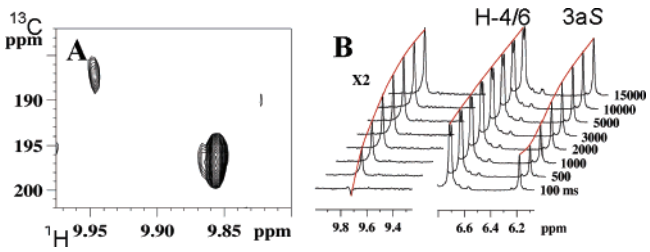


Figure 3. From the ^{1}H−^{13}C HSQC spectrum of jadomycin B in acetone-d_6/H_2O (A, pH 8.0), the two new peaks are from protons that are directly bonded to aldehydic carbons at chemical shifts of 187 and 196 ppm. Note: a search of the literature revealed that ^{13}C chemical shifts of >185 ppm are very typical of aldehydes, whereas pyridinium carbons are typically <155 ppm.[9,10] From selective inversion exchange experiments (B, pH 11.0), these aldehyde protons are in slow chemical exchange with H-3a*S* and H-3a*R* (as opposed to, for example, the H-4/6 protons for which no effect is observed). The red line joins the tops of the peaks in B.

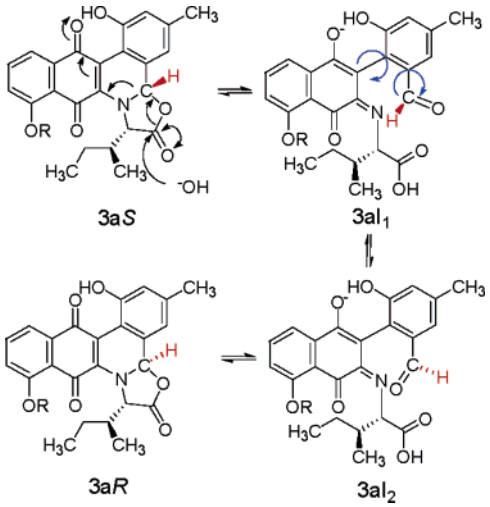
and Supporting Information). Thus, our spectroscopic data provides clear evidence for an aldehyde intermediate, rather than an iminium ion such as species B. In addition, for a

(8) Yang, K.; Han, L.; Ayer, S. W.; Vining, L. C. *Microbiology* **1996**, *142*, 123.

jadomycin B sample that was dissolved in D_2O (no acetone) at pH 8.0, exchange cross-peaks between 3a*R* and 3a*S*, and the two aldehyde signals were observed in both dimensions within ROSEY/NOESY spectra (Supporting Information), indicating that the 3a*R* and 3a*S* forms of jadomycin interconvert via these two aldehydic intermediates. This sample of jadomycin B was subsequently acidified (to pH 4.0) and the resultant precipitate redissolved in acetone-d_6. The spectrum obtained was virtually identical to the initial spectrum of jadomycin B dissolved in acetone-d_6 (Supporting Information, Figures 1S A−C), demonstrating that these intermediate jadomycin B species form under neutral/basic conditions and convert back into jadomycin B when acidified.

The intermediates between the 3a*S* and 3a*R* forms of the jadomycins are likely formed by nucleophilic attack of hydroxide ions at the carbonyl center of the oxazolone ring, with subsequent opening of the oxazolone and dihydropyridine rings forming an aldehyde (Scheme 2). Rotation about either the 7a−7b or 3a−3b C−C bond results in two forms of the aldehyde ($3aI_1$ and $3aI_2$). Chemical shift differences of substitutents fixed on opposite sides of aromatic rings have been observed for biphenyl derivatives.[11] Interestingly, the structure of these intermediates is highly analogous to one of the reactive intermediates proposed by Vining and coworkers for the biosynthesis of jadomycin B.[8] Upon the reverse ring closure reaction, the *R* or *S* diastereomer can reform depending on the rotational position (about the 3a−3b bond) of the aldehyde group.

Scheme 2. Interconversion of Jadomycin Diastereomers[a]



[a] The interconversion between the 3a*S* and 3a*R* configuration is a result of an opening of the oxazolone and dihydropyridine rings by addition of hydroxide ions at the carbonyl center. Rotation about a C−C bond results in the observation of two signals in the NMR spectrum for the aldehyde species ($3aI_1$ and $3aI_2$).

The unique dynamic equilibrium between the jadomycin B diastereomers and their corresponding ring-open forms offers a potentially novel mechanism for a bioactive molecule to traverse cell membranes. The ring-closed hydrophobic species will more easily pass through a lipophilic cell membrane and subsequently ring-open to the hydrophilic form inside a cell. In addition, the ring-open species formed at physiological pH may have a significant influence on the antibiotic and cytotoxic activity of the jadomycins, particularly in view of the different acidic environments of different cell types. The impact of the dynamic diastereomeric interconversion on the biological activity and function of the jadomycins is currently being investigated.

We repeatedly observed, and subsequently isolated, a less polar red compound from *S. venezuelae* ISP5230 cultures using L-threonine as the amino acid, in addition to isolating jadomycin T[5]. The compound was isolated when the reversed-phase C18 resin used to capture metabolites from the culture extract was washed with aqueous buffer (pH 7.0) prior to elution with organic solvent. No evidence for this red compound was observed in cultures extracted with ethyl acetate. The presence of a distinctive OMe signal at 3.52 ppm which correlated to H-1 in the HMBC initially alerted us to the change in the oxazolone ring. COSY correlations between H-2 and both H-1 and 2-CH_3 further supported the proposed structure (Figure 4), now called dalomycin T. High-

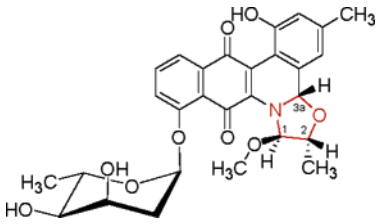


Figure 4. Dalomycin T contains the same angucycline framework and dideoxysugar as jadomycin B. Dalomycin T is distinguished from jadomycin B by the oxazolidine ring outlined in red.

resolution electrospray ionization mass spectral data also supported the proposed structure. Comparison of the 100 (B), 200 (C), 300 (D), and 400 (E) ms 1D selective NOESY spectra (obtained by inversion of H-3a, Figure 10S) of dalomycin T showed connections from H-3a to H-4, H-2, 1-OCH_3, and 1′-CH_3. The NOE transfer from H-3a to H-2 appears after 100 ms whereas the NOE transfer from H-3a to 2-CH_3 shows up after 400 ms confirming that dalomycin T exists exclusively in the 3a*S* configuration. From the *J*-coupling between the H-2 and H-1 (1.5 Hz which is indicative of a 90° dihedral angle) and the NOE transfer of H-3a to the 1-OCH_3, the stereochemistry at H-1 was determined to be *S*. The absolute configurations of centers 1 and 3a in dalomycin T are based on the assumption that the stereochemistry at center 2 was retained from L-Thr. Subsequently, the less polar aglycone of dalomycin T was also isolated and characterized. The five-membered ring derived from L-threonine in dalomycin T is not susceptible

(9) Stark, L. M.; Fin, X. F.; Flippin, L. A. *J. Org. Chem.* **2000**, *65*, 3227.
(10) Marek, R.; Tousek, J.; Dostal, J.; Slavik, J.; Dommisse, R.; Sklenar, V. *Magn. Reson. Chem.* **1999**, *37*, 781.
(11) Clayden, J. *Chem. Commun.* **2004**, *2*, 127.

to base hydrolysis (Figure 2D,E), confirming that the carbonyl group of the oxazolone is essential for ring opening (Scheme 2). In an attempt to prepare a related dalomycin T congener, we fed *S. venezuelae* ISP5230 L-threonine methyl ester, anticipating that the secondary hydroxyl functionality would potentially cyclize with the aldimine intermediate to form a substituted oxazolidine ring. However, no significant color was observed in the culture growths, and by TLC, no candidate species were observed. This observation, coupled with our evidence that we only observed dalomycin T after treatment of culture supernatants at basic pH on the C18 reversed-phase resin, likely indicates a chemical, rather than an enzymatic formation, of dalomycin T during the isolation process. We speculate that the formation of dalomycin T, occurs through an as yet uncharacterized series of chemical steps potentially including decarboxylation, hydroxylation, and *O*-methylation.

In summary, we have characterized the novel aldehyde intermediates that are formed during the interconversion of the two diastereomeric forms of both jadomycin A and B. We have isolated for the first time novel and diastereomerically pure derivatives dalomycin T and dalomycin T aglycone that do not undergo interconversion.

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Supporting Information Available: Experimental growth conditions for *S. venezuelae* ISP5230 cultures, characterization data of novel compounds, and a description of NMR experimental results. This material is available free of charge via the Internet at http://pubs.acs.org.

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